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G-protein coupled receptors involved in the resolution of inflammation: ligands and therapeutic perspectives.

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ABSTRACT

Dysregulated inflammation is a central pathological process in diverse disease states, including neurodegenerative disorders. The recent concept of “resolution of inflammation” is offering a conceptual change for the diagnosis and the development of new therapeutic approaches for chronic inflammatory diseases. Resolution of inflammation terminates the inflammatory response promoting return to tissue homeostasis through the action of several classes of mediators, termed specialized pro-resolving lipid mediators (SPMs), that include lipoxins, resolvins, protectins, and maresins. SPMs provide “stop signals” that reduce the number of immune cells at the site of insult and increase clearance of apoptotic cells through phagocytosis. SPMs elicit their effects through the interaction with specific G-protein coupled receptors (GPCRs). The elucidation of the pathways downstream the GPCRs involved in the resolution of chronic inflammation is opening novel opportunities to generate novel anti-inflammatory agents. This review focuses on the SPMs and the receptors through which their effects are mediated. The medicinal chemistry of the modulators of the GPCRs involved in the resolution of inflammation will be illustrated, by highlighting the potential for developing new anti-inflammatory drugs.

KEYWORDS: resolution of inflammation, pro-resolving specialized mediators, GPCRs, FPR2, GPR32, ChemR23, GPR18.

1. INTRODUCTION

Inflammation is a protective reaction of the body driven by infection and tissue injury with the final aim to eliminate the inciting stimulus, promote tissue repair, and, in case of infection, to establish immune memory. The inflammatory response is a complex and highly orchestrated sequence of events that involves molecular, cellular and physiological modifications. The inflammatory response starts with the release of soluble mediators, such as chemokines, cytokines, vasoactive amines, leukotrienes, and prostaglandins by resident immune cells in the injured or infected tissue. The consequence is the immediate influx of polymorphonuclear leukocytes (PMNs) into the tissue with the final aim to engulf and degrade pathogens.[1,2] This is a well-characterized phase of the inflammatory response and is routinely targeted by non-steroidal anti-inflammatory drugs or by biotechnological drugs targeting pro-inflammatory cytokines, which block the actions of pro-inflammatory mediators.

The period between inflammatory cells infiltration, clearance of these cells from the tissue, and restoration of functional homeostasis is defined as resolution.[3,4] This phase of inflammatory response has been traditionally considered a passive event during which inflammation simply fades away. Instead, the resolution of inflammation is an actively regulated process that involves the biosynthesis of an array of mediators as diverse as those that initiate inflammation.[3,5] Interestingly, it is initiated at early stage of the inflammatory response to halt the influx of PMNs at a level appropriate for the insult. In this step, the synthesis of pro-inflammatory mediators is suspended and the infiltrated immune cells undergo local apoptosis or necrosis. This induces the influx of monocytes-macrophages which ingest and degrade dead cells and debris, thus promoting tissue homeostasis and repair functions.[6,7] Abnormalities at any point of this process, such as prolonged leukocytes recruitment, impairments in apoptotic cell removal, alterations in

macrophage phenotype switching, could potentially lead to chronic inflammation with subsequent tissue damage.

As discussed above, the resolution of inflammation is an active process and it is orchestrated by several classes of mediators including proteins/peptides, such as Annexin A1, and lipid mediators. The latter have been termed specialized pro-resolving mediators (SPMs) and include lipoxins (LX), resolvins (Rv), protectins/neuroprotectins (PD/NPD), and maresins (Mar), which signal through the interaction with specific receptors to allow a careful balance between a sufficiently strong response to the injury and a limited damage to endogenous tissues.[3,8,9] SPMs act as immuneresolvents by combining both anti-inflammatory and pro-resolving activities. In fact, SPMs limit neutrophil infiltration, inhibit proinflammatory cytokines while inducing the production of anti-inflammatory mediators, enhance macrophage uptake, and stimulate nonphlogistic clearance of apoptotic cells and cellular debris by efferocytosis. In addition, SPMs act as strong attenuators of oxidative stress not only by reducing oxygen and nitrogen reactive species production but also by potentiating several naturally occuring antioxidant systems (for more detailed information on biological activities of SPMs and their role in the resolution of inflammation the reader is referred to excellent reviews).[10-12]

The genesis of resolution relies on the biosynthesis of SPMs by the same cells that initiate the inflammation: innate immune cells, platelets, and hypoxic endothelial cells. During acute inflammation, these cells use ω-6 polyunsaturated fatty acids (PUFAs), mostly arachidonic acid (AA), as the main source for the biosynthesis of the pro-inflammatory leukotrienes and prostanoids. In the resolution phase of inflammation, the same cells undergo a lipid mediator class switch during which the AA metabolism is switched to the production of LXs that are thought to start resolution.[6] In addition, the ω-3 PUFAs eicosapentaenoic acid (EPA) and docosapentaenoic acid (DHA) become precursors for the biosynthesis of E-series (RvE1-3) and D-series (RvD1-6) of resolvins,

protectins/neuroprotectins (PD1/NPD1 and PDX) and maresins (MaR1 and MaR2), respectively (Figure 1).[5]

Interestingly, SPMs are produced at different times and in different amounts with LXs appearing in the earliest phase of inflammation while the others reach their highest levels at the peak of inflammation (6-12 h) or are produced at the later stages (RvD3).[8] The actions of SPMs are mediated through the activation of specific G protein-coupled receptors (GPCRs) that display a certain level of redundancy. In fact, not only each receptor is often engaged by different SPMs, but also a single SPM can act through the activation of different receptors. Since the production of SPMs is time-dependent, also the activation of SPM-dedicated GPCRs is time- and cell-dependent and this makes pro-resolving mediators different from anti-inflammatory or blocking mediators.[13,14] To date only a handful of SPM-dedicated GPCRs have been identified and several of them are orphan receptors. Over the past years, pro-resolution based approaches have proved to have potential for the treatment of numerous inflammatory conditions in different animal models. Thus, the development of drug-like pro-resolving agents would be beneficial to treat a broad range of inflammatory diseases, including acute inflammation, infections, neurodegenerative disorders and, possibly, cancer.[15-18]

In this review, we will focus on “small-molecule” ligands which have been developed for SPM-dedicated GPCRs by discussing their potential for the development of anti-inflammatory and pro-resolving drugs.

