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Substituted Pyridazin-3(2*H*)-ones as Highly Potent and Biased Formyl Peptide Receptors Agonists

Girdhar Singh Deora,[†#] Cheng Xue Qin,[‡Δ#] Elizabeth A. Vecchio,[‡] Aaron J. DeBono,[†] Daniel L. Priebbenow,[†] Ryan M. Brady,[†] Julia Beveridge,[†] Silvia C. Teguh,[†] Minh Deo,[‡] Lauren T. May,[◊] Guy Krippner,[‡] Rebecca H. Ritchie,*[‡ΔΠ] Jonathan B. Baell*[†§]

[§]School of Pharmaceutical Sciences, Nanjing Tech University, Nanjing, 211816, People's Republic of China

[†]Medicinal Chemistry, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, Victoria 3052, Australia

[‡]Baker Heart and Diabetes Institute, 75 Commercial Road, Melbourne, Victoria 3004, Australia

[Δ]Department of Pharmacology and Therapeutics, University of Melbourne, Melbourne, Victoria 3010, Australia

[◊]Drug Discovery Biology, Monash Institute of Pharmaceutical Science, Monash University, Parkville, Vic 3052, Australia

[Π]Department of Diabetes (Central Clinical School), Monash University, Melbourne, VIC 3004, Australia.

KEYWORDS: Biased GPCR agonist, Formyl peptide receptor, Biased agonism, Pyridazinones, ERK1/2

ABSTRACT: Herein we describe the development of a focused series of functionalized pyridazin-3(2*H*)-one-based formyl peptide receptors (FPRs) agonists which demonstrate high potency and biased agonism. The compounds described demonstrated biased activation of pro-survival signaling - ERK1/2 phosphorylation – through diminution of the detrimental FPR1/2-mediated intracellular calcium (Ca_i^{2+}) mobilization. Compound **50** showed an EC_{50} of 0.083 μM for phosphorylation of ERK1/2 and an approximate 20-fold bias away from Ca_i^{2+} mobilization at the hFPR1.

INTRODUCTION

Formyl peptide receptors (FPR) are a group of Class A G-protein-coupled receptors (GPCRs) involved in the regulation and resolution of inflammation.[1, 2] They have attracted significant scientific attention as novel therapeutic targets for a range of inflammatory diseases including arthritis, myocardial infarction and Alzheimer's disease. There are 3 isoforms of human FPRs, hFPR1, hFPR2, and hFPR3.[3] These isoforms have been shown to interact with a range of structurally–diverse ligands including proteins, peptides, lipids and small-molecules. Interestingly, these ligands can mediate opposing biological responses. For example, bacterial-derived *N*-formyl peptides mediate pro-inflammatory responses, whereas annexin-A1 promotes anti-inflammatory signaling and is cardioprotective.[4] This cannot be explained by receptor subtype selectivity, but rather is proposed to arise from a new paradigm-shifting phenomenon called biased signaling.[5, 6]

Biased agonism reflects the ability of a ligand to stabilize different active GPCR conformational states, leading to the engagement of an alternative subset of intracellular effectors and distinct cellular outcomes.[5-7] Thus, the development of biased agonists offers the elusive therapeutic opportunity to promote beneficial signal transduction in the absence of on-target adverse effects,[8] the utility of which is already apparent in the clinical context of acute heart failure.[9] Our recent work revealed the first proof-of-concept evidence that FPRs exhibit biased signaling, potentially favoring cardiomyocyte survival, associated with beneficial outcomes *in vivo*.[10]

