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

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Abstract

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(2H)-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 (EC₅₀ = 0.6 μM) was the mixed FPR/FPRL1 agonist **14h**.

Keywords

Formyl peptide receptors; receptor agonists; neutrophils; Ca²⁺ mobilization; chemotaxis; 3(2H) pyridazinones; structure-activity relationship

Introduction

Innate immunity is a very important mechanism in defending humans against infectious microbes.¹ Thus, immune dysregulation due to viral infections (i.e. AIDS) and immunosuppression treatments following transplantation expose patients to life-threatening

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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>.

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 dysregulation 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, 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 anti-bacterial 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-aminoacid form of amyloid β (A β 42), 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 a prospective target for therapeutic intervention¹⁵. 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 peptides¹⁷ and small molecule non-peptide 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 arylisocyanate 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 CH₂Cl₂ 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**²¹ 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 (CH₂O + NH₃). 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**, **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 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 N₂H₄ 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 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 gave 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 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-methylenedioxy 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 butoxy 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 ($\text{EC}_{50} = 2.4 \mu\text{M}$) 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 CH₃, 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 Ca²⁺ 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 Ca²⁺ flux in human neutrophils, with EC₅₀ values in the range 0.8–21.7 μM.

The most potent chemotactic compound was **14h** (EC₅₀ = 0.6 μM), followed by **14j** (EC₅₀ = 0.9 μM), which were both nonselective agonists. The FPRL1-selective agonist (**14x**) showed lower potency (EC₅₀ = 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.

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). ¹H 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 Na₂SO₄, 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 CH₂Cl₂ (3 × 20 mL). Removal of the solvent gave a residue that was purified by column chromatography using CH₂Cl₂/CH₃OH 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). ¹H NMR (CDCl₃) δ 2.44 (s, 3H, CH₃), 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. (C₁₈H₁₅IN₄O₂) 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). ¹H NMR (CDCl₃) δ 2.43 (s, 3H, CH₃), 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. (C₁₈H₁₅BrN₄O₂) C, H, N.

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 (CDCl₃) δ 2.44 (s, 3H, CH₃), 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. (C₁₈H₁₅ClN₄O₂) 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 CH₂Cl₂ (3 × 20 mL). After removal of the solvent, the residue was purified by column chromatography using absolute EtOH/CH₂Cl₂/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 (CDCl₃) δ 1.01 (t, 3H, CH₂CH₃, *J* = 7.4 Hz), 1.52 (sext, 2H, CH₂CH₃, *J* = 7.4 Hz), 1.79 (quint, 2H, CH₂CH₂CH₂, *J* = 7.0 Hz), 2.40 (s, 3H, 6-CH₃), 3.95 (t, 2H, OCH₂, *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. (C₂₂H₂₄N₄O₃) 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: SOCl₂ (20.7 mmol) was added and the stirred suspension was cooled to 0 °C and treated with Et₃N (0.99 mmol). After 4 h of reflux, the mixture was evaporated in vacuo and the residue was washed 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 Et₃N (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 CH₂Cl₂ (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 (CDCl₃) δ 1.01 (t, 3H, CH₂CH₃, *J* = 7.4 Hz), 1.53 (sext, 2H, CH₂CH₃, *J* = 7.5 Hz), 1.82 (quint, 2H, CH₂CH₂CH₂, *J* = 7.0 Hz), 2.45 (s, 3H, 6-CH₃), 4.06 (t, 2H, CH₂O, *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(2H)-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(2H)-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-6H-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.

[5-(3-Methoxybenzyl)-3-methyl-6-oxo-6H-pyridazin-1-yl]-acetic Acid (13°)

Yield = 97%; mp = 194–195 °C (EtOH). 1H 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-6H-pyridazin-1-yl]-acetic Acid (13b)

Yield = 96%; mp = 144–145 °C (EtOH). ^1H NMR (CDCl_3) δ 2.26 (s, 3H, 3-CH₃), 3.84 (s, 3H, OCH₃), 3.87 (s, 2H, CH₂-Ar), 4.93 (s, 2H, NCH₂CO), 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), Et₃N (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 CH₂Cl₂ (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/NH₄OH/EtOH/CH₂Cl₂/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 CH₂Cl₂/CH₃OH 9.5:0.5 for compound **14u**.

