A non-peptide receptor inhibitor with selectivity for one of the neutrophil formyl peptide receptors, FPR 1

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The neutrophil formyl peptide receptors (FPR1 and FPR2) are members of the G-protein coupled receptor family. The signals generated by occupied FPRs are both pro-inflammatory and anti-inflammatory. Accordingly, these receptors have become a therapeutic target for the development of novel drugs that may be used to reduce injuries in inflammatory diseases including asthma, rheumatoid arthritis, Alzheimer’s disease and cardiovascular diseases. To support the basis for a future pharmacological characterization, we have identified a small molecular non-peptide inhibitor with selectivity for FPR1. We used the FPR1 and FPR2 specific ligands fMLF and WKYMVM, respectively, and an earlier described ratio technique, to determine inhibitory activity combined with selectivity. We show that the compound 3,5-dichloro-N-(2-chloro-5-methyl-phenyl)-2-hydroxy-benzamide (BVT173187) fulfills the criteria for an FPR1 inhibitor selective for FPR1 over FPR2, and it inhibits the same functional repertoire in neutrophils as earlier described peptide antagonists. Accordingly, the new inhibitor reduced neutrophil activation with FPR1 agonists, leading to mobilization of adhesion molecules (CR3) and the generation of superoxide anion from the neutrophil NADPH-oxidase. The effects of a number of structural analogs were determined but these were either without activity or less active/specific than BVT173187. The potency of the new inhibitor for reduction of FPR1 activity was the same as that of the earlier described FPR1 antagonist cyclosporine H, but signaling through the CSAR and CXCR (recognizing IL8) was also affected by BVT173187.

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1. Introduction

Professional phagocytes including neutrophil granulocytes and monocytes/macrophages play important roles in host defense against invading microbes, and they are also key regulators in the fine-tuning and resolution of inflammatory reactions. The functional repertoires of these cells are triggered and regulated through binding of different cytokines and chemoattractants, and these are in most instances recognized by specific receptors exposed on the cell surface of resting or primed cells [1,2]. One family of receptors, the G-protein coupled seven transmembrane receptors (GPCRs), is abundant on phagocytes. The basic functions and regulatory roles of one of these, the pattern recognition chemoattractant receptor, FPR1, which binds formylated peptides, have been extensively studied. This receptor is a member of the chemoattractant receptor subfamily and recognizes N-formylated peptides of microbial or mitochondrial origin [3–6]. Since such agonists of mitochondrial origin act as “danger signals”, it has been proposed that a primary function of FPR1 is to promote trafficking of phagocytic myeloid cells to infected or damaged tissues [7]. The FPR1 expressing cells that leave the blood stream and enter the tissues, thus, exert antibacterial effector functions and clear cell debris. Accordingly the prototype agonist formylmethionyl-leucyl-phenylalanine (fMLF) is a high-affinity FPR1 agonist produced by bacteria and it triggers a variety of biologic activities in neutrophils, including granule secretion, and superoxide release, the latter generated through an activation of an electron transporting NADPH-oxidase [8,9]. Both neutrophils and monocytes/macrophages have the ability to recognize and respond to a number of additional molecules that bind FPRs and serve as “danger signals”, and the list of such more or less well characterized agonists has steadily grown [6]. Many of the defined agonists are proteins or peptides of different length, but through the use of small molecule screening approaches, non-peptide agonists for the FPR sub-family have been identified during the last couple of years [10–12]. The importance of FPR1 and the closely related FPR2 is obvious as they both have, in
different animal models, been linked to chronic inflammation of systemic and auto-immune diseases, and through this type of research a few inhibitors/antagonists have been identified and characterized [13,14]. Small molecule FPR1 and FPR2 selective antagonists belonging to different chemical series were also recently described [15,16]. The expectation is, that once identified, such molecules may form the basis for development of clinically useful anti-inflammatory drugs. The most potent and receptor specific FPR1 antagonist described so far, is the fungal peptide metabolite cyclosporin H [13,17]. We have earlier described a number of FPR1 specific receptor agonists, identified through a screen with a small molecular library [10]. This screen was also accomplished in antagonist mode and one inhibitory compound was identified.

