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Discovery of small molecule human FPR1 receptor antagonists

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ABSTRACT

The identification of two novel series of formyl peptide receptor 1 (FPR1) antagonists are reported, represented by methionine benzimidazole **6** and diamide **7**. Both series specifically inhibited the binding of labelled fMLF to hrFPR1 and selectively antagonized FPR1 function in human neutrophils, making them useful in vitro validation tools for the target.

FPR1 is a member of the formyl peptide receptor family, a class A G-protein coupled receptor (GPCR). It is functionally expressed on a variety of cell phenotypes, especially neutrophils and macrophages, but also hepatocytes and platelets.[1,2] Related receptors FPR2 and FPR3 complete the FPR family and have significant sequence homology and overlapping pharmacology to FPR1.[3]

FPRs were originally identified by their ability to bind and be stimulated by *N*-formyl peptides, such as *N*-formylmethionine, produced by infecting bacteria.[4,5] Subsequently, FPR1 was identified and cloned in 1990 from differentiated HL-60 cells.[6] Recently the FPR1 ligand repertoire has expanded with the inclusion of mitochondrial genome-encoded *N*-formyl peptides/proteins, released by damaged host cell mitochondria.[7,8]

Agonism of FPR1 leads to activation of neutrophil and macrophage pro-inflammatory functions as part of the innate immune response, leading to chemotaxis, superoxide generation and degranulation required for efficient bacterial clearance. Consistent with the pro-inflammatory action of FPR1 agonists, this receptor and its ligands have been implicated in the pathogenesis of a range of inflammatory disorders, such as COPD, where bacterial infection and potentially smoke-driven lung damage (e.g., mobilisation of mitochondrial *N*-formyl peptides) are linked to exacerbations of symptoms and long term lung damage. Hence pharmaceutical interest in this area is driven by the potential therapeutic development of a FPR1 antagonist. We report in this paper the discovery of two novel series of small molecule with such profiles.

There are only a few examples of FPR1 antagonists in the literature (see Fig. 1) including phenylbutazone analogues **1**,[9,10] group E **2**[11] cyclosporins **3**[12] to *t*-Boc-peptides **4**.[13,14] Recently potent FPR1 probes (e.g., **5**) were identified by library screening by Young et al.[15], but their precise mechanism of action appears unclear. However, all these chemical starting points are unattractive for lead generation due to potentially chemically reactive functionality, weak potency, no species cross-over, high lipophilicity, flat SAR or having a complex peptidic structure.

To identify new chemical starting points, a High Throughput Screen (HTS) of the corporate AstraZeneca collection (800K) was run using Fluorometric Micro volume Assay Technology (FMAT™).[16] Compound inhibition of Alexa647-labelled fMLFK specific binding to HEK-293 cells expressing human recombinant FPR1 (hrFPR1) and hrG-protein $G_{\alpha 16}$ was measured. A HTS active rate of 2.6% was obtained. As the target was categorised as a challenging GPCR, in terms of known chemical tractability, a more lenient work up than normal was undertaken. Compounds with an inhibition >50% and a lipophilicity range $-1.5 < c\log P < 5.5$ were evaluated after the removal of unwanted chemotypes. Frequent HTS hitters were also removed prior to testing for FMAT™ technology blockers using a comparable parallel hrCCR1 FMAT™ assay.

The final set of active compounds were tested for FPR1 antagonism in a human neutrophil FPR1 Fluorescence Imaging Plate Reader (FLIPR)[17] assay and counter screened in the same assay

