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Antagonism of Human Formyl Peptide Receptor 1 with Natural Compounds and their Synthetic Derivatives

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Abstract

Formyl peptide receptor 1 (FPR1) regulates a wide variety of neutrophil functional responses and plays an important role in inflammation and the pathogenesis of various diseases. To date, a variety of natural and synthetic molecules have been identified as FPR1 ligands. Here, we review current knowledge on natural products and natural product-inspired small-molecules reported to antagonize and/or inhibit the FPR1-mediated responses. Based on this literature, additional screening of selected commercially available natural compounds for their ability to inhibit *f*MLF-induced Ca^{2+} mobilization in human neutrophils and FPR1 transfected HL-60 cells, and pharmacophore modeling, natural products with potential as FPR1 antagonists are considered and discussed in this review. The identification and characterization of natural products that antagonize FPR1 activity may have potential for the development of novel therapeutics to limit or alter the outcome of inflammatory processes.

Keywords

Natural compound; formyl peptide receptor 1; neutrophil; receptor antagonist; superoxide anion radical; neutrophil elastase; Ca^{2+} flux; molecular modeling

1. Introduction

Forty years ago, Schiffmann *et al.* [1] reported that *N*-formylated peptides are potent chemotactic agents for human neutrophils. Further study of the biological targets of formylated peptides led to the identification and subsequent cloning of human formyl peptide receptor 1 (FPR1) [2, 3]. Two other relatively conserved low-affinity *f*MLF receptors, now termed as FPR2 and FPR3, were subsequently cloned (reviewed in [4]).

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FPR1 is a key regulator of the inflammatory environment. However, the expression of FPRs in various nonphagocytic cells suggests that these receptors also participate in functions other than innate immunity and may represent unique targets for therapeutic drug design [5–7]. Such drugs may have the potential to treat many inflammatory diseases, including rheumatoid arthritis, asthma, other auto-immune diseases, and stimulate wound healing [6, 8–10].

Since FPRs represent potentially important therapeutic targets, much attention has been focused over the last two decades on the identification of natural and synthetic compounds that interact with these receptors and/or interfere with FPR-dependent pathways. To date, several reviews summarizing the research efforts on FPR1 and FPR2 agonists, including natural ligands, have been published [4, 11–16]. However, less attention has focused on natural FPR1 antagonists.

Natural products traditionally have played an important role in drug discovery and were the basis of most early medicines [17]. The potential of natural products as sources for new drugs is still largely unexplored, and only a small fraction of the products present in existing plants, fungi, microorganisms, and animals have been investigated so far. Previously, we reported that many FPR1 antagonists contain OH groups, which can serve as H-bond donors and/or acceptors upon binding to the receptor, and that this feature is much more characteristic of FPR1 antagonists than agonists [16]. Because natural compounds in general incorporate more oxygen atoms than synthetic compounds and drugs [18], this feature makes them attractive for screening as FPR1 antagonists. Furthermore, natural products contain more fused rings, but fewer rotatable bonds than synthetic medicinal compounds [18], and this particular feature is also characteristic of FPR1 antagonists [16, 19].

FPR1 activation stimulates multiple signal transduction pathways responsible for various neutrophil functions, such as adhesion, chemotaxis, phagocytosis, exocytosis of secretory granules, and superoxide anion radical ($O_2^{-\cdot}$) production, which contribute to the physiological inflammatory response associated with bacterial infection and tissue damage (reviewed in [4, 20, 21]). The main molecular events and mediators involved in FPR1 activation are summarized in Figure 1. The FPR1 ligand *f*MLF is known to activate phospholipases C (PLC) and A_2 (PLA_2) and release of intracellular Ca^{2+} stores. The second messengers resulting from FPR1 activation regulate various intracellular kinases, including phosphatidylinositol-3-kinase (PI3K), protein kinase C (PKC), and mitogen-activated protein (MAP) kinases p38 and extracellular signal related kinase 1 and 2 (ERK 1/2). Activation of the FPR1 stimulates an additional protein kinase C-independent pathway through the Src-related tyrosine kinase, Lyn, in human neutrophils [22]. Likewise, downstream activation of Rho GTPases, particularly Rac1 and Rac2, plays a key role in neutrophil NADPH oxidase assembly, chemotaxis, and degranulation [23]. Activation of these signal-transduction pathways is known to be responsible for the various biochemical responses that contribute to physiological defense against pathogens and the inflammatory response to cellular damage. Although the intrinsic functional redundancy in the chemoattractant/chemokine system may make blocking a single receptor problematic as a therapeutic approach [24], various approaches to inhibit the FPR1 signaling have nevertheless been considered for therapeutic development. For example, natural compounds

have been evaluated for their ability to inhibit leukocyte chemotaxis, reactive oxygen species (ROS) production, and human neutrophil elastase (HNE) release by targeting *f*MLF-induced signaling cascades. Since excess NADPH-oxidase generated ROS, HNE, and other neutrophil-derived proteases can promote inflammation and numerous pathological conditions [25–27], this approach could have significant clinical benefit if effective and specific inhibitors were identified.

Of particular interest is the possibility that FPR1 antagonists that can transiently inhibit neutrophil responses to formylated peptides could be therapeutic agents in the treatment of inflammatory diseases. In efforts to discover new anti-inflammatory agents from natural sources, inhibitory activity of several hundred compounds derived from bacteria, higher plants, algae, and marine corals have been screened for their ability to inhibit *f*MLF-induced functional responses in neutrophils. These products include a wide range of compound classes from peptides to secondary metabolites, such as flavonoids, coumarins, quinones, naphtalenones, lignans, terpenoids (sesquiterpene lactones, diterpenes, triterpenes), steroids, alkaloids and other compounds. Some of these compounds blocked FPR1 directly, while others inhibited downstream *f*MLF-induced pathways.

In this review, we will summarize the outcome of previous screening efforts and reconsider these studies with a specific focus on FPR1 antagonists. For some of these previously reported natural compounds, we also conducted additional screening of analogs and similar compounds for their ability to antagonize FPR1 activation by *f*MLF in human neutrophils and FPR1-transfected cells. Because a high possibility exists that compounds capable of inhibiting *f*MLF signaling may target FPR1 as one of their molecular mechanisms of action, we also used pharmacophore modeling and molecular docking studies to predict how well various natural compounds fit the FPR1 antagonist pharmacophore and their potential for binding to FPR1.

2. Natural peptides and their derivatives as FPR1 antagonists

2.1. Cyclosporines and other cyclic peptides

The hydrophobic cyclic peptides, cyclosporine A (CsA) and H CsH), are among most potent and receptor-specific FPR1 antagonists described so far [28, 29]. Both cyclosporines were first isolated as undecapeptides from the fungus *Tolypocladium inflatum*. Although CsA and CsH dose-dependently displaced [^{3}H]-*f*MLF in FPR1-transfected rat basophilic leukemia (RBL) cells with IC_{50} values of 1.8 μM and 100 nM, respectively [30], they did not exhibit any obvious inhibitory effects on FPR2-mediated cellular functions [29, 30]. Loor *et al.* [31] conducted comprehensive structure–activity relationship (SAR) analysis of sixty naturally occurring or biosynthetically produced cyclosporine analogs for their ability to inhibit *f*MLF-induced responses in differentiated HL-60 cells. Some of these cyclosporines, including naturally occurring FR901459 and SDZ 214-103, inhibited *f*MLF-induced *N*-acetyl-β-D-glucosaminidase release with nanomolar IC_{50} values [31]. Cyclosporine FR901459 was nearly 20-fold more potent than CsA but 4-fold less potent than CsH for inhibiting the FPR1-dependent response [32]. Extensive chemical modification of the cyclosporine scaffold led to the discovery of various non-immunosuppressive cyclophilin

inhibitors for the treatment of hepatitis C infection and other diseases [33, 34]. However, the effects of these synthetic cyclosporine analogs on FPR functions have not been reported.

The mechanism responsible for the cyclosporines ability to act as FPR1 antagonists is still unclear, although it has been suggested that they may occupy an area of the receptor that covers or overlaps with the FPR1 ligand binding site or that they can produce an allosteric effect that causes reduced binding at this receptor [31]. It has also been suggested that cyclosporines may act as inverse agonists of FPR1 by recruiting different inactive FPR1 conformers [31, 35]. Zhou *et al.* [36] found that FPR1 amino acid 101 may be part of the binding site for CsA and CsH. Compared with the Val101 variant, variants of FPR1 with Leu101 displayed significantly improved receptor affinity, thereby enhancing cyclosporine antagonism of FPR1-mediated functions [36]. On the other hand, when we performed molecular docking of CsH into the structures of both Val101 and Leu101 variants, we found that the docking pose of CsH overlapped well with that of *f*MLF for both FPR1 variants. Closer inspection, however, showed that CsH binds the Val101 FPR1 variant in quite a different pose compared to the Leu101 variant and is located closer to channel **C** of the protein surface (Figure 2; orthosteric FPR1 binding site regions are designated as in our previous publication [37]). Although CsH docking with either the Leu101 or Val101 FPR1 variant formed H-bonds with Arg205 and Tyr257, CsH also formed an H-bond with Cys98 of the Val101 variant, which is located in the vicinity of channel **C**. Possibly, this H-bond constrains CsH in a relatively unfavorable position that reduces its antagonist activity. Note that our molecular modeling assumed that peptides with up to 11 amino acids could fit into the orthosteric binding site, which is consistent with the size of CsH. This assumption is in contrast to older studies suggesting that the FPR1 binding pocket could accommodate no more than six amino acids [38]. Clearly, further studies are necessary to explore this issue. Although CsH has a good docking pose inside the FPR1 ligand binding site, allosteric effects could also occur via cyclosporine insertion deep into the FPR1 transmembrane–extracellular domain interface.

In animal experiments, cyclosporine H blocked *f*MLF-induced analgesia [39] and attenuated the acute inflammatory response evoked by cigarette smoke [40]. However, *in vivo* studies of cyclosporines should be interpreted carefully because their main therapeutic effects appear to involve signaling pathways unrelated to FPR1 [36]. Indeed, CsA, a relatively large molecule, inhibited the T-cell receptor signal transduction pathway via the formation of a CsA-cyclophilin complex, which in turn bound to and inhibited the Ca^{2+}-calmodulin dependent phosphatase calcineurin [41]. CsA also inhibited formation and opening of the mitochondrial permeability transition pore [42]. Although CsH does not bind to immunophilin, this peptide is a potent inhibitor of Ca^{2+}-calmodulin-dependent phosphorylation of elongation factor 2 (EF-2) [43].

