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Identification, Synthesis, and Biological Evaluation of 6-[(6*R*)-2-(4-Fluorophenyl)-6-(hydroxymethyl)-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidin-3-yl]-2-(2-methylphenyl)pyridazin-3(2*H*)-one (AS1940477), a Potent p38 MAP Kinase Inhibitor

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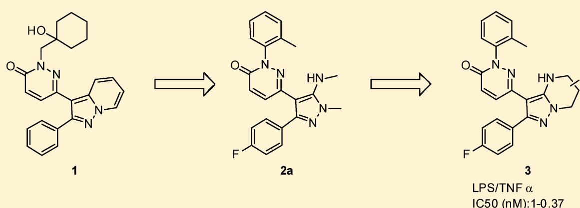
Ⓢ *Supporting Information*

ABSTRACT: Several p38 MAPK inhibitors have been shown to effectively block the production of cytokines such as IL-1β, TNFα, and IL-6. Inhibitors of p38 MAP kinase therefore have significant therapeutic potential for the treatment of autoimmune disease. Compound **2a** was identified as a potent TNFα production inhibitor in vitro but suffered from poor oral bioavailability. Structural modification of **2a** led to the discovery of tetrahydropyrazolopyrimidine derivatives, exemplified by compound **3**, with an improved pharmacokinetic profile. We found that blocking metabolism at the methyl group of the amine and constructing the tetrahydropyrimidine core were important to obtaining compounds with good biological profiles and oral bioavailability. Pursuing the structure−activity relationships of this series led to the discovery of AS1940477 (**3f**), with excellent cellular activity and a favorable pharmacokinetic profile. This compound represents a highly potent inhibitor of p38 MAP kinase with regard to in vivo activity in an adjuvant-induced arthritis model.



■ INTRODUCTION

Tumor necrosis factor α (TNFα) is a proinflammatory cytokine that plays important roles both in normal immune function and in disturbances leading to autoimmune diseases.[1−3] Overexpression of TNFα can lead to the progression of inflammatory diseases such as rheumatoid arthritis (RA), multiple sclerosis, chronic obstructive pulmonary disease (COPD), psoriasis, and inflammatory bowel disease (IBD). To date, many research programs have focused on the inhibition of TNFα production, antagonism of TNFα, or deletion of TNFα from the cell surface. TNFα has therefore attracted considerable attention as a molecular target for the treatment of these conditions.

The recent clinical success of anti-cytokine biological therapeutics such as etanercept,[4] infliximab,[5−7] and adalimumab[8] provides a strong rational for the targeting of TNFα for inflammatory diseases such as RA, COPD, and IBD. However, biologics are proteins and have the general disadvantages associated with protein drugs: poor stability, poor oral absorption and subcutaneous or intravenous administration, and high costs of manufacturing. These characteristics point to the urgent need for orally active small molecules that are safe, efficacious, and inexpensive.

Mitogen-activated protein kinase (MAPK) p38 is a serine/threonine kinase originally isolated from lipopolysaccharide (LPS) stimulated monocytes. p38 MAPK positively regulates a variety of genes involved in inflammation, such as TNFα, IL-1, IL-6, and IL-8. Because of the broad proinflammatory role of p38 MAPK in several in vitro systems, inhibition of this pathway has been advocated as novel therapeutic strategy for inflammatory disease. On the basis of this idea, many groups have investigated whether p38 MAPK inhibitors effectively block the production of proinflammatory cytokines and have demonstrated prominent efficacy in vitro and in animal models of acute inflammation and arthritis.[9−18] To date, many p38 MAPK inhibitors such as BIRB-796 or VX-745 have advanced to clinical studies for RA, but their development has been discontinued because of undesirable safety profiles.[19−21] Two of these compounds exhibited low efficacy, requiring high dosing, which may have led to adverse side effects,[13,14] and their reported side effects may have been related to their effective dose.

