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Anti-Inflammatory Therapeutic Potential of 6,8,10-Gingerols against Cox Pathway by Molecular Docking Analysis

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Abstract: While traditionally tied to infections and the immune system, inflammation is now recognized to manifest distinctive markers across a broader array of diseases, as suggested by recent evidence. It encompasses a series of cellular and microvascular reactions aimed at eliminating damaged tissue and promoting the generation of new, healthy tissue. Ginger (Zingiber officinale Roscoe), a member of the Zingiberaceae family, has earned widespread popularity as a spice with ancient roots. Ginger contains predominantly gingerols, shogaols, and paradols as its main phenolic compounds. The primary phenolic compounds present in fresh ginger are gingerols, with 6-gingerol being the predominant form, accompanied by 4-, 5-, 8-, 10-, and 12-gingerols. Gingerols, acting as inhibitors of COX (Cyclooxygenase enzymes), have proven effective in a diverse array of pharmacological activities. Concerning a diverse spectrum of biological activities and documented mechanisms, the intricate interplay among three pivotal events—namely, inflammation, oxidative stress, and immunity—seems to contribute significantly to the myriad pharmacological effects of this compound. Through the inhibition of protein kinase B (Akt) and nuclear factor kappa B (NF-κB) signaling pathways, gingerols exhibit the capacity to mitigate inflammation, resulting in a reduction of proinflammatory cytokines and an elevation of anti-inflammatory cytokines. Through molecular modeling simulations, it was observed that gingerols preferentially interact with COX (cyclooxygenase enzymes) with a significant binding energy of -7 Kcal/mol. Exploration of hit compounds involves the application of tools such as ADMET@SAR, Discovery Studio, ADME/toxicity profiling, and molecular docking simulations. In conclusion, we utilized a computational technique to analyze interactions with drug targets.

Keywords: Inflammation; Gingerols; Cyclooxygenase; Molecular docking; Pharmacokinetics; Modulating signaling pathways

Introduction

Inflammation is an integral aspect of the body's defense mechanism. It encompasses the immune system's recognition and elimination of harmful or foreign stimuli, initiating the subsequent healing process. At the cellular level, the inflammatory response encompasses a sequence of events, including the release of signaling molecules, heightened blood flow to the impacted region, and the activation of immune cells. Inflammation may manifest as either acute or chronic. Tissue damage resulting from trauma, microbial invasion, or exposure to harmful substances can trigger acute inflammation. It begins promptly, escalates quickly, and symptoms may endure for a brief period, as illustrated by conditions such as cellulitis or acute pneumonia. The period between acute and chronic inflammation is characterized as subacute inflammation, lasting approximately 2 to 6 weeks. Persistent inflammation, commonly known as chronic inflammation, is characterized by its slow, prolonged duration, lasting for several months to years. The scope and impact of chronic inflammation typically fluctuate based on the nature of the injury and the body's capacity to heal and recover from the damage [1,2,3]. Chronic inflammatory diseases stand as the leading cause of global mortality, with

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the World Health Organization (WHO) identifying them as the foremost threat to human health. The incidence of diseases related to chronic inflammation is projected to continue its upward trend for the next three decades in the United States. In the year 2000, nearly 125 million Americans were coping with chronic conditions, and 61 million (21%) were dealing with more than one such condition. Recent estimates from the Rand Corporation in 2014 indicated that nearly 60% of Americans had at least one chronic condition, 42% had multiple conditions, and 12% of adults were dealing with five or more chronic conditions. On a global scale, three out of every five people perish as a result of chronic inflammatory diseases [4,5,6]. Essential for maintaining optimal health is the comprehension of the delicate balance between the body's need for inflammation to protect itself and the potential harm posed by chronic inflammation. Continual research persists in unraveling the intricate mechanisms of inflammation, providing valuable insights into potential treatments and preventive strategies for diverse inflammatory conditions. Products derived from nature that contain bioactive phytochemicals represent potentially significant reservoirs for anti-inflammatory drugs [7]. Traditional medicine heavily relies on medicinal plants, serving as a fundamental resource, particularly in less developed countries where people regularly incorporate these plants into their healthcare practices. These medicinal plants are regarded as a valuable reservoir of ingredients that can be utilized in the development and synthesis of drugs. Furthermore, these plants play a crucial role in influencing the development of human cultures across the globe. The roots of traditional medical systems, existing for millennia, have been predominantly derived from plants. The ongoing contribution of plants involves providing humanity with new medicines. Ginger stands out as a natural product renowned for its therapeutic applications in treating inflammatory disorders [8]. Ginger, derived from the dried rhizome of the Zingiber officinale Roscoe plant (Zingiberaceae), is a widely utilized spice globally. For thousands of years, ginger has been recognized as a traditional remedy. Certain regulatory authorities categorize ginger as a safe herbal supplement [9] and Ginger has been incorporated into both complementary and alternative medicine for various purposes, such as managing fevers, colds, and headaches. It serves as an appetite stimulant, antibacterial, antiviral, antidiarrheal, choleretic, anti-emetic, and expectorant, among other applications [10,11,12,13,14,15]. Ginger is comprised of more than 200 identified compounds, featuring bioactive elements like tannins, anthocyanins, terpenes (e.g., α-zingiberene, β-bisabolene, β-sesquiphellandrene, arcurcumene, or (E, E)-α-farnesene), and phenolic compounds (gingerols, paradols, shogaols, and zingerone) [16,17,18]. The major pungent compounds in ginger are known as gingerols [19]. Several studies have explored ginger extracts and the phenolic compounds derived from them, demonstrating a range of pharmacological effects, with a primary emphasis on 6-gingerol. These encompass antiemetic, anti-inflammatory, antinociceptive, antioxidant, antimicrobial, anti-cancer, anti-hyperglycemic, anti-arteriosclerotic, rubefacient, digestive, and laxative effects [16,20,21,22,23,24,25,26,27,28,29]. The analgesic and anti-inflammatory effects of bioactive compounds, particularly phenolic compounds such as [4,6,8,10,12]-gingerols, are achieved through the inhibition of COX2 (PDB ID:5IKT) and LOX pathways (protein, Lipooxygenase, PDB ID:3V92), consequently preventing arachidonic acid metabolism [30]. Ginger's impact has been demonstrated to resemble that of the NSAIDs family; nevertheless, it does not exert a detrimental effect on the stomach mucosa. We are aware that ginger does not influence the mucosa, as evidenced by an increase in mucosal prostaglandin synthesis observed after ginger intake. This is due to its non-inhibitory effect on COX1(PDB ID: 6Y3C) [31]. Additionally, a study with an intervention of less than two weeks using oral ginger supplements in osteoarthritis patients demonstrated ginger's effectiveness as a pain reliever and anti-inflammatory compound. The evaluation of muscle pain and plasma PGE2 levels confirmed its specificity to the COX2(PDB ID: 5IKT) enzyme [32]. The immunomodulatory effects of these compounds have garnered interest due to their impact on the immune system [20,21]. Initiating macrophage activation is a crucial step in orchestrating an inflammatory response. Activated macrophages play a significant role in both antigen-dependent and antigen-independent inflammatory pathways [33]. The Toll-like receptors (TLRs) expressed on these cells are accountable for triggering the activation of inflammatory pathways. The interaction

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between TLRs and their ligands results in the activation of macrophages, consequently initiating an inflammatory reaction [34–37]. Gingerols have also demonstrated the ability to inhibit the activation of protein kinase B (Akt) and nuclear factor kappa B (NF-κB), leading to an elevation in anti-inflammatory cytokines and a reduction in proinflammatory cytokines [38,39]. Reactive oxygen species (ROS), encompassing superoxide radicals, hydroxyl radicals, singlet oxygen, and hydrogen peroxide, are commonly generated as byproducts of biological reactions or exposure to exogenous factors [40]. Reactive oxygen species (ROS) have been identified to play a crucial role in the initiation and/or progression of various diseases, including atherosclerosis, inflammatory injury, cancer, and cardiovascular disease [41]. Nitric oxide (NO) is a free radical generated by constitutive and inducible nitric oxide synthase (cNOS and iNOS) in multiple mammalian cells and tissues [42]. It serves as a crucial mediator involved in regulating physiological and pathophysiological mechanisms in cardiovascular, nervous, and immunological systems [43]. The constitutively expressed nitric oxide (NO) by neuronal NOS (nNOS) and endothelial NOS (eNOS) serves as a key regulator of homeostasis. Nevertheless, nitric oxide (NO) synthesized by inducible nitric oxide synthase (iNOS) is triggered by a range of stimuli, including oxidants, lipopolysaccharide (LPS), bacteria, viruses, and proinflammatory cytokines. Nitric oxide (NO) can exhibit direct cytotoxicity and also engage with superoxide anions, leading to the formation of peroxynitrite (ONOO−), the most reactive nitrogen species (RNS). The overproduction of reactive oxygen species (ROS), nitric oxide (NO), and reactive nitrogen species (RNS) can harm DNA, lipids, proteins, and carbohydrates, leading to impaired cellular functions and intensified inflammatory reactions. Therefore, in this study, we explored the effects of gingerols in scavenging DPPH, hydroxyl, and superoxide radicals, inhibiting ROS production in human polymorphonuclear neutrophils (PMN), and suppressing nitric oxide and PGE2 formation in mouse leukemic monocyte (RAW 264.7) macrophages to investigate potential structure–function relationships. The latest results reveal that the efficacy of gingerols and shogaol is influenced by the number of carbons and functional groups in their side chain.

Overview on Gingerols

Ginger is composed of bioactive components, with gingerols accounting for 23–25%, shogaols for 18–25%, and related ketone derivatives, as highlighted in reference [44]. Gingerols are aromatic phenolic structures, representing a series of structural analogs of 1-(3-methoxy-4-hydroxyphenyl) 3-oxo-5-hydroxy-hexane. These compounds exhibit diverse unbranched alkyl side chain lengths, as indicated in references [45,13,46,18,47]. Gingerol derivatives, including 4-, 6-, 7-, 8-, 10-, and 12-gingerols, are distinguished by variations in the length of their unbranched alkyl side chains. The most prevalent structure among gingerol derivatives is 6-Gingerol ((5S))-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl) decan-3-one, which holds the distinction of being the first isolated compound from the ginger rhizome. It is the primary constituent contributing to the pungent taste of fresh ginger, whereas the other gingerols are present in relatively smaller amounts. Nevertheless, the thermal instability of gingerols arises from the existence of a β-unsaturated ketone group in their structures. The process of drying or heating ginger rhizome induces the generation of shogaol through the dehydration of positions C-4 and C-5. Even with abundant evidence supporting the conversion of gingerols into shogaols, it's worth noting that isolated gingerols remain stable in an ethanolic solution for a period of five months when stored at 4°C. The transformation from the respective gingerols into shogaols occurs readily, as documented in references [13,48,46,49,50,51,52].

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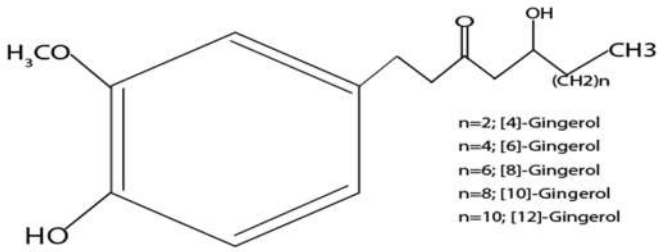

Figure 1. Chemical Structure of Gingerols.

Physicochemical Properties of Gingerol

The scent of ginger is linked to its volatile oil or oleoresin, containing monoterpenoids (like geraniol, curcumene, citral) and sesquiterpenoids (such as zerumbone, zingiberene, beta-bisabolene, zingiberol). Meanwhile, the non-volatile pungent character of ginger primarily results from the presence of gingerols, shogaols, and paradols, identified as vanillyl ketones or phenols. In the studies conducted by Jolad et al. [53,54], it was observed that both fresh ginger and commercially processed dry ginger contain more than 115 compounds. Among these, the primary constituents in fresh ginger are compounds related to gingerol, with 6-gingerol identified as the most abundant. Minor amounts of other gingerols, namely 4, 8, 10, and 12-gingerols, along with 6-gingerdione, can be found [55]. The thermal processing and drying of ginger lead to the formation of shogaols from the respective gingerols [56,57].

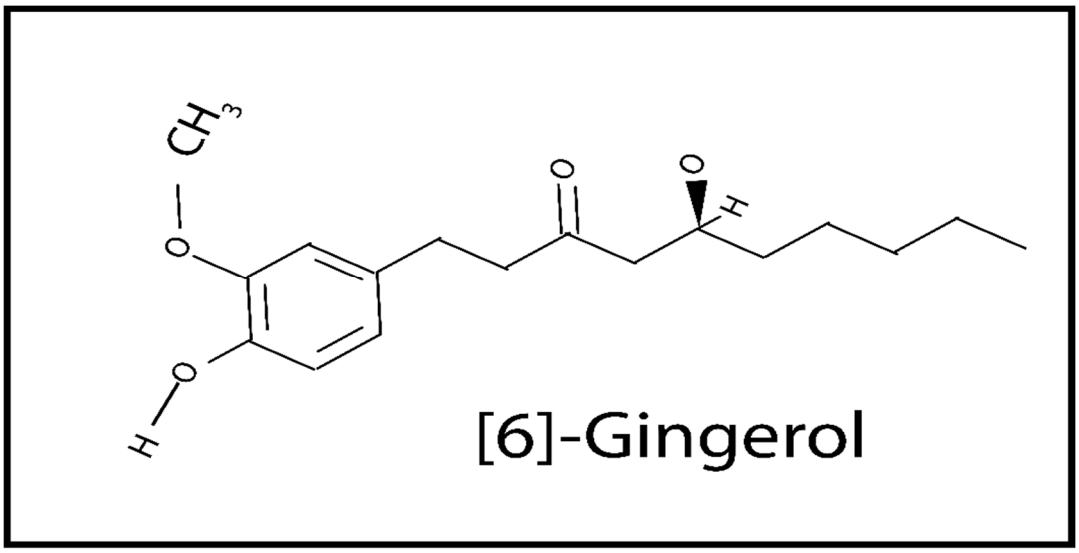

Figure 2. 2D structure of [6]-Gingerol.