2. SPM-DEDICATED GPCRs

2.1 FORMYL PEPTIDE RECEPTOR 2 (FPR2)

FPR2 belongs to the family of formyl peptide receptors, along with FPR1 and FPR3 [19] and is expressed in several immune cells (neutrophils, monocytes/macrophages, glial cells and astrocytes) and also in hepatocytes, dendritic cells, endocrine tissue and coronary arteries.[20]

FPR2 is functionally coupled with Gαi1, Gαi2, and Gαi3 and associates with G0, Gz, and Gα16.[21] This event triggers activation of a variety of signaling pathways, including fluxes in intracellular calcium and activation of phospholipases A2, C, and D, PI3K, and MAPK. Receptor activation would also cause rapid phosphorylation, leading to phospholipase C-mediated receptor desensitization and internalization.[22] FPR2 downstream activation of the MAPK pathway, in particular ERK1/2, regulates cell chemotaxis.[21] FPR2 is characterized by complex functional properties as it can be activated by different ligands, which stimulate different intracellular signalling pathways, depending on the chemical structure of ligand and/or the cell type involved. For instance, FPR2 mediates pro-inflammatory effects when activated by mitochondrial and bacterial N-formyl peptides or by serum amyloid A, β-amyloid, and prion protein $PrP_{106-126}$.[21] On the contrary, FPR2 mediates anti-inflammatory and pro-resolving effects when activated by LXA4,[23] RvD1,[24] RvD3,[25] and Annexin A1.[21] Several studies have attempted to elucidate the molecular basis of such heavily ligand-biased FPR2 pharmacology. It has been proposed that lipids and small peptides bind at discrete interaction sites facilitating the activation of different second messangers and downstream signaling pathways.[26] In another study it has been proposed that receptor dimerization is responsible, at least in part, for the complex functional properties of FPR2. In particular, LXA4 and annexin A1 induce FPR2 homodimerization, which leads to increased production of the anti-inflammatory interleukin IL-10 through the p38/MAPKAPK/Hsp27 pathway.[27] In bone marrow-derived macrophages, RvD1 suppresses calcium-sensing kinase calcium-calmodulin-dependent protein kinase II (CAMKII), most likely by blocking calcium entry into the cytosol, and subsequent inhibition of p38 mitogen-activated protein kinase (MAPK) phosphorylation.[28,29]

Annexin A1 limits neutrophil recruitment and promotes apoptotic neutrophil clearance by modulating monocyte recruitment and enhancing efferocytosis.[21] LXA4 binds FPR2 with high affinity and, through the interaction with FPR2, LXA4 regulates cytokines synthesis, reduces neutrophils infiltration, and increases phagocytosis.[30,31] All these actions are crucially dependent on LXA4 structure. In fact, the structural changes induced by endogenous metabolism, such as isomerization or reduction of double bonds, oxidation of alcohols, or change in chirality, translates in significant changes in potency.[32,33] The potent anti-inflammatory and pro-resolving functions of LXA4 elicited by activation of FPR2 have been studied in several preclinical disease models, including chronic obstructive pulmonary disease (COPD), cystic fibrosis, atherosclerosis, ischemia reperfusion, pain, and CNS disorders characterized by neuroinflammation. [17, 34-37] For example, in Tg2576 mice, a transgenic model of Alzheimer's Disease (AD), LXA4 reduced NF-κB activation and the levels of pro-inflammatory cytokines and chemokines and increased the levels of anti-inflammatory IL-10. These effects stimulated a pro-resolving phenotype of microglia which is responsible for enhanced phagocytic function associated with improved clearance of β-amyloid plaques, reduced synaptotoxicity, and improvements of cognition.[38]

As already mentioned above, FPR2 also mediates the anti-inflammatory effects of RvD1. In fact, RvD1 dose-dependently activate FPR2 with EC_{50} in the picomolar range.[24] RvD1 is a potent regulator of both human and murine PMN migration,[39] stimulates macrophage autophagy,[40] and regulates the levels of pro-inflammatory cytokines, such as TNF-α, IL-1β and IL-6, in different cellular models.[41,42] In vivo administration of RvD1 reduces neuroinflammation and improves recovery after focal brain damage and this effect is FPR2-mediated because the effect is abolished by intracerebroventricular administration of a FPR2 antibody.[43] Similarly, chronic administration of RvD1 in Syn rats, a model of Parkinson Disease, prevents central and peripheral inflammation, as well as neuronal dysfunction and motor deficits.[44]

Based on chemical and metabolic liability of SPMs, several attempts have been done to develop molecules with enhanced pharmacokinetic properties that might also be amenable to further development as potential drugs.

The first series of lipoxin analogues, also known as lipoxin mimetics, was based on modifications of the C_{15}-C_{20} portion of the lipoxin structure, which is subjected to metabolic oxidation. Compound **1** (Figure 2) was able to inhibit transmigration of human PMN at dose range comparable to LXA4.[32] In subsequent studies, the triene system of LXA4 has been replaced with a benzene ring (compounds **2-4**, figure 2).[45,46] These compounds were able to stimulate phagocytosis of apoptotic PMN by macrophages, to attenuate experimental renal fibrosis, and to reduce obesity-induced adipose inflammation disease at a dose range comparable to LXA4.[46,47] Subsequently, the benzene ring was replaced with heteroaromatic cores, such as pyridine, imidazole or oxazole, (compounds **5-7**, Figure 2) leading to the identification of less lipophilic LX mimetics, which are able to attenuate LPS-induced NF-kB activity with a potency similar to LXA4.[48,49] The most potent compounds **6**, (1*R*)-**7**, (1*S*)-**7** were tested in a model of zymosan-induced peritonitis and were able to reduce the inflammatory process.[48,49]