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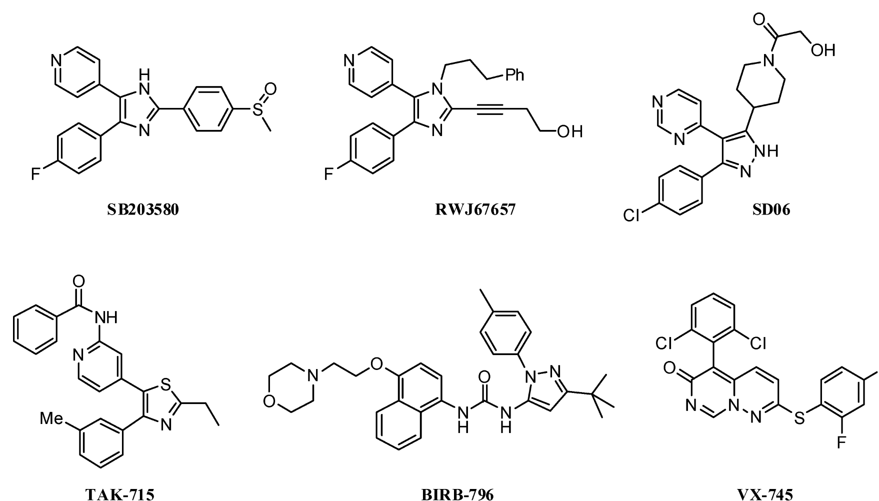


Figure 1. Structure of representative p38 MAP kinase inhibitors.

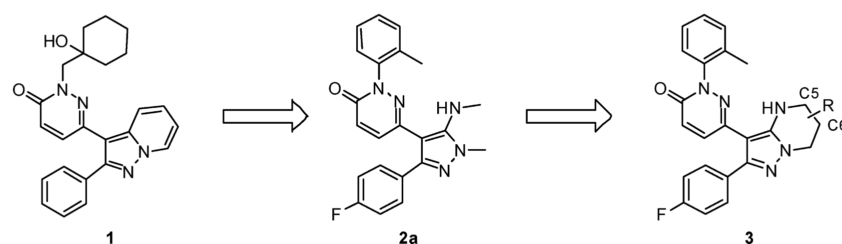
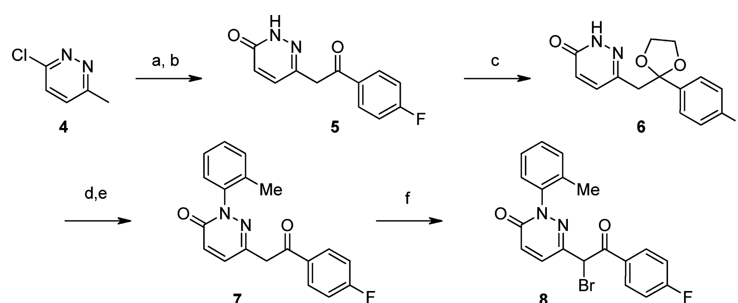


Figure 2. Leads **1** and **2** and derived target analogue **3**.

Scheme 1. Synthesis of α-Bromoketone 8[a]



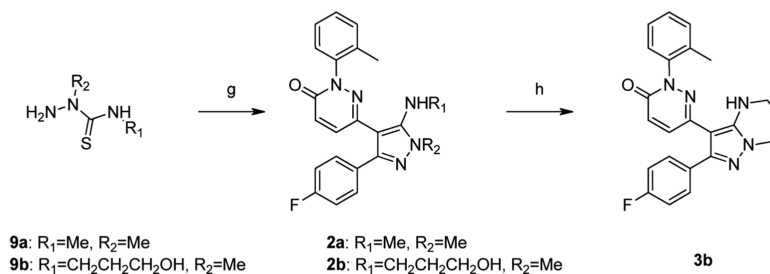
[a](a) LiHMDS, 4-fluorophenyl benzoate, THF, 15−23 °C, 83%; (b) NaOAc, HOAc, 110 °C, 83%; (c) ethylene glycol, TsOH, toluene, reflux, 100%; (d) 2-methylphenylboronic acid, $Cu(OAc)_2$, pyridine, DMF, air, 23 °C, 56%; (e) HCl, AcOH, 23 °C, 87%; (f) $PyBr_3$, AcOH, 23 °C, 99%.

