The Pharmacological Activities of Glycyrrhizinic Acid (“Glycyrrhizin”) and Glycyrrhetinic Acid

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Abstract

Glycyrrhizin or, more correctly, Glycyrrhizinic acid is a triterpenoid saponin obtained from the root and rhizome extracts of Licorice (*Glycyrrhiza glabra*), being commonly used as a sweetener, being reported as – at least – 30 times sweeter than sucrose. This natural product, along with its aglycone glycyrrhetinic

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acid, is known in the literature for its several pharmacological and biological activities. This chapter summarizes the activities reported in the literature for the saponin and its aglycone since 2010.

Keywords

Glycyrrhizinic acid • Glycyrrhizin • Glycyrrhetic acid • *Glycyrrhiza glabra* • Liquorice

1 Introduction

Glycyrrhizinic acid (**1**, Fig. 1), also known as Glycyrrhizin [1, 2], is a triterpenoid saponin obtained from the root and rhizome extracts of Liquorice (or “licorice” in US English) (*Glycyrrhiza glabra*). Normally, the dried licorice root extract may contain around 4–25 % of the saponin, along with other compounds such as polyphenols, saponins, triterpenes, etc. One of the triterpenes found in the extract is the triterpenic aglycone, glycyrrhetic acid, or “enoxolone” (**2**). Further purification using several extraction techniques gives usually the monoammonium glycyrrhetinate salt as the purified product [2].

The World Health Organization (WHO) report on Glycyrrhizin recommends that the compound must be named “Glycyrrhizinic acid.” According to the report, “Glycyrrhizin” is the name given, more correctly, to the Licorice extract and not the saponin itself, and “Glycyrrhizin” and “Glycyrrhizinic acid” should not be used as synonyms [1], although this is common practice in the literature. This chapter will follow this recommendation and use the second term as the saponin name.

Both the saponin (as a carboxylic acid) and its monoammonium salt are commonly employed as sweeteners, being reported as 30–50 [3] or 33–200 [2] times

Fig. 1 Chemical structures of Glycyrrhizinic (**1**) and Glycyrrhetic acid (**2**)

Fig. 2. Publications mentioning the terms “Glycyrrhizin” or “Glycyrrhetinic acid” in its titles and/or abstract in SciFinder database (year period: 2010–2015)

sweeter than sucrose. The saponin is also widely employed in several traditional Chinese, Tibetan, and Indian medicinal preparations [3]. “Stronger Neo-Minophagen C,” a glycyrrhetinic acid-containing i.v. preparation, is employed in the treatment of chronic hepatic diseases, being marketed in Japan, China, Korea, Taiwan, Indonesia, India, and Mongolia [4].

The intent of this chapter is to report the wide range of pharmacological activities reported in the literature for the saponin (1) and its aglycone (2). The inclusion of the aglycone in this chapter is justified due to the fact that the saponin has no oral bioavailability, being absorbed as glycyrrhetinic acid (2) after hydrolysis of its carbohydrate moiety by intestine bacteria [5]. Since there are several reports published regarding those compounds until *ca.* 2010 [3, 6, 7], the time period covered by this chapter is from 2010 onwards.

Using SciFinder scientific literature search service [8], several articles mentioning “Glycyrrhizin” or “Glycyrrhetinic acid” in its titles and/or abstracts were found (Fig. 2). As mentioned earlier, using “Glycyrrhizinic acid” as search term gives a reduced number of articles as response. Due to the enormous number of publications in the selected period, it was decided to focus this chapter in the publications reporting pharmacological activities to the saponin, its aglycone, and some closely related compounds. Several Japanese, Korean, and (mostly) Chinese patents found in the bibliographic research mentioning 1 are related to its use as a sweetener/

Scheme 1 Synthesis of 18α-epimers of Glycyrrhizinic acid

flavoring agent in herbal/drug preparations and, therefore, were left out of this review.