N-(4-Bromophenyl)-2-[5-(3-methoxybenzyl)-3-methyl-6-oxo-6H-pyridazin-1-yl]-acetamide (14a)

Yield = 86%; mp = 171–172 °C (EtOH). IR (cm^{-1}) 3300 (NH), 1708 (CO), 1644 (CO). ^1H NMR (CDCl_3) δ 2.20 (s, 3H, 3-CH₃), 3.70 (s, 3H, OCH₃), 3.73 (s, 2H, CH₂-Ar), 4.80 (s, 2H, NCH₂CO), 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. (C₂₁H₂₀BrN₃O₃) C, H, N.

N-(3-Bromophenyl)-2-[5-(3-methoxybenzyl)-3-methyl-6-oxo-6H-pyridazin-1-yl]-acetamide (14b)

Yield = 85%; mp = 73–75 °C (EtOH). IR (cm^{-1}) 3295 (NH), 1708 (CO), 1642 (CO). ^1H NMR (CDCl_3) δ 2.30 (s, 3H, 3-CH₃), 3.80 (s, 3H, OCH₃), 3.92 (s, 2H, CH₂-Ar), 4.95 (s, 2H, NCH₂CO), 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. (C₂₁H₂₀BrN₃O₃) C, H, N.

N-(2-Bromophenyl)-2-[5-(3-methoxybenzyl)-3-methyl-6-oxo-6H-pyridazin-1-yl]-acetamide (14c)

Yield = 80%; oil. IR (cm^{-1}) 3300 (NH), 1708 (CO), 1644 (CO). ^1H NMR (CDCl_3) δ 2.30 (s, 3H, 3-CH₃), 3.82 (s, 3H, OCH₃), 3.92 (s, 2H, CH₂-Ar), 5.00 (s, 2H, NCH₂CO), 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. (C₂₁H₂₀BrN₃O₃) C, H, N.

N-(4-Chlorophenyl)-2-[5-(3-methoxybenzyl)-3-methyl-6-oxo-6H-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-CH₃), 3.80 (s, 3H, OCH₃), 3.90 (s, 2H, CH₂-Ar), 4.95 (s, 2H, NCH₂CO), 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. (C₂₁H₂₀ClN₃O₃) 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 (CDCl₃) δ 2.30 (s, 3H, 3-CH₃), 3.79 (s, 3H, OCH₃), 3.88 (s, 2H, CH₂-Ar), 4.95 (s, 2H, NCH₂CO), 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. (C₂₁H₂₀IN₃O₃) 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 (CDCl₃) δ 2.30 (s, 3H, 3-CH₃), 3.80 (s, 3H, OCH₃), 3.90 (s, 2H, CH₂-Ar), 4.95 (s, 2H, NCH₂CO), 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. (C₂₁H₂₀FN₃O₃) 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 (CDCl₃) δ 2.28 (s, 3H, 3-CH₃), 3.81 (s, 3H, OCH₃), 3.91 (s, 2H, CH₂-Ar), 4.96 (s, 2H, NCH₂CO), 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. (C₂₁H₂₁N₃O₃) 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 (CDCl₃) δ 2.28 (s, 3H, 3-CH₃), 2.30 (s, 3H, CH₃-Ar), 3.81 (s, 3H, OCH₃), 3.90 (s, 2H, CH₂-Ar), 4.94 (s, 2H, NCH₂CO), 6.76 (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. (C₂₂H₂₃N₃O₃) 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 (CDCl₃) δ 1.30 (s, 9H, C-(CH₃)₃), 2.27 (s, 3H, 3-CH₃), 3.81 (s, 3H, OCH₃), 3.90 (s, 2H, CH₂-Ar), 4.96 (s, 2H, NCH₂CO), 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. (C₂₅H₂₉N₃O₃) 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 (CDCl₃) δ 2.28 (s, 3H, 3-CH₃), 3.80 (s, 3H, OCH₃), 3.81 (s, 3H, OCH₃), 3.91 (s, 2H, CH₂-Ar), 4.94 (s, 2H, NCH₂CO), 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. (C₂₂H₂₃N₃O₄) 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 (CDCl₃) δ 0.99 (t, 3H, O(CH₂)₃CH₃), 1.50 (sext, 2H, OCH₂CH₂CH₂, *J* = 7.5 Hz), 1.76 (quint, 2H, OCH₂CH₂CH₂, *J* = 7.0 Hz), 2.28 (s, 3H, 3-CH₃), 3.81 (s, 3H, OCH₃), 3.90 (s, 2H, CH₂-Ar), 3.93 (t, 2H, OCH₂CH₂CH₂, *J* = 6.5 Hz), 4.94 (s, 2H, NCH₂CO), 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-CH₃), 3.80 (s, 3H, OCH₃), 3.83 (s, 3H, OCH₃), 3.84 (s, 3H, OCH₃), 3.90 (s, 2H, CH₂-Ar), 4.95 (s, 2H, NCH₂CO), 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-CH₃), 3.81 (s, 3H, OCH₃), 3.89 (s, 2H, CH₂-Ar), 4.93 (s, 2H, NCH₂CO), 5.92 (s, 2H, O-CH₂-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-CH₃), 3.79 (s, 3H, OCH₃), 3.91 (s, 2H, CH₂-Ar), 5.00 (s, 2H, NCH₂CO), 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-CH₃), 3.78 (s, 3H, OCH₃), 3.89 (s, 2H, CH₂-Ar), 5.00 (s, 2H, NCH₂CO), 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-CH₃), 3.79 (s, 3H, OCH₃), 3.92 (s, 2H, CH₂-Ar), 5.01 (s, 2H, NCH₂CO), 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-CH₃), 3.80 (s, 3H, OCH₃), 3.91 (s, 2H, CH₂-Ar), 4.98 (s, 2H, NCH₂CO), 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-6H-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 (CDCl₃) δ 2.21 (s, 3H, 3-CH₃), 3.32 (s, 3H, CH₃N), 3.82 (s, 3H, OCH₃), 3.86 (s, 2H, CH₂-Ar), 4.64 (s, 2H, NCH₂CO), 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. (C₂₂H₂₂BrN₃O₃) C, H, N.

