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4-Aroyl-3-hydroxy-5-phenyl-1*H*-pyrrol-2(5*H*)-ones as *N*-formyl peptide receptor 1 (FPR1) antagonists

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ABSTRACT

Formyl peptide receptors (FPRs) are expressed on a variety of leukocytes and play important roles in inflammation. Thus, FPR antagonists may represent novel therapeutics for modulating innate immunity and treating inflammatory diseases. Previously, 1*H*-pyrrol-2(5*H*)-ones were reported to be potent and competitive FPR1 antagonists. In the present studies, 42 additional 1*H*-pyrrol-2(5*H*)-one analogs were evaluated for FPR1 antagonist activity. We identified a number of novel competitive FPR1 antagonists that inhibited *N*-formylmethionyl-leucyl-phenylalanine (*f*MLF)-induced intracellular Ca^{2+} mobilization in FPR1-transfected HL60 cells and effectively competed with WKYMVm-FITC for binding to FPR1 in FPR1-transfected RBL cells. The most active pyrroles inhibited human neutrophil Ca^{2+} flux, chemotaxis, and adhesion to human epithelial cells, with the most potent being compounds **14** (4-benzoyl-1-hexyl-3-hydroxy-5-(4-hydroxy-3-methoxyphenyl)-2,5-dihydro-1*H*-pyrrol-2-one) and **17** (4-benzoyl-5-(2,5-dimethoxyphenyl)-3-hydroxy-1-(2-methoxyethyl)-2,5-dihydro-1*H*-pyrrol-2-one). In addition, these FPR1 antagonists inhibited *f*MLF-induced phosphorylation of extracellular signal-regulated kinases (ERK1/2) in FPR1-RBL cells, differentiated HL-60 cells, and human neutrophils. Most of the antagonists were specific for FPR1 and did not inhibit WKYMVM/WKYMVm-induced intracellular Ca^{2+} mobilization in FPR2-HL60 cells, FPR3-HL60 cells, or interleukin 8-induced Ca^{2+} flux in human neutrophils. Moreover, molecular modeling showed that the active pyrroles had a significantly higher degree of similarity with the FPR1 antagonist pharmacophore template as compared to inactive analogs. Thus, the 4-aroyl-3-hydroxy-5-phenyl-1*H*-pyrrol-2(5*H*)-one scaffold represents an important backbone for the development of novel FPR1 antagonists and could provide important clues for understanding the molecular structural requirements of FPR1 antagonists.

1. Introduction

Formyl peptide receptors (FPRs) are G protein-coupled receptors (GPCR) that play an important role in leukocyte activation and chemotaxis [1]. In humans, there are three FPR isoforms: FPR1, FPR2, and FPR3 [1]. FPR1 is expressed on a variety of cell types, including neutrophils, macrophages, natural killer (NK) cells, immature dendritic cells, astrocytes, microglial cells, hepatocytes, and bone marrow-derived mesenchymal stem cells [1–6]. FPR1 was originally identified as a receptor for *N*-formyl peptides, which are produced by bacteria but can also be released from damaged mitochondria during tissue injury [2,7,8]. In phagocytes, FPR1 activation induces cell migration, the release of reactive oxygen

Abbreviations: BCECF, 2′,7′-Bis-(carboxyethyl)-5(6)-carboxyfluorescein; CXCR, chemokine (C-X-C motif) receptor; DMEM, Dulbecco's Modified Eagle Medium; DMSO, dimethyl sulfoxide; ERK1/2, p44/42 mitogen-activated protein kinases 1 and 2; FBS, fetal bovine serum; *f*MLF, *N*-formyl-methionyl-leucyl-phenylalanine; FPR, formyl peptide receptor; FITC, fluorescein isothiocyanate; IL-8, interleukin 8; HEPES, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid; $HBSS^-$, Hanks' balanced salt solution without Ca^{2+} and Mg^{2+}; $HBSS^+$, Hanks' balanced salt solution containing 10 mM HEPES and Ca^{2+} and Mg^{2+}; WKYMVm, Trp-Lys-Tyr-Met-Val-D-Met; SAR, structure–activity relationship; WKYMVm, Trp-Lys-Tyr-Met-Val-L-Met-NH_2; WKYMVM, Trp-Lys-Tyr-Met-Val-D-Met-NH_2.