The aim of this study was to identify new inhibitors of FPRs in the small molecule library mentioned above; the hit and selected analogues of that were further analyzed in order to determine potency and receptor preference. A newly described method designed to identify agonists/antagonists for the FPR family of receptors [18] was used to determine the ability of the new inhibitors, to affect neutrophil generation of superoxide anions from the electron transporting NADPH-oxidase. In the present study, the compound 3,5-dichloro-N-(2-chloro-5-methyl-phenyl)-2-hydroxy-benzamide (BVT173187) was found to possess functional characteristics that are similar to those of earlier described FPR1 antagonists. The new inhibitor reduced the rise in intracellular calcium, granule mobilization, and production of superoxide anion triggered by FPR1 agonists, and it was found to be as potent as cyclosporin H in inhibiting FPR1 over FPR2. The new inhibitor reduced, however, also the activity induced by C5a and IL8, neutrophil chemoattractants that bind to two others GPCRs. We suggest that the identified small non-peptide compound (or rather future variants of it) could serve as good tools for enhanced understanding of the structure-function relationships of different GPCRs and for in vitro/in vivo therapeutic studies in which a stable inhibitor is required.

2. Material and methods

2.1. Materials

The chemical library as well as the assay used in the initial screen has been described earlier [10]. The purity and identity of the used compounds were verified using HPLC and mass spectroscopy. The compounds were dissolved in DMSO at a concentration of 10 mM and stored at −80 °C. Isoluminol, fMLF, catalase, H2O2 as well as C5a were obtained from Sigma Chemical Co. (St. Louis, MO, USA). IL-8 was from R&D systems (Minneapolis, MN, USA). The hexapeptide Trp-Lys-Tyr-Met-Val-Met-NH2 was synthesized and purified by HPLC by Alta Bioscience (University of Birmingham, Birmingham, UK). The FPR2 selective PSm2 peptide (fMWGLAGKIKVKSLEQFTGK) [19] in its N-formylated form was synthesized by American Peptide Company (Sunnyvale, CA, USA). The horse radish peroxidase (HRP) was from Roche Diagnostics (Bromma, Sweden). Dextran and Ficol-Paque were from Pharmacia (Uppsala, Sweden). The receptor antagonist Trp-Arg-Trp-Trp-Trp-CONH2 (WRW4) was from GenScript Corp. (Piscataway, NJ, USA) and cyclosporine H was kindly provided by Novartis Pharma (Basel, Switzerland). The peptides/receptor antagonists were dissolved in dimethyl sulfoxide to 10−2 M and stored at 80 °C until use. Further dilutions were made in KRG.

MPO was obtained from Calbiochem (Darmstadt, Germany). RPMI 1640, FCS, PEST and G418 were from PAA Laboratories GmbH (Pasching, Austria).

2.2. Isolation of human neutrophils

Neutrophil granulocytes were isolated from buffy coats obtained from healthy adults [21]. After dextran sedimentation at 1 × g, hypotonic lyses of the remaining erythrocytes, and centrifugation in a Ficoll-Paque gradient, the neutrophils were washed twice and resuspended (1 × 107/mL) in KRG, pH 7.3. The cells were stored on melting ice and used within 120 min of preparation.

2.3. Expression of formyl peptide receptors in HL-60 cells

The procedures used to obtain stable expression of FPR1 and FPR2 in undifferentiated HL60 cells have been previously described [22]. To prevent possible auto-differentiation due to the accumulation of differentiation factors in the culture medium, cells were passed twice a week before they reached a density of 2 × 106 cells/mL. At each passage, an aliquot of the cell culture was centrifuged, the supernatant was discarded and the cell pellet was resuspended in fresh medium RPMI 1640 containing FCS (10%), PEST (1%), and G418 (1 mg/mL).