N-(4-Bromobenzyl)-2-[5-(3-methoxybenzyl)-3-methyl-6-oxo-6H-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-CH₃), 3.73 (s, 3H, OCH₃), 3.76 (s, 2H, CH₂-Ar), 4.27 (d, 2H, CH₂Ar), 4.68 (s, 2H, NCH₂CO), 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. (C₂₂H₂₂BrN₃O₃) C, H, N.

[5-(3-Methoxybenzyl)-3-methyl-6-oxo-6H-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 (CDCl₃) δ 2.27 (s, 3H, 3-CH₃), 3.81 (s, 3H, OCH₃), 3.90 (s, 2H, CH₂-Ar), 5.10 (s, 2H, NCH₂COO), 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. (C₂₁H₁₉BrN₂O₄) C, H, N.

4-(3-Methoxybenzyl)-6-methyl-2-[2-(4-methylpiperazin-1-yl)-2-oxo-ethyl]-pyridazin-3(2H)-one (14u)

Yield = 62%; oil. IR (cm⁻¹) 1673 (CO), 1644 (CO). ¹H NMR (CDCl₃) δ 2.25 (s, 3H, 3-CH₃), 2.55 (s, 3H, CH₃N), 2.74–2.81 (m, 4H, Ar), 3.77–3.82 (m, 7H (4H, Ar; 3H, OCH₃)), 3.87 (s, 2H, CH₂-Ar), 4.96 (s, 2H, NCH₂CO), 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. (C₂₀H₂₆N₄O₃) C, H, N.

N-(4-Butoxyphenyl)-2-[5-(4-methoxybenzyl)-3-methyl-6-oxo-6H-pyridazin-1-yl]-acetamide (14v)

Yield = 60%; mp = 160–161 °C (EtOH). IR (cm⁻¹) 3300 (NH), 1707 (CO), 1644 (CO). ¹H NMR (CDCl₃) δ 0.98 (t, 3H, O(CH₂)₃CH₃, *J* = 7.4 Hz), 1.45–1.54 (m, 2H, OCH₂CH₂CH₂CH₃), 1.73–1.80 (m, 2H, OCH₂CH₂CH₂CH₃), 2.28 (s, 3H, 3-CH₃), 3.83 (s, 3H, OCH₃), 3.88 (s, 2H, CH₂-Ar), 3.94 (t, 2H, OCH₂CH₂CH₂CH₃, *J* = 6.5 Hz), 4.93 (s, 2H, NCH₂CO), 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. (C₂₅H₂₉N₃O₄) C, H, N.

