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Anti-inflammatory Principles of the Plant Family Amaryllidaceae

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ABSTRACT

There is considerable interest in the utilisation of plants against inflammation. Over 50 species of the plant family Amaryllidaceae are known for such usage in traditional medicine. This review was undertaken to identify the chemical principles responsible for these anti-inflammatory effects. It describes the findings from *in vitro, in vivo* and *in silico* studies, as well as the probes made on the mechanisms of action. The literature search returned over 600 hits, of which around 130 were chosen for their relevance to the text. Over 140 compounds have thus far been screened for anti-inflammatory effects. These were mostly isoquinoline alkaloids but also included other classes of secondary metabolites such as chromones, flavonoids and triterpenoids. *In vitro* studies were carried out in mononuclear cells such as lymphocytes, monocytes, neutrophils and macrophages, against which no serious side effects were observed. The constituents were also effective against inflammation induced by physical and chemical stimuli in a variety of murine test subjects. Chief among the compounds were the isoquinoline alkaloids lycorine and narciclasine, which displayed potent effects against pain, swelling, asthma and arthritis, amongst others. From a mechanistic perspective, several of the compounds were shown to mediate in inflammatory pathways, notably via the modulation of both pro-inflammatory (such as NF-κB, TNF-α and IL-1) and anti-inflammatory (such as IL-10 and TGF-β) factors. Useful insights also emerged from active-site docking studies of some of the compounds. The Amaryllidaceae affords a rich and diverse platform for the discovery of potential anti-inflammatory drugs.

Introduction

As a primary host defence response, inflammation serves as a crucial tool to fend off injury and disease [1]. It seeks to eliminate the source of the attack, flush out affected cells and promote tissue repair [1]. The process involves blood vessels, immune cells and different molecular factors operating in tandem against inflammatory stimuli such as irritants, pathogens and damaged cells [2]. Acute inflammation is an instantaneous reaction to inducements such as shock, physical injury, burns and pathogens [1]. Its characteristic features include increased blood flow, vasodilation, capillary permeability and migration of neutrophils into infected tissue [2]. Chronic inflammation, as the name would suggest, is inflammation that occurs over an extended period of time [1]. The features seen with the acute form also persist during chronic inflammation, but in which case the composition of white blood cells (WBCs) changes so that neutrophils are superseded by macrophages and lymphocytes [2]. The simultaneous breakdown and regeneration of tissue is also a defining feature of chronic inflammation [2]. Common diseases that abound as a consequence of chronic inflammation include diabetes, asthma and arthritis [1].

Although the inflammatory response is contingent upon the nature and location of the initial assault, there is consensus in terms of the stages through which it proceeds [3]. These may be be summarised as follows: (i) recognition of harmful stimuli via cell surface pattern receptors, (ii) activation of inflammatory pathways, (iii) release of inflammatory markers and (iv) recruitment of inflammatory cells [3]. At the first stage, microbial structures referred to as pathogen-associated molecular patterns (PAMPs) trigger a response by activation of pattern-recognition receptors (PRRs) that are expressed in both immune and non-im-

mune cells [3]. A variety of endogenous signals produced during tissue or cell damage, known as danger-associated molecular patterns (DAMPS), can also be recognised by some PRRs [4]. Classes of PRR families include Toll-like receptors (TLRs), NOD-like receptors (NLRs), C-type lectin receptors (CLRs) and retinoic acid-inducible gene (RIG)-I-like receptors (RLRs) [4]. At stage two, primary inflammatory stimuli such as microbial products and cytokines (IL-1β, IL-6 and TNF-α) promote inflammation by interacting with their respective TLRs (IL-1R, IL-6R and TNFR) [4]. Receptor activation serves as a trigger for the inflammatory pathways, such as the MAPK, NF-κB, JAK and STAT signalling pathways [4]. During the third stage, there is inducible expression of pro-inflammatory cytokines (such as IL-1β, IL-6 and TNF-α), as well as other inflammatory proteins and enzymes, which can serve as biomarkers in disease diagnosis, prognosis and treatment [4]. For example, irregular activation of HMGB1, SOD, GPx, NADPH oxidase, iNOS and COX are thought to play significant roles in inflammation-related diseases such as cardiovascular disease and cancer [3]. Overproductions of ROS, MDA, 8-hydroxy-2-deoxyguanosine (8-OHdG) and isoprostanes as a consequence of oxidative stress can also serve as useful biomarkers since they are tied in with the activation of NF-κB, AP-1, p53 and STAT [3]. Inflammatory cytokine and protein expression facilitate the activation of the fourth stage in the inflammatory cascade, which is the recruitment of effector cells [4]. Neutrophils are the first cells to arrive at the site of inflammation, followed by monocytes, lymphocytes (comprising NK cells, T cells and B cells) and mast cells [4]. Neutrophils migrate towards sites of infection or inflammation via chemotaxis, where they play a key role in phagocytosis [4]. Inflammation resolution is necessary to avert the progression of acute inflammation to chronic inflammation [3]. It involves reduction in tissue infiltration by neutrophils, counter-regulation of chemokines and cytokines, macrophage transformation and initiation of healing [3].

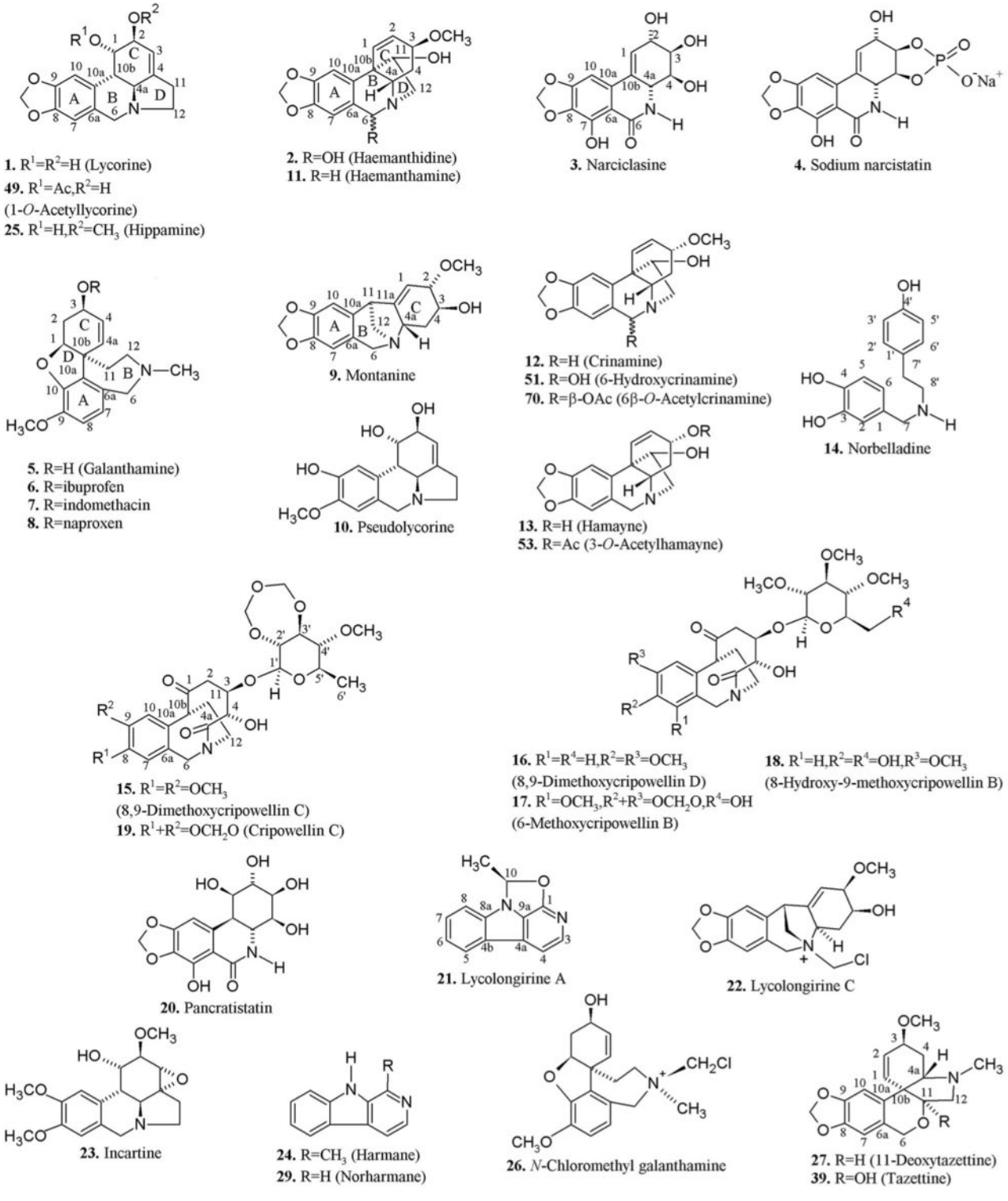
As a multi-billion dollar enterprise today, anti-inflammatory drugs are amongst the most common medicines dispensed via prescription or over-the-counter scheduling [2]. They are largely classified into steroidal (such as cortisone and prednisone) or non-steroidal anti-inflammatory drugs (NSAIDs) (such as aspirin, ibuprofen and naproxen) [2]. Herbal medicine has played a significant role in drug discovery in the area [5]. For example, the discoveries of morphine and aspirin stem from the traditional usage of their respective source plants (*Papaver somniferum* L. and *Salix alba* L.) for pain management [6]. The utilisation of members of the plant family Amaryllidaceae J. St.-Hil. against inflammatory diseases is a popular theme in traditional medicine (TM) [7]. As such, over 50 of its species from nearly 40 countries (notably in Africa and Asia) were identified with such traditional remediatory functions [7]. The extracts of over 70 species were examined in around 40 inflammation-based assays, where their activities *in vitro* and *in vivo* were shown to be significant [7]. Given this formidable background, we here set out to establish the chemical basis of such effects. The plausible mechanisms of action are also considered, as are pieces of information gathered from molecular models of study.

Alleviation of Pain

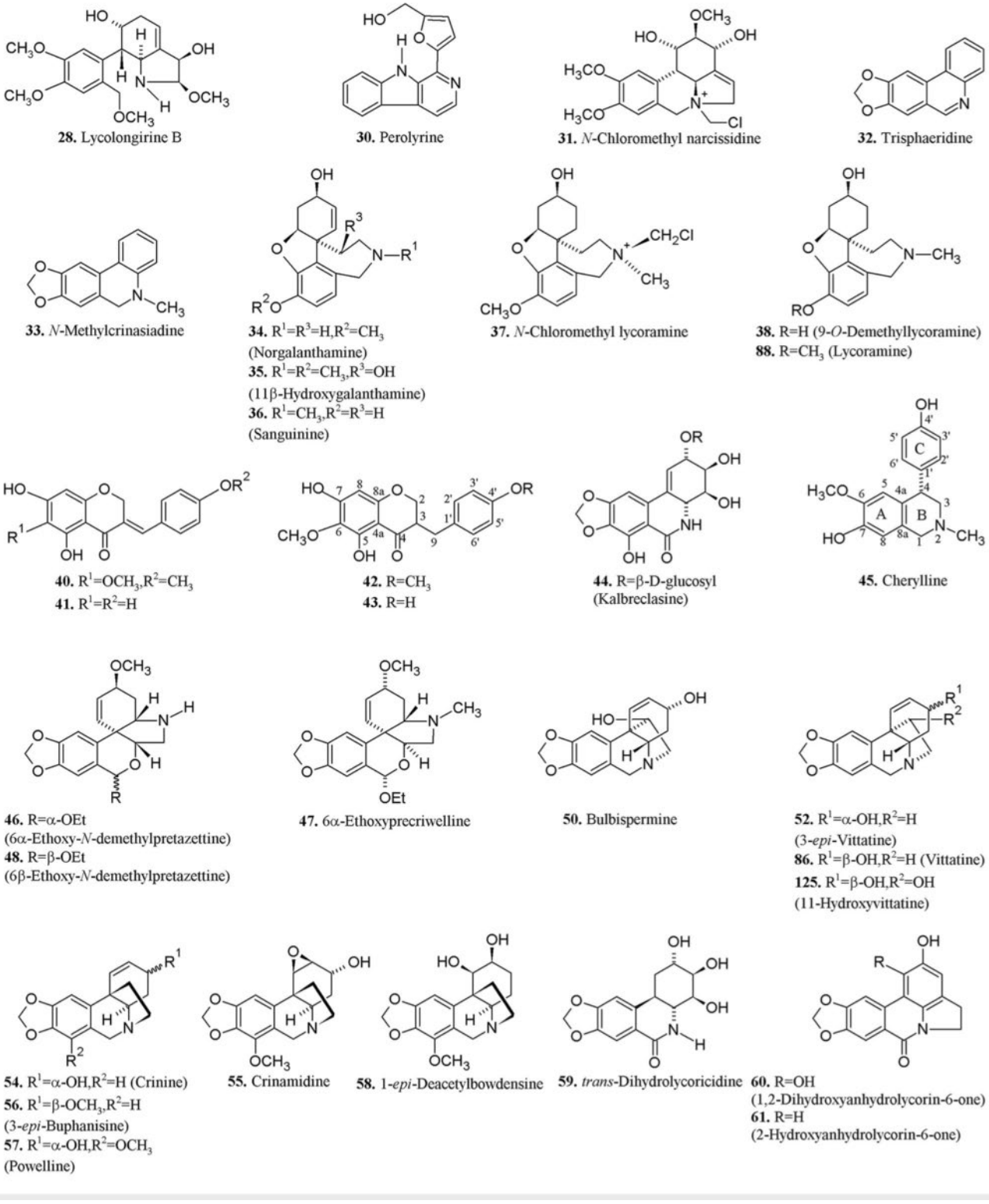
Pain has been defined as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" [8]. The sensation of pain is felt when specific nerves (called nociceptors) detect tissue damage and relay the information via the spinal cord to the brain [8]. The interpretation of such signals and the efficiency of the communication between the nociceptors and the brain will determine the severity of the sensation [8]. In terms of the ability of Amaryllidaceae alkaloids to alleviate pain, lycorine (**1**) and haemanthidine (**2**) (▶ **Fig. 1**) from an EtOH bulb extract of the Turkish medicinal plant *Sternbergia clusiana* (Ker Gawl.) Ker Gawl. ex Spreng. have been screened for analgesic effects [9]. Dose-dependent (25, 50 and 100 mg/kg) responses were observed via Koster's test for both compounds in albino mice, with the analgesic effects shown to be 58.5% and 48.8%, respectively, at the highest tested concentration [9]. These were much better than the effect of aspirin (28.8%) on test animals at the same concentration [9]. Furthermore, lycorine was well tolerated by test animals, with acute toxicity measurements showing it to be significant only at doses > 200 mg/kg [9]. Further pain-related effects were established for lycorine following its isolation from a bulb EtOH extract of *Sternbergia fischeriana* (Herb.) Roem. (synonymous with *Sternbergia vernalis* (Mill.) Gorer and J. H. Harvey) [10]. In this regard, its antinociceptive activity was probed via formalin-induced tail flicking and acetic acid-induced writhing models in Swiss albino mice [10]. Intraperitoneally administered lycorine (1.0 mg/kg) decreased by 62.7% abdominal constrictions (relative to aspirin, 44.5%, 300 mg/kg, oral) in animals exposed to acetic acid (6%, 60 mg/kg, intraperitoneal) [10]. In the tail flick test, lycorine (1.0 mg/kg) showed significant antinociceptive activity (30%) at the 90 min mark compared to aspirin (> 30% at each of the three tested time intervals) [10]. The ability of lycorine to alleviate pain could be related to its COX-inhibitory and prostaglandin-modulating effects, as discussed elsewhere in the text.

Alleviation of Swelling

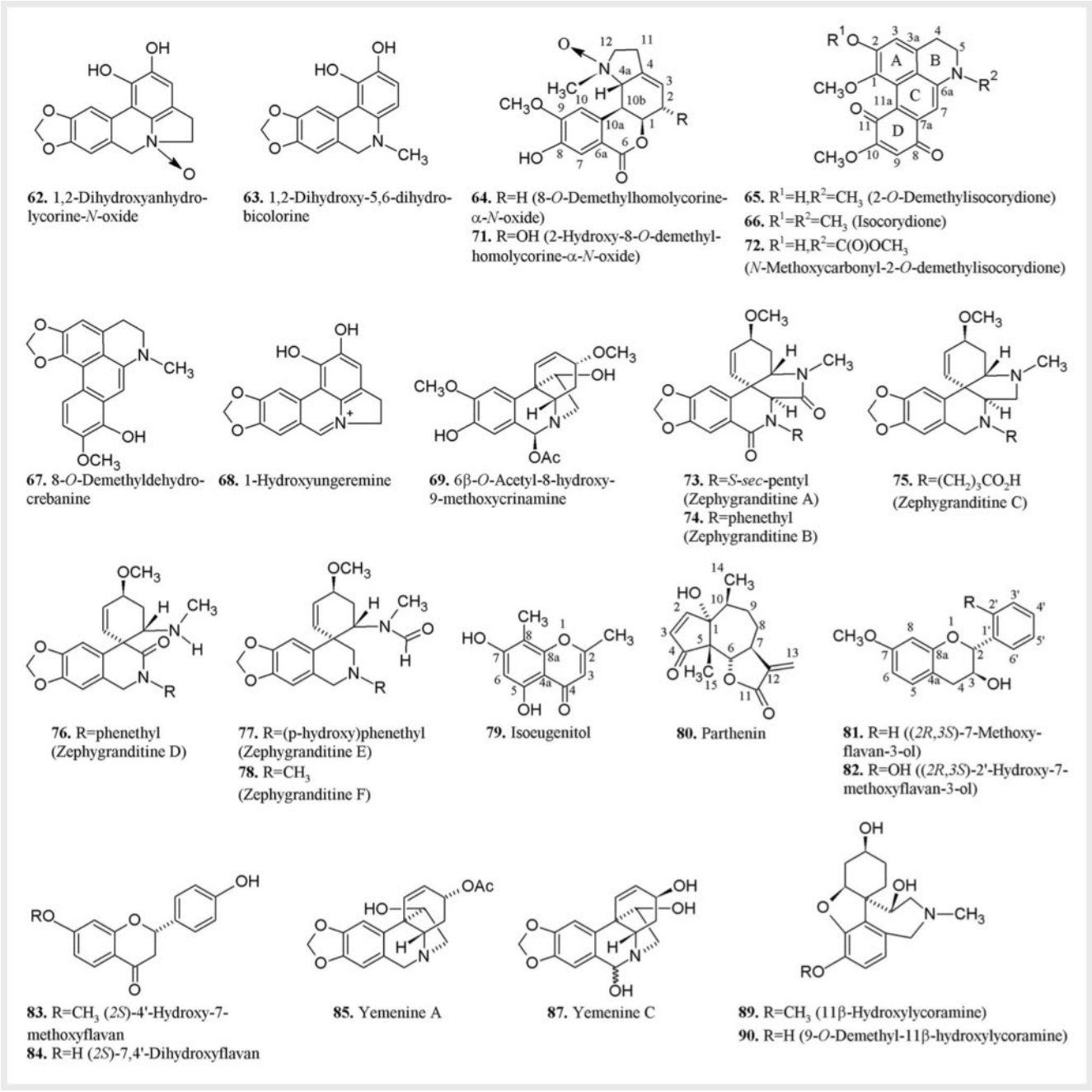
Swelling (or oedema) occurs when a part of the body increases in size, typically as a consequence of injury, inflammation or fluid retention [11]. It can occur in the skin, joints and other tissues and organs of the body [11]. Examined for such effects, lycorine (**1**) and haemanthidine (**2**) from *Sternbergia clusiana* dose-dependently inhibited (at 25, 50 and 100 mg/kg) carrageenan-induced paw oedema in mice, with 20.1% and 36.5% inhibition observed at the highest tested dosage, respectively (▶ **Fig. 6**) [12]. At this concentration, the inhibition by standard indomethacin (29.1%) lay between those observed for lycorine and haemanthidine [12]. Lycorine (1 mg/kg) also showed moderate activity (53.5% inhibition) in the self-same model of Sprague–Dawley rats, where indomethacin (95.7% inhibition, 3 mg/kg) was used for comparison purposes [10]. A sodium phosphate salt of narciclasine (**3**), referred to as sodium narcistatin (**4**), was shown to dose-dependently (1.75, 3.5 and 5.0 mg/kg/day/intraperitoneal) reduce (up to 28 days) dorsoplantar footpad swelling in male Lewis rats that was induced by CFA (complete Freund's adjuvant) (▶ **Table 1**) [13].



▶ **Fig. 1** Anti-inflammatory principles identified in members of the plant family Amaryllidaceae.



▶ **Fig. 2** Anti-inflammatory compounds found in members of the Amaryllidaceae.

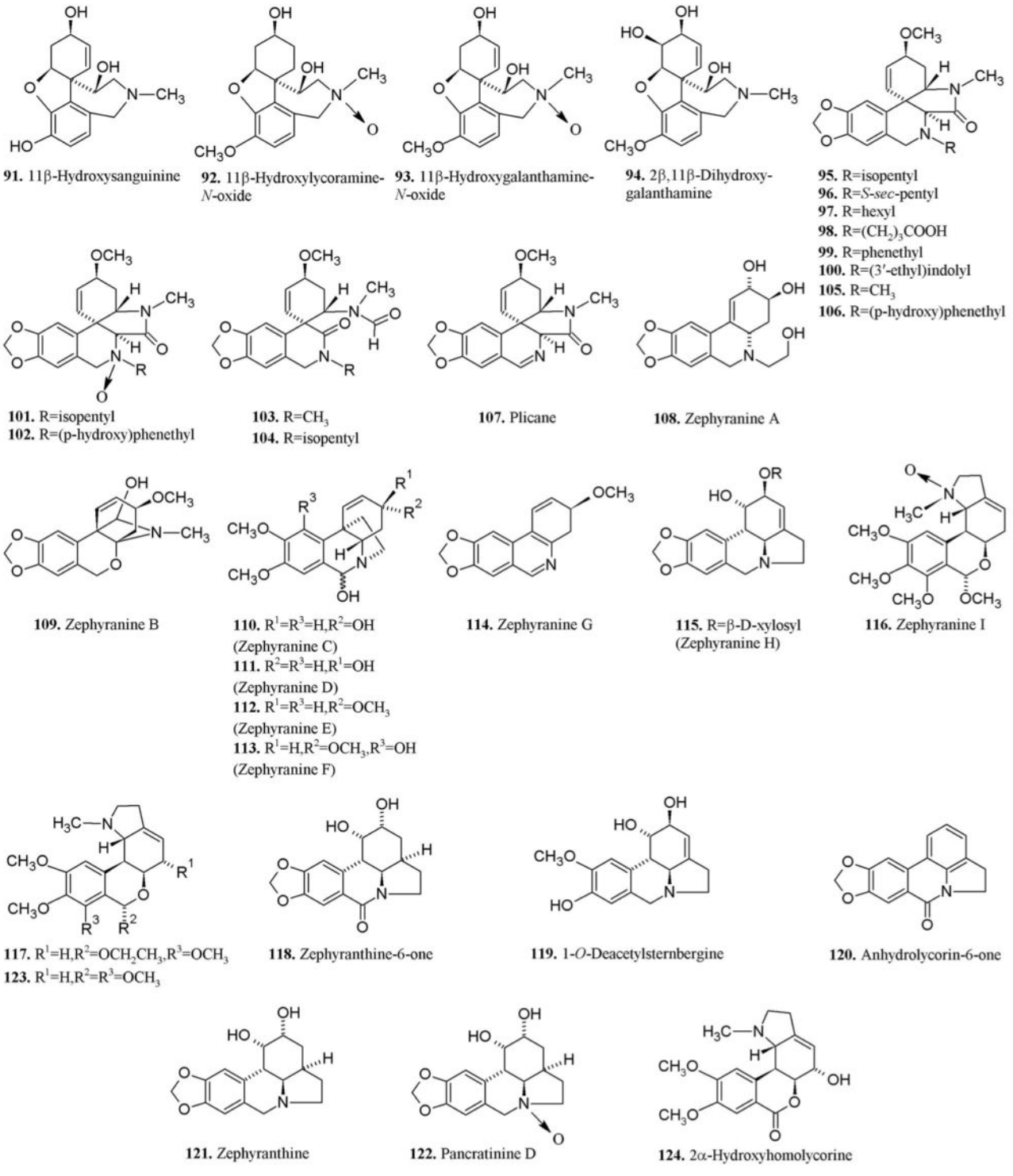


▶ **Fig. 3** Anti-inflammatory substances isolated from the Amaryllidaceae.

Crinumin was identified as a 67.7 kDa glycosylated serine protease from the leaf latex of *Crinum asiaticum* L. [14]. Its anti-inflammatory activity was ascertained via carrageenan-induced paw oedema in adult male Wistar rats [14]. Paw oedema was significantly reduced over 24 hr by crinumin (injected at 25 µg/kg), with the paw volume (0.273 mL) seen to be far lower than that in untreated controls (1.115 mL) [14]. Diclofenac-treated animals (50 µg/kg) showed a paw volume of 0.118 mL [14]. The anti-inflammatory effect of crinumin is here noteworthy since the proteinaceous constituents of the Amaryllidaceae (particularly its lectins) are strongly linked to antiviral effects.