2 Pharmacological Activities of Glycyrrizinic Acid ("Glycyrrhizin") and Related Compounds

2.1 Anticancer

Several findings were reported in the last few years regarding the anticancer properties of Glycyrrhizinic acid. Wang et al. reported the in vitro activity of **1** against gastric cancer cell line BCG-823. The saponin was able to prevent cell proliferation, adhesion, and migration at a 40 μM dose and they observed also that the expression of β-catenin, Bcl-2, CyclinD1, and survivin was significantly decreased [9].

In order to evaluate the carbohydrate moiety importance in the anticancer properties of **1**, Yang et al. synthesized two monoglucuronides from **1** by means of a glucuronidase from *Aspergillus* sp. (Scheme 1). The authors reported that 18α-monoglucuronide (**5**) obtained was more effective against HepG2, HeLa, and A549 cancer cell lines (IC₅₀ values: 6.67, 7.43, and 15.76 μM, respectively) than 18β-monoglucuronide (**3**), **1**, and 18α-glycyrrhizinic acid (**4**). The monoglucuronide **3** was also the best Epidermal Growth Factor Receptor (EGFR) inhibitor, with a IC₅₀

of 0.028 μM . Molecular docking simulations indicate that **3** also is able to bind with EGFR, suggesting that the anticancer properties of this glucuronide are related to EGFR inhibition [10].

Huang et al., in order to clarify the mechanism of the anticancer activity of **1** against lung adenocarcinoma A549 cell lines, evaluated the expression of several enzymes in an in vitro model and found that the anticancer activity of **1** against this cancer cell line can be, according to the authors, related to the inhibition of Thromboxane Synthase by **1**. The same results were also found in an in vivo mouse model using a western blot assay [11].

The inhibition of High-Mobility Group Box 1 (HMGB1), a protein that acts outside the cellular milieu as a proinflammatory cytokine, can also be related to the anticancer properties of **1**. Smolarczyk et al. reported that, when employing in an in vivo tumor model, the use of **1** inhibited cell growth and proliferation in mice. The authors concluded that the cell growth inhibition was probably due to the inhibition of HMGB1 by **1** in the extracellular media [12].

2.2 Anti-inflammatory

Wang et al. determined that **1** was able to attenuate levels of Tumor Necrosis Factor- α (TNF- α), interleukin 1 β (IL-1 β), and activation of the HMGB1/NF κ B (Nuclear factor- κ B) signaling pathway in the hippocampus of neonatal rats after exposure to isoflurane (a general anesthetic drug). Moreover, treatment with **1** also prevented the deficits in spatial memory related to isoflurane [13].

Using a pancreatic trauma model in male Wistar rats, Xiang et al. reported that treatment with **1**, 15 min after inducing the trauma, led to lower serum levels of HMGB-1, TNF- α , and IL-6 when compared to the control group. The treatment with the saponin also increased the seven-day survival rate of the animal subjects in this experimental model.

Zhang et al. reported that **1** has a neuroprotective effect in the postischemic brain in mice through the HMGB1-TLR4-IL-17A pathway [14].

Glycyrrhizinic acid (**1**) and Licorice flavonoids, tested in vitro in separate groups, were found by Zhao et al. to modulate the secretion of several cytokines by macrophages (RAW 264.7 cells) induced by lipopolysaccharides (LPS) [15]. Fu et al. reported that **1** presented its anti-inflammatory activity in LPS-stimulated RAW 264.7 cells by disrupting lipid rafts and inhibiting Toll-like receptor-4 (TLR-4) translocation to those rafts [16].

Wang et al. reported that **1** was able to reduce xylene, carrageenan, and acetic acid-induced rat paw edema and the nociceptions induced by formalin and acetic acid, but it had no effects in the hot plate test. The authors also observed the downregulation of levels of TNF- α , IL-6, inducible Nitric Oxide Synthase (iNOS), and Cyclooxygenase-2 (COX-2) [17].