Furthermore, Adam et al. reported that a p38 MAPK inhibitor containing a pyridyl or pyrimidinyl moiety led to potent inhibition of hepatic cytochrome p450 isozymes in vitro.[17,22] These results indicate that the development of p38 MAPK inhibitors for the treatment of chronic disease depends on both highly potent TNFα inhibition and the avoidance of CYP inhibition.

Prototypical p38 MAPK inhibitors, such as SB-203580, SD06, RWJ67657, and TAK-715 (Figure 1),[11,13] have been reported to be effective antiarthritic drugs in rodents. Unfortunately, SD06 and TAK-715[17,23] were unsuccessful in clinical use because of a lack of efficacy or poor pharmacokinetics. Also, as investigations have proceeded, compounds structurally different from SB-203580 have been reported as p38 inhibitors, including BIRB-796 and VX-745, which represent other p38 inhibitors and which are reported to have a potent TNFα inhibitory activity.

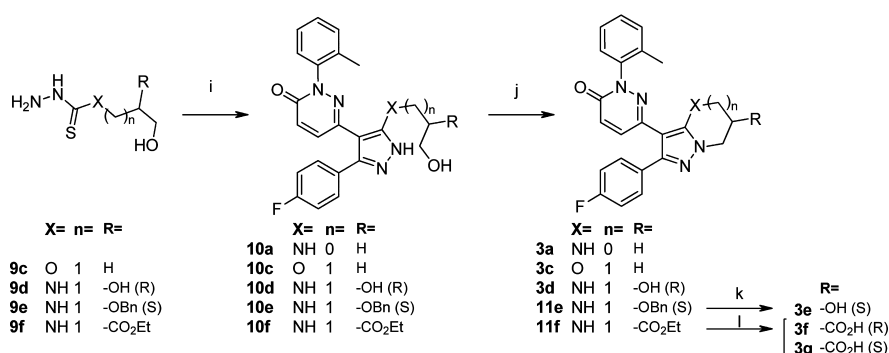
In our research program for TNFα production inhibitors, we identified pyrazolopyrimidine **1** (Figure 2) with suitable properties as optimizing leads. During the optimization process, a series of terahydropyrazolopyrimidine derivatives **3** were synthesized as potential inflammatory modulators that were demonstrated to have inhibitory activity against LPS-induced TNFα production both in vitro and in vivo. Several compounds of this series exhibited potent anti-inflammatory effects in an adjuvant-induced arthritis (AIA) model with ED_{30} values of less than 1 mg/kg on oral administration. We report the synthesis and structure−activity relationships of tetrahydropyrazolopyrimidine derivatives, leading to the discovery of the clinical

Scheme 2. Synthesis of Aminopyrazoles 2a, 2b, and Tetrahydropirimidinopyrazole 3b[a]



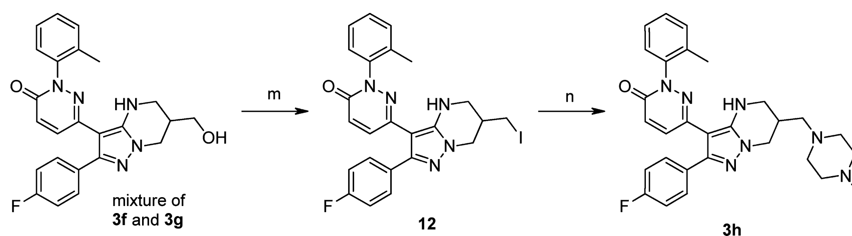
[a](g) **8**, AcOH, 60 °C, 78–85%; (h) DEAD, PPh_3, rt, 62%.