2.4. Measurement of superoxide anion production

The production of superoxide anion by the neutrophil NADPH-oxidase was measured by isoluminol-amplified chemiluminescence in a six-channel Biolumat LB 9505 (Berthold Co., Wildbad, Germany) and details about the technique and the precise oxygen metabolite measured has been described in detail earlier [23,24]. In short 2 × 107/mL neutrophils were mixed (in a total volume of 900 μL) with horse radish peroxidase (HRP, 4 U), and isoluminol (6 × 10−5 M) in KRG, pre-incubated at 37 °C after which the stimulus (100 μL) was added. The light emission was recorded continuously. The specific receptor inhibitors were included in the CL mixture for 5 min at 37 °C before stimulation.

2.5. Determination of changes in cytosolic calcium

Neutrophils at a density of 2 × 107 cells/mL in KRG without Ca2+ containing 0.1% BSA were loaded with 2 μM Fura 2-AM (Molecular Probes, Eugene, OR) for 30 min, at RT. Cells were then diluted to twice the original volume with RPMI 1640 culture medium without phenol red (PAA Laboratories GmbH, Austria) and centrifuged followed by washing once in KRG (with 1.0 mM Ca2+ from here on), and resuspended in KRG at a density of 2 × 106 cells/mL. Calcium measurements were carried out with a PerkinElmer fluorescence spectrophotometer (LC50).

The changes in cytosolic calcium levels were determined through measurement of the fluorescence, emitted at 510 nm, during excitation at 340 and 380 nm.

2.6. Cell surface receptor exposure by FACS analysis

To determine the effect of antagonists/inhibitors on cell surface CR3 expression, neutrophils (2 × 106 cells/mL) were incubated in the absence or presence of an inhibitor at 37 °C for 5 min, and the cells were then activated with a receptor agonist. The exposure of CR3 on the cell surface was determined through the binding of a PE-conjugated antibody against CR3. The amount of specifically bound rhodamine as described [20] was synthesized by K.J. Ross-Petersen (Holte, Denmark). The peptides/receptor antagonists were dissolved in dimethyl sulfoxide to 10−2 M and stored at −80 °C until use. Further dilutions were made in KRG.

MP0 was obtained from Calbiochem (Darmstadt, Germany). RPMI 1640, FCS, PEST and G418 were from PAA Laboratories GmbH (Pasching, Austria).
antibody after washing off the excess of unbound antibody was determined by flow cytometry.

To determine the effect of an inhibitor on ligand-binding, a FITC-conjugated formylated hexa peptide (FITC-fMLPNTL; 10⁻⁹ M final concentration) was added to neutrophils on ice. The peptide was added to neutrophils in the absence or presence of non-labeled fMLF (10⁻⁷ M). The cells were then incubated at 4°C for 10 min, and no washing was performed after labeling. The amount of specifically bound probes was determined as the mean fluorescence intensity (MFI) using an Accuri C6 flow cytometer (Accuri Cytometers Ltd. Camps, UK).

2.7. Inactivation using the myeloperoxidase/hydrogen peroxide system

The antagonists/inhibitors were incubated with MPO (1 μg/ml) at 37°C for 5 min and hydrogen peroxide (H₂O₂; 10 μM final concentration) was then added and the samples were incubated for another 10 min at ambient temperature. The activity of the MPO/ H₂O₂ treated molecules was determined through their remaining potential to inhibit the ROS release in neutrophils triggered with fMLF (10⁻⁷ M), an FPR1 selective agonist.