Table 1. Physical and Chemical Properties of [6]-Gingerol.

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Property	Property value	Reference
XLogP3-AA	4.2	Computed by XLogP3 3.0 (PubChem release 2021.10.14)
Hydrogen Bond Donor Count	2	Computed by Cactvs 3.4.8.18 (PubChem release 2021.10.14)
Hydrogen Bond Acceptor Count	4	Computed by Cactvs 3.4.8.18 (PubChem release 2021.10.14)
Rotatable Bond Count	12	Computed by Cactvs 3.4.8.18 (PubChem release 2021.10.14)
Exact Mass	322.21440943 g/mol	Computed by PubChem 2.2 (PubChem release 2021.10.14)
Monoisotopic Mass	322.21440943 g/mol	Computed by PubChem 2.2 (PubChem release 2021.10.14)
Topological Polar Surface Area	66.8Å²	Computed by Cactvs 3.4.8.18 (PubChem release 2021.10.14)
Heavy Atom Count	23	Computed by PubChem
Formal Charge	0	Computed by PubChem
Complexity	319	Computed by Cactvs 3.4.8.18 (PubChem release 2021.10.14)
Isotope Atom Count	0	Computed by PubChem
Defined Atom Stereocenter Count	1	Computed by PubChem
Undefined Atom Stereocenter Count	0	Computed by PubChem
Defined Bond Stereocenter Count	0	Computed by PubChem
Undefined Bond Stereocenter Count	0	Computed by PubChem
Covalently-Bonded Unit Count	1	Computed by PubChem
Compound Is Canonicalized	Yes	Computed by PubChem (release 2021.10.14)
Pubchem CID	168114	https://pubchem.ncbi.nlm.nih.gov/
Molecular Formula	C19H30O4	
IUPAC Name	(5S)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl) dodecan-3-one	

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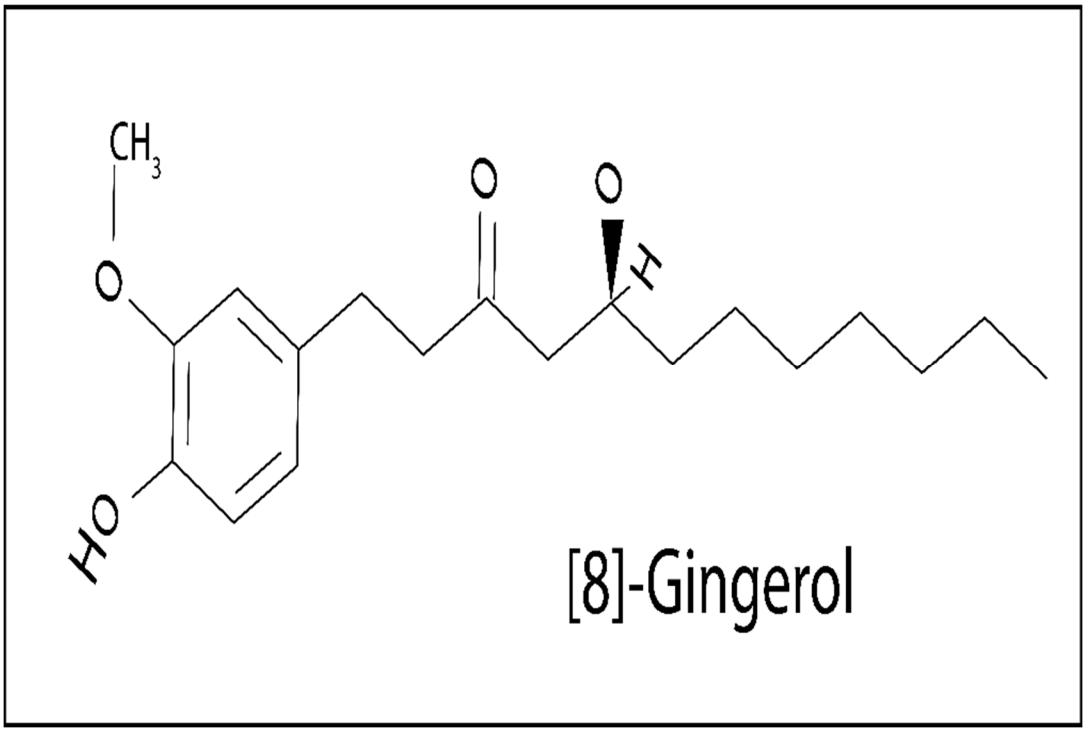

Figure 3. 2D structure of [8]-Gingerol.

Table 2. Physical and Chemical Properties of [8]-Gingerol.

Property	Property value	Reference
XLogP3-AA	4.2	Computed by XLogP3 3.0 (PubChem release 2021.10.14)
Hydrogen Bond Donor Count	2	Computed by Cactvs 3.4.8.18 (PubChem release 2021.10.14)
Hydrogen Bond Acceptor Count	4	Computed by Cactvs 3.4.8.18 (PubChem release 2021.10.14)
Rotatable Bond Count	12	Computed by Cactvs 3.4.8.18 (PubChem release 2021.10.14)
Exact Mass	322.21440943 g/mol	Computed by PubChem 2.2 (PubChem release 2021.10.14)
Monoisotopic Mass	322.21440943 g/mol	Computed by PubChem 2.2 (PubChem release 2021.10.14)
Topological Polar Surface Area	66.8Å²	Computed by Cactvs 3.4.8.18 (PubChem release 2021.10.14)
Heavy Atom Count	23	Computed by PubChem

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Formal Charge	0	Computed by PubChem
Complexity	319	Computed by Cactvs 3.4.8.18 (PubChem release 2021.10.14)
Isotope Atom Count	0	Computed by PubChem
Defined Atom Stereocenter Count	1	Computed by PubChem
Undefined Atom Stereocenter Count	0	Computed by PubChem
Defined Bond Stereocenter Count	0	Computed by PubChem
Undefined Bond Stereocenter Count	0	Computed by PubChem
Covalently-Bonded Unit Count	1	Computed by PubChem
Compound Is Canonicalized	Yes	Computed by PubChem (release 2021.10.14)
Pubchem CID	168114	https://pubchem.ncbi.nlm.nih.gov/
Molecular Formula	C19H30O4	
IUPAC Name	(5S)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl) dodecan-3-one	

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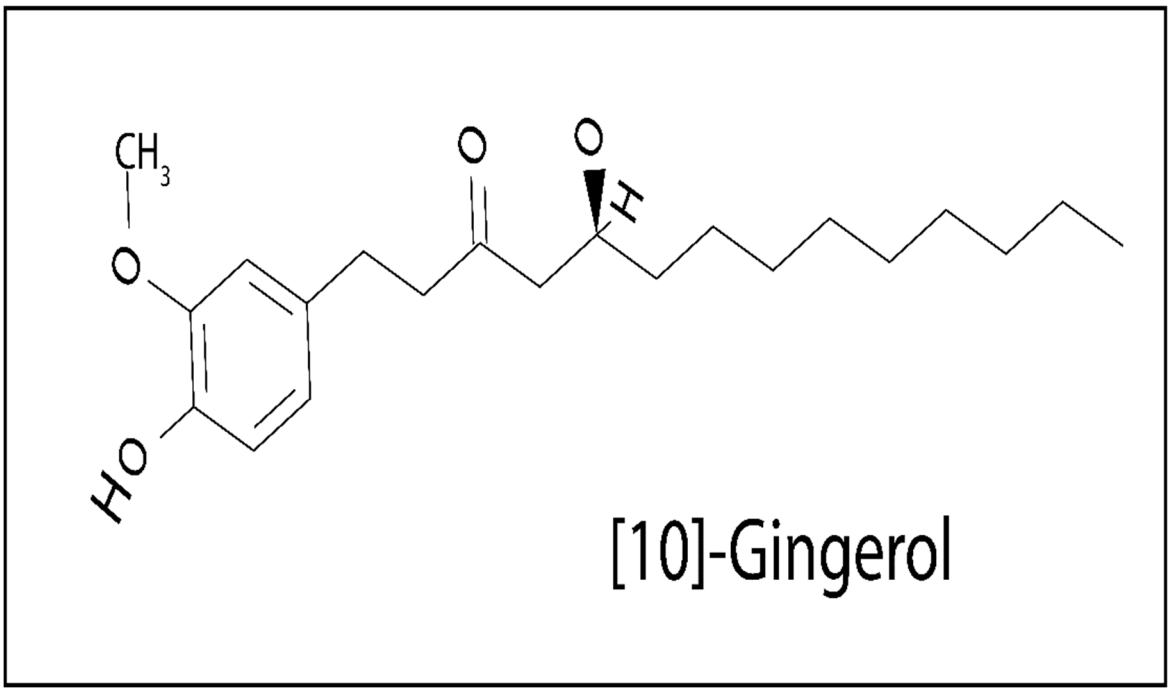

Figure 4. 2D structure of [10]-Gingerol.

Table 3. Physical and Chemical Properties of [10]-Gingerol.

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Property	Property value	Reference
XLogP3-AA	5.3	Computed by XLogP3 3.0 (PubChem release 2021.10.14)
Hydrogen Bond Donor Count	2	Computed by Cactvs 3.4.8.18 (PubChem release 2021.10.14)
Hydrogen Bond Acceptor Count	4	Computed by Cactvs 3.4.8.18 (PubChem release 2021.10.14)
Rotatable Bond Count	14	Computed by Cactvs 3.4.8.18 (PubChem release 2021.10.14)
Exact Mass	350.24570956 g/mol	Computed by PubChem 2.2 (PubChem release 2021.10.14)
Monoisotopic Mass	350.24570956 g/mol	Computed by PubChem 2.2 (PubChem release 2021.10.14)
Topological Polar Surface Area	66.8Å²	Computed by Cactvs 3.4.8.18 (PubChem release 2021.10.14)
Heavy Atom Count	25	Computed by PubChem
Formal Charge	0	Computed by PubChem
Complexity	345	Computed by Cactvs 3.4.8.18 (PubChem release 2021.10.14)
Isotope Atom Count	0	Computed by PubChem
Defined Atom Stereocenter Count	1	Computed by PubChem
Defined Atom Stereocenter Count	0	Computed by PubChem
Defined Bond Stereocenter Count	0	Computed by PubChem
Undefined Bond Stereocenter Count	0	Computed by PubChem
Covalently-Bonded Unit Count	1	Computed by PubChem

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Compound Is Canonicalized	Yes	Computed by PubChem (release 2021.10.14)
Pubchem CID	168115	https://pubchem.ncbi.nlm.nih.gov/
Molecular Formula	C21H34O4	
IUPAC Name	(5S)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl) tetradecan-3-one	

Pharmacokinetics of Gingerol and its Metabolism

The metabolic pathway of 6-gingerol after entering the bloodstream is not thoroughly understood. In a study, it was noted that the swift elimination of 6-gingerol from rat plasma occurred after intravenous administration of 3 mg/kg, with a total body clearance recorded at 16.8 ml/min/kg [58]. There are also findings suggesting that 6-gingerol undergoes enzymatic metabolism to gingerdiol in cell suspensions of rat liver [59]. Zick et al. [60] investigated the pharmacokinetics of 6, 8, 10-gingerol, and 6-shogaol in human subjects. The subjects were administered ginger in escalating doses ranging from 100 mg to 2 g, with distinct groups receiving varying amounts. None of the participants exhibited detectable levels of free 6, 8, 10-gingerol, or 6-shogaol in their serum. Instead, 6, 8, 10-gingerol, and 6-shogaol glucuronides were identified. Nakazawa and Ohsawa [61] conducted an investigation into the metabolic destiny of 6-gingerol. They identified a principal metabolite, (S)-6-gingerol-4′-O-b-glucuronide, in the bile of rats orally administered 6-gingerol. This finding implies the occurrence of conjugation and oxidation reactions involving its phenolic side chain. In a different study, it was demonstrated that 6, 8, and 10-gingerols obtained from natural ginger extract exhibited greater bioavailability when contrasted with gingerols in a synthetically prepared ginger mix. Furthermore, upon intravenous administration, the detection of gingerols in feces confirmed hepatobiliary elimination [62]. As reported by Bhattarai et al. [63], under acidic conditions, both 6-shogaol and 6-gingerol undergo reversible first-order dehydration and hydration reactions. This leads to the formation of 6-gingerol and 6-shogaol, respectively, in simulated gastric and intestinal fluids after prolonged incubation. Their research suggests that in the intestine, there is an interconversion between gingerol and shogaol. Their research suggests that in the intestine, there is an interconversion between gingerol and shogaol. After 21 days of incubation, it was observed that 6-gingerol displayed greater stability than 6-shogaol, with only 30% degradation for gingerol compared to 80% degradation for shogaol. In the process of drying or thermal processing, gingerols, being thermally labile due to the presence of a beta hydroxy group in the structure, either undergo dehydration to form the corresponding shogaols or undergo degradation through a retro-aldol reaction to produce zingerone and the corresponding aldehyde [58].

