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New 'chemical probes' to examine the role of the hFPRL1 (or ALXR) receptor in inflammation

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Abstract—We report the development of the novel N-substituted benzimidazole **11** as a potent and selective human formyl peptide receptor-like 1 (hFPRL1) agonist. This compound and its less active enantiomer **12** were identified as useful tools for studying receptor function in vitro.

The human formyl-peptide receptor-like 1 (hFPRL1 or ALXR) belongs to a family of G_i-protein-coupled receptors that is predominantly expressed on neutrophils and monocytes.[1] Recent literature suggests that its role in inflammation is complex; both pro- and anti-inflammatory functions have been reported. hFPRL1 is modulated by a wide variety of endogenous and exogenous ligands; for instance, *N*-formylated peptides such as the tripeptide *N*-formyl-Met-Leu-Phe (fMLP) are agonists and chemo-attractants for the receptor.[2] Since such peptide ligands are primary products of bacterial (or mitochondrial) translation, it has been postulated that hFPRL1 evolved as part of a defense mechanism against bacterial infections.[3] hFPRL1 has also been reported to mediate important pro-inflammatory processes in various neurodegenerative states. The 42-amino acid form of amyloid β ($A\beta_{42}$), which is believed to take part in the pathogenesis of Alzheimer's disease, can cause neuronal damage via recruitment and activation of mononuclear phagocytes (microglia) in the brain.[4] $A\beta_{42}$ is also a chemotactic agonist for hFPRL1 which suggests that the receptor plays a role in this disease. Interestingly, a 24-amino acid peptide called humanin blocks the cytopathic effect of $A\beta_{42}$ in neuroblast cells by interaction with hFPRL1.[5] In a different case serum amyloid A, a precursor to the amyloid fibril deposits of amyloidosis, is also a chemotactic hFPRL1 ligand (tested in transfected HEK 293 cells).[6] Moreover, the neurotoxic prion protein fragment $PrP_{106–126}$, which behaves in a similar manner to the pathologic isoform of prion protein, chemoattracts and separately induces cytokine secretion in human monocytes through interaction with hFPRL1.[7]

Separate studies suggest an integral role of hFPRL1 in host response to HIV-1 infection. Several synthetic peptide domains of HIV-1 envelope proteins have been shown to activate hFPRL1 in vitro, resulting in various downstream events.[8] Among these are the attenuation of cell response to some chemokines, which likely occurs via a cross-desensitization and down-regulation mechanism of the receptors CCR5 and CXCR4 (both in human monocytes).

In contrast to the reported pro-inflammatory effects of hFPRL1 modulators, Serhan and coworkers have described anti-inflammatory effects of Lipoxin A_4 (LXA_4) and analogs, another class of hFPRL1 agonists. The lipoxins, endogenously produced metabolites of arachidonic acid, have been shown to promote resolution of acute inflammation through agonism of hFPRL1.[9] This interaction results in the attenuation of immune cell chemotactic response towards pro-inflammatory mediators.[10] There is also a growing body of evidence suggesting that Annexin A1 (or Annexin-derived metabolites), a glucocorticoid-regulated protein, inhibits

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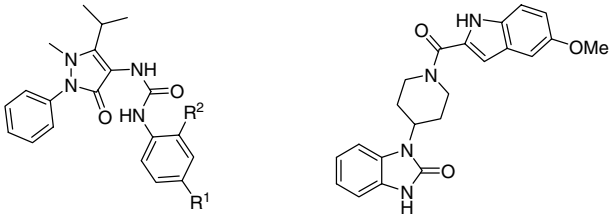
leukocyte migration/activation through agonism of hFPRL1.[11] In summary, hFPRL1 is clearly involved in inflammatory and pathogenic processes and therefore is a target of interest for drug discovery. Further studies will be required to understand how interference with hFPRL1 signaling might be of therapeutic benefit. We believe that small molecules, which selectively interact with hFPRL1, will provide valuable tools for such investigations.