The urokinase receptor-derived peptide ^{88}Ser-Arg-Ser-Arg-Tyr^{92} was previously reported as a mixed FPR1/FPR2 agonist [44]. Recently it was discovered that cyclized SRSRY had an opposite effect on cell migration, as compared to its linear form, and inhibited *f*MLF-induced monocyte locomotion with an IC_{50} value of 10 pM [45]. The cyclic peptide inhibited binding of fluorescein isothiocyanate (FITC)-labeled *f*MLF to FPR1 transfected cells [45], suggesting it might also be a receptor antagonist.

Yang *et al.* [46] found that an extract of bioactive secondary metabolites from a marine *Bacillus* sp. inhibited binding of a labeled formyl peptide to FPR1 in human neutrophils and FPR1-transfected HEK293 cells. However, the authors did not isolate the active metabolite(s). It should be noted that a large proportion of the secondary metabolites produced by the *Bacillus* isolates are cyclic lipopeptides belonging to three families, including iturins, fengycins, and surfactins [47]. Thus, these pure compounds may also represent novel FPR1 antagonists and should be evaluated.

2.2. Natural non-cyclic peptide FPR1 antagonists and their synthetic analogs

Chemotaxis inhibitory protein of *Staphylococcus aureus* (CHIPS), a 121-residue protein (14.1 kDa) excreted by several strains of *S. aureus*, has been reported to be an FPR1 and complement component 5a receptor (C5aR) antagonist [48]. Radiolabeled CHIPS bound FPR1 with an apparent K_d ~ 35 nM, indicating that the protein binds directly to FPR1 and blocks this receptor for ligation of *N*-formylated peptides [48]. Thus, inhibiting *f*MLF-induced neutrophil chemotaxis may be a significant virulence factor for the invasion of *S. aureus*, and antibodies targeting CHIPS may be useful in the treatment of this infection [49]. Further studies on the region of CHIPS responsible for FPR1-blocking activity showed that peptide FTFEPF (the first 6 *N*-terminal amino acids) exhibited FPR1 antagonist activity, although the activity was 10,000-fold less than CHIPS itself [50]. Additionally, this peptide was unable to inhibit the activation of neutrophils by C5a [50].

N-formylated peptides (such as *f*MLF) are present in bacteria, which formylate their proteins during synthesis. Mitochondrial proteins also contain *N*-formyl modifications, and these formylated proteins can be released from damaged mitochondria during tissue injury, leading to phagocyte recruitment (reviewed in [4]). Substitution of the formyl group with *t*-Boc, *i*-Boc, or a carbobenzoxy group transforms *f*MLF to peptides with FPR1 antagonist properties [51], with the most potent being *t*-Boc-MLF (usually designated as Boc-1) (Table 1). Several less potent FPR1 antagonists have also been synthesized based on the *f*MLF sequence [52]. Another potent FPR1/FPR2 pan-antagonist is synthetic peptide Boc-2; however, this peptide and other synthetic FPR1 antagonists [53, 54] have no closely related natural product homologues. Because Boc-1 is one of most potent and specific FPR1 antagonists described so far [29], Yuan *et al.* [55] created comparison docking poses of this peptide and *f*MLF using an homology FPR1 model that was based on structure of the chemokine receptor CXCR4 [55]. They found that the Phe residue of *f*MLF was engaged in a stable H-bond network, formed by the side chains of Arg84, Lys85 and Asp284, while a water molecule mediated H-bonding between the *f*MLF carbonyl group and Asp284. Similarly, the Boc-1 also formed H-bonds directly with Arg84 and Lys85. However, in contrast to *f*MLF, an H-bond of Boc-1 with Asp-284 was not created by a water molecule. Instead, the NH group of the peptide bond in the Phe residue of Boc-1 directly formed an H-bond with Asp284. This finding suggests an important role of the water molecule in discrimination between FPR1 agonists and antagonists.

Spinorphin (LVVYPWT), an endogenous anti-nociceptive non-cyclic peptide, was reported to be an FPR1 antagonist [56, 57]. However, this peptide also had direct FPR1 agonist activity [56], which may lead to desensensitization of this receptor rather than antagonism.

Saropeptate (aurantiamide acetate), a trypanocidal dipeptide composed of *N*-benzoylphenylalanine and phenylalaninol acetate (Table 1) isolated from various plants and fungi (e.g., [58, 59]), inhibited $O_2^{-\cdot}$ production by *f*MLF-stimulated neutrophils [60]. Twenty-four new dipeptide analogs of saropeptate were synthesized and evaluated for effects on ROS production and HNE release by *f*MLF-stimulated human neutrophils. Among them, seven *N*-(fluorenyl-9-methoxycarbonyl) (Fmoc)-dipeptides showed potent inhibitory effects [61]. Further SAR analysis based on synthesis of a series of Trp-containing dipeptides led to the discovery of several potent dipeptides that inhibited ROS production and HNE release by *f*MLF-stimulated neutrophils ~10–20-fold better than by WKYMVm-stimulated neutrophils, with the most potent being dipeptides **3** (Table 1) and **24a** [62]. Although the authors concluded these dipeptides were FPR1 antagonists, this conclusion requires further support by additional experiments, as direct activation and FPR1 binding were not assessed for these analogs. Indeed, the structures of these dipeptides resemble previously described Trp- and Phe-based derivatives that are potent FPR1/FPR2 agonists [63].

2.3. Pepducins

Mutagenesis studies [64] demonstrated that the third intracellular loop (i3) of GPCRs mediates a large part of the coupling between receptor and G protein, and synthesized lipidated peptides based on a GPCR i3 sequence are called “pepducins” to reflect their properties as GPCR-specific regulators of signal transduction [65]. The lipid moiety facilitates translocation across the plasma membrane, where pepducins can then allosterically modulate signaling of their cognate receptor. Many pepducins and related lipopeptides that can specifically modulate the activity of diverse GPCR, including FPR have been identified (for review [66]). However, pepducins are not always specific for their designated target receptor. For example, pepducin $F1Pal_{16}$, which contains a peptide sequence identical to the FPR1 i3, was found to inhibit FPR2-dependent functional responses, but had no effect on FPR1 signaling [67]. Another pepducin, $F2Pal_{16}$, containing 16 amino acids present in the FPR2 i3 and conjugated with palmitic acid, actually had agonist effects in human monocytes and neutrophils via FPR2 [68, 69]. Thus, to date there are no pepducins that able to specifically target FPR1.

3. Small-molecule non-peptide FPR1 antagonists and their synthetic derivatives

3.1. Flavonoids, isoflavones, and their synthetic derivatives

Flavonoids are an important class of synthetic and natural compounds that exhibit a broad range of biological activities [70]. Their closely related derivatives are isoflavones. Many flavonoids and isoflavones have inhibitory effects on *f*MLF-induced neutrophil responses. However, ROS scavenging activity of flavonoids and isoflavones may also contribute to the observed effects [71, 72], especially in tests of *f*MLF-induced ROS production (see Figure 1). For example, luteolin and apigenin apparently inhibited $O_2^{-\cdot}$ production in *f*MLF-stimulated neutrophils [73–75], but these flavonoids also inhibited neutrophil $O_2^{-\cdot}$ generation induced by phorbol-12-myristate-13-acetate (PMA) and tyrosyl phosphorylation

of proteins in *f*MLF-treated human neutrophils [76, 77], suggesting ROS scavenging activity rather than FPR1 antagonism.

Several synthetic isoflavone derivatives have been identified as small-molecule competitive FPR1 antagonists [19, 78, 79]. Recently, we evaluated 100 related 4*H*-chromen-4-ones, including synthetic and naturally occurring isoflavones, for their ability to antagonize FPR1-dependent signaling in neutrophils and FPR1-transfected HL-60 and RBL cells and identified novel and potent FPR1-specific antagonists [79]. Chemicals structures of several selected synthetic isoflavones (isoflavones **36**, **68**, and **73**) are shown in Table 1. However, none of the naturally occurring isoflavones that we tested, including genistein, daidzein, afrormosin, formononetin, and 7-hydroxyisoflavone inhibited *f*MLF-induced Ca^{2+} mobilization in human neutrophils. Although biochanin A (Table 1) inhibited Ca^{2+} flux in neutrophils and transfected FPR1-HL60 cells, this isoflavone did not inhibit binding of labeled ligand (WKYMVm-FITC) to FPR1-HL60 cells [79]. In addition, we found that biochanin A and its synthetic 2-trifluoromethyl derivative had direct agonist activity in FPR1-HL60 cells and primary neutrophils. Thus, biochanin A and its synthetic derivative may activate Ca^{2+} flux via pathways that lead to cross-desensensitization of FPR1 and/or cross-talk with the receptor. Indeed, several other synthetic and natural flavonoids, including apigenin, wogonin, genistein, kazinol B, and ugonin U, have been reported to stimulate a Ca^{2+} flux in various cell lines [80, 81] and neutrophils [82, 83].

We found that the isoflavone genistein did not antagonize *f*MLF-induced Ca^{2+} mobilization in FPR1-HL60 cells and human neutrophils [79]. Thus, the previously reported inhibitory effect of genistein on *f*MLF-stimulated degranulation, aggregation, and ROS production in human neutrophils [84–86] may be related to mechanisms downstream of Ca^{2+}-mobilization, such as ROS scavenger activity [87] and/or modulation of tyrosine kinase-dependent signaling events [84, 88]. Indeed, the broad modulatory activity of genistein on neutrophil activity suggests a non-receptor mode of action. For example, genistein inhibited neutrophil adhesion/adherence activated by tumor necrosis factor (TNF) [89] or agonists of protease-activated receptor 2 (PAR2) [90]. Moreover, pretreatment of neutrophils with genistein potentiated *f*MLF-induced cell transmigration [91]. Another isoflavone, afrormosin, inhibited *f*MLF-stimulated neutrophil degranulation at very high concentrations (IC_{50} ~67 μM) [92]. Similarly, although isopedicin, a flavanone derived from *Fissistigma oldhamii*, inhibited *f*MLF-induced neutrophil $O_2^{-\cdot}$ production and HNE release, this compound did not inhibit *f*MLF-induced Ca^{2+} flux in these cells [93].