The Impact of Gingerols on the Immune System

The immune system, responsible for maintaining homeostasis in a healthy organism, is influenced by diverse external and internal factors, leading to either immunosuppression or immunostimulation [64]. Several natural compounds impact the functions of immune cells or modulate antibody secretion to regulate infections and uphold immune equilibrium [65]. These compounds have been observed to activate innate immune components, such as stimulating macrophages and lymphocytes, adjusting cytokine profiles, lowering infection rates, and promoting the apoptosis process [66]. The anti-inflammatory characteristics of ginger, a herb utilized in Ayurvedic medicine for its diverse pharmacological properties over an extended period, have been recognized for centuries. Moreover, a significant proportion of traditional Chinese herbal remedies

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incorporates ginger. The suppressive effects of ginger on prostaglandin biosynthesis, initially observed in the early 1970s, have been consistently validated in subsequent research. The findings indicated that the naturally occurring compounds found in ginger demonstrate comparable pharmacological efficacy to non-steroidal anti-inflammatory drugs [67, 68, 69]. It has been elucidated that phosphatidylinositol-3-kinase (PI3K), Akt, and NF-κB (nuclear factor-kappa B) play a significant role in the mechanism underlying the anti-inflammatory effect [18, 70, 71, 72].

Molecular Targets of Gingerol in Modulating Signaling Pathways

6-Gingerol has been documented to exhibit potent anti-inflammatory activity, suppressing TNF-α production in female ICR mice and rats treated with TPA [73,74]. The activation of the TNF-α gene results in the release of proinflammatory cytokines, subsequently initiating the activation of the transcription factor NF-κB. Activation of NF-κB triggers the expression of various inflammatory cytokines, including COX-2(PDB ID:5IKT), LOX- pathways (PDB ID:6V92), other chemokines, and iNOS. This sequence of events contributes to inflammation and the onset of related diseases, highlighting the anti-inflammatory action. NF-κB and COX-2(PDB ID:5IKT) play roles in inflammatory processes, and a growing body of evidence suggests that persistent inflammation may contribute to the development of chronic diseases, including cancer in various organs such as skin, stomach, colon, breast, prostate, and pancreas [75,76]. NF-κB constitutes a family of closely related protein dimers with five subunits: p65 (RelA), c-Rel, RelB, p50/NF-κB1, and p52/NF-κB2. NF-kB exists in the cytoplasm, bound to specific inhibitory proteins known as the IkB families. Upon activation by cytokines (such as TNF-α), inflammatory stimuli, and other oxidative stress molecules, it can translocate into the nucleus. Once within the nucleus, NF-kB binds to a specific DNA region, activating numerous genes that facilitate cellular proliferation, transformation, invasion, metastasis, and genes that suppress apoptosis. This cascade contributes to the development of various chronic diseases, including cancer. Various dietary agents, including gingerols, have demonstrated potent inhibitory effects on NF-kB [75,76]. Cyclooxygenases (COX) function as prostaglandin H synthase, converting arachidonic acid (AA) into prostaglandins. There are two isoforms of COX, namely COX-1(PDB ID:6Y3C) and COX-2(PDB ID:5IKT). COX-2(PDB ID: 5IKT) has been identified as a molecular target for numerous anti-inflammatories and chemopreventive agents. Kim et al. [77] observed that 6-gingerol inhibits TPA-induced COX-2 expression in mouse skin by obstructing the p38 MAPK-NF-kB signaling pathway. This inhibition is accompanied by a decrease in Ik Bα degradation, p65 nuclear translocation, and its interaction with the cAMP response element-binding (CREB) protein, a transcriptional coactivator of NF-kB.

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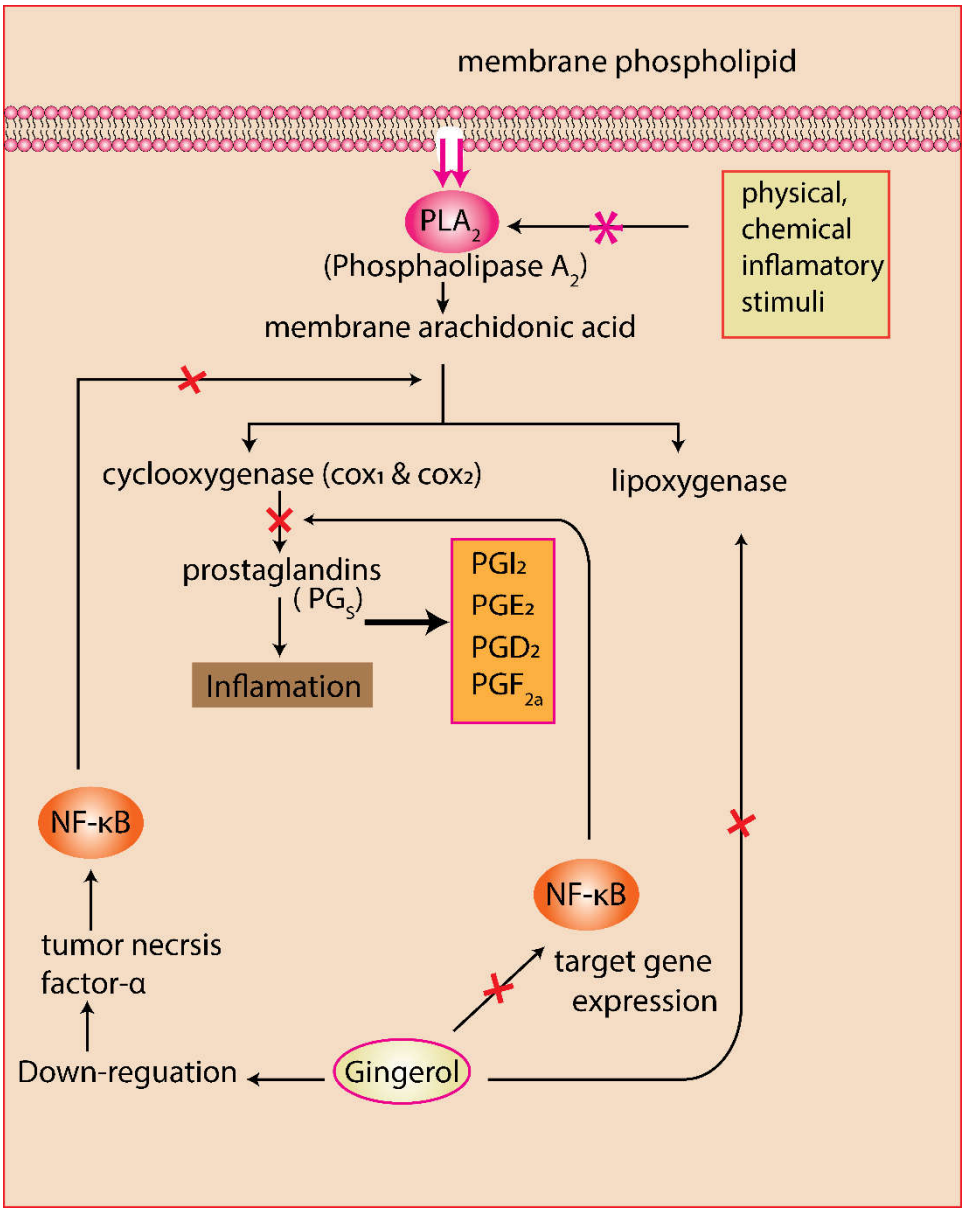

Figure 5: Upon activation by cell signaling or membrane injury/stress, cytoplasmic phospholipase A2 (PLA2) facilitates the conversion of membrane phospholipids into membrane arachidonic acid (MAA). Gingerol exerts inhibitory effects on both Cyclooxygenase (COX) and Lipoxygenase (LPO) expression by suppressing Nuclear Factor Kappa-B (NF-kB) activity, which is mediated via Tumor Necrosis Factor Alpha (TNF-α). Furthermore, gingerol contributes to the reduction of NF-kB target gene expression. This dual action underscores the potential anti-inflammatory properties of gingerol in modulating key pathways involved in inflammatory responses

Anti-inflammatory Effects of Gingerol

In traditional herbal remedies aimed at bolstering the body's immune response, ginger possesses the ability to diminish inflammation, swelling, and discomfort. In numerous countries, ginger and its derivatives are employed to enhance the immune system. Studies assessing the efficacy of ginger in

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patients with acute to chronic inflammatory conditions, such as osteoarthritis, yield controversial results [78]. Various researchers have reported that the 6-gingerol extract from dried ginger exhibits analgesic and potent anti-inflammatory effects [79,80]. A group of researchers investigated the efficacy of ginger supplementation in alleviating muscle pain over an 11-day period, involving 36 participants in their study. They asserted that the regular intake of both raw and heat-treated ginger led to significant reductions in muscle pain, with effects ranging from moderate to large [81]. Inflammation encompasses a series of cellular and molecular elements collectively known as inflammatory mediators. It can be categorized into pathways dependent on arachidonic acid (AA) and those independent of AA [82]. Mediators in the AA-dependent pathway encompass cyclooxygenase (COX), lipooxygenase (LOX), and phospholipase A2 (PLA2). On the other hand, AA-independent pathway mediators include nitric oxide synthase (NOS), NF-kB, peroxisome proliferator-activated receptors (PPAR), and NSAID-activated gene-1 (NAG-1) [83,82]. While the anti-inflammatory characteristics of ginger have been recognized for centuries, a substantial amount of scientific evidence has predominantly validated its anti-inflammatory properties in animal models of inflammation, with limited confirmation in human studies [84]. The anti-inflammatory attributes of gingerols were demonstrated through their inhibitory impact on the synthesis of prostaglandins and leukotrienes in RBL-1 cells [65]. Additionally, they were shown to mimic the actions of dual-acting nonsteroidal anti-inflammatory drugs (NSAIDs) in intact human leukocytes in vitro [85]. In their study, Kim et al. [86] demonstrated that a concentration of 30 μ M of [6]-gingerol led to a reduction in intracellular ROS levels induced by UVB. It also activated caspases 3, 8, and 9, along with Fas expressions. Furthermore, it stimulated the expression of COX-2(PDB ID;5IKT) and hindered the nuclear translocation of NF-kB from the cytosol in HaCaT cells, concomitant with the suppression of IkBk phosphorylation. When examined in vitro, gingerols and their derivatives have been documented to exhibit greater potency as anti-platelet and cyclooxygenase-1 (COX-1, PDB ID:6Y3C) inhibitors compared to aspirin [87]. In an independent study, Lantz and colleagues (reference [88]) demonstrated the significant inhibitory effects of ginger extracts on lipopolysaccharide (LPS)-induced prostaglandin E2 (PGE2) production and COX-2(DB ID:5IKT) expression in U937 cells. Notably, extracts with a predominance of either gingerols or shogaols exhibited high activity in these inhibitory processes. The generation of free radicals or reactive oxygen species (ROS) during metabolism exceeds the antioxidant capacity of a biological system, leading to oxidative stress. This oxidative stress is implicated in the progression of neurodegenerative diseases, cardiac disorders, cancer, and the aging process [89].

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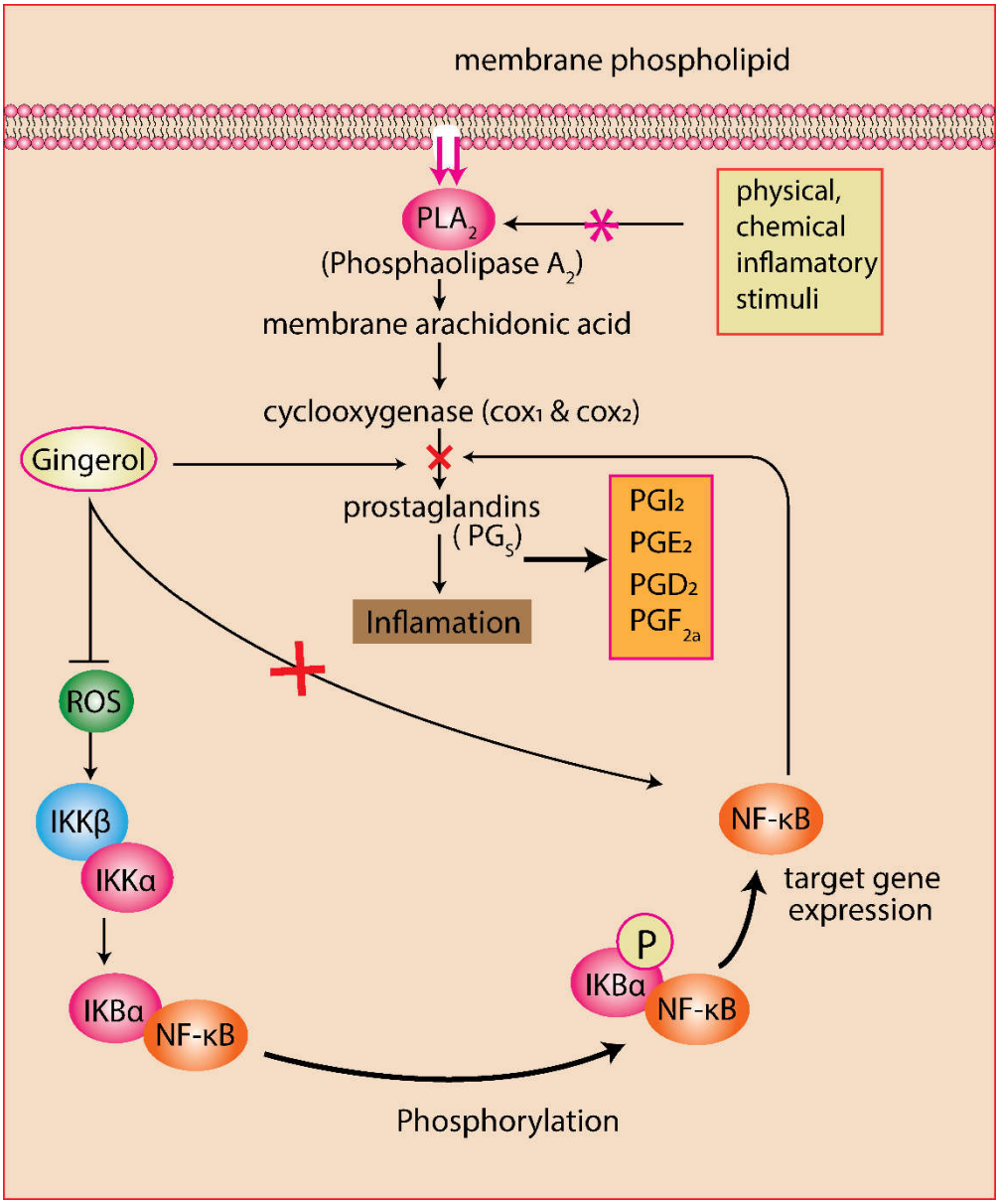