Recently, we reported the discovery of pyrazolone **1** as a potent and selective agonist of hFPRL1 (Fig. 1).[12] This molecule showed dose-dependent inhibition of polymorphonuclear human neutrophil (PMN) chemotaxis, whereas the hFPRL1-inactive isomer **2** did not have an effect.

Compound **1** also demonstrated promising efficacy in a murine model of inflammation. Given that structurally diverse agonists of hFPRL1 induce divergent downstream effects (as outlined above), we continued to study the pyrazolones and started to develop a second series of agonists. The goal was to generate another pair of active and inactive molecules, which are structurally related to each other, but diverse from the pyrazolones.

Our screening campaign using an aequorin-based bioluminescence assay (Ca^{2+} flux) revealed compound **3** (Fig. 1) to be a relatively weak agonist of hFPRL1 (EC_{50} = 6.39 μM).[13] Despite its moderate potency, this molecule seemed an attractive starting point for further optimization, since it showed modest selectivity over hFPR (no activity up to 10 μM) and potential for structural diversification in a modular fashion.



1: R^1 = Cl; R^2 = H
hFPRL1 (EC_{50}) = 0.044 μM
2: R^1 = H; R^2 = Cl
hFPRL1 (EC_{50}) >10 μM

3: hFPRL1 (EC_{50}) = 6.39 μM

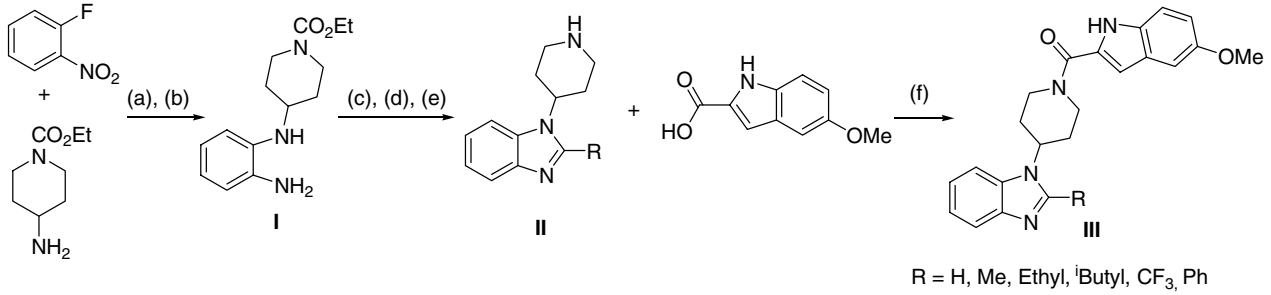
Figure 1.

Compounds were prepared according to the synthetic sequences outlined in Schemes 1 and 2. The benzimidazole core was constructed via S_NAr-type chemistry. For compounds **4–9,** ethyl 4-aminopiperidine-1-carboxylate was added to 1-fluoro-2-nitrobenzene by heating in the presence of potassium carbonate, which resulted in the corresponding 2-aminonitrobenzene derivative. This intermediate was converted to bis-aniline **I** under modified *Béchamp* conditions (Fe°, AcOH). Mono-acylation of this bis-aniline followed by dehydration of the resulting amide under microwave irradiation resulted in the corresponding benzimidazoles. The carbamate function was subsequently removed under basic conditions to give the unprotected piperidines (generic structure **II**) and amide coupling under standard conditions led to the final compounds. Pyrrolidine-containing compounds **10–18** were prepared using an analogous strategy according to Scheme 2.[14] Final compounds were in excess of 95% purity as measured by HPLC and ^1H NMR.[15]

The effect of structural modifications to the benzimidazolone moiety of compound **3** was investigated first. Formal replacement of this group by a benzimidazole resulted in a negligible increase in potency (compound **4**, Table 1). However, the transition from benzimidazolone to the benzimidazole core allowed modifications at C(2), which proved beneficial for potency. As shown in Table 1, benzimidazoles with small alkyl substituents such as ethyl at C(2) exhibited submicromolar activity. Increasing the size of this group from an ethyl group to ^{i}Bu, CF_3 or Ph did not result in further improvements. The ethyl-substituted benzimidazole fragment was therefore selected for subsequent efforts.