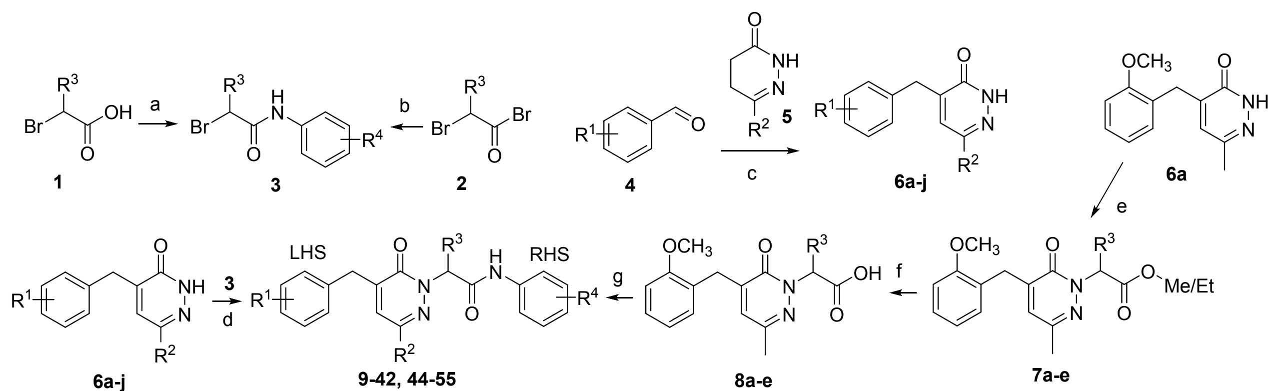
In cardiomyocytes, the reperfusion injury salvage kinase pathways such as extracellular signal-regulated kinase 1/2 (pERK1/2) and Akt signaling are tightly linked to post-ischemic cell survival.[4, 11-13] In contrast, increased intracellular calcium (Ca_i^{2+}) mobilization in the context of myocardial infarction (MI) injury promotes the influx of damaging inflammatory cells and further cardiomyocyte cell death.[4] We have demonstrated[14] that the small-molecule FPR agonist compound **17b** exhibits clear and biased signaling, favoring cell survival mechanisms whilst eschewing potentially deleterious pathways as a consequence of its ~30-fold bias away from intracellular calcium (Ca_i^{2+}) mobilization at both hFPR1 and hFPR2 relative to the reference ligand compound **43**.[10, 15] Interestingly, this biased signaling profile correlated with superior *in vitro* and *in vivo* cardioprotection of compound **17b** compared with the balanced, non-biased compound **43**.[10, 16]

Biased signaling structure-activity relationship (SAR) for the pyridazin-3(2*H*)-one chemotype has not previously been investigated. Here we report our focused medicinal chemistry efforts to establish clear SAR with the aim to generate potent FPR agonists biased away from intracellular calcium mobilization. This understanding will ultimately facilitate the future development of biased FPR agonists for the treatment of MI.

RESULTS AND DISCUSSION

The substituted pyridazin-3(2*H*)-ones (**9–42** and **44–55**) were synthesized as shown in **Scheme-1**. Multi-step synthetic procedures were employed, which involved construction of RHS bromoanilides (**3a–l**) using a variety of linkers and substituted anilines. For the synthesis of the LHS benzyl pyridazinone scaffold (**6a–j**), a condensation reaction between substituted aldehydes (**4**) and the 4,5-dihydropyridazinone moiety (**5**) were performed.[14] The synthesis of the final compounds (**9–42, 44–55**), was achieved by two synthetic routes, both involving *N*-alkylation at the 2-position of the pyridazinone core. The first route involved the direct *N*-alkylation of LHS moiety with the RHS moiety, while the second involved a 3-step route where the *N*-alkylation was performed employing a bromoester, which was then hydrolyzed to reveal the carboxylic acid motif which was subsequently coupled with an aniline under standard amide-coupling conditions.

Scheme 1. Representative synthetic schemes for the intermediates and final compounds.[a,b]



[a]**Reagents and conditions**. (a) i) COCl2, DMF (cat.), CH_2Cl_2, 0 °C, 1 h, ii) substituted anilines, Et_3N, 0 °C–r.t., 1–2 h; (b) substituted anilines, Et_3N, 0 °C–r.t. 4–6 h; (c) KOH/ethanol (5% w/v), reflux 4–6 h, 22–89%; (d) K_2CO_3, CH_3CN, 60 °C, 16 h, 58–99%; (e) $BrCH(R^3)CO_2R$, K_2CO_3, CH_3CN, 60 °C, 16–20 h, 86–92%; (f) LiOH, THF/H2O (1:1), r.t., 16 h, 37–88%; (g) substituted anilines, EDCI·HCl, HOBt, CH3CN 40 °C, 12–24 h, 4–87%. [b]Detailed experimental procedures and structures of the intermediates are provided in the Supporting Information.

We previously reported the biased nature of compound **17b,** which demonstrated bias away from the potentially detrimental FPR1/2-mediated Ca_i^{2+} mobilization, but retained pro-survival pERK1/2 signaling.[10, 15] These findings initiated further SAR elucidation for compound **17b** to identify more potent compounds with biased agonistic profiles. The synthesized compounds were assessed using Ca_i^{2+} mobilization and pERK1/2 signal readouts. The rational compound elaboration was primarily aimed at the development of a biased agonist, which selectively targets pERK1/2 whilst minimizing Ca_i^{2+} mobilization.