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species (ROS), and phagocytosis [1,4]. In addition to its role in phagocyte activation, FPR1 seems to have physiological roles in other cell types. For example, *N*-formylmethionyl-leucyl-phenylalanine (*f*MLF) induces osteoblast differentiation and upregulates expression of osteogenic markers [9]. Likewise, *f*MLF suppresses adipocyte differentiation in human mesenchymal stem cells [9]. Annexin A1 peptides can also activate FPR1 similar to *N*-formyl peptides and induce inflammatory responses [10]. Recently, it was found that FPR1 binds scolopendrasins, which are antimicrobial peptides from *Scolopendra subspinipes mutilans* [11,12]. Cytokine-like proteins FAM19A4 (family with sequence similarity 19 member A4) and FAM3D (family with sequence similarity 3 member D) were also reported as a novel FPR1 ligands [13,14], and FAM19A4 was shown to stimulate chemotactic migration, phagocytosis, and release of ROS in macrophages via FPR1 [13].

FPR1 has been reported to contribute to the pathogenesis of several diseases. For example, FPR1 expression is associated with tumor progression and survival in gastric cancer [15], and FPR1 mediates the tumorigenicity of human hepatocellular carcinoma cells [16]. High expression of FPR1 in neuroblastoma primary tumors corresponds with high-risk disease and poor patient survival [17]. Likewise, interaction of endogenous annexin A1 with FPR1 leads to transactivation of the epithelial growth factor receptor (EGFR), which promotes invasion and growth of glioma cells [18]. Gliadin, the immunogenic component within gluten and a trigger of celiac disease, induces neutrophil migration via engagement of FPR1 [19]. The efficacy of FPR1 blockade in hepatic ischemia-reperfusion injury was reported recently [20]. In addition, an aurantiamide analog HCH6-1 demonstrated protective effects on lipopolysaccharide-induced acute lung injury by blocking FPR1 in mice [21,22]. Thus, bioactive ligands acting as FPR1 antagonists might serve as useful therapeutics in host defense in order to reduce detrimental effects associated with inflammation and cancer [23].

Currently, the most potent FPR1-specific antagonists described are the fungal cyclic peptides, cyclosporine A and H [24]. However, *in vivo* studies of cyclosporines should be interpreted carefully, because their main therapeutic effects appear to involve signaling pathways unrelated to FPR1 [25–27]. However, growing evidence supporting the anti-inflammatory and tissue-protective effects of FPR1 antagonists led to the screening of natural products and commercial libraries for novel small-molecule FPR1 antagonists. As result of these screening efforts and/or structure–activity relationship (SAR)-directed design and synthesis, a number of synthetic non-peptide FPR1 antagonists with a wide range of chemical diversity have been identified (reviewed in [28,29]). In addition, a variety of natural molecules have been shown to be FPR1 antagonists [30]. Among the most potent and specific small-molecule FPR1 antagonists are compounds with a 4*H*-chromen-4-one scaffold [31]. Several FPR1 antagonists with a 4-aroyl-3-hydroxy-5-phenyl-1*H*-pyrrol-2(5*H*)-one scaffold were previously reported [32,33] (Fig. 1), but their activities in primary cells, SAR analysis of related compounds, as well as molecular modeling have not been described. It should be noted that compounds having the same scaffold were also reported as small molecule blockers of the interaction between S100A10 and annexin A2 [34]. Because both annexin A2 and FPR1 are involved in pathogenesis of tumor growth and invasion of neuroblastoma, glioma, and hepatocellular carcinoma [35–37], development of FPR1 antagonists based on the 4-aroyl-3-hydroxy-5-phenyl-1*H*-pyrrol-2(5*H*)-one scaffold could lead to promising dual functional agents for the treatment of these diseases.