Recently, we reported that FPR1 antagonists with a 4*H*-chromen-4-one scaffold were specific for FPR1 and did not inhibit FPR2, FPR3, or CXCR1-dependent responses. This specificity was supported by competition binding at FPR1 with the most potent compounds, including isoflavones **36**, **68**, and **73** (Table 1). SAR analysis of these compounds revealed the importance of a small hydrophobic group at position *2* and the nature of the substituent at position *7* of the 4*H*-chromen-4-one scaffold. These synthetic isoflavone derivatives were inactive as direct agonists of FPR1/FPR2 and mouse Fpr1 and did not activate Ca^{2+} flux in murine or human neutrophils. Molecular modeling showed a higher degree of similarity of the active isoflavones to the pharmacophore model of FPR1 antagonists, which was developed based on the structure of previously reported potent FPR1 antagonists with

diverse chemical scaffolds [79]. Thus, we concluded that 4*H*-chromen-4-one may represent a unique natural product-inspired chemical scaffold for the development of specific FPR1 antagonists that do not have undesirable off-target effects.

A natural chromone, cnidimol A (Table 1), which was isolated from *Cnidium monnieri* was found to inhibit *f*MLF-induced $O_2^{-\cdot}$ production and HNE release [94]. Likewise, the isoprenylated flavonoid ugonin **3** (Table 1) isolated from *Helminthostachys zeylanica* and several related ugonins potently inhibited similar *f*MLF-induced functional responses [95, 96]. However, a related ugonin (ugonin U) was found to directly activate PLC and Ca^{2+} flux in human neutrophils [97], suggesting possible receptor desensitization rather than antagonism. Binding of these compounds to FPR1 and effects on Ca^{2+} mobilization were not reported. It should be noted that some flavonoids and isoflavones can also interact with other cellular receptors and enzymes that cross-talk with FPR1 signaling (reviewed in [98–100]).

3.2. Coumarins

Coumarins represent an important type of naturally occurring and synthetic oxygen-containing heterocycle with a benzopyrone scaffold and a wide variety of biological activities. Some coumarins have been used in the clinic because of their anticoagulant and antineurodegenerative properties (reviewed in [101, 102]). Several coumarins, including isoheraclenin, imperatorin, osthenol, bergapten, xanthyletin, alloxanthoxyletin, and demethylauraptenol were found to be inhibitors of $O_2^{-\cdot}$ production and HNE release by *f*MLF-stimulated human neutrophils [94, 103–106]. Chemical structures of selected coumarins are shown in Table 1. Shen *et al.* [103] found that among 20 tested compounds, coumarins imperatorin, isoheraclenin, and osthol were the most potent inhibitors of *f*MLF-induced $O_2^{-\cdot}$ generation [103]. However, it was recently reported that imperatorin has complex effects on ion channels, including an inhibitory effect on voltage-gated K^+ channels and ATP-sensitive K^+ channels [107] and an agonist effect on transient receptor potential vanilloid 1 (TRPV1) ion channel [108]. Furthermore, we found that imperatorin directly activated Ca^{2+} flux in human neutrophils and transfected FPR1 HL-60 cells and that bergapten did not inhibit Ca^{2+} mobilization in *f*MLF-stimulated human neutrophils and FPR1 HL-60 cells (Table 2), thus excluding these compounds as possible FPR1 antagonists. It should be noted that osthol is a known plant-derived compound with anti-inflammatory activity and a broad spectrum of molecular targets [109, 110]. Osthol had a weak inhibitory effect on *f*MLF-induced HNE release [94]. Although a related coumarin, osthenol, had potent inhibitory activity on *f*MLF-induced $O_2^{-\cdot}$ production and HNE release [94], this compound was previously found to inhibit 5α-reductase type I, 5-lipoxygenase, and cyclooxygenase [111, 112], suggesting FPR-independent effects on cellular responses. Indeed, other cyclooxygenase inhibitors, such as celecoxib and indomethacin, have been reported to interfere with *f*MLF-induced functional responses, such as chemotaxis [113]. Similar to flavonoids, coumarins can also act as ROS scavengers [114] (see Figure 1), so additional studies are necessary to verify their possible direct effects on FPR1.

3.3. Quinones and naphthalenones

A large number of natural products contain quinone/naphthoquinone chromophores. These compounds have diverse biological properties and are able to modulate cellular signaling processes mainly via regulation of gene expression and receptor tyrosine kinases (reviewed in [115]). There are several publications on their effects in *f*MLF-treated neutrophils. For example, berryammone A and naphthalenone derivatives, berryammone B (Table 1) and 4-*O*-methylberryammone C, isolated from the stem of *Berrya ammonilla* exhibited a relatively potent inhibition of *f*MLF-induced $O_2^{-\cdot}$ generation and HNE release by neutrophils [116]. Likewise, a related methyl naphthalene carboxylate, lawsonaphthoate A (Table 1), and had potent inhibitory activity [73]. Although embelin, abruquinone A, and acetylshikonin inhibited *f*MLF-induced functional responses in neutrophils, these natural quinones also suppressed ionophore A23187-induced biosynthesis of eicosanoids in neutrophils [117–119], and embelin directly inhibited enzymatic activity of purified 5-lipoxygenase [119]. Finally, ehretiquinone isolated from the root of *Ehretia longiflora* potently inhibited *f*MLF-induced $O_2^{-\cdot}$ production, although effects on *f*MLF-induced HNE release and other functional activities were not evaluated [120]. Whether these compounds actually antagonize FPR1 is still unknown.

3.4. Lignans

Lignans are naturally occurring plant phenols that are derived biosynthetically from phenylpropanoids. Lignans are of clinical interest because of their numerous pharmacological properties, such as the antitumor, hepatoprotective, estrogenic, antihypertensive, antifungal, sedative, and antioxidant activities, as well as their ability to inhibit platelet activating factor (PAF) (reviewed in [121]). Several plant-derived lignans have been evaluated for their ability to inhibit *f*MLF-induced functional responses in neutrophils. For example, (7*S**,8*R**,7′*R**,8′*S**)-icariol A_2-9-*O*-β-xylopyranoside and (+)-9′-*O*-(*Z*)-feruloyl-5,5′-dimethoxylariciresinol isolated from *Hygroryza aristata* and *Zanthoxylum avicennae*, respectively, inhibited both $O_2^{-\cdot}$ production and HNE release by *f*MLF-stimulated neutrophils [122, 123]. Among the five lignans isolated from *Zanthoxylum ailanthoides* [(−)-syringaresinol, 5′,5″-didemethoxypinoresinol, (+)-episesamin, glaberide I, and (−)-dihydrocubebin], only (+)-episesamin (Table 1) inhibited both $O_2^{-\cdot}$ production and HNE release by *f*MLF-stimulated neutrophils [104]. However, we found that in addition to inhibiting *f*MLF-induced Ca^{2+} flux, (+)-episesamin also directly activated Ca^{2+} flux in human neutrophils and FPR1 HL-60 cells (Table 2), suggesting the inhibitory effects may be due to FPR1 desensitization. Several biphenyl-type neolignans isolated from *Magnolia officinalis*, including 5-allyl-5′-(1″-hydroxyallyloxy)biphenyl-2,2′-diol, 4-allyl-2-(2′-methylbenzofuran-5′-yl)phenol, magnolol, magnaldehyde D, randaiol (Table 1), and randainal, inhibited $O_2^{-\cdot}$ production and HNE release by *f*MLF-induced human neutrophils with varying levels of potency [124]. Magnolol also inhibited *f*MLF-induced human neutrophil chemotaxis [125] and was a weak inhibitor of *f*MLF-stimulated $O_2^{-\cdot}$ generation in rat neutrophils [126]. However, magnolol and related lignans also directly activated Ca^{2+} flux, $O_2^{-\cdot}$ production, and HNE release by neutrophils [124, 127]. Thus, the inhibitory effects of magnolol on *f*MLF-induced responses are also likely related to FPR1 desensitization or possible cross-desensitization, as magnolol can also bind to the $GABA_A$ receptor [128]. Although honokiol inhibited *f*MLF-induced $O_2^{-\cdot}$ production, cathepsin G

release, Ca^{2+} flux, and neutrophil migration [129–131], further analysis suggested that honokiol is not a competitive/allosteric FPR1 antagonist. Rather, it can specifically modulate *f*MLF-mediated neutrophil activation by inhibiting Lyn activation, which subsequently interferes with the activation of PLCγ2, AKT, p38, protein kinase C, and membrane localization of $p47^{phox}$ [129].

Nordihydroguaiaretic acid (NDGA) is a lignan from the creosote bush (*Larrea tridentata*) [132]. Although NDGA can effectively inhibit *f*MLF-induced ROS production and degranulation in human neutrophils [133–135], this lignan is a potent inhibitor of 5-, 12-, and 15-lipoxygenases [136]. Another lignan, di-*O*-methyltetrahydrofuroguaiacin B, had a bi-phasic effect on *f*MLF-induced ROS production in human neutrophils [137]. Lignan MSF-2 (5-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-3,7-dimethoxy-4*H*-chromen-4-one) purified from the fruit of *Melicope semecarpifolia* inhibited *f*MLF-induced $O_2^{-\cdot}$ production, cathepsin G release, and cell chemotaxis, while the intracellular Ca^{2+} release was only partially reduced in human neutrophils [138]. Because MSF-2 did not interfere with *f*MLF binding, the authors suggested that this lignan has targets downstream of FPR1, possibly PI3K.

Among the lignans reported to inhibit *f*MLF-induced responses in neutrophils, only PP-6 [(2*R*, 3*R*)-2-(3′,4′-dihydroxybenzyl)-3-(3″,4″-dimethoxybenzyl)butyrolactone] (Table 1), which was isolated from *Piper philippinum*, blocked *f*MLF binding to FPR1 (IC_{50} ~ 1.5 μM) [139]. It also inhibited *f*MLF-induced intracellular Ca^{2+} mobilization, $O_2^{-\cdot}$ production, and phosphorylation of ERK 1/2, Akt and p38 [139], suggesting direct antagonist activity.