Figure 6. Upon activation through cell signaling or membrane injury/stress, cytoplasmic phospholipase A2 (PLA2) initiates the conversion of membrane phospholipids into membrane arachidonic acid (MAA). The subsequent metabolism of MAA is catalyzed by Cyclooxygenase (COX) isoforms 1 and 2, leading to the synthesis of specific prostaglandins (PGs) such as PGI2, PGE2, PGD2, and PGF2a. These prostaglandins serve dual roles as local homeostatic regulators and secreted mediators involved in processes like pain, fever, vascular tone, and inflammatory responses associated with various diseases. In the context of inflammatory regulation, gingerol has been observed to reduce NF-kB target gene expression by suppressing NF-kB activity. This is achieved through the stabilization of inhibitory protein IkBα and the degradation of IkBα kinase (IKK) activity. The mechanism involves the inhibition of reactive oxygen species (ROS) generation, NF-kappa B activation, and subsequent cyclooxygenase-2 (COX-2) induction. This suggests a potential therapeutic role for gingerol in mitigating inflammatory pathways and associated conditions.

In silico studies Collection and preparation of identified ligands

PubChem serves as an online chemical compound database on the internet. [https://pubchem.ncbi.nlm.nih.gov/]. The diverse biochemical compound known as Gingerols, exhibits inhibitory effects on COX (Cyclooxygenase-1, PDB ID:6Y3C, and Cyclooxygenase-2, PDB

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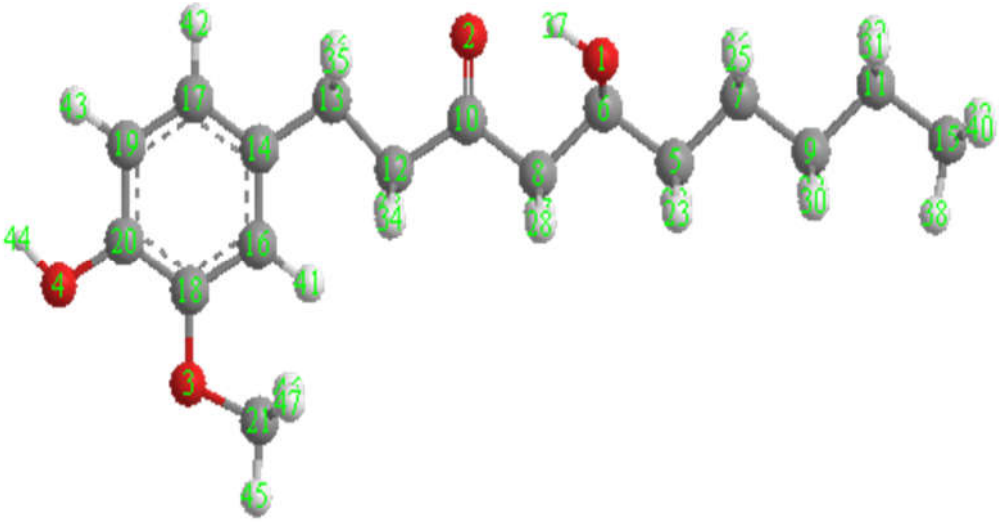
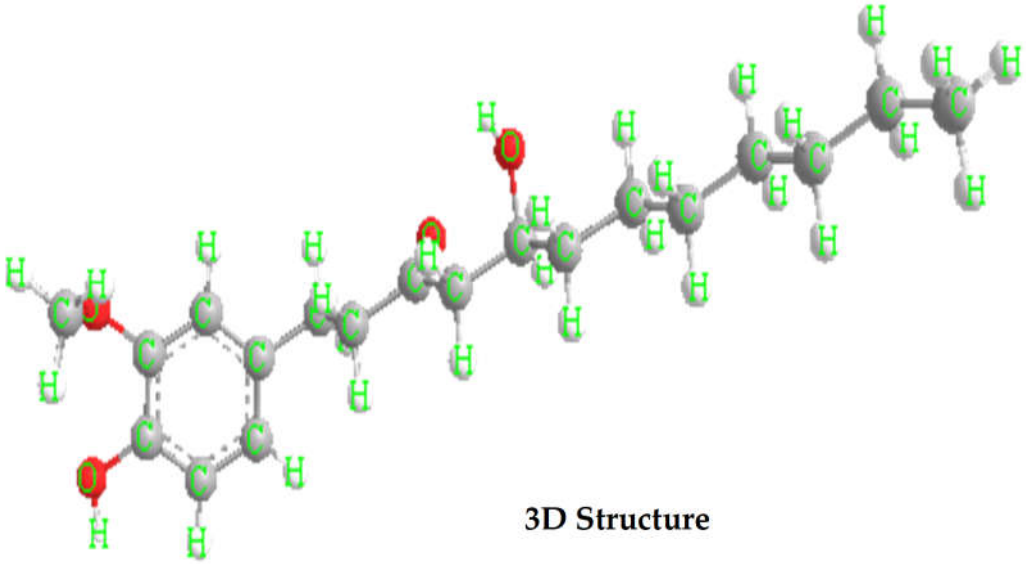
ID:5IKT and LOX (Lipooxygenase, PDB ID: 3V92), was input into the search field of the PubChem database. We collected 3D conformers in SDF format for these compounds, encompassing 6-gingerol (CID: 442793), 8-gingerol (168114), and 10-gingerol (168115). Within the standard input field, there existed a similarity search option. Users have the option to employ the similarity search feature, enabling them to discover chemical compounds with structures akin to the one they are searching for. In the initial search, PubChem delivered results for a total of 1000 chemicals. Subsequently, we employed the Chem3D 16.0 software tool to minimize the 3D conformers of the chemical agents, saving them as SDF files before converting them to MOL files for molecular docking and pharmacokinetic prediction.

A



3D Structure

B



3D Structure

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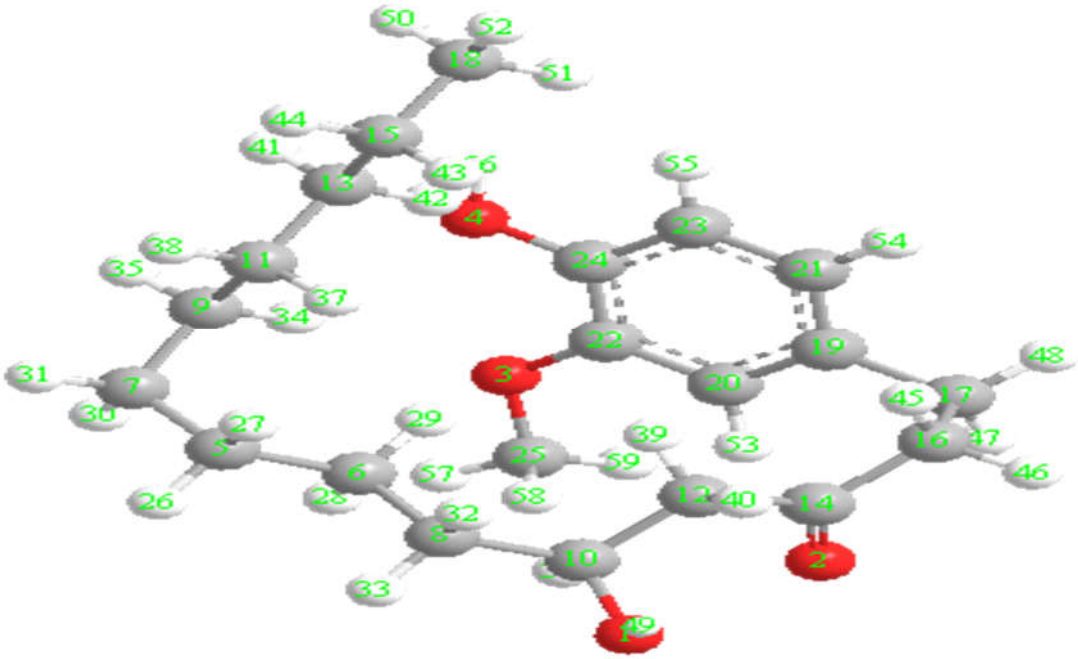
C



3D Structure

Figure 7. 3D Structure of 6,8,10-Gingerol (A, B, C).

Protein Collection and Preparation

In our literature review, our primary focus was on two proteins (receptors), specifically Cox1, Cox2, and LOX, which play a significant role in promoting inflammation. The RCSB Protein Data Bank (PDB) serves as an extensive repository of experimentally determined X-ray crystallographic structures. Maintained by the Collaborative Horizontal Integration for Systemic Bioinformatics (RCSB), the PDB is a readily accessible resource providing detailed information on the three-dimensional (3D) configurations of various entities, including genetic assistance proteins. In the investigation, an open-access database was utilized to search for experimentally solved x-ray crystallographic structures of specific proteins COX1(PDB ID: 6Y3C) and COX2(PDB ID:5IKT) LOX (PDB ID:6V92), resulting in multiple entries of 3D structures for each protein(https://www.rcsb.org/). The PyMOL program package (v2.4.1) was employed to eliminate extraneous molecules, including lipids, water molecules, and heteroatoms, from the protein sequence after data collection. This optimization of receptors was done to prevent interference during docking. GROMOS 96 force field was utilized for this purpose, and the PDB file was saved to facilitate molecular docking. Subsequently, the swissPDB Viewer software was utilized for energy minimization and geometry optimization of the receptors.

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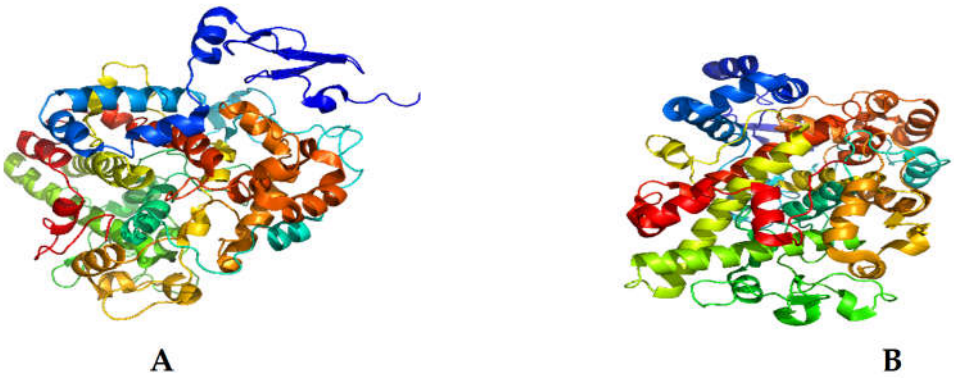

Figure 8. Structure of protein Cox1(A, PDB ID:6Y3C) And Cox2(B, PDB ID:5IKT).

Toxicity Assessment of the Identified Ligands

PreADMET was developed to assess ligand toxicity by employing methodologies from aquatic toxicology tests. Its purpose is to provide descriptive and analytical evidence of the adverse effects on aquatic animals. The table indicates that the toxicological estimation for all ligands is now below 1.0, suggesting that they are potentially non-toxic. Nevertheless, all ligands have exhibited negative results for both mouse and rat carcinogenicity tests, indicating a lack of significant toxic effects or carcinogenic potential(https://preadmet.webservice.bmdrc.org/).

Table 5. Toxicity testing using PreADME/T Profile.

Factors	[6]-Gingerol	[8]-Gingerol	[10]-Gingerol
algae_at	0.0128678	0.00685787	0.00363078
daphnia_at	0.0607967	0.0259264	0.0117833
medaka_at	0.00572286	0.00112928	0.00025129
minnow_at	0.00900351	0.00174438	0.000331235
Carcino_Mouse	negative	negative	negative
Carcino_Rat	negative	negative	negative

Molecular Docking Operation

Utilizing the PyRx software tool, molecular docking was conducted to assess the active binding potential of the targeted ligands against inflammation-related proteins (receptors). The calculation involved 2000 steps, and the grid box size for molecular docking was adjusted according to the X, Y, and Z directions. The docking outcomes were saved in .csv format, and the ligand-protein complex was stored in .pdb format for subsequent conversion to pdb-qt format. Medications were transformed into ligands, and proteins were optimized as macromolecules. The best drug ligands and optimal proteins as biomaterials were identified based on their binding affinity.