N-(4-Butoxyphenyl)-2-[5-(4-chlorobenzyl)-3-methyl-6-oxo-6H-pyridazin-1-yl]-acetamide (14w)

Yield = 70% mp = 146–147 °C (EtOH). IR (cm⁻¹) 3298 (NH), 1708 (CO), 1642 (CO). ¹H NMR (CDCl₃) δ 0.99 (t, 3H, O(CH₂)₃CH₃, *J* = 7.4 Hz), 1.49 (sext, 2H, OCH₂CH₂CH₂CH₃, *J* = 7.5 Hz), 1.76 (quint, 2H, OCH₂CH₂CH₂CH₃, *J* = 7.0 Hz), 2.29 (s, 3H, 3-CH₃), 3.88 (s, 2H, CH₂-Ar), 3.93 (t, 2H, CH₂-O, *J* = 6.5 Hz), 4.94 (s, 2H, NCH₂CO), 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. (C₂₄H₂₆ClN₃O₃) 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 (CDCl₃) δ 2.29 (s, 3H, 3-CH₃), 3.83 (s, 3H, OCH₃), 3.88 (s, 2H, CH₂-Ar), 4.94 (s, 2H, NCH₂CO), 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. (C₂₁H₂₀BrN₃O₃) 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 (CDCl₃) δ 1.26 (t, 3H, OCH₂CH₃, *J* = 7.1 Hz), 2.22 (s, 3H, 3-CH₃), 2.84 (t, 2H, NCH₂CH₂COO, *J* = 7.2 Hz), 3.83 (s, 3H, OCH₃), 3.87 (s, 2H, CH₂-Ar), 4.17 (q, 2H, OCH₂CH₃, *J* = 7.1 Hz), 4.45 (t, 2H, NCH₂CH₂COO, *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 (CDCl₃) δ 1.22 (t, 3H, OCH₂CH₃, *J* = 7.1 Hz), 1.65 (d, 3H, CH₃CHN, *J* = 7.2 Hz), 2.21 (s, 3H, 3-CH₃), 3.78 (s, 3H, OCH₃), 3.84 (s, 2H, CH₂-Ar), 4.19 (q, 2H, OCH₂CH₃, *J* = 6.7 Hz), 5.51 (q, 1H, CH₃CHN, *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 CH₂Cl₂ (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 (CDCl₃) δ 2.22 (s, 3H, 3-CH₃), 2.89 (t, 2H, NCH₂CH₂COO, *J* = 7.2 Hz), 3.81 (s, 3H, OCH₃), 3.87 (s, 2H, CH₂-Ar), 4.46 (t, 2H, NCH₂CH₂COO, *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. ¹H NMR (CDCl₃) δ 1.71 (d, 3H, CH₃CHN, *J* = 7.2 Hz), 2.25 (s, 3H, 3-CH₃), 3.81 (s, 3H, OCH₃), 3.88 (s, 2H, CH₂-Ar), 5.54 (q, 1H, CH₃CHN, *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). ¹H NMR (CDCl₃) δ 2.27 (s, 3H, 3-CH₃), 3.00 (t, 2H, NCH₂CH₂COO, *J* = 6.3 Hz), 3.80 (s, 3H, OCH₃), 3.88 (s, 2H, CH₂-Ar), 4.54 (t, 2H, NCH₂CH₂COO, *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. (C₂₂H₂₂BrN₃O₃) 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). ¹H NMR (CDCl₃) δ 1.71 (d, 3H, CH₃CHN, *J* = 7.1 Hz), 2.31 (s, 3H, 3-CH₃), 3.80 (s, 3H, OCH₃), 3.90 (s, 2H, CH₂-Ar), 5.71 (q, 1H, CH₃CHN, *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. (C₂₂H₂₂BrN₃O₃) 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). ¹H NMR (CDCl₃) δ 2.23 (s, 3H, 6-CH₃), 3.81 (s, 3H, OCH₃), 3.86 (s, 2H, CH₂-Ar), 5.25 (s, 2H, NCH₂Ar), 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. (C₂₀H₁₉BrN₂O₂) 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). ¹H NMR (CDCl₃) δ 2.25 (s, 3H, 6-CH₃), 3.83 (s, 3H, OCH₃), 3.89 (s, 2H, CH₂-Ar), 5.52 (s, 2H, NCH₂CO), 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. (C₂₁H₁₉BrN₂O₃) C, H, N.