Furfuran type lignans, such as matairesinol, lariciresinol and pinoresinol, are widely distributed in edible plants, and most of these dietary lignans are converted by intestinal microbiota to the lignans enterolactone (Table 1) and enterodiol. These mammalian lignans have been suggested to have anti-carcinogenic activity [140]. Enterolactone is an isomer of PP-6 and can also inhibit ROS production by *f*MLF-stimulated human neutrophils [133]. We evaluated enterolactone and found that it did not inhibit Ca^{2+} flux in *f*MLF-stimulated human neutrophils and FPR1 HL-60 cells and also did not directly activate these cells (Table 2), indicating its target is likely not FPR1.

3.5. Triterpenes, steroids, and saponins

The triterpenes are one of the most numerous and diverse groups of natural plant products. These compounds have 4- or 5-ring skeletons and hydroxyl, carboxyl, or oxo groups and are biosynthesized in plants via squalene cyclization. Several triterpenoids isolated from the stems of *Microtropis fokienensis* inhibited *f*MLF-induced $O_2^{-\cdot}$ generation and HNE release by neutrophils, with the most potent being 30-hydroxy-2,3-*seco*-lup-20(29)-ene-2,3-dioic acid (triterpene **17**; Table 1), bearing two carboxylic groups [141]. Oleanolic (Table 1), ursolic, and betulinic acids are known triterpenes with anti-inflammatory and immunomodulatory properties [142]. Oleanolic acid has been reported to inhibit *f*MLF-induced responses, including $O_2^{-\cdot}$ and HNE production by neutrophils [143–146]. The activity of this triterpene on $O_2^{-\cdot}$ production was ~8-fold higher in neutrophils stimulated with *f*MLF compared to those stimulated with PMA [145]. We found that oleanolic acid has a relatively high inhibitory activity on Ca^{2+} flux in *f*MLF-stimulated human neutrophils and

FPR1 HL-60 cells without a direct agonist effect in these cells (Table 2), suggesting that this compound could be a true FPR1 antagonist. Epibetulinic acid (Table 1) isolated from *Pachycentria formosana* demonstrated similar inhibitory effects as oleanolic acid [143], and its derivative, betulinic acid, also inhibited *f*MLF-induced $O_2^{-\cdot}$ production by neutrophils. However, this triterpene did not affect *f*MLF-induced release of HNE [116], suggesting that it has an FPR1-independent mechanism of action. Although α-amyrin, uvaol, ursolic acid, 19α-hydroxy ursolic acid, and 19α, 24-dihydroxy ursolic acid inhibited *f*MLF-induced $O_2^{-\cdot}$ production, all of these triterpenes also inhibited $O_2^{-\cdot}$ generation stimulated by arachidonic acid [147], suggesting nonspecific effects of these compounds and/or interaction with post-FPR1 pathways.

Steroids are derivatives of triterpene in both plants and animals. Bile acids are steroid acids found predominantly in the bile of mammals and other vertebrates. These endogenous anionic detergents are produced by the liver and contain a rigid steroidal hydrophobic scaffold. Although bile acids were considered previously as catabolic products of cholesterol involved in the solubilization of various lipids and lipid-soluble compounds in the gut, they have recently been described as signaling molecules involved in many physiological functions. At least two these acids, deoxycholic acid (DCA) and chenodeoxycholic acid (CDCA) have been reported to antagonize FPR1 and inhibit ^{3}H-*f*MLF binding at high concentrations (≥ 100 μM) [148, 149]. Chen *et al.* [148] reported that CDCA inhibited *f*MLF-induced Ca^{2+} flux in human monocytes with an IC_{50} of 40 μM [148]. We found that DCA and CDCA were both weak inhibitors of Ca^{2+} mobilization in *f*MLF-stimulated human neutrophils and FPR1 HL-60 cells but that they also directly activated Ca^{2+} flux in these cells (Table 2). Thus, DCA and CDCA may activate Ca^{2+} flux via FPR1-dependent and/or independent pathways, leading to FPR1 desensensitization and/or cross-talk with the receptor.

Another bile acid, tauroursodeoxycholic acid also inhibited *f*MLF-induced Ca^{2+} mobilization at relatively high concentrations (IC_{50} ~ 0.5 mM) [150]. Note, however, that the synthetic anionic detergent sodium dodecyl sulfate has also been reported to antagonize FPR1 and reduce binding of labeled peptide to this receptor [151]. Thus, allosteric effects could also occur via interaction of the bile acids/detergents with FPR1 at the transmembrane-extracellular interface (see Figure 1), similar to the proposed mechanism for cyclosporines.

A pharmacophore model for bile acid recognition by FPR1 and docking poses of DCA and CDCA into the rhodopsin homology-based FPR1 structure were reported by Ferrari *et al.* [152]. This model is based on four anchor points, including two hydrophobic centers, one H-bonding donor site, and one H-bonding acceptor/negative site. In the docked pose, three stabilizing H-bond interactions are observed between DCA and Thr177, Tyr257, and Thr265. While the interaction with Tyr257 is crucial for anchoring of the DCA and CDCA carboxylic group to FPR1, H-bonding with Tyr177 and Thr265 determines the potency of the bile acids [152].

Bile acids have multiple physiological functions, including activation of the farnesoid X receptor (FXR), Takeda G-protein-coupled receptor (TGR5), and G protein-coupled bile

acid receptor 1 (GPBAR1) [153, 154]. Both FXR and TGR5 demonstrate pleiotropic functions, including immune modulation. Recently it was found that bile acids can also activate ryanodine receptors [155], which are the major cellular mediator of calcium-induced calcium release. Although current studies shown a role of FPR in liver injury (e.g., [156]), involvement of bile acids as FPR1 ligands in liver pathology is still unknown. Indeed, endogenous bile acids can reduce liver neutrophil infiltration and prevent hepatotoxicity, which is similar to effects of the FPR1 antagonist Boc-1 [157].

Plant steroids have not been well studied for their effects on *f*MLF-stimulated functional responses in leukocytes. High concentrations (30–100 μM) of stigmast-4-ene-3,6-dione and hecogenin isolated from *Polygonum chinensis* inhibited $O_2^{-\cdot}$ production by *f*MLF-stimulated rat neutrophils, whereas stigmastane-3,6-dione was non-active [60]. Further work is necessary to assess the specificity and receptor targets of these plant steroids.

Triterpenoids and steroids containing one or more sugar moieties attached to the triterpenoid/steroid aglycones are called saponins. Several saponins have been evaluated for their ability to inhibit *f*MLF-induced functional responses by neutrophils. For example, among the five saponins of oleanolic acid isolated from the rhizome of *Anemone raddeana*, OTS-3 inhibited *f*MLF-induced $O_2^{-\cdot}$ production and did not suppress this response stimulated by PMA or arachidonic acid [158]. Five saponins, including taibaienoside I, chikusetsusaponin-Ib, and chikusetsusaponin-Iva, isolated from the roots of *Panax japonicus* var. *major* inhibited *f*MLF-stimulated neutrophils [146]. Likewise, 3-*O*-[β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl]-hederagenin from the buds of *Aralia elata* suppressed $O_2^{-\cdot}$ generation induced by *f*MLF in a concentration-dependent manner [159], and both drangustoside A and B from *Dracaena angustifolia* Roxb inhibited HNE release by *f*MLF-stimulated neutrophils [160].

Although none of these triterpenes and their saponins have been evaluated for direct FPR1 binding activity, SMG-1 (hederagenin 3-*O*-(3,4-*O*-di-acetyl-α-L-arabinopyranoside)-(1→3)-α-l-rhamnopyranosyl-(1→2)-α-l-arabinopyranoside) blocked binding of fluorescent FPR1 ligand in human neutrophils and inhibited *f*MLF-induced $O_2^{-\cdot}$ production and HNE release by these cells [161]. Because the saponinogen of SMG-1 was not studied, we evaluated the effect of hederagenin and found that this compound inhibited *f*MLF-induced Ca^{2+} flux in human neutrophils and FPR1 HL-60 cells. Importantly, hederagenin did not have direct agonist effects (Table 2), suggesting that it could potentially be an FPR1 antagonist. However, further studies are necessary to address this issue.

3.6. Diterpenes and sesquiterpene lactones

Sesquiterpene lactones and diterpenes are primary natural secondary metabolites. The trinorditerpene flueggrene A, which is isolated from the roots of *Flueggea virosa*, and the clerodane diterpenoid PL3S (Table 1) from *Polyalthia longifolia* var. *pendula*were were both found to be potent inhibitors of $O_2^{-\cdot}$ production and HNE release by *f*MLF-stimulated neutrophils [162, 163]. Moreover, PL3S also inhibited *f*MLF-induced Ca^{2+} mobilization in neutrophils [163]. The tetracyclic diterpenoid *ent*-kaurane **1** (16β,17-dihydroxy-*ent*-kauran-19-oic acid; Table 1) isolated from *Annona squamosa* inhibited *f*MLF-induced $O_2^{-\cdot}$ production, HNE release, and Ca^{2+} mobilization in human neutrophils without ROS

scavenging or direct inhibition of HNE activity [164, 165]. Among several clerodane diterpenoids isolated from *Polyalthia longifolia* var. *pendula*, 16-oxocleroda-3,13(14)*E*-dien-15-oic acid methyl ester was found to be a potent inhibitor of *f*MLF-induced $O_2^{-\cdot}$ production by neutrophils; however, the effects of this diterpene on other *f*MLF-stimulated functional responses were not studied [166].

Various diterpenoids isolated from the soft corals *Sarcophyton tortuosum*, *Cladiella krempfi*, *Cladiella krempfi, Cespitularia* taeniata, and *Junceella fragilis* have also been evaluated for their ability to inhibit *f*MLF-induced $O_2^{-\cdot}$ production and HNE release. Most of the coral-derived diterpenoids had low activity or were inactive [167–176]; however, the eunicellin-based diterpenoid klymollin M (Table 1) isolated from the soft coral *Klyxum molle* was a potent inhibitor of *f*MLF-induced responses [177]. Again, the mechanisms involved have not been defined.