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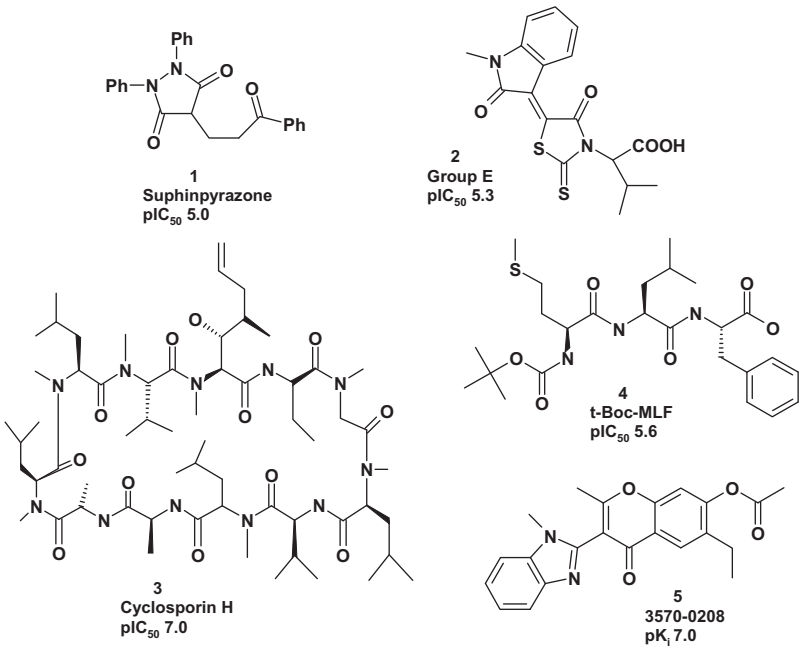
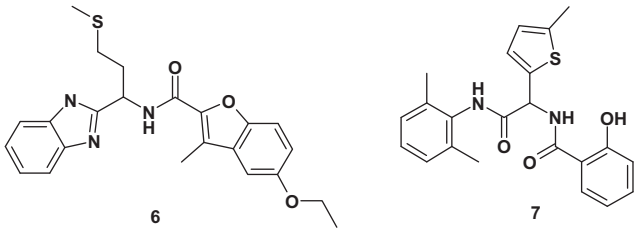


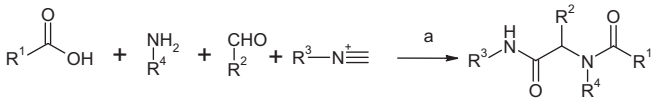
Figure 1. A selection of known FPR1 antagonists.

against FPR2 (also called FPRL1) (see Table 5), where >30-fold selectivity was the required progression criteria. This led to the identification a small number of chemical series, two of which are described in this Letter.



Benzimidazole **6** was a member of a small set (3) of external compounds, derived from methionine, whereas diamide **7** was from an Ugi library and subsequently formed part of a large cluster, providing early hints of Structure–Activity Relationship (SAR). The similarity of both these series to known FPR1 modulators is easy to visualise (e.g., fMLF, fMLFF, *t*BocMLF). This raises the distinct possibility that these compounds interact with FPR1 in a similar manner to the known peptidic modulators.

The syntheses of both templates are straightforward and shown in Schemes 1 and 2. The diamides were isolated in acceptable yields using a one pot multi-component reaction in methanol.[18] The benzimidazoles were prepared from the corresponding aromatic diamines employing standard amide coupling chemistry, followed by dehydration under acidic conditions to generate the imidazole ring.



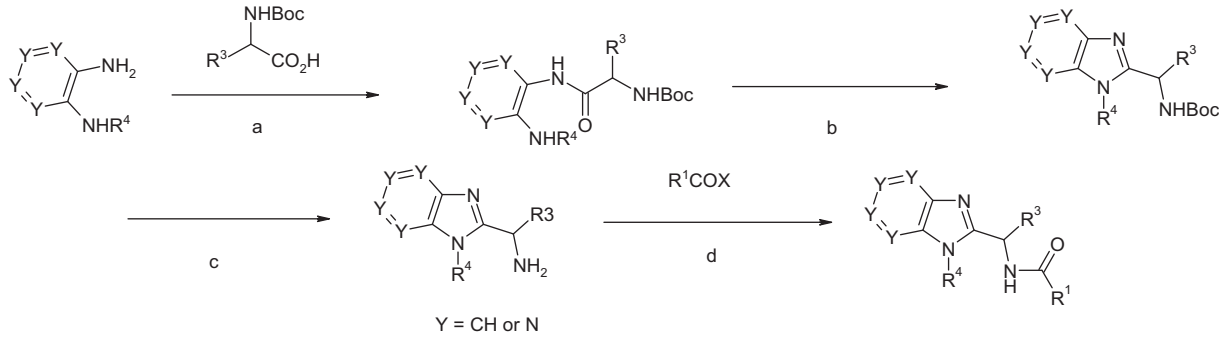
Scheme 1. Synthesis of diamides. Reagent and condition: (a) methanol 40 °C.

The diamide SAR was rapidly explored utilising Ugi related chemistry, which allowed multiple variations of R^{1-4} to be incorporated, generating broad data sets. Key results are highlighted in Table 1.