Anti-Irritant Effects

While irritants are typically thought of as chemical entities, irritation can be caused by mechanical, thermal and radiative stimuli [15]. Irritation can also be induced via an allergic response as in contact dermatitis, pruritus and irritation of the mucosal membranes [15]. Up-regulation of choline and use of cholinergic agonists such as AChE inhibitors have been shown to suppress pro-inflammatory cytokine release [16]. Furthermore, AChE plays a crucial (and often overlooked) role in systemic inflammation [16]. The Alzheimer's drug galanthamine (**5**), a potent inhibitor of



91. 11β-Hydroxysanguinine

92. 11β-Hydroxylycoramine-*N*-oxide

93. 11β-Hydroxygalanthamine-*N*-oxide

94. 2β,11β-Dihydroxy-galanthamine

95. R=isopentyl
96. R=*S*-*sec*-pentyl
97. R=hexyl
98. R=$(CH_2)_3COOH$
99. R=phenethyl
100. R=(3′-ethyl)indolyl
105. R=CH_3
106. R=(p-hydroxy)phenethyl

101. R=isopentyl
102. R=(p-hydroxy)phenethyl

103. R=CH_3
104. R=isopentyl

107. Plicane

108. Zephyranine A

109. Zephyranine B

110. R^1=R^3=H,R^2=OH (Zephyranine C)
111. R^2=R^3=H,R^1=OH (Zephyranine D)
112. R^1=R^3=H,R^2=OCH_3 (Zephyranine E)
113. R^1=H,R^2=OCH_3,R^3=OH (Zephyranine F)

114. Zephyranine G

115. R=β-D-xylosyl (Zephyranine H)

116. Zephyranine I

117. R^1=H,R^2=OCH_2CH_3,R^3=OCH_3
123. R^1=H,R^2=R^3=OCH_3

118. Zephyranthine-6-one

119. 1-*O*-Deacetylsternbergine

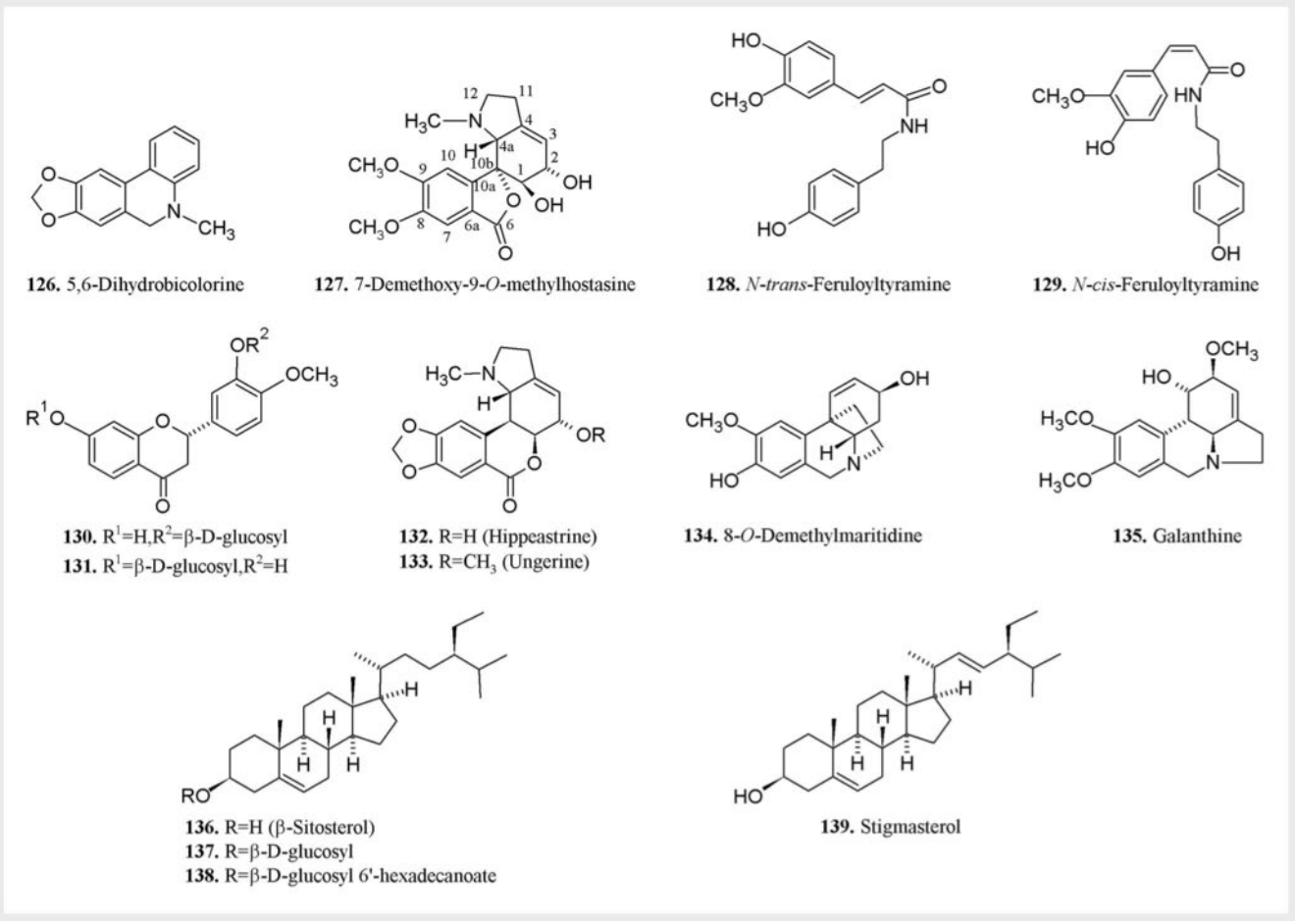
120. Anhydrolycorin-6-one

121. Zephyranthine

122. Pancratinine D

124. 2α-Hydroxyhomolycorine

▶ **Fig. 4** Anti-inflammatory entities described from the Amaryllidaceae.

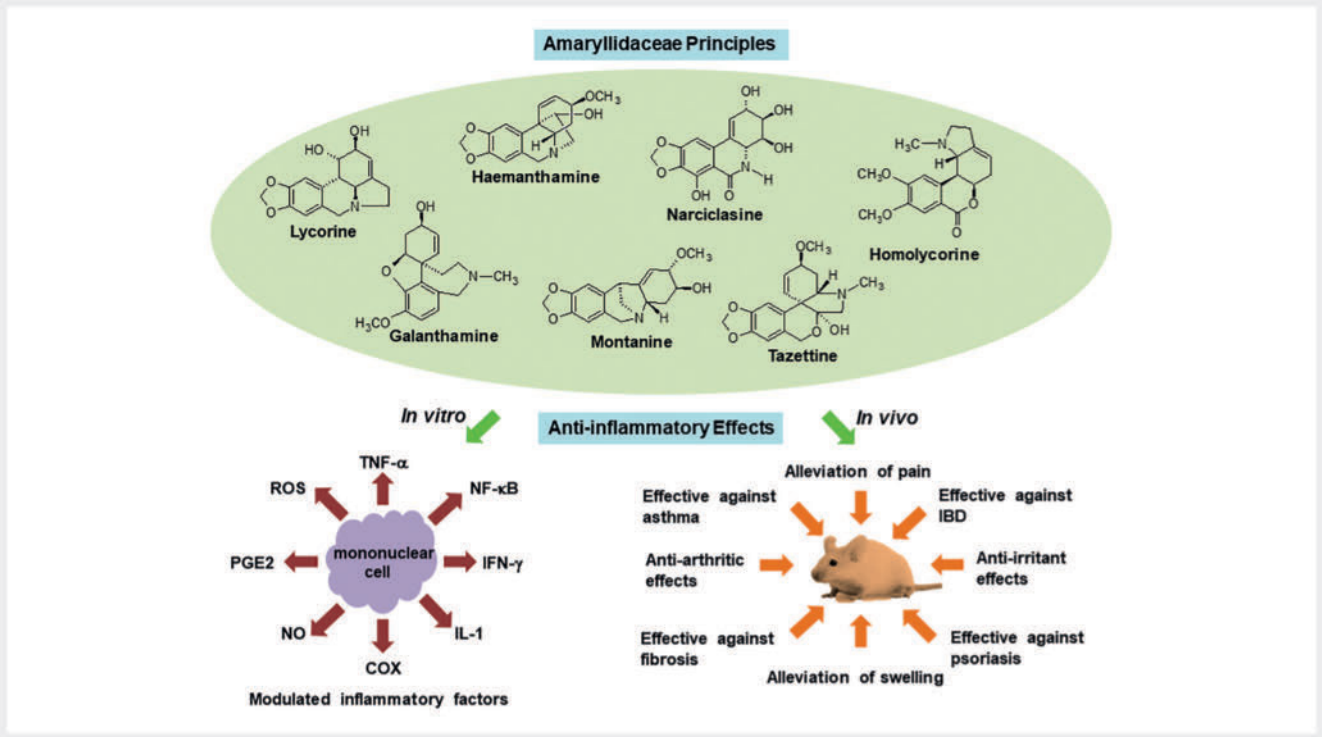


▶ **Fig. 5** Anti-inflammatory compounds detected in the Amaryllidaceae.

AChE, significantly reduces circulating levels of various pro-inflammatory cytokines [16]. Some NSAID-linked AChE inhibitors exhibit more potent anti-inflammatory and anti-vesicant effects against various irritant substrates than the NSAIDs alone [17]. The benefit of these lies with their ability to target multiple pathways, such as regulating acetylcholine levels on one hand and inhibiting COX on the other [17]. In this regard, ibuprofen, indomethacin and naproxen adducts of galanthamine (**6–8**) were examined (at 1.5 µM) in a mouse ear vesicant model against 2-chloroethyl ethyl sulphide (CEES) and 12-*O*-tetradecanoylphorbol-13-acetate (TPA) [18]. The ibuprofen–galanthamine (**6**) adduct was seen to be unresponsive in both models, whilst the indomethacin–galanthamine (**7**) and naproxen–galanthamine (**8**) adducts showed significant activity only against CEES onslaught (75% and 86% reductions, respectively) [18]. This is somewhat perplexing given that ibuprofen and naproxen are structurally similar members of the propionic acid class of NSAIDs. Of the three NSAID standards examined by themselves, indomethacin reduced the effects of CEES and TPA by 46% and 55%, respectively, while the reduction in effect of TPA by naproxen was 104% [18]. Interestingly, none of the three galanthamine adducts (**6–8**) were able to potentiate the AChE inhibitory effect of galanthamine itself (IC_{50} 1.12 µM) [18]. This therefore underlined the importance of the C-3 free hydroxyl group in galanthamine as a part of its AChE-inhibitory pharmacophore.

Anti-Arthritic Effects

Arthritis is a common disease that causes pain, swelling and stiffness in joints [19]. There are several types of arthritis, which affect people of all ages [19]. Some forms of the condition are more common in older people [19]. An adjuvant-induced arthritis model in male Wistar–Lewis rats was used to see if Amaryllidaceae constituents could intervene in such circumstances (▶ **Fig. 6**) [20]. While lycorine (**1**) (5 mg/kg/day over 10 days) relative to hydrocortisone did not suppress arthritis, narciclasine (**3**) was able to achieve this at the relatively low dose of 1 mg/kg/day [20]. Further study on the anti-arthritic effect of narciclasine was undertaken via its sodium phosphate salt (sodium narcistatin **4**), which holds the phosphate moiety in a bridged disposition via the C-3/C-4 hydroxy groups of ring-C [13]. Exposed to rats suffering from adjuvant-induced arthritis, the phosphate salt (**4**) dose-dependently (1.75, 3.5 and 5.0 mg/kg/day/intraperitoneal) manifested several positive effects over 4 weeks relative to saline-treated con-



▶ **Fig. 6** Inflammation-related effects of Amaryllidaceae constituents *in vivo* and the molecular targets associated with their effects *in vitro*.

trols (▶ **Table 1**) [13]. This saw less bone loss, less periosteal bone formation, less narrowing of joint spaces and increased bone density in the hind feet of drug-treated subjects [13]. Studies undertaken several years later indicated that lycorine (**1**) did possess anti-arthritic potential [21]. Progressive cartilage deterioration is one of the key features of osteoarthritis [22]. Cartilage consists of chondrocytes and extracellular matrix (ECM) [22]. Although the aetiology of cartilage impairment is not fully understood, an imbalance in the anabolism and catabolism of cartilage ECM is thought to be one of the factors behind cartilage degradation [22]. The protective effect of lycorine on cartilage was thus examined in a mouse anterior cruciate ligament transection (ACLT) model [21]. Control C57BL/6 mice that had been subjected to ACLT and treated with the vehicle (PBS) showed severe cartilage damage and fibrillation in the superficial and mid layers of cartilage, which was also compounded by matrix discontinuity [21]. These deleterious effects were effectively overcome by lycorine at 2.5 mg/kg/day over the 4-week treatment schedule [21].

More recently, lycorine (**1**) has been examined for anti-arthritic effects in the CFA-induced arthritis model of C57BL/6 J mice [23]. Treatment with lycorine (10 mg/kg) increased mechanical pain sensitivity, suppressed spontaneous pain and promoted recovery of motor co-ordination relative to CFA exposure (10 µL) [23]. It also reduced spinal cord expression levels of NF-κB and IL-1β [23]. Reductions were also seen for spinal astrocytic activation (based on GFAP levels) and NLRP3 inflammasome activity [23]. Astrocyte activation is an important channel for inflammatory cytokines, while the NLRP3 inflammasome mediates in caspase-1 and IL-1β secretion [23]. Oxidative stress was ascertained from levels of the antioxidant element Nrf2, as well as SOD activity, both of which were increased by lycorine compared to assessments of untreated animals [23]. Lycorine treatment also inhibited spinal GSK-3β activity [23].

A gene-based study was undertaken to verify the link between osteoarthritis and hypoxia [24]. GSE48556, GSE55235 and GSE55457 were identified as three expression profiles for osteoarthritis in blood samples from sufferers of this inflammatory malady [24]. Heat-map analysis indicated further that there were nine up-regulated and two down-regulated genes associated with fifteen hypoxia gene expression signatures in GSE48556 [24]. Correlation analysis showed that these genes were prominent in a number of signalling pathways including p53 signalling, cell senescence, NF-κB signalling, ubiquitin-mediated proteolysis and apoptosis [24]. Given these, as well as the fact that narciclasine (**3**) is known to attenuate ROS production, it was suggested it may have potential as a drug for osteoarthritis [24, 25].

Montanine (**9**) (▶ **Fig. 1**) (from an EtOH bulb extract of *Rhodophiala bifida* (Herb.) Traub) has been probed for anti-arthritic effects in the antigen-induced arthritis (AIA) model of BALB/c mice [26]. AIA is an immune-mediated (T cell-dependent) joint inflammation, the inducing antigen of which is methylated bovine serum albumin (mBSA) [26]. The histopathology of the condition displays several similarities with rheumatoid arthritis [26]. Intraperitoneal, twice-daily administration of montanine over 22 days produced significant relief from joint pain at all tested dosages (0.3, 1.0 and 3.0 mg/kg, respectively) [26]. Furthermore, total leukocyte migration was significantly reduced at all three doses, compared to control subjects that received saline solution [26].

▶ **Table 1** *In vivo* anti-inflammatory effects of Amaryllidaceae constituents.

Compound (No.)	Dose	Route[1]	Anti-inflammatory activity	Ref.
Lycorine (**1**)	100 mg/kg	i. p.	Analgesic effect in mice shown to be 58.5% via Koster's test.	[9]
	100 mg/kg	oral	20.1% inhibition of carrageenan-induced paw oedema in mice.	[12]
	5 mg/kg	i. p.	Did not suppress arthritis in rats over 10 days.	[20]
	5 mg/kg	i. p.	Protected mice from CCl_4-induced hepatotoxicity via antioxidant effects.	[60]
	1 mg/kg	i. p.	53.5% inhibition of carrageenan-induced paw oedema in Sprague–Dawley rats.	[10]
	1 mg/kg	i. p.	Reduced acetic acid-induced abdominal constrictions in mice by 62.7%.	[10]
	1 mg/kg	i. p.	30% antinociceptive activity demonstrated in the tail flick test in mice.	[10]
	5 mg/kg	oral	Countered CCl_4-induced oxidative stress in mice by stimulating ATP synthase.	[61]
	2.5 mg/kg	i. p.	Minimised cartilage damage, fibrillation and ECM discontinuity in the ACLT mouse model.	[21]
	6 mg/kg	i. p.	Overcame LPS-induced bone loss in mice.	[43]
	6 mg/kg	i. p.	Diminished surface area and number of osteoclasts in LPS-treated mice.	[43]
	0.5 mg/kg	i. p.	Protected mice from murine ovariectomy-induced osteoporosis and titanium particle-induced osteolysis.	[41]
	20 mg/kg	i. p.	Diminished alveolar congestion, alveolar wall thickening and oedema during LPS-induced ALI in mice.	[38]
	20 mg/kg	i. p.	Reduced MDA levels in lung tissue of ALI-affected BALB/c mice.	[38]
	10 mg/kg	i. p.	Reduced alveolar haemorrhage, oedema, thickness of alveolar septa and infiltration of inflammatory cells during bleomycin-induced IPF in mice.	[111]
	5 mg/kg	i. p.	Reduced isoproterenol-induced elevation of MDA and SOD levels in heart tissue of C57BL/6 J mice.	[36]
	0.5 mg/kg	i. p.	Ameliorated thioacetamide-induced liver fibrosis in rats by attenuating the expression of hepatic hydroxyproline, smooth muscle actin and transforming growth factor 1.	[45]
	0.5 mg/kg	i. p.	Reduced MDA levels, replenished GSH content and boosted SOD activity during thioacetamide-induced liver fibrosis in rats.	[45]
	10 mg/kg	i. p.	Increased mechanical pain sensitivity, suppressed spontaneous pain and promoted recovery of motor co-ordination during CFA-induced arthritis in mice.	[23]
	10 mg/kg	i. p.	Reduced spinal astrocytic activation and NLRP3 inflammasome activity during CFA-induced arthritis in mice.	[23]
	10 mg/kg	i. p.	Increased levels of Nrf2, as well as SOD activity, during CFA-induced arthritis in mice.	[23]
	10 mg/kg	i. p.	Inhibited spinal cord GSK-3β activity during CFA-induced arthritis in mice.	[23]
Haemanthidine (**2**)	100 mg/kg	i. p.	Analgesic effect in mice shown to be 48.8% via Koster's test.	[9]
	100 mg/kg	oral	36.5% inhibition of carrageenan-induced paw oedema in mice.	[12]

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▶ **Table 1** *Continued*

Compound (No.)	Dose	Route[1]	Anti-inflammatory activity	Ref.
Narciclasine (**3**)	1 mg/kg	i. p.	Suppressed arthritis in rats over 10 days.	[20]
	1 mg/kg	s. c.	Reduced weight shift towards the front paws in a zymosan-induced peritonitis model in mice.	[31]
	1 mg/kg	s. c.	Reduced infiltration of the peritoneum with neutrophils and monocytes in zymosan-induced peritonitis in C57BL/6 N mice.	[31]
	0.7 mg/kg	s. c.	Ameliorated LPS-induced acute lung injury in neonatal Sprague–Dawley rats.	[25]
	0.7 mg/kg	s. c.	Reduced ICAM-1, VCAM-1 and MCP-1 levels in rats subjected to LPS-induced acute lung injury.	[25]
	0.7 mg/kg	s. c.	Reduced ROS activity in lung tissue of rats subjected to LPS-induced acute lung injury.	[25]
	0.7 mg/kg	s. c.	Reduced expression of LDH, SOD and MPO in lung tissue of rats subjected to LPS-induced acute lung injury.	[25]
	0.1 mg/kg	i. p.	Improved the survival of neonatal rats subjected to *E. coli*-induced sepsis.	[33]
	0.1 mg/kg	i. p.	Reduced plasma levels of S100A8/A9 and suppressed its expression in liver and lung tissue of sepsis neonatal rats.	[33]
	0.1 mg/kg	i. p.	Reduced plasma levels of IFN-γ in sepsis neonatal rats.	[33]
	0.1 mg/kg	i. p.	Prevented Iκβα degradation in liver tissue of septic rats.	[33]
	0.1 mg/kg	oral	Attenuated LPS-induced increase in MPO activity in cardiomyocyte lysates of C57BL/6 mice.	[35]
	1 mg/kg	s. c.	Reduced lymphocyte, monocyte and neutrophil levels in the BALF of neonatal rats subjected to ovalbumin-induced asthma.	[47]
	1 mg/kg	s. c.	Attenuated increases to nitrotyrosine levels and the percentage of infiltrated cells in the BALF of neonatal rats subjected to ovalbumin-induced asthma.	[47]
	1 mg/kg	s. c.	Reduced ovalbumin-specific IgE in the BALF and serum of neonatal rats subjected to ovalbumin-induced asthma.	[47]
	0.5 mg/kg	i. p.	Pre-treatment protected C57BL/6 mice from conA-induced liver injury.	[39]
	0.5 mg/kg	i. p.	Protected C57BL/6 mice from conA-induced liver injury by reducing T cell migration and neutrophil infiltration into hepatocytes.	[39]
	2 mg/kg	i. d.	Reversed the severity of lesions and reduced PASI score during IMQ-induced psoriasis in mice.	[51]
	2 mg/kg	i. d.	Reduced the spleen index during IMQ-induced psoriasis in mice.	[51]
Sodium narcistatin (**4**)	1.75 mg/kg	i. d.	Reduced CFA-induced dorsoplantar footpad swelling in rats.	[13]
	1.75 mg/kg	i. d.	Reduced bone loss, periosteal bone formation and narrowing of joint spaces in hind feet of arthritic rats.	[13]
	1.75 mg/kg	i. d.	Increased bone density in hind feet of arthritic rats.	[13]
	5.0 mg/kg	i. d.	Reduced IFN-γ production in DLN cells of arthritic rats.	[13]
Galanthamine (**5**)	2.5 mg/kg	oral	Increased TAC and Nrf2 levels in diabetic rats.	[68]
	2.5 mg/kg	oral	Depleted MDA levels in diabetic rats.	[68]
	2 mg/kg	s. c.	Reversed the deleterious effects of DMH on the antioxidant markers MDA, GSH, SOD and catalase in rats.	[69]
	2 mg/kg	s. c.	Attenuated LOX levels in rats subjected to the toxic effects of DMH for 6 weeks.	[69]
	0.25 mg/kg	oral	Reduced acetic acid-induced ulceration in male Wistar rats as an indicator of anti-colitis activity.	[29]

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▶ **Table 1** *Continued*

Compound (No.)	Dose	Route[1]	Anti-inflammatory activity	Ref.
Ibuprofen-galanthamine (**6**)	1.5 µM	topical	Unresponsive in CEES and TPA mouse ear vesicant models.	[18]
Indomethacin-galanthamine (**7**)	1.5 µM	topical	75% reduction in the effect of CEES in the mouse ear vesicant model.	[18]
	1.5 µM	topical	Unresponsive in the TPA mouse ear vesicant model.	[18]
Naproxen-galanthamine (**8**)	1.5 µM	topical	86% reduction in the effect of CEES on the mouse ear vesicant model.	[18]
	1.5 µM	topical	Unresponsive in the TPA mouse ear vesicant model.	[18]
Montanine (**9**)	0.3 mg/kg	i. p.	Produced relief from joint pain in the AIA model of BALB/c mice.	[26]
	0.3 mg/kg	i. p.	Reduced total leukocyte migration in the AIA model of BALB/c mice.	[26]
	0.25 mg/kg	i. p.	Reduced clinical scores for CIA in DBA/1 J mice.	[26]
	0.01 µM	i. p.	Did not alter the viability of lymphocytes from lymph nodes of BALB/c mice.	[26]
Pseudolycorine (**10**)	40 mg/kg	i. p.	Alleviated MOG_{35-55}-induced autoimmune encephalomyelitis in C57BL/6 mice.	[49]
Kalbreclasine (**44**)	20 µg	n. i.	Caused proliferation of splenic lymphocytes in adult DBA/2 mice.	[72]
Crinumin	25 µg/kg	i. p.	Reduced carrageenan-induced paw oedema in rats.	[14]

[1]Route of administration; Abbreviations: i. d., intradermal; i. p., intraperitoneal; n. i., not indicated; s. c., subcutaneous

Promising results were also observed with montanine in the collagen-induced arthritis (CIA) model of DBA/1 J mice using bovine type II collagen as the inducing agent [26]. Administered twice daily for 15 days via the intraperitoneal route, montanine (at 0.25 and 0.5 mg/kg) significantly reduced the clinical scores for the disease from day 8 of the schedule [26]. Furthermore, treatment at the higher dosage improved all of the joint-associated histological parameters, except for synovial hyperplasia [26]. The effect of montanine (**9**) (from a *Rhodophiala bifida* EtOH bulb extract) on the invasiveness of fibroblast-like synoviocytes (FLS), a key process in cartilage and bone pannus invasion and articular destruction, was examined *in vitro* using a Matrigel transwell system [27]. In this regard, montanine at 1 µM reduced FLS (obtained from DBA/1 J mice with CIA) invasion by 54% (▶ **Table 2**) [27]. Of the more than 20 alkaloid groups known in the Amaryllidaceae, anti-arthritic effects have thus far only been described for the lycorine, narciclasine and montanine groups. It thus remains to be ascertained whether the other groups are similarly disposed towards such effects.