General Procedure for 19a,b

A mixture of **11a** (0.32 mmol), 40% formaldehyde (3 mL), and 33% NH₃ (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 CH₂Cl₂ (3 × 15 mL). The organic layer was dried with Na₂SO₄ and evaporated to afford an oil.

For compound **19a**, the residual oil was dissolved in 2 mL of anhydrous CH₂Cl₂ 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 CH₂Cl₂ 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-6H-pyridazin-1-ylmethyl]-urea (19a)

Yield = 27%; mp = 112–114 °C (EtOH). IR (cm⁻¹) 3270 (NH), 3265 (NH), 1708 (CO), 1630 (CO). ¹H NMR (CDCl₃) δ 2.25 (s, 3H, 3-CH₃), 3.82 (s, 3H, OCH₃), 3.88 (s, 2H, CH₂-Ar), 6.13 (s, 2H, NCH₂N), 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. (C₂₁H₂₁BrN₄O₃) C, H, N.

4-Bromo-*N*-[5-(3-methoxybenzyl)-3-methyl-6-oxo-6H-pyridazin-1-ylmethyl]-benzamide (19b)

Yield = 30%; oil. IR (cm⁻¹) 3290 (NH), 1708 (CO), 1640 (CO). ¹H NMR (CDCl₃) δ 2.26 (s, 3H, 3-CH₃), 3.83 (s, 3H, OCH₃), 3.90 (s, 2H, CH₂-Ar), 6.29 (s, 2H, NCH₂N), 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. (C₂₁H₂₀BrN₃O₃) C, H, N.

2-(2-Hydroxyethyl)-4-(3-methoxybenzyl)-6-methyl-pyridazin-3(2H)-one (20)

To a refluxed mixture of compound **12a** (0.47 mmol) and NaBH₄ (2.64 mmol) in anhydrous THF (6 mL), CH₃OH (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 CH₂Cl₂ (3 × 15 mL). Evaporation of the solvent afforded the final compound (**20**). Yield = 95%; oil. ¹H NMR (CDCl₃) δ 2.23 (s, 3H, 6-CH₃), 3.80 (s, 3H, OCH₃), 3.86 (s, 2H, CH₂-Ar), 4.00 (t, 2H, NCH₂CH₂OH, *J* = 5.1 Hz), 4.35 (t, 2H, NCH₂CH₂OH, *J* = 5.0 Hz), 4.66 (exch br s, 1H, CH₂OH), 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(2H)-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). ¹H NMR (CDCl₃) δ 2.24 (s, 3H, 6-CH₃), 3.82 (s, 3H, OCH₃), 3.87 (s, 2H, CH₂-Ar), 4.36 (t, 2H, NCH₂CH₂O, *J* = 5.9 Hz), 4.54 (t, 2H, NCH₂CH₂O, *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. (C₂₁H₂₁BrN₂O₃) C, H, N.