Scheme 3. Synthesis of Tetrahydropirimidinopyrazoles 3a, 3c–g[a]



[a](i) **8**, AcOH, 60 °C, 54–78%; (j) MsCl, DIPEA, MeCN, 80 °C, 10–88%; (k) $Pd(OH)_2$, EtOH, 50 °C, 33%; (l) $LiBH_4$, THF, rt, 82%.

Scheme 4. Synthesis of Tetrahydropirimidinopyrazole 3h[a]



[a](m) PPh_3, I_2, THF, 0 °C, 79%; (n) methylpiperazine, 60 °C, 22%.

candidate AS1940477 (**3f**),[24] a p38 MAPK inhibitor with novel structure. Compound **3f** showed highly potent in vitro activity and a significant anti-inflammatory effect in the AIA model.

■ RESULTS AND DISCUSSION

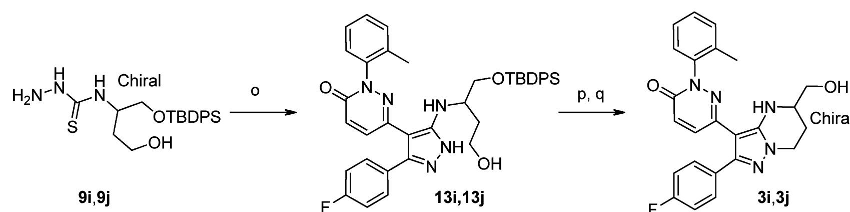
Chemistry. The synthesis of the 6-substituted tetrahydropyrazolopyrimidine analogues was conducted through cyclization of the α-bromoketone **8** with appropriately substituted thiosemicarbazides (Scheme 2). The requisite α-bromoketone **8** was synthesized by the method reported by Colletti et al.[16] as shown in Scheme 1. Synthesis of α-bromoketone **8** began with treatment of commercially available methylpyridazine **4** with 4-fluorophenyl benzoate under basic conditions, followed by hydrolysis. Acetal protection of **5** in toluene at reflux gave **6**, which was converted into **7** by oxidative Chan–Evans–Lam coupling[25] with $Cu(OAc)_2$ and pyridine in air and deprotection under acidic conditions with hydrochloric acid. Pyridinium hydrobromide perbromide in acetic acid gave α-bromoketone **8** at room temperature in high yields.

Scheme 2 describes the synthesis to obtain aminopyrazoles **2a** and **2b** and tetrahydropyrazolopyrimidine **3b**. Thiosemicarbazides **9a** and **9b** were reacted with α-bromoketone **8** under acidic conditions to afford the aminopyrazoles **2a** and **2b**,[26] followed by cyclization under Mitsunobu reaction condition to give tetrahydropyrazolopyrimidine **3b**.

The synthesis route from **8** to tetrahydropyrazolopyrimidines **3a–g** is shown in Scheme 3. The 5-substituted pyrazoles **10a** and **10c–f** were prepared from thiosemicarbazides **9c–f**) as described above for Scheme 2. Intramolecular cyclization with methanesulfonyl chloride and Hunig's base afforded the 6-substituted tetrahydropyrazolopyrimidines **3a**, **3c**, **3d**, **11e**, **11f**. Compound **3e** was obtained by hydrogenolysis of compound **11e** with catalytic palladium hydroxide under hydrogen gas. Reduction of ester **11f** with $LiBH_4$ afforded the racemic alcohol in high yield. The mixture of enantiomers was separated using chiral HPLC to afford hydroxymethyl derivatives **3f** and **3g**.