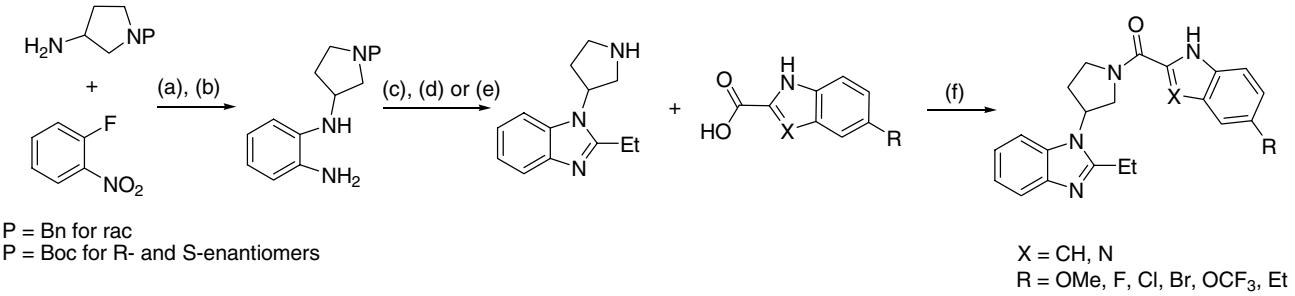
We then turned our attention to the piperidine linker element (Table 2) and observed that contraction of the piperidine ring of **6** to a pyrrolidine **10** (racemic) resulted in a five fold increase in potency. Application of the synthetic sequence (Scheme 2) utilizing enantiomerically pure 3-aminopyrrolidines yielded the individual antipodes **11** and **12**, of which the *R*-isomer **11** proved significantly more potent than *S*-enantiomer **12**.

The effects of compounds with modified indole groups on hFPRL1-mediated Ca^{2+} mobilization are summa-



R = H, Me, Ethyl, ^{i}Butyl, CF_3, Ph

Scheme 1. Reagents and conditions: (a) K_2CO_3, DMF, 100 °C, 96%; (b) Fe°, THF/H_2O/AcOH (5:5:1), 68%; (c) RCOCl, NEt_3, CH_2Cl_2, 0 °C or trifluoroacetic anhydride, pyridine, CH_2Cl_2; (d) CH_3COOH, 150 or 180 °C, microwave irradiation, 15 min; (e) H_2NNH_2, KOH, 2-propanol, 85 °C or 2 N NaOH, reflux, 29–87% for 3 steps; (f) EDC, DMF or CH_2Cl_2, 29–88%.



Scheme 2. Reagent and conditions: (a) K_2CO_3, DMF, 60 °C, 46–65%; (b) Pd/C, H_2, MeOH, 90–97%; (c) propionyl chloride, NEt_3, CH_2Cl_2, 0 °C to room temperature, 59% for rac; (d) Pd/C, NH_4CO_2H, EtOH, reflux, 70% for rac; (e) HCl (2 equiv), dioxane, 80 °C, 80% over 2 steps for 'R', 97% over 2 steps for 'S'; (f) EDC, DMF or CH_2Cl_2, 32–86%.

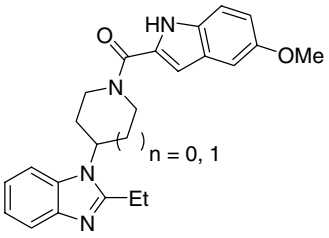
Table 1. Influence of benzimidazoles **4**–**9** (general structure **III**, Scheme 1) on hFPRL1-mediated Ca^{2+} mobilization