Methanesulfonic Acid 2-[5-(3-Methoxybenzyl)-3-methyl-6-oxo-6H-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 CH₂Cl₂ (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 CH₂Cl₂ (3 × 15 mL): evaporation of the solvent afforded the desired compound. Yield = 85%; oil. ¹H NMR (CDCl₃) δ 2.22 (s, 3H, 3-CH₃), 2.98 (s, 3H, CH₃SO₃), 3.80 (s, 3H, OCH₃), 3.86 (s, 2H, CH₂-Ar), 3.87–3.90 (m, 2H, NCH₂CH₂O), 4.45 (t, 2H, NCH₂CH₂O, *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(2H)-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 CH₂Cl₂ (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). ¹H NMR (CDCl₃) δ 2.24 (s, 3H, 6-CH₃), 3.58 (t, 2H, NCH₂CH₂NHAr, *J* = 5.7 Hz), 3.82 (s, 3H, OCH₃), 3.86 (s, 2H, CH₂-Ar), 4.46 (t, 2H, NCH₂CH₂NHAr, *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(2H)-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. 1H 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-6H-pyridazin-1-yl]-ethyl}-urea (25a)

Yield = 15%; mp = 114–115 °C (EtOH). IR (cm^{-1}) 3270 (NH), 3265 (NH), 1705 (CO), 1630 (CO). 1H 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-6H-pyridazin-1-yl]-ethyl}-benzamide (**25b**) Yield = 13%; oil. IR (cm^{-1}) 3300 (NH), 1707 (CO), 1643 (CO). 1H 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(2H)-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). 1H 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(2H)-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). 1H 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). ¹H NMR (CDCl₃) δ 2.40 (s, 3H, CH₃), 3.82 (s, 3H, OCH₃), 5.32 (s, 2H, CH₂), 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. (C₂₀H₁₈BrN₃O₃) 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 CH₂Cl₂ (3 × 15 mL), and the solvent was evaporated in vacuo. Compound **30** was purified by column chromatography using first CH₂Cl₂ 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). ¹H NMR (CDCl₃) δ 2.12 (s, 3H, CH₃), 3.57 (s, 3H, OCH₃), 5.08 (s, 2H, CH₂), 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. (C₂₀H₁₉BrN₄O₃) 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 Ca²⁺ and Mg²⁺ (HBSS⁻). Neutrophil preparations were routinely >95% pure, as determined by light microscopy, and >98% viable, as determined by trypan blue exclusion.

Ca²⁺ Mobilization Assay

Changes in intracellular Ca²⁺ 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 Ca²⁺ and Mg²⁺ (HBSS⁺), and aliquotted into the wells of a flat-bottomed, half-area-well black microtiter plates (2 × 10⁵ 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 μ L 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) $\times$ 100. EC_{50} values were determined by nonlinear regression analysis of the dose–response curves generated using Prism 5 software.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Scheme 1. 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) Et₃N, RCOCl, toluene, 110°C, 4 h; (d) Cu (Ac)₂, 4-butoxyphenylboronic acid, Et₃N, rt, 12 h.

Scheme 2. Reagents and conditions

(a) aromatic aldehyde, 5% KOH, EtOH, reflux, 2-5 h; (b) ethyl bromoacetate, K₂CO₃, anhydrous CH₃CN, reflux, 1-6 h; (c) NaOH, 60-80°C, 1-4 h; (d) ethyl chloroformate, anhydrous THF, Et₃N, appropriate substituted aryl(cycloalkyl)amine (or 4-bromophenol for compound **14t**), 12 h, rt.

Scheme 3. Reagents and conditions

(a) appropriate alkyl halide, K₂CO₃, anhydrous CH₃CN, reflux, 3 h; (b) NaOH, 60°C, rt; (c) ethyl chloroformate, anhydrous THF, Et₃N, 4-bromoaniline, 12 h, rt; (d) appropriate alkyl halide, K₂CO₃, anhydrous CH₃CN, reflux, 2-4 h; (e) 40% CH₂O, 33% NH₃, dioxane, CH₂Cl₂, 4-bromophenyl isocyanate (for compound **19a**) or 4-bromobenzoyl chloride (for compound **19b**), 0°C-rt, 6-12 h.

Scheme 4. Reagents and conditions

(a) anhydrous THF, NaBH₄, CH₃OH, 60°C, 1 h; (b) Cu(Ac)₂, 4-bromophenylboronic acid, anhydrous CH₂Cl₂, Et₃N, rt, 20 h; (c) pyridine, CH₂Cl₂, methanesulfonyl chloride, 0°C-rt, 4 h; (d) 2-propanol, 4-bromoaniline, 60°C, 6 h; (e) 33% NH₃, isopropanol, 60°C, 3 h; (f) anhydrous toluene, 4-bromophenyl isocyanate, 110°C, 5 h (for compound **25a**); anhydrous CH₂Cl₂, Et₃N, 4-bromobenzoyl chloride, 0°C, 6 h (for compound **25b**).