Lead optimization resulted in the synthesis of a focused library of analogues exploring the SAR of this series (**Table 1**). The effects of a variety of substituents at the 2-and 4-positions of the pyridazinone core were explored based on the prototype compound **17b**. The first generation compounds explored the inclusion of various substituents on both the left and right aryl rings. These substituents include fluoro and methoxy groups at different positions on the LHS ring, and halogens, cyano, nitro, methyl, methoxy and ethoxy groups at various positions on the RHS ring. These changes were subsequently introduced with variations at the linker region, which demonstrated significant impacts on the agonistic activity and biased signaling of these compounds.

Compounds with no substitutions on the linker. All discussion on bioactivity refers to pERK1/2 signaling at the hFPR1 and all activity comparisons are made with 17b unless otherwise specified. The initial compounds of this series had no substituents at the α-carbon of the amide linker. Initially, the *meta*-methoxy substituent (as in compound **17b**) was preserved whilst we probed the RHS, for which the effect of a chloro group at the *ortho*-, *meta*- or *para*- positions on the RHS ring was simultaneously determined. The *para*-chloro derivative (**11**) demonstrated a substantial improvement in activity whilst the *ortho*- and *meta*-chloro derivatives (**9**, **10**) led to reduced activity with pEC_{50}values <4.00. Similarly, derivatives with electron-withdrawing groups, including a nitrile (**12–14**), trifluoromethoxy (**15**) and fluoro substituent (**19**, **20**), gave inactive compounds with pEC_{50} values <4.00. The activities of derivatives with halogen-substituents at the *para*-position of the RHS phenyl remained consistent with compound **17b** (with the LHS phenyl bearing a *meta*-methoxy group); these analogues retained activity and selectivity. Interestingly the incorporation of one or two fluoro substituents on the RHS phenyl containing a *para*-chloro group (**34**, **35**) demonstrated a significant loss of activity.

Methyl substituted linker. Compounds that demonstrated improved activity from our initial SAR study were further functionalized with the introduction of a methyl substituent at the α-position of the linker. In all cases, the compounds were tested as racemates.

Table 1. Structures and FPR1/2 activity data of compounds.