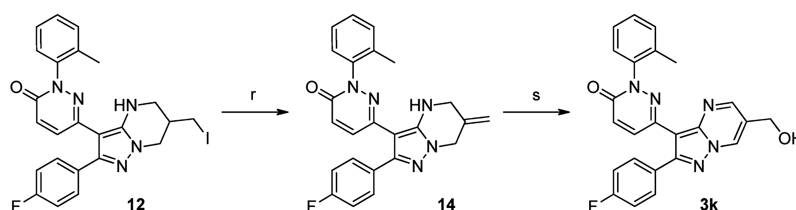
Synthesis of the methylpiperazine analogue **3h** is shown in Scheme 4. Iodide **12** was provided by iodination of alcohols **3f**

Scheme 5. Synthesis of Tetrahydropyrimidinopyrazoles 3i and 3j[a]



[a](o) **8**, AcOH, 60 °C, 77−79%; (p) MsCl, DIPEA, MeCN, 80 °C, 60−76%; (q) TBAF, THF, rt, 91−93%.

Scheme 6. Synthesis of Pyrimidinopyrazoles 3k[a]



[a](r) NaOMe, MeOH, reflux, 92%; (s) OsO_4, NMM, H_2O, acetone, MeCN, rt, 24%.

and **3g** as a racemic mixture. Methylpiperazine analogue **3h** was obtained in low yields through nucleophilic displacement of iodide with methylpiperazine under neat conditions.

The 5-substituted tetrahydropyrazolopyrimidine analogues **3i** and **3j** were prepared from chiral aspartic acids as shown in Scheme 5.[27] Thiosemicarbazide **9i** or **9j** was readily synthesized from phenyl chlorothioformate and the corresponding amine by a similar method reported by Arora et al.[28] Cyclization of the thiosemicarbazide **9i** or **9j** with α-bromoketone **8** afforded the aminopyrazole **13i** or **13j**. The formation of the tetrahydropyrimidine rings to give the 5-substituted analogues (**3i**, **3j**) proceeded in quantitative yields, followed by desilylation with tetrabutylammonium fluoride.

Elimination of iodide **12** led to the exomethylene analogue **14** in high yield as shown in Scheme 6. Oxidative treatment of exomethylene **14** with OsO_4 allowed for facile aromatization to give pyrazolopyrimidine **3k** at ambient temperature.

Structure−Activity Relationships. Pyrazolopyridine **1**,[29] identified from our chemical library, showed 14 nM potency against LPS-induced TNFα production in vitro. Although encouraged by the in vitro potency of this hit, compound **1** was also shown to have low solubility and poor metabolic stability, which may have contributed to its limited in vivo efficacy. Preliminary SAR and scaffold modifications revealed that the aminopyrazole structures typified by **2a** might have been suitable for the development of novel TNFα production inhibitors. Although compound **2a**[29] showed more potent in vivo activity than **1**, these 1-methyl-5-(methylamino)pyrazole analogues displayed high in vitro clearance in rat liver homogenates. The low metabolic stability of compound **2a** and its analogues resulted in short half-lives (0.66 h for compound **2a**). To improve stability, we turned our attention to investigating alternative scaffolds without metabolically labile groups. In preliminary studies, demethylation of **2a** was observed as a major metabolic pathway in rats in vitro. As can be seen from the lower rate of intrinsic clearance in Table 1, the metabolic stability of **2a** was improved by converting the methyl(methylamino)-pyrazole to tetrahydropyrazolopyrimidine structure **3**. Furthermore,

Table 1. Data Comparison of Lead Compounds 1, 2, and 3

compd	THP-1 LPS/TNFα,[a] IC_{50} (nM)	TNFα production[b] inhibition (%)	MLM stability,[c] CL_{int} (mL min^{-1} kg^{-1})
1	14	43[d]	194.8
2a	6	86	212.9
3b	1	93	94.0

[a]Effect on LPS-induced TNFα production from THP-1 cells. [b]Effect on LPS-induced TNFα production at 1 mg/kg po in rats. Percent inhibition was calculated relative to the vehicle group. [c]In vitro metabolism with rat liver microsomes in the presence of the NADPH-generating system (n = 2). [d]Effect on LPS-induced TNFα production at 3.2 mg/kg po in rats.

a series of tetrahydropyrazolopyrimidine derivatives based on **3** significantly inhibited TNFα production in vivo.