Substitution at R[2] and R[3] are tolerated. R[2] does appear to have the greatest scope for modification. Polar heterocycles do improve lipophilic ligand efficiencies (LLE)[20], but the most potent analogues identified (i.e. pIC_{50} >7) possessed more lipophilic R[2] substituents. Variation of the R[3] group was the least explored region and there appears to be scope to vary this further. Replacement of hydrogen as the R[4] motif with methyl resulted in a loss of FPR1 potency. For R[1] the ortho phenol was essential to maintain FPR1 activity and despite varying this motif broadly, only compound **17** showed any sign of FPR1 activity without R[1] being a specific 2-hydroxyphenyl analogue. Even in this case there is still a strong similarity to a phenolic type pharmacophore. This initial SAR suggested that in order to generate good FPR1 biological profiles, the series would require a number of parameters that would be challenging to successfully remove/modify in latter phases of the drug discovery process. Accordingly further work, including testing of enantiomers, was not undertaken.

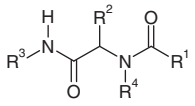
Spot test screening of available near neighbours of benzimidazole **6** identified additional active analogues, suggesting chemical scope for broader SAR evaluation. Overall the compounds are lipophilic, leading to unfavourable metabolic stability and modest LLE. Therefore, the main priority in expanding the series was to reduce lipophilicity whilst attempting to increase potency.

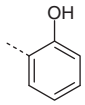
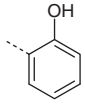
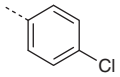
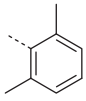
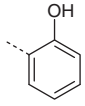
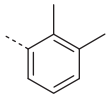
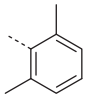
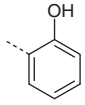
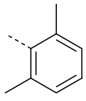
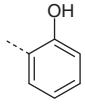
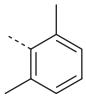
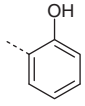
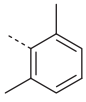
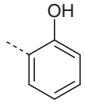
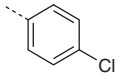
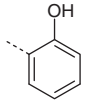
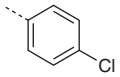
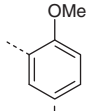
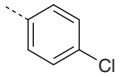
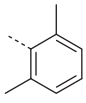
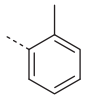
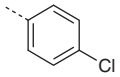
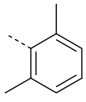
Preliminary exploration of the SAR of R[1] was achieved through parallel synthesis, with profiles for key compounds highlighted in Table 2. Modification of the benzofuran suggests no specific part dominates potency for FPR1. Removal of the ethoxy group reduces activity, whilst maintaining ligand efficiency. Disrupting the planarity of the amide/aromatic ring may have some benefit, but initial data was inconclusive. The R[1] substituent does not need to be aromatic, with the cyclohexyl motif being equiactive to phenyl. Insertion of an alkyl linker between the amide and cyclic motif did however reduce potency. The lipophilicity of the aromatic group can be reduced with only limited impact on potency and ligand efficiency, allowing LLEs to be increased to >3.



Scheme 2. Synthesis of benzimidazoles. Reagents and conditions: (a) 2-propanephosphonic acid anhydride (T3P), dimethylfomamide; (b) acetic acid; (c) trifluoroacetic acid, dichloromethane; (d) X = OH, T3P, dimethylformamide, X = Cl, dichloromethane, triethylamine.

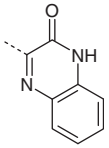
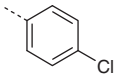
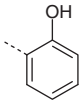
Table 1
Diamide SAR



Compound	R^1	R^2	R^3	R^4	FPR1 pIC_{50}[a]	*c* log *P*	LE[b]	LLE[c]
7				H	6.5	3.9	0.23	2.6
8				H	6.1	4.5	0.21	1.6
9				H	7.1	4.7	0.24	2.4
10				H	5.9	3.6	0.19	2.3
11				H	5.8	1.6	0.21	4.2
12				H	6.1	4.6	0.22	1.5
13				H	5.3	4.8	0.19	0.5
14				H	5.6	5.1	0.2	0.5
15				H	NA	4.0	--	--
16				H	NA	4.0	--	--

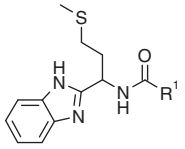
(*continued on next page*)

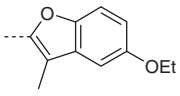
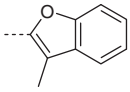
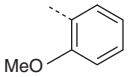
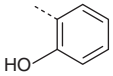
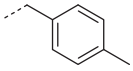
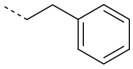
Table 1 (*continued*)

