5044 J. Med. Chem. 2009, 52, 5044–5057
DOI: 10.1021/jm900592h

Journal of Medicinal Chemistry Article

6-Methyl-2,4-Disubstituted Pyridazin-3(2*H*)-ones: A Novel Class of Small-Molecule Agonists for Formyl Peptide Receptors

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Received May 7, 2009

Following a ligand-based drug design approach, a potent mixed formyl peptide receptor 1 (FPR1) and formyl peptide receptor-like 1 (FPRL1) agonist (**14a**) and a potent and specific FPRL1 agonist (**14x**) were identified. These compounds belong to a large series of pyridazin-3(2*H*)-one derivatives substituted with a methyl group at position 6 and a methoxy benzyl at position 4. At position 2, an acetamide side chain is essential for activity. Likewise, the presence of lipophilic and/or electronegative substituents in the position para to the aryl group at the end of the chain plays a critical role for activity. Affinity for FPR1 receptors was evaluated by measuring intracellular calcium flux in HL-60 cells transfected with FPR1, FPRL1, and FPRL2. Agonists were able to activate intracellular calcium mobilization and chemotaxis in human neutrophils. The most potent chemotactic agent ($EC_{50} = 0.6\,\mu M$) was the mixed FPR/FPRL1 agonist **14h**.

Introduction

Innate immunity is a very important mechanism in defending humans against infectious microbes.[1] Thus, immune disregulation due to viral infections (i.e., AIDS) and immunosuppression treatments following transplantation expose patients to life-threatening risks. Moreover, in the context of increasing aging of the population in more developed countries, it was observed that older people exhibit a natural immune function disregulation, which may be exacerbated in chronic stress conditions.[2] In addition, the ongoing emergence of bacterial and viral strains resistant to multiple classes of chemotherapeutics increases morbidity, mortality, and costs associated with nosocomial infections.[3] Thus, identification and development of bioactive molecules that selectively stimulate the innate immune response is an important challenge both for biologists and for chemists.[4]

The human formyl peptide receptor (FPR) family belongs to a group of G-protein coupled receptors that are predominantly expressed on neutrophils and monocytes.[5] Three FPR subtypes (FPR1[a], FPRL1, and FPRL2) have been identified in humans, while eight FPR-related receptors have been characterized in mice.[6] Evidence for an important role of FPRs both in infective and inflammatory processes, has been well documented in the literature. The finding that bacteria are the major natural source of chemotactic formyl peptides (i.e., *N*-formylmethionine-leucine-phenylalanine, fMLF), which are high affinity agonists for FPR1, strongly supports the hypothesis that these receptors work as antibacterial receptors.[7] According to this idea, Fpr1-knockout mice show reduced resistance to infections provoked by some types of bacteria.[8] Moreover, a key role of FPRL1 has been proposed in host response to HIV-1 infection, and synthetic peptide domains of HIV-1 envelope proteins are able to activate FPRL1 leading to attenuation of cell response to chemokines through cross-desensitization and down-regulation of the monocyte receptors CCR5 and CXCR4.[9] The potent in vitro anti-HIV-1 activity of the synthetic gp41 peptide T2 (DP178), which is an agonist of FPR, strongly supports this view.[10]

Evidence for a pro-inflammatory role of FPRL1 in various neurodegenerative diseases was also found. In fact, the 42 amino acid form of amyloid β (AB42), which is believed to play a key role in Alzheimer disease-induced neuronal damage by recruitment and activation of mononuclear phagocytes, is a chemotactic agonist of FPRL1.[11] Likewise, the neurotoxic protein fragment PrP106−126, which behaves as the pathologic isoform of prion protein, was chemotactic and independently induced cytokine secretion in human monocytes through interaction with FPRL1.[12] On the other hand, anti-inflammatory properties are displayed by different types of FPRL1 ligands. For example, lipoxin A4 (LXA4) is an endogenous arachidonic acid metabolite that has been shown to promote resolution of inflammation through FPRL1 agonism.[13] Likewise, annexin A1, a glucocorticoid-regulated protein that is particularly abundant in neutrophils, is able to inhibit leucocytes migration/activation through hFPRL1 agonism.[14] Taken together, these data clearly support a key role of FPRL1 in a variety of acute inflammatory and infective pathologies. Thus, this receptor has recently become

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[a] Abbreviations: FPRs, formyl peptide receptors; FPR1, formyl peptide receptor 1; FPRL1, formyl peptide receptor-like 1; FPRL2, formyl peptide receptor-like 2; fMLF, *N*-formylmethionine-leucine-phenylalanine; LXA4, lipoxin A4; WT, wild-type; HBD, hydrogen bond donor; HBA, hydrogen bond acceptor.

 Published on Web 07/29/2009

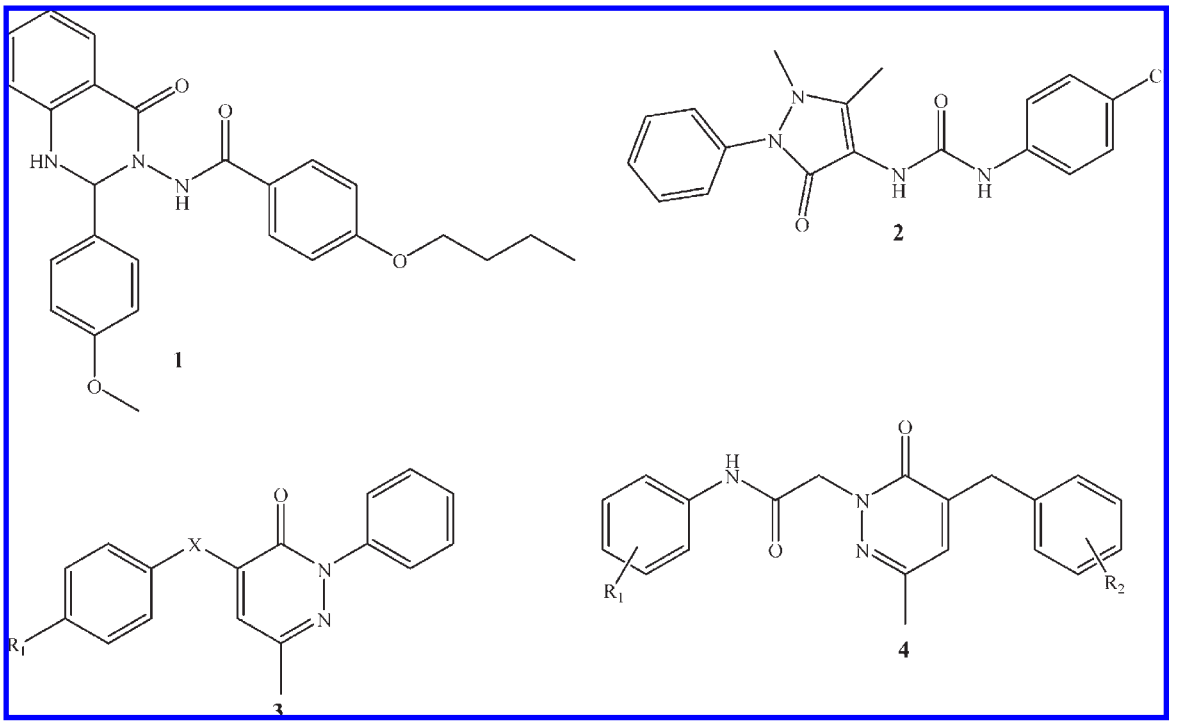


Figure 1. Structures of FPRL1 agonists.

a prospective target for therapeutic intervention.[15] In addition, the identification of novel agonists and antagonists may be a useful tool to clarify the FPR-mediated (patho)-physiological effects.

In addition to natural peptides like the bacterial fMLF,[16] synthetic peptides[17] and small molecule nonpeptide ligands of FPRL1 have been recently reported. Quin-C1[18,19] (compound **1**, Figure 1) was the first small-molecule FPRL1-selective agonist shown to induce chemotaxis in peripheral blood neutrophils, with a relative potency 1000-fold lower than the synthetic peptide agonist WKYMVm. More recently, researchers from Amgen reported a series of pyrazolone derivatives displaying nanomolar selective affinity for FPRL1.[20] These types of agonists, of which **2** is an example, are characterized by the presence of an arylurea substructure linked to the heterocyclic backbone and served us as starting point to develop novel chemotypes of FPR ligands. Thus we report here the synthesis and evaluation of two series of functionalized pyridazinones of general structure **3** and **4**.

Chemistry

The synthetic pathways employed to prepare the final compounds are depicted in Schemes 1−5.

Compound **5**[21] in anhydrous toluene was refluxed with the appropriate aryl isocyanate to afford the urea derivatives **6a**−**c** (Scheme 1). The 4-butoxy analogue **7** was synthesized with an alternative procedure starting from the same precursor and using triphosgene in anhydrous THF, followed by treatment with the appropriate aniline. The aromatic amide **8** was obtained from **5** by treatment with the opportune aroyl chloride in toluene at reflux. Finally, coupling of the amine **5** with 4-butoxyphenylboronic acid in CH_2Cl_2 in presence of $Cu(Ac)_2$ gave the aromatic amine **9**.

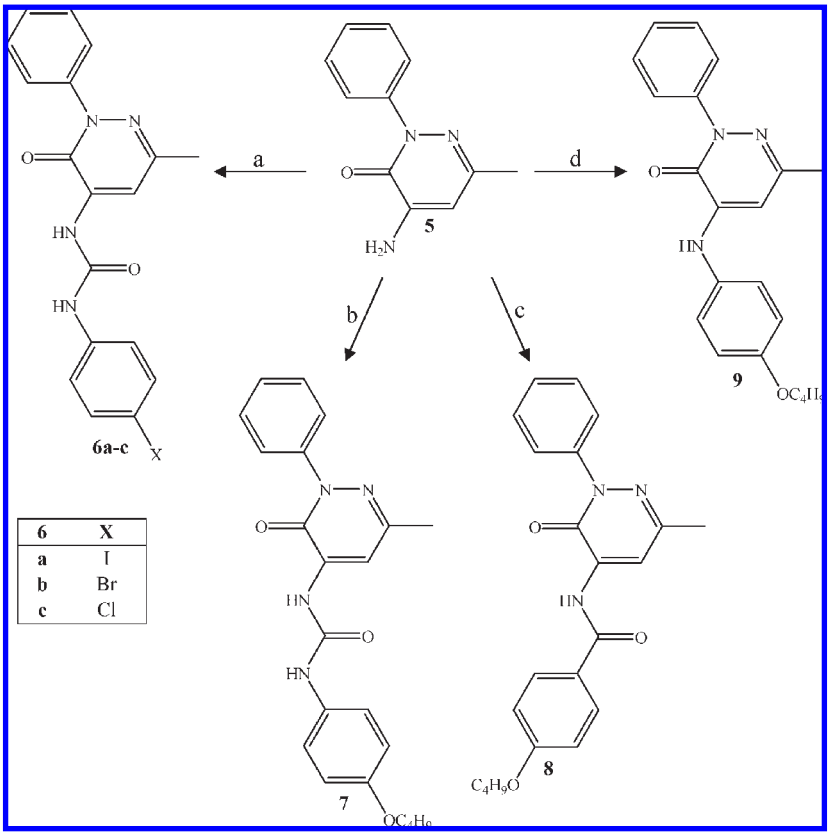
In Scheme 2 is depicted the synthesis of compounds **14a**−**x** (Table 2). The dihydropyridazinone **10**[22] was converted into the previously described 4-benzyl derivatives **11b**,**c**[23,24] and the new **11a** by condensation with the appropriate aromatic aldehyde in the presence of KOH. Compounds **11**, in turn, were alkylated with ethyl bromoacetate to give the esters **12a**−**c**, whose **12b**,**c** were previously reported.[24,25] Alkaline hydrolysis of compounds **12** gave the known **13c**[23] and the new carboxylic acids **13a**,**b**. These compounds were treated with ethyl chloroformate in THF in presence of triethylamine, affording the intermediate mixed anhydrides, which, in turn, were transformed into the final amides **14a**−**s** and **14u**−**x** by treatment with the appropriate aryl or cycloalkyl amine. To obtain the ester **14t**, the mixed anhydride was treated with 4-bromophenol.

Scheme 3 outlines the synthetic procedure for compounds **15**−**19**: the precursor **11a** was alkylated with the appropriate bromo ester to give compounds **15a**,**b**, which, in turn, were converted into the corresponding acids **16a**,**b**. The final step was the transformation of these compounds to the final amides **17a**,**b** using the same procedure described for compounds **14**. Compounds **18a**,**b** were obtained by alkylation of **11a** with the appropriate halide in standard conditions.

Moreover the precursor **11a** was converted into the final **19a**,**b** through a Mannich reaction (CH_2O + NH_3). The intermediate amine was not isolated and was converted into the urea **19a** by treatment with 4-bromophenyl isocyanate and into the amide **19b** with 4-bromobenzoyl chloride.

The final compounds **21**, **23**, and **25** were synthesized as shown in Scheme 4. The intermediate **12a** was reduced with sodium borohydride in THF/MeOH to generate the primary alcohol **20**. This compound was the starting material for the synthesis both of the ether **21**, through a coupling reaction with the 4-bromophenylboronic acid in presence of $Cu(Ac)_2$, and of compound **23**, through the mesylate **22**, which was converted into the final compound **23** by nucleophilic replacement with 4-bromo aniline. Treatment of **22** with ammonia gave the intermediate **24** from which the amide **25b** and the urea **25a** were obtained using 4-bromobenzoyl chloride or 4-bromophenyl isocyanate, respectively, using the same

Scheme 1[a]



[a] Reagents and conditions: (a) anhydrous toluene, aryl isocyanate, reflux, 3−7 h; (b) THF, anhydrous sodium acetate, triphosgene 70 °C, 2 h, 4-butoxyaniline, 12 h, rt; (c) Et_3N, RCOCl, toluene, 110 °C, 4 h; (d) $Cu(Ac)_2$, 4-butoxyphenylboronic acid, Et_3N, rt, 12 h.

reaction conditions described for the analogues **7** and **8** in Scheme 1.

In Scheme 5 is depicted the synthesis of compounds **29** and **30**. Treatment of the commercially available 6-methylpyridazinone **26** with *m*-methoxybenzyl chloride in acetonitrile resulted in compound **27**, which, in turn, gave the corresponding 4-amino derivative **28** by heating with N_2H_4 under hard conditions. The amide **29** and the urea **30** were obtained from **28** by treatment with the proper aroyl chloride or the aryl isocyanate by following the same conditions reported for **25a** and **25b**.