Effects on Miscellaneous Inflammatory Diseases

Inflammatory bowel disease (IBD), which includes ulcerative colitis and Crohn's disease, is a well-known gastrointestinal disorder [28]. Current drugs for these conditions are restricted to corticosteroids, 5-aminosalicylates and immuno-suppressives [28]. There is also significant interest in the use of TMs against IBD [28]. Acetic-acid-induced ulceration in male Wistar rats was used to ascertain the anti-colitis properties of galanthamine (**5**) [28]. At oral doses of 0.25, 1.25 or 2.5 mg/kg/day over 5 days, it, alone or in combination with dicyclomine (5 mg/kg/day), diminished the ulcer index, total colitis index and myeloperoxidase (MPO) activity scores, which are important biomarkers for colitis [29]. Furthermore, dicyclomine itself was not effective on these parameters, and its co-administration with galanthamine did not reverse the anti-ulcerative effect of the latter [29].

Peritonitis refers to the inflammation of the peritoneum, symptoms of which include severe pain, swelling of the abdomen, fever and weight loss [30]. Causes for this disease include perforation of the intestinal tract, pancreatitis, pelvic inflammatory disease, stomach ulcer, cirrhosis, a ruptured appendix or even a perforated gallbladder [30]. The mainstay treatment options are antibiotics, intravenous fluids, pain medication and surgery [30]. Narciclasine (**3**) (Carl Roth, Karlsruhe, Germany) was probed for possible therapeutic benefits in a zymosan-induced peritonitis model in C57BL/6 N mice [31]. Vehicle-treated animals showed a pronounced weight shift towards the front paws, which is an indicator of inflammatory abdominal pain [31]. By comparison, subcutaneous administration of narciclasine (1 mg/kg) prior to zymosan (2 mg/mL, intraperitoneal) produced a significant reduction in weight shift [31].

Sepsis is a life-threatening illness caused by a virulent systemic inflammatory response to infection [32]. It starts with dysregulated inflammation, followed by the systemic inflammatory response, which leads to severe sepsis and septic shock [32]. It is the most common cause of death in critically ill patients in non-coronary high-care units [32]. Sepsis is also one of the leading causes of morbidity and mortality among babies in neonatal high-care wards [32]. Narciclasine (**3**) (Tocris Bioscience, Minneapolis, USA) dose-dependently (0.1, 1 and 3 mg/kg) improved

▶ **Table 2** Miscellaneous anti-inflammatory effects of Amaryllidaceae constituents.

Compound (No.)	Anti-inflammatory activity	Ref.
Lycorine (**1**)	Inhibited calprotectin-induced toxicity in MM46 cells (80% reduction in MTT at 5 µg/mL).	[120]
	IC_{50} against calprotectin-induced toxicity in MM46 cells determined as 0.1 µg/mL.	[20]
	Reduced AAPH-induced haemolysis in human erythrocytes (at 0.01–0.05 mg/mL).	[56]
	Reduced (at 5 µM) PGE2 release in LPS-stimulated RAW264.7 macrophages.	[76]
	IC_{50} > 0.8 µg/mL against PGE2 release in LPS-stimulated RAW264.7 macrophages.	[87]
	Diminished secretion of MMP-1, MMP-2 and MMP-7 in HCT-116 and LoVo cells (at 6 µM).	[107]
	No effect on MMP-13 secretion in HCT-116 and LoVo cells (at 6 µM).	[107]
	Attenuated (at 1.6 µM) LPS-induced increase in number and activity of osteoclasts *in vitro*.	[43]
	Inhibited (0.1–0.4 µM) RANKL-induced osteoclastogenesis in bone marrow macrophages via inhibition of MAPK.	[41]
	MAPK agonist anisomycin attenuated inhibitory effect on RANKL-induced osteoclastogenesis.	[41]
	Suppressed (at 10 µM) LPS/nigericin- or LPS/ATP-induced NLRP3 inflammasome activation in BMDMs.	[111]
Narciclasine (**3**)	Inhibited calprotectin-induced toxicity in MM46 cells (IC_{50} 0.001–0.01 µg/mL).	[20]
	Dose-dependently (0.003–1 µM) inhibited ICAM-1 up-regulation in HUVECs.	[97]
	Dose-dependently (30, 100 and 300 nM) attenuated LPS-induced reduction in the viabilities of NRCMs.	[35]
	Dose-dependently (0.1, 0.2 and 0.3 µM) inhibited PGE2 release during LPS-induced neuro-inflammation in BV-2 microglia.	[78]
	Diminished secretion of MMP-1, MMP-2 and MMP-7 in HCT-116 and LoVo cells (at 0.08 µM).	[107]
	No effect on MMP-13 secretion in HCT-116 and LoVo cells (at 0.08 µM).	[107]
	Inhibited (at 0.003–3 µM) HaCaT keratinocyte proliferation by inducing cell cycle arrest.	[51]
	Reduced (at 0.003–3 µM) secretion of CCL1/2/20 and CXCL1/10 in HaCaT keratinocytes, which reduced the recruitment of T cells and neutrophils.	[51]
Montanine (**9**)	Did not facilitate migration of human leukocytes in solution.	[54]
	Had no effect (at 1.0 µM) on lymphocyte proliferation stimulated by LPS.	[26]
	Significantly decreased (at 1.0 µM) lymphocyte proliferation stimulated by conA.	[26]
	Reduced by 54% (at 1 µM) FLS invasion into Matrigel.	[27]
Haemanthamine (**11**)	Diminished secretion of MMP-1, MMP-2 and MMP-7 in HCT-116 and LoVo cells (at 6 µM).	[107]
	No effect on MMP-13 secretion in HCT-116 and LoVo cells (at 6 µM).	[107]
Kalbreclasine (**44**)	Stimulation index of 3.1 for mitogenic activation of splenic lymphocytes.	[72]
trans-Dihydrolycoricidine (**59**)	Dose-dependently (0.1, 0.3 and 0.5 µM) suppressed PGE2 release in LPS-stimulated BV-2 cells.	[79]
Hippeastrine (**132**)	Inhibited calprotectin-induced toxicity in MM46 cells (IC_{50} 10–100 µg/mL).	[20]
Ungerine (**133**)	Inhibited calprotectin-induced toxicity in MM46 cells (IC_{50} 10–100 µg/mL).	[20]
Narcin	Produced a nearly fourfold increase in IgE (at 10 µg/mL) in PBMCs of healthy human subjects.	[108]
	Increased production of IFN-γ (at 10 µg/mL) in CD4+ T cells from PBMCs.	[108]

the survival of neonatal rats subjected to *E. coli*-induced sepsis (to 16.7%, 50% and 66.7%, respectively) [33]. In addition to its modulation of various inflammatory markers during *E. coli*-induced sepsis, as discussed elsewhere in the text, narciclasine also reduced the bacterial load in the blood, peritoneal fluid, liver and lungs of affected animals [33].

Often presenting as a secondary manifestation to sepsis, acute myocardial injury (AMI) is associated with severe cardiac inflammation [34]. The over-production of inflammatory cytokines, particularly TNF-α, IL-1α, IL-1β and IL-6, has been linked to cardiac dysfunction in sepsis [34]. Targeting the inflammatory response is thus considered a viable therapeutic option towards sepsis-induced cardiomyopathy [34]. Given the observations made for narciclasine (**3**) in *E. coli*-induced sepsis above, a study was carried out to probe its effect on sepsis-induced myocardial injury [35]. It was shown that LPS-induced reduction in the viabilities of neonatal rat

cardiomyocytes (NRCMs) was overcome by narciclasine in a dose-dependent manner (30, 100 and 300 nM), without it being detrimental to the cells (▶ **Table 2**) [35]. Narciclasine also dose-dependently suppressed LPS-induced release of the inflammatory cytokines TNF-α, IL-1β and IL-6 from NRCMs [35]. *In vivo*, the LPS-induced (6 mg/kg) increase in MPO activity, an indicator of neutrophil infiltration, in cardiomyocyte lysates of C57BL/6 mice was effectively abrogated by narciclasine (at 0.1 mg/kg/day/7 days) [35]. It also attenuated the LPS-induced increases in mRNA expression levels of TNF-α, IL-1β, IL-6 and VEGF [35].

An abnormal inflammatory response in heart tissue contributes to isoproterenol-induced cardiac injury [36]. Lycorine (**1**) (Aladdin Biochemical Technology, Shanghai, China) was probed as to whether it could mediate in such circumstances [36]. Protein and mRNA levels of IL-1β, IL-6 and TNF-α were significantly upregulated in heart tissue of C57BL/6 J mice exposed to isoproterenol (at 10 mg/kg) daily for 2 weeks [36]. However, co-administration of lycorine (at 5 mg/kg/day) produced a significant reduction in these inflammatory cytokines [36]. Lycorine also suppressed isoproterenol-induced phosphorylation of NF-κB, suggesting that it in part attenuated cardiac inflammation by inhibiting activation of the NF-κB signalling pathway [36]. Furthermore, elevation of MDA and SOD levels by isoproterenol was negated by lycorine, suggesting that it protected against cardiac dysfunction by inhibiting oxidative stress [36].

Acute lung injury (ALI) is a life-threatening condition with significant incidence and mortality [37]. It is characterised by excessive airway inflammation, oedema, hypoxemia, impaired lung tissue, increased permeability, alveolar congestion, haemorrhage and atelectasis [37]. LPS-induced (2 mg/kg) ALI in neonatal Sprague–Dawley rats over 72 hr was characterised by increased inflammation, haemorrhage, alveolar congestion and alveolar wall oedema in lung tissue [25]. Exposure to narciclasine (0.7 mg/kg) (Carl Roth, Karlsruhe, Germany) 12 hr prior to induction of ALI by contrast was shown to ameliorate all of these LPS-related ill effects [25]. In the toxicity analysis, the effect of narciclasine (**3**) alone was shown to be the same as that of normal saline [25]. Studies on LPS-induced ALI were also undertaken with lycorine (**1**) (Aladdin Biochemical Technology, Shanghai, China) (▶ **Fig. 1**) in BALB/c mice [38]. In this regard, it dose-dependently decreased alveolar congestion, alveolar wall thickening and oedema when administered intraperitoneally (at 20 and 40 mg/kg) 1 hr prior to LPS (2 mg/kg) [38]. Studies were also carried out to ascertain the effect of narciclasine (Med Chem Express, New Jersey, USA) on acute liver injury brought about by concanavalin A (conA) in C57BL/6 mice [39]. It was shown that pre-treatment with narciclasine dose-dependently (0.5–10 mg/kg) protected animals from conA-induced liver injury [39]. This was achieved by decreasing T cell migration and neutrophil infiltration into hepatocytes [39]. Narciclasine (5 mg/kg) also attenuated conA-induced increases in serum levels of IFN-γ, TNF-α, IL-2, IL-6, IL-10 and IL-17 [39].

Osteoclastogenesis refers to the formation of bone-resorbing cells, also called osteoclasts, from precursor cells of myeloid origin [40]. Physical contact between precursor cells with osteoblasts (cells that form bone tissue) or other mesenchymal cells, such as stromal or synovial cells, is necessary for the process [40]. Excessive osteoclast activity has been linked to osteolytic diseases such as osteoporosis and periprosthetic osteolysis [40]. Bone homeostasis is thus maintained through a dynamic balance between osteoclastic bone resorption on one hand and osteoblastic bone formation on the other [40]. Osteoclast formation is enhanced by pro-inflammatory cytokines such as TNF-α, IL-1β and IL-17 [40]. T cells, stromal cells and synoviocytes also enhance osteoclast formation through the expression of RANKL (receptor activator of nuclear factor-κB ligand) [40]. *In vitro* studies showed that RANKL-induced osteoclastogenesis could be inhibited by lycorine (0.1–0.4 µM) (Sigma Aldrich, St Louis, USA) in bone marrow macrophages, which ensued via the inhibition of MAPK [41]. The involvement of MAPK was demonstrated via the introduction of the MAPK agonist anisomycin, which attenuated the inhibitory effect of lycorine (**1**) [41]. *In vivo*, lycorine (at 0.5 or 2.5 mg/kg) played a protective role against murine ovariectomy-induced osteoporosis and titanium-particle-induced osteolysis in C57BL/6 female mice [41]. Inflammatory osteolysis is characterised by irreversible bone erosion due to the aberrant stimulation of osteoclasts, either directly or indirectly via osteoblast/stromal cell stimulation [42]. Since osteoclasts play a key role in LPS-induced bone loss, a study was undertaken to establish whether lycorine (**1**) (Chengdu Biopurity Phytochemicals Ltd., Chengdu, China) could suppress such loss by acting on osteoclasts after LPS stimulation [43]. It was shown that while LPS (5 mg/kg) caused significant bone loss in C57BL/6 J mice, this could be overcome by intraperitoneal administration of lycorine (6 mg/kg/thrice weekly) over a three-week period [43]. Furthermore, the increased surface area and number of osteoclasts seen in LPS-treated mice were notably diminished by lycorine, suggesting that osteoclasts were involved in the bone-sparing effect of lycorine [43]. *In vitro*, the increased number and activity of osteoclasts induced by LPS (50 ng/mL) were reduced by lycorine (1.6 µM) [43].

Fibrosis is a wound-healing process whereby normal parenchymal tissue is replaced by connective tissue [44]. If left unchecked, it can lead to tissue remodelling and the formation of permanent scar tissue [44]. A study was undertaken to investigate the possible antifibrotic effect of lycorine (**1**) (at 0.5 or 1 mg/kg/day) against liver fibrosis induced by thioacetamide (200 mg/kg/thrice weekly/4 weeks) in male Wistar rats [45]. Lycorine (Molport, Riga, Latvia) ameliorated fibrosis in a dose-dependent manner by attenuating the expression of hepatic hydroxyproline, smooth muscle actin (SMA) and transforming growth factor 1 (TGF-1) [45]. It also improved the oxidative status of infected animals by reducing MDA levels, replenishing GSH content and boosting SOD activity in hepatic tissue homogenates [45]. Furthermore, while thioacetamide produced significant increases in the pro-inflammatory mediators TNF-α, IL-6 and IL-1β, these were attenuated by lycorine in a dose-dependent manner [45].

Asthma is an inflammatory disorder characterised by the chronic hyper-responsiveness of the respiratory system that results in obstruction to the flow of air [46]. In spite of significant advances in therapies involving both steroidal and non-steroidal entities, the prognoses for asthma remain poor [46]. As immune cells infiltrate lung tissue, there is remodelling of the airways and considerable lung inflammation during asthma [46]. Increases in inflammatory cytokines lead to alterations in bronchial airway tissue, which also facilitate the progression of asthma [46]. It is com-

monly seen in neonates with mothers that are afflicted with lung infection [46]. Based on this, the ameliorative effect of narciclasine was evaluated in an ovalbumin-induced asthma model in neonatal Wistar rats [47]. The BALF of asthmatic neonates showed significant increases in nitrotyrosine levels and the percentage of infiltrated cells compared to control animals, both of which were attenuated by narciclasine in a dose-dependent manner (1 and 3 mg/kg) [47]. While the number of lymphocytes, monocytes and neutrophils in the BALF of asthma subjects was higher than in the control group, narciclasine reduced these at both tested dosages [47]. It also reduced the ovalbumin-specific IgE in the BALF and serum of asthmatic rats [47]. There were also reductions in the cytokines IL-4, IL-6, IL-17 and IL-21 [47]. Whilst the observations from the ovalbumin model are insightful, it would be interesting to gauge what effects narciclasine (or other Amaryllidaceae alkaloids) have on the α2 and β2 adrenergic receptors, as well as on AChE, which are common targets for asthma medications.

Myeloid-derived suppressor cells (MDSCs) originate from common myeloid progenitor cells [48]. Their differentiation and proliferation are facilitated by the myeloid-specific growth factors GM-CSF, G-CSF and M-CSF and the pro-inflammatory cytokines IL-6 and IL-11 [48]. MDSCs promote the differentiation of Th17 (T helper 17) cells and potentiate a variety of Th17-cell-mediated autoimmune diseases [48]. For example, collagen-induced arthritis is alleviated by MDSC depletion, which suppresses Th17 and IL-17A without affecting regulatory T (Treg) cells [48]. During autoimmune encephalomyelitis, the proliferation of MDSCs is known to induce Th17 cell differentiation [48]. The lycorine analogue pseudolycorine (**10**) (from an EtOH bulb extract of *Narcissus tazetta* L. var. *chinensis*) was examined as to whether it could mediate during this inflammatory condition [49]. *In vitro*, pseudolycorine dose-dependently (0.67, 2 and 6 µM) inhibited (relative to solcitinib) IL-6- and GM-CSF-induced MDSC proliferation, expansion and differentiation into monocyte-like MDSCs [49]. Pseudolycorine (at 40 mg/kg) also alleviated MOG_{35-55}-induced autoimmune encephalomyelitis in C57BL/6 mice relative to FK-506 [49]. It achieved this by attenuating the severity of the disease, reducing monocyte-like MDSC infiltration into the spinal cord and inhibiting Th17 cell differentiation and IL-17A secretion [49].

Psoriasis is a chronic condition whereby the immune system becomes overactive, leading to the rapid multiplication of skin cells [50]. It is characterised by scaly and inflamed patches on the skin, particularly on the scalp, elbows and knees [50]. Although the causes of psoriasis are not fully understood, it is believed that a combination of genetic and environmental factors may be at play [50]. The therapeutic effect of narciclasine (**3**) (Medchem Express, Shanghai, China) was assessed in an IMQ-induced psoriasis model in BALB/c mice [51]. Topical application of narciclasine (2 mg/kg/day) for 7 days was seen to reverse the severity of lesions produced by IMQ, which was accompanied by a reduced PASI (psoriasis area and severity index) score [51]. Furthermore, the spleen index (spleen weight to body weight ratio), which is an indicator of the severity of inflammation, was also reduced by narciclasine [51]. The abnormal proliferation and apoptosis of keratinocytes is a major feature of psoriasis-related skin lesions [50]. In this regard, narciclasine dose (0.003–3 µM)- and time-dependently (0–96 hr) inhibited *in vitro* HaCaT keratinocyte proliferation by inducing cell cycle arrest [51]. It also reduced the secretion of CCL1/2/20 and CXCL1/10, which in turn reduced the recruitment of T cells and neutrophils [51].

Antioxidant Effects

Although reactive oxygen species (ROS) are produced to a limited extent in the body, they are crucial in the maintenance of cell homeostasis and the regulation of signal transduction, gene expression and activation of various receptors [52]. Their negative effects, on the other hand, are kept in check by the endogenous antioxidant system [52]. Any imbalance between the production and elimination of ROS can cause oxidative stress, which in turn can lead to inflammation [52]. This occurs via the activation of the transcription factors of various genes associated with inflammatory pathway signalling [52]. Oxidative stress is believed to be responsible for several chronic diseases including, cardiovascular, motorneuron and autoimmune diseases as well as cancer and diabetes [52]. Some of the most effective antioxidant substances (such as ascorbic acid and β-carotene) are derived from natural sources such as fruits and vegetables, hence their advocation in dietary measures towards good health and well-being [53]. Studies on the Brazilian Amaryllidaceae member *Rhodophiala bifida* led to the identification of montanine (**9**) as its chief antioxidant principle [54]. Isolated from a fresh bulb EtOH extract, it displayed 37% DPPH radical scavenging ability relative to the flavonol antioxidant rutin (EC_{50} 4.2 µg/mL) (▶ **Table 3**) [54]. Four alkaloids were described from an EtOH bulb extract of the Nigerian medicinal plant *Crinum ornatum* (Aiton) Herb., which were lycorine (**1**), haemanthamine (**11**), crinamine (**12**) and hamayne (**13**) [55]. DPPH radical scavenging analysis showed that their effects (at 1 mg/mL) relative to rutin were weak, with antioxidant activities in each case determined to be < 15% [55].

Lycorine (**1**) (from a *Crinum asiaticum* EtOH leaf extract) has also been examined for antioxidant effects via free radical scavenging and erythrocyte protective activities [56]. At the highest tested concentration (0.05 mg/mL), its potential against lipid peroxidation was deemed to be poor, as indicated by 19.3% inhibition of ferric thiocyanate (FTC) radical oxidation of linoleic acid [56]. Its DPPH and superoxide radical as well as H_2O_2 scavenging effects relative to α-tocopherol were determined to be 20.9%, 16.3% and 23.1% at the highest tested concentration, respectively [56]. Furthermore, dose- and time-dependent effects were observed for lycorine (at 0.01–0.05 mg/mL) in its ability to reduce AAPH-induced haemolysis in human erythrocytes [56]. This is significant since AAPH-induced erythrocytic haemolysis affords a convenient way to assess *ex vivo* free radical cell membrane damage [56]. The superoxide radical scavenging ability of lycorine (**1**) (from a *Lycoris radiata* (L'Hér.) Herb. EtOH bulb extract) in LPS-stimulated RAW264 murine macrophages was shown to be poor (IC_{50} > 1 mM) relative to the polyphenolic butein (IC_{50} 0.043 mM) [57]. The same result was obtained for narciclasine (**3**), also an isolate of *Lycoris radiata* [57]. Narciclasine was shown to be effective at countering the symptoms of acute lung injury (induced by LPS) in neonatal Sprague–Dawley rats, as revealed above [25]. Its response also involved modulation of the inflammatory markers

▶ **Table 3** Antioxidant effects of Amaryllidaceae constituents.