The effects of sesquiterpene lactones on FPR1-mediated functional responses have been less studied, and only few reports have been published. For example, Arakawa *et al.* [178] found that the furanoheliangolide budlein A isolated from *Viguiera robusta* inhibited release of HNE by *f*MLF-stimulated neutrophils (IC_{50} = 16.8 μM), but the effect was not specific for FPR1, as this compound also inhibited degranulation of PAF-stimulated neutrophils. Six furanogermacrane sesquiterpenes isolated from *Neolitsea parvigemma* and the sesquiterpenoids hiiranlactone B and hiiranlactone D isolated from the leaves of *Neolitsea hiiranensis* exhibited mild/weak inhibitory activity against *f*MLF-induced $O_2^{-\cdot}$ production, although effects on other *f*MLF-induced neutrophil responses were not studied [179, 180]. Germacranolide isolated from *Dimerostemma brasilianum* had a similar profile of biological activity and suppressed HNE release by *f*MLF- and PAF-stimulated neutrophils with IC_{50} values of ~ 27.0 and 23.0 μM, respectively [181].

A well-known sesquiterpene lactone studied in clinical trials, artemisinin (Table 1) [182], was previously not tested for its effects on FPR1-mediated responses. Thus, we evaluated its effects on Ca^{2+} flux in human neutrophils and FPR1 HL-60 cells and found that artemisinin was inactive in these assays (Table 2), suggesting its target is likely not FPR1.

3.7. Miscellaneous compounds

Natural plant-derived compounds with different chemical scaffolds than those reviewed above have also been reported to inhibit *f*MLF-induced functional activities in human neutrophils or other FPR1 expressing cells. For example, benzophenone and phloroglucinol derivatives isolated from the fruits of *Garcinia multiflora* [183–185] and seeds of *Garcinia subelliptica* [186, 187] inhibited *f*MLF-induced responses, with the most potent being garcimultiflorone B (Table 1). Several phthalides isolated from *Pittosporum illicioides* had weak inhibitory activity on *f*MLF-induced $O_2^{-\cdot}$ production and HNE release by neutrophils [188]. Alkaloids aristolatams IIIa (Table 1) from *Aristolochia manshuriensis* and evofolin B, decarine, and ailanthamide from *Zanthoxylum ailanthoides* were also potent inhibitors of neutrophil $O_2^{-\cdot}$ production and HNE release [104, 106, 162, 189]. Similarly, phenanthrenedione pterolinus K (Table 1) isolated from *Pterocarpus santalinus* was a potent inhibitor of *f*MLF-induced $O_2^{-\cdot}$ production and HNE release [190]. Although rutaecarpine, an alkaloid isolated from *Evodia rutaecarpa*, was a mild inhibitor of *f*MLF-induced HNE

release and ROS production [191], this alkaloid has a broad spectrum of biological activity, including inhibition of cyclooxygenase-2 [192] and activation of the TRPV1 ion channel [193, 194], suggesting that this molecule could interact with downstream processes in the FPR1 pathway.

Several compounds with mild inhibitory effects on *f*MLF-induced responses in neutrophils, including 9-acetoxy-8,10-epoxythymol-3-*O*-tiglate, 10-acetoxy-8-hydroxy-9-*O*-angeloylthymol, and 1-[2-hydroxy-4-(hydroxymethyl)phenyl]ethan-1-one, have been isolated from *Eupatorium cannabinum* [195]. Lawsochylin A (Table 1) and (4*S*)-4-hydroxy-α-tetralone isolated from *Lawsonia inermis* also inhibited *f*MLF-induced responses [73]. Recently, we found that 6-methyl-3,5-heptadien-2-one, which is a minor constituent in essential oils from *Artemisia kotuchovii* [196] and oleoresins from paprika and tomato [197], could non-specifically inhibit all functional responses that we tested in human neutrophils and FPR1-transfected HL-60 cells, including *f*MLF-stimulated Ca^{2+} flux and chemotaxis, PMA-induced ROS production, and interleukin 8-induced Ca^{2+} flux. We concluded that this compound may interfere with intracellular signaling pathways common to both FPR1 and CXCR1/2 [196]. Viscolin and bractelactone, which are chalcones from *Viscum coloratum* and *Fissistigma bracteolatum*, respectively, also inhibited *f*MLF-induced $O_2^{-\cdot}$ production and HNE release by neutrophils; however, these compounds did not inhibit *f*MLF-induced Ca^{2+} flux [198, 199], suggesting targets other than FPR1. Likewise, inhibition of *f*MLF-stimulated rat neutrophil respiratory burst activity by broussochalcone A, a prenylated chalcone isolated from *Broussonetia papyrifera*, was found to be due to the suppression of PKC [200]. The oligomeric procyanidin F2 isolated from grape seeds inhibited *f*MLF-induced Ca^{2+} flux, chemotaxis, and ERK phosphorylation in human glioblastoma U-87 cells [201]. However, further studies showed that this procyanidin could directly induce Ca^{2+} flux and chemotaxis in U-87 cells [202], suggesting this compound is not an FPR1 antagonist.

Compounds of various chemical classes were also isolated from marine sources and evaluated for their ability to inhibit *f*MLF-induced functional responses in human neutrophils. Among of them, the tocopherol-derived metabolite hirsutocospiro A (Table 1) and methylfarnesylquinone isolated from the soft coral *Cladiella hirsute* and the brown alga *Homoeostrichus formosana*, respectively, inhibited $O_2^{-\cdot}$ production and HNE release [203, 204]. However, the specificity of these effects remains to be determined.

4. Prediction of FPR1 antagonists among natural products using molecular modeling

Natural-product-based drug discovery can be enhanced with computational methods [205]. Because most of the reported small-molecule natural inhibitors of *f*MLF-induced functional responses were not evaluated in competition binding assays, we used molecular modeling to predict how well these compounds fit the FPR1 antagonist pharmacophore and their potential for binding to FPR1. The FPR1 antagonists described so far represent a wide range of chemotypes, and it is consequently difficult to derive meaningful SAR on structural grounds [206]. Recently, we built a pharmacophore model of FPR1 antagonists [79] based on field point methodology developed by Cheeseright *et al.* [207, 208]. This approach allowed us to compare diverse molecules in terms of their electrostatic field similarity and

create an alignment of their bioactive conformations [206]. We found that three potent small-molecule synthetic non-peptide FPR1 antagonists that were identified previously using high-throughput screening and/or lead optimization [6-ethyl-2-methyl-3-(1-methyl-1*H*-benzimidazol-2-yl)-4-oxo-4*H*-chromen-7-yl acetate, *N*-(1*S*)(1-(benzoimidazol-2-yl)-3-(methylthio)propyl)-5-ethoxy-3-methylbenzofuran-2-carboxamide, and 6-benzyl-3-(2-chlorophenyl)-5-methyl-2-(trifluoromethyl)pyrazolo[1,5-a]pyrimidin-7(1*H*)-one (designated previously by us as compounds **1**, **5**, and **7**)] [78, 209] could be overlaid simultaneously with good correspondence of their molecular fields to form an FPR1 antagonist pharmacophore template [79]. Thus, we evaluated the ability of a given molecule to fit the FPR1 antagonist pharmacophore by construction of an extended 4-molecule template containing the molecule under investigation along with compounds **1**, **5**, and **7**. The possibility of forming an extended template can be regulated by the magnitude of μ (maximum score variation between molecule pairs), which is an inner parameter of the FieldTemplater program, with lower values of μ indicating templates of higher similarity. We found that the previously reported FPR1 antagonists (isoflavones **36**, **68**, **73**, and lignan PP-6) (see Table 1) gave extended templates with relatively low μ values (0.03 – 0.04), indicating high similarity with the FPR1 antagonist pharmacophore, while inactive isoflavones (compounds **54**, **58**, and **70**; compound numbers as designated previously [79]) generally had μ values >0.04 for obtaining an extended template. Based on the outcome of this small training set of 7 molecules, we utilized a simple rule of μ≤0.04 for assessing potential as an FPR1 antagonist based on similarity with the antagonist pharmacophore and used this rule to evaluate a set of 24 naturally occurring compounds that were initially selected from our review of the literature and additional screening because they inhibited of all *f*MLF-induced functional responses tested but did not exhibit any direct agonist activity or other undesirable effects (Tables 2 and 3).

As result of this modeling study, most natural compounds under investigation did not fit into a satisfactory common 4-molecule FPR1 antagonist template (i.e., μ>0.04). Although oleanolic acid and hederagenin showed promising results in our additional testing in human neutrophils and FPR1 HL-60 cells, these compounds were rejected as FPR1 antagonists by the modeling assessment of their ability to fit with the FPR1 antagonist pharmacophore. However, three compounds, in addition to PP-6 (i.e., garcimultiflorone B, cnidimol A, and PL3S), also had μ values ≤0.04 and can be regarded as potential FPR1 antagonists based on all of the available biochemical and molecular modeling criteria. It should be noted that this modeling study is focused on orthosteric interaction of a ligand with FPR1, so allosteric antagonism cannot be excluded with some of the other compounds in the set.

To further investigate possible ligand binding, the natural compounds with ability to form a high-quality common 4-molecular template of FPR1 antagonists were subjected to molecular docking into the FPR1 ligand binding site. The best docking poses obtained for garcimultiflorone B, cnidimol A, PL3S, and PP-6 showed that these molecules were oriented similarly to *f*MLF within the orthosteric FPR1 binding site of the Leu101 FPR1 variant (Figure 3A), again supporting their potential as FPR1 antagonists. All four natural compounds in their best docking poses were located in area **D** and cavity **B**, while garcimultiflorone B and *f*MLF also occupied the entrance of channel **A** (notation of the

FPR1 binging site regions according to [210]). Analysis of H-bond energies formed by the docked molecules (Table 4) indicated that PP-6 and cnidimol A formed much stronger H-bonds with FPR1 than PL3S and garcimultiflorone B. We found that H-bonds with Thr265 are formed for three of the four natural antagonists investigated by our docking methodology (Table 4). In addition, strong H-bonding interactions of cnidimol A and PP-6 were found with Asp106 and Arg205, respectively. As an example, all three H-bonds for PP-6 in the FPR1 ligand binding site are shown in Figure 3B.