3. Results

3.1. Identification of a novel non-peptide molecule, 3,5-dichloro-N-(2-chloro-5-methyl-phenyl)-2-hydroxy-benzamide (BVT173187), that changes the ratio between the fMLF and WKYMVM induced oxidase activity

We used an earlier described ratio technique [18] to determine if small molecule candidates, earlier identified from a compound library and a screening procedure for FPR agonists [10], showed any receptor selectivity when interacting with neutrophils. We have earlier shown that the ratio between the FPR1 (activated with the peptide fMLF) and FPR2 (activated with the peptide WKYMVM) triggered respiratory burst is close to 1 and very constant, and that a change in the ratio, calculated from the amount of radical release in neutrophils triggered with FPR1 and FPR2 specific agonists, can be used as a valuable tool to find/identify receptor specific/ selective agonists as well as inhibitors/antagonists (shown for cyclosporine H and PBP10 in Fig. 1). Many of the classical FPR inhibitors such as cyclosporine H (structure shown in Fig. 2) retain their receptor specificity/selectivity when present in low μM concentrations [13], and accordingly, concentrations corresponding to this [10] were chosen to identify new inhibitors.

We identified one inhibitor and the chemical structure of this compound, 3,5-dichloro-N-(2-chloro-5-methyl-phenyl)-2-hydroxy-benzamide (BVT173187), is shown in Fig. 2A. We found that BVT173187, decreased the ratio between the responses induced by fMLF and WKYMVM, respectively (Fig. 2B), suggesting a preference of the inhibitor for FPR1. The FPR1 preference over FPR2 was confirmed using stably transfected HL-60 cells expressing either FPR1 or FPR2. The dual receptor agonist WKYMVm that is recognized by both FPR1 and FPR2 [25] was used to activate the cells and BVT173187 inhibited the response induced by the peptide in FPR1 expressing cells (Supplementary Fig. 1A left) but not in FPR2 expressing cells (Supplementary Fig. 1A right).

Moreover, the FPR1 preference over FPR2 was not agonist dependent, as illustrated by the fact that the same pattern of inhibition was obtained when fMLF was replaced by the more potent FPR1 agonist fMLF (Supplementary Fig. 1B left), and when WKYMVM was replaced by PSMα2 another FPR2 agonist (Supplementary Fig. 1B right) [19].

![Fig. 1. Effects of the PBP 10 (an FPR2 selective inhibitor) and cyclosporine H (an FPR1 selective antagonist) on the ratio induced by an FPR1 (fMLF) and an FPR2 (WKYMVM) agonist. Neutrophils were pre-incubated at 37°C for 5 min with or without the inhibitors. The concentration of cyclosporine chosen (1 μM final concentration) inhibited the fMLF induced activity by more than 90% (A) and PBP10 inhibited the WKYMVM induced activity to the same degree (B). Neutrophils were challenged with the FPR1 specific agonist fMLF (10⁻⁷ M final concentration; A) or the FPR2 specific agonist WKYMVM (10⁻⁷ M; B). The extracellular release of superoxide anion was monitored. The agonists were added at the start of the measurement and the amounts of superoxide produced are given in arbitrary units (relative light emission expressed in cpm x 10⁻⁶).](image-url)
The shift in the ratio was, thus due to a selective effect on the fMLF-induced response and the IC₅₀ value was determined to around 0.1 μM. A working solution of 0.3 μM was chosen for the basic characterization of the inhibitory compound, and with this concentration the fMLF-induced NADPH-oxidase activity (mediated through FPR1) was routinely inhibited by around 90%, whereas there was no effect on the WKYMVM induced response (mediated through FPR2).

The inhibitory activity of 44 analogues to BVT173187 was investigated using one single concentration (0.3 μM; Supplementary Fig. 2).

3.2. FPR1 is the preferred over FPR2 but the IL-8 and C5a receptors are also affected by BVT173187

Neutrophils express in addition to FPR1 and FPR2 a number of other GPCR/chemoattractant receptors. In order to determine if the activity mediated by other receptors was affected by BVT173187, we used IL-8 and C5a, two well known receptor specific agonists binding to CXCR and C5aR, respectively. The triggering through CXCR (the IL-8 receptor), showed reduced oxidase activity in the presence of BVT173187 (Fig. 3A and C) and this was true also for the response triggered through the C5aR (Fig. 3B and D). The IC₅₀ value for the IL-8 induced response was >1 μM and for the C5a induced response around 0.5 μM.