Compound (no.)	Antioxidant activity	Ref.
Lycorine (**1**)	14.7% DPPH radical scavenging ability at 1 mg/mL.	[55]
	20.9% DPPH radical scavenging ability at 0.05 mg/mL.	[56]
	19.3% inhibition of FTC radical oxidation of linoleic acid at 0.05 mg/mL.	[56]
	16.3% inhibition of superoxide radical scavenging activity at 0.05 mg/mL.	[56]
	23.1% inhibition of H_2O_2 scavenging activity at 0.05 mg/mL.	[56]
	Protected SH-SY5Y neuroblastoma from H_2O_2-induced oxidative injury (cell viability 59.6% at 6.25 µM).	[67]
	Reduced (at 10 µM) MDA levels in MLE-12 cells following exposure to LPS (2 µg/mL).	[38]
Haemanthidine (**2**)	Protected SH-SY5Y neuroblastoma from H_2O_2-induced oxidative injury (cell viability 78.1% at 12.5 µM).	[67]
Narciclasine (**3**)	Low superoxide radical scavenging ability in LPS-activated RAW264 macrophages (IC_{50} > 1 mM).	[57]
Galanthamine (**5**)	Good scavenging activities against oxygen, hydroxyl and HOCl radicals (EC_{50}s 15, 83 and 25 µM).	[65]
	Protected SH-SY5Y neuroblastoma from H_2O_2-induced oxidative injury (cell viability 71.7% at 25 µM).	[67]
Montanine (**9**)	37% DPPH radical scavenging ability relative to rutin (EC_{50} 4.2 µg/mL).	[54]
Haemanthamine (**11**)	8.8% DPPH radical scavenging ability at 1 mg/mL.	[55]
Crinamine (**12**)	1.8% DPPH radical scavenging ability at 1 mg/mL.	[55]
Hamayne (**13**)	1.2% DPPH radical scavenging ability at 1 mg/mL.	[55]
Norbelladine (**14**)	At 10 µM, it quenched the DPPH radical by 31% and reduced superoxide radicals by 33%.	[58]
8,9-Dimethoxycripowellin C (**15**)	IC_{50} 124.0 µM against DPPH radical scavenging.	[59]
	IC_{50} 104.7 µM against ABTS radical scavenging.	[59]
8,9-Dimethoxycripowellin D (**16**)	IC_{50} 80.1 µM against DPPH radical scavenging.	[59]
	IC_{50} 73.4 µM against ABTS radical scavenging.	[59]
6-Methoxycripowellin B (**17**)	IC_{50} 62.1 µM against DPPH radical scavenging.	[59]
	IC_{50} 52.2 µM against ABTS radical scavenging.	[59]
8-Hydroxy-9-methoxycripowellin B (**18**)	IC_{50} 69.9 µM against DPPH radical scavenging.	[59]
	IC_{50} 67.3 µM against ABTS radical scavenging.	[59]
Cripowellin C (**19**)	IC_{50} 130.7 µM against DPPH radical scavenging.	[59]
	IC_{50} 125.6 µM against ABTS radical scavenging.	[59]
Pancratistatin (**20**)	Induced apoptosis (at 1 µM) in LNCaP adenocarcinoma cells, accompanied by increased ROS production and destabilisation of MMP.	[63]
Lycolongirine A (**21**)	Protected SH-SY5Y neuroblastoma from H_2O_2-induced oxidative injury (cell viability 94.5% at 12.5 µM).	[67]
Lycolongirine C (**22**)	Protected SH-SY5Y neuroblastoma from H_2O_2-induced oxidative injury (cell viability 90.4% at 6.25 µM).	[67]
Incartine (**23**)	Protected SH-SY5Y neuroblastoma from H_2O_2-induced oxidative injury (cell viability 93.7% at 12.5 µM).	[67]
Harmane (**24**)	Protected SH-SY5Y neuroblastoma from H_2O_2-induced oxidative injury (cell viability 91.7% at 25 µM).	[67]
Hippamine (**25**)	Protected SH-SY5Y neuroblastoma from H_2O_2-induced oxidative injury (cell viability 95.4% at 25 µM).	[67]
N-Chloromethyl galanthamine (**26**)	Protected SH-SY5Y neuroblastoma from H_2O_2-induced oxidative injury (cell viability 93.4% at 12.5 µM).	[67]
11-Deoxytazettine (**27**)	Protected SH-SY5Y neuroblastoma from H_2O_2-induced oxidative injury (cell viability 92.9% at 12.5 µM).	[67]
Lycolongirine B (**28**)	Protected SH-SY5Y neuroblastoma from H_2O_2-induced oxidative injury (cell viability 60.9% at 6.25 µM).	[67]
Norharmane (**29**)	Protected SH-SY5Y neuroblastoma from H_2O_2-induced oxidative injury (cell viability 60% at 12.5 µM).	[67]
Perlolyrine (**30**)	Protected SH-SY5Y neuroblastoma from H_2O_2-induced oxidative injury (cell viability 62.2% at 12.5 µM).	[67]
N-Chloromethyl narcissidine (**31**)	Protected SH-SY5Y neuroblastoma from H_2O_2-induced oxidative injury (cell viability 78.1% at 25 µM).	[67]

continued next page

▶ **Table 3** *Continued*

Compound (no.)	Antioxidant activity	Ref.
Trisphaeridine (**32**)	Protected SH-SY5Y neuroblastoma from H_2O_2-induced oxidative injury (cell viability 75.5% at 12.5 µM).	[67]
N-Methylcrinasiadine (**33**)	Protected SH-SY5Y neuroblastoma from H_2O_2-induced oxidative injury (cell viability 75.5% at 12.5 µM).	[67]
Norgalanthamine (**34**)	Protected SH-SY5Y neuroblastoma from H_2O_2-induced oxidative injury (cell viability 77.5% at 12.5 µM).	[67]
11β-Hydroxy galanthamine (**35**)	Protected SH-SY5Y neuroblastoma from H_2O_2-induced oxidative injury (cell viability 79.2% at 25 µM).	[67]
Sanguinine (**36**)	Protected SH-SY5Y neuroblastoma from H_2O_2-induced oxidative injury (cell viability 76.8% at 50 µM).	[67]
N-Chloromethyl lycoramine (**37**)	Protected SH-SY5Y neuroblastoma from H_2O_2-induced oxidative injury (cell viability 85.5% at 100 µM).	[67]
9-*O*–Demethyllycoramine (**38**)	Protected SH-SY5Y neuroblastoma from H_2O_2-induced oxidative injury (cell viability 73.5% at 50 µM).	[67]
Tazettine (**39**)	Protected SH-SY5Y neuroblastoma from H_2O_2-induced oxidative injury (cell viability 79.8% at 50 µM).	[67]
3-Benzylidenechromanone (**40**)	No DPPH radical scavenging activity.	[70]
3-Benzylidenechromanone (**41**)	No DPPH radical scavenging activity.	[70]
3-Benzylchromanone (**42**)	Weak DPPH radical scavenging activity (IC_{50} 371.54 µg/mL).	[70]
3-Benzylchromanone (**43**)	Weak DPPH radical scavenging activity (IC_{50} 288.40 µg/mL).	[70]

TNF-α, IL-6 and IL-1β [25]. Furthermore, its alleviatory action was shown to be associated with the modulation of oxidative stress [25]. While there was a fivefold increase in ROS in lung tissue of LPS-treated animals (2 mg/kg), narciclasine (at 0.7 mg/kg) was able to return this to the level of the untreated controls over the 72 hr monitoring period [25]. Similar observations were made in the expressions of the antioxidant enzymes LDH, SOD and MPO [25]. Lycorine (**1**) was also shown to be effective against LPS-induced ALI in BALB/c mice as outlined above [38]. Examined as to whether its activity was related to the modulation of oxidative stress, it was seen that it dose-dependently (20 and 40 mg/kg) reduced MDA levels in lung tissue of affected animals [38]. At the higher dosage, this was around four times less than that seen with LPS alone (at 2 mg/kg) [38]. Similarly, lycorine (10 µM) reduced MDA levels in cultured mouse lung epithelial MLE-12 cells following exposure to LPS (2 µg/mL) [38]. Norbelladine (**14**), a precursor in the biosynthesis of Amaryllidaceae alkaloids, has also been screened for antioxidant effects via DPPH and superoxide radical scavenging [58]. Obtained via a synthetic protocol, it (at 10 µM) was able to quench the DPPH radical by 31% and reduce superoxide radicals from xanthine oxidase by 33%, relative to the standards vanillic acid and *p*-hydroxybenzoic acid [58]. Five alkaloids (8,9-dimethoxycripowellin C **15**, 8,9-dimethoxycripowellin D **16**, 6-methoxycripowellin B **17**, 8-hydroxy-9-methoxycripowellin B **18** and cripowellin C **19**) described from the EtOH bulb extract of *Crinum latifolium* L. were evaluated for antioxidant activities via DPPH and ABTS free radical scavenging assays [59]. With IC_{50}s of 62.1–130.7 µM against DPPH radical scavenging, none of the compounds were able to better the effect of trolox (IC_{50} 43.2 µM) [59]. Nonetheless, three of the alkaloids (**16, 17** and **18**) were shown to be better than trolox (IC_{50} 80.9 µM) against ABTS radical scavenging [59].

Lycorine (**1**) (Sigma Aldrich, Bangalore, India) has also been examined for its ability to moderate CCl_4-induced hepatotoxicity in Swiss albino mice [60]. Oral administration of CCl_4 (1 mL/kg, twice weekly) produced a notable increase in the levels of the lipid peroxidation product malondialdehyde (MDA) over an eight-week period, as well as significant decreases in the non-enzymatic antioxidant markers glutathione and ascorbic acid [60]. This was accompanied by reduction in activities of the enzymatic antioxidant markers superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase and glutathione reductase [60]. However, these deleterious effects were normalised in test animals following the co-administration of lycorine (5 mg/kg), suggesting that its buffering against hepatotoxicity was mediated via antioxidant pathways [60]. Furthermore, CCl_4-induced oxidative stress in Swiss albino mice was shown to involve, amongst other liver proteins, the differential expression of ATP synthase [61]. CCl_4 enhances ROS production, which leads to mitochondrial dysfunction and reduced ATP production (via diminished ATP synthase activity) [61]. By contrast, lycorine, when administered via the intraperitoneal route (at 5 mg/kg/day) over eight weeks, was able to counter this effect and actually stimulate ATP synthase activity [61].

The intrinsic apoptotic pathway, which involves the loss of mitochondrial membrane potential, permeabilisation of the outer mitochondrial membrane and release of pro-apoptotic proteins into the cytosol, can be induced by increased ROS production [62]. Mitochondrial respiratory chain (MRC) complexes I and III are the prime locations for cellular ROS production [62]. While excessive ROS activity is detrimental from a homeostatic point of view, it can be of immense benefit during tumourigenesis [63]. The cytotoxic effect of the Amaryllidaceae alkaloid pancratistatin (**20**) (from an EtOH bulb extract of *Hymenocallis littoralis* Salisb.) (at 1 µM) on LNCaP prostate adenocarcinoma cells was manifested by apoptosis induction, which was accompanied by increased production of ROS and destabilisation of the mitochondrial membrane potential (▶ **Table 3**) [63]. Furthermore, no such action was observable in normal human fibroblast NHF cells, highlighting the high selectivity of pancratistatin [63].

The Alzheimer's drug galanthamine (**5**) is a scavenger of ROS and manifests its neuroprotective effects by modulating oxidative neuronal damage [64]. It also attenuates the overproduction of ROS by increasing acetylcholine levels via the inhibition of AChE and allosteric potentiation of the nicotinic acetylcholine receptor α7 nAChR [64]. Galanthamine exhibited EC_{50} values of 15, 83 and 25 µM for its scavenging activities of oxygen, hydroxyl and HOCl radicals in the NBT spectrophotometric test [65]. AD is typified by cerebral degeneration, neuronal cell death and the buildup of amyloid-β (Aβ) in plaques and tau tangles of nerve cells in affected areas of the brain [66]. The transgenic *Caenorhabditis elegans* CL4176 is characterised by its unique Aβ-expressing ability [66]. When human $A\beta_{1-42}$ is induced in this nematode, by raising the temperature of the growth medium from 16 °C to 23 °C, it leads to paralysis [66]. This can be overcome by the use of secondary metabolites, such as the isoflavone glycitein, which protected CL4176 worms from Aβ-induced toxicity via antioxidative action [66]. Examined for such effects with alkaloids from an EtOH bulb extract of *Lycoris radiata*, it was seen that 8 % of worms exposed to galanthamine (**5**) (10 µM) were still alive after 36 h [66]. The survival rates were improved to 32 % and 42 % by 30 and 50 µM of galanthamine, respectively, 34 h post the temperature upshift [66]. By contrast, there was 100 % mortality in controls 34 h after the temperature adjustment [66]. Haemanthidine (**2**) (at 10, 30 and 50 µM), in contrast, reduced mortalities in CL4176 by 11 %, 14 % and 14 %, respectively, 34 h after the temperature change had been made [66]. It was demonstrated further that attenuation of Aβ toxicity by haemanthidine (**2**) and galanthamine (**5**) in the CL4176 model occurred mostly via the inhibition of AChE gene expression and not via any antioxidant effects [66]. Nonetheless, it was also shown that the anti-Aβ effect may also ensue via the modulation of inflammation and stress-related genes [66]. In this regard, haemanthidine (**2**) and galanthamine (**5**) both (at 50 µM) significantly reduced (by 4.8 and 2.2 times, respectively) the gene expression of the TNFAIP1 (TNFα-induced protein 1) homolog *F22E5.6* [66].

The neuroprotective effects of Amaryllidaceae constituents were probed further via studies carried out in SH-SY5Y neuroblastoma cells [67]. Alkaloid entities from the EtOH bulb extract of *Lycoris longituba* Y. C.Hsu & G. J.Fan were examined for their abilities to alleviate oxidative neuronal damage brought about by H_2O_2 [67]. These were gauged by cell viability assessments made via the MTT staining method [67]. The compounds that produced the best viabilities were lycolongirine A (**21**, 94.5 % at 12.5 µM), lycolongirine C (**22**, 90.4 % at 6.25 µM), incartine (**23**, 93.7 % at 12.5 µM), harmane (**24**, 91.7 % at 25 µM), hippamine (**25**, 95.4 % at 25 µM), *N*-chloromethyl galanthamine (**26**, 93.4 % at 12.5 µM) and 11-deoxytazettine (**27**, 92.9 % at 12.5 µM) [67]. These were all seen to be better than the effect vitamin E had on H_2O_2-exposed SH-SY5Y cells (83.5 % viability at 100 µM) [67]. Activities for the remaining isolates were mostly lower than those of the reference standard [67]. These included lycorine (**1**), haemanthidine (**2**), galanthamine (**5**), lycolongirine B (**28**), norharmane (**29**), perlolyrine (**30**), *N*-chloromethyl narcissidine (**31**), trisphaeridine (**32**), *N*-methylcrinasiadine (**33**), norgalanthamine (**34**), 11β-hydroxygalanthamine (**35**), sanguinine (**36**), *N*-chloromethyl lycoramine (**37**), 9-*O*-demethyllycoramine (**38**) and tazettine (**39**) [67].

The cholinergic anti-inflammatory pathway is considered a putative link between diabetes and Alzheimer's disease [68]. The n5-STZ rat model was thus engaged to verify the potential antidiabetic effect of galanthamine [68]. Diabetic animals treated orally with galanthamine (10 mg/kg/day) for 4 weeks showed (relative to vildagliptin) lowering of n5-STZ-induced elevation in body weight, food/water intake, serum levels of glucose, fructosamine and ALT/AST, as well as AChE in the tested organs [68]. The antioxidant potential of galanthamine (**5**) was demonstrated via its ability to alter the effect of n5-STZ on Nrf2 (nuclear factor erythroid 2-related factor 2), TAC (total antioxidant capacity) and MDA (malondialdehyde) [68]. TAC and Nrf2 levels were dose-dependently increased by galanthamine (at 2.5, 5.0 and 10.0 mg/kg) in both liver and muscle tissue, while there were corresponding depletions in MDA at each of the tested doses [68]. The latter is noteworthy since MDA is a common analytical marker for oxidative stress [68]. Nrf2 is involved in the regulation of the cellular defence response to toxic and oxidative insults [68]. The antioxidant effect of galanthamine (Sun Pharma Laboratories, Jammu, India) was probed during 1,2-dimethylhydrazine (DMH)-induced toxicity in Wistar albino rats [69]. Subcutaneous administration of DMH (20 mg/kg/week) over six weeks caused an increase in MDA compared to saline-treated controls [69]. DMH also produced declines to levels of the other antioxidant markers GSH, SOD and catalase [69]. In contrast, galanthamine (**5**) administered peritoneally at 2 mg/kg/day was seen to reverse all of the deleterious effects brought about by the carcinogen DMH [69]. There has also been interest in the contributions of non-alkaloid principles towards the antioxidant effects of Amaryllidaceae [70]. In this regard, four homoisoflavanones (**40–43**) were described from a CH_3OH extract of the 'Knysna lily' *Cyrtanthus obliquus* (L. f.) Aiton [70]. Measurements made via the FRAP assay showed all four compounds to have antioxidant potential [70]. Further analysis with the DPPH free radical scavenging reagent revealed that the 3-benzylidenechromanones (**40, 41**) were inactive, while the 3-benzylchromanones (**42, 43**) (▶ **Fig. 2**) were weakly active (IC_{50}s 371.54 and 288.40 µg/mL) relative to ascorbic acid (IC_{50} 27.54 µg/mL) [70].

Effects on Mononuclear Cells

Peripheral blood mononuclear cells (PBMCs) (or mononuclear cells) are blood cells with rounded nuclei that originate from haematopoietic stem cells (HSCs) located in bone marrow [71]. HSCs give rise to all blood cells of the immune system through a process called haematopoiesis [71]. As HSCs progress through the various stages of haematopoiesis, they generate the myeloid (monocytes, macrophages, granulocytes, megakaryocytes, dendritic cells and erythrocytes) and lymphoid (T cells, B cells and NK cells) lineages [71]. The first compound from the Amaryllidaceae to be examined in an inflammation-related assay was the phenanthridone glucoside, kalbreclasine (**44**) [72]. Described from root material of *Haemanthus kalbreyeri* Baker (synonymous with *Scadoxus multiflorus* (Martyn) Raf.), kalbreclasine at doses of 20 µg and above produced substantial proliferation of splenic lymphocytes in adult DBA/2 mice [72]. The mitogenic activation observed for kalbreclasine (at 20 µg) was comparable to that of the known mitogen

conA (at 5 µg), as shown via stimulation indices of 3.1 and 3.0, respectively [72].

Chemotaxis has been shown to be a convenient way to gauge the anti-inflammatory response of a particular substance [73]. It is based on the premise that various motile plant and animal cells will gravitate towards or away from foci from which certain chemical substances are diffusing [73]. This has been demonstrated on mammalian leukocytes, which are known to actively migrate towards clumps of bacteria or starch grains [73]. In the investigation to establish the anti-inflammatory activity of *Rhodophiala bifida*, it was found that there was not significant migration of human leukocytes in solution towards montanine (**9**) that was obtained from bulbs of the plant [54]. Further studies on montanine showed that it (at 0.01, 0.1 or 1.0 µM) did not alter the viability of lymphocytes cultured from the lymph nodes of BALB/c mice [26]. While no significant differences between controls and montanine-treated cells were seen with the treatment at 10 µM, diminished cell viabilities were observed at doses higher than 100 µM [26]. Montanine (1.0 µM) had no effect on lymphocyte proliferation stimulated by LPS but significantly decreased proliferation stimulated by conA [26]. LPS acts primarily on the B cell receptor and the Toll-like receptor 4 (TLR4), which are molecules present on the surface of B lymphocytes [26]. ConA by comparison acts on several receptors containing glycoproteins or lipoproteins, stimulating both lymphocytes but acting preferentially on T lymphocytes [26]. The data obtained for montanine thus suggests it has preference for T lymphocytes [26].

As noted above, narciclasine (**3**) displayed considerable therapeutic benefits in a zymosan-induced peritonitis model of C57BL/6 N mice [31]. Further assessment of the peritoneal lavage from control animals revealed that zymosan had also induced notable infiltration of the peritoneum with neutrophils and monocytes [31]. Animals that had prior exposure to narciclasine (1 mg/kg) by contrast showed significant reductions to these mononuclear cells [31]. The study also set out to establish whether narciclasine could impair the interaction of leukocytes with ECs *in vivo* [31]. This was carried out by intravital microscopic observation of rolling, adhesion and transmigration of leukocytes in the mouse cremaster muscle following activation with TNF (300 ng) [31]. Narciclasine (at 1 mg/kg) decreased the number of rolling leukocytes by 50% and fully blocked the processes of adhesion and transmigration [31].

Effects on Cyclooxygenases

The cyclooxygenase isoenzymes COX-1 and COX-2 catalyse the formation of prostaglandins, thromboxane and levuloglandins [74]. As autocoid mediators, prostaglandins mediate in almost all known physiological and pathological processes via their reversible interaction with G-protein-coupled membrane receptors [74]. COX enzymes are of clinical relevance since they can be inhibited by aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) [74]. The inhibition of COX confers symptomatic relief from inflammatory, pyretic, thrombotic, neurodegenerative and oncological maladies [74]. Since Amaryllidaceae plant extracts demonstrated cyclooxygenase-inhibitory activities, there has been considerable interest in the effects of their chemical constituents [75]. The first of such studies involved a screen of 15 alkaloids from 4 alkaloid groups (found in *Crinum bulbispermum* (Burm.f.) Milne-Redh. & Schweick. and *Crinum moorei* Hook.f.) for responses to the cyclooxygenase enzymes COX-1 and COX-2 [75]. Overall, the results relative to indomethacin (mean inhibition 62% at 5 µM and 200 µM, respectively) were poor, ranging from 0% (cherylline **45**) to 32% (6*α*-ethoxy-*N*-demethylpretazettine **46**) against COX-1 at tested dosages of 500 µM, respectively (▶ **Table 4**) [75]. Nevertheless, the alkaloids were shown to be selective inhibitors since of the entire library, only 6*α*-ethoxyprecriwelline (**47**) was active against COX-2 (14% at 500 µM) [75]. COX-1 activities for the other tazettine-related compounds were 11% (6*α*-ethoxyprecriwelline **47**) and 24% (6*β*-ethoxy-*N*-demethylpretazettine **48**) [75]. In addition, 1-*O*-Acetyllycorine (**49**), the only lycorine representative in the library, exhibited a low 4% inhibition [75]. Crinane alkaloids formed the bulk of the targets, with only bulbispermine (**50**) (23%) and 6-hydroxycrinamine (**51**) (21%) exhibiting meaningful activities [75]. Activities for the remaining crinane alkaloids were as follows: 3-*epi*-vittatine (**52**) (17%), 3-*O*-acetylhamayne (**53**) (17%), crinine (**54**) (16%), crinamidine (**55**) (10%), crinamine (**12**) (9%), 3-*epi*-buphanisine (**56**) (4%), powelline (**57**) (4%) and 1-*epi*-deacetylbowdensine (**58**) (3%) [75]. *In vitro* studies carried out on lycorine (**1**) (Aladdin Biochemical Technology, Shanghai, China) in RAW264.7 macrophages showed that lycorine was capable of inhibiting endogenous COX-2 expression [76]. It was seen that while there was increased expression of COX-2 in LPS-stimulated (100 ng/mL) cells, lycorine dose-dependently (1–5 µM) inhibited this elevation relative to the glucocorticoid dexamethasone [76]. For comparative purposes, no data are available on exogenous COX activation by lycorine. Narciclasine (**3**) (from a *Lycoris radiata* EtOH bulb extract) was likewise examined for COX-2 inhibitory effects in LPS-stimulated RAW264.7 murine macrophages, where it dose-dependently (0.008 and 0.016 µM) inhibited COX-2 protein expression relative to dexamethasone [77]. This was supported by its suppressive effect on COX-2 mRNA expression at both of the tested concentrations [77]. Narciclasine dose-dependently (0.1, 0.2 and 0.3 µM) prevented LPS-induced neuro-inflammation in BV-2 microglia [78]. The involvement of COX was demonstrated by the fact that it significantly reduced the protein expression of COX-2 as well as COX-2 mRNA levels, both of which were shown to be elevated via LPS treatment alone [78]. Similarly, COX-2 expression was markedly suppressed by the narciclasine derivative *trans*-dihydrolycoricidine (**59**) (from an EtOH bulb extract of *Lycoris chejuensis* K.Tae & S.C.Ko) (at 0.1, 0.3 and 0.5 µM) in LPS-stimulated (100 ng/mL) BV-2 cells [79]. The fact that COX-2, which is not normally present in microglial cells, was induced during the neuro-inflammatory process suggests that it may be a valuable target in attempts to slow the progression of neurodegeneration [79]. Furthermore, LPS (2.5 mg/kg/day/8 days) induced over-expression of COX-2 in the cerebral cortex of C57BL/6 N mice and was attenuated by *trans*-dihydrolycoricidine (relative to genipin) in a dose-dependent manner (0.5, 1 and 1.5 mg/kg) [79]. Attenuation of COX-2 over-expression was also observed in Tg2576 Swedish mutant APP transgenic mice, which contain two mutations associated with early-onset AD, following *trans*-dihydrolycoricidine exposure (at 0.5, 1 and 1.5 mg/kg) [79].

▶ **Table 4** Effects of Amaryllidaceae constituents on cyclooxygenase enzymes.