Investigating the effects of **1** in the subarachnoid hemorrhagic (SAH) rat model, Chang et al. reported that the saponin exerted anti-inflammatory effects reducing SAH-induced vasospasm, mostly through the attenuation of peroxisome

proliferator-activating receptor gamma (PPAR- γ) [18]. The same research group reported that, in the same SAH model, treatment with **1** was also able to attenuate the ultrashort time expression of Toll-like receptors (TLR) 2 and 4 [19].

The saponin was also subject of some clinical trials regarding its anti-inflammatory activity. Xiao et al. conducted a randomized trial with 39 children presenting Henoch-Schonlein purpura (HSP) in order to verify the functions of regulatory T-cells (Treg) and Th17 cells in the peripheral blood of those patients. The group treated with **1** had significantly different Interleukin-17 (IL-17) serum levels after the treatment but no difference in Transforming Growth Factor-beta (TGF- β) and Interleukin-10 (IL-10) serum levels after the treatment. The authors concluded that **1** was able to reduce Th17 function, without noticeable effects in Treg function [20].

Glycyrrhizinic acid was also trialed in patients with chronic urticaria. Eighty four patients with the disease were randomized in two groups: one with **1** (50 mg oral tablets, three times per day) and the other with levocetirizine (an antiallergic drug). Both compounds were administered during a four-week period. The authors concluded that **1** was superior to levocetirizine and could be used to treat chronic urticaria [21]. It is worth noticing that **1** has no oral bioavailability, being hydrolyzed to form glycyrrhetic acid (**2**) before being absorbed [1]. Therefore, the observed pharmacological activity is probably due to the known anti-inflammatory activity of **2** (see section 3 of this chapter) rather than **1**.

2.3 Hepatoprotective Effects

Magnesium isoglycyrrhizinate, the magnesium salt of 18 α -glycyrrhizinic acid (**4**), was found by Luo et al. to be an effective treatment against hepatitis E with severe jaundice during a clinical trial with 78 patients. The patients received an intravenous injection of 150 mg of the magnesium once a day during 6 weeks [22].

Zhang et al. conducted a clinical trial with 84 patients with digestive tract cancer in order to verify the hepatoprotective activity of **1** during standard cancer chemotherapy. The authors observed that the group undergoing chemotherapy and treated with the saponin (160 mg i.v. once a day) presented significantly lower liver transaminase levels and increased levels of neutrophile, granulocytes, and white blood cells when compared with the control group (standard chemotherapy only) [23].

Also while investigating the hepatoprotective activity of **1**, Hsiang et al. investigated the gene expression of HepG2 cells treated in vitro independently with **1**, silymarin, and ursodeoxycholic acid, natural products also known by their hepatoprotective properties. The authors concluded that the compounds inhibited NF- κ B activities in a dose-dependent manner and that those compounds regulated the expression of genes related to oxidative stress and apoptosis in those cells [24].

Meng et al. conducted a meta-analysis in 12 randomized clinical trials regarding the hepatoprotective activity of **1** in patients with alcoholic liver disease. Their analysis concluded that in those trials the levels of alanine and aspartate

aminotransferases, as well as γ -glutamyl transpeptidase were lower than the control groups after treatment with the saponin [25].

During an investigation with patients with chronic hepatitis B, Yin et al. found that a three-week treatment with **1** did not reduce the expressions of inflammatory cytokine IL-17 and antinuclear antibodies (ANA) in the treated patients when compared to the control group [26].

Treating chronic hepatitis B with either Magnesium isoglycyrrhizinate or **1** was compared in a clinical trial reported by Cai et al. The trial involved 64 patients which were divided in two groups (one group being treated intravenously with the Magnesium salt and the other with the saponin, also i.v., once a day for 4 weeks). In both cases liver function was improved in the patients, but the authors found no statistical difference between both treatments [27].

Ding et al. investigated the effect of 18 α -glycyrrhizinic acid (**4**) in rats with carbon tetrachloride (CCl₄)-induced liver fibrosis. It was found that treatment with **4** increased the activity of enzymes glutathione peroxidase and superoxide dismutase, which reduced lipid peroxidation and the levels of malondialdehyde and hydroxynonenal in the liver, protecting it from damage caused by the aldehydes [28].