3.3. The FPR1 inhibitor BVT173187 affects mobilization of complement receptor 3 (CR3)

Neutrophil activation by chemoattractants is associated with an induction of granule secretion, and such a process leads to a mobilization of new receptors to the cell surface. To characterize BVT173187 with respect to its ability to inhibit granule mobilization, we examined the effect of surface exposure of CR3, a marker protein localized in easily mobilized granules in neutrophils (i.e. secretory vesicles, gelatinase granules, and to some extent specific granules), upon stimulation with fMLF and WKYMVM, FPR1 and FPR2 specific agonists, respectively. These receptor agonists induce an increase in CR3 exposure (Fig. 4A), and BVT173187 inhibited to a large extent this increase when induced by fMLF but was without effect of the WKYMVM induced CR3 exposure (Fig. 4B).

3.4. Comparison between the inhibitory effects of cyclosporine H and BVT173187

The cyclic undecapeptide, cyclosporine H, is a potent FPR1 selective receptor antagonist [17]. Accordingly, this antagonist inhibits fMLF-induced superoxide anion secretion in human neutrophils (see Fig. 1). Our results as well as those of others [13,26,27] show that cyclosporine H is a more potent FPR1 selective antagonist than other earlier described antagonists such as the peptides BocPLPLP and BocMLF. We have now compared the inhibitory effect of BVT173187 with that of cyclosporine H. The IC₅₀ values for the two inhibitors were the same (Fig. 5), and the new inhibitor is, thus, a just as potent FPR1 inhibitor as cyclosporine H. We have earlier shown that cyclosporine H is without effect on FPR2 induced neutrophil activity in concentrations up to 1 μM [13], and this was true also for BVT173187 (Fig. 5).

To determine the effect of BVT173187 on peptide binding, a FITC-conjugated formylated peptide (FITC-FLNLNPTL; 10⁻⁹ M final concentration) was added and the amount of bound probe (mean fluorescence intensity; MFI) was determined by flow cytometry. The binding was specific, illustrated by the fact that an excess of non-labeled fMLF (0.1 μM final concentration) but not of WKYMVM reduced the binding of FITC-FLNLNPTL (data not shown). The presence of BVT173187 (from 0.3 μM up to 1 μM final concentration) during
Fig. 3. BVT173187 inhibits the activity triggered by agonists that bind C5aR or CXCR. Neutrophils were incubated with BVT173187 (0.3 μM) or cyclosporine H (0.3 μM) at 37 °C for 5 min and then challenged with IL-8 (100 ng/ml; A and summarized in C) or C5a (100 ng/ml; B and summarized in D). The amounts of superoxide (peak values) produced are expressed as percent of controls (IL-8 and C5a) without any antagonist (mean values ± S.E.M.). The results from six experiments are analyzed using one-way ANOVA test, followed by Bonferroni’s multiple comparison test. Probabilities of less than 0.05 were considered significant.

Fig. 4. BVT173187 inhibits mobilization of complement receptor 3 (CR3) induced by the FPR1 agonist fMLF. Surface exposure of CR3 analyzed by antibody binding and flow cytometry and expressed as increase (percent) of the mean fluorescence intensity (MFI) compared to control cells incubated without agonist. Neutrophils were activated by fMLF (10 nM) or WKYMVM (20 nM) in the presence and absence of either BVT173187 (0.3 μM) or cyclosporine H (1 μM). (A) Fluorescence curves from one representative experiment. (B) The results from three experiments are summarized (mean values ± S.E.M.) and analyzed using a one-way ANOVA test followed by Bonferroni’s multiple comparison test. Probabilities of less than 0.05 were considered significant.
the interaction did not affect the neutrophil binding of FITC–FNLPTNL (specific binding with BVT173187; mean fluorescence value = 318, compared to control without any antagonist; mean fluorescence value = 246), whereas the presence of non-labeled formyl peptide inhibited binding totally. Cyclosporin H (from 0.3 μM up to 1 μM final concentration) was also without effect the neutrophil binding of FITC–FNLPTNL (data not shown).