Results and Discussion

All synthesized compounds were evaluated for their ability to induce intracellular Ca^{2+} flux in HL-60 cells transfected with FPR1, FPRL1, or FPRL2, and results were reported in Tables 1 and 2. All compounds were also evaluated in WT (wild-type nontransfected HL-60 cells) and were inactive. Moreover, both EC_{50} values and relative efficacy compared to the peptide agonists, fMLF and WKYMVm, were determined.

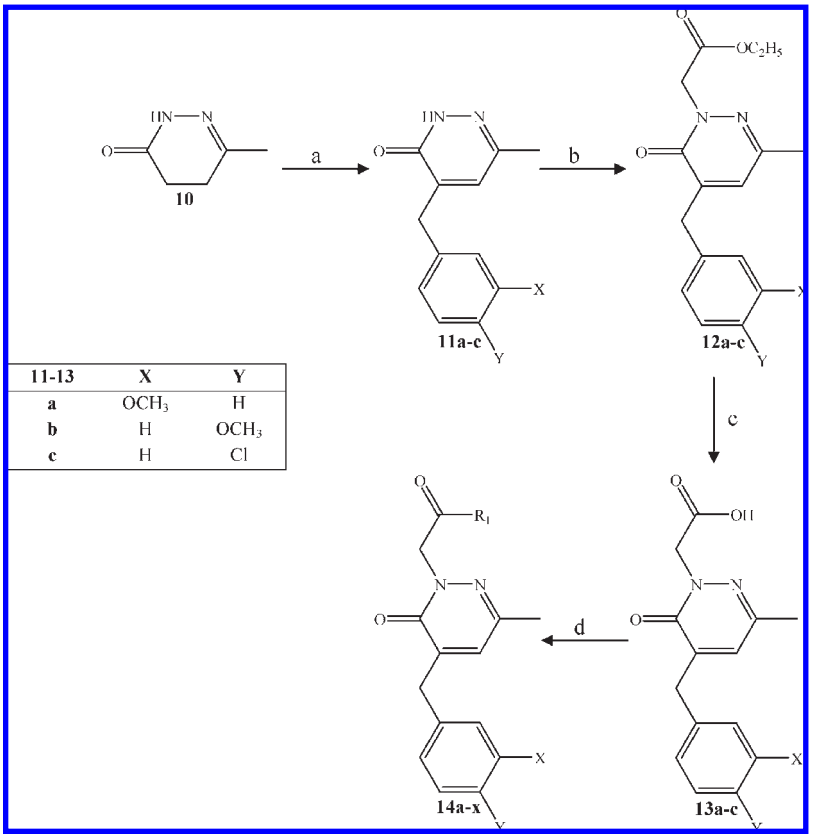
In the early phase of our project, we synthesized pyridazin-3(2*H*)-ones bearing a phenyl group at position 2 and a functionalized side chain at position 4 (compounds **6a**−**c**, **7**, **8**, and **9**), which may be regarded as enlarged analogues of **2** (Figure 1). Because these compounds failed to show activity (see Table 1), we turned our attention to analogues with a substituted benzyl at position 4 and a similar functionalized chain at N-2 (compounds **4**, Figure 1). In this series, we were able to identify compound **14a**, a potent mixed FPR1 and FPRL1 agonist.

Thus **14a** was selected as lead and extensive structure−activity relationship (SAR) studies on this prototype were performed by modifying the nature and the length of the functionalized side chain and by replacing Br with a variety of substituents. Elongation of carbon chain from one to two methylene groups gave compound **17a**, which resulted less potent at FPR1 and FPRL1 of **14a** (Table 1). Branching the carbon chain resulted in compound **17b**, which did not activate FPRL1 but retained activity for FPR1 similar to that of **14a**. Thus, **17b** is an FPR1-specific agonist. Modifications more substantial of the functionalized chain were obtained with compounds **18a**,**b** and were completely detrimental. Further inactive compounds were the urea derivative **19a**, the inverse amide **19b**, and their superior homologues **25a** and **25b**. Replacement of CONH with an ether group and a secondary amine gaves compounds **21** and **23**, respectively, which resulted also inactive. Moreover, compounds **29** and **30**, in which the substituents at position 2 and 4 were interchanged, were inactive.

Other modifications of the functionalized chain were performed with compounds **14r**−**u** (Table 2). When the NH was converted to NCH_3 (compound **14r**), the activity disappeared. Compound **14s**, where the CONH is in the middle of the spacer, was also inactive. Replacement of CONH with COO (**14t**) or inclusion of amidic nitrogen in piperazine nucleus (**14u**) gave the same effect.

The nature and the position of the substituent on the phenyl group at the end of the chain also played a crucial role in ligand activity. Moving Br to positions meta (**14b**) and ortho (**14c**) resulted in a complete loss of FPR1/FPRL1 activity. Among halo-derivatives, the 4-chloro analogue **14d** exhibited

Scheme 2[a]



[a] Reagents and conditions: (a) aromatic aldehyde, 5% KOH, EtOH, reflux, 2−5 h; (b) ethyl bromoacetate, K_2CO_3, anhydrous CH_3CN, reflux, 1−6 h; (c) NaOH, 60−80 °C, 1−4 h; (d) ethyl chloroformate, anhydrous THF, Et_3N, appropriate substituted aryl(cycloalkyl)amine (or 4-bromophenol for compound **14t**), 12 h, rt.

the same profile as **14a**. The corresponding 4-iodo derivative **14e** was 2 times less potent at FPRL1. For this compound, a weak effect at FPRL2 was also observed. The 4-fluoro analogue **14f** was less potent compared to **14a**, but specificity for FPR1 was evident. The elimination of Br (compound **14g**) was associated with loss of activity. Replacement of Br in **14a** with substituents having similar steric properties, such as *t*-But (**14i**), OCF_3 (**14o**), and CN (**14q**), led to loss of activity at both FPR1 and FPRL1. The 4-trifluoromethyl and the 4-nitro analogues (**14n** and **14p**, respectively) as well as compound **14h**, bearing a methyl group at position 4, had relatively low activity. Introduction of alkoxy groups gave interesting results: the 4-methoxy derivative **14j** and the 3,4-dimethoxy derivative **14l** had low activity at both receptors, whereas the 3,4-methylendioxy derivative **14m** was specific for FPR1. It is worth noting that compounds **14v**, **14w**, and **14k**, where Br in the para position was substituted with the typical buthoxy group characteristic of Quin-C1, were found to be completely devoid of activity.

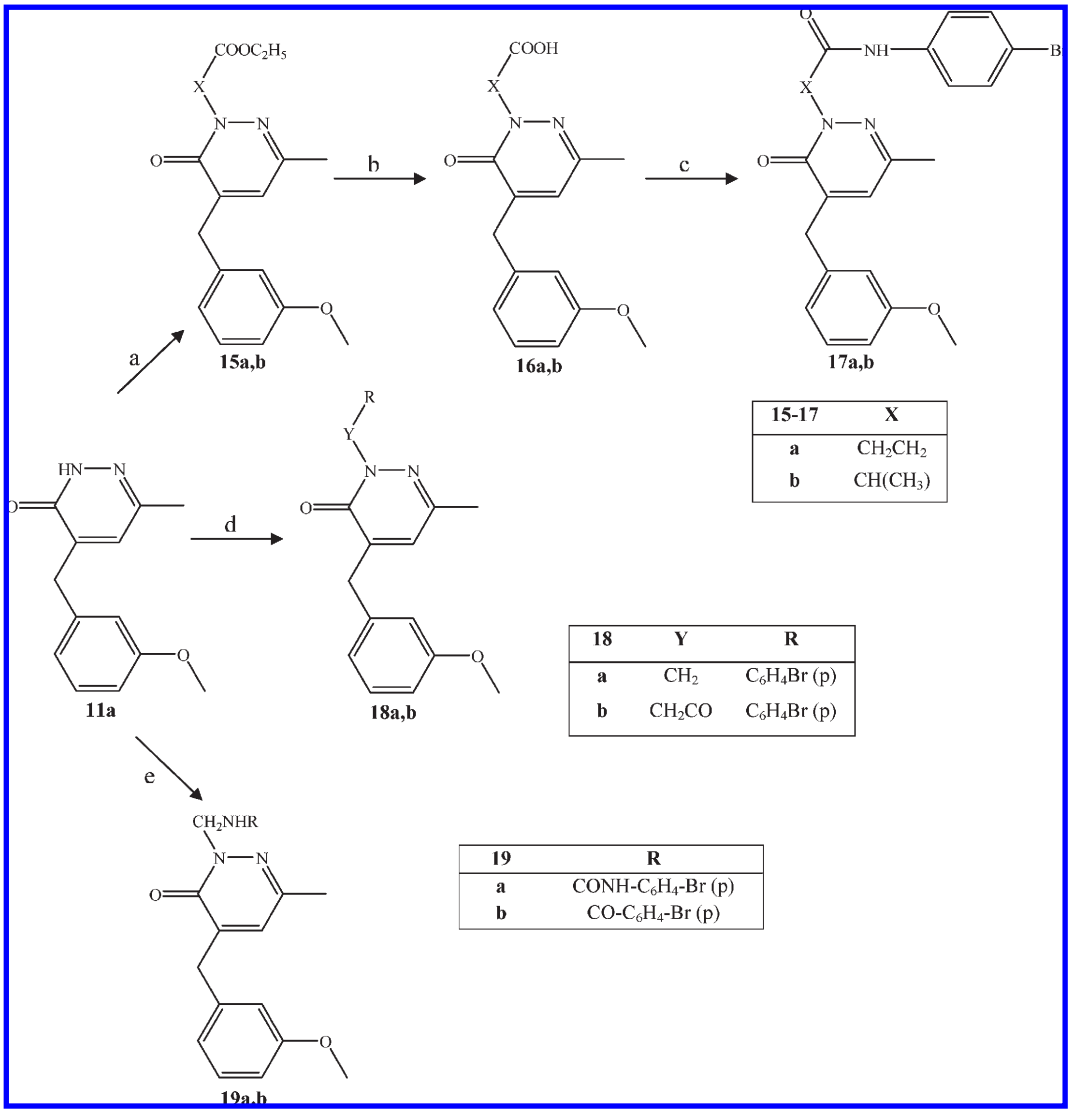
Only a few substitutions were performed at the level of the aromatic system in the benzyl group. Moving OCH_3 from the meta to the para position (compound **14x**) resulted in high activity (EC_{50} = 2.4 μM) and selectivity for FPRL1. This exciting result prompted us to design several additional analogues, which are currently under investigation. Taken together, these data suggest that regarding the aromatic system at the end of the functionalized chain in position 2, the presence of a lipophilic and/or electronegative substituent, such as F, Br, or CH_3, in position para is an essential requirement for potency and/or selectivity. Likewise, the presence of an acetamide spacer at pyridazine N-2 also plays a crucial role in specificity and potency. The role of both CO and NH in the side chain seems to indicate that a hydrogen bond donor (HBD) neighboring an acceptor (HBA) system is also an essential requirement for binding at FPRs, according to the Quin-C1 and pyrazolone structures **2**, where the presence of an urea is a common structural element. Moreover, this HBD\HBA system must be placed at an appropriate distance from both the aromatic and the heterocyclic scaffold.

For compound **14a** were also calculated dose−response curves (Figure 2). Note that no response was observed in control, undifferentiated HL-60 cells, which do not express FPRs. Furthermore, the effect of selected agonists on Ca^{2+} flux in human neutrophils was also determined to verify the HL-60 results in primary phagocytes (Table 3). We found that both selective and nonselective agonists identified in HL-60 cell assays also induced Ca^{2+} flux in human neutrophils, with EC_{50} values in the range 0.8−21.7 μM.

The most potent chemotactic compound was **14h** (EC_{50} = 0.6 μM), followed by **14j** (EC_{50} = 0.9 μM), which were both nonselective agonists. The FPRL1-selective agonist (**14x**) showed lower potency (EC_{50} = 13.1 μM) as a chemotactic agent.

In conclusion, we have identified a novel chemotype of interesting and selective FPR1 or FPRL1 agonists and mixed FPR/FPRL1 agonists active in the low micromolar range in human neutrophils. Moreover, some of these compounds proved to be able to stimulate chemotaxis at submicromolar concentrations.

Scheme 3[a]



[a] Reagents and conditions: (a) appropriate alkyl halide, K_2CO_3, anhydrous CH_3CN, reflux, 3 h; (b) NaOH, 60 °C, rt; (c) ethyl chloroformate, anhydrous THF, Et_3N, 4-bromoaniline, 12 h, rt; (d) appropriate alkyl halide, K_2CO_3, anhydrous CH_3CN, reflux, 2−4 h; (e) 40% CH_2O, 33% NH_3, dioxane, CH_2Cl_2, 4-bromophenyl isocyanate (for compound **19a**), or 4-bromobenzoyl chloride (for compound **19b**), 0 °C to rt, 6−12 h.