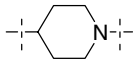
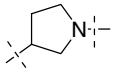
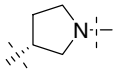
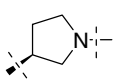
Compound	R	hFPRL1 EC_{50}[a] (μM)	hFPR EC_{50}[a] (μM)
4	H	5.10 ± 0.73	>10
5	Me	1.02 ± 0.27	>10
6	Et	0.69 ± 0.28	>10
7	^{i}Bu	0.83	>10
8	CF_3	1.52	>10
9	Ph	2.71	>10

[a] EC_{50} values have been determined by Ca^{2+} flux in CHO recombinant cells co-expressing hFPRL1, $G_{\alpha 15}$ protein, and aequorin. Values lacking standard deviation are the average of two data points.

rized in Table 3. A brief survey centering around the nature of the C(5)-substituent revealed that C(5) OMe is optimal for in vitro potency. The C(5)-bromo and -ethyl substituted analogs **15** and **17** were the most potent alternatives, but lost significant activity (EC_{50} =

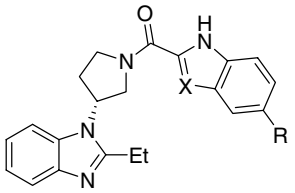
Table 2. Influence of amine ring modification on hFPRL1-mediated Ca^{2+} mobilization



Compound	Amine ring	hFPRL1 EC_{50}[a] (μM)	hFPR EC_{50}[a] (μM)
6		0.69 ± 0.28	>10
10		0.13	>10
11		0.034 ± 0.010	>10
12		3.50	>10

[a] EC_{50} values have been determined by Ca^{2+} flux in CHO recombinant cells co-expressing hFPRL1, $G_{\alpha 15}$ protein, and aequorin. Values lacking standard deviation are the average of two data points.

Table 3. Influence of indole substituents/replacement on hFPRL1-mediated Ca^{2+} mobilization



Compound	R	X	hFPRL1 EC_{50}[a] (μM)	hFPR EC_{50}[a] (μM)
11	OMe	CH	0.034 ± 0.010	>10
13	F	CH	1.49	>10
14	Cl	CH	14.1	>10
15	Br	CH	0.81	>10
16	OCF_3	CH	12.2	>10
17	Et	CH	0.27	>10
18	OMe	N	1.87	>10

[a] EC_{50} values have been determined by Ca^{2+} flux in CHO recombinant cells co-expressing hFPRL1, $G_{\alpha 15}$ protein, and aequorin. Values lacking standard deviation are the average of two data points.

0.81 and 0.27 μM, respectively) compared to **11**. Similarly, the indole core appeared to be necessary for efficient downstream signaling; for example, the corresponding benzimidazole analog **18** proved less active (EC_{50} = 1.87 μM). Other indole replacements have been studied in a slightly different series, but no equipotent replacement of the 5-methoxy-indole unit has been identified (data not shown). None of the tested compounds showed significant activity against hFPR below 10 μM concentrations.[16]

In conclusion, optimization of the initial benzimidazolone **3** (EC_{50} = 6.39 μM) for hFPRL1-mediated activity in a cell-based system yielded benzimidazole **11** (EC_{50} = 0.034 μM) and its enantiomer **12**, which was roughly 100-fold less active (EC_{50} = 3.50 μM). Our next goal was to utilize these two molecules alongside the initially reported pyrazolones (**1** and **2**) in order to interrogate the functional consequence of hFPRL1 activation. We first tested the impact of these compounds on polymorphonuclear human neutrophil (PMN) chemotaxis.

For this, freshly collected human PMN's were preincubated for 45 min with compound or vehicle and subse-

Table 4. Comparison of Ca^{2+} mobilization and inhibition of neutrophil (PMN) migration by compounds **1**, **2**, **11**, and **12**

Compound	Ca^{2+} flux	Migration[a]	
	hFPRL1 EC_{50}[b] (μM)	fMLP IC_{50} (μM)	IL-8 IC_{50} (μM)
11	0.034 ± 0.010	0.22 ± 0.22	1.71 ± 2.19
12	3.50	>10	>10
1	0.044 ± 0.005	0.64 ± 0.20	0.24 ± 0.05
2	>10	>10	>10