Compound	R^1	R^2	R^3	R^4	FPR1 pIC_{50}[a]	*c* log *P*	LE[b]	LLE[c]
17				H	5.6	3.2	0.16	2.4
18				Me	5.0	3.3	0.17	1.7

[a] Inhibition of fMLF stimulated intracellular calcium mobilisation in human neutrophils. pIC_{50} values are the means of at least two experiments. NA: Not active (<25% inhibition) at 50 μM.
[b] LE (Ligand efficiency). [19]Values are calculated as pIC_{50}/Heavy atom count.
[c] LLE (Lipophilic ligand efficiency). [20]Values are calculated as pIC_{50}–*c* log *P*.

Table 2
Benzimidazole R^1 SAR



Compound	R^1	FPR1 pIC_{50}	*c* log *P*	LE	LLE
6		6.3	4.5	0.21	1.8
19		5.4	3.9	0.2	1.5
20		5.4	2.5	0.23	2.9
21		5.3	3.1	0.23	2.2
22		NA	3.3	––	––
23		5.4	3.8	0.23	1.6
24		5	2	0.22	3
25		5.6	3.5	0.24	2.1
26		5	1.5	0.22	3.5
27		5.7	3	0.25	2.7
28		5	3.7	0.21	1.3
29		NA	3.6	––	––

However, despite making a number of heterocyclic R[1] analogues, none were more potent than **6**.

Key modifications around the benzimidazole motif are shown in Table 3. Limited SAR for substitution of the heterocyclic nitrogen was available. Lipophilic motifs were generally equipotent to hydrogen, irrespective of the size of substituent. Introduction of a cyano group (**31**) interestingly did give a log unit increase in potency compared to **30**, but this compound still has a *c* log *P* >4. Incorporation of heteroatoms into the phenyl ring of the benzimidazole had variable effects on FPR1 profiles depending on the substitution pattern. Whilst all reduced lipophilicity and gave superior LLEs to **6**, potency and ligand efficiency (LE) [19] were reduced and for **32** stimulation of intracellular calcium mobilisation was observed. This apparent agonism (pEC_{50} 5.8, intrinsic activity (IA) 0.81) was shown to be mediated by FPR1 as **7** antagonized the compound's effects in the neutrophil (pA_2 6.3). This finding is not without precedent as benzimidazole-based agonists of FPR1 and FPR2 have been recently described.[21] Replacement of the benzimidazole could be achieved, with the 4-phenylimidazole analogue, **35**, showing slightly reduced FPR1 activity compared to **27**, although reductions in LE and LLE were more pronounced.

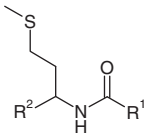
The SAR for the amino acid substituent is summarised in Table 4. The ethylthiomethyl side chain (R[3]) appears to be required for FPR1 potency, with the (*S*) isomer preferentially inhibiting intracellular calcium mobilisation. Replacement of the side chain with a butyl group (**40**) maintained FPR1 potency, but this analogue mobilised intracellular calcium, suggesting it was an FPR1 agonist. This is supported by the finding that its potency as an agonist (pEC_{50} 6.3, IA 0.7) was in good accordance with the pIC_{50} value estimated from its blockade of the effect of fMLF. Other variants, such as aromatic substituents were all inactive, whilst the sulphone analogue showed reduced potency and LE, though LLE was improved.

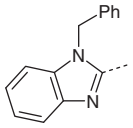
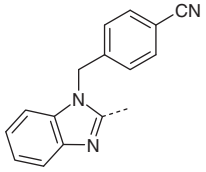
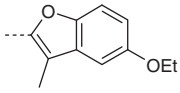
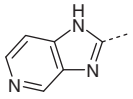
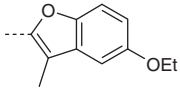
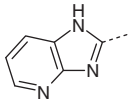
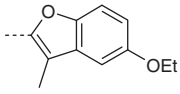
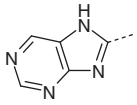
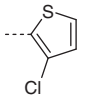
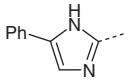
The initial data shows SAR in this region is likely to be limited. This is in agreement with data generated for formylated methionine peptide analogues, where modification of the thioether side chain hasn't been tolerated.[22] Compounds **38** and **41** show improvement in metabolic stability in rodent and human systems compared to **6** (human microsomal intrinsic clearances 3, 14, 37 μL/min/mg, respectively) demonstrating the thioether, as expected, to be the major metabolic liability. Finding a suitable replacement would be essential if the series were to have any chance to progress towards Lead Optimisation.