Scheme 5. 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.

Figure 1.
Structures of FPRL1 agonists.

Figure 2.

Analysis of Ca²⁺ 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 Ca²⁺ 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 Ca²⁺ 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 Ca²⁺ flux was monitored for the indicated times. The data are from one experiment that is representative of three independent experiments.

Table 1

Activity of the Compounds in HL-60 cells Expressing Human FPR1, FPR1, or FPR2

Compd	X	Y	R ₁	R ₂	Ca ²⁺ Mobilization EC ₅₀ (μM) and Efficacy (%) ^a		
					FPR1	FPR1	FPR2
6a	NHCONH	-	I	-	N.A.	N.A.	N.A.
6b	NHCONH	-	Br	-	N.A.	N.A.	N.A.
6c	NHCONH	-	Cl	-	N.A.	N.A.	N.A.
7	NHCONH	-	OC ₄ H ₉	-	N.A.	N.A.	N.A.
8	NHCO	-	OC ₄ H ₉	-	N.A.	N.A.	N.A.
9	NH	-	OC ₄ H ₉	-	N.A.	N.A.	N.A.
14a	CH ₂	CH ₂ CONH	3-OCH ₃	4-Br	3.4 ± 1.6 (75)	3.8 ± 1.5 (70)	N.A.
17a	CH ₂	(CH ₂) ₂ CONH	3-OCH ₃	4-Br	9.7 ± 2.7 (30)	5.4 ± 1.2 (25)	N.A.
17b	CH ₂	CH(CH ₃)CONH	3-OCH ₃	4-Br	3.2 ± 1.5 (90)	N.A.	N.A.
18a	CH ₂	CH ₂	3-OCH ₃	4-Br	N.A.	N.A.	N.A.
18b	CH ₂	CH ₂ CO	3-OCH ₃	4-Br	N.A.	N.A.	N.A.
19a	CH ₂	CH ₂ NHCONH	3-OCH ₃	4-Br	N.A.	N.A.	N.A.
19b	CH ₂	CH ₂ NHCO	3-OCH ₃	4-Br	N.A.	N.A.	N.A.
21	CH ₂	(CH ₂) ₂ O	3-OCH ₃	4-Br	N.A.	N.A.	N.A.
23	CH ₂	(CH ₂) ₂ NH	3-OCH ₃	4-Br	N.A.	N.A.	N.A.
25a	CH ₂	(CH ₂) ₂ NHCONH	3-OCH ₃	4-Br	N.A.	N.A.	N.A.
25b	CH ₂	(CH ₂) ₂ NHCO	3-OCH ₃	4-Br	N.A.	N.A.	N.A.

14a-30

6-9

Compd	X	Y	R ₁	Ca ²⁺ Mobilization EC ₅₀ (μM) and Efficacy (%) ^a		
				R ₂	FPRL1	FPRL2
29	NHCO	CH ₂	4-Br	3-OCH ₃	N.A.	N.A.
30	NHCONH	CH ₂	4-Br	3-OCH ₃	N.A.	N.A.
fMLF					0.01	20.4
WKYMVM					0.5	0.01

^aN.A., no activity was observed (no response was observed during first 2 min after addition of compounds under investigation). The EC₅₀ values are presented as the mean ± S.D. of three independent experiments, in which median effective concentration values (EC₅₀) 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 (FPRL1) or 5 nM WKYMVM (FPRL1 and FPRL2).