In the present study, we evaluated forty-two 1*H*-pyrrol-2(5*H*)-ones for their ability to antagonize FPR-dependent signaling in neutrophils and FPR-transfected cells and identified novel and relatively potent FPR1 antagonists. Most of these antagonists were specific for FPR1 and did not inhibit FPR2-, FPR3-, or chemokine (C-X-C motif) receptor (CXCR) 1/2-dependent responses. SAR analysis of these compounds revealed the importance of a small hydrophobic group at position R_4 of the 4-aroyl-3-hydroxy-5-phenyl-1*H*-pyrrol-2(5*H*)-one scaffold. In addition, molecular modeling showed a high degree of similarity for low-energy conformations of these antagonists with the pharmacophore model of FPR1 antagonists.

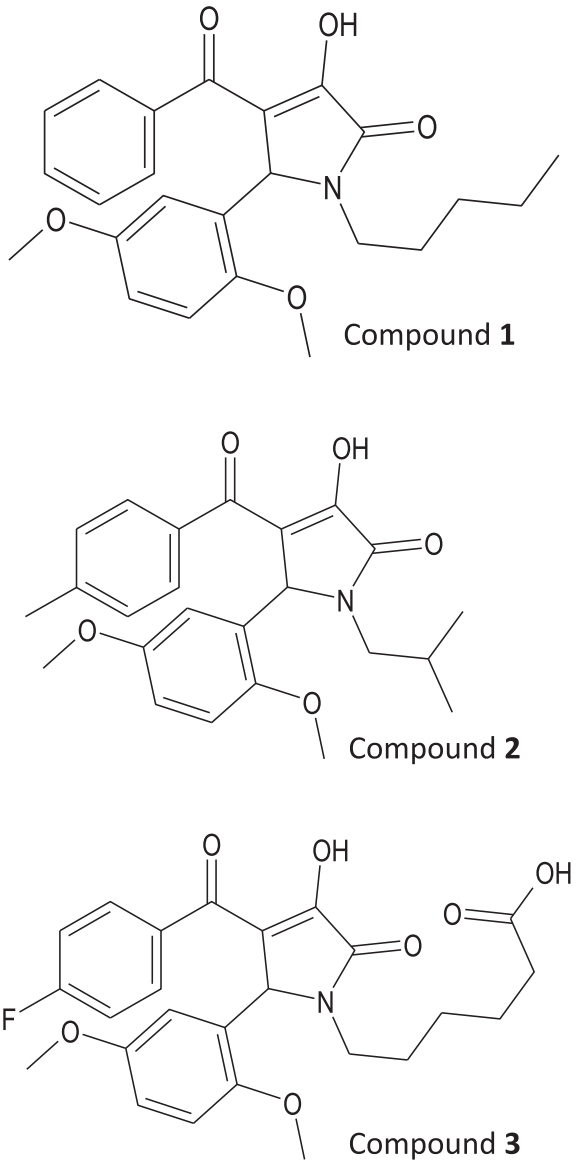

Fig. 1. Previously reported FPR1 antagonists **1**–**3** with a 4-benzoyl-3-hydroxy-5-phenyl-1*H*-pyrrol-2(5*H*)-one scaffold.

2. Materials and methods

2.1. Materials

Dimethyl sulfoxide (DMSO), *f*MLF, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), and Histopaque 1077 were from Sigma Chemical Co. (St. Louis, MO, USA). Dulbecco's Modified Eagle Medium (DMEM), Roswell Park Memorial Institute (RPMI) 1640 medium and penicillin–streptomycin solution were from Mediatech (Herdon, VA, USA). DMEM/F12 was from Lonza (Walkersville, MD, USA). Fetal bovine serum (FBS) was from Atlas Biolog-