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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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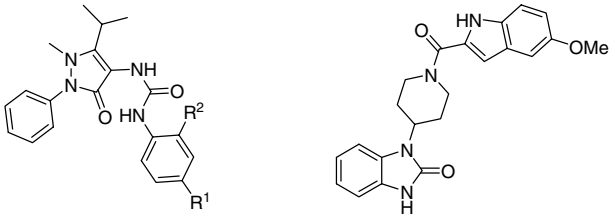
leukocyte migration/activation through agonism of hFPRL1.[11] In summary, hFPRL1 is clearly involved in inflammatory and pathogenic processes and therefore is a target of interest for drug discovery. Further studies will be required to understand how interference with hFPRL1 signaling might be of therapeutic 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

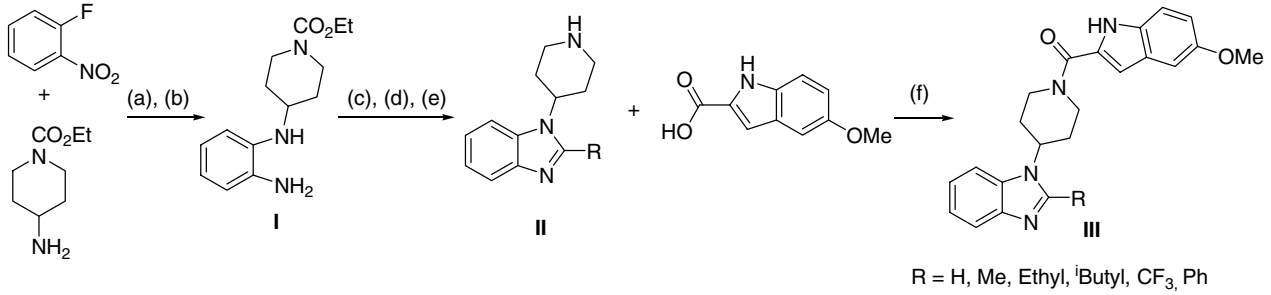
Figure 1.

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%.