[a]Mean IC_{50} (μM) values determined using PMNs from at least three donors. fMLP or IL-8 were used as chemo-attractants (each 10 nM). Final [DMSO] = 0.1%.
[b] hFPRL1 activity for comparison.

quent migration against a gradient of chemo-attractant (either IL-8 or fMLP) was followed by fluorescence measurement according to Martin and coworkers.[17] The result of this experiment is summarized in Table 4. For both structural series, it is clear that the hFPRL1 agonist inhibits neutrophil migration, whereas the structurally related isomer/enantiomer, which does not modulate hFPRL1, had no effect on migration. These results strongly suggest that the observed inhibition of neutrophil migration, which is anti-inflammatory in nature, is indeed hFPRL1-mediated.

We then tested the effect of the same four molecules on secretion of IL-6 from human whole blood.[18] In this experiment, 50% human blood was incubated with IL-1β both in the presence and absence of test compound for 18 h. The secretion of IL-6 was then detected by fluorescence measurement. As exemplified in Figure 2, we observed that the hFPRL1 agonists **1** and **11** stimulate IL-6 secretion above levels induced by IL-1β alone (defined as 100 POC). The less potent analogs did not cause an effect. This stimulatory effect is clearly pro-inflammatory in nature.

In summary, optimization of a weakly potent hFPRL1 agonist **3** resulted in a structurally novel, optically active hFPRL1 agonist **11** with an in vitro activity well below micromolar concentrations (EC_{50} = 34 nM). Its enantiomer **12** was about 100-fold less active in the Ca^{2+} mobilization assay. Subsequently, the pair of enantiomers was studied in functional cell-based assays of inflammation. We have found that the hFPRL1-active enantiomer **11** inhibits neutrophil migration in a dose-dependent manner, while its 'hFPRL1-inactive' analog **12** did not show an effect. In contrast, we observed that the active molecule significantly stimulates IL-6 secretion in human whole blood; again, the compounds that did not modulate

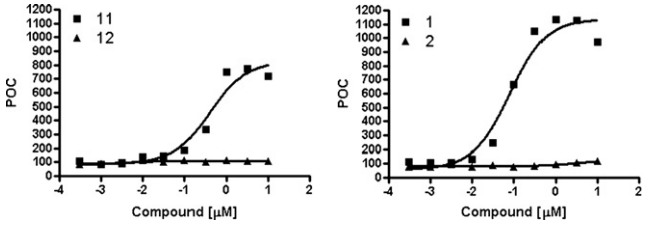


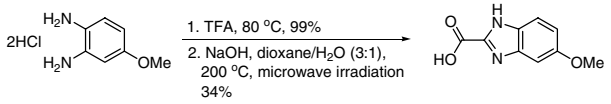
Figure 2. Stimulation of IL-6 secretion by hFPRL1 agonists in 50% human whole blood.

hFPRL1 had no effect. The previously reported, structurally diverse pyrazolones (**1** and **2**) showed the same trend in the migration and cytokine secretion assays. From these results, we conclude that the anti-inflammatory inhibition of neutrophil migration of compounds **1** and **11** as well as their pro-inflammatory stimulation of IL-6 secretion are likely to be hFPRL1-mediated. Clearly, more studies will be required to understand the complex role of hFPRL1 in vivo and how it might serve as a therapeutic target. We believe that the two pairs of structurally distinct active and 'inactive' hFPRL1 agonists (benzimidazoles **11** and **12**; pyrazolones **1** and **2**) will provide excellent tools to study this receptor in more detail.