Experimental Section

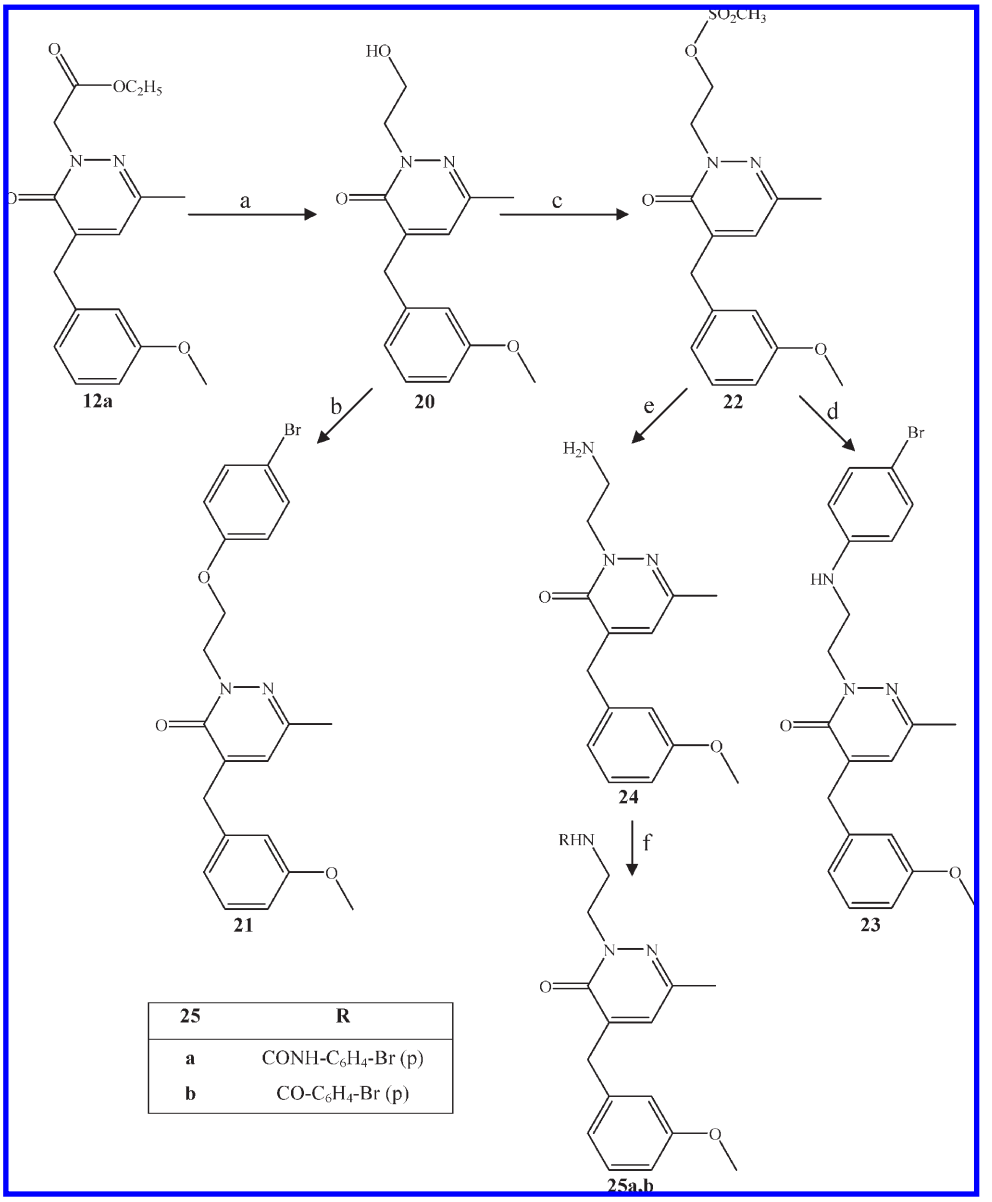
Chemistry. All melting points were determined on a Buchi apparatus and are uncorrected. IR spectra were measured as Nujol mulls with a PerkinElmer spectrometer (FT-IR, Spectrum 1000). ^{1}H NMR spectra were recorded with Avance 400 instruments (Bruker Biospin Version 002 with SGU). Chemical shifts are reported in ppm, using the solvent as internal standard. Mass spectra (*m*/*z*) were recorded on a ESI-TOF mass spectrometer (Bruker Micro TOF). Extracts were dried over Na_2SO_4, and the solvents were removed under reduced pressure. Merck F-254 commercial plates were used for analytical TLC to follow the course of reaction. Silica gel 60 (Merck 70−230 mesh) was used for column chromatography. Microanalyses were performed with a Perkin-Elmer 260 elemental analyzer for C, H, N, and the results were within ±0.4% of the theoretical values unless otherwise stated. Reagents and starting materials were commercially available.

General Procedure for 6a−c. To a stirred solution of compound **5**[21](0.35 mmol) in anhydrous toluene (2 mL), the proper aryl isocyanate (0.40 mmol) was added. The mixture was refluxed for 4−7 h. After cooling, the precipitate was collected by suction and purified by crystallization from toluene (**6b** and **6c**). In the case of **6a**, a second batch of product was obtained from evaporation of the filtrate. The residue, after treatment with cold water, was extracted with CH_2Cl_2 (3 × 20 mL). Removal of the solvent gave a residue that was purified by column chromatography using CH_2Cl_2/CH_3OH 9.9:0.1 as eluent.

1-(4-Iodophenyl)-3-(6-methyl-3-oxo-2-phenyl-2,3-dihydropyridazin-4-yl)urea (6a). Yield = 45%; mp = 264−265 °C (EtOH/toluene). IR (cm^{-1}) 3270 (NH), 3256 (NH), 1708 (CO), 1627 (CO). ^{1}H NMR ($CDCl_3$) δ 2.44 (s, 3H, CH_3), 6.50 (d, 2H, Ar, J = 8.7 Hz), 7.21 (t, 1H, Ar, J = 7.5 Hz), 7.36−7.41 (m, 4H, Ar), 7.68 (d, 2H, Ar, J = 7.7 Hz), 8.15 (s, 1H, Ar), 8.41 (exch br s, 1H, NH), 9.38 (exch br s, 1H, NH). MS (ESI) *m*/*z* 447.03 [M + H]$^+$. Anal. ($C_{18}H_{15}IN_4O_2$) C, H, N.

1-(4-Bromophenyl)-3-(6-methyl-3-oxo-2-phenyl-2,3-dihydropyridazin-4-yl)urea (6b). Yield = 50%; mp = 263−265 °C (toluene). IR (cm^{-1}) 3271 (NH), 3250 (NH), 1704 (CO), 1625 (CO). ^{1}H NMR ($CDCl_3$) δ 2.43 (s, 3H, CH_3), 6.60 (d, 2H, Ar, J = 8.6 Hz), 7.19−7.22 (m, 3H, Ar), 7.38 (t, 2H, Ar, J = 7.8 Hz), 7.69 (d, 2H, Ar, J = 7.9 Hz), 8.16 (s, 1H, Ar), 8.44 (exch, br, s, 1H, NH), 9.40 (exch br s, 1H, NH). MS (ESI) *m*/*z* 399.05 [M + H]$^+$. Anal. ($C_{18}H_{15}BrN_4O_2$) C, H, N.

Scheme 4[a]



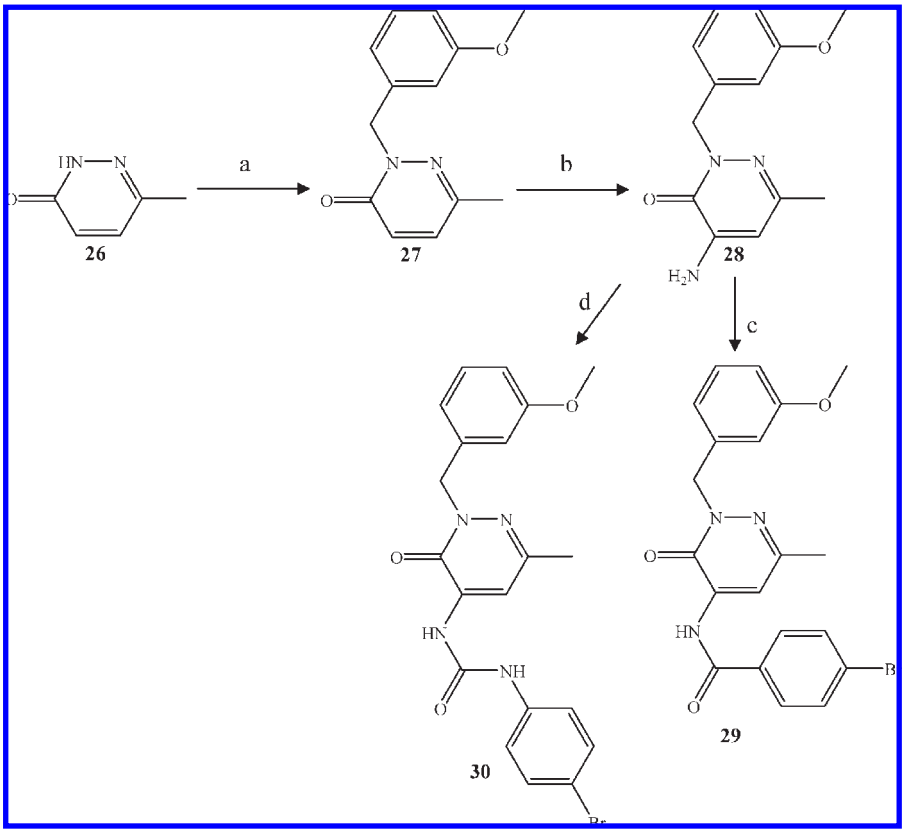
[a] Reagents and conditions: (a) anhydrous THF, $NaBH_4$, CH_3OH, 60 °C, 1 h; (b) $Cu(Ac)_2$, 4-bromophenylboronic acid, anhydrous CH_2Cl_2, Et_3N, rt, 20 h; (c) pyridine, CH_2Cl_2, methanesulfonyl chloride, 0 °C to rt, 4 h; (d) 2-propanol, 4-bromoaniline, 60 °C, 6 h; (e) 33% NH_3, isopropanol, 60 °C, 3 h; (f) anhydrous toluene, 4-bromophenyl isocyanate, 110 °C, 5 h (for compound **25a**); anhydrous CH_2Cl_2, Et_3N, 4-bromobenzoyl chloride, 0 °C, 6 h (for compound **25b**).

1-(4-Chlorophenyl)-3-(6-methyl-3-oxo-2-phenyl-2,3-dihydropyridazin-4-yl)urea (6c). Yield = 85%; mp = 274−276 °C (toluene). IR (cm^{-1}) 3269 (NH), 3255 (NH), 1705 (CO), 1626 (CO). ^1H NMR ($CDCl_3$) δ 2.44 (s, 3H, CH_3), 6.65 (d, 2H, Ar, J = 8.8 Hz), 7.07 (d, 2H, Ar, J = 8.8 Hz), 7.21 (t, 1H, Ar, J = 7.5 Hz), 7.38 (t, 2H, Ar, J = 7.9 Hz), 7.69 (d, 2H, Ar, J = 7.6 Hz), 8.16 (s, 1H, Ar), 8.43 (exch br s, 1H, NH), 9.40 (exch br s, 1H, NH). MS (ESI) m/z 355.10 $[M + H]^+$. Anal. ($C_{18}H_{15}ClN_4O_2$) C, H, N.

1-(4-Butoxyphenyl)-3-(6-methyl-3-oxo-2-phenyl-2,3-dihydropyridazin-4-yl)urea (7). To a cooled and stirred suspension of **5** (0.5 mmol) and anhydrous sodium acetate (1.19 mmol) in anhydrous THF (5 mL), triphosgene (1.18 mmol) was added. The mixture was stirred for an additional 10 min at 0 °C and refluxed for 2 h. The solvent was removed in vacuo, and the residue was dissolved in anhydrous THF (2 mL). 4-Butoxyaniline (1.25 mmol) was added, and the mixture was stirred at room temperature for 12 h. After dilution with cold water, the suspension was extracted with CH_2Cl_2 (3 × 20 mL). After removal of the solvent, the residue was purified by column chromatography using absolute EtOH/CH_2Cl_2/petroleum ether/toluene/33% ammonia 3.3:19.7:6.5:70:0.5 as eluent. Yield = 31%; mp = 189−191 °C (EtOH). IR (cm^{-1}) 3270 (NH), 3255 (NH), 1705 (CO), 1625 (CO). ^1H NMR ($CDCl_3$) δ 1.01 (t, 3H, CH_2CH_3, J = 7.4 Hz), 1.52 (sext, 2H, CH_2CH_3, J = 7.4 Hz), 1.79 (quint, 2H, $CH_2CH_2CH_2$, J = 7.0 Hz), 2.40 (s, 3H, 6-CH_3), 3.95 (t, 2H, OCH_2, J = 6.6 Hz), 6.70 (s, 4H, Ar), 7.17 (t, 1H, Ar, J = 7.5 Hz), 7.32 (t, 2H, Ar, J = 7.8 Hz), 7.61 (d, 2H, Ar, J = 7.9 Hz), 8.11 (exch br s, 1H, NH), 8.14 (s, 1H, Ar), 9.33 (exch br s, 1H, NH). MS (ESI) m/z 393.19 $[M + H]^+$. Anal. ($C_{22}H_{24}N_4O_3$) C, H, N.

4-Butoxy-*N*-(6-methyl-3-oxo-2-phenyl-2,3-dihydropyridazin-4-yl)benzamide (8). 4-Butoxybenzoic acid (0.36 mmol) was converted to the corresponding chloride as follows: $SOCl_2$ (20.7 mmol) was added and the stirred suspension was cooled to 0 °C and treated with Et_3N (0.99 mmol). After 4 h of reflux, the mixture was evaporated in vacuo and the residue was washed

Scheme 5[a]



[a] Reagents and conditions: (a) 3-methoxybenzyl chloride, anhydrous CH_3CN, anhydrous K_2CO_3, 80 °C, 2 h; (b) hydrazine hydrate, 180 °C, 11 h; (c) 4-bromobenzoyl chloride, Et_3N, 0 °C, 4 h; (d) 4-bromophenyl isocyanate, anhydrous toluene, reflux, 7 h.

with cyclohexane (3 × 5 mL). The residue was dissolved in anhydrous toluene (5 mL), and the solution was treated with a solution of compound **5** (0.40 mmol) in toluene (1 mL) and Et_3N (1.48 mmol). After 4 h of reflux, the solvent was removed in vacuo and the residue was treated with cold water and extracted with CH_2Cl_2 (3 × 20 mL). After removal of the solvent, the residue was purified by column chromatography (eluent: cyclohexane/ethyl acetate 3:1). Yield = 37%; mp = 95−97 °C (EtOH). IR (cm^{-1}) 3269 (NH), 1682 (CO), 1644 (CO). ^1H NMR ($CDCl_3$) δ 1.01 (t, 3H, CH_2CH_3, J = 7.4 Hz), 1.53 (sext, 2H, CH_2CH_3, J = 7.5 Hz), 1.82 (quint, 2H, $CH_2CH_2CH_2$, J = 7.0 Hz), 2.45 (s, 3H, 6-CH_3), 4.06 (t, 2H, CH_2O, J = 6.5 Hz), 6.99 (d, 2H, Ar, J = 8.8 Hz), 7.43 (t, 1H, Ar, J = 7.4 Hz), 7.52 (t, 2H, Ar, J = 7.8 Hz), 7.65 (d, 2H, Ar, J = 7.7 Hz), 7.91 (d, 2H, Ar, J = 8.8 Hz), 8.24 (s, 1H, Ar), 9.46 (exch br s, 1H, NH). MS (ESI) m/z 378.18 $[M + H]^+$. Anal. ($C_{22}H_{23}N_3O_3$) C, H, N.