Once preliminary SAR had been established, more specific follow up rounds of synthesis were undertaken as shown in Figure 2, looking to expand on the initial observations.

These campaigns failed to generate suitable FPR1 potency or LLE improvements. A large number of these analogues possessed *c* log *P* values in the range of 2–3.5, generating compounds with acceptable Lead Identification in vitro DMPK and physicochemical

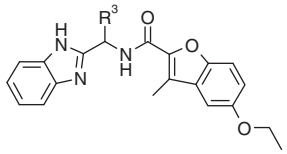
Table 3
Benzimidazole R^2 SAR

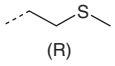
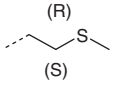
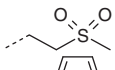
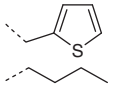
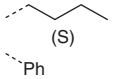
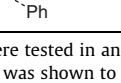


Compound	R^1	R^2	FPR1 pIC_{50}	FPR1 agonism pEC_{50}[a]	*c* log *P*	LE	LLE
30			5.6	NA	4.7	0.19	0.9
31			6.5	NA	4.1	0.2	2.4
32			6.0	5.8 (IA = 0.8)	3.6	0.2	2.4
33			5.5	NA	3.6	0.19	1.9
34			5.3	NA	2.8	0.18	2.5
35			5.2	NA	3.5	0.21	1.7

[a] Apparent stimulation of intracellular calcium mobilisation in human neutrophils antagonized by a selective FPR1 antagonist, **7**.

Table 4
Benzimidazole R^3 SAR



Compound	R^3	FPR1 pIC_{50}	*c* log *P*	LE	LLE
36	 (R)	<4.6	4.5	––	––
37	 (S)	6.2	4.5	0.21	1.8
38		5	2.6	0.16	2.4
39		NA	5.1	––	––
40	 (S)	6[b]	5.5	0.2	0.5
41	 Ph	NA	5	––	––

All compounds were tested in an FPR1 agonism assay.
[b] Compound **40** was shown to possess equivalent activity in both assays.

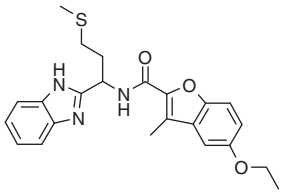
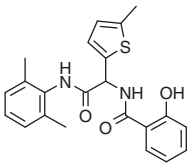
parameters, but the majority possessed inadequate FPR1 potency. The expanded data set clearly showed a disconnection in preferred substituents for both benzimidazole and diamide series (R[1] and R[3] in particular) suggesting possible differences in how they interact with the FPR1 receptor.

Although reduction of lipophilicity could be achieved in certain regions around the benzimidazole template, reviewing the overall data demonstrated a lipophilicity/potency correlation with a clear leading edge for the more potent compounds (Fig. 3). This indicated that consistently achieving LLEs >3 where FPR1 pIC_{50} >6 would be a challenge. The trend was similar for the diamides (see Supplementary data) leading to the conclusion that options to improve FPR1 profiles for either series in subsequent phases would be difficult.

Complete profiles of **6** and **7** are highlighted in Table 5. Both were shown to be reversible antagonists and exhibited no FPR1 agonism in the human neutrophil FLIPR assay. Additional selectivity profiling, showed these compounds to be inactive at 30 μM against other human GPCRs, such as FPR2, C5aR and CXCR2. Species cross-over was examined in cell calcium mobilisation assays expressing recombinant rat and mouse FPR1. Both series demonstrated limited cross-over to these rodent receptors. FPR1 agonism was only observed in the previously described benzimidazole analogues and not by **6** or **7** or in any other diamides tested (data not shown).