References and notes

1. For reviews, see (a) Migeotte, I.; Communi, D.; Parmentier, M. *Cytokine Growth Factor Rev.* **2006**, *17*, 501; (b) Selvatici, R.; Falzarano, S.; Mollica, A.; Spisani, S. *Eur. J. Pharmacol.* **2006**, *534*(1–3), 1; (c) Fu, H.; Karlsson, J.; Bylund, J.; Movitz, C.; Karlsson, A.; Dahlgren, C. *J. Leukocyte Biol.* **2006**, *79*, 247; (d) Iribarren, P.; Zhou, Y.; Hu, J.; Le, Y.; Wang, J. M. *Immunol. Res.* **2005**, *31*, 165; (e) Le, Y.; Zhou, Y.; Tao, H.; Wang, J. M. *Clin. Exp. Allergy Rev.* **2004**, *4*(2 suppl), 155; (f) Le, Y.; Murphy, P. M.; Wang, J. M. *Trends Immunol.* **2002**, *23*, 541; (g) Le, Y.; Oppenheim, J. J.; Wang, J. M. *Cytokine Growth Factor Rev.* **2001**, *12*, 91.
2. Ye, R. D.; Cavanagh, S. L.; Quehenberger, O.; Prossnitz, E. R.; Cochrane, C. G. *Biochem. Biophys. Res. Commun.* **1992**, *184*, 582.
3. For a lead reference, see Devchand, P. R.; Arita, M.; Hong, S.; Bannenberg, G.; Moussignac, R.-L.; Gronert, K.; Serhan, C. N. *FASEB J.* **2003**, *17*, 652.
4. Le, Y.; Gong, W.; Tiffany, H. L.; Tumanov, A.; Nedospasov, S.; Shen, W.; Dunlop, N. M.; Gao, J.-L.; Murphy, P. M.; Oppenheim, J. J.; Wang, J. M. *J. Neurosci.* **2001**, *21 RC123*, 1.
5. Ying, G.; Iribarren, P.; Zhou, Y.; Gong, W.; Zhang, N.; Yu, Z.-X.; Le, Y.; Cui, Y.; Wang, J. M. *J. Immunol.* **2004**, *172*, 7078.
6. Su, S. B.; Gong, W.; Gao, J.-L.; Shen, W.; Murphy, P. M.; Oppenheim, J. J.; Wang, J. M. *J. Exp. Med.* **1999**, *189*, 395.
7. Le, Y.; Yazawa, H.; Gong, W.; Yu, Z.; Ferrans, V. J.; Murphy, P. M.; Wang, J. M. *J. Immunol.* **2001**, *166*, 1448.
8. See [1g] and references therein.
9. For a recent review, see (a) Chiang, N.; Serhan, C. N.; Dahlén, S.-E.; Drazen, J. M.; Hay, D. W. P.; Rovati, G. E.; Shimizu, T.; Yokomizo, T.; Brink, C. *Pharmacol. Rev.* **2006**, *58*, 463; (b) Fiore, S.; Ryeom, S. W.; Weller, P. F.; Serhan, C. N. *J. Biol. Chem.* **1992**, *267*, 16168; (c) Serhan, C. N. *Prostaglandins Other Lipid Mediat.* **2002**, *68–69*, 433; (d) Fiore, S.; Maddox, J. F.; Perez, H. D.; Serhan, C. N. *J. Exp. Med.* **1994**, *180*, 253; (e) Takano, T.; Fiore, S.; Maddox, J. F.; Brady, H. R.; Petasis, N. A.; Serhan, C. N. *J. Exp. Med.* **1997**, *185*, 1693; (f) Maddox, J. F.; Hachicha, M.; Takano, T.; Petasis, N. A.; Fokin, V. V.; Serhan, C. N. *J. Biol. Chem.* **1997**, *272*, 6972; (g) Brenner, P. S.; Krakauer, T. *Curr. Med. Chem. Anti-Inflammatory & Anti-Allergy Agents* **2003**, *2*, 274; (h) Christie, P. E.; Spur, B. W.; Lee, T. H. *Am. Rev. Respir. Dis.* **1992**, *145*, 1281; (i) Fiorucci, S.; Wallace, J. L.; Mencarelli, A.; Distrutti, E.; Rizzo, G.; Farneti, S.; Morelli, A.; Tseng, J.-L.; Suramanyam, B.; Guilford, W. J.; Parkinson, J. F. *Proc. Natl. Acad. Sci.* **2004**, *101*, 15736.