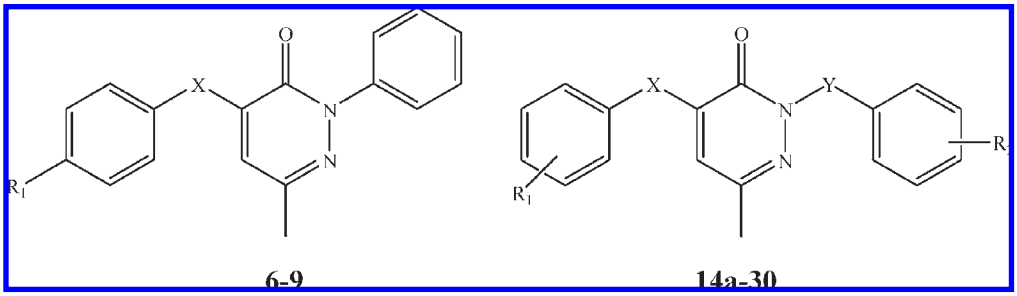
4-(4-Butoxyphenylamino)-6-methyl-2-phenylpyridazin-3(2*H*)-one (9). To a suspension of **5** (0.55 mmol), copper acetate (0.82 mmol) and 4-butoxyphenylboronic acid (1.08 mmol) in CH_2Cl_2 (2 mL), Et_3N (1.08 mmol) was added and the mixture was stirred at room temperature for 12 h. The suspension was extracted with 15% aqueous ammonia (10 mL), and the organic layer was washed with 10 mL of water and dried over sodium sulfate. After removal of the solvent, the residue was purified by column chromatography using as eluent cyclohexane/ethyl acetate 3:1. The analytical sample was obtained from a further purification performed on a silica gel preparative column (eluent: cyclohexane/ethyl acetate 3:1). Yield = 16%; mp = 137−139 °C (EtOH). IR (cm^{-1}) 3275 (NH), 1633 (CO). ^1H NMR ($CDCl_3$) δ 1.02 (t, 3H, CH_2CH_3, J = 7.4 Hz), 1.54 (sext, 2H, CH_2CH_3, J = 7.0 Hz), 1.81 (quint, 2H, $CH_2CH_2CH_2$), 2.31 (s, 3H, 6-CH_3), 4.01 (t, 2H, CH_2O, J = 6.5 Hz), 6.40 (s, 1H, Ar), 6.97 (d, 2H, Ar, J = 8.9 Hz), 7.20 (d, 2H, Ar, J = 8.8 Hz), 7.41 (t, 1H, Ar, J = 7.4 Hz), 7.51 (t, 3H, Ar, J = 7.7 Hz), 7.64 (d, 2H, Ar, J = 8.0 Hz). MS (ESI) m/z 350.19 $[M + H]^+$. Anal. ($C_{21}H_{23}N_3O_2$) C, H, N.

4-(3-Methoxybenzyl)-6-methylpyridazin-3(2*H*)-one (11a). To a solution of **10** (1.79 mmol) in 7 mL of a solution of KOH 5% (w/v) in absolute EtOH, 3-methoxybenzaldehyde (1.79 mmol) was added and the mixture was refluxed under stirring for 3 h. After cooling, the sample was concentrated in vacuo, diluted with cold water (10−15 mL), and acidified with 2 N HCl. The suspension was extracted with CH_2Cl_2 (3 × 15 mL). Removal of the solvent afforded compound **11a**, which was purified by flash chromatography using cyclohexane/ethyl acetate 1:1 as eluent. Yield = 61%; mp = 133−134 °C (EtOH). ^1H NMR ($CDCl_3$) δ 2.26 (s, 3H, 6-CH_3), 3.82 (s, 3H, OCH_3), 3.90 (s, 2H, CH_2-Ar), 6.73 (s, 1H, Ar), 6.82−6.86 (m, 3H, Ar), 7.276−7.28 (m, 1H, Ar).

[5-(3-Methoxybenzyl)-3-methyl-6-oxo-6*H*-pyridazin-1-yl]-acetic acid ethyl ester (12a). A mixture of **11a** (1.13 mmol), K_2CO_3 (2.26 mmol), and ethyl bromoacetate (3.40 mmol) in CH_3CN (3 mL) was refluxed under stirring for 6 h. The mixture was then concentrated in vacuo, diluted with cold water, and extracted with CH_2Cl_2 (3 × 15 mL). The solvent was evaporated in vacuo, and compound **12a** was purified by column chromatography using cyclohexane/ethyl acetate 1:1 as eluent. Yield = 98%; oil. ^1H NMR ($CDCl_3$) δ 1.29t, 3H, CH_2CH_3, J = 7.1 Hz), 2.22 (s, 3H, 3-CH_3), 3.80 (s, 3H, OCH_3), 3.86 (s, 2H, CH_2-Ar), 4.22−4.25 (m, 2H, OCH_2CH_3), 4.85 (s, 2H, NCH_2CO), 6.67 (s, 1H, Ar), 6.78−6.82 (m, 3H, Ar), 7.26 (t, 1H, Ar, J = 7.8 Hz).

General Procedure for 13a,b. A suspension of the appropriate derivatives **12a** or **12b**,[24] respectively (0.5−1.11 mmol), and 6 N NaOH (6−8 mL) in ethanol (2 mL) was stirred at rt to 80 °C for 1−4 h. The mixtures were then concentrated in vacuo, diluted with cold water, and acidified with 6 N HCl, and the final products were filtered off with suction and recrystallized from ethanol.

Table 1. Activity of the Compounds in Human HL-60 Cells Expressing Human FPR1, FPRL1, or FPRL2



compd	X	Y	R_1	R_2	Ca^{2+} mobilization EC_{50} (μM) and efficacy (%)[a] FPR1	FPRL1	FPRL2
6a	NHCONH		I		NA	NA	NA
6b	NHCONH		Br		NA	NA	NA
6c	NHCONH		Cl		NA	NA	NA
7	NHCONH		OC_4H_9		NA	NA	NA
8	NHCO		OC_4H_9		NA	NA	NA
9	NH		OC_4H_9		NA	NA	NA
14a	CH_2	CH_2CONH	3-OCH_3	4-Br	3.4 ± 1.6 (75)	3.8 ± 1.5 (70)	NA
17a	CH_2	$(CH_2)_2CONH$	3-OCH_3	4-Br	9.7 ± 2.7 (30)	5.4 ± 1.2 (25)	NA
17b	CH_2	$CH(CH_3)CONH$	3-OCH_3	4-Br	3.2 ± 1.5 (90)	NA	NA
18a	CH_2	CH_2	3-OCH_3	4-Br	NA	NA	NA
18b	CH_2	CH_2CO	3-OCH_3	4-Br	NA	NA	NA
19a	CH_2	$CH_2NHCONH$	3-OCH_3	4-Br	NA	NA	NA
19b	CH_2	CH_2NHCO	3-OCH_3	4-Br	NA	NA	NA
21	CH_2	$(CH_2)_2O$	3-OCH_3	4-Br	NA	NA	NA
23	CH_2	$(CH_2)_2NH$	3-OCH_3	4-Br	NA	NA	NA
25a	CH_2	$(CH_2)_2NHCONH$	3-OCH_3	4-Br	NA	NA	NA
25b	CH_2	$(CH_2)_2NHCO$	3-OCH_3	4-Br	NA	NA	NA
29	NHCO	CH_2	4-Br	3-OCH_3	NA	NA	NA
30	NHCONH	CH_2	4-Br	3-OCH_3	NA	NA	NA
fMLF					0.01	20.4	1.9
WKYMVm					0.5	0.001	0.01

[a] NA, no activity was observed (no response was observed during first 2 min after addition of compounds under investigation). The EC_{50} values are presented as the mean ± SD of three independent experiments, in which median effective concentration values (EC_{50}) were determined by nonlinear regression analysis of the dose−response curves (5−6 points) generated using GraphPad Prism 5 with 95% confidential interval ($p < 0.05$). Efficacy (in bracket) is expressed as percent of the response induced by 5 nM fMLF (FPR1) or 5 nM WKYMVm (FPRL1 and FPRL2).

[5-(3-Methoxybenzyl)-3-methyl-6-oxo-6*H*-pyridazin-1-yl]-acetic Acid (13a). Yield = 97%; mp = 194−195 °C (EtOH). ^{1}H NMR (DMSO) δ 2.21 (s, 3H, 3-CH_3), 3.72 (s, 3H, OCH_3), 3.76 (s, 2H, CH_2-Ar), 4.69 (s, 2H, NCH_2CO), 6.79−6.85 (m, 3H, Ar), 7.07 (s, 1H, Ar), 7.22 (t, 1H, Ar, J = 7.8 Hz).

[5-(4-Methoxybenzyl)-3-methyl-6-oxo-6*H*-pyridazin-1-yl]-acetic Acid (13b). Yield = 96%; mp = 144−145 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 2.26 (s, 3H, 3-CH_3), 3.84 (s, 3H, OCH_3), 3.87 (s, 2H, CH_2-Ar), 4.93 (s, 2H, NCH_2CO), 6.69 (s, 1H, Ar), 6.91 (d, 2H, Ar, J = 8.5 Hz), 7.16 (d, 2H, Ar, J = 8.5 Hz).

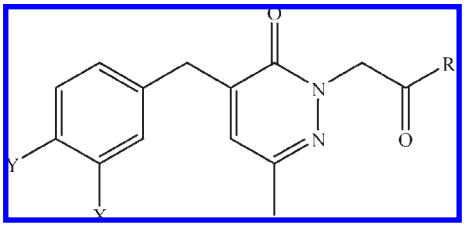
General Procedure for 14a−x. To a cooled (−5 °C) and stirred solution of compound **13a** (0.35 mmol) in anhydrous tetrahydrofuran (3−5 mL), Et_3N (1.22 mmol) was added. After 30 min, the mixture was allowed to warm up to 0 °C, and ethyl chloroformate (0.38 mmol) was added. After 1 h, the appropriate substituted aryl(cycloalkyl)amine (for compounds **14a−s** and **14u−x**) or 4-bromophenol (for compound **14t**), commercially available (0.7 mmol), was added. The reactions were carried out at room temperature for 12 h. The mixtures were then concentrated in vacuo, diluted with cold water (20−30 mL), and extracted with CH_2Cl_2 (3 × 15 mL). The solvent was evaporated to afford final compounds **14a−x**, which were purified by column chromatography using cyclohexane/ethyl acetate 1:1 as eluent for compounds **14a**, **14d**, **14f−m**, **14q**, **14w**; toluene/NH_4OH/EtOH/CH_2Cl_2/petroleum ether 7:0.05:0.30:2:0.65 for compounds **14b**,**c**; **14x**; cyclohexane/ethyl acetate 1:2 for compounds **14e**, **14n−p**, **14s**, 14v; cyclohexane/ethyl acetate 2:1 for compounds **14r**,**t** and CH_2Cl_2/CH_3OH 9.5:0.5 for compound **14u**.

***N*-(4-Bromophenyl)-2-[5-(3-methoxybenzyl)-3-methyl-6-oxo-6*H*-pyridazin-1-yl]-acetamide (14a).** Yield = 86%; mp = 171−172 °C (EtOH). IR (cm^{-1}) 3300 (NH), 1708 (CO), 1644 (CO). ^{1}H NMR ($CDCl_3$) δ 2.20 (s, 3H, 3-CH_3), 3.70 (s, 3H, OCH_3), 3.73 (s, 2H, CH_2-Ar), 4.80 (s, 2H, NCH_2CO), 6.77−6.82 (m, 3H, Ar), 7.13 (s, 1H, Ar), 7.20 (t, 1H, Ar, J = 8.0 Hz), 7.47 (s, 4H, Ar). MS (ESI) m/z 442.08 $[M + H]^+$. Anal. ($C_{21}H_{20}BrN_3O_3$) C, H, N.

***N*-(3-Bromophenyl)-2-[5-(3-methoxybenzyl)-3-methyl-6-oxo-6*H*-pyridazin-1-yl]-acetamide (14b).** Yield = 85%; mp = 73−75 °C (EtOH). IR (cm^{-1}) 3295 (NH), 1708 (CO), 1642 (CO). ^{1}H NMR ($CDCl_3$) δ 2.30 (s, 3H, 3-CH_3), 3.80 (s, 3H, OCH_3), 3.92 (s, 2H, CH_2-Ar), 4.95 (s, 2H, NCH_2CO), 6.80−6.86 (m, 4H, Ar), 7.14 (t, 1H, Ar, J = 8.0 Hz), 7.22 (m, 1H, Ar, J = 8.2 Hz), 7.27−7.31 (m, 1H, Ar), 7.39 (d, 1H, Ar, J = 8.0 Hz), 7.77 (s, 1H, Ar), 9.05 (exch br s, 1H, NH). MS (ESI) m/z 271.11 $[M + H]^+$. Anal. ($C_{21}H_{20}BrN_3O_3$) C, H, N.

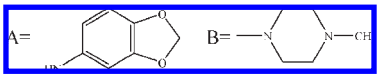
***N*-(2-Bromophenyl)-2-[5-(3-methoxybenzyl)-3-methyl-6-oxo-6*H*-pyridazin-1-yl]-acetamide (14c).** Yield = 80%; oil. IR (cm^{-1}) 3300 (NH), 1708 (CO), 1644 (CO). ^{1}H NMR ($CDCl_3$) δ 2.30 (s, 3H, 3-CH_3), 3.82 (s, 3H, OCH_3), 3.92 (s, 2H, CH_2-Ar), 5.00 (s, 2H, NCH_2CO), 6.76 (s, 1H, Ar), 6.80−6.86 (m, 3H, Ar), 7.00 (t, 1H, Ar, J = 7.7 Hz), 7.27−7.34 (m, 2H, Ar), 7.52 (d, 1H, Ar, J = 8.0 Hz), 8.36 (d, 1H, Ar, J = 8.2 Hz), 8.54 (exch br s, 1H, NH). MS (ESI) m/z 442.08 $[M + H]^+$. Anal. ($C_{21}H_{20}BrN_3O_3$) C, H, N.