Lead compounds **2a** and **2b** exhibited promising in vitro activity profiles but displayed low oral bioavailability. This poor oral bioavailability of **2a** and **2b** might be attributable to low metabolic stability, as indicated by the high total clearance. We therefore investigated strategies for decreasing metabolic oxidation of the substituent on the pyrazole scaffold. In general, compared to linear alkyl functions, the corresponding cyclic groups are more stable.[30] To probe this concept, we synthesized several cyclic compounds fused to a pyrazole. Compounds were evaluated in the inhibition of LPS-induced TNFα production in THP-1 cells and in vivo at 1 mg/kg po in rat. The results shown in Table 2 are based on the average of three independent experiments. Although the five-membered ring analogue **3a** fused to the pyrazole was substantially less potent than **2a** in the cellular assay, the six-membered ring analogue **3b** exhibited impressive inhibitory activity against TNFα production both in vitro and in vivo. The corresponding alkoxypyrazole **3c** showed attenuated inhibition in THP-1 cells. Next, to investigate whether introduction of polar substituents to the tetrahydropyrazolopyrimidine backbone would improve in vivo activity, hydroxyl or amino groups were attached at several positions about a fused pyrimidine core of the scaffold. Both compounds **3d** and **3e** with a hydroxyl group showed superior

Table 2. TNFα Inhibitory Activity of Aminopyrazole Derivatives

2a, b; 3a-3h; 3i (*R*), 3j (*S*); 3k

compd	X	*n*	R_1	R_2	THP-1 LPS/TNFα,[a] IC_{50} (nM)	inhibition of TNFα release[b] (%)	PAMPA[c] (pH 6.5[d]), $\times 10^{-6}$ cm/s	ClogP
2a			−Me	−Me	6	86	30	3.11
2b	NH	1	$-CH_2CH_2CH_2OH$	−H	1.1	87	17	3.22
3a	NH	0	H		106	NT[e]	NT[e]	3.00
3b	NH	1	H		1	93	30	3.15
3c	O	1	H		10	33	NT[e]	3.32
3d	NH	1	−OH (*R*)		0.85	94	16	2.45
3e	NH	1	−OH (*S*)		1.8	89	17	2.45
3f	NH	1	$-CH_2OH$ (*R*)		0.6	92	20	2.09
3g	NH	1	$-CH_2OH$ (*S*)		0.95	92	NT[e]	2.09
3h	NH	1	$-CH_2$-methylpiperazine		1.1	88	21	1.69
3i	NH	1			0.46	98	23	2.30
3j	NH	1			0.37	98	22	2.30
3k					23	11	NT[e]	2.29

[a]Effect on LPS-induced TNFα production from THP-1 cells. IC_{50} values are shown as the mean of triplicate experiments. [b]Effect on LPS-induced TNFα production at 1 mg/kg po in rats (n = 3−4). Percent inhibition was calculated relative to the vehicle group. [c]pION membrane lipid was used. [d]pH of donor buffer. [e]NT = not tested.

potency against TNF release. In particular, alcohol **3d** showed an IC_{50} of 0.85 nM in vitro. These data suggest that introduction of a polar group such as a hydroxyl group or amino group provided stronger inhibitory activity against TNFα release in vivo due to improved permeability. Furthermore, analogues **3f** and **3g** with a methanol group at the C-6 position were more potent than **3b** in the cellular assay. Analogue **3h** with a methylpiperazyl group at the C-6 position showed a slight decrease in potency in vitro and in vivo due to increased hydrophilicity (**3h**, ClogP = 1.69) relative to that of **3b**. Changing the hydroxymethyl substituent from the C-6 to the C-5 position for alcohols **3i** and **3j**, which possessed a hydroxymethyl group at the C-5 position, strongly inhibited TNFα production in vitro with IC_{50} values of 0.46 and 0.37 nM, respectively. It was therefore unsurprising that both compounds inhibited LPS-induced TNF release by 98%. Finally, aromatization of the tetrahydropyrazolopyrimidine afforded pyrazolopyrimidine **3k**, which exhibited a 38-fold decrease in cell potency with respect to **3f**.