Entry	R[1]	R[2]	R[3]	R[4]	pEC$_{50}$ (FPR1)		pEC$_{50}$ (FPR2)	
					pERK1/2 (SEM)	Ca$_i^{2+}$ (SEM)	pERK1/2 (SEM)	Ca$_i^{2+}$ (SEM)
9	2-OCH$_3$	CH$_3$	H	2-Cl	<4.00	<4.00	n.d	n.d.
10	2-OCH$_3$	CH$_3$	H	3-Cl	<4.00	<4.00	n.d	n.d.
11	2-OCH$_3$	CH$_3$	H	4-Cl	5.91 (0.13)	<4.00	n.d	n.d.
12	2-OCH$_3$	CH$_3$	H	2-CN	<4.00	<4.00	n.d	n.d.
13	2-OCH$_3$	CH$_3$	H	3-CN	<4.00	<4.00	n.d	n.d.
14	2-OCH$_3$	CH$_3$	H	4-CN	<4.00	<4.00	n.d	n.d.
15	2-OCH$_3$	CH$_3$	H	4-OCF$_3$	<4.00	<4.00	n.d	n.d.
16	2-OCH$_3$	CH$_3$	H	4-OCH$_3$	5.1 (0.23)	<4.00	n.d	n.d.
17	2-OCH$_3$	CH$_3$	H	4-C$_2$H$_5$	5.3 (0.12)	<4.00	n.d	n.d.
18	2-OCH$_3$	CH$_3$	H	2,4-di-Cl	<4.00	<4.00	n.d	n.d.
19	2-OCH$_3$	CH$_3$	H	2-F,4-Cl	<4.00	<4.00	n.d	n.d.
20	2-OCH$_3$	CH$_3$	H	2,6-di-F, 4-Cl	<4.00	<4.00	n.d	n.d.
21	2-OCH$_3$	**CF$_3$**	H	4-Cl	<4.00	<4.00	n.d	n.d.
22	3-OCH$_3$	CH$_3$	H	4-Cl	5.65 (0.13)	<4.00	n.d	n.d.
23	3-OCH$_3$	CH$_3$	H	4-Br	5.22 (0.13)	<4.00	n.d	n.d.
24	2-OCH$_3$	CH$_3$	CH$_3$	4-Cl	5.86 (0.16)	4.76 (0.23)	n.d	n.d.
25	2-OCH$_3$	CH$_3$	CH$_3$	4-Br	6.07 (0.11)	<4.00	n.d	n.d.
26	2-F	CH$_3$	CH$_3$	4-Br	5.75 (0.12)	<4.00	n.d	n.d.
27	3-OCH$_3$	CH$_3$	CH$_3$	2-Cl	<4.00	<4.00	n.d	n.d.
28	3-OCH$_3$	CH$_3$	CH$_3$	3-Cl	<4.00	<4.00	n.d	n.d.
29	3-OCH$_3$	CH$_3$	CH$_3$	4-Cl	5.47 (0.12)	4.60 (0.63)	n.d	n.d.
30	3-OCH$_3$	CH$_3$	CH$_3$	2-F	<4.00	<4.00	n.d	n.d.
31	3-OCH$_3$	CH$_3$	CH$_3$	3-F	5.12 (0.38)	<4.00	n.d	n.d.
32	3-OCH$_3$	CH$_3$	CH$_3$	4-F	5.07 (0.18)	<4.00	n.d	n.d.
33	3-F	CH$_3$	CH$_3$	4-Br	5.68 (0.16)	<4.00	n.d	n.d.
34	3-OCH$_3$	CH$_3$	H	2-F, 4-Cl	5.25 (0.13)	<4.00	n.d	n.d.
35	3-OCH$_3$	CH$_3$	H	2,6-di-F, 4-Cl	<4.00	<4.00	n.d	n.d.
36	4-OCH$_3$	CH$_3$	CH$_3$	4-Br	5.58 (0.14)	<4.00	n.d	n.d.
37	4-F	CH$_3$	CH$_3$	4-Br	5.74 (0.13)	<4.00	n.d	n.d.
38	4-F	CH$_3$	H	4-Br, 2-NO$_2$	<4.00	<4.00	n.d	n.d.
39	4-F	CH$_3$	H	4,6-di-F, 2-NO$_2$	<4.00	<4.00	n.d	n.d.
40	2,3-di-OCH$_3$	CH$_3$	H	4-Cl	6.77 (0.05)	6.65 (0.50)	n.d	n.d.
41	2,5-di-OCH$_3$	CH$_3$	H	4-Cl	5.0 (0.37)	5.2 (0.22)	n.d	n.d.
42	2,6-di-OCH$_3$	CH$_3$	H	4-Cl	5.58 (0.02)	5.55 (0.08)	n.d	n.d.
44	2,3-di-OCH$_3$	CH$_3$	CH$_3$	4-Cl	6.84 (0.21)	6.81 (0.22)	n.d	n.d.
45	2-OCH$_3$	CH$_3$	Et	4-Cl	5.73 (0.26)	<4.00	n.d	n.d.
46	2-OCH$_3$	CH$_3$	*n*Pr	4-Cl	5.2 (0.19)	5.01 (0.06)	n.d	n.d.
47	2-OCH$_3$	CH$_3$	*i*Pr	4-Cl	5.55 (0.15)	<4.00	n.d	n.d.
48	2-OCH$_3$	CH$_3$	*cyclo*Pr	4-Cl	<4.00	<4.00	n.d	n.d.
49	2-OCH$_3$	CH$_3$	*n*Bu	4-Br	6.48 (0.21)	<4.00	n.d	n.d.
50	2-OCH$_3$	CH$_3$	*n*Bu	4-Cl	7.08 (0.03)	6.05 (0.21)	7.15 (0.25)	7.29 (0.15)
51	3-OCH$_3$	CH$_3$	*n*Bu	4-Br	6.83 (0.12)	4.94 (0.23)	n.d	n.d.
52	3-OCH$_3$	CH$_3$	*n*Bu	4-Cl	6.90 (0.14)	5.28 (0.32)	n.d	n.d.
53	2-OCH$_3$	CF$_3$	*n*Bu	4-Cl	<4.00	<4.00	n.d	n.d.
54	2,3-di-OCH$_3$	CH$_3$	*n*Bu	4-Cl	7.03 (0.08)	7.26 (0.14)	7.21 (0.06)	7.28 (0.05)
55	2,3-di-OCH$_3$	CH$_3$	*n*Bu	4-Br	6.61 (0.06)	8.19 (0.24)	n.d	n.d.
17b	3-OCH$_3$	CH$_3$	CH$_3$	4-Cl	6.22 (0.18)	5.21 (0.14)	5.02(0.16)	<4.00
43	-	-	-	-	7.29 (0.08)	7.49 (0.17)	7.39 (0.05)	7.02 (0.15)

These methyl-substituted compounds were synthesized with either *ortho*-, subs*m eta*- or *para*-methoxy substituents on the LHS phenyl ring whilst maintaining a halogen substituent on the RHS phenyl ring. Similar to the first generation of analogues, compounds presenting a *meta*-methoxy group on the LHS ring and a *meta*-/*ortho*-chloro (**27**, **28**) or fluoro substitution on the RHS (**30**, **31**) ring were found to be inactive. While, a *para*-chloro or *para*-bromo substituent with an *ortho*-methoxy group on the LHS ring, i.e. compounds **24** and **25**, retained pERK1/2 potency in the micromolar range. Replacement of the pyridazinone methyl in **11** with a trifluoromethyl group (**21**) resulted in a dramatic loss of activity. Further substitutions at the *para*-position on LHS ring i.e. methoxy (**36**) or fluoro (**37**), generated compounds with low micromolar potency.