Table 2. Activity of the Compounds in Human HL-60 Cells Expressing Human FPR1, FPRL1, or FPRL2



				Ca^{2+} mobilization EC_{50} (μM) and efficacy (%)[a]		
compd	X	Y	R_1	FPR1	FPRL1	FPRL2
14a	OCH_3	H	NH-C_6H_4-Br (p)	3.4 ± 1.6 (75)	3.8 ± 1.5 (70)	NA
14b	OCH_3	H	NH-C_6H_4-Br (m)	NA	NA	NA
14c	OCH_3	H	NH-C_6H_4-Br (o)	NA	NA	NA
14d	OCH_3	H	NH-C_6H_4-Cl (p)	2.6 ± 0.3 (110)	4.0 ± 1.6 (35)	NA
14e	OCH_3	H	NH-C_6H_4-I (p)	2.8 ± 0.2 (90)	6.8 ± 2.2 (40)	13.0 ± 3.1 (30)
14f	OCH_3	H	NH-C_6H_4-F (p)	7.6 ± 0.2 (40)	NA	NA
14g	OCH_3	H	NH-C_6H_5	NA	NA	NA
14h	OCH_3	H	NH-C_6H_4-CH_3 (p)	7.2 ± 2.2 (120)	10.9 ± 3.4 (50)	NA
14i	OCH_3	H	NH-C_6H_4-tC_4H_9 (p)	NA	NA	NA
14j	OCH_3	H	NH-C_6H_4-OCH_3 (p)	7.7 ± 2.5 (65)	14.4 ± 2.0 (35)	NA
14k	OCH_3	H	NH-C_6H_4-OC_4H_9 (p)	NA	NA	NA
14 L	OCH_3	H	NH-C_6H_4-$(OCH_3)_2$ (m, p)	15.5 ± 2.9 (25)	16.8 ± 3.2 (25)	NA
14m	OCH_3	H	A[b]	2.3 ± 1.1 (50)	NA	NA
14n	OCH_3	H	NH-C_6H_4-CF_3 (p)	5.7 ± 1.8 (50)	8.8 ± 2.3 (95)	NA
14o	OCH_3	H	NH-C_6H_4-OCF_3 (p)	NA	NA	NA
14p	OCH_3	H	NH-C_6H_4-NO_2 (p)	10.5 ± 2.9 (60)	12.3 ± 2.5 (55)	NA
14q	OCH_3	H	NH-C_6H_4-CN (p)	NA	NA	NA
14r	OCH_3	H	N(CH_3)-C_6H_4-Br (p)	NA	NA	NA
14s	OCH_3	H	$NHCH_2$-C_6H_4-Br (p)	NA	NA	NA
14t	OCH_3	H	O-C_6H_4-Br (p)	NA	NA	NA
14u	OCH_3	H	B[b]	NA	NA	NA
14v	H	OCH_3	NH-C_6H_4-OC_4H_9 (p)	NA	NA	NA
14w	H	Cl	NH-C_6H_4-OC_4H_9 (p)	NA	NA	NA
14x	H	OCH_3	NH-C_6H_4-Br (p)	NA	2.4 ± 0.9 (70)	NA
fMLF				0.01	20.4	1.9
WKYMVm				0.5	0.001	0.01

[a] NA, no activity was observed (no response was observed during first 2 min after addition of compounds under investigation). The EC_{50} values are presented as the mean ± SD of three independent experiments, in which median effective concentration values (EC_{50}) were determined by nonlinear regression analysis of the dose−response curves (5−6 points) generated using GraphPad Prism 5 with 95% confidential interval ($p < 0.05$). Efficacy (in bracket) is expressed as percent of the response induced by 5 nM fMLF (FPR1) or 5 nM WKYMVm (FPRL1 and FPRL2). [b]



***N*-(4-Chlorophenyl)-2-[5-(3-methoxybenzyl)-3-methyl-6-oxo-6*H*-pyridazin-1-yl]-acetamide (14d).** Yield = 86%; mp = 170−171 °C (EtOH). IR (cm^{-1}) 3296 (NH), 1705 (CO), 1644 (CO). ^1H NMR ($CDCl_3$) δ 2.30 (s, 3H, 3-CH_3), 3.80 (s, 3H, OCH_3), 3.90 (s, 2H, CH_2-Ar), 4.95 (s, 2H, NCH_2CO), 6.79 (s, 1H, Ar), 6.80−6.85 (m, 3H, Ar), 7.16 (d, 2H, Ar, J = 8.8 Hz), 7.25−7.29 (m, 1H, Ar), 7.38 (d, 2H, Ar, J = 8.8 Hz), 9.28 (exch br s, 1H, NH). MS (ESI) m/z 398.13 $[M + H]^+$. Anal. ($C_{21}H_{20}ClN_3O_3$) C, H, N.

***N*-(4-Iodophenyl)-2-[5-(3-methoxybenzyl)-3-methyl-6-oxo-6*H*-pyridazin-1-yl]-acetamide (14e).** Yield = 99%; mp = 179−180 °C (EtOH). IR (cm^{-1}) 3300 (NH), 1707 (CO), 1645 (CO). ^1H NMR ($CDCl_3$) δ 2.30 (s, 3H, 3-CH_3), 3.79 (s, 3H, OCH_3), 3.88 (s, 2H, CH_2-Ar), 4.95 (s, 2H, NCH_2CO), 6.78−6.84 (m, 4H, Ar), 7.17 (d, 2H, Ar, J = 8.7 Hz), 7.24−7.28 (m, 1H, Ar), 7.45 (d, 2H, Ar, J = 8.7 Hz), 9.41 (exch br s, 1H, NH). MS (ESI) m/z 490.06 $[M + H]^+$. Anal. ($C_{21}H_{20}IN_3O_3$) C, H, N.

***N*-(4-Fluorophenyl)-2-[5-(3-methoxybenzyl)-3-methyl-6-oxo-6*H*-pyridazin-1-yl]-acetamide (14f).** Yield = 97%; mp = 145−147 °C (EtOH). IR (cm^{-1}) 3300 (NH), 1707 (CO), 1643 (CO). ^1H NMR ($CDCl_3$) δ 2.30 (s, 3H, 3-CH_3), 3.80 (s, 3H, OCH_3), 3.90 (s, 2H, CH_2-Ar), 4.95 (s, 2H, NCH_2CO), 6.80−6.84 (m, 4H, Ar), 6.90 (t, 2H, Ar, J = 8.7 Hz), 7.25−7.29 (m, 1H, Ar), 7.39−7.42 (m, 2H, Ar), 9.21 (exch br s, 1H, NH). MS (ESI) m/z 382.16 $[M + H]^+$. Anal. ($C_{21}H_{20}FN_3O_3$) C, H, N.

2-[5-(3-Methoxybenzyl)-3-methyl-6-oxo-6*H*-pyridazin-1-yl]-*N*-phenyl-acetamide (14g). Yield = 97%; oil. IR (cm^{-1}) 3295 (NH), 1708 (CO), 1644 (CO). ^1H NMR ($CDCl_3$) δ 2.28 (s, 3H, 3-CH_3), 3.81 (s, 3H, OCH_3), 3.91 (s, 2H, CH_2-Ar), 4.96 (s, 2H, NCH_2CO), 6.78−6.86 (m, 4H, Ar), 7.08 (t, 1H, Ar, J = 7.4 Hz), 7.26−7.30 (m, 3H, Ar), 7.50 (d, 2H, Ar, J = 8.0 Hz), 8.93 (exch br s, 1H, NH). MS (ESI) m/z 364.16 $[M + H]^+$. Anal. ($C_{21}H_{21}N_3O_3$) C, H, N.

2-[5-(3-Methoxybenzyl)-3-methyl-6-oxo-6*H*-pyridazin-1-yl]-*N*-4-tolyl-acetamide (14h). Yield = 78%; mp = 142−144 °C (EtOH). IR (cm^{-1}) 3300 (NH), 1708 (CO), 1644 (CO). ^1H NMR ($CDCl_3$) δ 2.28 (s, 3H, 3-CH_3), 2.30 (s, 3H, CH_3-Ar), 3.81 (s, 3H, OCH_3), 3.90 (s, 2H, CH_2-Ar), 4.94 (s, 2H, NCH_2CO), 6.76

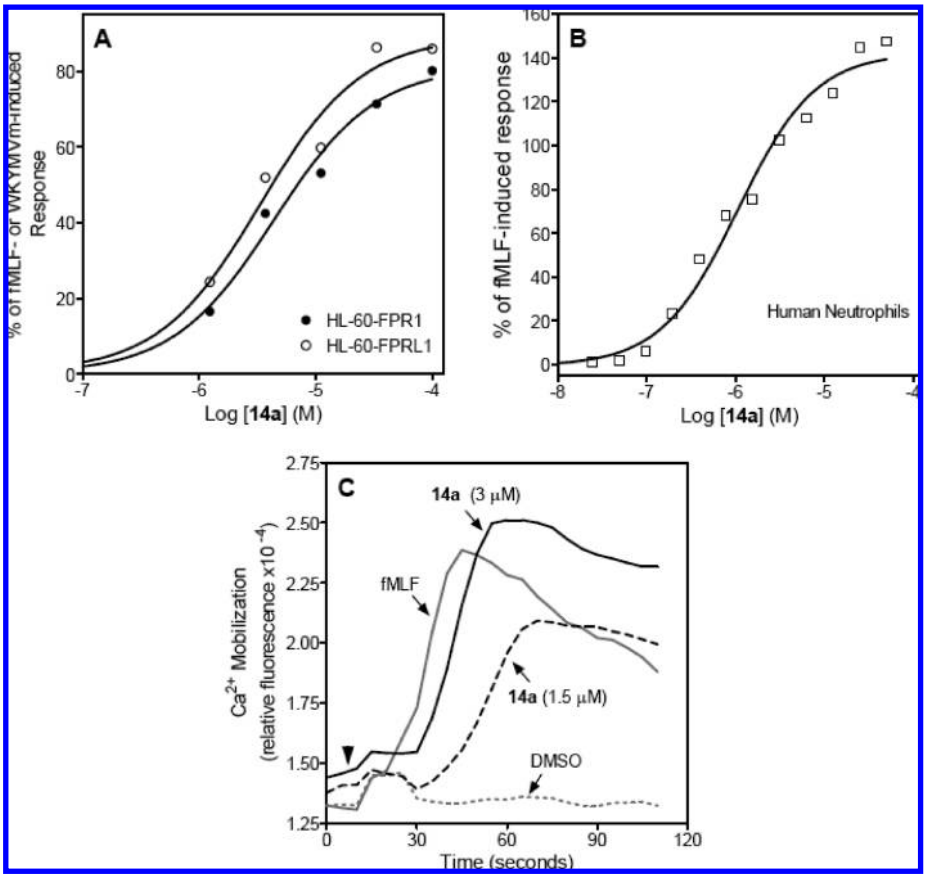


Figure 2. Analysis of Ca^{2+} mobilization in phagocytes treated with compound **14a**. HL-60-FPR1 and HL-60-FPRL1 cells (A) or human neutrophils (B) were loaded with FLIPR calcium 3 dye, and Ca^{2+} flux was analyzed, as described. Responses were normalized to the response induced by 5 nM fMLF for HL-60-FPR1 cells and neutrophils, or 5 nM WKYMVm for HL-60-FPRL1 cells, which were assigned a value of 100%. (C) Representative kinetics of Ca^{2+} mobilization after treatment with compound **14a** or fMLF. Human neutrophils were treated with the compound **14a** (1.5 and 3 μM), 5 nM fMLF (positive control), or 1% DMSO (negative control), and Ca^{2+} flux was monitored for the indicated times. The data are from one experiment that is representative of three independent experiments.

Table 3. Ca^{2+} Mobilization and Chemotactic Activity in Human Nneutrophils Treated with Selected FPR1/FPRL1 Agonists[a]

	EC_{50} (μM)	
compd	Ca^{2+} mobilization	chemotaxis
14a	2.6 ± 0.3	2.1 ± 0.8
14d	6.7 ± 1.1	1.6 ± 0.2
14e	3.2 ± 1.2	1.8 ± 0.3
14f	3.9 ± 0.6	8.2 ± 1.4
14h	3.2 ± 0.3	0.6 ± 0.3
14j	1.6 ± 0.8	0.9 ± 0.2
14l	1.1 ± 0.6	1.1 ± 0.6
14m	3.6 ± 1.0	1.2 ± 0.6
14n	3.6 ± 0.8	4.5 ± 2.5
14p	21.7 ± 4.2	1.9 ± 0.6
14x	4.3 ± 1.1	13.1 ± 2.3
17a	11.3 ± 2.8	11.8 ± 2.6
17b	0.8 ± 0.2	0.6 ± 0.4

[a] The data are presented as the mean ± SD of three independent experiments with cells from different donors, in which median effective concentration values (EC_{50}) were determined by nonlinear regression analysis of the dose−response curves (5−6 points) generated using GraphPad Prism 5 with 95% confidential interval ($p < 0.05$).

(s, 1H, Ar), 6.80−6.86 (m, 3H, Ar), 7.08 (d, 2H, Ar, J = 8.3 Hz), 7.26−7.30 (m, 1H, Ar), 7.38 (d, 2H, Ar, J = 8.4 Hz), 8.82 (exch br s, 1H, NH). MS (ESI) m/z 378.18 $[M + H]^+$. Anal. ($C_{22}H_{23}N_3O_3$) C, H, N.

***N*-(4-*tert*-Butylphenyl)-2-[5-(3-methoxybenzyl)-3-methyl-6-oxo-6*H*-pyridazin-1-yl]-acetamide (14i).** Yield = 95%; mp = 54−56 °C (EtOH). IR (cm^{-1}) 3296 (NH), 1705 (CO), 1644 (CO). ^1H NMR ($CDCl_3$) δ 1.30 (s, 9H, C-$(CH_3)_3$), 2.27 (s, 3H, 3-CH_3), 3.81 (s, 3H, OCH_3), 3.90 (s, 2H, CH_2-Ar), 4.96 (s, 2H, NCH_2CO), 6.76 (s, 1H, Ar), 6.80 (s, 1H, Ar), 6.81−6.86 (m, 2H, Ar), 7.26−7.30 (m, 3H, Ar), 7.43 (d, 2H, Ar, J = 8.6 Hz), 8.88 (exch br s, 1H, NH). MS (ESI) m/z 420.22 $[M + H]^+$. Anal. ($C_{25}H_{29}N_3O_3$) C, H, N.

2-[5-(3-Methoxybenzyl)-3-methyl-6-oxo-6*H*-pyridazin-1-yl]-*N*-(4-methoxyphenyl)-acetamide (14j). Yield = 75%; mp = 65−67 °C (EtOH). IR (cm^{-1}) 3300 (NH), 1705 (CO), 1644 (CO). ^1H NMR ($CDCl_3$) δ 2.28 (s, 3H, 3-CH_3), 3.80 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.91 (s, 2H, CH_2-Ar), 4.94 (s, 2H, NCH_2CO), 6.77 (s, 1H, Ar), 6.80 (s, 1H, Ar), 6.81−6.86 (m, 4H, Ar), 7.27−7.30 (m, 1H, Ar), 7.42 (d, 2H, Ar, J = 8.9 Hz), 8.71 (exch br s, 1H, NH). MS (ESI) m/z 394.18 $[M + H]^+$. Anal. ($C_{22}H_{23}N_3O_4$) C, H, N.