Compound (no.)	COX inhibitory activity	Ref.
Lycorine (**1**)	Dose-dependently (1–5 μM) inhibited COX-2 expression in LPS-challenged RAW264.7 macrophages.	[76]
Narciclasine (**3**)	Dose-dependently (0.008 and 0.016 μM) inhibited COX-2 protein and COX-2 mRNA expression in LPS-stimulated RAW264.7 macrophages.	[77]
	Attenuated (at 0.7 mg/kg) LPS-induced increase in COX-2 expression in ALI mice.	[25]
	Dose-dependently (0.1, 0.2 and 0.3 μM) reduced the protein expression of COX-2, as well as COX-2 mRNA levels, during LPS-induced neuro-inflammation in BV-2 microglia.	[78]
Galanthamine (**5**)	Attenuated COX levels (at 2 mg/kg/day/6 weeks) in rats subjected to the toxic effects of DMH.	[69]
Crinamine (**12**)	9% inhibition of COX-1 at 500 μM.	[75]
Norbelladine (**14**)	At 0.25 μM inhibited COX-1 and COX-2 by 51% and 25%, respectively.	[58]
8,9-Dimethoxycripowellin C (**15**)	69.7% and 93.6% inhibition of COX-1 and COX-2 at 100 μM, respectively.	[59]
8,9-Dimethoxycripowellin D (**16**)	66.8% and 92.7% inhibition of COX-1 and COX-2 at 100 μM, respectively.	[59]
6-Methoxycripowellin B (**17**)	70.2% and 91.1% inhibition of COX-1 and COX-2 at 100 μM, respectively.	[59]
8-Hydroxy-9-methoxycripowellin B (**18**)	67.9% and 95.2% inhibition of COX-1 and COX-2 at 100 μM, respectively.	[59]
Cripowellin C (**19**)	64.1% and 90.4% inhibition of COX-1 and COX-2 at 100 μM, respectively.	[59]
Cherylline (**45**)	No inhibition of COX-1 or COX-2 at 500 μM.	[75]
6*α*-Ethoxy-*N*-demethylpretazettine (**46**)	32% inhibition of COX-1 at 500 μM.	[75]
6*α*-Ethoxyprecriwelline (**47**)	11% and 14% inhibition of COX-1 and COX-2 at 500 μM, respectively.	[75]
6*β*-Ethoxy-*N*-demethylpretazettine (**48**)	24% inhibition of COX-1 at 500 μM.	[75]
1-*O*-Acetyllycorine (**49**)	4% inhibition of COX-1 at 500 μM.	[75]
Bulbispermine (**50**)	23% inhibition of COX-1 at 500 μM.	[75]
6-Hydroxycrinamine (**51**)	21% inhibition of COX-1 at 500 μM.	[75]
3-*epi*-Vittatine (**52**)	17% inhibition of COX-1 at 500 μM.	[75]
3-*O*-Acetylhamayne (**53**)	17% inhibition of COX-1 at 500 μM.	[75]
Crinine (**54**)	16% inhibition of COX-1 at 500 μM.	[75]
Crinamidine (**55**)	10% inhibition of COX-1 at 500 μM.	[75]
3-*epi*-Buphanisine (**56**)	4% inhibition of COX-1 at 500 μM.	[75]
Powelline (**57**)	4% inhibition of COX-1 at 500 μM.	[75]
1-*epi*-Deacetylbowdensine (**58**)	3% inhibition of COX-1 at 500 μM.	[75]
trans-Dihydrolycoricidine (**59**)	Dose-dependently (0.1, 0.3 and 0.5 μM) suppressed COX-2 activity in LPS-stimulated BV-2 cells.	[79]
	Dose-dependently (0.5, 1 and 1.5 mg/kg) attenuated LPS-induced over-expression of COX-2 in the cerebral cortex of C57BL/6 N mice.	[79]
	Dose-dependently (0.5, 1 and 1.5 mg/kg) attenuated over-expression of COX-2 in the cerebral cortex of APP transgenic mice.	[79]
1,2-Dihydroxyanhydro-lycorin-6-one (**60**)	< 0% and 22.3% inhibition of COX-1 and COX-2 at 100 μM, respectively.	[80]
2-Hydroxyanhydro-lycorin-6-one (**61**)	< 0% inhibition of COX-1 and COX-2 at 100 μM, respectively.	[80]
1,2-Dihydroxyanhydrolycorine-*N*-oxide (**62**)	< 0% and 24.7% inhibition of COX-1 and COX-2 at 100 μM, respectively.	[80]
1,2-Dihydroxy-5,6-dihydrobicolorine (**63**)	12.9% and 27.6% inhibition of COX-1 and COX-2 at 100 μM, respectively.	[80]

continued next page

▶ **Table 4** *Continued*

Compound (no.)	COX inhibitory activity	Ref.
8-*O*-Demethylhomo-lycorine-α-*N*-oxide (**64**)	13.4% and 45.3% inhibition of COX-1 and COX-2 at 100 µM, respectively.	[80, 81]
2-*O*-Demethylisocorydione (**65**)	36.7% and 96.5% inhibition of COX-1 and COX-2 at 100 µM, respectively.	[80]
Isocorydione (**66**)	45.5% and 93.2% inhibition of COX-1 and COX-2 at 100 µM, respectively.	[80]
8-*O*-Demethyldehydrocrebanine (**67**)	38.5% and 95.3% inhibition of COX-1 and COX-2 at 100 µM, respectively.	[80]
1-Hydroxyungeremine (**68**)	36.7% and 96.5% inhibition of COX-1 and COX-2 at 100 µM, respectively.	[81]
6β-*O*-Acetyl-8-hydroxy-9-methoxycrinamine (**69**)	12.9% and 27.6% inhibition of COX-1 and COX-2 at 100 µM, respectively.	[81]
6β-*O*-Acetylcrinamine (**70**)	12.9% and 27.6% inhibition of COX-1 and COX-2 at 100 µM, respectively.	[81]
2-Hydroxy-8-*O*-demethylhomolycorine-α-*N*-oxide (**71**)	< 0% and 22.3% inhibition of COX-1 and COX-2 at 100 µM, respectively.	[81]
N-Methoxycarbonyl-2-*O*-demethylisocorydione (**72**)	45.5% and 93.2% inhibition of COX-1 and COX-2 at 100 µM, respectively.	[81]
Zephygranditine A (**73**)	50.2% and 88.7% inhibition of COX-1 and COX-2 at 100 µM, respectively.	[82]
Zephygranditine B (**74**)	48.3% and 85.1% inhibition of COX-1 and COX-2 at 100 µM, respectively.	[82]
Zephygranditine C (**75**)	40.1% and 69.7% inhibition of COX-1 and COX-2 at 100 µM, respectively.	[82]
Zephygranditine D (**76**)	37.1% and 64.1% inhibition of COX-1 and COX-2 at 100 µM, respectively.	[82]
Zephygranditine E (**77**)	36.1% and 55.2% inhibition of COX-1 and COX-2 at 100 µM, respectively.	[82]
Zephygranditine F (**78**)	35.6% and 52.7% inhibition of COX-1 and COX-2 at 100 µM, respectively.	[82]
Isoeugenitol (**79**)	Weak activity against COX-1 (IC_{50} 262 µM).	[83]

Neonatal Sprague–Dawley rats subjected to LPS-induced (2 mg/kg) ALI were spared from fatality by pre-treatment with narciclasine (0.7 mg/kg) [25]. In addition to reducing levels of TNF-α, IL-6, IL-1β and MCP-1 in lung tissue of stricken rats, narciclasine also attenuated the LPS-induced increase in COX-2 expression [25]. Norbelladine (**14**), an open-chain precursor molecule of Amaryllidaceae alkaloids, at 0.25 µM also inhibited both COX-1 and COX-2 enzymes by 51% and 25%, respectively [58]. These were shown to be comparable to the reference standards aspirin (for COX-1) and NS-398 (for COX-2) at equimolar concentrations (▶ **Table 4**) [58]. The five alkaloids (8,9-dimethoxycripowellin C **15**, 8,9-dimethoxycripowellin D **16**, 6-methoxycripowellin B **17**, 8-hydroxy-9-methoxycripowellin B **18** and cripowellin C **19**) described from EtOH bulb extracts of *Crinum latifolium* were analysed for COX-1 and COX-2 inhibitory effects [59]. Their responses were shown to be highly selective towards COX-2 (90.4–95.2% inhibition) relative to NS-398 (97.0%), all at the tested dosage of 100 µM [59]. The best activity measured was that of 8-hydroxy-9-methoxycripowellin B (**18**) [59]. The COX-1 inhibitions ranged from 64.1% (for cripowellin C **19**) to 70.2% (for 6-methoxycripowellin B **17**), against which SC-560 (63.5% inhibition) was utilised as a reference standard [59].

Studies on the Asian medicinal plant *Lycoris aurea* (L'Hér.) Herb. led to the isolation of eight alkaloids (**60–67**) from its EtOH bulb extract [80]. The last three of these (2-*O*-demethylisocorydione **65**, isocorydione **66** and 8-*O*-demethyldehydrocrebanine **67**) belonged to the aporphine subgroup of isoquinoline alkaloids, whose members have rarely been found in Amaryllidaceae plants [80]. In the screen for COX-inhibitory action (at 100 µM), better overall results were seen against COX-2 than COX-1 [80]. All three aporphine alkaloids (**65–67**) (relative to NS-398) were noted for their potent anti-COX-2 activities (> 93% inhibition) [80]. Of the Amaryllidaceae alkaloids, the best COX-2 inhibitor was shown to be the homolycorine analogue (8-*O*-demethylhomolycorine-*α*-*N*-oxide **64**), with 45.3% inhibition [80]. In contrast, only the aporphine alkaloid isocorydione (**66**) (relative to SC-560) was able to achieve a level of COX-1 inhibition that was greater than 40% [80]. Activities for the Amaryllidaceae alkaloids (**60–64**) against COX-1 ranged from < 0% to only 13.4% [80]. Further studies of the genus *Lycoris* involved the 'red spider lily' *Lycoris radiata*, a popular herb in Chinese and Japanese medicinal culture [81]. A bulbous EtOH extract of the plant afforded six alkaloids (8-*O*-demethylhomolycorine-*α*-*N*-oxide **64**, 1-hydroxyungeremine **68**, 6*β*-*O*-acetyl-8-hydroxy-9-methoxycrinamine **69**, 6*β*-*O*-acetylcrinamine **70**, 2-hydroxy-8-*O*-demethylhomolycorine-*α*-*N*-oxide **71** and *N*-methoxycarbonyl-2-*O*-demethylisocorydione **72**), the last of which (as also seen with *Lycoris aurea* above) was characterised by its aporphine-based isoquinoline structural features [81]. Their COX-1 activities relative to SC-560 (63.2% inhibition at 100 µM) were poor, ranging from < 0% (for **71**) to 45.5% (for **72**) [81]. The lycorine analogue (**68**), as well as the aporphine alkaloid (**72**), displayed potent effects against the COX-2 enzyme with inhibitions (96.5% and 93.2%, respectively) that matched that of the standard NS-398 (97.1%), all at 100 µM [81]. These responses were al-

so highly selective as the corresponding COX-1 inhibitions were found to be 36.7% and 45.5%, respectively [81]. The anti-COX-2 effects of the remaining isolates ranged from 22.3% to 45.3% [81].

Galanthamine (**5**) was seen to reverse the deleterious effects of the carcinogen DMH on the antioxidant markers MDA, GSH, SOD and catalase in rats [69]. A further result from the study was that while DMH (20 mg/kg/week/six weeks) produced an increase in COX levels, this could be attenuated with the inclusion of galanthamine (2 mg/kg/day) [69]. Whole plants of *Zephyranthes grandiflora* Lindl. (synonymous with *Zephyranthes minuta* (Kunth) D. Dietr.) after EtOH extraction yielded six plicamine-type alkaloids (zephygranditine A **73**, zephygranditine B **74**, zephygranditine C **75**, zephygranditine D **76**, zephygranditine E **77** and zephygranditine F **78**) [82]. Better overall activities were observed against COX-2 (52.7–88.7% inhibition) than against COX-1 (35.6–50.2% inhibition) based on measurements carried out at 100 µM [82]. Compounds **73** and **74** were the most active against COX-1 (50.2% and 48.3% inhibition, respectively) and COX-2 (88.7% and 85.1% inhibition, respectively), wherein SC-560 (63.3% inhibition) and NS-398 (96.7% inhibition) were the respective control standards [82]. There has also been some interest in the non-alkaloid entities of the Amaryllidaceae that could explain the anti-inflammatory effects for its members [83]. Amongst the first studies to do so was the investigation of *Gethyllis ciliaris* (Thunb.) Thunb., from which the chromone isoeugenitol (**79**) (▶ **Fig. 3**) was described from a CH_3OH bulb/root extract [83]. However, its activity against COX-1 (IC_{50} 262 µM) relative to indomethacin (IC_{50} 3.1 µM) proved to be disappointing [83]. This is not surprising, given that natural chromone compounds are not known for their COX-inhibitory activities [84]. Nonetheless, the chromone nucleus has been used to good effect in the development of semi-synthetic chromone analogues, some of which have displayed potent activities against COX [84].

Effects on Lipoxygenases

Lipoxygenases (LOXs) are dioxygenase enzymes that catalyse the formation of hydroperoxides from polyunsaturated fatty acids such as linoleic acid and arachidonic acid [85]. They are expressed in immune, epithelial and tumour cells and thus perform physiological roles related to inflammation, skin disorders and tumourigenesis [85]. Six isoforms of LOX are known to occur in humans and mice [85]. Of these, the 5-LOX isoform is a key role player in asthma and inflammation [85]. It causes constriction of bronchioles in response to cysteinyl leukotrienes such as LTC4, thus leading to asthma [85]. As well, 5-LOX also induces neutrophilic inflammation in response to LTB4 [85]. The study of DMH-induced toxicity in rats discussed above showed that galanthamine (**5**) was able to negate the deleterious effects of the toxin on the antioxidant markers MDA, GSH, SOD and catalase [69]. Galanthamine also reversed the effect of DMH on cyclooxygenase activity [69]. Further analysis showed that there were increased levels of LOX in DMH-treated (20 mg/kg/week) animals, which could nevertheless be normalised by galanthamine (2 mg/kg/day) over the six-week treatment period [69].

Effects on Prostaglandins

Lycorine (**1**) has been examined for its mediatory action on prostaglandin release in RAW264.7 murine macrophages [76]. The results showed that lycorine (5 µM) produced a significant reduction in PGE2 in cells that had been stimulated by LPS (100 ng/mL) [76]. PGE2 synthesis is initiated with the activation of arachidonic acid by phospholipase A2, following which it is oxygenated by COX to form prostaglandin endoperoxides [86]. Prostaglandin G2 (PGG2) is modified by the peroxidase moiety of COX to produce prostaglandin H2 (PGH2), which is then converted to PGE2 [86]. PGE2 contributes to inflammation by enhancing oedema and leukocyte infiltration, through increased vascular permeability, particularly when acting on its receptor EP2 [76]. NSAIDs such as aspirin and ibuprofen can effectively block COX-2, thereby decreasing production of PGE2, which serves to alleviate fever and inflammation [76]. The IC_{50} for lycorine (from an EtOH leaf extract of *Crinum asiaticum*) against PGE2 release in RAW264.7 macrophages under LPS stimulation (0.08 µg/mL) was later determined to be > 0.8 µg/mL, markedly higher than that seen with prednisolone (IC_{50} 0.07 µg/mL) [87]. Narciclasine (**3**) dose-dependently (at 0.1, 0.2 and 0.3 µM) alleviated LPS-induced (100 ng/mL) neuro-inflammation in BV-2 microglia [78]. There was significant release of PGE2 in the LPS-activated microglia, a scenario that was dose-dependently attenuated by narciclasine [78]. Similar reductions in PGE2 were seen with the narciclasine derivative *trans*-dihydrolycoricidine (**59**) (at 0.1, 0.3 and 0.5 µM) in LPS-stimulated (100 ng/mL) BV-2 cells [79]. Notably, this response was in accompaniment of the *trans*-dihydrolycoricidine-mediated suppression of COX-2, an up-regulator of PGE2, as seen above [79].

Effects on NF-κB

Phytochemical studies of the Vietnamese medicinal plant *Crinum ensifolium* Roxb. (synonymous with *Crinum viviparum* (Lam.) R.Ansari & V.J.Nair) led to the description of the sesquiterpene lactone parthenin (**80**) from an aerial part EtOH extract [88]. Examined for nuclear factor effects, it was found to strongly inhibit NF-κB activation in TNF-α stimulated HeLa cervical adenocarcinoma cells (IC_{50} 1.82 µM) (▶ **Table 5**) [88]. NF-κB belongs to a family of inducible transcription factors that have various functions in the immune response [89]. Primarily through activation of NF-κB, cytokines of the TNF family induce transcription of genes that regulate inflammation, cell survival, cell proliferation and cell differentiation [89]. Protracted (or aberrant) activation of NF-κB has come to be associated with several inflammatory diseases, hence the appeal of selective NF-κB inhibitors in drug discovery [90].

ROS is also intimately involved with NF-κB activation, which in turn is closely associated with COX-2 expression [89]. Given that the truncated Amaryllidaceae alkaloid norbelladine (**14**) as noted above displayed both antioxidant and COX-2 inhibitory effects, it was also probed for effects pertaining to NF-κB activation [58]. In the event, norbelladine dose-dependently (1, 10 and 20 µM) inhibited NF-κB activation in THP-1 (acute monocytic leukemia) cells following stimulation with LPS (0.5 µg/mL) [58]. The best effect (30% inhibition) was observed at the highest tested concentration [58]. Modulation of NF-κB activity by Amaryllidaceae alkaloids has

▶ **Table 5** Effects of Amaryllidaceae constituents against NF-κB activation.

Compound (no.)	Activity against NF-κB	Ref.
Lycorine (**1**)	Inhibited (at 0.4 μM) IL-1β-induced phosphorylation of JNK and activation of the NF-κB pathway.	[21]
	Inhibited (at 20 and 40 mg/kg) LPS-induced (2 mg/kg) activation of the HMGB1/TLRs/NF-κB pathway during ALI in mice.	[38]
	Inhibited (at 10 μM) LPS-induced (2 μg/mL) activation of the HMGB1/TLRs/NF-κB pathway in MLE-12 cells.	[38]
	Suppressed (at 5 mg/kg/day) isoproterenol-induced phosphorylation of NF-κB in heart tissue of C57BL/6 J mice.	[36]
	At 10 mg/kg, reduced spinal cord expression levels of NF-κB and IL-1β during CFA-induced arthritis in mice.	[23]
Narciclasine (**3**)	Did not affect (at 7.2 nM) translocation of NF-κB into the nucleus nor expression of COX-2 in TNF-α-stimulated HUVECs.	[99]
	Suppressed translocation of the NF-κB subunit p65 to the nucleus in LPS-stimulated RAW264.7 macrophages.	[77]
	Blocked (at 300 nM) TNF-triggered NF-κB promotor activity and p65 nuclear translocation in HUVECs.	[31]
	Reversed LPS-induced reduction in cytosolic NF-κB p65 and increase in nuclear NF-κB p65 in rats.	[25]
	Dose-dependently (0.1, 1 and 3 mg/kg) suppressed NF-κB p65 phosphorylation in liver tissue of septic rats.	[33]
	Weakly reduced translocation of NF-κB from the cytoplasm to the nucleus (at 0.1, 0.2 and 0.3 μM) during LPS-induced neuro-inflammation in BV-2 microglia.	[78]
	Dose-dependently (0.1, 0.2 and 0.3 μM) inhibited phosphorylation of NF-κB during LPS-induced neuro-inflammation in BV-2 microglia.	[78]
Galanthamine (**5**)	Depleted NF-κB levels (at 2.5, 5.0 and 10.0 mg/kg) in liver and muscle tissue of diabetic rats.	[68]
	Down-regulated molecules in NF-κB pathway that were activated in TRAPS.	[105]
Norbelladine (**14**)	Dose-dependently (1, 10 and 20 μM) inhibited NF-κB activation in THP-1 cells following LPS challenge.	[58]
Parthenin (**80**)	Inhibited NF-κB activation in TNF-α stimulated HeLa cells (IC_{50} 1.82 μM).	[88]
(*2R,3S*)-7-Methoxy-flavan-3-ol (**81**)	Dose-dependently (3.125–50 μM) inhibited NF-κB activation in LPS-stimulated RAW264.7 macrophages.	[91]
(*2R,3S*)-2′-Hydroxy-7-methoxyflavan-3-ol (**82**)	Dose-dependently (3.125–50 μM) inhibited NF-κB activation in LPS-stimulated RAW264.7 macrophages.	[91]
(*2S*)-4′-Hydroxy-7-methoxyflavan (**83**)	Dose-dependently (3.125–50 μM) inhibited NF-κB activation in LPS-stimulated RAW264.7 macrophages.	[91]
(*2S*)-7,4′-Dihydroxyflavan (**84**)	Dose-dependently (3.125–50 μM) inhibited NF-κB activation in LPS-stimulated RAW264.7 macrophages.	[91]
Crinumin	Suppressed NF-κB levels (at 25 or 50 mg/kg) in rats subjected to carrageenan-induced paw oedema.	[14]

also been pursued with narciclasine (**3**) (from *Lycoris radiata*) in LPS-stimulated RAW264.7 murine macrophages [77]. Translocation of the NF-κB subunit p65 to the nucleus under LPS (1 μg/mL) stimulation indicated the activation of NF-κB in these macrophage cells [77]. However, exposure of stimulated cells to narciclasine (0.016 μM) led to the effective suppression of p65 translocation [77]. Similarly, two flavanols ((*2R,3S*)-7-methoxyflavan-3-ol **81** and (*2R,3S*)-2′-hydroxy-7-methoxyflavan-3-ol **82**) and two flavans ((*2S*)-4′-hydroxy-7-methoxyflavan **83** and (*2S*)-7,4′-dihydroxyflavan **84**) from *Crinum asiaticum* dose-dependently (3.125–50 μM) inhibited NF-κB activation in LPS-stimulated (100 ng/mL) RAW264.7 macrophages [91]. This too was achieved by suppression of p65 translocation [91].

The alleviation of LPS-induced (100 ng/mL) neuro-inflammation in BV-2 microglia proceeded in a manner that was dose-dependent on narciclasine (0.1, 0.2 and 0.3 μM) [78]. This was shown to ensue via the inhibition of the pro-inflammatory factors TNF-α, IL-6, IL-18, NO and PGE2 as referred to elsewhere in the text [78]. Probed as to whether there was any involvement of NF-κB, it was seen that, while narciclasine weakly reduced translocation of NF-κB from the cytoplasm to the nucleus, it did inhibit the phosphorylation of NF-κB [78]. Furthermore, narciclasine inhibited NF-κB signalling by inhibiting the Akt/IKK/IκB signal transduction cascade [78]. Neonatal rats experienced relief from *E. coli*-induced sepsis following exposure to narciclasine (**3**) as indicated above [33]. Further analysis revealed that, while there was in-

creased NF-κβ p65 phosphorylation in the liver tissue of untreated septic animals, narciclasine produced a dose-dependent (0.1, 1 and 3 mg/kg) suppression of this reaction [33]. Iκβα expression in untreated rats was also notably down-regulated, a situation that was modulated by narciclasine, which prevented Iκβα degradation [33].

Narciclasine dose-dependently (10–300 nM) suppressed TNF-induced (10 ng/mL) expression of cell adhesion molecules ICAM-1, VCAM-1 and E-selectin in cultured HUVECs [31]. It also blocked (at 300 nM) crucial steps of the TNF-triggered NF-κB activation cascade, including NF-κB promotor activity, p65 nuclear translocation and IκBα phosphorylation and degradation, as well as IκBα kinase β and TGF-β-activated kinase 1 phosphorylation (▶ **Table 5**) [31]. Alleviation of LPS-induced (2 mg/kg) ALI in neonatal Sprague–Dawley rats by narciclasine (0.7 mg/kg) was seen to involve modulation of TNF-α, IL-6, IL-1β, MCP-1 and COX-2 [25]. It was shown that LPS also reduced expression of cytosolic NF-κB p65 but increased the level of nuclear NF-κB p65 [25]. This was effectively reversed via pre-treatment with narciclasine [25]. Lycorine (**1**) (at 20 and 40 mg/kg) was shown to be effective against LPS-induced (2 mg/kg) ALI in BALB/c mice as referenced above [38]. Attenuation of MDA, TNF-α, IL-6 and IL-1β levels that had seen elevation with LPS were some of the reasons behind this response [38]. Furthermore, lycorine pre-treatment produced a decrease in protein levels of HMGB1, TLR4, TLR5, MyD88, P-p65 and P-IκBα, as well as an increase in IκBα in lung tissue of affected animals [38]. This suggested that lycorine inhibited LPS-induced activation of the HMGB1/TLRs/NF-κB pathway during ALI [38]. Similar observations were made for lycorine *in vitro* (at 10 µM) in MLE-12 cells following the LPS challenge (at 2 µg/mL) [38].

The antidiabetic effect of galanthamine (**5**) in the n5-STZ rat model described above showed that the cholinergic anti-inflammatory pathway can serve as a link between diabetes and Alzheimer's disease [68]. The results indicated that galanthamine also modulated the effect of n5-STZ on NF-κB expression [68]. While n5-STZ alone produced marked increases in NF-κB in liver and muscle tissue of control animals, these were dose-dependently depleted by galanthamine at daily oral doses (2.5, 5.0 or 10.0 mg/kg) over 4 weeks [68]. Examined for its influence on the catabolic activation of rat chondrocytes, lycorine (**1**) as described below was shown to reverse the effect of IL-1β on the expression of the metalloproteinases MMP-3 and MMP-13 [21]. These enzymes are known to have important functions in cartilage destruction that manifests in the progression of osteoarthritis [22]. The study also shed light on the effect of lycorine on IL-1β-induced activation of the NF-κB pathway [21]. In this regard, rat articular chondrocytes incubated with lycorine (0.4 µM) and then exposed to IL-1β (10 ng/mL) showed reduced levels of p-JNK, indicating that it significantly attenuated the phosphorylation of JNK [21]. In contrast, the phosphorylation of ERK and p38 were not affected to any great extent by lycorine [21]. IκB-α protein levels were reduced following IL-1β exposure, which indicated the degradation of IκB-α and activation of the NF-κB signalling pathway [21]. Such changes were not observed in cells that had pre-exposure to lycorine, suggesting that it inhibited IL-1β-induced phosphorylation of JNK and activation of the NF-κB pathway [21]. ERKs, JNKs and p38/SAPKs are the three main families of proteins identifiable within the MAP kinases, which are vital in signal transduction pathways that regulate cell proliferation, cell differentiation and cell death in eukaryotes [92]. The ERKs such as ERK1, ERK2 and ERK5 are more closely associated with cell growth, differentiation and development [92]. The JNKs and p38, in addition to roles in cell growth and differentiation, are also important in inflammation and apoptosis [92]. The glycosylated protease crinumin from the latex of *Crinum asiaticum* inhibited carrageenan-induced paw oedema in rats as noted above [14]. Test subjects that received crinumin (25 or 50 mg/kg/day) over seven days also showed significant suppression of NF-κB levels in paw tissue [14]. Carrageenan activates the NF-κB pathway, thereby stimulating the release of TNF-α and allowing PMN filtration [14]. Carrageenan also stimulates vasodilation by increasing NF-κB-mediated release of NO [14]. Given that NF-κB is a key mediator in the inflammatory response, surprisingly little has thus far been undertaken to probe the effects of Amaryllidaceae constituents (particularly alkaloids) on this protein. Such studies, which have only come to fore in the past decade, will certainly gain traction in efforts going forward.