Wang et al. found that the treatment of HepG2 cells with **1** caused the increase in both CYP3A mRNA and protein levels. The CYP3A gene encodes monooxygenases responsible for drug metabolism and steroid biosynthesis [29]. The authors also found that the induction of CYP3A was mediated through the activation of Pregnane X receptor (PXR), resulting in the induction of CYP3A11 expression and CYP7A1 inhibition [30].

The hepatoprotective effect of **1** was also studied using HL-7702 (normal human liver cell line) through acetaminophen-induced damage. According to the report by Chen et al., **1**, liquiritin, and isoliquirtigenin (two polyphenols also found in the Liquorice root extract) were effective as hepatoprotecting agents against acetaminophen-induced damage [31].

Gwak et al., investigating the role of HMGB-1 in hepatocyte apoptosis, found that **1**, known as a HMGB-1 inhibitor, was able to prevent HMGB-1-induced apoptosis, cytochrome C release, and p38 activation in Huh-BAT (human hepatocellular carcinoma transfected with bile acid transporter) cell line [32].

2.4 Antiviral

Glycyrrhizinic acid (**1**) is known in the literature as an antiviral agent against clinically relevant viruses, such as HIV and the SARS-Coronavirus [3, 6]. Duan et al. reported that **1** is active against porcine reproductive and respiratory syndrome virus (PRRSV), relevant in veterinary medicine and in the swine industry. The saponin was able to inhibit mainly the penetration stage of the virus cycle [33].

Investigating the known anti-HIV activity of the saponin, Li et al. found that **1**, among 27 compounds isolated from Liquorice extract, has the high binding constant for R15K, the conserved core sequence in the V3 loop region of gp120. This region,

Scheme 2 Synthesis of glycyrrhetic acid 3-*O*-hemiphtalate

according to the authors, is where the binding of glycopospholipids occurs, leading to the virus entry in the host cell, which can indicate that the saponin is having anti-HIV activity by inhibiting the virus entry stage [34].

Using a Hepatitis C virus (HCV)-infected Huh7 cell model, Matsumoto et al. reported that **1** inhibits the release of HCV particles. Suppression of viral entry and reproduction stages were not observed [35]. Ashfaq et al., also investigating the anti-HCV activity of the saponin, reported that treatment of HCV-infected hepatic cells led to the suppression of HCV 3a core gene both at mRNA and protein levels. Coadministration of **1** with interferon alpha-3a caused a synergistic effect [36].

2.5 Other Activities

Xiao and Zhou reported that **1** reduced the Human neutrophil elastase-induced Mucin 5 AC (MUC5AC) overproduction in human bronchial epithelial cell culture (16HBE) [37].

The interaction of **1** with human hemoglobin (Hb) was studied by Sil et al. The authors reported that the interaction between the saponin and the protein reduces Hb-mediated oxidative damage without affecting the oxygen binding properties of Hb [38].

The antithrombin activity of **1** was already known since 1997 [39]. Glycyrrhizinic acid was found to be a weak *in vitro* and *in vivo* thrombin inhibitor but, unlike other antithrombin agents, the saponin acts as an allosteric inhibitor [40]. Glycyrrhetic acid (**2**), however, has no thrombin inhibiting activity, indicating that the carbohydrate moiety of the saponin is important to the allosteric site interaction. With these results in mind, Paula et al. synthesized some derivatives aiming to simplify the carbohydrate scaffold. From all the synthesized compounds, the hemiphtalate **6** (Scheme 2) presented better *in vitro* antithrombin activity against thrombin and increased the thrombin time, indicating that the carboxylic acid moiety present in the saponin (and in the phthalate) is important to the recognition at the allosteric site [41].

Investigating the free-radical scavenging abilities of **1**, Imai et al. reported that **1** and glycyrrhetinic acid (**2**) were able to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals but not hydroxyl and superoxide anion radicals [42].