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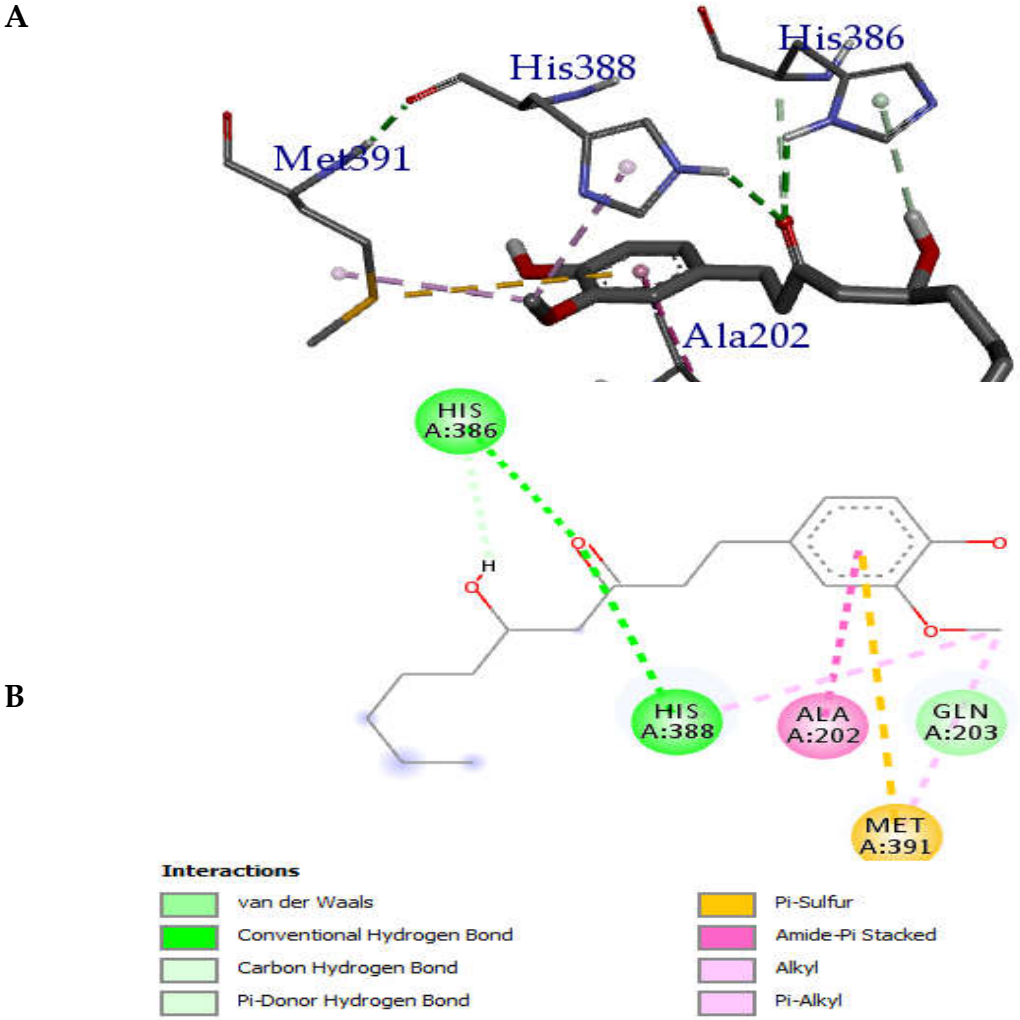

Figure 9. The best possible interaction for molecular docking of the COX1(PDB ID: 6Y3C) receptor with [6]-Gingerol with 2D and 3D dimension (A&B).

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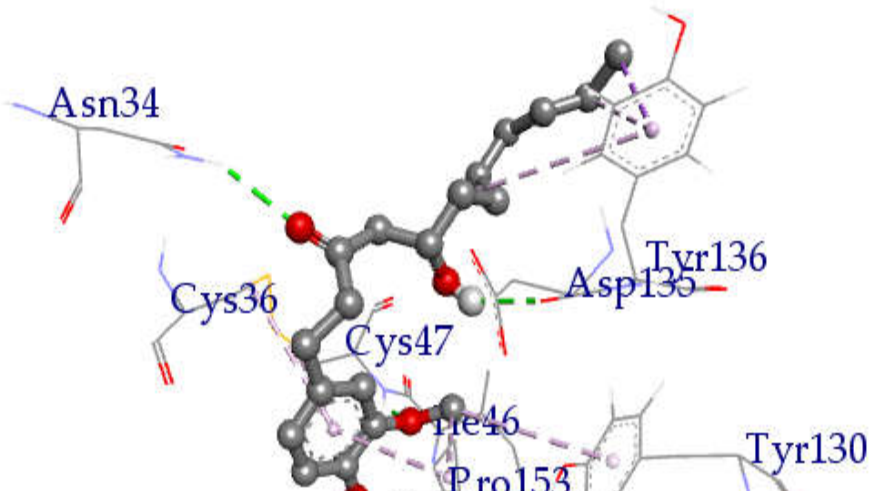
A



B

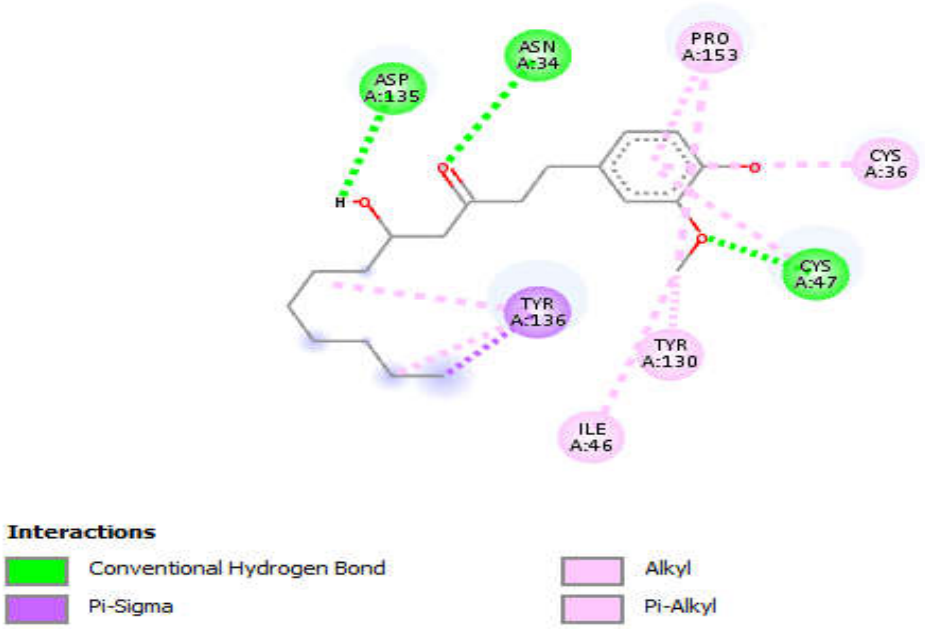


Figure 10. The best possible interaction for molecular docking of the COX2(PDB ID: 5IKT) receptor towards [6]-Gingerol with 2D and 3D dimension (A&B).

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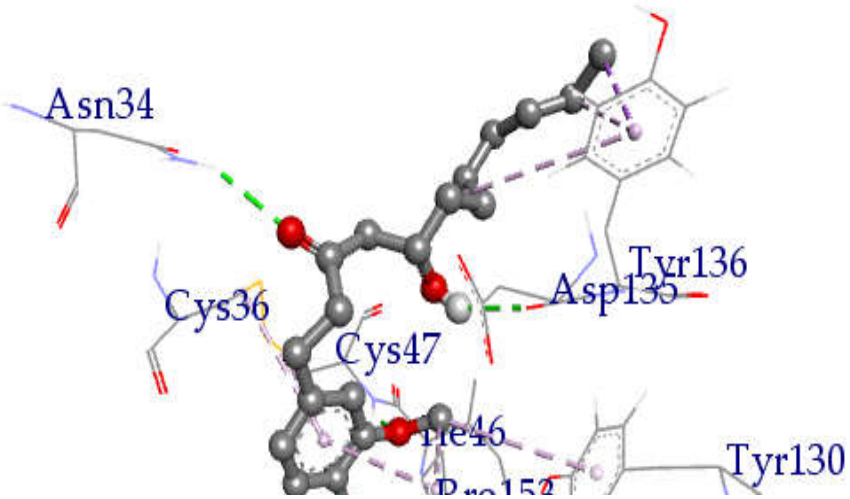
A



B

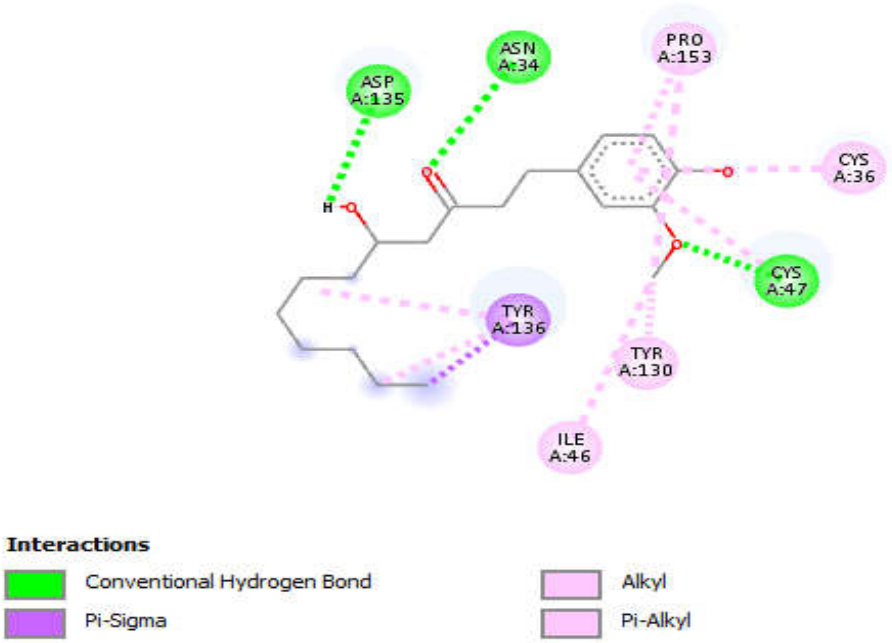


Figure 11. The best possible interaction for molecular docking of the COX1(PDB ID: 6Y3C) receptor towards [8]-Gingerol with 3D & 2D dimension (A & B).

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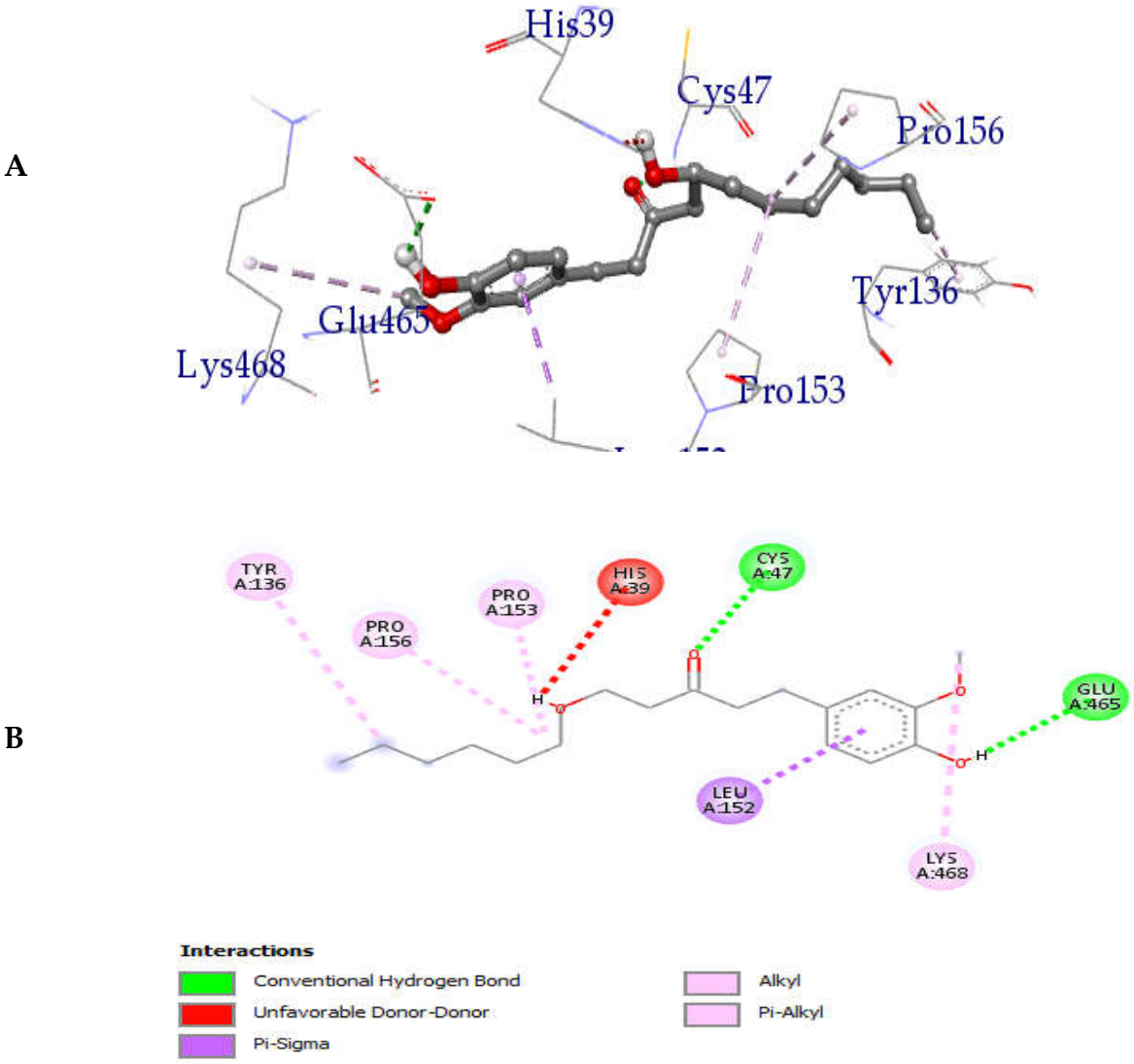

Figure 12. The best possible interaction for molecular docking of the COX2(PDB ID:5KT) receptor towards [8]-Gingerol with 3D & 2D dimension (A & B).