Effects on Tumour Necrosis Factor

TNFs are a class of proinflammatory cytokines that participate in tumour regression, septic shock and cachexia [93]. Cellular death signals conveyed by TNF-α binding to its receptor (TNF-R1) or the Fas ligand binding the Fas receptor, are the key ways in which TNF is involved in apoptosis [93]. Probes into how lycorine (Latoxan, Rosans, France) and narciclasine (**3**) (from a *Sternbergia lutea* (L.) Ker Gawl. ex Spreng. bulb CH_3OH extract) manifested their anti-inflammatory effects also involved the tumour necrosis factor TNF-α (▶ **Table 6**) [94]. TNF-α production in murine RAW264 macrophages stimulated by lipopolysaccharide (LPS) was strongly inhibited by both alkaloids (ID_{50} s 0.2 and 0.002 µg/ml, respectively) [94]. It was demonstrated further that inhibition of TNF-α by narciclasine was due mainly to the inhibition of protein biosynthesis [94]. Lycorine on the other hand inhibited TNF-α production at concentrations that were lower than those required to inhibit ^{35}S-cysteine/^{35}S-methionine incorporation into macrophages [94]. Studies carried out later revealed that the IC_{50} values for lycorine (**1**) and narciclasine (**3**) (from *Lycoris radiata*) against TNF-α production in LPS-activated RAW264 macrophages were 2.1 and 0.020 µM, respectively [57]. These effects were superior to those of the reference polyphenol butein (IC_{50} 8 µM) [57]. However, butein (ED_{50} > 30 µM) turned out to be less detrimental to the macrophages than either lycorine (ED_{50} 8 µM) or narciclasine (ED_{50} 0.3 µM) [57]. Lycorine (**1**) (5 µM) also inhibited LPS-induced (100 ng/mL) TNF-α release in RAW264.7 macrophages [76]. The IC_{50} for lycorine (from *Crinum asiaticum*) against TNF-α production in LPS-stimulated (5 ng/mL) RAW264.7 macrophages was later shown to be > 0.8 µg/mL, against which prednisolone (IC_{50} 0.07 µg/mL) was used as reference [87].

Similarly, narciclasine dose-dependently (0.001–0.016 µM) inhibited TNF-α activity in LPS-stimulated RAW264.7 macrophages (relative to dexamethasone), with 92 % inhibition observed at the highest dosage [77]. Since they can release considerable amounts of neurotoxic factors, over-activation of microglia has been impli-

▶ **Table 6** Effects of Amaryllidaceae constituents on TNF.

Compound (no.)	Activity against TNF	Ref.
Lycorine (**1**)	Inhibited TNF-α production in LPS-stimulated murine RAW264 macrophages (ID_{50} 0.2 µg/ml).	[94]
	Inhibited TNF-α production in LPS-stimulated murine RAW264 macrophages (IC_{50} 2.1 µM).	[57]
	Inhibited TNF-α production in LPS-stimulated murine RAW264.7 macrophages (IC_{50} > 0.8 µg/mL).	[87]
	Dose-dependently (20 and 40 mg/kg) reduced TNF-α levels in BALB/c mice subjected to LPS-induced (2 mg/kg) acute lung injury.	[38]
	Reduced (at 10 µM) TNF-α levels in MLE-12 cells following exposure to LPS (2 µg/mL).	[38]
	Reduced (at 5 mg/kg/day) protein and mRNA levels of IL-1β, IL-6 and TNF-α in heart tissue of C57BL/6 J mice exposed to isoproterenol.	[36]
	Attenuated (at 0.5 or 1 mg/kg/day) increases in TNF-α, IL-6 and IL-1β during thioacetamide-induced liver fibrosis in rats.	[45]
	Favourable binding energies ascertained in its interactions with TGF-β1, GSK-3, TNF-α and IL-1β (− 5.63, − 6.08, − 3.96 and − 4.05 kcal/mol, respectively).	[126]
Haemanthidine (**2**)	Reduced gene expression of the TNFAIP1 homolog *F22E5.6* by 4.8 times (at 50 µM).	[66]
Narciclasine (**3**)	Inhibited TNF-α production in LPS-stimulated mouse macrophages (ID_{50} 0.002 µg/ml).	[94]
	Inhibited TNF-α production in LPS-stimulated RAW264 macrophages (IC_{50} 0.020 µM).	[57]
	92% inhibition of TNF-α production in LPS-stimulated RAW264.7 macrophages (at 0.016 µM).	[77]
	Depleted E-selectin levels (at 7.2 nM) in HUVECs activated by TNF-α (10 ng/mL).	[99]
	Reduced adhesion (at 7.2 nM) of THP-1 monocytes to TNF-α-stimulated HUVECs by blocking ICAM-1 expression.	[99]
	Induced 50% reduction in TNF levels in peritoneal lavage during zymosan-induced peritonitis in C57BL/6 N mice.	[31]
	Blocked (at 300 nM) TNF-triggered IκBα phosphorylation and degradation, as well as IκBα kinase β and TGF-β-activated kinase 1 phosphorylation, in HUVECs.	[31]
	Blocked (at 300 nM) TNFR1 activity in HUVECs.	[31]
	At 1 mg/kg, decreased the number of rolling leukocytes by 50% and fully blocked the processes of adhesion and transmigration in the mouse cremaster muscle following activation with TNF (300 ng).	[31]
	Diminished (at 0.7 mg/kg) TNF-α levels in rats subjected to LPS-induced (2 mg/kg) acute lung injury.	[25]
	Produced a 10-fold depreciation in TNF-α (at 3 mg/kg) in blood plasma of neonatal rats subjected to *E. coli*-induced sepsis.	[33]
	Dose-dependently (30, 100 and 300 nM) suppressed LPS-induced release of the inflammatory cytokines TNF-α, IL-1β and IL-6 from NRCMs.	[35]
	Attenuated (at 0.1 mg/kg/day/7 days) LPS-induced increases in mRNA expression levels of TNF-α, IL-1β, IL-6 and VEGF in cardiomyocyte lysates of C57BL/6 mice.	[35]
	Dose-dependently (0.1, 0.2 and 0.3 µM) reduced TNF-α levels during LPS-induced neuro-inflammation in BV-2 microglial cells.	[78]
	Attenuated (at 5 mg/kg) conA-induced increases in serum levels of IFN-γ and TNF-α in C57BL/6 mice.	[39]
Sodium narcistatin (**4**)	Blocked elevation of TNF-α levels in PBMCs and splenocytes of arthritic rats (at 5.0 mg/kg/day).	[13]
	Did not modulate TNF-α production in DLN cells of arthritic rats.	[13]
Galanthamine (**5**)	Reduced TNF levels when administered (at 0.1 mg/kg) prior to the initiation of endotoxemia with LPS in BALB/c mice.	[16]
	Atropine sulphate negated the suppressive effect of galanthamine on TNF in mice (both at 4 mg/kg).	[16]
	At 4 mg/kg did not suppress TNF in α7 nAChR-knockout C57BL/6 mice.	[16]
	Reduced gene expression of the TNFAIP1 homolog *F22E5.6* by 2.2 times (at 50 µM).	[66]
	Depleted serum levels of TNF-α (at 2.5, 5.0 and 10.0 mg/kg) in diabetic rats.	[68]

continued next page

▶ **Table 6** *Continued*

Compound (no.)	Activity against TNF	Ref.
trans-Dihydrolycoricidine (**59**)	Dose-dependently (0.1, 0.3 and 0.5 µM) attenuated expression of TNF-α and IL-6 in LPS-stimulated BV-2 cells.	[79]
	Attenuated (0.5, 1 and 1.5 mg/kg/day) LPS-mediated cortical over-expression of TNF-α in C57BL/6 N mice relative to genipin.	[79]
β-Sitosterol (**136**)	Favourable binding energies ascertained in its interactions with TGF-β1, GSK-3, TNF-α and IL-1β (− 6.39, − 5.03, − 4.87 and − 5.04 kcal/mol, respectively).	[126]
Sitosterol 3-*O*-β-D-glucopyranoside (**137**)	Favourable binding energies ascertained in its interactions with TGF-β1, GSK-3, TNF-α and IL-1β (− 8.58, − 4.75, − 5.33 and − 5.33 kcal/mol, respectively).	[126]
Sitosterol 3-*O*-β-D-glucopyranoside-6-*O*-hexadecanoate (**138**)	Favourable binding energies ascertained in its interactions with TGF-β1, GSK-3, TNF-α and IL-1β (− 8.49, − 8.31, − 7.01 and − 6.22 kcal/mol, respectively).	[126]
Stigmasterol (**139**)	Favourable binding energies ascertained in its interactions with TGF-β1, GSK-3, TNF-α and IL-1β (− 5.46, − 5.13, − 4.97 and − 4.81 kcal/mol, respectively).	[126]

cated in the pathogenesis of neurodegenerative diseases [95]. Thus, inhibiting microglial activation may represent an attractive approach for preventing neuro-inflammatory disorders [95]. In this regard, a study was undertaken to examine the effect of narciclasine on LPS-induced neuro-inflammation in BV-2 microglial cells [78]. Narciclasine dose-dependently (0.1, 0.2 and 0.3 µM) over 12 hr reduced TNF-α levels that had been elevated by LPS (100 ng/mL) [78]. These effects were manifested without significant toxicity at concentrations up to 0.3 µM, in the presence or absence of LPS [78]. Similar prior observations had been made with the narciclasine analogue *trans*-dihydrolycoricidine (**59**), an isolate of the Korean medicinal plant *Lycoris chejuensis* [79]. It dose-dependently (0.1, 0.3 and 0.5 µM) attenuated expression of the TNF-α that had been raised by LPS (100 ng/mL) [79]. This was attained without deleterious effects on BV-2 cells at concentrations up to 2 µM in the absence or presence of LPS [79]. Furthermore, C57BL/6 N mice treated intraperitoneally with LPS (2.5 mg/kg/day/8 days) showed significant cortical over-expression of TNF-α, which *trans*-dihydrolycoricidine (relative to genipin) dose-dependently (0.5, 1 and 1.5 mg/kg) reversed by means of oral exposure [79].

Narciclasine (**3**) was effective in the zymosan-induced peritonitis model of C57BL/6 N mice as described above [31]. Further information to emerge from the study was the fact that, whereas zymosan alone (at 2 mg/mL) produced an increase in TNF (to 140 pg/mL) in the peritoneal lavage, this was halved by narciclasine at subcutaneous doses of 1 mg/kg (▶ **Table 6**) [31]. Narciclasine was shown to be capable of negating the symptoms of acute lung injury (induced by LPS) in neonatal rats as discussed above [25]. The study also reported observations on the effect of this alkaloid on the expression of various inflammatory factors (including TNF-α) during the progression of the disease [25]. It was seen that, whereas LPS at 2 mg/kg produced a marked increase in serum TNF-α to 60 ng/mL, pre-treatment with narciclasine (0.7 mg/kg) reduced this to 20 ng/mL over 72 hr [25]. Similar reductions were observed with the endothelial cell adhesion molecules ICAM-1 and VCAM-1 [25]. Narciclasine pre-treatment also caused significant reduction in the CC chemokine MCP-1, from around 2000 ng/L (with LPS alone) to 500 ng/L [25]. Lycorine (**1**) also negated the deleterious effects of LPS-induced ALI in BALB/c mice as noted above [38]. It achieved this (at 20 and 40 mg/kg) by attenuating the increase in TNF-α in lung tissue brought about by exposure to LPS (2 mg/kg) [38]. Similarly, lycorine (at 10 µM) reduced TNF-α levels in cultured mouse lung epithelial MLE-12 cells that had been stimulated by LPS (2 µg/mL) [38]. By lowering bacterial loads, narciclasine (**3**) as alluded to above improved the survival of neonatal rats subjected to *E. coli*-induced sepsis [33]. Examined as to whether it could also mediate in the intensive inflammatory component of the disease, narciclasine was shown to be capable of dose-dependently reducing systemic levels of TNF-α in affected animals [33]. In this regard, a tenfold depreciation in TNF-α was measured in the blood plasma of narciclasine-treated subjects (at 3 mg/kg) compared to untreated control animals [33].

Dietary regimens geared towards inhibiting the production of pro-inflammatory cytokines and mediators (such as TNF-α) are potentially beneficial in the treatment of chronic inflammatory conditions [57]. While there has been significant interest in polyphenolic substances (such as butein) in dietary interventions against inflammation, considerably less is known about such actions for alkaloid entities [57]. The results seen with the medicinal herb *Lycoris radiata* suggested that alkaloids (Amaryllidaceae alkaloids in particular) may indeed be of significant benefit in such circumstances [57]. The sodium phosphate derivative of narciclasine (sodium narcistatin **4**) has been examined for *ex vivo* modulation of TNF-α in PBMCs derived from arthritic male Lewis rats [13]. To this extent, while cells obtained from saline-treated animals reflected a pronounced elevation in TNF-α levels (> 200 %), this was effectively blocked in the PBMCs of rats that had been exposed to sodium narcistatin (**4**) (5.0 mg/kg/day) [13]. Similar observations were made for **4** in splenocytes from the adjuvant-induced arthritic rat subjects [13]. By contrast, there were no significant differences between the levels of TNF-α released by DLN (draining lymph node) cells from arthritic or non-arthritic rats [13]. Increased expression of endothelial cell adhesion molecules (such as ICAM-1, VCAM-1 and E-selectin) in response to pro-inflammatory mediators such as TNF-α is an important indicator for the ex-

travasation of leukocytes into inflamed tissue [96]. Examined for such effects, narciclasine (**3**) (isolated from an EtOH bulb extract of *Haemanthus coccineus* L.) was seen to dose-dependently inhibit ICAM-1 up-regulation in HUVECs in the range of 0.003 to 1 µM [97, 98]. HUVECs pre-treated with narciclasine showed a notable depreciation in ICAM-1 expression (IC_{50} 50 nM) following 24 hours of exposure to TNF-α [97, 98]. Later, it was shown that narciclasine (from the *Cyrtanthus contractus* CH_3OH bulb extract) depleted E-selectin levels (at 7.2 nM) in human endothelial (HUVEC) cells that had been activated by TNF-α (10 ng/mL) [99]. At the same concentration, it also reduced the adhesion of THP-1 monocytes to TNF-α-stimulated HUVECs by blocking expression of the endothelial cell adhesion molecule ICAM-1 [99]. E-selectin plays a key role in the recruitment of leukocytes to the site of inflammation [100]. The release of cytokines TNF-α and IL-1 by macrophages in inflamed tissue induces the over-expression of E-selectin on endothelial cells of blood vessels [100]. TNF-α triggers the translocation of NF-κB from the cytoplasm into the nucleus, thereby facilitating the transcription of target genes [96]. TNF-α stimulation of the vascular endothelium increases the expression of cell adhesion molecules such as ICAM-1, VCAM-1 and E-selectin [96]. It was seen that, while narciclasine (**3**) reduced E-selectin and ICAM-1 expression, it did not affect translocation of NF-κB into the nucleus nor expression of COX-2 [99]. This suggested that narciclasine may be involved with mRNA production, shuttling or stability but not with accumulation of NF-κB in the nucleus [99]. Narciclasine suppressed TNF-induced expression of cell adhesion molecules ICAM-1, VCAM-1 and E-selectin, as well as crucial steps of the NF-κB activation cascade in cultured HUVECs [31]. These effects were shown to be a consequence of narciclasine-triggered (300 nM) loss of TNF receptor 1 (TNFR1) activity [31].

Excessive production of TNF and other pro-inflammatory cytokines and their release into the bloodstream from immune cells are intimately associated with the pathophysiology of inflammation [101]. Cytokine production in turn is stimulated by neural input via an inflammatory reflex [101]. Efferent vagus nerve activity, which constitutes the motor arm of the inflammatory reflex, regulates cytokine production via α7 nAChR signalling [102]. Stimulation of this pathway (also referred to as the 'cholinergic anti-inflammatory pathway') during endotoxemia suppresses systemic levels of TNF and other pro-inflammatory cytokines [102]. Acetylcholinesterase (AChE) is responsible for the breakdown of acetylcholine (ACh) in the synaptic cleft leading to a reduction in cholinergic neurotransmission via the degeneration of cholinergic neurons and loss of nicotinic acetylcholine receptor (nAChR) functions [103]. Since AChE inhibition can modulate cholinergic neurotransmission, inhibitors such as galanthamine (**5**) have been shown to be effective in the symptomatic treatment of Alzheimer's disease (AD) [103]. It is conceivable that AChE may also play a role in regulating the vagus-nerve-based cholinergic anti-inflammatory pathway [104]. To probe this, a study was carried out to establish if suppression of the cytokine response during endotoxemia was linked to brain cholinergic transmission via the inhibition of brain AChE [16]. To this extent, galanthamine (**5**) (Calbiochem, San Diego, USA) (0.1, 1.0 or 4.0 mg/kg) administered 1 hr prior to the endotoxin LPS (6 mg/kg) significantly reduced TNF serum levels in BALB/c mice [16]. Furthermore, the effect was manifested via vagus nerve signalling and also served to buffer against murine fatality during endotoxemia [16]. However, prior administration of the centrally acting muscarinic receptor antagonist atropine sulphate (4 mg/kg) negated the suppressive effect on TNF by galanthamine [16]. This suggested that suppression of AChE activity via central muscarinic receptors was involved in the control of peripheral cytokine responses [16]. Galanthamine (at 4 mg/kg) also failed to suppress TNF in C57BL/6 mice containing the α7 nAChR knockout gene [16]. This indicated that the α7 nAChR-mediated cholinergic anti-inflammatory pathway is required for the anti-inflammatory effect of galanthamine [16].

The cholinergic anti-inflammatory pathway also serves as a link between diabetes and Alzheimer's disease [68]. This was ratified via studies probing the antidiabetic effect of galanthamine (**5**) in the n5-STZ rat model as outlined above [68]. Additional information to emerge from the study was the fact that galanthamine also modulated the effect of n5-STZ on TNF-α expression [68]. While n5-STZ caused a pronounced increase in serum TNF-α in test animals (to ~ 170 pg/mL), this was attenuated by galanthamine at all tested oral dosages (2.5, 5 and 10 mg/kg/day) over 4 weeks [68]. The best reduction (~ 50 pg/mL) was observed at the 5 mg/kg level of testing [68]. Since galanthamine can modulate TNF production, a study was carried out to ascertain its effect on the TNF receptor, TNFR1 [105]. At the basis of the investigation was the auto-inflammatory disease TRAPS (TNF-receptor-associated periodic syndrome), which arises as a consequence of mutations in TNFR1 (TNF Receptor 1) [105]. The authors sought to identify drugs for repurposing as anti-inflammatories based on their capacities to down-regulate molecules linked to inflammatory signalling pathways that were activated in TRAPS [105]. Galanthamine appeared amongst the top 35 (out of 1300 approved drugs screened) that produced changes in pro-inflammatory pathways caused by a TRAPS-associated C33Y-mutant TNFR1 [105]. These observations were made in the human liver adenocarcinoma SK-Hep-1 endothelial-like cell line (which expresses low levels of TNFR1), which was stably transfected with wild-type or C33Y mutant TNFRSF1A gene constructs [105]. The four inflammatory signalling pathways activated in TRAPS included the NF-κB, PI3K/Akt, MAPK and JAK/STAT3 pathways, from which 40 diagnostic signalling molecules were detected by reverse-phase protein microarray (RPPA) analysis [105]. Galanthamine was seen to be involved with the NF-κB pathway wherein TRAF2, p-RIP2(S176), p-IκB alpha, p-NFκBp65, A20/TNFAIP3 and c-IAP1 were identified as the key signalling molecules [105].

Effects on Interleukins

Investigation into the effect of lycorine (**1**) on interleukins showed that it (at 5 µM) significantly inhibited IL-6 release in RAW264.7 macrophages that were stimulated by LPS (100 ng/mL) (▶ **Table 7**) [76]. The human genome codes for more than 50 interleukins and related proteins [106]. Interleukins (ILs) constitute a group of cytokines that are expressed mainly by white blood cells (leukocytes), which perform key functions in the regulation of the immune system [106]. Rare deficiencies of a number of ILs have been reported, all of which are associated with autoimmune diseases or immune deficiency [106]. The majority of ILs are synthes-

▶ **Table 7** Effects of Amaryllidaceae constituents against interleukins.

Compound (no.)	Activity against interleukins	Ref.
Lycorine (**1**)	Reduced (at 5 μM) IL-6 release in LPS-activated RAW264.7 macrophages.	[76]
	Dose-dependently (0.05–0.4 μM) down-regulated matrix metalloproteinases MMP-3 and MMP-13 in rat chondrocytes that had been exposed to IL-1β.	[21]
	Reduced IL-8 levels in HCT-116 and LoVo cells (at 6 μM).	[107]
	Dose-dependently (20 and 40 mg/kg) reduced IL-6 and IL-1β levels in BALB/c mice subjected to LPS-induced (2 mg/kg) acute lung injury.	[38]
	Reduced (at 10 μM) IL-6 and IL-1β levels in MLE-12 cells following exposure to LPS (2 μg/mL).	[38]
	Reduced (at 10 mg/kg/day/3 weeks) levels of cleaved caspase-1 and cleaved IL-1β during bleomycin-induced IPF in mice.	[111]
Narciclasine (**3**)	98% inhibition of IL-6 production in LPS-stimulated RAW264.7 macrophages at 0.016 μM.	[77]
	40% inhibition of IL-1β production in LPS-stimulated RAW264.7 macrophages at 0.016 μM.	[77]
	Induced 50% reduction in IL-6 and IL-1β levels in peritoneal lavage during zymosan-induced peritonitis in C57BL/6 N mice.	[31]
	Diminished (at 0.7 mg/kg) IL-6 and IL-1β levels in rats subjected to LPS-induced (2 mg/kg) acute lung injury.	[25]
	Dose-dependently (0.1, 1 and 3 mg/kg) reduced IL-1α, IL-1β, IL-2 and IL-6 levels in blood plasma of sepsis rats.	[33]
	Dose-dependently (0.1, 1 and 3 mg/kg) increased levels of IL-4 and IL-10 in blood plasma of sepsis rats.	[33]
	Dose-dependently (0.1, 0.2 and 0.3 μM) reduced levels of IL-6 and IL-18 during LPS-induced neuro-inflammation in BV-2 microglia.	[78]
	Dose-dependently (0.1, 0.2 and 0.3 μM) increased levels of IL-10 and TGF-β1 during LPS-induced neuro-inflammation in BV-2 microglia.	[78]
	No effect on IL-8 production in HCT-116 and LoVo cells (at 0.08 μM).	[107]
	Reduced (at 1 and 3 mg/kg) levels of IL-4, IL-6, IL-17 and IL-21 in the BALF of neonatal rats subjected to ovalbumin-induced asthma.	[47]
	Attenuated (at 5 mg/kg) conA-induced increases in serum levels of IL-2, IL-6, IL-10 and IL-17 in C57BL/6 mice.	[39]
Sodium narcistatin (**4**)	Blocked elevation of IL-1, IL-2 and IL-10 levels in PBMCs of arthritic rats (at 5.0 mg/kg/day).	[13]
	Blocked the increase in IL-6 in the splenocytes of arthritic rats (5.0 mg/kg/day).	[13]
	Blocked the release of IL-1 from DLN cells of arthritic rats (5.0 mg/kg/day).	[13]
Pseudolycorine (**10**)	Dose-dependently (0.67, 2 and 6 μM) inhibited IL-6- and GM-CSF-induced MDSC proliferation, expansion and differentiation into monocyte-like MDSCs.	[49]
	Alleviated autoimmune encephalomyelitis in mice by reducing monocyte-like MDSC infiltration into the spinal cord and inhibiting Th17 cell differentiation and IL-17A secretion.	[49]
Haemanthamine (**11**)	No effect on IL-8 production in HCT-116 and LoVo cells (at 6 μM).	[107]
trans-Dihydrolycoricidine (**59**)	Dose-dependently (0.1, 0.3 and 0.5 μM) potentiated expression of IL-10 in LPS-stimulated BV-2 cells.	[79]
(*2R,3S*)-7-Methoxy-flavan-3-ol (**81**)	Dose-dependently (3.125–50 μM) reduced IL-6 production in LPS-stimulated RAW264.7 macrophages.	[91]
(*2R,3S*)-2′-Hydroxy-7-methoxyflavan-3-ol (**82**)	Dose-dependently (3.125–50 μM) reduced IL-6 production in LPS-stimulated RAW264.7 macrophages.	[91]
(*2S*)-4′-Hydroxy-7-methoxyflavan (**83**)	Dose-dependently (3.125–50 μM) reduced IL-6 production in LPS-stimulated RAW264.7 macrophages.	[91]
(*2S*)-7,4′-Dihydroxyflavan (**84**)	Dose-dependently (3.125–50 μM) reduced IL-6 production in LPS-stimulated RAW264.7 macrophages.	[91]
Narcin	Increased IL-10 production (at 10 μg/mL) by 2% in $CD4^+$ T cells from PBMCs.	[108]
	Increased IL-4 production (at 10 μg/mL) by 2.6% in $CD4^+$ T cells from PBMCs.	[108]
	Increased IL-13 production (at 10 μg/mL) by 3.5% in $CD4^+$ T cells from PBMCs.	[108]

ised by CD4 helper T-lymphocytes, as well as by macrophages, monocytes and endothelial cells [106]. In addition to demonstrating its protective effect on cartilage in the mouse ACLT model above, lycorine (**1**) was also probed for its effect on the catabolic activation of rat chondrocytes [21]. These cells and their ECM are the two main components of cartilage, gradual damage to which is known to be of relevance in osteoarthritis [22]. In particular, ECM-degrading enzymes, notably matrix metalloproteinases, are crucial for cartilage destruction during the progression of osteoarthritis [22]. It was seen that there was significant up-regulation of mRNA levels of the matrix metalloproteinases MMP-3 and MMP-13 in chondrocytes that had been exposed to IL-1β (10 ng/mL) [21]. This was reversed by lycorine (0.05–0.4 µM), leading to the dose-dependent down-regulation of the two proteinases [21]. Apart from this, the secretion of MMP-1, MMP-2 and MMP-7 were markedly diminished in colon HCT-116 carcinoma and LoVo adenocarcinoma cells by lycorine (**1**) (6 µM), narciclasine (**3**) (0.08 µM) and haemanthamine (**11**) (6 µM) (from *Sternbergia lutea* and *Narcissus pseudonarcissus* L. EtOH bulb extracts) [107]. None of these compounds had any effect on MMP-13, although both cell lines appeared to secrete this MMP [107]. Of the three, only lycorine produced significant reduction in IL-8 levels in both cells [107].