Table 2

Activity of the Compounds in HL-60 cells Expressing Human FPR1, FPRL1, or FPRL2

Compound	X	Y	R1	Ca ²⁺ Mobilization EC ₅₀ (μM) and Efficacy (%) ^a		
				FPRL1	FPRL2	
14a	OCH ₃	H	NH-C ₆ H ₄ -Br (p)	3.4 ± 1.6 (75)	3.8 ± 1.5 (70)	N.A.
14b	OCH ₃	H	NH-C ₆ H ₄ -Br (m)	N.A.	N.A.	N.A.
14c	OCH ₃	H	NH-C ₆ H ₄ -Br (o)	N.A.	N.A.	N.A.
14d	OCH ₃	H	NH-C ₆ H ₄ -Cl (p)	2.6 ± 0.3 (110)	4.0 ± 1.6 (35)	N.A.
14e	OCH ₃	H	NH-C ₆ H ₄ -I (p)	2.8 ± 0.2 (90)	6.8 ± 2.2 (40)	13.0 ± 3.1 (30)
14f	OCH ₃	H	NH-C ₆ H ₄ -F (p)	7.6 ± 0.2 (40)	N.A.	N.A.
14g	OCH ₃	H	NH-C ₆ H ₅	N.A.	N.A.	N.A.
14h	OCH ₃	H	NH-C ₆ H ₄ -CH ₃ (p)	7.2 ± 2.2 (120)	10.9 ± 3.4 (50)	N.A.
14i	OCH ₃	H	NH-C ₆ H ₄ -C ₄ H ₉ (p)	N.A.	N.A.	N.A.
14j	OCH ₃	H	NH-C ₆ H ₄ -OCH ₃ (p)	7.7 ± 2.5 (65)	14.4 ± 2.0 (35)	N.A.
14k	OCH ₃	H	NH-C ₆ H ₄ -OC ₄ H ₉ (p)	N.A.	N.A.	N.A.
14l	OCH ₃	H	NH-C ₆ H ₄ -(OCH ₃) ₂ (m, p)	15.5 ± 2.9 (25)	16.8 ± 3.2 (25)	N.A.
14m	OCH ₃	H	A ^b	2.3 ± 1.1 (50)	N.A.	N.A.
14n	OCH ₃	H	NH-C ₆ H ₄ -CF ₃ (p)	5.7 ± 1.8 (50)	8.8 ± 2.3 (95)	N.A.
14o	OCH ₃	H	NH-C ₆ H ₄ -OCF ₃ (p)	N.A.	N.A.	N.A.

Compound	X	Y	R1	Ca ²⁺ Mobilization EC ₅₀ (μM) and Efficacy (%) ^a		
				FPRL1	FPRL2	
14p	OCH ₃	H	NH-C ₆ H ₄ -NO ₂ (p)	10.5 ± 2.9 (60)	12.3 ± 2.5 (55)	N.A.
14q	OCH ₃	H	NH-C ₆ H ₄ -CN (p)	N.A.	N.A.	N.A.
14r	OCH ₃	H	N(CH ₃)-C ₆ H ₄ -Br (p)	N.A.	N.A.	N.A.
14s	OCH ₃	H	NHCH ₂ -C ₆ H ₄ -Br (p)	N.A.	N.A.	N.A.
14t	OCH ₃	H	O-C ₆ H ₄ -Br (p)	N.A.	N.A.	N.A.
14u	OCH ₃	H	B ^b	N.A.	N.A.	N.A.
14v	H	OCH ₃	NH-C ₆ H ₄ -OC ₄ H ₉ (p)	N.A.	N.A.	N.A.
14w	H	Cl	NH-C ₆ H ₄ -OC ₄ H ₉ (p)	N.A.	N.A.	N.A.
14x	H	OCH ₃	NH-C ₆ H ₄ -Br (p)	N.A.	2.4 ± 0.9 (70)	N.A.
fMLF				0.01	20.4	1.9
WKYMVM				0.5	0.001	0.01

^aN.A., no activity was observed (no response was observed during first 2 min after addition of compounds under investigation); The EC₅₀ values are presented as the mean ± S.D. of three independent experiments, in which median effective concentration values (EC₅₀) 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 (FPRL1) or 5 nM WKYMVM (FPRL1 and FPRL2).

^b

Table 3

Ca²⁺ Mobilization and Chemotactic Activity in Human Neutrophils Treated with Selected FPR1/FPRL1 Agonists^a

Compound	EC ₅₀ (μM)	
	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

^aThe data are presented as the mean ± SD of three independent experiments with cells from different donors, in which median effective concentration values (EC₅₀) were determined by nonlinear regression analysis of the dose-response curves (5-6 points) generated using GraphPad Prism 5 with 95% confidential interval ($p < 0.05$).