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Longer alkyl chain substitutions on the linker. Compounds with both *ortho*- or *meta*-methoxy groups on the LHS and a *para*-chloro/bromo group on the RHS ring were further modified. Longer alkyl chains at the α-position of the linker region were investigated. Interestingly the propyl derivative (**46**) demonstrated no marked improvement in activity or pathway selectivity, while the butyl derivatives resulted in a significant improvement in pERK1/2 potency relative to Ca_i^{2+} mobilization. Compounds with a n-butyl-substituted linker, and either an *ortho*- or a *meta*-methoxy group on the LHS, and a *para*-chloro group on the RHS (**50**, **52**), exhibited a pERK1/2 pEC_{50} of 7.08 and 6.90 respectively, and **50** demonstrated an approximate 20-fold bias away from Ca_i^{2+} signaling at the FPR1 (Table S1, supporting information). In addition, **50** behaves as a partial agonist with reduced maximal response in Ca_i^{2+} mobilization at the FPR1 (**Figure 2B**). Moreover, replacement of this *para*-chloro group in **50** and **52** with a *para*-bromo group (**49** and **51** respectively) resulted in a slight reduction in pERK1/2 potency with considerably good selectivity over Ca_i^{2+} mobilization.

Cyclic or branched substitutions on the linker. Apart from the methyl and longer alkyl chain substituents on the linker, compounds possessing branched and cyclic moieties were also synthesized and biologically evaluated. However, activity data revealed that these substitutions were not tolerated within the linker region. Compound with branched (**47**) or cyclic (**48**) substitution as a part of the linker showed a pERK1/2 pEC_{50} of 5.55 and <4.00, respectively.

Dimethoxy substitutions on the LHS ring. Compounds pertaining a 2,3-dimethoxy substitution on the LHS ring with or without a methyl substituent at the α-position of the linker (**40**, **44**) improved the potency of the agonists. Similarly, 2,3-dimethoxy substitution along with a butyl substituent at the α-position of the linker (**54**, **55**) showed significant improvement in ERK1/2 potency. Surprisingly, unlike the other active compounds in the series, **54** and **55** potently stimulates Ca_i^{2+} mobilization (pEC_{50}, 7.26 and 8.19, respectively), whilst, 2,5 or 2,6-di-methoxy derivatives (**41**, **42**) did not show any improvement in potency or pathway selectivity.

Given that the prototype molecule **17b** activates both hFPR1 and hFPR2 in engineered CHO cells,[10, 15] we then evaluated the two most active novel compounds (**50**, **54**) at the hFPR2 subtype. Similar to activity at hFPR1, both **50, 54** demonstrated significant improvement in ERK1/2 potency (pEC_{50}, 7.15, 7.21 respectively), and in stimulating Ca_i^{2+} mobilization (pEC50, 7.29, 7.28 respectively) (Table 1). In contrast to **17b**, which elicited minimal activity with respect to Ca_i^{2+} mobilization at hFPR2,[10, 15] both **50** and **54** displayed a non-significant trend for a bias towards Ca_i^{2+} mobilization in hFPR2-CHO cells (**Figure 2F;** Table S1, supporting information). This is in contrast to the bias profile observed at the hFPR1 subtype, whereby **50** is biased away from Ca_i^{2+} mobilization and **54** behaves as a non-biased (i.e. balanced) agonist. These divergent signaling profiles will hopefully enable **50** and **54** to be used as complementary tool compounds to further interrogate the biological relevance of FPR signaling bias in disease settings in our future studies.