***N*-(4-Butoxyphenyl)-2-[5-(3-methoxybenzyl)-3-methyl-6-oxo-6*H*-pyridazin-1-yl]-acetamide (14k).** Yield = 73%; oil. IR (cm^{-1}) 3300 (NH), 1708 (CO), 1645 (CO). ^1H NMR ($CDCl_3$) δ 0.99 (t, 3H, $O(CH_2)_3CH_3$), 1.50 (sext, 2H, $OCH_2CH_2CH_2$, J = 7.5 Hz), 1.76 (quint, 2H, $OCH_2CH_2CH_2$, J = 7.0 Hz), 2.28 (s, 3H, 3-CH_3), 3.81 (s, 3H, OCH_3), 3.90 (s, 2H, CH_2-Ar), 3.93 (t, 2H, $OCH_2CH_2CH_2$, J = 6.5 Hz), 4.94 (s, 2H, NCH_2CO), 6.76 (s, 1H, Ar), 6.80−6.86 (m, 5H, Ar), 7.26−7.30 (m, 1H, Ar), 7.40 (d, 2H, Ar, J = 8.9 Hz), 8.76 (exch br s, 1H, NH). MS (ESI) m/z 436.23 $[M + H]^+$. Anal. ($C_{25}H_{29}N_3O_4$) C, H, N.

***N*-(3,4-Dimethoxyphenyl)-2-[5-(3-methoxybenzyl)-3-methyl-6-oxo-6*H*-pyridazin-1-yl]-acetamide (14l).** Yield = 74%; mp = 57−59 °C (EtOH). IR (cm^{-1}) 3298 (NH), 1708 (CO), 1640 (CO). ^1H NMR ($CDCl_3$) δ 2.29 (s, 3H, 3-CH_3), 3.80 (s, 3H, OCH_3),

3.83 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.90 (s, 2H, CH_2-Ar), 4.95 (s, 2H, NCH_2CO), 6.72 (d, 1H, Ar, J = 8.6 Hz), 6.78 (s, 2H, Ar), 6.82−6.88 (m, 3H, Ar), 7.25−7.29 (m, 1H, Ar), 7.32 (d, 1H, Ar, J = 2.2 Hz), 8.93 (exch br s, 1H, NH). MS (ESI) m/z 424.19 $[M + H]^+$. Anal. ($C_{23}H_{25}N_3O_5$) C, H, N.

***N*-Benzo[1,3]dioxol-5-yl-2-[5-(3-methoxybenzyl)-3-methyl-6-oxo-6*H*-pyridazin-1-yl]-acetamide (14m).** Yield = 98%; oil. IR (cm^{-1}) 3300 (NH), 1707 (CO), 1643 (CO). ^1H NMR ($CDCl_3$) δ 2.28 (s, 3H, 3-CH_3), 3.81 (s, 3H, OCH_3), 3.89 (s, 2H, CH_2-Ar), 4.93 (s, 2H, NCH_2CO), 5.92 (s, 2H, O-CH_2-O), 6.65 (d, 1H, Ar, J = 8.3 Hz), 6.77−6.85 (m, 5H, Ar), 7.21 (d, 1H, Ar, J = 2.0 Hz), 7.25−7.29 (m, 1H, Ar), 9.04 (exch br s, 1H, NH). MS (ESI) m/z 408.16 $[M + H]^+$. Anal. ($C_{22}H_{21}N_3O_5$) C, H, N.

2-[5-(3-Methoxybenzyl)-3-methyl-6-oxo-6*H*-pyridazin-1-yl]-*N*-(4-trifluoromethylphenyl)-acetamide (14n). Yield = 80%; mp = 175−176 °C (EtOH). IR (cm^{-1}) 3297(NH), 1708 (CO), 1646 (CO). ^1H NMR ($CDCl_3$) δ 2.33 (s, 3H, 3-CH_3), 3.79 (s, 3H, OCH_3), 3.91 (s, 2H, CH_2-Ar), 5.00 (s, 2H, NCH_2CO), 6.81−6.85 (m, 3H, Ar), 6.89 (s, 1H, Ar), 7.25−7.29 (m, 1H, Ar), 7.38 (d, 2H, Ar, J = 8.7 Hz), 7.47 (d, 2H, Ar, J = 8.7 Hz), 9.62 (exch br s, 1H, NH). MS (ESI) m/z 432.16 $[M + H]^+$. Anal. ($C_{22}H_{20}F_3N_3O_3$) C, H, N.

2-[5-(3-Methoxybenzyl)-3-methyl-6-oxo-6*H*-pyridazin-1-yl]-*N*-(4-trifluoromethoxyphenyl)-acetamide (14o). Yield = 87%; mp = 168−169 °C (EtOH). IR (cm^{-1}) 3300 (NH), 1708 (CO), 1644 (CO). ^1H NMR ($CDCl_3$) δ 2.31 (s, 3H, 3-CH_3), 3.78 (s, 3H, OCH_3), 3.89 (s, 2H, CH_2-Ar), 5.00 (s, 2H, NCH_2CO), 6.79−6.83 (m, 3H, Ar), 6.87 (s, 1H, Ar), 7.00 (d, 2H, Ar, J = 8.6 Hz), 7.24−7.28 (m, 1H, Ar), 7.41 (d, 2H, Ar, J = 9.0 Hz), 9.54 (exch br s, 1H, NH). MS (ESI) m/z 448.15 $[M + H]^+$. Anal. ($C_{22}H_{20}F_3N_3O_4$) C, H, N.

2-[5-(3-Methoxybenzyl)-3-methyl-6-oxo-6*H*-pyridazin-1-yl]-*N*-(4-nitrophenyl)-acetamide (14p). Yield = 49%; mp = 165−166 °C (EtOH). IR (cm^{-1}) 3298 (NH), 1708 (CO), 1644 (CO). ^1H NMR ($CDCl_3$) δ 2.34 (s, 3H, 3-CH_3), 3.79 (s, 3H, OCH_3), 3.92 (s, 2H, CH_2-Ar), 5.01 (s, 2H, NCH_2CO), 6.80−6.84 (m, 3H, Ar), 6.94 (s, 1H, Ar), 7.25−7.28 (m, 1H, Ar), 7.49 (d, 2H, Ar, J = 9.2 Hz), 7.99 (d, 2H, Ar, J = 9.2 Hz), 9.92 (exch br s, 1H, NH). MS (ESI) m/z 409.15 $[M + H]^+$. Anal. ($C_{21}H_{20}N_4O_5$) C, H, N.

***N*-(4-Cyanophenyl)-2-[5-(3-methoxybenzyl)-3-methyl-6-oxo-6*H*-pyridazin-1-yl]-acetamide (14q).** Yield = 55%; mp = 156−158 °C (EtOH). IR (cm^{-1}) 3285 (NH), 2221 (CN), 1716 (CO), 1644 (CO). ^1H NMR ($CDCl_3$) δ 2.32 (s, 3H, 3-CH_3), 3.80 (s, 3H, OCH_3), 3.91 (s, 2H, CH_2-Ar), 4.98 (s, 2H, NCH_2CO), 6.80−6.88 (m, 4H, Ar), 7.26−7.30 (m, 1H, Ar), 7.50−7.57 (m, 4H, Ar), 9.60 (exch br s, 1H, NH). MS (ESI) m/z 389.16 $[M + H]^+$. Anal. ($C_{22}H_{20}N_4O_3$) C, H, N.

2-[5-(3-Methoxybenzyl)-3-methyl-6-oxo-6*H*-pyridazin-1-yl]-*N*-(4-methoxyphenyl)-*N*-methyl-acetamide (14r). Yield = 45%; mp = 125−127 °C (EtOH). IR (cm^{-1}) 1709 (CO), 1644 (CO). ^1H NMR ($CDCl_3$) δ 2.21 (s, 3H, 3-CH_3), 3.32 (s, 3H, CH_3N), 3.82 (s, 3H, OCH_3), 3.86 (s, 2H, CH_2-Ar), 4.64 (s, 2H, NCH_2CO), 6.62 (s, 1H, Ar), 6.77 (s, 1H, Ar), 6.80−6.85 (m, 2H, Ar), 7.26−7.30 (m, 3H, Ar), 7.60 (d, 2H, Ar, J = 8.4 Hz). MS (ESI) m/z 456.09 $[M + H]^+$. Anal. ($C_{22}H_{22}BrN_3O_3$) C, H, N.

***N*-(4-Bromobenzyl)-2-[5-(3-methoxybenzyl)-3-methyl-6-oxo-6*H*-pyridazin-1-yl]-acetamide (14s).** Yield = 97%; mp = 184−185 °C (EtOH). IR (cm^{-1}) 3300 (NH), 1708 (CO), 1644 (CO). ^1H NMR (DMSO-d_6) δ 2.21 (s, 3H, 3-CH_3), 3.73 (s, 3H, OCH_3), 3.76 (s, 2H, CH_2-Ar), 4.27 (d, 2H, CH_2Ar), 4.68 (s, 2H, NCH_2CO), 6.80−6.86 (m, 3H, Ar), 7.05 (s, 1H, Ar), 7.23−7.26 (m, 3H, Ar), 7.51 (d, 2H, Ar, J = 8.3 Hz), 8.63 (exch br t, 1H, NH, J = 5.8 Hz). MS (ESI) m/z 456.09 $[M + H]^+$. Anal. ($C_{22}H_{22}BrN_3O_3$) C, H, N.

[5-(3-Methoxybenzyl)-3-methyl-6-oxo-6*H*-pyridazin-1-yl]-acetic Acid 4-Bromo-phenyl Ester (14t). Yield = 97%; mp = 111−112 °C (EtOH). IR (cm^{-1}) 3300 (NH), 1745 (CO), 1644 (CO). ^1H NMR ($CDCl_3$) δ 2.27 (s, 3H, 3-CH_3), 3.81 (s, 3H, OCH_3), 3.90 (s, 2H, CH_2-Ar), 5.10 (s, 2H, NCH_2COO), 6.73 (s, 1H, Ar), 6.80 (s, 1H, Ar), 6.83−6.86 (m, 2H, Ar), 7.05 (d, 2H, Ar, J = 8.7 Hz), 7.26−7.30 (m, 1H, Ar), 7.50 (d, 2H, Ar, J = 8.7 Hz). MS (ESI) m/z 443.06 $[M + H]^+$. Anal. ($C_{21}H_{19}BrN_2O_4$) C, H, N.

4-(3-Methoxybenzyl)-6-methyl-2-[2-(4-methylpiperazin-1-yl)-2-oxo-ethyl]-pyridazin-3(2*H*)-one (14u). Yield = 62%; oil. IR (cm^{-1}) 1673 (CO), 1644 (CO). ^1H NMR ($CDCl_3$) δ 2.25 (s, 3H, 3-CH_3), 2.55 (s, 3H, CH_3N), 2.74−2.81 (m, 4H, Ar), 3.77−3.82 (m, 7H (4H, Ar; 3H, OCH_3)), 3.87 (s, 2H, CH_2-Ar), 4.96 (s, 2H, NCH_2CO), 6.69 (s, 1H, Ar), 6.80 (s, 1H, Ar), 6.82−6.85 (m, 2H, Ar), 7.26−7.30 (m, 1H, Ar). MS (ESI) m/z 371.21 $[M + H]^+$. Anal. ($C_{20}H_{26}N_4O_3$) C, H, N.

***N*-(4-Butoxyphenyl)-2-[5-(4-methoxybenzyl)-3-methyl-6-oxo-6*H*-pyridazin-1-yl]-acetamide (14v).** Yield = 60%; mp = 160−161 °C (EtOH). IR (cm^{-1}) 3300 (NH), 1707 (CO), 1644 (CO). ^1H NMR ($CDCl_3$) δ 0.98 (t, 3H, $O(CH_2)_3CH_3$, J = 7.4 Hz), 1.45−1.54 (m, 2H, $OCH_2CH_2CH_2CH_3$), 1.73−1.80 (m, 2H, $OCH_2CH_2CH_2CH_3$), 2.28 (s, 3H, 3-CH_3), 3.83 (s, 3H, OCH_3), 3.88 (s, 2H, CH_2-Ar), 3.94 (t, 2H, $OCH_2CH_2CH_2CH_3$, J = 6.5 Hz), 4.93 (s, 2H, NCH_2CO), 6.74 (s, 1H, Ar), 6.82 (d, 2H, Ar, J = 9.0 Hz), 6.90 (d, 2H, Ar, J = 8.6 Hz), 7.17 (d, 2H, Ar, J = 8.5 Hz), 7.39 (d, 2H, Ar, J = 9.0 Hz), 8.67 (exch br s, 1H, NH). MS (ESI) m/z 436.23 $[M + H]^+$. Anal. ($C_{25}H_{29}N_3O_4$) C, H, N.

***N*-(4-Butoxyphenyl)-2-[5-(4-chlorobenzyl)-3-methyl-6-oxo-6*H*-pyridazin-1-yl]-acetamide (14w).** Yield = 70% mp = 146−147 °C (EtOH). IR (cm^{-1}) 3298 (NH), 1708 (CO), 1642 (CO). ^1H NMR ($CDCl_3$) δ 0.99 (t, 3H, $O(CH_2)_3CH_3$, J = 7.4 Hz), 1.49 (sext, 2H, $OCH_2CH_2CH_2CH_3$, J = 7.5 Hz), 1.76 (quint, 2H, $OCH_2CH_2CH_2CH_3$, J = 7.0 Hz), 2.29 (s, 3H, 3-CH_3), 3.88 (s, 2H, CH_2-Ar), 3.93 (t, 2H, CH_2-O, J = 6.5 Hz), 4.94 (s, 2H, NCH_2CO), 6.76 (s, 1H, Ar), 6.81 (d, 2H, Ar, J = 9.0 Hz), 7.18 (d, 2H, Ar, J = 8.4 Hz), 7.32 (d, 2H, Ar, J = 8.4 Hz), 7.38 (d, 2H, Ar, J = 9.0 Hz), 8.69 (exch br s, 1H, NH). MS (ESI) m/z 440.17 $[M + H]^+$. Anal. ($C_{24}H_{26}ClN_3O_3$) C, H, N.

***N*-(4-Bromophenyl)-2-[5-(4-methoxybenzyl)-3-methyl-6-oxo-6*H*-pyridazin-1-yl]-acetamide (14x).** Yield = 73%; mp = 139−141 °C (EtOH). IR (cm^{-1}) 3300 (NH), 1709 (CO), 1644 (CO). ^1H NMR ($CDCl_3$) δ 2.29 (s, 3H, 3-CH_3), 3.83 (s, 3H, OCH_3), 3.88 (s, 2H, CH_2-Ar), 4.94 (s, 2H, NCH_2CO), 6.78 (s, 1H, Ar), 6.90 (d, 2H, Ar, J = 8.6 Hz), 7.17 (d, 2H, Ar, J = 8.6 Hz), 7.40 (s, 4H, Ar), 9.01 (exch br s, 1H, NH). MS (ESI) m/z 442.08 $[M + H]^+$. Anal. ($C_{21}H_{20}BrN_3O_3$) C, H, N.