5. Discussion and concluding remarks

FPR1 is considered to be a promising drug target for treating inflammatory and immunological diseases. Thus, natural compounds that could block and/or regulate FPR1 activity may be an important source of novel therapeutics for modulating inflammatory processes. Here, we reviewed the literature and conclude that only a few natural products and their synthetic derivatives are characterized well enough to be defined as FPR1 antagonists. CsH, CHIPS, cyclic peptide SRSRY, Boc-1, several synthetic isoflavones, PP-6, and SMG-1 are FPR1 antagonists with quantified FPR1 binding affinity. Conversely, numerous other natural compounds with the ability to suppress $O_2^{-\cdot}$ production and HNE release and/or other functional activities in *f*MLF-stimulated neutrophils that have been reported have not been evaluated for direct FPR1 binding activity and/or had off-target effects, suggesting that they could not be bona fide receptor antagonists.

In an effort to uncover additional natural products that may represent potential FPR1 antagonists, we used this review of the literature and additional screening of selected compounds for their ability to inhibit Ca^{2+} flux in *f*MLF-stimulated human neutrophils to define a set of potential FPR1 antagonists. For a compound to be considered, it had to: *(a)* inhibit all *f*MLF-induced functional responses studied; *(b)* not directly inhibit known processes downstream of FPR1 that could interfere with the functional responses tested, including inhibition of ion channels and eicosanoid biosynthesis; and *(c)* not directly activate functional responses in neutrophils. Based on all of these restrictions, we selected a prospective set of 24 natural products from the literature that were all relatively potent inhibitors of *f*MLF-induced signaling (IC_{50} <30 μM) and conducted molecular modeling to see if these compounds fit the structural requirements of an FPR1 antagonist. Four natural compounds (cnidimol A, PP-6, PL3S, and garcimultiflorone B) met this additional requirement, suggesting they may be FPR1 antagonists. Indeed, one of these compounds (PP-6) has already been shown to compete with *f*MLF for binding to FPR1 [139]. Thus, further investigation of the binding of cnidimol A, PL3S, and garcimultiflorone B to the FPR1 ligand binding site will be important to evaluate.

Cnidimol A has a 4*H*-chromen-4-one scaffold, which is similar to recently reported isoflavone FPR1 antagonists [79]. Thus, the high similarity of cnidimol A to the FPR1 pharmacophore model suggests 4*H*-chromen-4-one may represent an important scaffold for developing FPR1 antagonists. Although we predict garcimultiflorone B could be an FPR1 antagonist, it is also possible that this natural product could inhibit *f*MLF-induced functional activity via downstream pathways, as some natural compounds related to garcimultiflorone B, such as garcinol and hyperforin, inhibited 5-lipoxygenase, a key enzyme in leukotriene

biosynthesis [211, 212]. Our docking study showed that PP-6 formed three H-bonds with FPR1. This lignan is structurally related to the mammalian lignans enterolactone and prestegane B. Various mammalian-type lignan derivatives are now commercially available and study of their FPR1-regulatory activity would be desirable. Importantly, key chemical moieties of these natural compounds could provide leads for the development of effective natural compound-inspired small molecule FPR1 antagonists.

Since our molecular modeling only evaluated orthosteric interaction of a ligand with FPR1, possible allosteric mechanisms for various compounds cannot be excluded. It is now recognized that GPCRs possess spatially distinct allosteric sites, and G protein-GPCR interactions, including β-arrestin-GPCR interactions, can involve allosteric interactions [213]. Thus, compounds that are able to interfere with these FPR1 interactions or directly insert into the FPR1 transmembrane-extracellular interface could modify FPR1-dependent signal transduction pathways. Finally, FPR1 molecules can interact laterally in the plasma membrane [214] and, for example, bile acids, plant steroids, and saponins may alter lateral allosteric regulation, modifying the membrane environment responsible for the receptor dimerization, FPR1 coupling with its G proteins, and other scaffolding/accessory proteins.

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Abbreviations

Cs	Cyclosporine
CHIPS	chemotaxis inhibitory protein of *Staphylococcus aureus*
FPR	formyl peptide receptor
GPCR	G-protein coupled receptor
HNE	human neutrophil elastase
PAF	platelet activating factor
PMA	phorbol-12-myristate-13-acetate
ROS	reactive oxygen species
SAR	structure–activity relationship
FITC	fluorescein isothiocyanate
NDGA	nordihydroguaiaretic acid

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Highlights

- We review natural products reported to antagonize or inhibit FPR1-mediated responses.
- Results of additional screening of natural compounds for their ability to antagonize FPR1 are presented and discussed.
- We also use molecular modeling to predict how well natural compounds fit the FPR1 antagonist pharmacophore.
- Identification and characterization of natural products that antagonize FPR1 activity may have potential for the development of novel therapeutics.

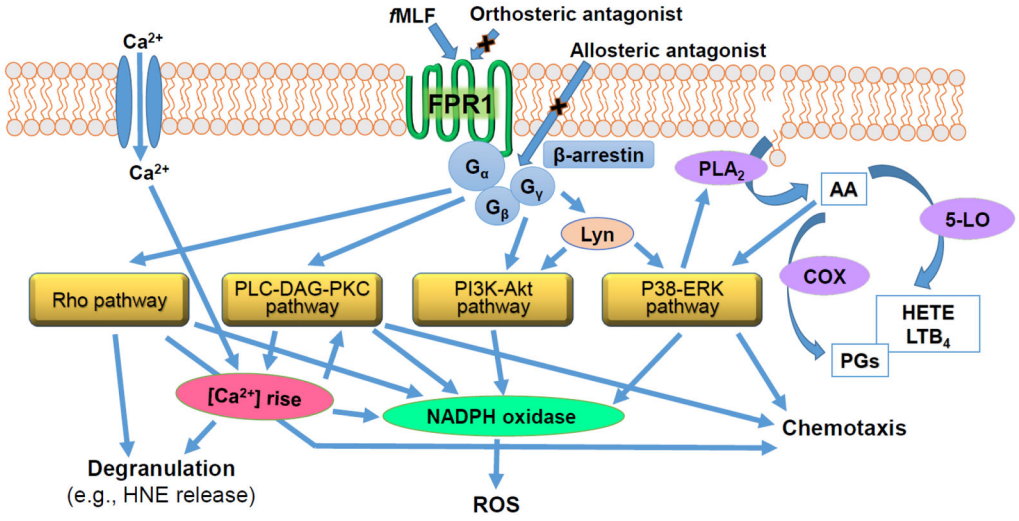


Figure 1.

Summary of *f*MLF-induced signaling events in human neutrophils. Upon *f*MLF binding, trimeric G proteins are uncoupled from formyl peptide receptor 1 (FPR1), and a series of signal transductions events ensue, resulting in neutrophil degranulation, including release of human neutrophil elastase (HNE), activation of the NADPH oxidase to produce reactive oxygen species (ROS), such as superoxide anion ($O_2^{-\cdot}$), and chemotaxis. FPR1 activation can be blocked by either orthosteric or allosteric antagonists. The second messengers resulting from FPR1 activation regulate three main intracellular kinase pathways, including phosphatidylinositol-3-kinase (PI3K), protein kinase C (PKC), and mitogen-activated protein (MAP) kinases p38/extracellular signal related kinases 1 and 2 (ERK 1/2), leading to the array of functional responses listed. In addition, PKC-independent pathways can be activated through the Src-related tyrosine kinase, Lyn. Rho GTPase pathways also play key roles in regulating a variety of neutrophil functional responses. PKC-independent pathways can be activated through the Src-related tyrosine kinase, Lyn. Activation of protein kinase C (PKC) and mitogen-activated protein kinase (MAPK) and elevation in intracellular Ca^{2+} ($[Ca^{2+}]_i$) also results in the activation phospholipase A_2 (PLA_2), leading to arachidonic acid (AA) release. AA is metabolized by 5-lipoxygenase (5-LO) and cyclooxygenase (COX), and products of this metabolism [hydroxyeicosatetraenoic acid (HETE), leukotriene B_4 (LTB_4), prostaglandins (PGs), etc.] can act in an autocrine manner. As discussed in the text, various natural compounds can interfere with receptor binding and downstream intracellular signaling events.

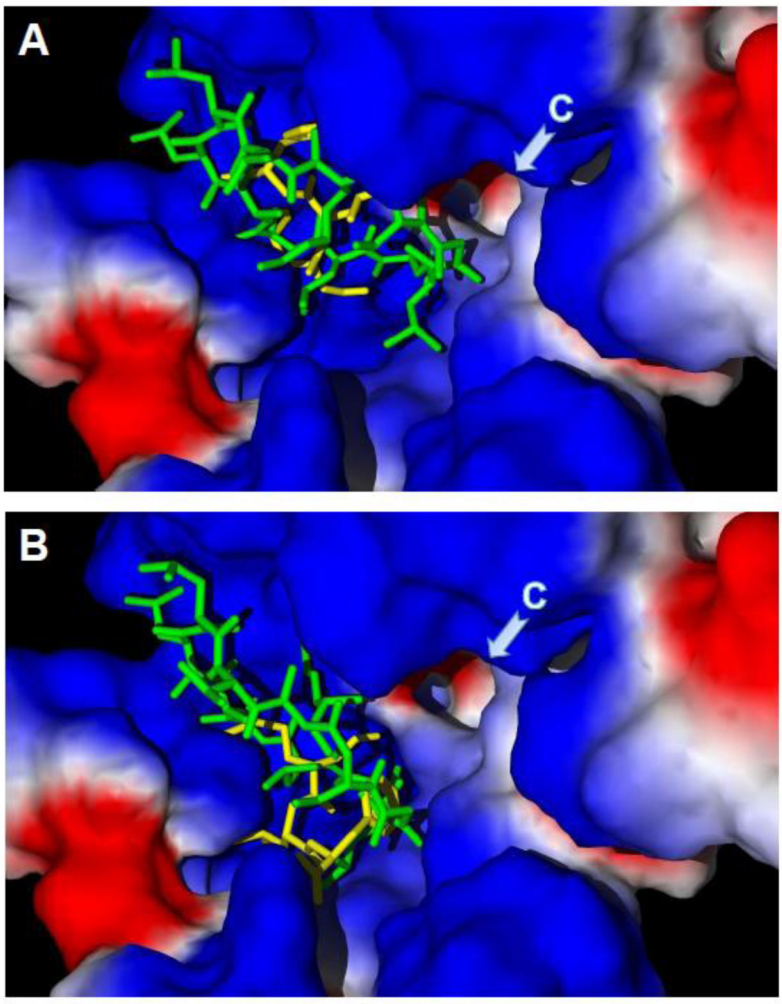


Figure 2.