3 Pharmacological Activities of Glycyrrhetinic Acid (**2**) and Related Compounds

As mentioned before, **2** is the compound absorbed in the human blood (due to the hydrolysis of **1** by bacterial metabolism) after oral administration of the saponin [1]. It was decided, therefore, to report the new pharmacological properties described for the aglycone in the literature below.

3.1 Anticancer Activity

There are several reports regarding the design, synthesis, and pharmacological evaluation of glycyrrhetinic acid derivatives towards compounds with improved anticancer properties. Since this chapter is focused mainly in the pharmacological properties of **1** and **2** itself (along with some closely related derivatives), only reports regarding the anticancer activity of **2** itself will be detailed below. Readers interested in more in-depth articles regarding chemical modifications in the aglycone structure are recommended to the review article by Czuk [43] as well as other review articles previously mentioned in the chapter [3, 6].

Kim et al. reported that **2** presented in vitro cytotoxic activity against A549 (human lung adenocarcinoma) cell line and also was able to reduce in vivo tumors induced by the same cell culture injected in Balb/c nu/nu mice [44].

Employing r/m HM-SFCE-1 cells (highly metastatic ras/myc-transformed serum-free mouse embryo), Yamaguchi et al. found that the cytotoxic effect of **2** in this model was due to the downregulation of glutathione, disrupting the redox balance in those cells [45].

Xu et al., using the human myeloma cell line (U266) reported that **2** induced in vitro apoptosis in this cell line by downregulating the survivin gene expression and arresting the cells in G₀/G₁ phase [46].

Aiming to study the potential use of **2** as a chemoprotective agent, Kowsalya et al. induced buccal pouch carcinogenesis in hamsters using 7,12-dimethylbenz(a)anthracene (DMBA) as a tumor initiator. Oral administration of **2** (45 mg/kg) along with DMBA prevented tumor formation in this animal model [47].

Charma et al. reported that the apoptotic effect of **2** in human breast cancer cell line MCF-7 was due to the activation of caspase-9 and modulation of the Protein Kinase B/Forkhead box O 3a transcription factor (Akt/FOXO3a) pathway [48].

Working with hepatic stellate cells (HSC), cells with immunosuppressive capabilities and related to the development of hepatocellular carcinomas, Kuang et al. found that **2** is able to reduce in vitro HSC-mediated immunosuppression by

reducing T-cell apoptosis and regulatory T (Treg) cell expression, which in turn lead to an increase in T-cell activity against hepatocellular carcinoma cells [49].

Xie et al. reported that **2** inhibited the epidermal growth factor (EGF)-induced proliferation of HaCaT cells (human keratinocyte cell line), likely by suppressing the extracellular signal-regulated kinase (ERK1/2) signaling pathway [50].

An in vitro study with prostate cancer cell line LNCaP conducted by Li et al. indicates that the activity of **2** in those cells is probably due to the suppression of 17 β -hydroxysteroid dehydrogenase type III (17 β -HSD3) mRNA expression via activation of eukaryotic initiation factor 2 α (eIF2 α) [51].

Wang et al. investigated the cytotoxic effect of **2** in pituitary adenoma cancer cell lines MMQ and GH3. The authors reported that the triterpene induced apoptosis in those cells by activating mitochondria-mediated reactive oxygen species (ROS)/mitogen-activated protein kinase (MAPK) pathways [52].

Huang et al. reported that **2** is able to suppress proliferation of non-small cell lung cancer (NSCLC) lines cells A549 and NCI-H460 through inhibition of thromboxane synthase and activation of ERK/CREB signaling [53].

Investigating the effect of **2** in the metastatic and cell-invading ability of several cancer cell lines, Jayasooriya et al. found that **2** is capable of downregulating matrix metalloproteinase-9 (MMP-9) and vascular endothelial growth factor (VEGF) via inhibition of phosphatidylinositol 3 kinase (PI3K)/Akt-dependent NF- κ B activity [54].

3.2 Hepatoprotective Activity

As reported with Glycyrrhizinic acid (**1**), **2** also presents hepatoprotective activity. Mahmoud et al. observed that **2** was able to counteract cyclophosphamide-induced hepatotoxicity in rats via activation of nuclear factor-erythroid 2 (NF-E2) related factor 2 (Nrf2) and PPAR- γ [55].