On the basis of the results of these studies, we selected compounds **3d**, **3e**, **3f**, **3g**, and **3j** for further in vivo evaluation. Selected compounds were evaluated in PK assay (po and iv) in rats, and their pharmacokinetic parameters are shown in Table 3. The results indicated that tetrahydropyrimidinopyrazole derivatives displayed better oral bioavailability and had a longer plasma half-life in rats than acyclic compounds such as **2a**. Cyclization of the methylpyrazole had a significant impact on total clearance and bioavailability of the analogues (**2a** vs **3d** or **3e**). Extension of the hydroxyl group on the tetrahydropyrazolopyrimidine ring increased systemic plasma exposure (**3d** or **3e** vs **3f** or **3j**). Shifts from the C-6 position to the C-5 position on the tetrahydropyrazolopyrimidine ring showed similar pharmacokinetics (**3f** vs **3j**). Compounds **3d**, **3e**, **3f**, **3g**, and **3j** were found to exhibit exceptionally high in vitro and in vivo potency with a relatively long duration of action.

Table 3. Pharmacokinetic Parameters of Selected TNFα Release Inhibitors in Rat[a]

	iv (1.0 mg/kg)[b]			po (1.0 mg/kg)[b]	
	CL_{tot} (mL min^{-1} kg^{-1})	V_{ss} (L/kg)	$t_{1/2}$ (h)	AUC (ng·h/mL)	*F* (%)
2a	20.64	0.65	0.66	235	28.6
3d	4.80	1.23	3.89	763	67.7
3e	9.53	4.21	9.01	457	81.0
3f	2.17	0.77	5.15	1518	61.8
3g	4.56	1.11	3.82	1217	103.6
3j	3.80	0.98	5.95	4502	100.5

[a]Pharmacokinetics were determined at a dose of 1 mg/kg iv and po in SD rats. [b]Results expressed as the mean ± SD of n = 3.

These tetrahydropyrazolopyrimidine derivatives (**2a**, **3d**, **3e**, **3f**, **3g**, and **3j**) were evaluated in adjuvant-induced arthritis (AIA) rat models. As can be seen in Table 4, **3d**, **3f**, and **3j** provided a significant reduction in the measured outcome of disease progression relative to the vehicle group when dosed orally at 1 mg/kg once a day for a period of 15−24 days. Although these derivatives all displayed good pharmacokinetics properties, **3f** displayed the most potent inhibition in the AIA model.

Compound **3f** was further investigated to confirm its in vivo efficacy against inflammation. As shown in Table 5, **3f** and representative compounds that are reported to be p38 MAP kinase inhibitors were compared in vivo in a rat LPS-induced TNFα model as an acute model and in the AIA model as a chronic disease model. **3f** displayed excellent in vivo efficacy in both models and represents one of most potent p38 inhibitor reported to date.[31]

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Table 4. Compound Efficacy in the Adjuvant-Induced Arthritis Model after po Dosing of 1 mg/kg

compd	AIA, % suppression[a]
2a	37.0**
3d	61.4**
3e	51.0**
3f	71.3**
3g	59.7**
3j	62.3**

[a]Edema volume of foot pads in rats with the development of AIA. Edema of the hind paw was calculated as the mean percentage of the control value (n = 6). Double asterisks indicate statical significance at P < 0.01 vs 0.5% MC control by Dunnett's multiple comparison test.

Table 5. Comparative Studies of in Vivo Efficacy Assays

compd	rat LPS-induced TNF ED_{50} (mg/kg)[a]	AIA ED_{30} (mg/kg)[a]
3f	0.05	0.15
BIRB-796	2.60	5.80
VX-745	39.9	92

[a]The results are reported as the average of at least four separate determinations.