General Procedure for 15a,b. Compounds **15a**,**b** were obtained starting from **11a** following the general procedure described for **12a**, using the appropriate alkyl halide in the place of ethyl bromoacetate.

3-[5-(3-Methoxybenzyl)-3-methyl-6-oxo-6*H*-pyridazin-1-yl]-propionic Acid Ethyl Ester (15a). Yield = 86%; oil. ^1H NMR ($CDCl_3$) δ 1.26 (t, 3H,OCH_2CH_3, J = 7.1 Hz), 2.22 (s, 3H, 3-CH_3), 2.84 (t, 2H, NCH_2CH_2COO, J = 7.2 Hz), 3.83 (s, 3H, OCH_3), 3.87 (s, 2H, CH_2-Ar), 4.17 (q, 2H, OCH_2CH_3, J = 7.1 Hz), 4.45 (t, 2H, NCH_2CH_2COO, J = 7.3 Hz), 6.64 (s, 1H, Ar), 6.80 (s, 1H, Ar), 6.83 (d, 2H, Ar, J = 7.9 Hz), 7.26−7.30 (m, 1H, Ar).

2-[5-(3-Methoxybenzyl)-3-methyl-6-oxo-6*H*-pyridazin-1-yl]-propionic Acid Ethyl Ester (15b). Yield = 85%; oil. ^1H NMR ($CDCl_3$) δ 1.22 (t, 3H,OCH_2CH_3, J = 7.1 Hz), 1.65 (d, 3H, CH_3CHN, J = 7.2 Hz), 2.21 (s, 3H, 3-CH_3), 3.78 (s, 3H, OCH_3), 3.84 (s, 2H, CH_2-Ar), 4.19 (q, 2H, OCH_2CH_3, J = 6.7 Hz), 5.51 (q, 1H, CH_3CHN, J = 7.2 Hz), 6.64 (s, 1H, Ar), 6.77−6.81 (m, 3H, Ar), 7.22−7.26 (m, 1H, Ar).

General Procedure for 16a,b. Compounds **16a**,**b** were obtained starting from **15a**,**b** following the same general procedure described for **13a**,**d**. After dilution with cold water and acidification with 6N HCl, the mixtures were extracted with CH_2Cl_2 (3 × 15 mL) and the solvent evaporated in vacuo.

3-[3-(3-Methoxybenzyl)-5-methyl-2-oxo-2*H*-pyridin-1-yl]-propionic Acid (16a). Yield = 96%; mp = 86−88 °C (EtOH). ^1H NMR ($CDCl_3$) δ 2.22 (s, 3H, 3-CH_3), 2.89 (t, 2H, NCH_2CH_2COO, J = 7.2 Hz), 3.81 (s, 3H, OCH_3), 3.87 (s, 2H, CH_2-Ar), 4.46 (t, 2H, NCH_2CH_2COO, J = 7.2 Hz), 6.66 (s, 1H, Ar),

6.78 (s, 1H, Ar), 6.81−6.85 (m, 2H, Ar), 7.25−7.29 (m, 1H, Ar), 9.99 (exch br s, 1H, OH).

2-[5-(3-Methoxybenzyl)-3-methyl-6-oxo-6*H*-pyridazin-1-yl]-propionic Acid (16b). Yield = 85%; oil. ^{1}H NMR ($CDCl_3$) δ 1.71 (d, 3H, CH_3CHN, J = 7.2 Hz), 2.25 (s, 3H, 3-CH_3), 3.81 (s, 3H, OCH_3), 3.88 (s, 2H, CH_2-Ar), 5.54 (q, 1H, CH_3CHN, J = 7.2 Hz), 6.67 (s, 1H, Ar), 6.79−6.85 (m, 3H, Ar), 7.26−7.29 (m, 1H, Ar).

General Procedure for 17a,b. Compounds **17a**,**b** were obtained starting from **16a**,**b** following the general procedure described for **14a**−**x**. Purification of the final compounds was performed by column chromatography using cyclohexane/ethyl acetate 1:2 as eluent for compound **17a**, cyclohexane/ethyl acetate 2:1 for compound **17b**.

***N*-(4-Bromophenyl)-3-[3-(3-methoxybenzyl)-5-methyl-2-oxo-2*H*-pyridazin-1-yl]-propionamide (17a).** Yield = 82%; mp = 123−125 °C (EtOH). IR (cm^{-1}) 3297 (NH), 1710 (CO), 1644 (CO). ^{1}H NMR ($CDCl_3$) δ 2.27 (s, 3H, 3-CH_3), 3.00 (t, 2H, NCH_2CH_2COO, J = 6.3 Hz), 3.80 (s, 3H, OCH_3), 3.88 (s, 2H, CH_2-Ar), 4.54 (t, 2H, NCH_2CH_2COO, J = 6.3 Hz), 6.76−6.84 (m, 4H, Ar), 7.25 (t, 1H, Ar, J = 7.9 Hz), 7.40 (d, 2H, Ar, J = 8.8 Hz), 7.50 (d, 2H, Ar, J = 8.8 Hz), 9.29 (exch br s, 1H, NH). MS (ESI) m/z 456.09 [M + H]$^+$. Anal. ($C_{22}H_{22}BrN_3O_3$) C, H, N.

***N*-(4-Bromophenyl)-2-[5-(3-methoxybenzyl)-3-methyl-6-oxo-6*H*-pyridazin-1-yl]-propionamide (17b).** Yield = 53%; oil; IR (cm^{-1}) 3300 (NH), 1709 (CO), 1643 (CO). ^{1}H NMR ($CDCl_3$) δ 1.71 (d, 3H, CH_3CHN, J = 7.1 Hz), 2.31 (s, 3H, 3-CH_3), 3.80 (s, 3H, OCH_3), 3.90 (s, 2H, CH_2-Ar), 5.71 (q, 1H, CH_3CHN, J = 7.0 Hz), 6.79−6.85 (m, 4H, Ar), 7.25−7.29 (m, 1H, Ar), 7.35−7.36 (m, 4H, Ar), 9.18 (exch br s, 1H, NH). MS (ESI) m/z 456.09 [M + H]$^+$. Anal. ($C_{22}H_{22}BrN_3O_3$) C, H, N.

General Procedure for 18a,b. Compounds **18a**,**b** were obtained starting from **11** following the procedure described for **12a**. The final compounds were purified by column chromatography using cyclohexane/ethyl acetate 2:1 as eluent for compound **18a** and cyclohexane/ethyl acetate 1:1 for compound **18b**.

2-(4-Bromobenzyl)-4-(3-methoxybenzyl)-6-methyl-pyridazin-3(2*H*)-one (18a). Yield = 68%; mp = 112−114 °C (EtOH). IR (cm^{-1}) 1638 (CO). ^{1}H NMR ($CDCl_3$) δ 2.23 (s, 3H, 6-CH_3), 3.81 (s, 3H, OCH_3), 3.86 (s, 2H, CH_2-Ar), 5.25 (s, 2H, NCH_2Ar), 6.64 (s, 1H, Ar), 6.78−6.85 (m, 3H, Ar), 7.26−7.30 (m, 1H, Ar), 7.35 (d, 2H, Ar, J = 8.4 Hz), 7.45−7.47 (m, 2H, Ar). MS (ESI) m/z 399.07 [M + H]$^+$. Anal. ($C_{20}H_{19}BrN_2O_2$) C, H, N.

2-[2-(4-Bromophenyl)-2-oxo-ethyl]-4-(3-methoxybenzyl)-6-methyl-pyridazin-3(2*H*)-one (18b). Yield = 95%; oil. IR (cm^{-1}) 1715 (CO), 1644 (CO). ^{1}H NMR ($CDCl_3$) δ 2.25 (s, 3H, 6-CH_3), 3.83 (s, 3H, OCH_3), 3.89 (s, 2H, CH_2-Ar), 5.52 (s, 2H, NCH_2CO), 6.72 (s, 1H, Ar), 6.80 (s, 1H, Ar), 6.84 (d, 2H, Ar, J = 7.9 Hz), 7.27−7.31 (m, 1H, Ar), 7.66 (d, 2H, Ar, J = 8.6 Hz), 7.88 (d, 2H, Ar, J = 8.5 Hz). MS (ESI) m/z 427.07 [M + H]$^+$. Anal. ($C_{21}H_{19}BrN_2O_3$) C, H, N.

General Procedure for 19a,b. A mixture of **11a** (0.32 mmol), 40% formaldehyde (3 mL), and 33% NH_3 (1.5 mL) in dioxane (1.5−2 mL) was heated at 50 °C for 1 h. The solvent was then evaporated in vacuo, and the residue was extracted with CH_2Cl_2 (3 × 15 mL). The organic layer was dried with Na_2SO_4 and evaporated to afford an oil.

For compound **19a**, the residual oil was dissolved in 2 mL of anhydrous CH_2Cl_2 and 4-bromophenyl isocyanate (0.35 mmol) was added. The mixture was stirred at room temperature for 12 h and then the solid residue was filtered off and the solution was evaporated in vacuo to afford compound **19a**, which was purified by flash chromatography using cyclohexane/ethyl acetate 3:1 as eluent.

For compound **19b**, the residual oil was dissolved in 3 mL of anhydrous CH_2Cl_2 and, after cooling (0 °C), 4-bromobenzoyl chloride (0.44 mmol) was added and the mixture was stirred at 0 °C for 6 h. Finally, the residue was washed with cold 0.5 N NaOH (3 × 10 mL) and with cold water (2 × 10 mL). Evaporation of the organic layer afforded compound **19b**, which was purified by flash chromatography using cyclohexane/ethyl acetate 3:1 as eluent.

1-(4-Bromophenyl)-3-[5-(3-methoxybenzyl)-3-methyl-6-oxo-6*H*-pyridazin-1-ylmethyl]-urea (19a). Yield = 27%; mp = 112−114 °C (EtOH). IR (cm^{-1}) 3270 (NH), 3265 (NH), 1708 (CO), 1630 (CO). ^{1}H NMR ($CDCl_3$) δ 2.25 (s, 3H, 3-CH_3), 3.82 (s, 3H, OCH_3), 3.88 (s, 2H, CH_2-Ar), 6.13 (s, 2H, NCH_2N), 6.68 (s, 1H, Ar), 6.78−6.81 (m, 1H, Ar), 6.83−6.86 (m, 2H, Ar), 6.99 (exch br s, 1H, NH), 7.27−7.31 (m, 3H, Ar), 7.41 (m, 2H, Ar, J = 8.8 Hz). MS (ESI) m/z 458.07 [M + H]$^+$. Anal. ($C_{21}H_{21}BrN_4O_3$) C, H, N.

4-Bromo-*N*-[5-(3-methoxybenzyl)-3-methyl-6-oxo-6*H*-pyridazin-1-ylmethyl]-benzamide (19b). Yield = 30%; oil. IR (cm^{-1}) 3290 (NH), 1708 (CO), 1640 (CO). ^{1}H NMR ($CDCl_3$) δ 2.26 (s, 3H, 3-CH_3), 3.83 (s, 3H, OCH_3), 3.90 (s, 2H, CH_2-Ar), 6.29 (s, 2H, NCH_2N), 6.69 (s, 1H, Ar), 6.81−6.87 (m, 3H, Ar), 7.28−7.32 (m, 1H, Ar), 7.58 (d, 2H, Ar, J = 8.6 Hz), 7.94 (d, 2H, Ar, J = 8.6 Hz). MS (ESI) m/z 443.06 [M + H]$^+$. Anal. ($C_{21}H_{20}BrN_3O_3$) C, H, N.

2-(2-Hydroxyethyl)-4-(3-methoxybenzyl)-6-methyl-pyridazin-3(2*H*)-one (20). To a refluxed mixture of compound **12a** (0.47 mmol) and $NaBH_4$ (2.64 mmol) in anhydrous THF (6 mL), CH_3OH (1.45 mL) was slowly added. After stirring for 1 h at 60 °C, the mixture was concentrated in vacuo, diluted with cold water (10−15 mL), and extracted with CH_2Cl_2 (3 × 15 mL). Evaporation of the solvent afforded the final compound (**20**). Yield = 95%; oil. ^{1}H NMR ($CDCl_3$) δ 2.23 (s, 3H, 6-CH_3), 3.80 (s, 3H, OCH_3), 3.86 (s, 2H, CH_2-Ar), 4.00 (t, 2H, NCH_2CH_2OH, J = 5.1 Hz), 4.35 (t, 2H, NCH_2CH_2OH, J = 5.0 Hz), 4.66 (exch br s, 1H, CH_2OH), 6.68 (s, 1H, Ar), 6.78−6.84 (m, 3H, Ar), 7.25−7.29 (m, 1H, Ar).

2-[2-(4-Bromophenoxy)-ethyl]-4-(3-methoxybenzyl)-6-methyl-pyridazin-3(2*H*)-one (21). Compound **21** was obtained starting from **20** following the general procedure described for **9**. Compound **21** was purified by flash chromatography using toluene/ethyl acetate 8:2 as eluent. Yield = 26%; mp = 82−83 °C (EtOH). IR (cm^{-1}) 1643 (CO). ^{1}H NMR ($CDCl_3$) δ 2.24 (s, 3H, 6-CH_3), 3.82 (s, 3H, OCH_3), 3.87 (s, 2H, CH_2-Ar), 4.36 (t, 2H, NCH_2CH_2O, J = 5.9 Hz), 4.54 (t, 2H, NCH_2CH_2O, J = 5.8 Hz), 6.66 (s, 1H, Ar), 6.79−6.86 (m, 5H, Ar), 7.27−7.35 (m, 1H, Ar), 7.35−7.37 (m, 2H, Ar). MS (ESI) m/z 431.08 [M + H]$^+$. Anal. ($C_{21}H_{21}BrN_2O_3$) C, H, N.

Methanesulfonic Acid 2-[5-(3-Methoxybenzyl)-3-methyl-6-oxo-6*H*-pyridazin-1-yl]-ethyl Ester (22). To a cooled (0 °C) and stirred solution of **20** (0.47 mmol) and pyridine (0.5 mmol) in anhydrous CH_2Cl_2 (2 mL), methanesulfonyl chloride (0.61 mmol) was added dropwise, and the mixture was stirred at room temperature for 4 h. Then ice cold water was added and the mixture was extracted with CH_2Cl_2 (3 × 15 mL): evaporation of the solvent afforded the desired compound. Yield = 85%; oil. ^{1}H NMR ($CDCl_3$) δ 2.22 (s, 3H, 3-CH_3), 2.98 (s, 3H, CH_3SO_3), 3.80 (s, 3H, OCH_3), 3.86 (s, 2H, CH_2-Ar), 3.87−3.90 (m, 2H, NCH_2CH_2O), 4.45 (t, 2H, NCH_2CH_2O, J = 6.6 Hz), 6.66 (s, 1H, Ar), 6.78 (s, 1H, Ar), 6.82 (d, 2H, Ar, J = 8.1 Hz), 7.24−7.28 (m, 1H, Ar).