The factor Nrf2 is also involved in the chemoprotective mechanism of **2** against carbon tetrachloride-induced liver fibrosis in mice. Chen et al. reported that the triterpene is able to upregulate Nrf2 and increase the activity of antioxidant enzymes in the liver [56].

Glycyrrhetinic acid is also a chemoprotective agent against 2-acetylaminofluorene-induced hepatotoxicity in Wistar rats via attenuation of oxidative stress, inflammation, and hyperproliferation, according to Hasan et al [57].

3.3 Antiparasitic Activity

Investigating the antileishmanial activity of **2** in experimental visceral leishmaniasis models, Gupta et al. reported that this activity occurs through nitric oxide (NO) upregulation, proinflammatory cytokine expression and NF- κ B activation through p38 kinase [58]. The same research group later reported that this activity also depends on phosphatase-dependent modulation of cellular MAP kinases [59].

Fig. 3. Chemical structure of 18 α -glycyrrhetinic acid (7)

Kalani et al., in an effort to find new affordable antimalarial agents, reported that **2** has in vitro anti-*P. falciparum* activity, with $IC_{50} = 1.69 \mu\text{g/mL}$, and in vivo activity during an 8-day course treatment [60]. The same research group found that **2** is also an antifilarial agent, being active in vitro against microfilariae but not against adult worms. Some **2**-related amides synthesized by the group were able to affect both microfilariae and adult worms [61].

3.4 Antibacterial Activity

Long et al. reported that **2** is active against Methicilin-resistant *Staphylococcus aureus* (MRSA), a major source of complicated infections in hospital environments. According to the authors, **2** presented in vitro bactericidal activity at $0.223 \mu\text{M}$ and, in sublethal concentrations, it is able to reduce the expression of genes related to the *S. aureus* virulence, such as *saeR* and *hla* [62].

Investigating the effect of 18 α -glycyrrhetinic acid (**7**, Fig. 3) on the periodontal pathogen *Porphyromonas gingivalis*, Kim et al. reported that **7** reduces the bacterial LPS-induced permeability by suppressing repressing NF- κ B-dependent endothelial IL-8 production [63].

3.5 Anti-inflammatory Activity

Glycyrrhetinic acid (**2**) is also known for its anti-inflammatory activity, acting as a 11β -hydroxysteroid dehydrogenase inhibitor. This enzyme is responsible for the conversion of cortisol (**8**, Fig. 4) to cortisone and, as a result, **2** is an indirect glucocorticoid anti-inflammatory agent by extending cortisol half-life [1, 6]. Carbenexolone (**9**), the hemisuccinate ester of **2**, is used as an anti-inflammatory drug in United Kingdom [3, 6]. The chemical structures of **2**, **8**, and **9** are shown in Fig. 4.

Since there are several reports regarding the design, synthesis, and pharmacological evaluation of glycyrrhetinic acid (**2**) derivatives towards new anti-inflammatory agents with improved properties regarding the natural product, it was decided, in

Fig. 4. Structures of Glycyrrhetic acid (**2**), Cortisol (**8**), and Carbenexolone (**9**)

order to not extend this review size, to focus in the new reports of the anti-inflammatory activity of **2** itself.

Puchner et al. tested the efficacy of **2** in a model of rheumatoid arthritis (hTNFtg mice) and found that the triterpene was not effective in this model when compared with the positive control (TNF inhibitors) [64].

Investigating the anti-inflammatory activity of **2** in HepG2 cells, Chen et al. reported that it suppresses TNF-induced inflammation in this cell culture by diminishing NF- κ B activation [65].

Wang et al. reported that using **2** in RAW264.7 macrophages induced with LPS did not alter cell viability and suppressed the activity of NF- κ B and PI3K, inhibiting the expression of several proinflammatory genes and attenuating the production of NO, prostaglandin E₂ (PGE₂), and reactive oxygen species (ROS) [66].