Among small-molecule ligands, the quinazolinone derivative Quin-C1 (**8**) (Figure 3) was reported as a highly selective FPR2 agonist able to induce Ca^{2+} mobilization (EC_{50}= 15nM). In a mouse model of bleomycin-induced lung injury, Quin-C1 reduced the expression of TGF-β1, IL-1β, TNF-α and CXCL10 through FPR2 activation,[50] highlighting anti-inflammatory properties of this compound. In 2006, the pharmaceutical company Amgen identified the pyrazolone **9** (Figure 3) as a FPR2 agonist within a high-throughput screening campaign on their compound library.[51] Further structure-activity relationship (SAR) studies focused on phenylurea moiety led to the identification of the potent mixed FPR1/FPR2 agonist chloropyrazolone **10**, often referred in the literature as “compound 43” (EC_{50}= 44 nM) (Figure 3). The SAR studies evidenced that: i) the steric and the electronic nature of

the para-substituent on the phenylurea moiety greatly influenced FPR2 activity; ii) the introduction of alkyl substituents at C(5) of pyrazole ring forced the molecule to adopt a U-shaped conformation, which was preferred in the interaction with FPR2; iii) modifications of the N(2)-substituent were relatively well tolerated. Beside inducing Ca^{2+} mobilization, derivative **9** and "compound 43" were able to dose-dependently inhibit PMN migration after stimulation with the chemoattractants IL-8 or fMLP. In addition, "compound 43" showed acceptable in vivo pharmacokinetic properties. The anti-inflammatory properties of "compound 43" were confirmed in in vivo models of inflammation. In fact, "compound 43" reduced the edema by 58% in a mouse ear inflammation model and inhibited cell trafficking in the air-pouch and zymosan peritonitis models, two model of acute inflammation, at dose range comparable to LXA4.[31,51]

The interest of pharmaceutical companies in developing molecules able to modulate FPR2 as new anti-inflammatory agents is witnessed by the extensive patent literature reported to date. The pharmaceutical company Allergan disclosed a series of phenylurea derivatives as potent FPR2 modulators (compounds **11** and **12**, Figure 4). The compounds were characterized as FPR2 agonists and were claimed to be useful for the treatment of peripheral and central inflammatory diseases. However, no experimental data were reported in the patent application.[52,53] The pharmaceutical company Actelion filed several patent application describing FPR2 agonists with an aminopyrazole or aminotriazole core, exemplified by compounds **13** and **14** (Figure 4), respectively. The compounds were claimed to be beneficial for the treatment of peripheral and central inflammatory diseases, but no experimental data were reported in the patent application.[54,55] The efforts in the drug development process were aimed at identifying suitable cores that could replace the 4-substituted aniline moiety, a common feature of FPR2 agonists, which is known to possibly lead to genotoxicity. Compound **14**, also known as ACT-389949, proved to be safe and well tolerated in phase I clinical trials.[56]

In a recent study, Qin et al compared the FPR2 agonists “compound 43” (Figure 3) and the optically active pyridazin-3(2*H*)-one derivative **15**, also known as “compound 17b” (Figure 5) and found biased-agonist properties.[57] In fact, in CHO cells overexpressing FPR2 and in primary cardiomyocytes, “compound 17b” exhibited a marked biased effect as it induced a robust phosphorylation of ERK1/2 and Akt1/2/3 along with 30-fold bias away from intracellular Ca^{2+} mobilization relative to “compound 43”. Moreover, “compound 17b” reduced necrosis in isolated cardiomyocytes and inhibited the release of pro-inflammatory IL-1β after stimulation with TGF-β.[57] These effects were confirmed also in in vivo model, suggesting that biased agonism can be a promising strategy in the treatment of ischaemia-reperfusion injury because activation of ERK1/2-Akt signalling elicits cardioprotective effects, whereas increased Ca^{2+} may contribute to cardiomyocytic damage and unfavourable consequences. In a subsequent study, the scaffold of “compound 17b” was modified to elucidate the SAR needed to generate potent FPR agonists biased away from intracellular calcium mobilization.[58] The effect of structural modifications on both left- and right-hand side of the molecule as well as the replacement of the methyl substituent on the linker with longer alkyl groups was studied. The study led to the identification of compound **16** (Figure 5), which showed similar signaling bias away to Ca^{2+} mobilization in comparison to “compound 17b” for FPR1 and balanced signaling at FPR2. In addition, compound 16 showed improved in vitro pharmacokinetic properties, but no data on in vivo pro-resolving effects of compound **16** were reported in the paper.[58]

We contributed to the field by developing a series of ureidopropanamide-based FPR2 agonists with anti-inflammatory properties. The investigation started from the observation that that the bombesin receptor 2 antagonists PD168368 and PD176252 behaved as potent FPR1/FPR2 agonists.[59] Then, structural modifications of the ureidopropanamido scaffold of PD168368 and PD176252 led to the identification of compound **17** (Figure 6), a moderately potent and selective

ureidopropanamide FPR2 agonist. Unfortunately, **17** showed high liability to oxidative metabolism, which made this molecule not suitable for in vivo studies.[60-62] In a subsequent study, **17** was modified by replacing the (5-methoxypyridin-2-yl) cyclohexylmethyl moiety with the phenylcyclopropylmethyl moiety (which is more synthetically accessible) and the tryptophan core with non-natural amino acids, with the aim to improve metabolic stability. The exploration of structure-activity relationships of this class of FPR2 agonists evidenced that the right-hand part of the molecule was crucial in the interaction with FPR2 because structural modifications in that part of the molecule greatly influenced agonist potency. On the other hand, the steric hindrance of the same part of the molecule was an important requisite for metabolically stable compounds. The study led to the identification of compound **18** (Figure 6), which showed a good balance of agonist potency at FPR2 and metabolic stability. In addition, **18** was able to reduce the release of the pro-inflammatory mediators IL-1β and TNF-α in LPS-stimulated rat primary microglial cultures, an in vitro model of neuroinflammation.[63]

2.2 G-PROTEIN RECEPTOR 32 (GPR32)

The orphan receptor GPR32 has been recently identified as involved in the pro-resolving effect of RvD1. Using a luciferase-based reporter assay Krishnamoorthy et al found that RvD1 was able to significantly reduce the TNFα-stimulated NF-κB response in cells overexpressing GPR32.[64] In addition, RvD1 reduced actin polymerization, a key event in neutrophil migration. This event was GPCR-mediated since it was significantly reduced by treatment with pertuxin.[64] Activation of GPR32 by RvD1 leads to the regulation of a number of miRNA involved in the regulation of acute inflammation, such as miR-146b, miR208-a, and miR-219.[24,65] GPR32 also mediates the pro-resolving effects of RvD5 in the context of bacterial infections. The activation of GPR32 leads to enhanced bacterial phagocytosis in human macrophages and to a down-regulation of several pro-

inflammatory genes, including NF-κB and TNF-α.[66] In addition, GPR32 is involved in the regulation of macrophage phenotype and function, where it controls the production of inflammatory cytokines IL-1β and IL-8. GPR32 mediates the inhibitory effect of RvD1 on macrophage chemotaxis induced by chemerin and fMLF.[67] In AD patients, RvD1 improved phagocytosis of fluorescently-labeled β-amyloid (Aβ) in macrophages and reduced Aβ-induced apoptosis.[68]