The sodium phosphate derivative of narciclasine (sodium narcistatin **4**) has been examined *ex vivo* for modulation of interleukins in PBMCs of arthritic male Lewis rats [13]. In this regard, increases in IL-1, IL-2 and IL-10 were effectively blocked in PBMCs exposed to **4** (at 5.0 mg/kg/day), relative to cells obtained from saline-treated animals, which reflected pronounced elevations of these cytokines [13]. Low levels of IL-4 and IL-6 were released from the PBMCs of non-arthritic animals, but these were nonetheless shown to be similar in all of the treatment groups [13]. Analysis of the splenocytes of arthritic rats showed that, while **4** (5.0 mg/kg/day) blocked the increase in IL-6, it had no effect on IL-1, compared with cells from saline-treated animals [13]. In contrast, phosphate-treated arthritic rat splenocytes produced more IL-4 than non-arthritic or saline-treated arthritic animals [13]. Furthermore, there were no significant treatment- or disease-related effects on IL-2 or IL-10 levels [13]. The anti- to pro-inflammatory macrophage cytokine production ratio (IL-10/TNF-α) indicated a shift towards the former in **4**-treated arthritic rats compared to their saline-treated arthritic counterparts [13]. While adjuvant-induced (CFA) arthritis accelerated the release of IL-1 from DLN cells (relative to non-arthritic controls), this was reversed by narciclasine phosphate (**4**) [13]. There were no significant differences in the levels of IL-2, IL-4, IL-6 or IL-10 released by DLN cells from arthritic and non-arthritic rats (▶ **Table 7**) [13]. Narciclasine (**3**) was also involved with *in vitro* modulation of interleukins [77]. In this regard, it displayed potent activity against IL-6 inhibition (98% at 0.016 µM) in LPS-stimulated RAW264.7 macrophages [77]. Furthermore, dose-dependent (0.001–0.016 µM) effects were noted for their inhibition of IL-1β, with 40% inhibition observed at the highest tested concentration relative to dexamethasone [77]. LPS-induced neuro-inflammation in BV-2 microglia was markedly overcome by narciclasine [78]. The effect was shown to involve dose-dependent (0.1, 0.2 and 0.3 µM) reductions in levels of the pro-inflammatory factors IL-6 and IL-18 that had been increased by LPS (100 ng/mL) [78]. Accompanying these were increases in the anti-inflammatory cytokines IL-10 and TGF-β1 [78]. The narciclasine analogue *trans*-dihydrolycoricidine (**59**) likewise dose-dependently (0.1, 0.3 and 0.5 µM) diminished expression of IL-6 in LPS-stimulated (100 ng/mL) BV-2 cells without observable cytotoxic effects [79]. It concurrently also raised levels of the anti-inflammatory cytokine IL-10 at each of the tested dosages [79].

At 1 mg/kg, narciclasine alleviated the ill effects of zymosan in a peritonitis model of C57BL/6 N mice as referred to above [31]. A further facet of the study was the fact that narciclasine also diminished IL-6 and IL-1β levels in the peritoneal lavage of affected animals by at least 50% to that of zymosan alone [31]. IL-6 and IL-1β were also significantly reduced in neonatal Sprague–Dawley rats subjected to LPS-induced acute lung injury [25]. Serum levels of both these cytokines were around 600 ng/L following exposure to LPS alone (2 mg/kg) but were diminished to around 200 ng/L with narciclasine (0.7 mg/kg) over 72 hr [25]. IL-6 and IL-1β levels were also diminished by lycorine (**1**) (at 20 or 40 mg/kg) during ALI induced by LPS exposure (2 mg/kg) [38]. Lycorine (at 10 µM) reduced IL-6 and IL-1β production in mouse lung epithelial MLE-12 cells that had been stimulated by LPS (2 µg/mL) [38]. *E. coli*-induced sepsis in neonatal rats as detailed above was effectively mitigated by exposure to narciclasine (**3**) [33]. Examined as to whether the effect involved the modulation of pro-inflammatory factors, it was shown that narciclasine dose-dependently (0.1, 1 and 3 mg/kg) reduced levels of IL-1α, IL-1β, IL-2 and IL-6 in the blood plasma of affected animals [33]. There was also an increase in the anti-inflammatory cytokines IL-4 and IL-10 in narciclasine-treated subjects relative to untreated controls [33].

Two flavanols ((*2R,3S*)-7-methoxyflavan-3-ol **81** and (*2R,3S*)-2′-hydroxy-7-methoxyflavan-3-ol **82**) and two flavans ((*2S*)-4′-hydroxy-7-methoxyflavan **83** and (*2S*)-7,4′-dihydroxyflavan **84**) from *Crinum asiaticum* (whole-plant CH_3OH extract) dose-dependently (3.125–50 µM) reduced IL-6 production in LPS-stimulated (100 ng/mL) RAW264.7 macrophages [91]. This proceeded without detriment to the macrophages, which were only affected by doses > 50 µM [91]. In addition to its allergenic properties, the lectin narcin from *Narcissus tazetta* was also examined for whether it could mediate in IL synthesis [108]. A small (but significant) increase in IL-10 production (2%) was observed in $CD4^+$ T cells (from PBMCs) in response to stimulation by narcin (10 µg/mL) [108]. This was found to be slightly higher for IL-4 (2.6%) and IL-13 (3.5%) [108]. Of these three ILs, IL-13 is a pleiotropic cytokine that is important in the regulation of the inflammatory and immune responses [106]. It inhibits inflammatory cytokine production and synergises with IL-2 in regulating IFN-γ synthesis [106].

Effects on Interferons

Interferons are a group of cytokines that are produced in response to infection or various inflammatory stimuli [109]. They activate immune cells such as macrophages and natural killer (NK) cells [109]. IFNs also heighten host defence responses by up-regulating antigen presentation via increased expression of the major histocompatibility complex (MHC) antigens [109]. More than 20 distinct IFN genes and proteins have been identified in humans and animals, which have been divided into three classes: Type I IFNs,

Type II IFNs and Type III IFNs [109]. In relation to the modulation of IFNs by Amaryllidaceae constituents, the phosphate derivative of narciclasine (sodium narcistatin **4**) (at 5.0 mg/kg/day) was examined for modulation of interleukins in adjuvant-induced (CFA) arthritic Lewis rats [13]. Analysis of cultured PBMCs indicated that low levels of IFN-γ were released *ex vivo*, but in which case, these were similar in all treatment groups, including arthritic and non-arthritic subjects exposed to **4** or to a saline solution [13]. Furthermore, while there were no significant differences in IFN-γ release by DLN cells from untreated arthritic and non-arthritic rats, exposure to **4** reduced IFN-γ production in the former compared to its saline-treated counterparts [13]. IFN-γ is a dimerised soluble cytokine, the only member of the Type II class of interferons that is encoded by the IFNG gene [109]. It plays important roles in both innate and adaptive immunity and is secreted primarily by adaptive immune cells, notably $CD4^+$ T helper 1 (Th1) cells, NK cells and $CD8^+$ cytotoxic T cells [109]. Narciclasine (**3**) alleviated *E. coli*-induced sepsis in neonatal rats as discussed above [33]. It was also examined as to whether it was capable of modulating interferon levels during sepsis [33]. Narciclasine dose-dependently (0.1, 1 and 3 mg/kg) reduced IFN-γ levels in the blood plasma of affected animals [33]. In particular, 500 ng/mL of IFN-γ was measured at the highest narciclasine (**3**) dosage, three times less than that found in the plasma of untreated subjects [33]. In addition to its allergenic effects, the *Narcissus tazetta* lectin narcin was also examined for the extent to which it could mediate in IFN-γ synthesis [108]. It was shown that IFN-γ production was over 3 times higher in $CD4^+$ T cells of narcin-treated PBMCs (10 µg/mL) compared to those tested without stimulation [108].

Effects on Inflammasomes

Inflammasomes are large, cytosolic multi-protein complexes that are assembled in response to infection or various stress-related stimuli [110]. This leads to caspase-1-mediated inflammatory responses, including cleavage and secretion of IL-1β and IL-18, as well as initiation of pyroptosis (or inflammation-related cell death) [110]. They have been implicated in the progression of a variety of chronic diseases, such as gout, atherosclerosis and metabolic syndrome [110]. A study was undertaken to ascertain the effect of lycorine (**1**) (98%, TargetMol, Shanghai, China) on bleomycin-induced idiopathic pulmonary fibrosis (IPF) and NLRP3 inflammasome activation in C57BL/6 mice [111]. Bleomycin-treated animals (1.2 U/kg) reflected severe alveolar haemorrhage, oedema, thicker alveolar septa and infiltration of inflammatory cells, which were all markedly reduced by lycorine (10 mg/kg/day/3 weeks) relative to the IPF drug nintedanib [111]. Furthermore, bleomycin- or saline-treated subjects showed higher levels of cleaved caspase-1 and cleaved IL-1β than those exposed to lycorine or the caspase-1 antagonist Z-VAD-FMK [111]. LDH release in the bronchoalveolar lavage fluid was also much higher following exposure to bleomycin than to lycorine or Z-VAD-FMK [111]. These observations suggested that lycorine (**1**) produced *in vivo* suppression of inflammasome activation and pyroptosis [111]. *In vitro* studies were then carried out in BMDMs (bone marrow-derived macrophages) that were first primed with LPS (100 ng/mL) and then exposed to nigericin (10 µM) or ATP (3 mM) to effect NLRP3 inflammasome activation [111]. Western blotting showed that only with nigericin or ATP stimulation was there any release of cleaved caspase-1 [111]. Pre-treatment with lycorine (10 µM), in contrast, significantly reduced cleaved caspase-1 levels both in the cells and supernatants [111]. Lycorine (**1**) also significantly inhibited LPS/nigericin- or LPS/ATP-induced IL-1β release [111]. This suggested that lycorine was capable of suppressing LPS/nigericin- or LPS/ATP-induced NLRP3 inflammasome activation [111].

Effects on nitric oxide

Nitric oxide (NO) is an inorganic free radical that is produced oxidatively from L-arginine by the enzyme nitric oxide synthase (NOS) [112]. NO has been linked to several physiological and pathological processes, including cancer [112]. The NO family of synthases is comprised of inducible NOS (iNOS), endothelial NOS (eNOS) and neuronal NOS (nNOS) [112]. iNOS is involved in processes where there is excess production of NO and can be expressed in response to pro-inflammatory elements such as interleukin-1β (IL-1β) and TNF-α, as well as LPS stimulation of macrophages, endothelial cells and smooth muscle [112]. Phytochemical investigation of *Crinum yemense* Deflers (synonym for *Crinum album* (Forssk.) Herb.) led to the identification of several alkaloids from its CH_3OH bulb extract [113]. Subsequent analysis of their effects on NO production in LPS-activated mouse peritoneal macrophages indicated lycorine (**1**, IC_{50} 2.5 µM), crinamine (**12**, IC_{50} 1.8 µM), 6-hydroxycrinamine (**51**, IC_{50} 5.4 µM) and yemenine A (**85**, IC_{50} 4.9 µM) to be better inhibitors than guanidinoethyl disulphide, a selective iNOS inhibitor (IC_{50} 7.4 µM) (▶ **Table 8**) [113]. Bulbispermine (**50**) exhibited an IC_{50} of 24 µM, with the remaining compounds (trisphaeridine **32**, vittatine **86** and yemenine C **87**) shown to be inactive [113]. Furthermore, it was demonstrated that NO inhibition was a consequence of the inhibition of iNOS in LPS-activated macrophages [113]. Lycorine (**1**) and narciclasine (**3**) from *Lycoris radiata* relative to butein (IC_{50} 5 µM) were effective in the suppression of NO production in LPS-activated RAW264 murine macrophages, with IC_{50}s of 1.2 and 0.01 µM, respectively [57]. The IC_{50} for lycorine (from *Crinum asiaticum*) against NO production in LPS-stimulated (5 ng/mL) RAW264.7 macrophages was determined to be 0.4 µg/mL, where prednisolone (IC_{50} 1.1 µg/mL) was used as reference standard [87]. Narciclasine dose-dependently (0.004–0.016 µM) inhibited (relative to dexamethasone) NO production in LPS-stimulated RAW264.7 macrophages, with 75% inhibition observed at the highest concentration [77]. Narciclasine (**3**) also suppressed (at 0.008 and 0.016 µM) LPS-induced elevation of mRNA expression levels of iNOS, suggesting that it suppressed NO synthesis by inhibiting iNOS activity [77]. *In vitro* studies were undertaken to establish whether lycorine (**1**) could inhibit endogenous expression of iNOS [76]. It was shown that while there was increased expression of iNOS in LPS-stimulated (100 ng/mL) RAW264.7 macrophages, lycorine relative to the glucocorticoid dexamethasone inhibited this increase at all levels of testing (1–5 µM) [76]. The inhibition of iNOS was also accompanied by the inhibition of NO release in the macrophages [76]. Studies into whether narciclasine (Carl Roth, Karlsruhe, Germany) was involved in eNOS modulation indicated that it did not alter the activity of the enzyme in HUVECs [114].

▶ **Table 8** Effects of Amaryllidaceae constituents on nitric acid production.

Compound (No.)	Activity against NO production	Ref.
Lycorine (**1**)	Inhibited NO production in LPS-activated mouse peritoneal macrophages (IC_{50} 2.5 µM).	[113]
	Inhibited NO production in LPS-activated RAW264 macrophages (IC_{50} 1.2 µM).	[57]
	Inhibited NO production in LPS-activated RAW264.7 macrophages (IC_{50} 0.4 µg/mL).	[87]
	Dose-dependently (1–5 µM) inhibited iNOS expression in LPS-challenged RAW264.7 macrophages.	[76]
	Displayed favourable binding energy (− 8.5 kcal/mol) for the NOS active site relative to cromolyn sodium (− 10.7 kcal/mol).	[125]
Haemanthidine (**2**)	Inhibited NO production (IC_{50} 12.2 µM) in LPS-stimulated RAW264.7 macrophages.	[116]
	Inhibited NO production in LPS-activated RAW264 macrophages (IC_{50} 0.01 µM).	[57]
	Inhibited NO production by 75% in LPS-activated RAW264.7 macrophages at 0.016 µM.	[77]
	Suppressed (at 0.008 and 0.016 µM) LPS-induced elevation of mRNA expression levels of iNOS.	[77]
	Did not modulate eNOS activity in HUVECs.	[114]
	Did not alter the release of NO from HUVECs.	[114]
	Dose-dependently (0.1, 0.2 and 0.3 µM) inhibited NO production during LPS-induced neuro-inflammation in BV-2 microglia.	[78]
	Dose-dependently (0.1, 0.2 and 0.3 µM) reduced the protein expression of iNOS, as well as iNOS mRNA levels, during LPS-induced neuro-inflammation in BV-2 microglia.	[78]
Galanthamine (**5**)	Weak inhibition of NO production (IC_{50} > 200 µM) in LPS-stimulated RAW264.7 macrophages.	[115]
Haemanthamine (**11**)	Inhibited NO production (IC_{50} 4.6 µM) in LPS-stimulated RAW264.7 macrophages.	[116]
Crinamine (**12**)	Inhibited NO production in LPS-activated mouse peritoneal macrophages (IC_{50} 1.8 µM).	[113]
	Displayed favourable binding energy (− 9.2 kcal/mol) for the NOS active site relative to cromolyn sodium (− 10.7 kcal/mol).	[125]
Trisphaeridine (**32**)	Did not inhibit NO production in LPS-activated mouse peritoneal macrophages.	[113]
	Weak inhibition of NO production (IC_{50} > 200 µM) in LPS-stimulated RAW264.7 macrophages.	[116]
11*β*-Hydroxygalanthamine (**35**)	Weak inhibition of NO production (IC_{50} > 200 µM) in LPS-stimulated RAW264.7 macrophages.	[115]
Sanguinine (**36**)	Weak inhibition of NO production (IC_{50} > 200 µM) in LPS-stimulated RAW264.7 macrophages.	[115]
9-*O*-Demethyllycoramine (**38**)	Weak inhibition of NO production (IC_{50} > 200 µM) in LPS-stimulated RAW264.7 macrophages.	[115]
Tazettine (**39**)	Weak inhibition of NO production (IC_{50} > 200 µM) in LPS-stimulated RAW264.7 macrophages.	[116]
Bulbispermine (**50**)	Inhibited NO production in LPS-activated mouse peritoneal macrophages (IC_{50} 24 µM).	[113]
6-Hydroxycrinamine (**51**)	Inhibited NO production in LPS-activated mouse peritoneal macrophages (IC_{50} 5.4 µM).	[113]
trans-Dihydrolycoricidine (**59**)	Dose-dependently (0.1, 0.3 and 0.5 µM) inhibited NO production in LPS-stimulated BV-2 cells.	[79]
	Dose-dependently (0.1, 0.3 and 0.5 µM) attenuated iNOs activity in LPS-stimulated BV-2 cells.	[79]
Zephygranditine A (**73**)	IC_{50} 17.3 µM against NO production in RAW264.7 macrophages.	[82]
Zephygranditine B (**74**)	IC_{50} 20.1 µM against NO production in RAW264.7 macrophages.	[82]
Zephygranditine C (**75**)	IC_{50} 35.6 µM against NO production in RAW264.7 macrophages.	[82]
Zephygranditine D (**76**)	IC_{50} 67.4 µM against NO production in RAW264.7 macrophages.	[82]
Zephygranditine E (**77**)	IC_{50} 75.1 µM against NO production in RAW264.7 macrophages.	[82]
Zephygranditine F (**78**)	IC_{50} 67.1 µM against NO production in RAW264.7 macrophages.	[82]
(*2R,3S*)-7-Methoxy-flavan-3-ol (**81**)	Inhibited LPS-induced NO production in RAW264.7 mouse macrophages (IC_{50} 17.3 µM).	[117]
	Dose-dependently inhibited NO release (IC_{50} 11.6 µM) in LPS-stimulated RAW264.7 macrophages.	[91]
(*2R,3S*)-2′-Hydroxy-7-methoxyflavan-3-ol (**82**)	Dose-dependently inhibited NO release (IC_{50} 12.3 µM) in LPS-stimulated RAW264.7 macrophages.	[91]

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▶ **Table 8** *Continued;*

Compound (No.)	Activity against NO production	Ref.
(*2S*)-4′-Hydroxy-7-methoxy-flavan (**83**)	Dose-dependently inhibited NO release (IC_{50} 12.4 µM) in LPS-stimulated RAW264.7 macrophages.	[91]
(*2S*)-7,4′-Dihydroxyflavan (**84**)	Dose-dependently inhibited NO release (IC_{50} 11.2 µM) in LPS-stimulated RAW264.7 macrophages.	[91]
Yemenine A (**85**)	Inhibited NO production in LPS-activated mouse peritoneal macrophages (IC_{50} 4.9 µM).	[113]
Vittatine (**86**)	Did not inhibit NO production in LPS-activated mouse peritoneal macrophages.	[113]
Yemenine C (**87**)	Did not inhibit NO production in LPS-activated mouse peritoneal macrophages.	[113]
Lycoramine (**88**)	Weak inhibition of NO production (IC_{50} > 200 µM) in LPS-stimulated RAW264.7 macrophages.	[115]
11*β*-Hydroxylycoramine (**89**)	Weak inhibition of NO production (IC_{50} > 200 µM) in LPS-stimulated RAW264.7 macrophages.	[115]
9-*O*-Demethyl-11*β*-hydroxyly-coramine (**90**)	Weak inhibition of NO production (IC_{50} > 200 µM) in LPS-stimulated RAW264.7 macrophages.	[115]
11*β*-Hydroxysanguinine (**91**)	Weak inhibition of NO production (IC_{50} > 200 µM) in LPS-stimulated RAW264.7 macrophages.	[115]
11*β*-Hydroxylycoramine *N*-oxide (**92**)	Weak inhibition of NO production (IC_{50} > 200 µM) in LPS-stimulated RAW264.7 macrophages.	[115]
11*β*-Hydroxygalanthamine *N*-oxide (**93**)	Weak inhibition of NO production (IC_{50} > 200 µM) in LPS-stimulated RAW264.7 macrophages.	[115]
2*β*,11*β*-Dihydroxygalan-thamine (**94**)	Weak inhibition of NO production (IC_{50} > 200 µM) in LPS-stimulated RAW264.7 macrophages.	[115]
N-Isopentyl-5,6-dihydroplicane (**95**)	Weak inhibition of NO production (IC_{50} > 200 µM) in LPS-stimulated RAW264.7 macrophages.	[115]
N-(*S*)-*s*-Pentyl-5,6-dihydro-plicane (**96**)	Weak inhibition of NO production (IC_{50} > 200 µM) in LPS-stimulated RAW264.7 macrophages.	[115]
N-Hexyl-5,6-dihydroplicane (**97**)	Weak inhibition of NO production (IC_{50} > 200 µM) in LPS-stimulated RAW264.7 macrophages.	[115]
N-(4′-Hydroxycarbonyl) butyl-5,6-dihydro-plicane (**98**)	Inhibited NO production (IC_{50} 18.8 µM) in LPS-stimulated RAW264.7 macrophages.	[115]
N-Phenethyl-5,6-dihydro-plicane (**99**)	Inhibited NO production (IC_{50} 10.2 µM) in LPS-stimulated RAW264.7 macrophages.	[115]
N-(3′-Ethyl)indolyl-5,6-dihydroplicane (**100**)	Inhibited NO production (IC_{50} 18.1 µM) in LPS-stimulated RAW264.7 macrophages.	[115]
N-Isopentyl-5,6-dihydroplicane *N*-oxide (**101**)	Weak inhibition of NO production (IC_{50} > 200 µM) in LPS-stimulated RAW264.7 macrophages.	[115]
Bliquine *N*-oxide (**102**)	Inhibited NO production (IC_{50} 7.5 µM) in LPS-stimulated RAW264.7 macrophages.	[115]
N-Methyl-11,12-*seco*-5,6-dihydroplicane (**103**)	Weak inhibition of NO production (IC_{50} > 200 µM) in LPS-stimulated RAW264.7 macrophages.	[115]
N-Isopentyl-11,12-*seco*-5,6-dihydroplicane (**104**)	Inhibited NO production (IC_{50} 23.6 µM) in LPS-stimulated RAW264.7 macrophages.	[115]
N-Methyl-5,6-dihydroplicane (**105**)	Weak inhibition of NO production (IC_{50} > 200 µM) in LPS-stimulated RAW264.7 macrophages.	[115]
Obliquine (**106**)	Weak inhibition of NO production (IC_{50} > 200 µM) in LPS-stimulated RAW264.7 macrophages.	[115]
Plicane (**107**)	Weak inhibition of NO production (IC_{50} > 200 µM) in LPS-stimulated RAW264.7 macrophages.	[115]
Zephyranine A (**108**)	Weak inhibition of NO production (IC_{50} > 200 µM) in LPS-stimulated RAW264.7 macrophages.	[116]
Zephyranine B (**109**)	Inhibited NO production (IC_{50} 21.3 µM) in LPS-stimulated RAW264.7 macrophages.	[116]
Zephyranine C (**110**)	Weak inhibition of NO production (IC_{50} > 200 µM) in LPS-stimulated RAW264.7 macrophages.	[116]
Zephyranine D (**111**)	Weak inhibition of NO production (IC_{50} > 200 µM) in LPS-stimulated RAW264.7 macrophages.	[116]
Zephyranine E (**112**)	Weak inhibition of NO production (IC_{50} > 200 µM) in LPS-stimulated RAW264.7 macrophages.	[116]