2-[2-(4-Bromophenylamino)-ethyl]-4-(3-methoxybenzyl)-6-methyl-pyridazin-3(2*H*)-one (23). A solution of **22** (0.4 mmol) and 4-bromoaniline (0.8 mmol) in 2-propanol (2 mL) was heated under stirring for 6 h at 60 °C. After the mixture was concentrated in vacuo and cold water (30 mL) was added, the suspension was extracted with CH_2Cl_2 (3 × 15 mL). Evaporation of the solvent afforded the final compound **23**, which was purified by column chromatography using toluene/ethyl acetate 8:2 as eluent. Yield = 58%; mp = 86−88 °C (EtOH). IR (cm^{-1}) 3350 (NH), 1643 (CO). ^{1}H NMR ($CDCl_3$) δ 2.24 (s, 3H, 6-CH_3), 3.58 (t, 2H, NCH_2CH_2NHAr, J = 5.7 Hz), 3.82 (s, 3H, OCH_3), 3.86 (s, 2H, CH_2-Ar), 4.46 (t, 2H, NCH_2CH_2NHAr, J = 5.8 Hz), 6.65−6.68 (m, 3H, Ar), 6.79−6.86 (m, 3H, Ar), 7.27−7.31 (m, 3H, Ar). MS (ESI) m/z 428.10 [M + H]$^+$. Anal. ($C_{21}H_{22}BrN_3O_2$) C, H, N.

2-(2-Aminoethyl)-4-(3-methoxybenzyl)-6-methyl-pyridazin-3(2*H*)-one (24). A mixture of **22** (0.43 mmol) and 33% NH_3 (3 mL) in isopropanol (2 mL) was stirred at 60 °C for 3 h. After concentration of the solvent and dilution with cold water (20 mL), the mixture was extracted with CH_2Cl_2 (3 × 15 mL). Evaporation of the solvent afforded desired compound **24**. Yield = 68%; oil. ^{1}H NMR ($CDCl_3$) δ 2.25 (s, 3H, 6-CH_3), 3.82 (s, 3H, OCH_3), 3.88 (s, 2H, CH_2-Ar), 4.02−4.04 (m, 2H, $NCH_2CH_2NH_2$), 4.37 (t, 2H, $NCH_2CH_2NH_2$, J = 4.9 Hz), 5.38 (exch br s, 2H, NH_2), 6.69 (s, 1H, Ar), 6.79−6.85 (m, 3H, Ar), 7.26−7.30 (m, 1H, Ar).

General Procedure for 25a,b. Compounds **25a**,**b** were obtained by starting from **24**. For compound **25a**, the general procedure reported for **6a**−**c** was followed, and the final compound was purified by flash chromatography using cyclohexane/ethyl acetate 1:1 as eluent. For compound **25b**, Et_3N (1.8 mmol) and 4-bromobenzoyl chloride (1.43 mmol) were added to a cooled (0 °C) and stirred solution of **24** (0.73 mmol) in anhydrous CH_2Cl_2 (2 mL), and the mixture was stirred at 0 °C for 6 h. The solid residue was filtered off, and the solution was washed with 6 N NaOH (3 × 10 mL) and then with cold water (2 × 10 mL). The organic layer was dried with Na_2SO_4 and evaporated in vacuo to afford compound **25b**, which was purified by flash chromatography using CH_2Cl_2/MeOH 99:1 as eluent.

1-(4-Bromophenyl)-3-{2-[5-(3-methoxybenzyl)-3-methyl-6-oxo-6*H*-pyridazin-1-yl]-ethyl}-urea (25a). Yield = 15%; mp = 114−115 °C (EtOH). IR (cm^{-1}) 3270 (NH), 3265 (NH), 1705 (CO), 1630 (CO). ^{1}H NMR ($CDCl_3$) δ 2.23 (s, 3H, 3-CH_3), 3.81 (s, 3H, OCH_3), 3.87 (s, 2H, CH_2-Ar), 4.47 (t, 2H, NCH_2CH_2NH, J = 5.2 Hz), 4.57 (t, 2H, NCH_2CH_2NH, J = 5.1 Hz), 6.69 (s, 1H, Ar), 6.78−6.84 (m, 3H, Ar), 7.04 (exch br s, 1H, NH), 7.25−7.28 (m, 3H, Ar), 7.41 (d, 2H, Ar, J = 8.8 Hz). MS (ESI) m/z 472.08 $[M + H]^+$. Anal. ($C_{22}H_{23}BrN_4O_3$) C, H, N.

4-Bromo-*N*-{2-[5-(3-methoxybenzyl)-3-methyl-6-oxo-6*H*-pyridazin-1-yl]-ethyl}-benzamide (25b). Yield = 13%; oil. IR (cm^{-1}) 3300 (NH), 1707 (CO), 1643 (CO). ^{1}H NMR ($CDCl_3$) δ 2.18 (s, 3H, 3-CH_3), 3.82 (s, 3H, OCH_3), 3.87 (s, 2H, CH_2-Ar), 4.55 (t, 2H, NCH_2CH_2NH, J = 5.4 Hz), 4.70 (t, 2H, NCH_2CH_2NH, J = 5.4 Hz), 6.67 (s, 1H, Ar), 6.68−6.85 (m, 3H, Ar), 7.26 (d, 1H, Ar, J = 7.8 Hz), 7.56 (d, 2H, Ar, J = 8.5 Hz), 7.86 (d, 2H, Ar, J = 8.5 Hz). MS (ESI) m/z 457.08 $[M + H]^+$. Anal. ($C_{22}H_{22}BrN_3O_3$) C, H, N.

2-(3-Methoxybenzyl)-6-methylpyridazin-3(2*H*)-one (27). Compound **27** was obtained starting from **26** following the general procedure described for **12a**. Compound **27** was purified by flash chromatography using CH_2Cl_2/MeOH 9.9:0.1 as eluent. Yield = 86%; mp = 53−55 °C (cyclohexane). ^{1}H NMR ($CDCl_3$) δ 2.32 (s, 3H, CH_3), 3.80 (s, 3H, OCH_3), 5.26 (s, 2H, CH_2), 6.81−6.84 (m, 1H, Ar), 6.87 (d,1H, Ar, J = 9.4 Hz), 6.97−7.05 (m, 2H, Ar), 7.07 (d, 1H, Ar, J = 9.4 Hz), 7.22−7.26 (m, 1H, Ar).

4-Amino-2-(3-methoxybenzyl)-6-methylpyridazin-3(2*H*)-one (28). A suspension of **27** (0.78 mmol) and hydrazine hydrate (322 mmol) was stirred in a sealed tube at 180 °C for 12 h. After cooling, ice-cold water was added, the suspension was kept at 0 °C for 2 h, and the precipitate was filtered off. The solution was saturated with NH_4Cl and extracted with CH_2Cl_2 (3 × 25 mL). Removal of the solvent afforded a second batch of crude product. Yield = 89%; mp = 96−98 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 2.23 (s, 3H, CH_3), 3.81 (s, 3H, OCH_3), 4.85 (exch br s, 2H, NH_2), 5.27 (s, 2H, CH_2), 6.16 (s, 1H, Ar), 6.81−6.85 (m, 1H, Ar), 6.97−7.02 (m, 2H, Ar), 7.23−7.28 (m, 1H, Ar).

4-Bromo-*N*-[2-(3-methoxybenzyl)-6-methyl-3-oxo-2,3-dihydropyridazin-4-yl]benzamide (29). Compound **29** was obtained from compound **28** following the procedure described for **25b**. The final compound was purified by flash chromatography using cyclohexane/ethyl acetate 3:1 as eluent. Yield = 28%; mp = 162−164 °C (EtOH). IR (cm^{-1}) 3300 (NH), 1709 (CO), 1644 (CO). ^{1}H NMR ($CDCl_3$) δ 2.40 (s, 3H, CH_3), 3.82 (s, 3H, OCH_3), 5.32 (s, 2H, CH_2), 6.84−6.87 (m, 1H, Ar), 6.98−7.02 (m, 2H, Ar), 7.26−7.30 (m, 1H, Ar), 7.66 (d, 1H, Ar, J = 8.7 Hz), 7.80 (d, 2H, Ar, J = 8.6 Hz), 8.14 (s, 1H, Ar), 9.37 (exch br s, 1H, NH). MS (ESI) m/z 428.06 $[M + H]^+$. Anal. ($C_{20}H_{18}BrN_3O_3$) C, H, N.

1-(4-Bromophenyl)-3-[2-(3-methoxybenzyl)-6-methyl-3-oxo-2,3-dihydropyridazin-4-yl]urea (30). Compound **30** was obtained from compound **28** following the general procedure reported for **6a**−**c**. After dilution with cold water, the mixture was extracted with CH_2Cl_2 (3 × 15 mL), and the solvent was evaporated in vacuo. Compound **30** was purified by column chromatography using first CH_2Cl_2 to remove the 4-bromophenyl urea, followed by cyclohexane/ethyl acetate 2:1 as eluent. Yield = 56%; mp = 207−209 °C (EtOH). IR (cm^{-1}) 3270 (NH), 3265 (NH), 1705 (CO), 1630 (CO). ^{1}H NMR ($CDCl_3$) δ 2.12 (s, 3H, CH_3), 3.57 (s, 3H, OCH_3), 5.08 (s, 2H, CH_2), 6.51−6.58 (m, 3H, Ar), 6.97−7.04 (m, 3H, Ar), 7.23 (d, 2H, Ar, J = 8.7 Hz), 7.86 (s, 1H, Ar), 8.70 (exch br s, 1H, NH), 8,94 (exch, br,s, 1H, NH). MS (ESI) m/z 443.07 $[M + H]^+$. Anal. ($C_{20}H_{19}BrN_4O_3$) C, H, N.

Biological Assays. Cell Culture. Human HL-60 cells stably transfected with FPR1 (HL-60-FPR1), FPRL1 (HL-60-FPRL1), or FPRL2 (HL-60-FPRL2) were as previously described.[25] The transfected cells were cultured in RPMI supplemented with 10% heat inactivated fetal calf serum, 10 mM HEPES, 100 μg/mL streptomycin, 100 U/mL penicillin, and G418 (1 mg/mL), as described previously.[26] Parent (wild-type) HL-60 cells were cultured under the same conditions but without G418.

Isolation of Human Neutrophils. Blood was collected from healthy donors in accordance with a protocol approved by the Institutional Review Board at Montana State University. Neutrophils were purified from the blood using dextran sedimentation, followed by Histopaque 1077 gradient separation and hypotonic lysis of red blood cells, as described previously.[27] Isolated neutrophils were washed twice and resuspended in HBSS without Ca^{2+} and Mg^{2+} ($HBSS^-$). Neutrophil preparations were routinely >95% pure, as determined by light microscopy, and >98% viable, as determined by trypan blue exclusion.

Ca^{2+} Mobilization Assay. Changes in intracellular Ca^{2+} were measured with a FlexStation II scanning fluorometer using a FLIPR 3 calcium assay kit (Molecular Devices, Sunnyvale, CA) for human neutrophils and HL-60 cells. All active compounds were evaluated in parent (wild-type) HL-60 cells for supporting that the agonists are inactive in nontransfected cells. Human neutrophils or HL-60 cells, suspended in $HBSS^-$ containing 10 mM HEPES, were loaded with Fluo-4 AM dye (Invitrogen) (1.25 μg/mL final concentration) and incubated for 30 min in the dark at 37 °C. After dye loading, the cells were washed with $HBSS^-$ containing 10 mM HEPES, resuspended in HBSS containing 10 mM HEPES and Ca^{2+} and Mg^{2+} ($HBSS^+$), and aliquotted into the wells of a flat-bottomed, half-area-well black microtiter plates (2 × 10^5 cells/well). The compound source plate contained dilutions of test compounds in $HBSS^+$. Changes in fluorescence were monitored (λ_{ex} = 485 nm, λ_{em} = 538 nm) every 5 s for 240 s at room temperature after automated addition of compounds. Maximum change in fluorescence, expressed in arbitrary units over baseline, was used to determine agonist response. Responses were normalized to the response induced by 5 nM fMLF (Sigma Chemical Co., St. Louis, MO) for HL-60-FPR1 and neutrophils, or 5 nM WKYMVm (Calbiochem, San Diego, CA) for HL-60-FPRL1, which were assigned a value of 100%. Curve fitting (5−6 points) and calculation of median effective concentration values (EC_{50}) were performed by nonlinear regression analysis of the dose−response curves generated using Prism 5 (GraphPad Software, Inc., San Diego, CA).

Chemotaxis Assay. Neutrophils were suspended in $HBSS^+$ containing 2% (v/v) fetal bovine serum (FBS) (2 × 10^6 cells/mL), and chemotaxis was analyzed in 96-well ChemoTx chemotaxis chambers (Neuroprobe, Gaithersburg, MD), as described previously.[27] In brief, lower wells were loaded with

30 μL of HBSS$^+$ containing 2% (v/v) FBS and the indicated concentrations of test compound, DMSO (negative control), and 1 nM fMLF as a positive control. The number of migrated cells was determined by measuring ATP in lysates of transmigrated cells using a luminescence-based assay (CellTiter-Glo; Promega, Madison, WI), and luminescence measurements were converted to absolute cell numbers by comparison of the values with standard curves obtained with known numbers of neutrophils. The results are expressed as percentage of negative control and were calculated as follows: (number of cells migrating in response to test compounds/spontaneous cell migration in response to control medium) × 100. EC_{50} values were determined by nonlinear regression analysis of the dose−response curves generated using Prism 5 software.

Acknowledgment. We thank Dr. Francois Boulay (Laboratoire de Biochimie et Biophysique des Systèmes Intégrés, CEA-Grenoble, France) for help in obtaining HL-60 cells transfected with FPR subtypes. This work was supported in part by NIH contract HHSN266200400009C and P20 RR020185. We also thank Dr. Brian Bothner and Navid Mohaved (Department of Chemistry & Biochemistry, Montana State University, Bozeman, MT) for their help in mass spectrometry analysis.

Supporting Information Available: Elemental analysis results for all target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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