GPR32 is expressed in innate immune cells, including neutrophils, lymphocytes, and macrophages, and in adipose tissue.[67,69]

RvD1 analogues, exemplified by benzo-diacetylenic-17R-RvD1-methyl ester (BDA-RvD1, compound **19**, Figure 7), have been recently reported.[68] The compounds showed improved chemical stability as compared to RvD1 and retained agonist activity at GPR32 and proresolving properties, including inhibition of leukocyte infiltration and stimulation of phagocytosis.[70]

Using a chemiluminscent cell-reporting assay which measures recruitment of β-arrestin to GPR32 upon agonist activation, Chiang et al have recently identified different chemotypes that can activate GPR32 using a high-throughput screening of small-molecule libraries. Among them, the serotonin 5-HT_{1A} receptor agonists p-MPPF (**20**) and p-MPPI (**21**), were identified as GPR32 agonists (Figure 7). In addition, the chemotypes C1A (**22**) and C2A (**23**) have been identified as GPR32 agonits (Figure 7). These compounds acted as RvD1 mimetics because they were able to stimulate phagocytosis of serum-treated zymosan in the same concentration range as RvD1. Interestingly, biased agonism at GPR32 was observed as pMPPI activated $G_{\alpha s}$ (similarly to RvD1), whereas pMPPF, C1A and C2A activated $G_{\alpha i}$ upon ligand interaction.[71]

2.3 CHEMERIN RECEPTOR 23 (CHEMR23)

ChemR23, also known as chemokine-like receptor 1 (CMKLR1), was initially identified as the RvE1 receptor because RvE1 was able to inhibit TNF-α stimulated NF-κB activation in HEK-293

overexpressing ChemR23 and co-transfected with NF-κB response element-luciferase reporter plasmid.[72] Subsequently, the affinity of RvE1 for ChemR23 was confirmed using radioligand binding assay.[72] RvE1 increased the phosphorylation of ERK and MAP kinase in both peripheral blood monocytes and in ChemR23-transfected HEK cells. These effects were abolished by treatment with pertussin toxin suggesting coupling to Gαi/o protein for the signal transduction.[72] ChemR23 is expressed on dendritic cells, resident macrophages and natural killer cells as well as on adipocytes, endothelial cells, peripheral blood leukocytes (monocytes, neutrophils, T lymphocytes), brain, kidney and myeloid tissues.[73] In macrophages, RvE1 signalling via ChemR23 stimulated efferocytosis and inhibited TNF-α, IFN-γ and IL-12p40 production.[74] These actions were attributed to RvE1-stimulated S6 phosphorylation downstream of the PI3K/Akt and Raf/ERK pathways. In a murine model of zymosan-induced peritonitis, RvE1 regulated leukocyte infiltration and stimulated macrophage phagocytosis of apoptotic PMN.[75] Of note, ChemR23 also binds chemerin, a peptide able to enhance chemotaxis of immature dendritic cells and macrophages and involved in several inflammatory diseases, including rheumatoid arthritis and psoriasis.[76,77] Radioligand binding studies suggest that RvE1 and chemerin share the same recognition sites on ChemR23 as chemerin peptide was able to displace [^{3}H]-RvE1 (almost 70% of specific binding). Of note, ChemR23 activation from the two ligands resulted in different signalling activities.[78] In fact, chemerin is more potent in G protein activation and controls cells migration, whereas RvE1 was an order of magnitude more potent in NF-κB activation.[75]

It has been proposed that impaired/defective signaling of ChemR23 in diabetes may be a component in the etiopathogenesis of the disease. In fact, ChemR23 expression is upregulated in human diabetic patients, although the biological effects of RvE1 in these patients are blunted.[79]

Recently, Deyama et al. demonstrated that intracerebroventricular injections of RvE1 and RvE2 produced dose-dependent antidepressant effects in a murine LPS-induced model of depression

using the tail suspension test and forced swim test. The antidepressant effect of RvE1 was more pronounced than that observed with RvE2, confirming that RvE1 is a full agonist, while RvE2 is a partial agonist of ChemR23.[80]

As for RvE1 mimetics, compound RX-10045 (compound **24**, Figure 8) is currently under evaluation in clinical trials for the treatment of dry eye syndrome (NCT01675570). RX-10045 showed in vitro potent anti-inflammatory effect as it inhibited the release of pro-inflammatory mediators from corneal epithelial cells.[81]

To date, only few small molecules able to interact with ChemR23 have been identified. Imaizumi et al identified compound **25** (figure 8) as a hit for the development of new compounds able to block ChemR23.[82] Compound **25** was able to inhibit, in the micromolar range, calcium signaling in CAL-1 cells, a dendritic cell (DC)-like cell line, established from a patient with blastic natural killer cell lymphoma. The insertion of a methyl group on the benzyl of compound **25** led to a 10-fold increase of potency (compound **26**, Figure 8). A further increase of the potency was obtained by introducing an N-methylcarboxamide group in 5-position of benzoxazole ring (compound **27**, Figure 8). Compounds **26** and **27** proved to be able to block chemerin-induced chemotaxis in CAL-1 cells, suggesting that the compounds may block the infiltration of human pDCs into inflammatory lesions and may inhibit the production of cytokines, especially IFN-α, in the inflamed tissue. These inhibitors were proposed as therapeutic agents for systemic lupus erythematosus and psoriasis.[82]