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▶ **Table 8** *Continued;*

Compound (No.)	Activity against NO production	Ref.
Zephyranine F (**113**)	Weak inhibition of NO production (IC_{50} > 200 µM) in LPS-stimulated RAW264.7 macrophages.	[116]
Zephyranine G (**114**)	Weak inhibition of NO production (IC_{50} > 200 µM) in LPS-stimulated RAW264.7 macrophages.	[116]
Zephyranine H (**115**)	Weak inhibition of NO production (IC_{50} > 200 µM) in LPS-stimulated RAW264.7 macrophages.	[116]
Zephyranine I (**116**)	Weak inhibition of NO production (IC_{50} > 200 µM) in LPS-stimulated RAW264.7 macrophages.	[116]
6-*O*-Ethylnerinine (**117**)	Weak inhibition of NO production (IC_{50} > 200 µM) in LPS-stimulated RAW264.7 macrophages.	[116]
Zephyranthine-6-one (**118**)	Weak inhibition of NO production (IC_{50} > 200 µM) in LPS-stimulated RAW264.7 macrophages.	[116]
1-*O*-Deacetylsternbergine (**119**)	Weak inhibition of NO production (IC_{50} > 200 µM) in LPS-stimulated RAW264.7 macrophages.	[116]
Anhydrolycorin-6-one (**120**)	Weak inhibition of NO production (IC_{50} > 200 µM) in LPS-stimulated RAW264.7 macrophages.	[116]
Zephyranthine (**121**)	Weak inhibition of NO production (IC_{50} > 200 µM) in LPS-stimulated RAW264.7 macrophages.	[116]
Pancratinine D (**122**)	Weak inhibition of NO production (IC_{50} > 200 µM) in LPS-stimulated RAW264.7 macrophages.	[116]
6-*O*-Methylnerinine (**123**)	Weak inhibition of NO production (IC_{50} > 200 µM) in LPS-stimulated RAW264.7 macrophages.	[116]
2*α*-Hydroxyhomolycorine (**124**)	Weak inhibition of NO production (IC_{50} > 200 µM) in LPS-stimulated RAW264.7 macrophages.	[116]
11-Hydroxyvittatine (**125**)	Inhibited NO production (IC_{50} 5.6 µM) in LPS-stimulated RAW264.7 macrophages.	[116]
5,6-Dihydrobicolorine (**126**)	Weak inhibition of NO production (IC_{50} > 200 µM) in LPS-stimulated RAW264.7 macrophages.	[116]
7-Demethoxy-9-*O*-methyl-hostasine (**127**)	Inhibited NO production (IC_{50} 17.4 µM) in LPS-stimulated RAW264.7 macrophages.	[116]
N-*trans*-Feruloyltyramine (**128**)	Weak inhibition of NO production (IC_{50} > 200 µM) in LPS-stimulated RAW264.7 macrophages.	[116]
N-*cis*-Feruloyltyramine (**129**)	Weak inhibition of NO production (IC_{50} > 200 µM) in LPS-stimulated RAW264.7 macrophages.	[116]
(2*S*)-7-Hydroxy-4′-methoxy-flavan 3′-*O*-*β*-D-glucopyrano-side (**130**)	Inhibited LPS-induced NO production in RAW264.7 mouse macrophages (IC_{50} 16.1 µM).	[117]
(2*S*)-7-Hydroxy-4′-methoxy-flavan 7-*O*-*β*-D-glucopyrano-side (**131**)	Inhibited LPS-induced NO production in RAW264.7 mouse macrophages (IC_{50} 21.5 µM).	[117]

Furthermore, it was found that narciclasine (**3**) did not alter the release of NO from these ECs [114]. LPS-induced neuro-inflammation in BV-2 microglia was dose-dependently overcome by narciclasine (at 0.1, 0.2 and 0.3 µM) [78]. While LPS (100 ng/mL) stimulated microglia to release significant quantities of NO, this was dose-dependently reversed by exposure to narciclasine [78]. The involvement of NOS was demonstrated by the fact that narciclasine (**3**) also diminished the protein expression of iNOS, as well as iNOS mRNA levels, both of which were seen to be potentiated by LPS [78]. Similar observations were made previously with the narciclasine derivative *trans*-dihydrolycoricidine (**59**), which inhibited NO production in LPS-stimulated (100 ng/mL) BV-2 cells in a dose-specific manner (0.1, 0.3 and 0.5 µM) without obvious cytotoxic effects [79]. LPS also induced the expression of iNOS, the activity of which was significantly attenuated by *trans*-dihydrolycoricidine [79].

Whole plants of *Zephyranthes candida* (Lindl.) Herb. yielded 24 alkaloidal substances (galanthamine **5**, 11*β*-hydroxygalanthamine **35**, sanguinine **36**, 9-*O*-demethyllycoramine **38**, lycoramine **88**, 11*β*-hydroxylycoramine **89**, 9-*O*-demethyl-11*β*-hydroxylycoramine **90**, 11*β*-hydroxysanguinine **91**, 11*β*-hydroxylycoramine *N*-oxide **92**, 11*β*-hydroxygalanthamine *N*-oxide **93**, 2*β*,11*β*-dihydroxygalanthamine **94**, *N*-isopentyl-5,6-dihydroplicane **95**, *N*-(*S*)-s-pentyl-5,6-dihydroplicane **96**, *N*-hexyl-5,6-dihydroplicane **97**, *N*-(4′-hydroxycarbonyl)butyl-5,6-dihydroplicane **98**, *N*-phenethyl-5,6-dihydroplicane **99**, *N*-(3′-ethyl) indolyl-5,6-dihydroplicane **100**, *N*-isopentyl-5,6-dihydroplicane *N*-oxide **101**, bliquine *N*-oxide **102**, *N*-methyl-11,12-*seco*-5,6-dihydroplicane **103**, *N*-isopentyl-11,12-*seco*-5,6-dihydroplicane **104**, *N*-methyl-5,6-dihydroplicane **105**, obliquine **106** and plicane **107**) following an EtOH extraction process, all of which were subsequently analysed for inhibition of NO production in LPS-stimulated (1 µg/mL) RAW264.7 macrophages [115]. Of these, five (**98, 99, 100, 102** and **104**) were noted for good inhibitory activities (IC_{50}s 7.5–23.6 µM) relative to dexamethasone (IC_{50} 0.6 µM) [115]. The other members of the library exhibited IC_{50}s that were in excess of 200 µM [115]. Later on, 26 alkaloid entities (haemanthidine **2**, haemanthamine **11**, trisphaeridine **32**, tazettine **39**, zephyranines A–I **108–116**, 6-*O*-ethylnerinine **117**, zephyranthine-6-one **118**, 1-*O*-deacetylsternbergine **119**, anhydrolycorin-6-one **120**, zephyranthine **121**, pancratinine D **122**, 6-*O*-methylnerinine **123**, 2*α*-hydroxyhomolycorine **124**, 11-hydroxyvittatine **125**, 5,6-dihydrobicolorine **126**,

7-demethoxy-9-*O*-methylhostasine **127**, *N*-*trans*-feruloyltyramine **128** and *N*-*cis*-feruloyltyramine **129**) were described from the bulb EtOH extract of *Zephyranthes candida* [116]. Examined for inhibitory effects on NO production in LPS-stimulated (1 µg/mL) RAW264.7 macrophages, five compounds (**2, 11, 109, 125** and **127**) exhibited good activities (IC_{50}s 4.6–21.3 µM) relative to dexamethasone (IC_{50} 0.6 µM), with the remaining 20 having IC_{50}s that were > 200 µM [116]. Interestingly, the three most active compounds (haemanthidine **2**, haemanthamine **11** and 11-hydroxyvittatine **125**) were all closely related structures belonging to the crinane alkaloid group of the Amaryllidaceae [116]. Furthermore, survival rates for the macrophages were all close to 100% when exposed to compounds **2, 11, 109, 125** and **127** (▶ **Fig. 4**) at dosages up to 200 µM, suggesting that general cytotoxicity was not a contributing factor towards their NO inhibitory effects [116]. The whole-plant EtOH extract of *Zephyranthes grandiflora* yielded six plicamine-type alkaloids (zephygranditines A–F **73–78**), which were screened for inhibitory effects towards LPS-stimulated NO production in RAW264.7 macrophages [82]. All of the compounds proved to be better inhibitors than indomethacin (IC_{50} 118.3 µM), with zephygranditines A and B (**73, 74**) showing the best activities (IC_{50}s 17.3 and 20.1 µM, respectively) (▶ **Table 8**) [82]. Selectivity index (SI) values for the compounds were ≥ 2, favouring their inhibitory effects towards NO production [82].

Examination of the EtOH extract of *Zephyranthes candida* also led to the isolation of three flavonoid compounds ((*2R,3S*)-7-methoxyflavan-3-ol **81**, (*2S*)-7-hydroxy-4′-methoxyflavan 3′-*O*-*β*-D-glucopyranoside **130** and (*2S*)-7-hydroxy-4′-methoxyflavan 7-*O*-*β*-D-glucopyranoside **131**) from a non-alkaloid fraction [117]. All were in possession of a flavan-related skeleton with variable substitutions in rings A and C [117]. The effects on LPS-induced NO production in RAW264.7 mouse macrophages were used to ascertain the *in vitro* anti-inflammatory activities of compounds **81, 130** and **131** [117]. All three compounds exhibited activities (IC_{50}s 17.3, 16.1 and 21.5 µM, respectively) that were around 30 times less than that observed for dexamethasone [117]. Furthermore, none of the flavans displayed significant cytotoxicities towards the macrophages at concentrations as high as 200 µM, with or without the inclusion of LPS (1 µg/mL) [117]. Two flavanols ((*2R,3S*)-7-methoxyflavan-3-ol **81** and (*2R,3S*)-2′-hydroxy-7-methoxyflavan-3-ol **82**) and two flavans ((*2S*)-4′-hydroxy-7-methoxyflavan **83** and (*2S*)-7,4′-dihydroxyflavan **84**) from *Crinum asiaticum* also displayed dose-dependent (3.125–50 µM) inhibitory effects towards NO release in LPS-stimulated (100 ng/mL) RAW264.7 macrophages [91]. The IC_{50}s, which ranged from 11.2 to 12.4 µM, were better than those seen for the positive control L-NMMA (IC_{50} 39.3 µM) [91]. Negligible cytotoxic effects were observed at the respective IC_{50} values, which only became apparent at concentrations > 50 µM [91].

Effects on Immunoglobulin

A 13 kDa lectin (narcin) has been described from bulbs of *Narcissus tazetta* and shown to be responsible for its allergenic properties [108]. Whilst mostly recognised for their nutritional, medicinal, environmental and aesthetic values, plants are also a major source of allergens [118]. Many of these display antigen-like properties, which are capable of eliciting an allergic response from the host via the production of immunoglobulin E (IgE) antibodies [118]. Such entities can enter the body by diffusion across mucosal surfaces and trigger the response, such as that seen with allergic rhinitis, rhinoconjunctivitis, allergic asthma and contact dermatitis [118]. Immunoglobulins (or antibodies) are large Y-shaped proteins that are used by the immune system to identify and eliminate foreign bodies, such as viruses and bacteria [118]. They can achieve this by one (or more) of the following reactions: neutralisation, agglutination, precipitation and fixation [118]. The last of these proceeds via lysis of the foreign body, as well as through stimulation of inflammation by chemotactically attracting inflammatory cells [118]. Haemagglutinins and lectins are also known for their allergenic effects, which are manifested via histamine release and interaction with IgE [118]. Examined for such effects, the *Narcissus tazetta* lectin narcin (at 10 µg/mL) produced a nearly fourfold increase in IgE in PBMCs derived from healthy human subjects [108].

Effects on Calprotectin

Calprotectin is a calcium-binding protein present in polymorphonuclear leukocytes (PMNs) and is known to inhibit growth and induce apoptosis in several tumour and normal cells [119]. It has been suggested that calprotectin may cause tissue damage during severe inflammation since it is released into extracellular spaces under such conditions [119]. For example, high levels of calprotectin are detectable in the synovial fluid of patients with rheumatoid arthritis [119]. Lycorine (**1**) from *Crinum asiaticum* (stem H_2O extract) was identified as the constituent responsible for negating the deleterious effects of calprotectin in MM46 cells [120]. Lycorine dose-dependently inhibited calprotectin-induced toxicity in MM46 cells but at markedly lower concentrations [120]. For example, an 80% reduction in MTT was observed at the highest tested concentration of 5 µg/mL [120]. A further alkaloid to display such effects was the phenanthridone narciclasine (**3**), an isolate of *Sternbergia lutea* CH_3OH bulb extracts, with an IC_{50} of 0.001–0.01 µg/mL against calprotectin toxicity towards MM46 cells [20]. Its response was 10 times better than that of lycorine (**1**) (IC_{50} 0.1 µg/mL), whilst those for hippeastrine (**132**) and ungerine (**133**) were far less impressive (IC_{50}s 10–100 µg/mL) [20]. Sepsis induced by *E. coli* in neonatal rats was effectively mitigated by narciclasine (**3**) as described above [33]. The excessive release of S100A8 and S100A9 (heterodimers that together make up calprotectin) in neonatal sepsis may be detrimental as they could exacerbate the inflammatory response [33]. It was found that narciclasine significantly reduced (at 0.1, 1 and 3 mg/kg) plasma levels of S100A8/A9 and also suppressed its expression in liver and lung tissue of sepsis-stricken neonatal rats [33].

Molecular Modelling

Computer-aided drug design has become a useful tool in drug discovery, particularly in conjunction with molecular topology [121]. For this purpose, a topological–mathematical model, accessed by linear discriminant analysis, was developed for the identification

▶ **Table 9** Molecular modelling of Amaryllidaceae constituents against inflammation-related targets.

Compound (no.)	Binding energy to molecular target	Ref.
Lycorine (**1**)	Favourable LibDock score (74.890) for binding to 5-LOX.	[123]
	Free radical stabilising ability predicted from VIP and BDE binding energies (174.0 and 106.7 kcal/mol).	[124]
	Displayed a favourable binding energy of − 7.0 kcal/mol during docking to the GSK-3β active site.	[23]
Galanthamine (**5**)	Free radical stabilising ability predicted from VIP and BDE binding energies (171.6 and 109.2 kcal/mol).	[124]
Crinamine (**12**)	Free radical stabilising ability predicted from VIP and BDE binding energies (172.4 and 104.4 kcal/mol).	[124]
Sanguinine (**36**)	Free radical stabilising ability predicted from VIP and BDE binding energies (171.4 and 84.6 kcal/mol).	[124]
Tazettine (**39**)	Free radical stabilising ability predicted from VIP and BDE binding energies (165.6 and 99.9 kcal/mol).	[124]
Vittatine (**86**)	Free radical stabilising ability predicted from VIP and BDE binding energies (172.1 and 106.6 kcal/mol).	[124]
Lycoramine (**88**)	Free radical stabilising ability predicted from VIP and BDE binding energies (171.6 and 107.0 kcal/mol).	[124]
8-*O*-Demethylmaritidine (**134**)	Free radical stabilising ability predicted from VIP and BDE binding energies (167.6 and 87.5 kcal/mol).	[124]
Galanthine (**135**)	Free radical stabilising ability predicted from VIP and BDE binding energies (177.1 and 106.8 kcal/mol).	[124]

of new anti-inflammatory agents of natural origin [121]. A library of 412 compounds from the MicroSource database, representing most classes of natural products, was used in the analysis [121]. The descriptors used in the model were TI1, ATS7m, ATS4v, ATS7v and ATS1p [121]. While TI1 was in regards to the first Mohar index (taking into consideration bond number), the other five related to the 2D Broto–Moreau autocorrelation for a topological structure weighted by atomic mass, Van der Waals volume or atomic polarisability [121]. Crinamine (**12**), with descriptor values of 78.42, 2.9, 3.48, 2.38 and 3.09, was shown to be unsuitable as an anti-inflammatory drug [121]. Gallic acid, a common anti-inflammatory agent, by comparison exhibited descriptor values of 0, 0, 1.93, 0 and 2.33, respectively [121].

Lipoxygenases (LOXs) are monomeric, non-heme iron-containing dioxygenases that play critical roles in cell differentiation, as well as in inflammatory and hyperproliferative diseases [122]. Of the various known isozymes of LOX, 5-LOX is considered to be key in the biosynthesis of leukotrienes that are associated with a number of diseases, including cancer [122]. The synthetic drug zileuton is the only known 5-LOX inhibitor in clinical use today, notably for the prophylaxis and chronic treatment of asthma [122]. The search and identification of new 5-LOX inhibitors have thus become important themes in contemporary drug discovery efforts [122]. In this regard, natural-product-based targets have afforded a vast resource for such endeavours [123]. In the search for such entities, docking simulations with 5-LOX were carried out on a library of 300 natural products, including 100 each of alkaloids, flavonoids and triterpenoids [123]. Amongst these was the Amaryllidaceae alkaloid lycorine (**1**), which emerged amongst the top eight candidates, with a LibDock score of 74.890 (relative to zileuton, 87.780) that favoured it as a potential 5-LOX inhibitor (▶ **Table 9**) [123]. This was further enhanced by its favourable ADMET (absorption, distribution, metabolism, excretion, toxicity) characteristics [123]. Lycorine also displayed the requisite physical and chemical properties that coincided with “Lipinski’s rule of five” [123].

Seven alkaloids of the Amaryllidaceae were examined for antioxidant effects via theoretical calculations of their free-radical stabilising capacities based on the thermodynamic parameters VIP (the energy needed to remove an electron) and BDE (the energy needed to release a hydrogen radical) [124]. Used as an antioxidant reference, quercetin exhibited values of 170.3 and 79.4 kcal/mol for its VIP and BDE energies, respectively [124]. The energy data for the alkaloids (ranked from best to worst) were as follows: sanguinine (**36**, 171.4 and 84.6 kcal/mol), 8-*O*-demethylmaritidine (**134**, 167.6 and 87.5 kcal/mol), tazettine (**39**, 165.6 and 99.9 kcal/mol), crinamine (**12**, 172.4 and 104.4 kcal/mol), vittatine (**86**, 172.1 and 106.6 kcal/mol), lycorine (**1**, 174.0 and 106.7 kcal/mol), galanthine (**135**, 177.1 and 106.8 kcal/mol), lycoramine (**88**, 171.6 and 107.0 kcal/mol) and galanthamine (**5**, 171.6 and 109.2 kcal/mol) [124]. Sanguinine and 8-*O*-demethylmaritidine had, in common with quercetin, a phenolic hydroxyl group, which would stabilise the electronic charge generated by the homolytic breakdown of the O-H moiety (from Ar-OH) [124].

Lycorine (**1**) (IC_{50} 2.5 µM) and crinamine (**12**) (IC_{50} 1.8 µM) as referenced above were shown to be better inhibitors of NO production in LPS-activated mouse peritoneal macrophages than guanidinoethyl disulphide (IC_{50} 7.4 µM) [113]. Consequently, docking studies were carried out with both compounds to establish their binding capacities at the NOS active site [125]. Crinamine and lycorine reflected binding energies (− 9.2 and − 8.5 kcal/mol, respectively) that were better than that of the NOS substrate L-arginine (− 5.9 kcal/mol) but slightly lower than that seen for the NOS inhibitor cromolyn sodium (− 10.7 kcal/mol) [125]. Both compounds penetrated deeply into the substrate metabolism site of NOS and established hydrogen bond interactions with Gly355 and Trp356, which could be rationalised in terms of their similar chemical scaffolds [125]. Crinamine (**12**) interacted hydrophobically with Phe353, while its aromatic ring was involved in π-stacking with Trp178 [125]. There were hydrophobic interactions for lycorine with Val185, Val418 and Ala423 [125].

Narcissus pseudonarcissus was shown to have significant wound-healing ability (53.6% closure for its EtOH bulb extract) as

determined via the scratch wound assay of WI-38 fibroblasts [126]. Phytochemical analysis led to the isolation of lycorine (**1**) together with four sterols (*β*-sitosterol **136**, sitosterol 3-*O*-*β*-D-glucopyranoside **137**, sitosterol 3-*O*-*β*-D-glucopyranoside-6-*O*-hexadecanoate **138** and stigmasterol **139**) from the same bulbous material [126]. These compounds were then subjected to docking studies to probe for interactions with four enzymes involved in the wound healing process, namely TGF-*β*1, GSK-3, TNF-*α* and IL-1*β* [126]. TGF-*β* represents a class of pluripotent cytokines that act as promoting factors during wound healing [127]. They influence almost all cell types involved in wound healing, particularly at the inflammatory, maturation and/or remodelling stages [127]. TGF-*β*1 is crucial for initiation of inflammation, cell migration and formation of granulation tissue [127]. GSK-3 is a serine/threonine kinase that mediates in several cellular processes, such as energy metabolism and neuronal development, as well as signalling and transport [128]. It is also involved in the proliferation phase of wound healing via *β*-catenin-dependent Wnt signalling [128]. As a critical inflammatory cytokine with manifold roles in both physiological and pathological processes, TNF-*α* production is ramped up by activated monocytes during acute inflammation [93]. However, increased levels of TNF-*α* also interrupt the operation of fibroblasts, thereby diminishing the amounts of collagen, hydroxyproline and granulation tissue, which impair wound healing [93]. IL-1*β* is also a pro-inflammatory cytokine released by various cell types of the innate immune system to initiate inflammation at the wound site [106]. However, it also sustains persistent inflammation and defective tissue repair, which adversely affect wound healing and skin appearance [106]. In the docking analysis, sitosterol 3-*O*-*β*-D-glucopyranoside (**137**) (▶ **Fig. 5**) displayed the best binding affinity towards TGF-*β*1 (− 8.58 kcal/mol) relative to the co-crystallised ligand (− 5.10 kcal/mol) [126]. Furthermore, H-bond donor/acceptor links were established for its interactions with Leu278 and Lys232 [126]. Sitosterol 3-*O*-*β*-D-glucopyranoside-6′-*O*-hexadecanoate (**138**) exhibited the best affinities for GSK-3 (− 8.31 kcal/mol), TNF-*α* (− 7.01 kcal/mol) and IL-1*β* (− 6.22 kcal/mol) relative to the ligands (− 6.06, -5.52 and − 4.25 kcal/mol, respectively) [126]. Notable interactions for it were with the Tyr59 and Gln149 amino acid residues at the TNF-*α* active site [126]. Its key interaction with IL-1*β* involved the Met148 residue [126]. Lycorine (**1**) demonstrated anti-arthritic effects in the CFA-induced arthritis model of mice as alluded to above [23]. As a part of this action, it also inhibited spinal cord GSK-3*β* activity [23]. A molecular docking analysis of lycorine (**1**) at the GSK-3*β* active site showed that it had a favourable binding affinity of − 7.0 kcal/mol (▶ **Table 9**) [23]. Molecular simulations of inflammation, which have only been explored in recent years, have indicated that Amaryllidaceae constituents are capable of interacting with key inflammatory mediators such as TNF-*α*, NOS and IL-1*β*. This information should prove to be useful in the design and modification of potential anti-inflammatory chemotherapeutics from the family.

Conclusions

The Amaryllidaceae provides a unique resource for the discovery of potential anti-inflammatory drugs. This has been reinforced by the large number of species available in the family and the significant levels of structural diversity discernible for its isoquinoline alkaloid principles, as well as the useful pieces of information emerging from traditional forms of medicine. While many of these efforts have focused on the isoquinoline alkaloids, interesting activities were also reported for members of other classes of natural products. This should be the focus of work going forward. A relatively small proportion of all alkaloids known in the Amaryllidaceae have to date been examined for anti-inflammatory activities, which should also be addressed in future endeavours. Ideally, this should involve targets from each alkaloid subgroup of the family. Lycorine and narciclasine were identified as the targets with the most potential, focus on which should thus open up the field, particularly in regards to structure–activity relationship perspectives. The mononuclear cells such as neutrophils, lymphocytes, macrophages and monocytes, while affording a close-up view of the *in vitro* anti-inflammatory activities, were themselves not affected by any deleterious effects of the Amaryllidaceae constituents. Similarly, no serious side effects were observed in the *in vivo* murine models of inflammation. Going forward, studies should also engage primate and human models to further verify the *in vivo* capacities of these compounds.

Contributors' Statement

J. J. Nair carried out the literature search, analysed the data and produced the first draft. J. van Staden was responsible for conceiving the topic, provided funding and supervision, as well as reviewed and edited the final draft.

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Conflict of Interest

The authors declare that they have no conflict of interest. The account herein is the product of findings made independently by the authors, no part of which may be deemed liable to any other individual or organisation.

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