The reversibility of the inhibitory effect of BVT173187 was determined in an experimental set up where a dense population of neutrophils (10^7/ml) were incubated with the inhibitor, and the cells (together with the inhibitor) were then diluted 1/100 and the response to the FPR1 agonist fMLF was determined. The inhibitory effect was fully reversible and this was true also when cyclosporine H replaced BVT173187 (data not shown).

3.5. The BVT173187 inhibitor is not inactivated by the MPO–H2O2–system

Many agonists that trigger an activation of the neutrophil NADPH-oxidase, trigger also their own inactivation through a mechanism that involves the neutrophil granule enzyme myeloperoxidase (MPO). MPO uses hydrogen peroxide (H2O2) to catalyze the reaction that causes the inactivation of agonist at the same time [10,28]. The same type of reaction could possibly inactivate also inhibitors, and we have determined the stability of BVT173187 to the MPO/H2O2 system. We found that the inhibitory properties of BVT173187 were retained after an exposure to MPO/H2O2 (Fig. 6), and this was true also for cyclosporine H (data not shown).

3.6. Changes in FPR1/FPR2 ratio induced by a subset of other small molecules

We determined the effects of 44 small molecules with some structural resemblance to the described inhibitor BVT173187, and we used the neutrophil NADPH-oxidase activity triggered by fMLF and WKYMVM as the read out system. None of the tested compounds had any agonistic effects; a set of 5 compounds were identified which affected the ratio between the responses induced by fMLF and WKYMVM (marked with an * in Supplementary Fig. 2). The ratios obtained are given together with the structure of the compound used (Supplementary Fig. 2), and all but one of the compounds that were active, had in common that they decreased the ratio, meaning that FPR1 was the preferred receptor. The exception being BVT016895, which inhibited both FPR1 and FPR2. All compounds with any activity were, however, much less potent than the receptor selective than BVT173187. The majority of compounds were without effect on the ratio between the responses induced by fMLF and WKYMVM.

4. Discussion

In this study we show that a non-peptide small molecule, 3,5-dichloro-N-(2-chloro-5-methyl-phenyl)-2-hydroxy-benzamide (BVT173187), inhibits FPR1 signaling. Due to the crucial roles of the FPR receptors in inflammatory reactions as well as in regulation of pain [29,30], significant effort has been made to identify receptor agonists as well as inhibitors/antagonists for the members of this receptor family. To date, very many agonists have been identified [3,6,10] whereas only a very limited number of inhibitors/antagonists have been described and that is true both for FPR2 [20,31], and for FPR1 [13,14,17,32,33]. The results from our small molecule screen [10] clearly show that the number of hits when the experiments are performed in antagonist mode is very limited compared to when it is performed in agonist mode. The reason for this is not known, but it is notable that the most potent inhibitors/antagonists (the PBP10 peptide for FPR2 and cyclosporine H for FPR1) have no obvious structural similarities with any of the known FPR1 or FPR2 agonists (for a review see [6]). There is, thus, no amino acid sequence similarity between the cyclic undecapeptide cyclosporine H and known FPR1 agonists, but this does not exclude the possibility that there might be similarities in the three dimensional features of these molecules and of the new inhibitor BVT173187. It might also be that they share important physicochemical properties. From a structural point of view it is worth to notice that a recently described FPR1 antagonists [15] contain a salicylic amide moiety in which the orthophenol is reported to be crucial. This is in accordance to our results showing that molecules included in our study that lacked the orthophenol (marked in Fig. 2A) displayed no antagonist activity what so ever (Supplementary Fig. 2).