2.4 G-PROTEIN RECEPTOR 18 (GPR18)

GPR18 is a G protein-coupled receptor belonging to the cannabinoid receptor family, although it is structurally different from cannabinoid receptors CB_1 and CB_2.[83] Until 2006, GPR18 was classified as an orphan receptor due to the lack of known endogenous ligand(s). Then, N-arachidonoylglycine, a carboxylic metabolite of the endocannabinoid anandamide, was reported as the endogenous

ligand of GPR18.[84] GPR18 signaling pathways are still not fully elucidated due to the lack of pharmacological tools and challenging heterologous expression.[85] Coupling to Gαi/o and Gαq has been reported and several groups have found that GPR18 has high constitutive activity.[86] In 2015, using an unbiased GPCR-β-arrestin–coupled screening, Chiang et al identified GPR18 as the functional receptor for RvD2 and the interaction of GPR18/RvD2 was further confirmed in radioligand binding studies.[87] In the same study, it was also demonstrated that in human macrophages RvD2 stimulated phagocytosis of Escherichia coli and apoptotic PMN and the effect was abolished by silencing the receptor. In vivo, RvD2 limited polymorphonuclear cell infiltration, enhanced phagocytosis, and accelerated resolution and these effects were not observed in GPR18-deficient mice.[87] A correlation between RvD2 levels in cerebrospinal fluid and the cognitive functions in AD patients has been reported, as decreased levels of RvD2 seem to be connected with the evolution of AD.[86] In ageing rats, the administration of DHA led to increased levels of RvD2 and this resulted in improved cognitive functions.[88]

As in the case of other SPMs, RvD2 has a limited chemical and metabolic stability.[89] For this reason, new compounds able to mimicking RvD2 actions are needed to explore the pro-resolving effects of GPR18 stimulation in pathological conditions. To date, only few small molecules able to interact with GPR18 have been reported, the majority being antagonists. As an example, the antagonist O-1918 (compound **28**, Figure 9) a synthetic analog of cannabidiol [90] increases migration in a concentration-dependent manner via the p44/42 MAPK pathway.[91] Another GPR18 antagonist is amauromine (compound **29**, Figure 9), a dipeptide isolated from the marine sponge-derived fungus *Auxarthron reticulatum*, which was able to inhibit β-arrestin recruitment induced by Δ^9-THC in CHO cells overexpressing GPR18. No data are available on the effect of amauromine in cellular models of inflammation.[92] Another class of GRP18 antagonists has been developed from the imidazothiazinone PSB-CB-5 (compound **30**, Figure 9), which was able to partially inhibit β-

arrestin recruitment induced by Δ^9-THC in CHO cells overexpressing GPR18. In addition, PSB-CB-5 showed appreciable affinity for CB2 receptor.[93] Further exploration of the SAR of compound **30** evidenced that large lipophilic substituents on benzyloxy moiety were important for GPR18 interaction. Accordingly, the elongation of the alkyl chain connecting the two phenyl rings improved the antagonistic potency at GPR18 leading to the identification of PBS-CB-27 (compound **31**, Figure 9). Compound **31** displayed lower IC_{50} value at GPR18 and improved selectivity versus CB2 receptors. When chlorine substituent on phenylhexyloxy moiety was replaced with a methyl group, the highest potency was observed but with a decrease of selectivity over GPR55 (compound **32**, Figure 9).[94] No data are available on the effect of this class of compounds in cellular models of inflammation.

3. CONCLUSION

It is becoming evident that resolution of inflammation is an active process and several studies have proved that the induction of resolution is possible in vivo and may be useful to control inflammatory responses. These findings provided support to the idea that promoting resolution of inflammation may open new therapeutic avenues to treat chronic inflammatory diseases. Pro-resolving drugs are expected to activate endogenous mechanisms that terminate inflammation and promote tissue repair. Differently from the current anti-inflammatory therapies which are “resolution toxic” (i.e. non steroidal anti-inflammatory drugs), pro-resolving drugs would elicit multiple positive effects, including reducing leukocytes trafficking, inducing PMN apoptosis and clearance, and restoring of microenvironment homeostasis. As a result, pro-resolving drugs would modulate the entire inflammatory response and would likely show a low burden of side effects.

Endogenous SPMs are difficult to synthesize in large scale, costly, and, most importantly, not chemically stable. Consequently, SPMs are unsuitable for use as drugs. Instead, small drug-like molecules able to mimic the pro-resolving effects of SPM may have the characteristics to possibly

exploit resolution-based therapeutic strategies. Thus, the development of such chemical entities is highly desired. Among the SPM-dedicated GPCRs, FPR2 is undoubtedly the most studied and the anti-inflammatory/protective role of FPR2 has been demonstrated in several in vitro and in vivo models of inflammatory diseases. A wide range of agonists for FPR2 have been developed so far. However, most of these agonists have been characterized only for their ability to induce Ca^{2+} mobilization. Two recent studies suggest that the identification of the signaling profile of the agonists will be needed to select which agonist is adequate to pursue the resolution of inflammation. In fact, Qin et al have showed that the FPR2 agonists compound 43 and compound 17b both increase intracellular Ca^{2+} mobilization but do activate different intracellular signaling pathways related to the resolution of inflammation. Another study has shown biased signaling of the GPR32 agonists p-MPPF and p-MPPI which exhibit different characteristics in the activation of pro-resolving pathways.

In conclusion, future efforts in this area will be devoted to the identification of small-molecule drug-like modulators of SPM-dedicated GPCRs and the detailed characterization of their signaling profile. The search of drug-like molecules to study SPM-dedicated GPCRs is still in its infancy and awaits well-tailored efforts to address the complexity of the topic.