Docking poses of cyclosporine H and *f*MLF into the FPR1 ligand binding site. Docking poses for CsH (green) and *f*MLF (yellow) were determined using Molegro software for the Val101 (**Panel A**) and Leu101 (**Panel B**) FPR1 variants. Briefly, the PDB structure of a rhodopsin-based homology model of FPR1 was imported into the Molegro program. To perform "mutation," Leu101 was changed into Val101, and the latter was optimized using a built-in module of the program. The search space was defined as a sphere of 11 Å, as described previously [210]. Side chains of 34 residues inside this sphere were treated as flexible during docking. CsH and *f*MLF structures were prepared using the HyperChem 7 package and optimized with the MM+ force field. Arrows indicate channel **C** (region designated as previously reported [210]). Surface coloring is based on electrostatic properties, where negatively and positively charged areas are shown in red and blue, respectively.

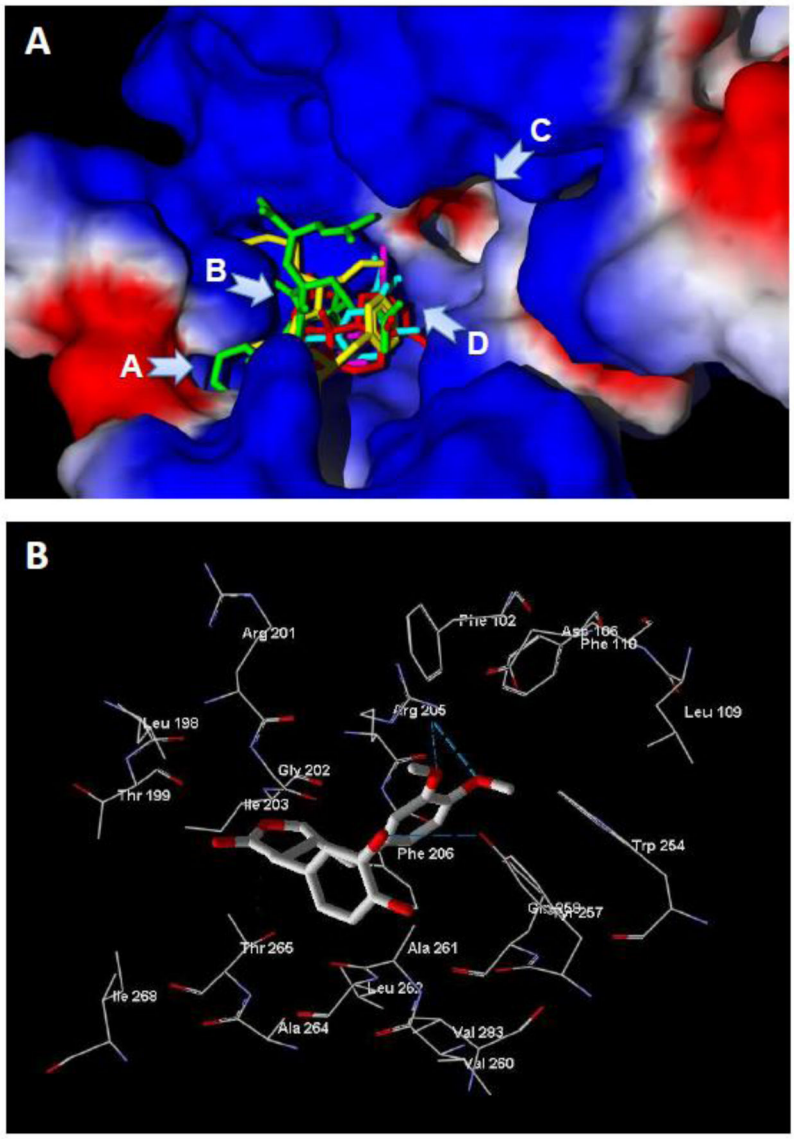
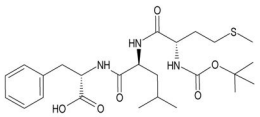
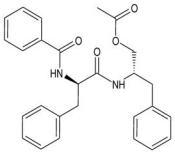
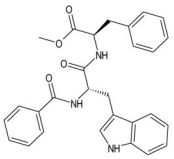
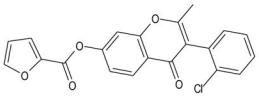
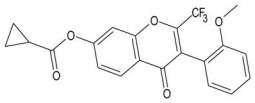
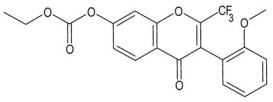
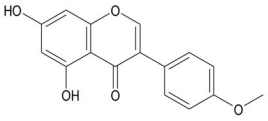
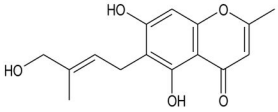
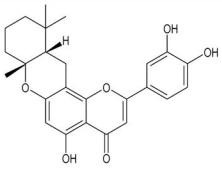


Figure 3.

Molecular docking of natural compounds with μ≤0.04 into the FPR1 ligand binding site. **Panel A**. Overlaid docking poses of PP-6 (light-blue), cnidimol A (pink), PL3S (red), garcimultiflorone B (green), and *f*MLF (yellow) into the Leu101 FPR1 variant. Structures of the docked compounds were prepared using the HyperChem 7 package and optimized with the MM+ force field. Arrows indicate: channel **A**; curved cavity **B** located behind the blue-colored ledge; channel **C**; and "bottom" **D** of the binding site between channels **A** and **C** (regions designated as previously reported [210]). Surface coloring is based on electrostatic properties, where negatively and positively charged areas are shown in red and blue, respectively. **Panel B**. H-bonding of PP-6 in the FPR1 ligand binding site. Residues within 6 Å of the docking pose are shown. H-bonds are indicated with dashed lines.

Table 1

Structures of selected natural compounds and their synthetic derivatives that inhibited *f*MLF-induced functional responses in neutrophils.

Di- ant tri-peptides		
 *t*-Boc-MLF	 Aurantiamide acetate	 Dipeptide **3**
4*H*-Chromen-4-ones		
 Isoflavone **36**	 Isoflavone **68**	 Isoflavone **73**
 Biochanin A	 Cnidimol A	 Ugonin **3**
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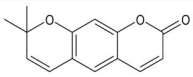
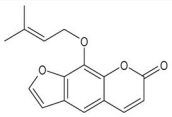
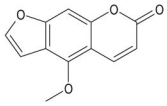
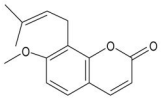
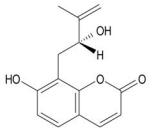
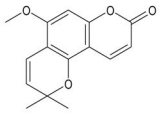
 Xanthyletin	 Imperatorin	 Bergapten
 Osthenol	 Demethylauraptenol	 Alloxanthoxyletin
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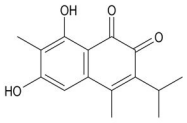
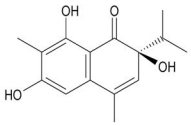
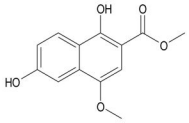
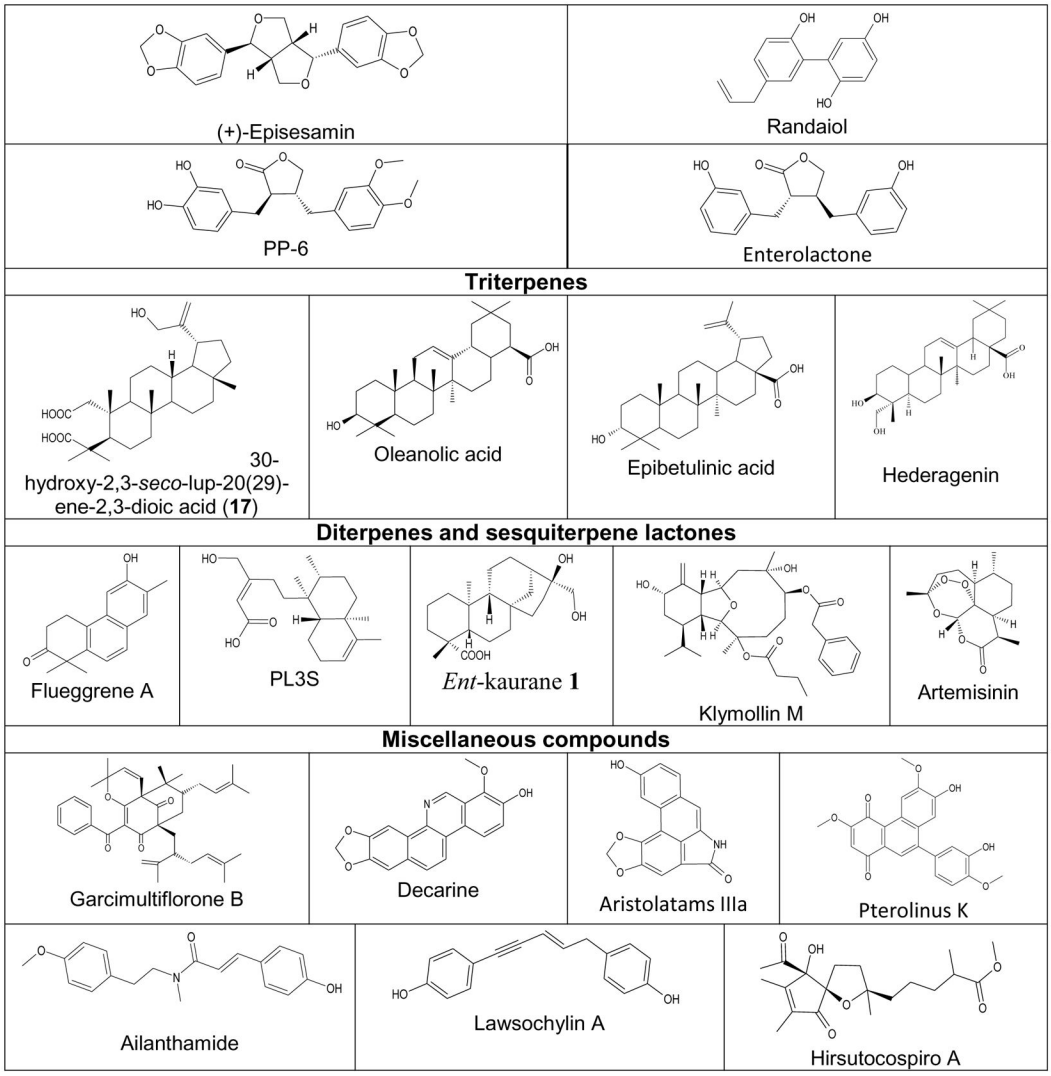
 Berryammone A	 Berryammone B	 Lawsonaphthoate A
Lignans		