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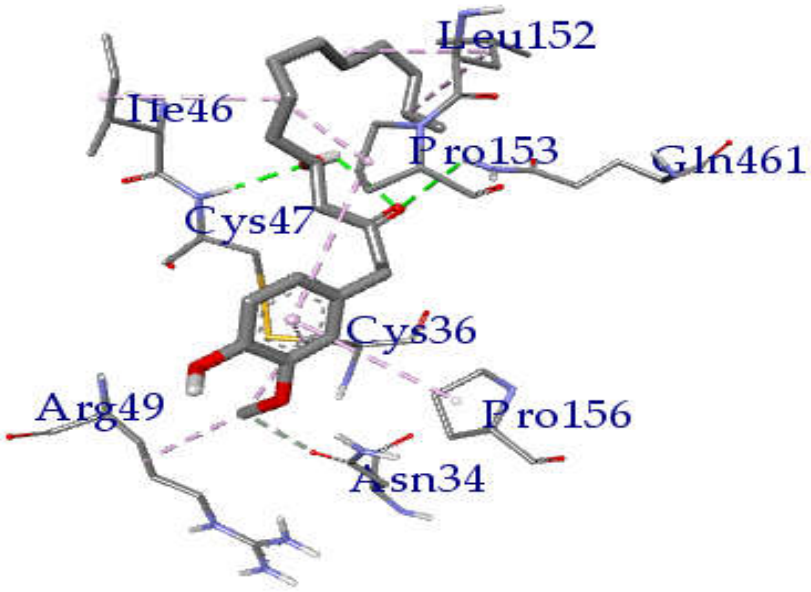
A



B

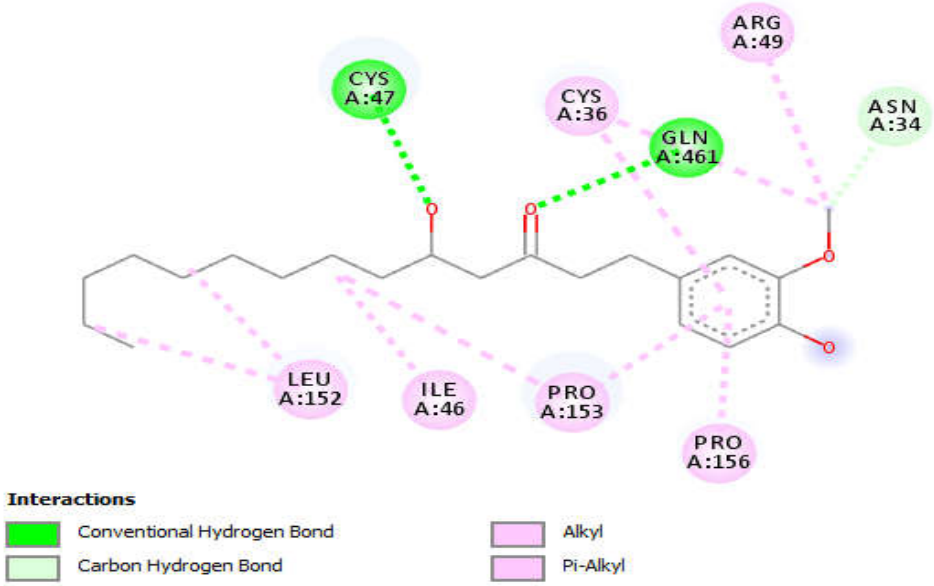


Figure 13. The best possible interaction for molecular docking of the COX1(PDB ID: 6Y3C) receptor towards [10]-Gingerol with 3D & 2D dimension (A & B).

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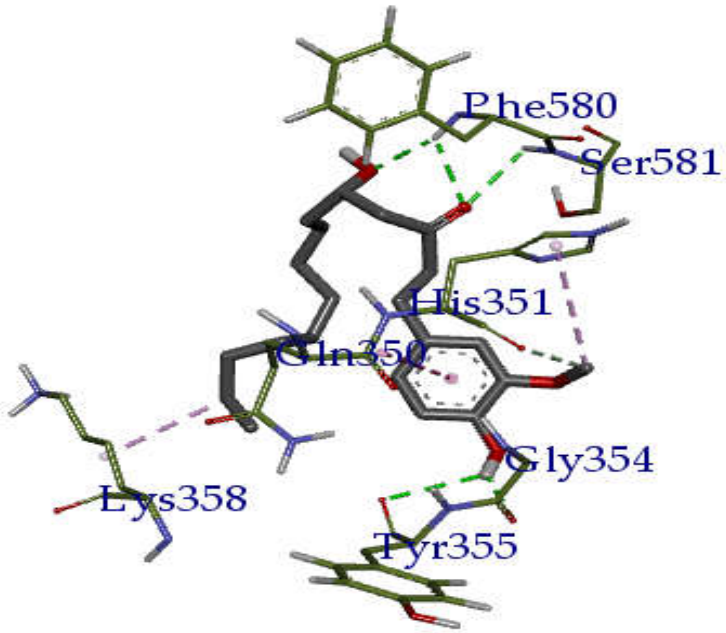
A



B

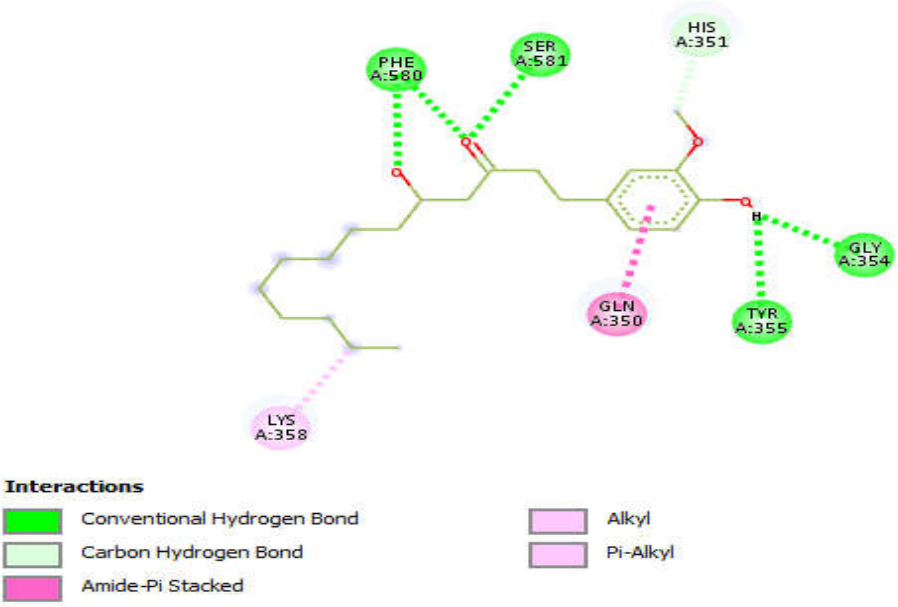


Figure 14. The best possible interaction for molecular docking of the COX2(PDB ID: 5IKT) receptor towards [10]-Gingerol with 3D & 2D dimension (A & B).

Non-Covalent Interactions within the receptor-ligand systems by Discovery Studio

The characteristics of non-bonded interactions play a crucial role in molecular recognition and binding during biological processes. In the research domain, determining and integrating non-bonded energy at the residue level has posed challenges. In this investigation, BIOVIA Discovery Studio was employed to compute non-bonded connections between membrane proteins and ligands.

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Compounds with binding scores most similar to the reference were selected and documented in the table. (SeeTable-6).

Table 6. Binding affinity kcal/mol and non-covalent interactions of Gingerols closely aligned with targeted receptors.

Compound	Receptor		Hydrogen Bond		Hydrophobic bond	
			Interacting Amino Acid	Distance (Å)	Interacting amino acid	Distance(Å)
[6]-Gingerol	COX1 (PDB ID:6Y3C)	-6.6	HIS386	2.60	ALA202	4.338
			HIS388	2.86	MET391	5.91
					GLN203	4.25
	COX2 (PDB ID: 5IKT)	-7	CYS47	2.089	HIS39	2.57
			CYS36	2.03	LYS468	4.99
			GLU465	2.47	PRO156	3.82
					LEU152	3.47
[8]-Gingerol	COX1 (PDB ID:6Y3C)	-5.8	ASP135	2.37	PRO153	4.03
			ASN34	2.52	TYR130	5.24
			CYS47	1.90	ILE46	4.07
					TYR136	5.31
	COX2 (PDB ID: 5IKT	-6.9	CYS47	2.44	TYR136	5.15
			GLU465	2.62	PRO156	4.55
					PRO153	4.54
					HIS39	2.66
					LYS468	4.72
[10]-Gingerol	COX1 (PDB ID:6Y3C)	-6.2	CYS47	2.34	CYS36	3.94
			GLN461	2.93	ARG49	3.94
					ASN34	3.51
					LEU152	5.17
					PRO153	4.94
					ILE46	4.27
					PRO156	5.11
	COX2 (PDB ID: 5IKT	-5.4	PHE580	2.35	HIS351	4.30
			SER581	2.65	GLN350	4.30
			GLY354	2.23	LYS358	3.86
			TYR355	2.91		

In silico investigation

Table 7. Binding affinity of Gingerols with cyclooxygenase (COX1, PDB ID: 6Y3C and Cox2, DB ID:5IKT).

Protein	Compound		
	[6]-Gingerol	[8]-Gingerol	[10]-Gingerol
COX1	-6.6	-5.8	-6.2
COX2	-7	-6.9	-5.4

Molecular docking

Molecular docking is conducted to anticipate interactions between ligands and receptors, as well as to predict their binding affinities towards the receptors. In this investigation, we conducted molecular docking of 6, 8, and 10-gingerol to evaluate their impact on the COX1 and COX2 receptors. The binding affinities of 6, 8, and 10-gingerol against the COX1 receptor were determined as -6.6 kcal/Mol, -5.8 kcal/Mol, and -6.2 kcal/mol, respectively. Additionally, the binding affinities of 6, 8,

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and 10-gingerol against the COX2 receptor were found to be -7 kcal/mol, -6.9 kcal/mol, and -5.4 kcal/mol, respectively. All binding affinity of the identified ligands against the targeted receptors is provided in

Visualization of Ligand-Receptor Interactions

6-gingerol demonstrated the highest binding affinity (-7 kcal/mol) towards the COX2 receptor (PDB ID: 5IKT) compared to the COX1 receptor (PDB ID: 6Y3C). This can be attributed to the formation of various types of hydrophobic bonds, including pi-sigma, pi-alkyl, Alkyl, Amide pi-stacked, unfavorable donor-donor, and Conventional Hydrogen bonds. 6-gingerol formed hydrogen bonds with amino acid residues, including HIS(A:386), HIS(A:388), while 8-gingerol established hydrogen bonds with ASP(A:135), ASN(A:34), CYS(A:47), and 10-gingerol exhibited hydrogen bonds with CYS(A:47), GLN(A:461) against the COX1 receptor (PDB ID: 6Y3C). 6-gingerol formed several hydrophobic bonds with amino acid residues such as ALA(A:202), MET(A:391), and GLN(A:203). On the other hand, 8-gingerol established hydrogen bonds with amino acid residues including PRO(A:153), TYR(A:130), ILE(A:46), and TYR(A:136). Additionally, 10-gingerol exhibited hydrogen bonds with amino acid residues CYS(A:36), ARG(A:49), ASN(A:34), LEU(A:152), ILE(A:46), PRO(A:153), PRO(A:156) against the COX1 receptor (PDB ID: 6Y3C). Similarly, 6-gingerol formed hydrogen bonds with amino acid residues, including CYS(A:47), CYS(A:36), and GLU(A:465), while 8-gingerol established hydrogen bonds with CYS(A:47), GLU(A:465). Additionally, 10-gingerol exhibited hydrogen bonds with PHE(A:580), SER(A:581), GLY(A:354), TYR (A:355) against the COX2 receptor (PDB ID: 5IKT). 6-gingerol formed several hydrophobic bonds with amino acid residues, including HIS(A:39), LYS(A:468), PRO(A:156), and LEU(A:152). Similarly, 8-gingerol exhibited hydrogen bonds with amino acid residues, including TYR(A:136), PRO(A:156), PRO(A:153), HIS(A:39), LYS(A:468). Furthermore, 10-gingerol exhibited hydrogen bonds with amino acid residues, including HIS(A:351), GLN(A:350), and LYS(A:358) against the COX2 receptor (PDB ID: 5IKT). All the hydrophobic and hydrogen bonds with their interacting amino acid residues and their corresponding bond distance are provided in the table-6

Conclusion

The medicinal benefits of ginger are more widely acknowledged than its reputation as a flavor-enhancing agent. The primary phytochemicals in fresh ginger rhizome are gingerols, which contribute to its pungent flavor. Among these, 6-gingerol stands out as the most extensively researched bioactive component. According to the pharmacokinetic study, gingerols tend to accumulate in the gastrointestinal tract, and in the liver, they undergo metabolism to form glucuronides. Extended incubation in the intestine can lead to the conversion of gingerol into shogaol. The predominant active constituents in ginger are gingerols, and currently, 31 gingerol-related compounds have been identified. 6-gingerol stands out as the primary pungent and bioactive component among these compounds. In addition to 4-, 6-, 8-, and 10-gingerols, the rhizome contains other bioactive components that exhibit anti-inflammatory activity. NF-κB activation serves as a primary mediator of inflammation in numerous diseases, including pulmonary diseases, cardiovascular diseases, type-2 diabetes, cancer, arthritis, Alzheimer's, neurological diseases, and autoimmune diseases. The suppression of NF-κB activation can effectively mitigate inflammation. Elevated expression of NF-κB, COX2(PDB ID:5IKT), 5-LOX (PDB ID:6V92), and iNOS contribute to inflammation and disorders associated with inflammation. Due to its robust NF-κB inhibitory effects, ginger effectively restrains the expression of COX-2(PDB ID:5IKT), 5-LOX (PDB ID:6V92), and iNOS, probably by downregulating NF-κB activation. Ginger's potential as an anti-inflammatory agent lies in its ability to inhibit NF-κB activation through the suppression of the proinflammatory cytokine TNF-α. The molecular docking analysis demonstrated the binding affinity of all compounds, highlighting that 6-gingerol, in particular, exhibits significant binding to the protein. This suggests its potential therapeutic efficacy against inflammation. Additionally, other related compounds were

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observed to be less hazardous and displayed improved pharmacokinetic behavior in ADME/T studies. This suggests their potential utilization in the treatment of inflammatory diseases.