LIST OF ABBREVIATIONS

AA: arachidonic acid

AD: Alzheimer's Disease

CNS: central nervous system

COPD: chronic obstructive pulmonary disease

DHA: docosapentaenoic acid

EPA: eicosapentaenoic acid

ERK: extracellular signal-regulated kinase

FPR: formyl peptide receptor

GPCRs: G-protein coupled receptors

GPR: G-protein receptor

LPS: lipopolysaccharide

LX: lipoxin

MAPK: mitogen-activated protein kinase

Mar: maresin

miRNA: micro RNA

PD:/NPD: protectin/neuroprotectin

PI3K: phosphoinositide 3-kinase

PMNs: polimorphonuclear leukocytes

PUFAs: polyunsaturated fatty acids

Rv: resolvin

SPMs: Specialized pro-resolving mediators

CONFLICT OF INTEREST

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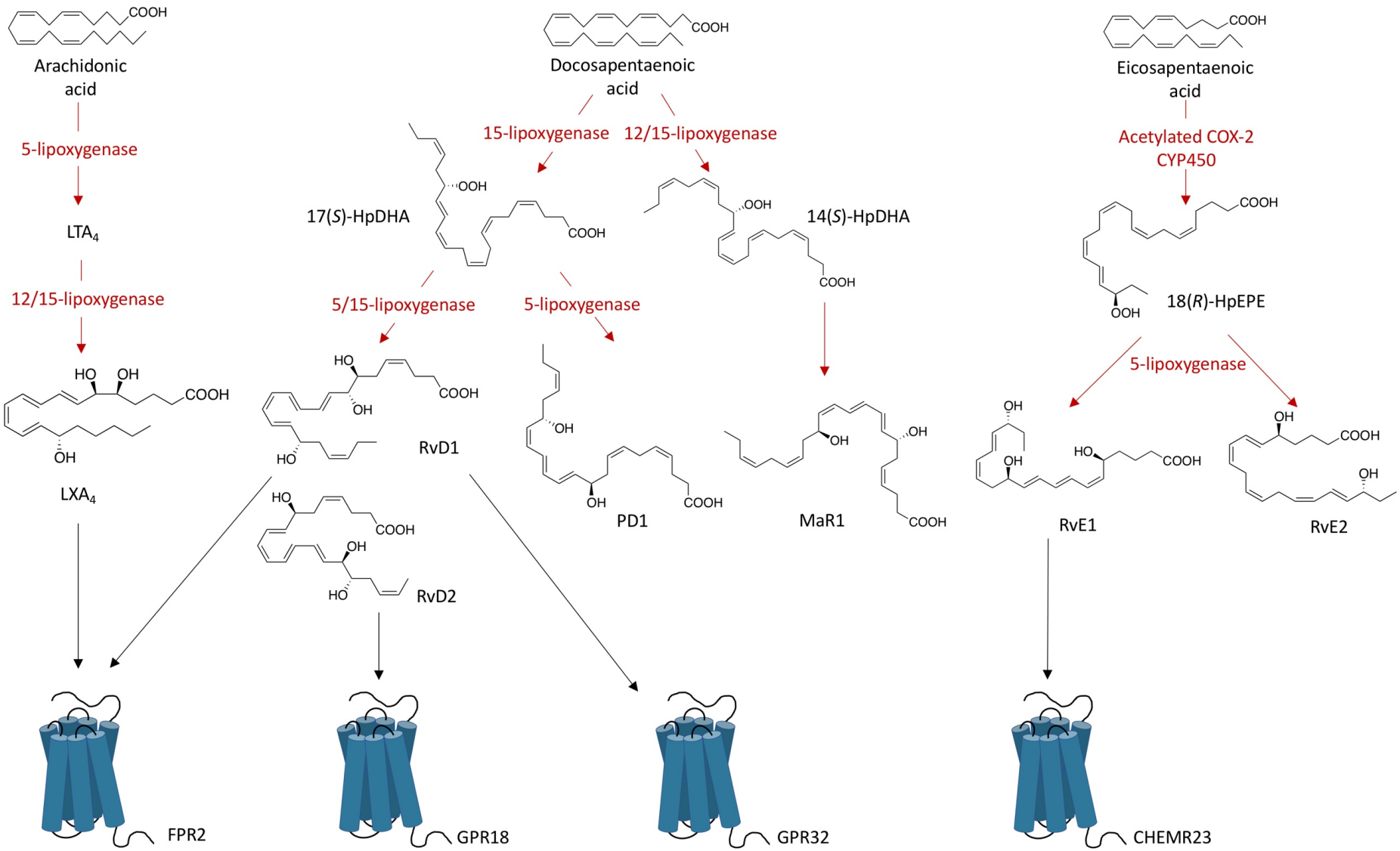
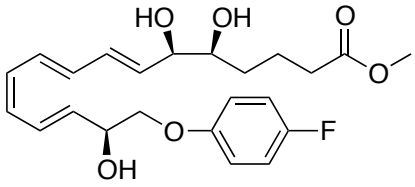

Arachidonic acid
5-lipoxygenase
LTA4
12/15-lipoxygenase
LXA4
Docosapentaenoic acid
15-lipoxygenase
12/15-lipoxygenase
17(S)-HpDHA
14(S)-HpDHA
5/15-lipoxygenase
5-lipoxygenase
RvD1
RvD2
PD1
MaR1
Eicosapentaenoic acid
Acetylated COX-2
CYP450
18(R)-HpEPE
5-lipoxygenase
RvE1
RvE2
FPR2
GPR18
GPR32
CHEMR23

Figure 1. Biosynthesis of SPMs. Lipoxins LXA4 and LXB4 are biosynthesized from AA through the combined action of 5-lipoxygenase (5-LOX) and 12- or 15-LOX. The E-series resolvins are biosynthesized starting from EPA, which is converted into 18R-hydroxyeicosapentanoic acid (18-R-HEPE) by acetylated cyclooxygenase-2 (COX-2) or cytochrome P450 enzymes in hypoxic endothelial cells. 18-R-HEPE is, next, converted into RvE1 or RvE2 by 5-LOX or RvE3 by 15-LOX. DHA is the precursor of the most heterogenous class of SPMs that includes D-series resolvins, protectins, and maresins. DHA is converted by 15-LOX to a 17-hydroperoxide intermediate (17-HpDHA), which can subsequently be converted to D-series resolvins via 5-LOX. Alternatively, 17-HpDHA can be converted to a 16.17-epoxydocosatriene intermediate that in turn is converted to protectin D1 (PD1). Additionally, DHA can be converted by 12-LOX into 14-HpDHA, which is converted into maresin 1 (Mar1) and maresin 2 (Mar2).

Figure 2. Structures of lipoxins mimetics.