In summary, exploration of the SAR of **17b**-related compounds focused on four distinct regions of the compound structure: the RHS and LHS aromatic rings, pyridazinone core, and modification of the linker between the pyridazinone core and the RHS ring. **Figure 1** represents the regions of the pharmacophore and the key structural changes that are either favorable or unfavorable. This SAR study suggests that optimal pERK1/2 potency is achieved by having a methoxy substituent on the LHS phenyl, combined with a *para*-chloro or *para*-bromo substituent on the RHS phenyl. Replacement of the methyl on the pyridazinone core with a trifluoromethyl group was detrimental and pERK1/2 potency diminished. Functionalization on the α-position of the linker resulted in substantial improvements of both the potency and biased activity of these compounds. Compounds with a methylene or methyl group α to the amide linker with a mono-substituted LHS phenyl were found to be inactive (pEC_{50}<4.0), whilst longer alkyl chains at the aforementioned position resulted in a great improvement in both activity and selectivity, producing a novel lead (**50**) for further biological assessment. In addition, this novel lead (**50**) display similar signaling bias away to Ca_i^{2+} mobilization at hFPR1, and distinct signaling bias towards Ca_i^{2+} mobilization at hFPR2, in comparison to **17b**.

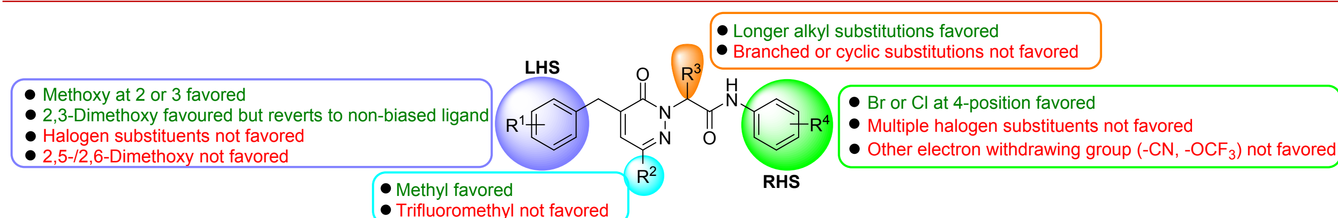

Figure 1. SAR summary of substituted pyridazin-3(2H)-ones analogues.

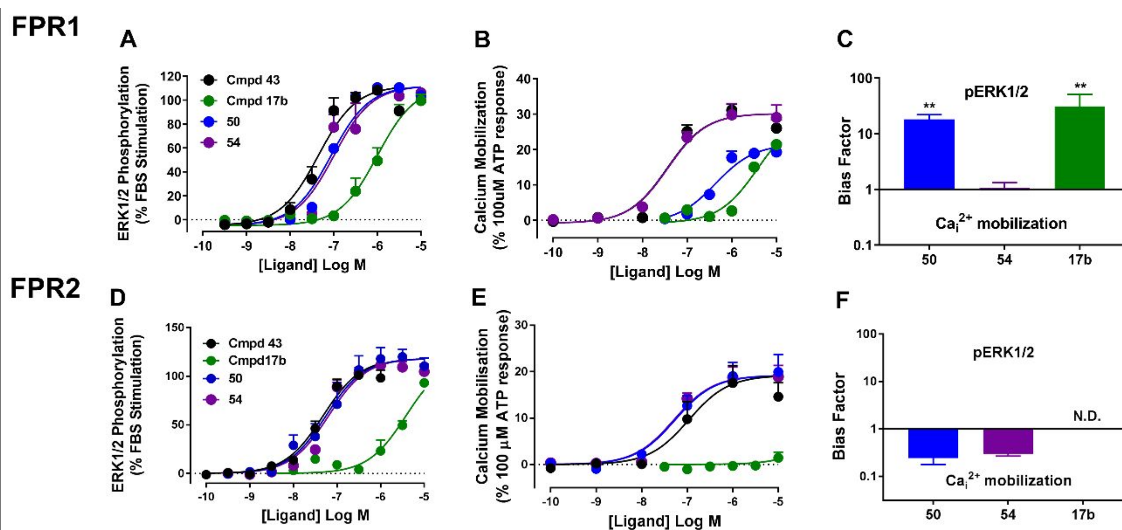

Figure 2. Agonist-stimulated pERK1/2 and Ca$_i^{2+}$ mobilization in CHO cells stably expressing hFPR1 (A, B; top panel) and hFPR2 (D, E; bottom panel). Bias factors at the hFPR1 (C) and hFPR2 (F). Compound **43** is used as a reference compound that is equipotent at both the pERK1/2 and Ca$_i^{2+}$ mobilization pathways.[16] Bias factors of **50**, **54** and **17b**, quantified relative to the reference ligand, compound **43** and the reference pathway, Ca$_i^{2+}$ mobilization, demonstrate that, like **17b**, compound **50** exhibits a significant bias towards pERK1/2 over Ca$_i^{2+}$ at hFPR1 (C), but toward Ca$_i^{2+}$ over pERK1/2 at hFPR2 (F). Compound **54** has a bias factor of 1, indicating it is non-biased towards the two pathways at hFPR1, but toward Ca$_i^{2+}$ over pERK1/2 at hFPR2. Bias of 17b at the hFPR2 couldn't be calculated due to incomplete curve in Ca$_i^{2+}$ mobilization **P < 0.01, unpaired t-test on ΔLog(τ/KA). Data represent the mean ± SEM from 4 independent experiments, performed in duplicate.