References

1. Michels da Silva D, Langer H, Graf T. Inflammatory and Molecular Pathways in Heart Failure-Ischemia, HFpEF and Transthyretin Cardiac Amyloidosis. Int J Mol Sci. 2019 May 10;20(9).
2. Zhang X, Wu X, Hu Q, Wu J, Wang G, Hong Z, Ren J., Lab for Trauma and Surgical Infections. Mitochondrial DNA in liver inflammation and oxidative stress. Life Sci. 2019 Nov 01;236:116464.
3. Fritsch J, Abreu MT. The Microbiota and the Immune Response: What Is the Chicken and What Is the Egg? Gastrointest Endosc Clin N Am. 2019 Jul;29(3):381-393.
4. Barcelos IP, Troxell RM, Graves JS. Mitochondrial Dysfunction and Multiple Sclerosis. Biology (Basel). 2019 May 11;8(2)
5. Tsai DH, Riediker M, Berchet A, Paccaud F, Waeber G, Vollenweider P, Bochud M. Effects of short- and long-term exposures to particulate matter on inflammatory marker levels in the general population. Environ Sci Pollut Res Int. 2019 Jul;26(19):19697-19704.
6. Deepak P, Axelrad JE, Ananthakrishnan AN. The Role of the Radiologist in Determining Disease Severity in Inflammatory Bowel Diseases. Gastrointest Endosc Clin N Am. 2019 Jul;29(3):447-470.
7. Raskin I, Ribnicky DM, Komarnytsky S, et al. Plants and human health in the twenty-first century. Trends Biotechnol 2002; 20:522.
8. Grzanna R, Lindmark L, Frondoza CG. Ginger: An herbal medicinal product with broad anti-inflammatory actions. J Med Food 2005;8:125.
9. Vemuri, S. K., Banala, R. R., Subbaiah, G. P. V., Srivastava, S. K., Gurava Reddy, A. V., and Malarvili, T. (2017). Anti-cancer potential of a mix of natural extracts of turmeric, ginger and garlic: A cell-based study. Egypt. J. Basic Appl. Sci. 4, 332–344. Doi: 10.1016/j.ejbas.2017.07.005
10. Dugasani, S., Pichika, M. R., Nadarajah, V. D., Balijepalli, M. K., Tandra, S., and Korlakunta, J. N. (2010). Comparative antioxidant and anti-inflammatory effects of [6]-gingerol, [8]-gingerol, [10]-gingerol and [6]-shogaol. J. Ethnopharmacol. 127 (2), 515–520. doi:10.1016/j.jep.2009.10.004
11. Embuscado, M. E. (2015). Spices and herbs: Natural sources of antioxidants-A mini review. J. Funct. Foods 18, 811–819. doi:10.1016/j.jff.2015.03.005
12. Konmun, J., Danwilai, K., Ngamphaiboon, N., Sripanidkulchai, B., Sookprasert, A., and Subongkot, S. (2017). A phase II randomized double-blind placebocontrolled study of 6-gingerol as an anti-emetic in solid tumor patients receiving moderately to highly emetogenic chemotherapy. Med. Oncol. 34 (4), 69. doi:10.1007/s12032-017-0931-4
13. de Menezes de Lima, R. M. T., Dos Reis, A. C., de Menezes, A. P. M., Santos, J. V. O., Filho, J. W. G. O., Ferreira, J. R. O., et al. (2018). A. Protective and therapeutic potential of ginger (Zingiber officinale) extract and [6]-gingerol in cancer: A comprehensive review. Phytother. Res. 32 (10), 1885–1907. doi:10.1002/ptr.6134
14. Liang, N., Sang, Y., Liu, W., Yu, W., and Wang, X. (2018). Anti-inflammatory effects of gingerol on lipopolysaccharide-stimulated RAW 264.7 cells by inhibiting NF-κB signaling pathway. Inflammation 41 (3), 835–845. doi:10.1007/s10753-018- 0737-3
15. Lashgari, N. A., Momeni Roudsari, N., Khayatan, D., Shayan, M., Momtaz, S., Roufogalis, B. D., et al. (2022). Ginger and its constituents: Role in treatment of inflammatory bowel disease. Biofactors 48 (1), 7–21. doi:10. 1002/biof.1808
16. Semwal, R. B., Semwal, D. K., Combrinck, S., and Viljoen, A. M. (2015). Gingerols and shogaols: Important nutraceutical principles from ginger. Phytochemistry 117, 554–568. doi:10.1016/j.phytochem.2015.07.012
17. Koch, W., Kukula-Koch, W., Marzec, Z., Kasperek, E., Wyszogrodzka-Koma, L., Szwerc, W., et al. (2017). Application of chromatographic and spectroscopic methods towards the quality assessment of ginger (Zingiber officinale) rhizomes from ecological plantations. Int. J. Mol. Sci. 18 (2), 452. doi:10.3390/ijms18020452
18. Mao, Q. Q., Xu, X. Y., Cao, S. Y., Gan, R. Y., Corke, H., Beta, T., et al. (2019). Bioactive compounds and bioactivities of ginger (Zingiber officinale Roscoe). Foods 8 (6), 185. doi:10.3390/foods8060185

 doi:10.20944/preprints202503.2176.v1

19. Ozkur, M., Benlier, N., Takan, I., Vasileiou, C., Georgakilas, A. G., Pavlopoulou, A., et al. (2022). Ginger for healthy ageing: A systematic review on current evidence of its antioxidant, anti-inflammatory, and anticancer properties. Oxid. Med. Cell. Longev. 2022, 4748447. doi:10.1155/2022/4748447
20. Hitomi, S., Ono, K., Terawaki, K., Matsumoto, C., Mizuno, K., Yamaguchi, K., et al. (2017). [6]-gingerol and [6]-shogaol, active ingredients of the traditional Japanese medicine hangeshashinto, relief oral ulcerative mucositis-induced pain via action on Na+ channels. Pharmacol. Res. 117, 288–302. doi:10.1016/j.phrs.2016.12.026
21. Samad, M. B., Mohsin, M. N. A. B., Razu, B. A., Hossain, M. T., Mahzabeen, S., Unnoor, N., et al. (2017). [6]-Gingerol, from Zingiber officinale, potentiates GLP-1 mediated glucose-stimulated insulin secretion pathway in pancreatic-cells and increases RAB8/RAB10-regulated membrane presentation of GLUT4 transporters in skeletal muscle to improve hyperglycemia in Leprdb/db type 2 diabetic mice. BMC Complement. Altern. Med. 17 (1), 395. doi:10.1186/ s12906-017-1903-0
22. Wang, S., Tian, M., Yang, R., Jing, Y., Chen, W., Wang, J., et al. (2018). 6-gingerol ameliorates behavioral changes and atherosclerotic lesions in apoe-/- mice exposed to chronic mild stress. Cardiovasc. Toxicol. 18 (5), 420–430. doi:10.1007/s12012- 018-9452-4
23. Ghasemzadeh, A., Jaafar, H. Z. E., Baghdadi, A., and Tayebi-Meigooni, A. (2018). formation of 6-8- and 10-shogaol in ginger through application of different drying methods: Altered antioxidant and antimicrobial activity. Molecules 23 (7), 1646. doi:10.3390/molecules23071646
24. Han, H. S., Kim, K. B., Jung, J. H., An, I. S., Kim, Y. J., and An, S. (2018). Antiapoptotic, antioxidant and anti-aging effects of 6-shogaol on human dermal fibroblasts. Biomed. Dermatol. 2, 27. doi:10.1186/s41702-018-0037-4
25. Ho, S. C., and Chang, Y. H. (2018). Comparison of inhibitory capacities of 6-8- and 10-gingerols/shogaols on the canonical NLRP3 inflammasome-mediated IL-1β secretion. Molecules 23 (2), 466. doi:10.3390/molecules23020466
26. Lee, E. B., Kim, J. H., Kim, Y. J., Noh, Y. J., Kim, S. J., Hwang, I. H., et al. (2018). Lifespan-extending property of 6-shogaol from Zingiber officinale Roscoe in Caenorhabditis elegans. Arch. Pharm. Res. 41, 743–752. doi:10.1007/s12272-018- 1052-0
27. Smith, N. C., Christian, S. L., Taylor, R. G., Santander, J., and Rise, M. L. (2018). Immune modulatory properties of 6-gingerol and resveratrol in Atlantic salmon macrophages. Mol. Immunol. 95, 10–19. doi:10.1016/j.molimm.2018.01.004
28. Han, X., Liu, P., Zheng, B., Zhang, M., Zhang, Y., Xue, Y., et al. (2022a). 6- Gingerol exerts a protective effect against hypoxic injury through the p38/Nrf2/HO1 and p38/NF-κB pathway in H9c2 cells. J. Nutr. Biochem. 104, 108975. doi:10.1016/ j.jnutbio.2022.108975
29. Lashgari, N. A., Momeni Roudsari, N., Khayatan, D., Shayan, M., Momtaz, S., Roufogalis, B. D., et al. (2022). Ginger and its constituents: Role in treatment of inflammatory bowel disease. Biofactors 48 (1), 7–21. doi:10.1002/biof.1808
30. Kravchenko, I.A.; Eberle, L.V.; Nesterkina, M.V.; Kobernik, A.O. Pharmacotherapy of inflammatory process by ginger extract (Zingiber officinale) ointment. J. Herb. Med. 2019, 8, 101–107. [CrossRef]
31. Drozdov, V.N.; Kim, V.A.; Tkachenko, E.V.; Varvanina, G.G. Influence of a specific ginger combination on gastropathy conditions in patients with osteoarthritis of the knee or hip. J. Altern. Complement. Med. 2012, 18, 583–588. [CrossRef]
32. Black, C.D.; Herring, M.P.; Hurley, D.J.; O'Connor, P.J. Ginger (Zingiber officinale) reduces muscle pain caused by eccentric exercise. J. Pain 2010, 11, 894–903. [CrossRef]
33. Fujiwara N, Kobayashi K. Macrophages in inflammation. Curr Drug Targets Inflamm Allergy 2005;4:281.
34. Lien E, Ingalls RR. Toll-like receptors. Crit Care Med 2002; 30(Suppl):S1.
35. Takeda K, Kaisho T, Akira S. Toll-like receptors. Annu Rev Immunol 2003;21:335.
36. Aderem A. Role of toll-like receptors in inflammatory response in macrophages. Crit Care Med 2001;29(Suppl):S16.
37. Heine H, Lien E. Toll-like receptors and their function in innate and adaptive immunity. Int Arch Allergy Immunol 2003;130: 180.