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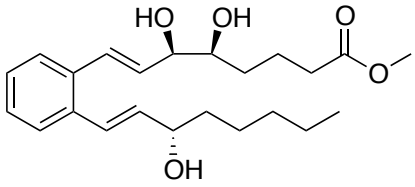

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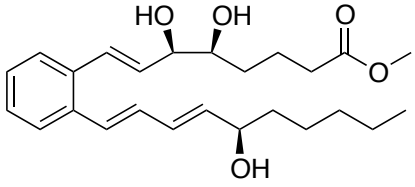

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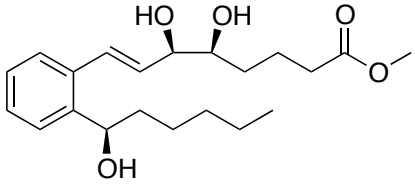

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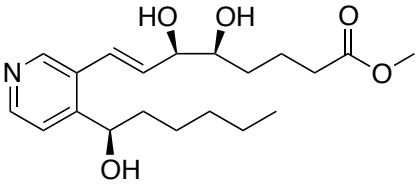

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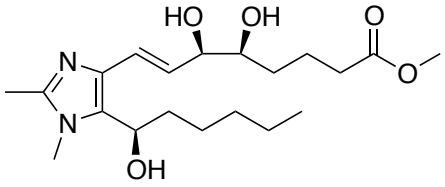

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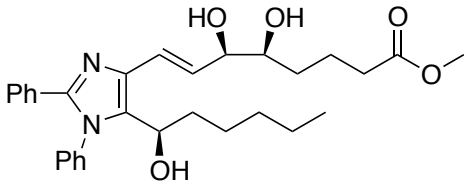
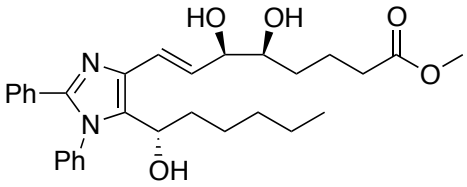
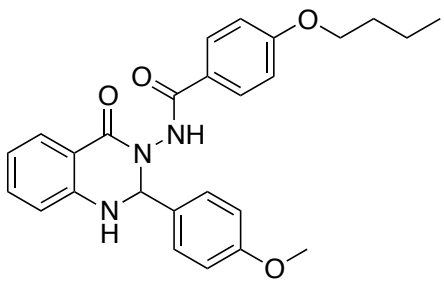


(1*R*)-**7**

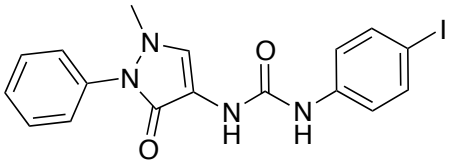


(1*S*)-**7**

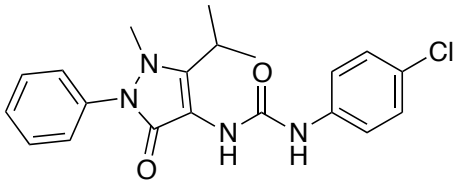
Figure 3. Structures of reference FPR2 agonists.



Quin C1 (**8**)
FPR2 EC_{50}= 15 nM

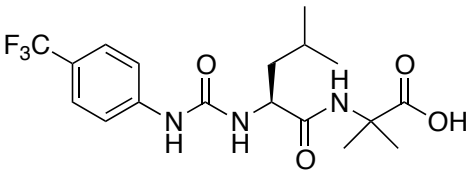


9
FPR2 EC_{50}= 30 nM

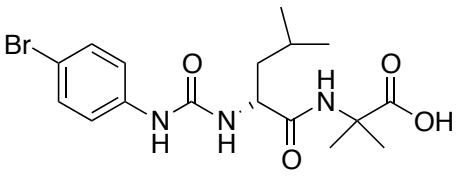


compound 43 (**10**)
FPR2 EC_{50}= 44 nM

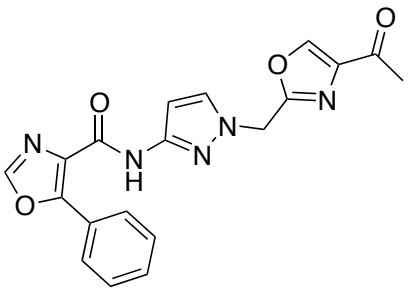
Figure 4. Structures of FPR2 agonists reported in patent literature.



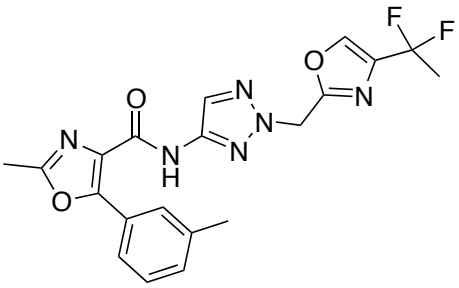
11
FPR2 EC_{50}= 2.3 nM



12
FPR2 EC_{50}= 1 nM

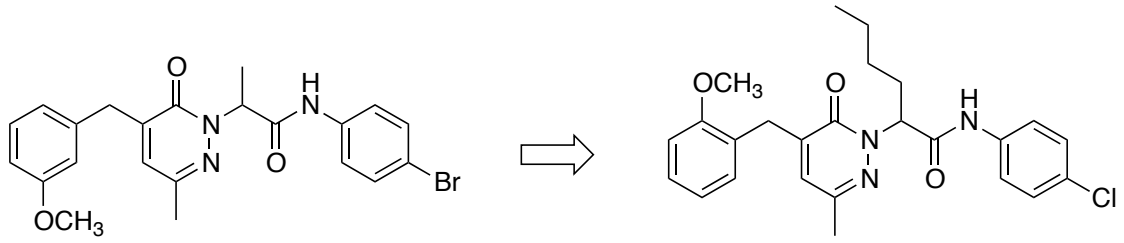


13
FPR2 EC_{50}= 10 nM



ACT-389949 (**14**)
FPR2 EC_{50}= 2.4 nM

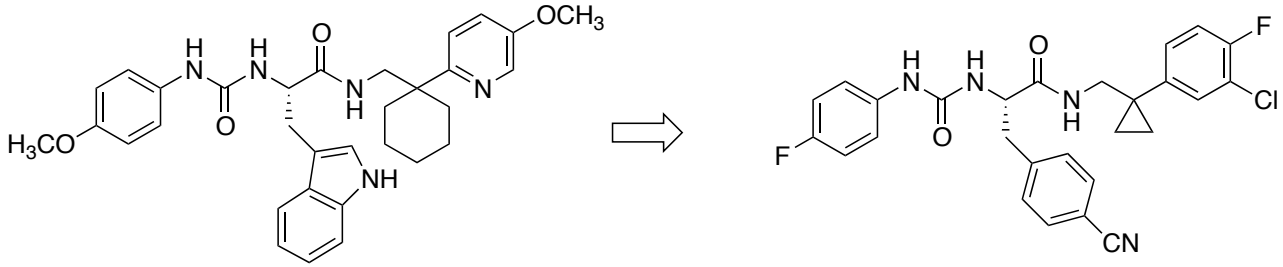
Figure 5. Structures of FPR2 agonists with the pyridazin-3(2*H*)-one core.