Biased agonism. An extension of the Black-Leff operational model of agonism was used to estimate agonist transduction coefficients (Log(τ/K_A)) for each signaling output.[17] Transduction coefficients were normalized to a reference ligand, compound **43**, and a reference pathway, Ca$_i^{2+}$ mobilization, as described in the Supporting Information to generate normalized transduction coefficients (ΔLog(τ/K_A)) and Log(bias factors). This provides a quantification of the relative bias of each agonist at a given pathway. Almost all of the compounds in the series were more potent in stimulating pERK1/2 than Ca$_i^{2+}$ mobilization (**Table 1**). The most active compound of the series, **50** showed a similar degree of bias (approx. 20-fold; Table S1, supporting information) to compound **17b** at the hFPR1, but conversely a trend towards bias favoring Ca$_i^{2+}$ mobilization at the hFPR2. This biased activity was abrogated by the introduction of the dimethoxy substituents at the *ortho*- and *meta*-position of the LHS phenyl ring. Similar to compound **43**, these dimethoxy analogues (**40**, **42** and **54**) were roughly equipotent for both pathways.

Physicochemical, plasma protein binding (PPB) and metabolic properties. Compound **17b** and **50** were further evaluated for their physicochemical, PPB and metabolic stability in liver microsomes. The observed poorer aqueous solubility of **50** compared with compound **17b** may be due to the increased lipophilicity as a result of the longer alkyl substituent in the linker region. Conversely, **50** showed slightly better metabolic stability compared with compound **17b** although the rate of microsomal metabolism remained relatively high (**Table 2**).

Involvement of FPRs in inflammation and its resolution. There is clear evidence that FPRs play an important role in the resolution of inflammation, as both we[4] and others[18-20] have reviewed. Much of the literature to date has focussed on FPR2 activation (rather than both FPR subtypes) as contributing to the resolution of inflammation. On this basis, FPR2 agonists (and perhaps also certain FPR1 ligands, both agonists and antagonists) have been postulated as potential therapeutic approaches for diseases associated with inflammation.[19-21] Prior to our observations of bias at this receptor family,[10] the ability of FPRs to interact with a diverse range of agonists capable of stimulating opposing cellular responses downstream of receptor activation (specific to cell type and ligand, but not FPR subtype selectivity), a known distinguishing feature of FPRs, was not well understood. It would be of interest for future studies to confirm the signaling bias profile in primary immune cells, in addition to receptor selectivity. Whether or not the signaling bias contributes to the extent of inflammatory response warrants further investigation especially given that FPRs are known to be "promiscuous" receptors, interacting with a range of structurally diverse ligands.[2, 4] Our studies provides a better understanding of the key pharmacophores that are critical for mediating signaling bias at the FPRs. Whether specific disease settings trigger phenotypic change of immune cells such as macrophages (e.g. from a pro-inflammatory, M1-like phenotype to a pro-resolving, M2-like phenotype), as a result of altered cytokine and lipid mediator profiles[18] and how this impacts on the signaling fingerprint of each ligand, also warrants investigation in future studies.

CONCLUSION

A pyridazin-3(2*H*)-one based series of FPR agonists was synthesized and evaluated at pERK1/2 and Ca$_i^{2+}$ mobilization signaling pathways. The most active

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compound (**50**) in the series was significantly more potent than the original molecule **17b** (EC_{50} in pERK1/2 of 0.083 μM and 0.603 μM, away from Ca_i^{2+} mobilization at hFPR1. Interestingly, this compound had an opposing bias profile with a trend towards Ca_i^{2+} mobilization at hFPR2. Compounds with a flexible butyl group attached to the linker moiety were found to be the most active compounds in the series, and **50** is the most potent biased FPR1 agonist reported to date. Interestingly, dimethoxy substitution at the *ortho*- and *meta*-position (**54**) increased potency in Ca_i^{2+} mobilization and abolished ligand bias at FPR1, but retained modest bias towards Ca_i^{2+} mobilization over pERK1/2 at the hFPR2.

Table 2. Physicochemical, PPB and metabolic properties of 50 and compound 17b.