In order to further characterise the nature of the receptor interaction and functional antagonism of both series, exemplars were tested and shown to inhibit [[3]H] fMLF binding to hrFPR1 in parallel with attenuation of fMLF-driven neutrophil functions such as degranulation. Both **6** and **7** are potent inhibitors of fMLF binding to FPR1 suggesting the compounds interact at the receptor level

Table 5
Hit profiles for **6** and **7**

Parameter	Lead criteria[23]	Compound **6**	Compound **7**
Structure			
FPR1Δ[Ca^{2+}]$_i$[a]	pIC_{50} >6	6.3	6.4
FPR1 agonism[b]		NA	NA
hrFPR1binding[c]	pIC_{50} >7	7.5	7.0
FPR2[d]		NA	NA
CXCR2[e]		NA	NA
C5aR[f]		NA	NA
hrFPR1Δ[Ca^{2+}]$_i$[g]		5.5	5.8
Rat rFPR1[h]		5.0	5.9
Mouse rFPR1[i]		NA	4.6
Degranulation[j]	pIC_{50} >6	6.8	6.4
Cytotoxicity[k]		4.0	4.1
c log *P*	<3.0	4.5	3.9
Log *D*	<3.0	4.8	4.1
Mol Wt	<450	423	395
LE		0.21	0.23
LLE		1.8	2.5
Solubility[l]	>10 μM	17	68
Hu Mics[m]	<30	37	43
Rat Heps[n]	<15	27	70
Chem stability[26]	$t_{½}$ >100 h	>260	>260

pIC_{50} values are the means of at least three experiments. NA: not active (<25% inhibition) at 50 μM.
[a] Inhibition of fMLF stimulated intracellular calcium mobilisation in human neutrophils.
[b] Apparent stimulation of intracellular calcium mobilisation in human neutrophils.
[c] Inhibition of [^{3}H]fMLF binding to CHO cell membranes expressing hrFPR1.
[d–f] Inhibition of [d]SHAAGtide, [e]hrGROα and [f]hrC5a-stimulated intracellular calcium mobilisation in human neutrophils.
[g–i] Inhibition of [g,h]fMLF or [i]fMLFF-stimulated intracellular calcium mobilisation in HEK cells expressing [g]human, [h]rat or [i]mouse recombinant FPR1.
[j] Inhibition of fMLF-stimulated human neutrophil degranulation.
[k] Inhibition of THP-1 cell viability.
[l] Solubility protocol (see Supplementary data).
[m] Human microsome metabolism intrinsic clearance (μL/min/mg).[24]
[n] Rat Sprague–Dawley hepatocyte metabolism intrinsic clearance (μL/min/10^6 cells).[25]

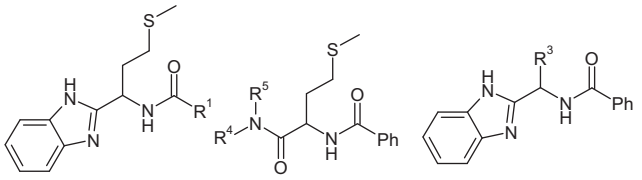


Figure 2. Follow up libraries.

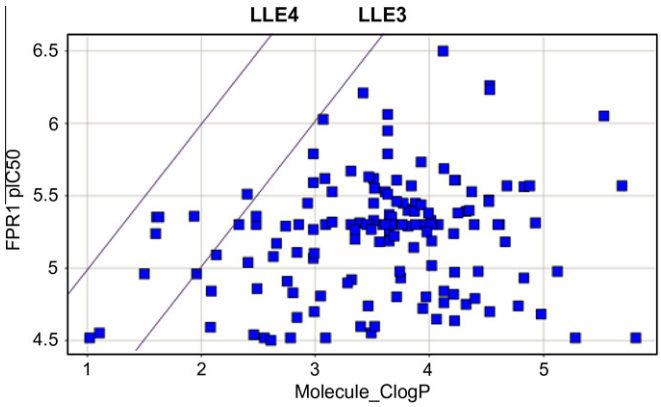


Figure 3. Plot of *c* log *P* vs FPR1 pIC_{50}.

to antagonize FPR1-driven cell function. Additionally the compounds were shown not to inhibit degranulation per se as they had no effect on PMA-stimulated neutrophil degranulation. Finally to identify any innate cytotoxicity in the hit series, compounds were shown to be weakly active (pIC_{50} ~4) in a standard 24-hour cell viability assay. Even though the measured thermodynamic solubility of **6** and **7** was below the top test concentrations employed, no evidence was observed for these compounds being insoluble under assay conditions.

In summary, HTS has provided two novel FPR1 antagonist hit series that are superior to any known antagonists. These are useful tools to help understand the role of FPR1 in various disease settings. More detailed studies of these compounds will subsequently be reported elsewhere. Literature data and our own findings suggest that identification of drug like FPR1 antagonists will be a challenge.

Acknowledgements

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.03.049.

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