compound 17b (**15**)
pERK1/2 pEC_{50}= 5.02
Ca^{2+} pEC_{50}< 4.00

16
pERK1/2 pEC_{50}= 7.15
Ca^{2+} pEC_{50}< 7.29

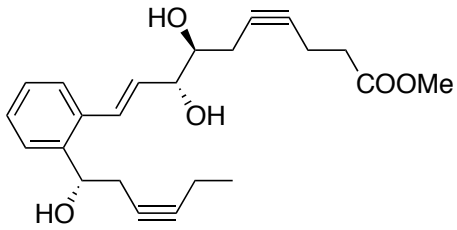
Figure 6. Structures of ureidopropamido-based FPR2 agonists.



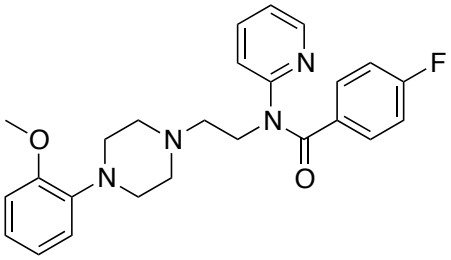
17
FPR2 EC_{50}= 0.19 μM

18
FPR2 EC_{50}= 0.19 μM

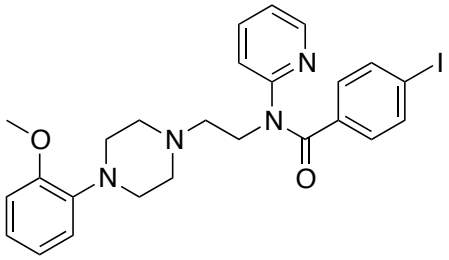
Figure 7. Structures of GPR32 agonists.



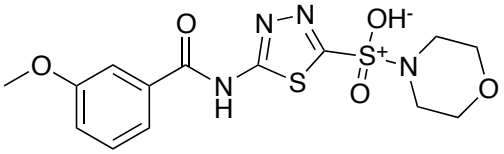
BDA-RvD1 (**19**)



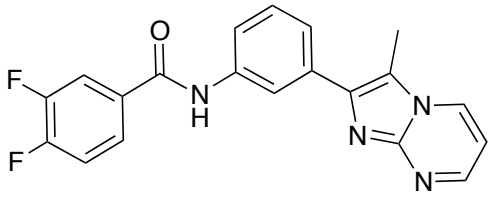
p-MPPF (**20**)



p-MPPI (**21**)

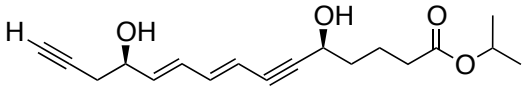


C1A (**22**)

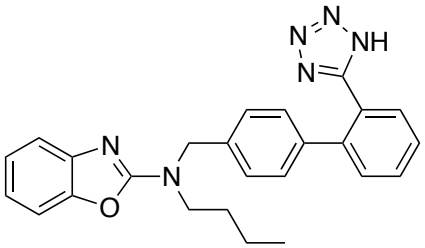


C2A (**23**)

Figure 8. Structures of ChemR23 ligands.



RX-10045 (**24**)



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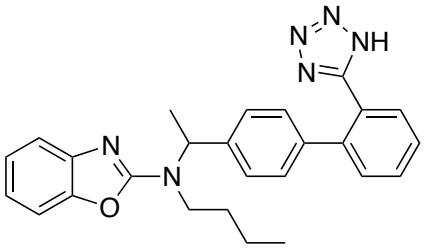

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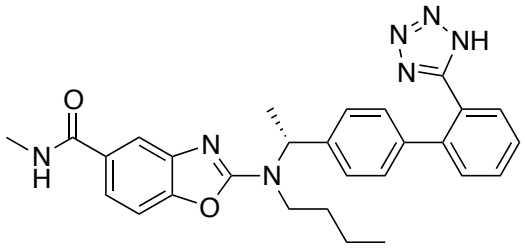
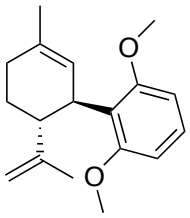
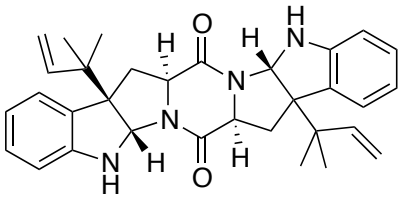


27

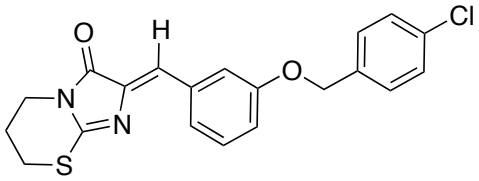
Figure 9. Structures of GPR18 ligands.



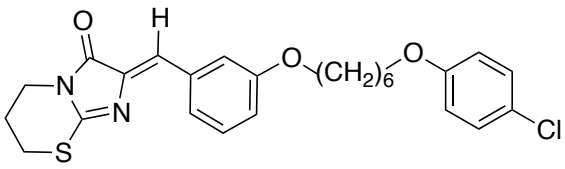
O-1918 (**28**)



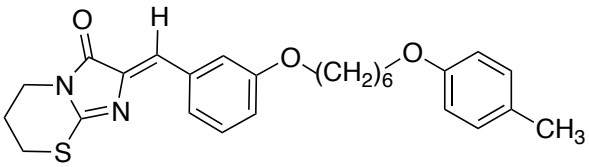
Amauromine (**29**)



PBS-CB-5 (**30**)
GPR18 IC_{50}= 0.279 μM
GPR55 IC_{50}= >10 μM



PBS-CB-27 (**31**)
GPR18 IC_{50}= 0.650 μM
GPR55 IC_{50}= >10 μM



32
GPR18 IC_{50}= 0.238 μM
GPR55 IC_{50}= 2.91 μM