Compound	*f*MLF-induced Responses Inhibited in Neutrophils	References
t-Boc-MLF	FPR1 binding, $O_2^{-\cdot}$ production, cell adhesion	[29, 51]
Aurantiamide acetate	$O_2^{-\cdot}$ production	[60]
Dipeptide **3**	$O_2^{-\cdot}$ production, HNE release	[62]
Isoflavone **36**	Ca^{2+} flux, FPR1 binding	[79]
Isoflavone **68**	Ca^{2+} flux, FPR1 binding	[79]
Isoflavone **73**	Ca^{2+} flux, FPR1 binding	[79]
Biochanin A	Ca^{2+} flux, FPR1 binding	[79]
Cnidimol A	$O_2^{-\cdot}$ production, HNE release	[94]
Ugonin **3**	$O_2^{-\cdot}$ production, HNE release	[95]
Xanthyletin	$O_2^{-\cdot}$ Production, HNE release	[104, 106]

Compound	*f*MLF-induced Responses Inhibited in Neutrophils	References
Imperatorin	$O_2^{-\cdot}$ production	[103]
Bergapten	$O_2^{-\cdot}$ production, HNE release	[94]
Osthenol	$O_2^{-\cdot}$ production, HNE release	[94]
Demethylauraptenol	$O_2^{-\cdot}$ production, HNE release	[94]
Alloxanthoxyletin	$O_2^{-\cdot}$ production, HNE release	[105]
Berryammone A	$O_2^{-\cdot}$ production, HNE release	[116]
Berryammone B	$O_2^{-\cdot}$ production, HNE release	[116]
Lawsonaphthoate A	$O_2^{-\cdot}$ production, HNE release	[73]
(+)-Episesamin	$O_2^{-\cdot}$ production, HNE release	[104]
Randaiol	$O_2^{-\cdot}$ production and HNE release	[124]
Lignan PP-6	FPR1 binding, Ca^{2+} flux, $O_2^{-\cdot}$ production, phosphorylation of ERK 1/2, Akt, and p38	[139]
Triterpene **17**	$O_2^{-\cdot}$ production, HNE release	[141]
Oleanolic acid	$O_2^{-\cdot}$ production, HNE release	[143–146]
Epibetulinic acid	$O_2^{-\cdot}$ production	[143]
Flueggrene A	$O_2^{-\cdot}$ production, HNE release	[162]
Diterpene PL3S	$O_2^{-\cdot}$ production, HNE release	[163]
Ent-kaurane **1**	$O_2^{-\cdot}$ production, HNE release, Ca^{2+} flux	[164, 165]
Klymollin M	$O_2^{-\cdot}$ production, HNE release	[177]
Garcimultiflorone B	$O_2^{-\cdot}$ production, HNE release	[183]
Decarine	$O_2^{-\cdot}$ production, HNE release	[104, 106]
Aristolatams IIIa	$O_2^{-\cdot}$ production, HNE release	[189]
Pterolinus K	$O_2^{-\cdot}$ production, HNE release	[190]
Ailanthamide	$O_2^{-\cdot}$ production, HNE release	[106]
Lawsochylin A	$O_2^{-\cdot}$ production, HNE release	[73]
Hirsutocospiro A	$O_2^{-\cdot}$ production, HNE release	[204]

Compound numbers for dipeptide **3**, isoflavones (**36**, **68**, and **73**), ugonin **3**, triterpene **17,** and *ent*-kaurane **1** are as designated in previous reports [62, 79, 95, 141, 165].

Table 2

Effects of selected natural compounds on Ca^{2+} mobilization in human neutrophils and FPR1 transfected HL-60 cells.

Compound	Human Neutrophils		FPR1 HL-60	
	Activation (EC_{50}, μM)	Inhibition (IC_{50}, μM)	Activation (EC_{50}, μM)	Inhibition (IC_{50}, μM)
Imperatorin	31.1 ± 3.6	31.2 ± 2.8	50.9 ± 4.7	33.9 ± 4.2
Bergapten	N.A.	N.A.	N.A.	N.A.
(+)-Episesamin	38.1 ± 4.2	30.4 ± 2.6	54.8 ± 5.2	45.4 ± 4.9
Enterolactone	N.A.	N.A.	N.A.	N.A.
Oleanolic acid	N.A.	1.5 ± 0.18	N.A.	14.5 ± 3.3
Hederagenin	N.A.	11.5 ± 1.4	N.A.	10.1 ± 2.6
Chenodeoxycholic acid (CDCA)	49.5 ± 5.1	21.4 ± 1.6	40.3 ± 4.5	19.9 ± 3.7
Deoxycholic acid (DCA)	49.0 ± 5.4	32.6 ± 3.3	40.9 ± 5.5	20.8 ± 2.9
Artemisinin	N.A.	N.A.	N.A.	N.A.

Imperatorin, bergapten, (+)-episesamin, and hederagenin were from Toronto Research Chemicals (Toronto, Canada); enterolactone was from Cayman Chemical (Ann Arbor, MI); chenodeoxycholic acid was from Chem-Impex (Wood Dale, IL); deoxycholic acid was from Alfa Aesar (Ward Hill, MA); oleanolic acid was from Seleckchem (Houston, TX); and artemisinin was from Biotrend Chemicals (Zurich, Switzerland). Compounds were evaluated for their ability to directly activate or inhibit *f*MLF-induced Ca^{2+} flux in human neutrophils and FPR1 transfected HL-60 cells. Changes in intracellular Ca^{2+} were measured with a FlexStation II scanning fluorometer, as described previously [79]. For evaluation of direct agonist activity, compounds of interest were added from a source plate just before plate reading. Antagonist activity and selectivity were evaluated after 30 min pretreatment with test compounds at room temperature, followed by addition of 5 nM *f*MLF. N.A., no activity was betobserved at the highest tested concentration (55 μM).

Table 3

Biological activity of selected natural compounds and their ability to contribute to a high-quality common 4-molecule pharmacophore template of orthosteric FPR1 antagonists.

Compound	Inhibitory effect on *f*MLF-induced			μ_a	Predicted FPR1 binding[b]
	$O_2^{-\cdot}$ production	HNE release IC_{50} (μM)	Ca^{2+} flux		
Aurantiamide acetate	~ 15.0	n.d.	n.d.	0.05	No
Cnidimol A	13.4	11.6	n.d.	**0.04**	**Yes**
Ugonin **3**	3.8	2.5	n.d.	0.06	No
Xanthyletin	18.4	24.1	n.d.	0.06	No
Demethylauraptenol	2.2	17.9	n.d.	0.06	No
Berryammone A	3.2	3.8	n.d.	0.05	No
Berryammone B	1.7	1.8	n.d.	0.05	No
Lawsonaphthoate A	8.5	20.6	n.d.	0.05	No
Randaiol	1.6	6.9	n.d.	0.05	No
Lignan PP-6	0.3	n.d.	<10	**0.03**	**Yes**
Triterpene **17**	0.12	2.2	n.d.	0.05	No
Oleanolic acid	1.4	0.75	1.5	0.06	No
Hederagenin	n.d.	n.d.	11.5	0.06	No
Flueggrene A	4.4	4.3	n.d.	0.05	No
Diterpene PL3S	~ 3.0	~ 3.0	n.d.	**0.04**	**Yes**
Ent-kaurane **1**	4.0	12.5	~ 30.0	0.06	No
Klymollin M	3.1	2.9	n.d.	0.06	No
Garcimultiflorone B	0.11	0.14	n.d.	**0.03**	**Yes**
Decarine	4.1	6.3	n.d.	0.05	No
Aristolatams IIIa	0.43	0.72	n.d.	0.06	No
Pterolinus K	0.99	4.2	n.d.	0.05	No
Ailanthamide	12.0	13.5	n.d.	0.05	No
Lawsochylin A	7.2	6.4	n.d.	0.06	No
Hirsutocospiro A	4.1	3.7	n.d.	0.05	No

[a] Maximum score variation per molecule pairs, which is an adjustable parameter of the template construction algorithm in the FieldTemplater program. Lower values of μ indicate templates of higher quality.

[b] If μ ≤ 0.04 for a common 4-molecule FPR1 template, the compound was predicted to bind FPR1. n.d., no data.

Table 4

H-bonding centers of FPR1 and docked natural molecules, as well as energies of H-bond interactions between the indicated centers for the best predicted docking poses.

Compound	Number of H-bonds	Ligand		FPR1 residues		Energy (*kcal/mol*)
		Center	Type	Center	Type	
PP-6	3	*m*-OH	Acceptor/Donor	Phenolic OH in Tyr257	Donor/Acceptor	1.79
		p-OCH_3	Acceptor	Terminal nitrogen in Arg205	Donor	2.06
		m-OCH_3	Acceptor	Terminal nitrogen in Arg205	Donor	1.18
Cnidimol A	2	CH_2OH	Donor	COOH in Asp106	Acceptor	2.37
		Endocyclic oxygen	Acceptor	OH in Thr265	Donor	2.24
Garcimultiflorone B	1	Endocyclic oxygen	Acceptor	OH in Thr265	Donor	2.50
PL3S	1	CH_2OH	Acceptor/Donor	OH in Thr265	Donor/Acceptor	2.41

Molecular modeling was performed using the Leu101 FPR1 variant.