The formyl peptide receptors, FPR1 and FPR2, share a large sequence identity and an even higher amino acid similarity (http://www.uniprot.org/blast/uniprot;20120129424N724LV7alignment=24), they induce almost indistinguishable cellular responses, and there are antagonists that can bind to both receptors. We have earlier shown that the hexapeptide WKYMVM selectively activates
neutrophils via FPR2 [25]. This peptide therefore emerges as a very useful agonist to study this receptor, without interference emanating from the activation of FPR1. An exchange of the carboxy terminal l-methionine in WKYMVM for the D-isomer generates a peptide that increases its binding to FPR2 but at the same time the d-methionine containing peptide is an agonist for FPR1. We used this dual agonist to show that the inhibition by BVT173187 was not linked to the agonist but to the receptor. This conclusion is drawn from the fact that the response induced by WKYMVM in stable transfectants expressing FPR1 was inhibited by BVT173187, but no such effect was obtained when the cells instead expressed FPR2. The same inhibitory profile was obtained also when the FPR1 agonist fMLF was replaced by another agonist, fMIF, that binds to the same receptor, and when WKYMVM was replaced by the newly described FPR2 agonist PSMe2, a bacterial peptide produced by virulent community associated methillin resistant strains of Staphylococcus aureus [19]. The dual agonist WKYMVM binds FPR1; this receptor is, however, not used unless signaling through FPR2 is blocked [34]. We used the earlier mentioned FPR2-specific inhibitor (gelsolin derived peptide PBPI0) to confirm the receptor preference for BVT173187. We show that the WKYMVM-induced neutrophil response was inhibited by BVT173187 only when the FPR2 signaling pathway was blocked; suggesting a specificity of the new inhibitor for FPR1 and that activation by WKYMVM involves different parts (binding sites) on FPR1 and FPR2, one being sensitive and the other insensitive. The same type of inhibition profile is obtained when the other FPR1-selective antagonists are used to inhibit the neutrophil response with WKYMVM as the agonist [14,34]; that is, they inhibit the response only when signaling through FPR2 is blocked. It should be noticed that the neutrophil response to C5a and IL-8 was partly inhibited by BVT173187, suggesting a somewhat broader specificity. Several Staphylococcus aureus strains produce and secrete a protein (CHIPS) that functions as a receptor antagonist and inhibits neutrophil chemotaxis [35,36]. This naturally occurring neutrophil receptor antagonist has the same inhibitory profile as BVT173187 as it has no effect on FPR2 but inhibits the neutrophil activity through both FPR1 and C5aR. This suggests that these two receptors have some type of common denominator that is missing in FPR2. It is clear that FPR1 and FPR2 differ not only in their extracellular agonist binding domains, but also in the intracellular signaling domains [20], and it is not clear at what level BVT173187 actually inhibit FPR1. The fact that BVT173187 has no effect on agonist binding (measured as inhibition of binding of an FITC-labeled FPR1 agonist) suggests that the inhibitory effect could be linked to signaling rather to a competitive binding. This gains support from the fact that molecules very similar to BVT173187, have been shown to affect intracellular signaling. This is obvious from results presented with IMD-0354, a low molecular weight compound with anti-allergic and anti-inflammatory activities, sharing the salicylalanilide moiety (Fig. 2A indicated by the extended frame) with BVT173187, showing that this is a potent inhibitor of NF-kB [37]. In accordance with this, another membrane permeable small molecule inhibitor with structural similarities with BVT173187, naphthol AS-E, has been shown to inhibit protein–protein interactions of importance for gene transcription [38]. It should, however, be noticed that the same result (no inhibition in binding) was obtained when BVT173187 was replaced by cyclosporine H, a receptor antagonist shown earlier to inhibit agonist binding to an FPR1 containing membrane fraction [27]. The binding technique used is obviously not suited to determine an inhibition in binding when the binding affinity of the agonist is much higher than that of the antagonist.

Taken together, we describe a non-peptide small molecule, BVT173187, which is a selective FPR1 inhibitor, that could be used as a tool for further characterization of the FPR family in host defense and inflammation and the BVT173187 molecule may also be used as a starting point for the development of a new class of inhibitors that could be used to regulate the inflammatory process.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bcp.2012.02.024.

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