Entry	Physicochemical properties					cPPB[c] (%)	in vitro CLint[d] (μL/min/mg protein)	microsome-predicted EH
	cLogP[a]	cLogD at pH 7.4	gLogD at pH 7.4	Sol2.0 (μg/mL)[b]	Sol6.5 (μg/mL)[b]			
17b	4.0	4.0	4.3	< 1.6	< 1.6	97.4	477[e]	0.92
50	5.2	5.2	5.1	< 0.78	< 0.78	98.4	233	0.83

[a]Measured chromatographically. [b]Kinetic solubility determined by nephelometry. [c]Human plasma protein binding estimated using a chromatographic method. [d]Mouse liver microsome. [e]For human liver microsomes, value was 273 μL/min/mg protein

Although metabolic stability would need to be improved to impart future therapeutic relevance, we promote **50** as an improved tool compound compared with compound **17b** for *in vitro* investigations focused on pharmacological intervention with FPR biased agonists. In addition, our data supports assessment of receptor signaling profile as well as receptor subtype selectivity in the rational drug design at FPRs.

EXPERIMENTAL SECTION

Pyridazinone *N*-alkylation (7a-e, 9–11, 15–23, 25–46, 51–55). *Method A*: Pyridazin-3(2*H*)-one in MeCN (ml/mmol) was treated with K_2CO_3 (3.0 eq), followed by the bromo anilide (1.3 eq). The reaction was warmed to reflux over 4-6 h. After this time, the mixture was cooled to rt and the solvent was removed *in vacuo*. The residual material was taken up in EtOAc and washed with water. The organic layer was collected, dried over $MgSO_4$ and concentrated *in vacuo*. The product was then purified by flash chromatography. *Method B*: To a solution of the pyridazinone in dry THF, sodium hydride (3 eq, 60% in mineral oil) was added, followed by the alkyl halide (1.3 eq), and the reaction mixture heated at 50 °C for 5 h, monitored by TLC and/or LC-MS. The reaction was quenched by the addition of NH_4Cl (sat. aq. solution) and extracted with ethyl acetate (3 × 10 mL). The combined organic phases were dried over $MgSO_4$, filtered then evaporated under reduced pressure. The product was then purified by column chromatography. Some alkylation reactions resulted in partial hydrolysis of the ester, in which case the crude material was progressed to the hydrolysis reaction without further purification (yields and spectral data are reported in the Supporting Information).

Ester hydrolysis (8a-e). The ester was dissolved in a 1:1 mixture of THF and H_2O (5 mL/mmol) and treated with LiOH (10 eq). The suspension was stirred at room temperature overnight. The reaction was quenched by the addition of 1M HCl and extracted with EtOAc. The organic layers were collected, dried using $MgSO_4$ and reduced *in vacuo*.

Amide coupling (12–14, 24, 47–50). To a solution of carboxylic acid in anhydrous CH_3CN, EDCI·HCl (1.3 eq) and HOAt (1.3 eq) were added. After 15 min the amine was added and the reaction stirred at 40 °C for 16 h. After cooling to room temperature, the reaction was quenched by the addition of NH_4Cl (sat. aq. solution) and extracted with ethyl acetate (3 × 10 mL). The combined organic phases were dried over $MgSO_4$, filtered, then concentrated under reduced pressure. Purification by column chromatography afforded the corresponding amide products (yields and spectral data are reported in the Supporting Information).

The identity and purity (>95% pure) of the target compounds were confirmed using HRMS, NMR and HPLC.

ASSOCIATED CONTENT

Supporting Information. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.XXXXXXX.

Synthetic experimental procedures, spectroscopic data for the intermediates and final compounds, biological (in vitro and quantification of biased agonism) assay methods and some data, Molecular formula strings and some data.

AUTHOR INFORMATION

Corresponding Author

*For J.B.B: E-mail, jonathan.baell@monash.edu; phone, +61 3 9903 9044.
*For R.H.R: E-mail, Rebecca.Ritchie@baker.edu.au; phone, +61 3 8532 1392.

Author Contributions

#G.S.D. and C.X.Q. contributed equally to this work.

ORCID

Girdhar Singh Deora: 0000-0001-9857-101X
Daniel L. Priebbenow: 0000-0002-7840-0405
Rebecca H. Ritchie: 0000-0002-8610-0058
Jonathan B. Baell: 0000-0003-2114-8242

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ABBREVIATIONS

Ca_i^{2+}, intracellular calcium; ERK1/2, extracellular signal-regulated kinase 1/2; FPR, formyl peptide receptor; GPCRs, G-protein coupled receptors; LHS, left hand side; MI, myocardial infarction; RHS, right hand side.

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