 doi:10.20944/preprints202503.2176.v1

38. Guleria, A., Kamboj, A., Kaushal, J., Anupam, K., and Bhatnagar, A. (2022). A molecular insight into significance of functional foods in better management of rheumatoid arthritis. Rev. Bras. Farmacogn. 2022. doi:10.1007/s43450-022-00266-6
39. Ley-Martínez, J. S., Ortega-Valencia, J. E., García-Barradas, O., JiménezFernández, M., Uribe-Lam, E., Vencedor-Meraz, C. I., et al. (2022). Active compounds in Zingiber officinale as possible redox inhibitors of 5-Lipoxygenase using an in-silico approach. Int. J. Mol. Sci. 23, 6093. doi:10.3390/ijms23116093
40. Cerrutti, P.A., 1991. Oxidant stress and carcinogenesis. European Journal of Clinical Investigation 21, 1–11.
41. Halliwell, B., 1997. Antioxidants and human diseases: a general introduction. Nutrition Reviews 55, S52
42. Nathan, C., Xie, Q.W., 1994. Nitric oxide synthase: roles, tolls, and controls. Cell 78, 915–918
43. Atkan, F., 2004. iNOS-mediated nitric oxide production and its regulation. Life Sciences 75, 639–653
44. Grzanna, R., Lindmark, L., and Frondoza, C. G. (2005). Ginger – An herbal medicinal product with broad anti-inflammatory actions. J. Med. Food 8 (2), 125–132. doi:10.1089/jmf.2005.8.125
45. Denniff, P., Macleod, I., and Whiting, D. A. (1980). Studies in the biosynthesis of [6]-gingerol, pungent principle of ginger (Zingiber officinale). J. Chem. Soc. Perkin 1 1, 2637–2644. doi:10.1039/p19800002637
46. Li, L. L., Cui, Y., Guo, X. H., Ma, K., Tian, P., Feng, J., et al. (2019). Pharmacokinetics and tissue distribution of gingerols and shogaols from ginger (Zingiber officinale Roscoe) in rats by UPLC-Q-exactive-HRMS. Molecules 24 (3), 512. doi:10.3390/molecules24030512
47. Alolga, R. N., Wang, F., Zhang, X., Li, J., Tran, L.-S. P., and Yin, X. (2022). Bioactive compounds from the Zingiberaceae Family with known antioxidant activities for possible therapeutic uses. Antioxidants 11, 1281. doi:10.3390/ antiox11071281
48. Habtemariam, S. (2019). "The chemical and pharmacological basis of ginger (Zingiber officinale Roscoe) as potential therapy for diabetes and metabolic syndrome," in Medicinal foods as potential therapies for type-2 diabetes and associated diseases, the chemical and pharmacological basis of their action (London, United Kingdom: Academic Press), 639–687. doi:10.1016/C2018-0- 02257-4
49. Liu, Y., Liu, J., and Zhang, Y. (2019). Research progress on chemical constituents of Zingiber officinale Roscoe. Biomed. Res. Int. 2019, 5370823. doi:10.1155/2019/ 5370823
50. Loung, C. Y., Rasmussen, A. N., and Hoskin, D. W. (2019). "The phenolic gingerols and gingerol-derived shogaols: Features and properties related to the prevention and treatment of cancer and chronic inflammation," in Polyphenols in plants isolation, purification and extract preparation. Editor R. Ross Watson (India: Academic Press), 395–405.
51. Kukula-Koch, W., and Czernicka, L. (2020). "Gingerols and shogaols from food," in Handbook of dietary phytochemicals. Editors J. Xiao, S. D. Sarker, and Y. Asakawa (Singapur: Springer), 1–31.
52. Unuofin, J. O., Masuku, N. P., Paimo, O. K., and Lebelo, S. L. (2021). Ginger from farmyard to town: Nutritional and pharmacological applications. Front. Pharmacol. 12, 779352. doi:10.3389/fphar.2021.779352
53. Jolad SD, Lantz RC, Chen GJ et al (2005) Commercially processed dry ginger (Zingiber officinale): composition and effects on LPS-stimulated PGE2 production. Phytochem 66 (13):1614–1635
54. Jolad SD, Lantz RC, Chen GJ et al (2005) Commercially processed dry ginger (Zingiber officinale): composition and effects on LPS-stimulated PGE2 production. Phytochem 66 (13):1614–1635.
55. Jiang H, Solyom AM, Timmermann BN et al (2005) Characterization of gingerol-related compounds in ginger rhizome (Zingiber officinale Rosc.) by high-performance liquid chromatography/ electrospray ionization mass spectrometry. Rapid Commun Mass Spectrom 19(20):2957–2964
56. Wohlmuth H, Leach DN, Smith MK et al (2005) Gingerol content of diploid and tetraploid clones of ginger (Zingiber officinale Roscoe). J Agric Food Chem 53:5772–5778
57. Zhang YX, Li JS, Chen LH et al (2012) Simultaneous determination of five gingerols in raw and processed ginger by HPLC. Chinese Pharm J 47:471–474
58. Ding GH, Naora K, Hayashibara M et al (1991) Pharmacokinetics of [6]-gingerol after intravenous administration in rats. Chem Phar Bull (Tokyo) 39:1612–1614
59. Surh YJ, Lee E, Lee JM (1998) Chemoprotective properties of some pungent ingredients present in red pepper and ginger. Mutat Res 402(1–2):259–267

 doi:10.20944/preprints202503.2176.v1

60. Zick SM, Djuric Z, Ruffin MT et al (2008) Pharmacokinetics of 6-, 8-, 10-gingerols and 6-shogaol and conjugate metabolites in healthy human subjects. Cancer Epidemiol Biomarkers Prev 17(8):1930–1936. doi:10.1158/1055-9965.EPI-07-2934
61. Nakazawa T, Ohsawa K (2002) Metabolism of [6]-gingerol in rats. Life Sci 70:2165–2175
62. Gundala SR, Mukkavilli R, Yang C et al (2014) Enterohepatic recirculation of bioactive ginger phytochemicals is associated with enhanced tumor growth-inhibitory activity of ginger extract. Carcinogenesis 35(6):1320–1329. doi:10.1093/carcin/bgu011
63. Bhattarai S, Tran VH, Duke CC (2007) Stability of [6]-gingerol and [6]-shogaol in simulated gastric and intestinal fluids. J Pharmaceut Biomed Anal 45:648–653
64. Jantan, I., Ahmad, W., and Bukhari, S. N. (2015). Plant-derived immunomodulators: An insight on their preclinical evaluation and clinical trials. Front. Plant Sci. 6, 655. doi:10.3389/fpls.2015.00655
65. Ortuño-Sahagún, D., Zänker, K., Rawat, A. K. S., Kaveri, S. V., and Hegde, P. (2017). Natural immunomodulators. J. Immunol. Res. 2017, 7529408. doi:10.1155/ 2017/7529408
66. 46. Nagoba, B., and Davane, M. (2018). Natural immunomodulators. J. Immunol. Microbiol. 2, 1–2.
67. Kumar, S., Saxena, K., Singh, U. N., and Saxena, R. (2013). Anti-inflammatory action of ginger: A critical review in anemia of inflammation and its future aspects. Int. J. Herb. Med. 1 (4), 16–20.
68. Aziz, D. M., Wsoo, M. A., and Ibrahim, B. M. (2015). Antimicrobial and antioxidant activities of extracts from medicinal plant ginger (Zingiber officinale) and identification of components by gas chromatography. Afr. J. Plant Sci. 9 (10), 412–420. doi:10.5897/AJPS2015.1345
69. Deng, M., Yun, X., Ren, S., Qing, Z., and Luo, F. (2022). Plants of the genus zingiber: A review of their ethnomedicine, phytochemistry and pharmacology. Molecules 27, 2826. doi:10.3390/molecules27092826
70. Zahoor, A., Yang, C., Yang, Y., Guo, Y., Zhang, T., Jiang, K., et al. (2020). 6- Gingerol exerts anti-inflammatory effects and protective properties on LTAinduced mastitis. Phytomedicine 76, 153248. doi:10.1016/j.phymed.2020. 153248
71. Zhang, M., Zhao, R., Wang, D., Wang, L., Zhang, Q., Wei, S., et al. (2021). Ginger (Zingiber officinale Rosc.) and its bioactive components are potential resources for health beneficial agents. Phytother. Res. 35 (2), 711–742. doi:10. 1002/ptr.6858
72. Zhou, X., Münch, G., Wohlmuth, H., Afzal, S., Kao, M. T., Al-Khazaleh, A., et al. (2022). Synergistic inhibition of pro-inflammatory pathways by ginger and turmeric extracts in RAW 264.7 cells. Front. Pharmacol. 202213, 818166. doi:10. 3389/fphar.2022.818166
73. Park KK, Chun KS, Lee SS, Surh YJ. Inhibitory effect of [6] - gingerol, a major pungent principle of ginger, on phorbol esterinduced inflammation, epidermal ornithine decarboxylase activity and skin tumor promotion in ICR mice. Cancer Lett 1998; 129:139- 44
74. Surh YJ. Cancer chemoprevention with dietary phytochemical.Nat Rev Cancer 2003; 3:768-80
75. Aggarwal BB, Shishodia S (2006) Molecular targets of dietary agents for prevention and therapy of cancer. Biochem Pharmacol 71:1397–1421
76. Maeda S, Omata M (2008) Inflammation and cancer: role of nuclear factor-kappa B activation. Cancer Sci 99:836–842
77. Kim SO, Chun KS, Kundu JK et al (2004) Inhibitory effects of [6]-gingerol on PMA-induced COX-2 expression and activation of NF-kB and p38 MAPK in mouse skin. BioFactors 21:27–31
78. Maghbooli M, Golipour F, Moghimi Esfandabadi A, Yousefi M. Comparison between the efficacy of ginger and sumatriptan in the ablative treatment of the common migraine. Phytotherapy Research. 2014;28(3):412-5.
79. Young H-Y, Luo Y-L, Cheng H-Y, Hsieh W-C, Liao J-C, Peng W-H. Analgesic and anti-inflammatory activities of [6]-gingerol. Journal of Ethnopharmacology. 2005;96(1- 2):207-10.
80. Minghetti P, Sosa S, Cilurzo F, Casiraghi A, Alberti E, Tubaro A, et al. Evaluation of the topical anti-inflammatory activity of ginger dry extracts from solutions and plasters. Planta Medica. 2007;73 (15):1525-30
81. Black CD, Herring MP, Hurley DJ, O'Connor PJ. Ginger (Zingiber officinale) reduces muscle pain caused by eccentric exercise. The Journal of Pain. 2010;11(9):894-903

 doi:10.20944/preprints202503.2176.v1

82. Yoon JH, Baek SJ (2005) Molecular targets of dietary polyphenols with anti-inflammatory properties. Yonsei Med J 46(5):585–596. doi:10.3349/ymj.2005.46.5.585
83. Issa AY, Volate SR, Wargovich MJ (2006) The role of phytochemicals in inhibition of cancer and inflammation: new directions and perspectives. J Food Compost Anal 19: 405–419
84. Wei QY, Ma JP, Cai YJ et al (2005) Cytotoxic and apoptotic activities of diarylheptanoids and gingerol-related compounds from the rhizome of Chinese ginger. J Ethnopharmacol 102:177–184
85. Flynn DL, Rafferty MF, Boctor AM (1968) Inhibition of human neutrophil 5-lipoxygenase activity by gingerdione, shogaol, capsaicin and related pungent compounds. Prostaglandins Leukot Med 24:195–198
86. Kim JK, Kim Y, Na KM et al (2007) [6]-Gingerol prevents UVB induced ROS production and COX-2 expression in vitro and in vivo. Free Rad Res 41:603–614
87. Nurtjahja-Tjendraputra E, Ammit AJ, Roufogalis BD et al (2003) Effective anti-platelet and COX-1 enzyme inhibitors from pungent constituents of ginger. Thromb Res 111(4–5):259–265
88. Lantz RC, Chen GJ, Sarihan M et al (2007) The effect of extracts from ginger rhizome on inflammatory mediator production. Phytomed 14:123–128
89. Sharifi-Rad M, Varoni EM, Salehi B, Sharifi-Rad J, Matthews KR, Ayatollahi SA, et al. Plants of the genus Zingiber as source of antimicrobial agents: From Tradition to Pharmacy;2017
90. Feixiong Cheng, Weihua Li, Yadi Zhou, Jie Shen, Zengrui Wu, Guixia Liu, Philip W. Lee, Yun Tang. admetSAR: a comprehensive source and free tool for evaluating chemical ADMET properties. J. Chem. Inf. Model., 2012, 52(11): 3099-3105. (This paper is the hot paper of JCIM in Dec. 2012).
91. Ficker, E., Zhao, S., Obejero-Paz, C. A., & Brown, A. M. (2002). The binding site for channel blockers that rescue misprocessed human long QT syndrome type 2 ether-a-gogorelated gene (HERG) mutations. Journal of Biological Chemistry, 277(7), 4989-4998
92. Lin, J. H., & Yamazaki, M. (2003). Role of P-glycoprotein in pharmacokinetics. Clinical pharmacokinetics, 42(1), 59-98.
93. Halgren, T. A. (1996). Merck molecular force field. II. MMFF94 van der Waals and electrostatic parameters for intermolecular interactions. Journal of Computational Chemistry, 17(5-6), 520-552.
94. Zhu, C., Gao, Y., Li, H., Meng, S., Li, L., Francisco, J. S., & Zeng, X. C. (2016). Characterizing hydrophobicity of amino acid side chains in a protein environment via measuring contact angle of a water nanodroplet on planar peptide network. Proceedings of the National Academy of Sciences, 113(46), 12946-12951
95. Payton, J. L., Morton, S. M., Moore, J. E., & Jensen, L. (2014). A hybrid atomistic electrodynamics–quantum mechanical approach for simulating surface-enhanced Raman scattering. Accounts of chemical research, 47(1), 88-99
96. Carrasco, M. J., Alishetty, S., Alameh, M. G., Said, H., Wright, L., Paige, M., ... & Buschmann, M. D. (2021). Ionization and structural properties of mRNA lipid nanoparticles influence expression in intramuscular and intravascular administration. Communications biology, 4(1), 1-15.
97. Schiebel, J., Gaspari, R., Wulsdorf, T., Ngo, K., Sohn, C., Schrader, T. E., ... & Klebe, G. (2018). Intriguing role of water in protein-ligand binding studied by neutron crystallography on trypsin complexes. Nature communications, 9(1), 1-15.