10. (a) Machado, F. S.; Johndrow, J. E.; Esper, L.; Dias, A.; Bafica, A.; Serhan, C. N.; Aliberti, J. *Nat. Med.* **2006**, *12*, 330; (b) Bandeira-Melo, C.; Bozza, P. T.; Diaz, B. L.; Cordiero, R. S. B.; Jose, P. J.; Martins, M. A.; Serhan, C. N. *J. Immunol.* **2000**, *164*, 2267; (c) Soyombo, O.; Spur, B. W.; Lee, T. H. *Allergy* **1994**, *49*, 230; (d) Lee, T. H.; Horton, C. E.; Kyan-Aung, U.; Haskard, D.; Crea, A. E.; Spur, B. W. *Clin. Sci.* **1989**, *77*, 195; (e) Ramstedt, U.; Serhan, C. N.; Nicolaou, K. C.; Webber, S. E.; Wigzell, H.; Samuelsson, B. *J. Immunol.* **1987**, *138*, 266.
11. (a) Gavins, F. N. E.; Yona, S.; Kamal, A. M.; Flower, R. J.; Perretti, M. *Blood* **2003**, *101*, 4140; (b) Perretti, M.; Chiang, N.; La, M.; Fierro, I. M.; Marullo, S.; Getting, S. J.; Solito, E.; Serhan, C. N. *Nat. Med.* **2002**, *8*, 1296.
12. Bürli, R. W.; Xu, H.; Zou, X.; Muller, K.; Golden, J.; Frohn, M.; Adlam, M.; Plant, M. H.; Wong, M.; McElvain, M.; Regal, K.; Viswanadhan, V.; Tagari, P.; Hungate, R. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3713.
13. Chinese hamster ovarian cells (CHO) were stably co-transfected with aequorin, $G_{\alpha 15}$ protein, and hFPRL1. In this assay, receptor activation by an agonist induces release of cytosolic Ca^{2+} storage. The increased cytosolic [Ca^{2+}] was monitored by luminescence emitted by aequorin.
14. All indole 2-carboxylates were obtained from commercial sources. The benzimidazole 2-carboxylate was prepared from 4-methoxy-*o*-phenylenediamine dihydrochloride:



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15. HPLC (Phenomenex, MAX RP, 4μ, 50× 2.0 mm, 1 mL/min, A: 0.1% TFA in H_2O, B: 0.1% TFA in MeCN, 10–100% B in 10 min), ^1H NMR (Bruker, 400 MHz) in DMSO-d_6 *or* $CDCl_3$.
16. For the counter screen, CHO cells were stably transfected with human formyl peptide receptor (hFPR), aequorin, and $G_{\alpha 15}$ protein. The assay was performed as described.
17. Frevert, C. W.; Wong, V. A.; Goodman, R. B.; Goodwin, R.; Martin, T. R. *J. Immunol. Methods* **1998**, *213*, 41.
18. Heparinized whole blood (0.10 mL) was plated in a 96-well polystyrene flat bottom micro titer tissue culture plate and pre-incubated with 4× compound for 1 h at 37 °C, 5% CO_2 in a tissue culture incubator. 4× IL-1β (300 pM final concentration) or control was then added and the plates were incubated at 37 °C, 5% CO_2 in a tissue culture incubator for 18 h. The final percent concentration whole blood was 50%. The final DMSO concentration was 0.1%. The total volume for all assays was 200 μL. After 18 h, supernatant/plasma was collected and assayed immediately or frozen at −80 °C using IL-6 detection MSD ECL based plates. Fifty microliters of supernatant was added to MSD plates and incubated for 1 h on a shaker. The plates were then washed once with 200 μL of PBS/0.1% Tween. Twenty-five microliters of detection antibody diluted in antibody diluent (1 μg/mL), and 125 μL of 2× Read Buffer P were added, and the plates were incubated for 1 h on a shaker, protected from light. ECL was measured using the SECTOR HTS Imager (MSD, Gaithersburg, MD).