Studying the proinflammatory cytokine effect of **1** and **2**, Kao et al. reported that both compounds are capable of suppressing cytokine production but by different mechanisms: while **1** work via PI3K/Akt/GSK3 β pathway, **2** acts by dissociating the glucocorticoid receptor (GR)-HSP90 complex [67].

3.6 Other Activities

Zhang et al. reported that **2** is a competitive Glyoxalase I inhibitor, with $K_i = 0.29 \mu\text{M}$. The authors also studied the crystal structure of the complex Glyoxalase I-glycyrrhetic acid, indicating that the carboxylic acid moiety of the triterpene is important for the inhibitor–enzyme interaction [68].

Inspired in previous reports regarding the chemoprotective effect of **1** and **2**, Wu et al. reported that **2** can act as a chemoprotective agent against cisplatin-induced nephrotoxicity in BALB/c mice. This effect is, according to the authors, related to upregulation of Nrf2 and downregulation of Nf- κ B [69].

Kong et al. reported that **2** can act as a skin protector against UV-induced photoaging in a mouse model. The authors also found that this protective effect is probably due to the anti-inflammatory and antioxidative properties of **2** [70].

Moon et al. investigated the effects of **2** in in vitro models of adipogenesis and found that the triterpene alters fat mass by affecting adipogenesis in maturing preadipocytes and lipolysis in matured adipocytes [71]. Also investigating the

antiobesity properties of Liquorice, Park et al. reported that **2** suppresses the activation of cannabinoid type 1 receptor (CB1R) induced by the endogenous agonist anandamide [72].

While researching the effects of **2** in the hemostasis, Jiang et al. found that glycyrrhetinic acid is a Factor Xa (FXa) inhibitor, with in vitro $IC_{50} = 32.6 \pm 1.24 \mu M$. The authors also tested the in vivo effect in rats using two protocols (tail bleeding and venous stasis models) and reported that **2** can increase prothrombin time and reduce thrombus weight (at a 50 mg/kg dose) when compared to the control group [73].

Hardy et al. reported that **2** was able to inhibit rotavirus replication in in vitro assays, while **1** was ineffective in this assay. The authors also reported that the antiviral activity of **2** occurs after the entry stage of the viral cycle [74].

4 Concluding Remarks

A great range of pharmacological and biological activities have been reported in the literature in the last 5 years, ranging from the exploration of known activities (such as the signaling pathways responsible for the anticancer and anti-inflammatory activity of **1** and **2**), scope broadening (the investigation of the antibacterial and antiviral properties against new, unreported species of virus and bacteria) and also new, unreported activities (such as the antiparasitic activities, glyoxalase I, factor Xa, etc.). Several clinical trials regarding the anti-inflammatory and hepatoprotective activity of **1** were also discussed.

The wide range of reported activities of **1** and **2** can lead to a very important question: how safe it is the use of **1** as a sweetener? Glycyrrhizinic acid is currently considered “Generally recognized as safe” by the US Food and Drug Administration, and there is a guideline for maximum permitted levels of the saponin in several preparations [75]. Also, the European Commission report on Glycyrrhizin states that the saponin is safe for consumption with the warning that the maximum daily dose of **1** should not be higher than 100 mg because of the glucocorticoid effects of glycyrrhetinic acid (**2**), given that the saponin is absorbed by the intestine as the aglycone, after hydrolysis of its carbohydrate moiety [2].

Regarding the reported properties for both products, most of the published articles explored in this chapter describe the in vitro or experimental in vivo activities, many of them in μM levels, which are considered too high to be useful as a drug [76] unless a very high dose is administered – way over the quantities permitted by the regulatory boards in both European Union [2] and USA [74]. Those activities should be regarded as potential ones, being useful as prototypes for the design and synthesis of new, improved compounds structurally related to **1** and/or **2**.

Glycyrrhizinic acid can be, described, eventually, as more than a sweetener. Along with glycyrrhetinic acid, it can be considered as scaffold molecule for the design and development of new bioactive compounds.

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