Further biological evaluation demonstrated that compound **3f** has strong inhibition of p38α/β and high selectivity against JNK2,[32] as shown in Table 6. Compound **3f** was also completely clean with regard to CYP enzyme inhibitory activities, indicating that it has very low potential for drug−drug interactions, as shown in Table 7.

Table 6. Kinase Profiling of 3f

compd	kinase assay IC_{50} (nM)[a]		
	p38α	p38β	JNK2
3f	11	36	>10000

[a]Assayed according to ref 30. See Experimental Section for assay details.

Table 7. In Vitro Profile of Compound 3f

	CYP inhibition, IC_{50} (μM)						
compd	1A2	2C19	2C9	2D6	3A4[a]	PAMPA,[b] P_e (10^{-6}cm/s) at pH 6.5	solubility (μg/mL) at pH 6.8
3f	>50	>50	19	>50	>100	19.7	42

[a]Midazolam was the substrate in CYP3A4 inhibition assay. [b]pION membrane lipid was used as a donor buffer, pH 6.5 (n = 2).

Compound **3f** showed an acceptable off-target profile in both CYP inhibition (IC_{50}) and hERG inhibition (Rb efflux assay, IC_{50} > 100 μM) in addition to desirable physicochemical properties (PAMPA,[33] solubility) and mutagenic profile (negative in the in vivo micronucleus test).

Finally, to clarify the role of the hydroxymethyl group, we have docked compound **3f** into the crystal structure of p38α MAP kinase using the docking program GOLD, version 3.2 (Figure 3). The pyridazinone ring forms three hydrogen bonds with hinge residues His 104, Met 106, and Gly 107. The methylphenyl ring and fluorophenyl ring occupy the hydrophobic front region and the hydrophobic back pocket, respectively. The hydroxymehyl group and aromatic nitrogen atom of the tetrahydropyrazolopyrimidine ring form hydrogen bonds with Asp 168 and Lys 53, respectively. Because the hydrogen bond between Asp 168 and the hydroxyl group is half-exposed to solvent, this hydrogen bond was predicted to have no substantial effect on binding affinity. In fact, there is little difference in TNFα inhibitory activity between the presence (compound **3f**) and absence of the hydroxymethyl group (compound **3b**). On the other hand, the hydroxyl group is thought to contribute to improving the physical properties of compound **3f**.

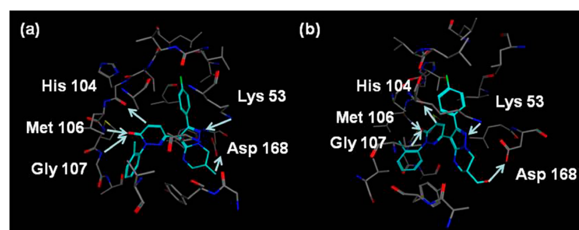

Figure 3. Docking model of compound **3f** binding to p38 MAP kinase: (a) front view; (b) side view. Light blue and gray indicate carbon atoms of compound **3f** and p38 MAP kinase, respectively. Red, blue, and green indicate oxygen, nitrogen, and fluorine atoms, respectively. Arrows indicate a hydrogen bond.

■ CONCLUSION

In summary, we explored several series of tetrahydropyrazolopyrimidine scaffolds as prototypical inhibitors of TNFα production. Among these series, analogues that contained a hydroxyl group showed improved metabolic stability compared to **2a** and displayed excellent potency in cellular and in vivo TNFα assays. After extensive SAR studies, compounds **3d**, **3e**, **3f**, **3g**, and **3j** were identified as potent inhibitors of TNFα production and were shown to potently inhibit the production of TNFα in vitro and in vivo. Evaluation of this series led to **3f**, which is orally bioavailable with a long duration of action and efficacious in an AIA model. On the basis of the overall profile, compound **3f** was selected as a clinical candidate. These findings suggested that **3f** represents an attractive therapeutic candidate for the treatment of inflammatory diseases.

■ EXPERIMENTAL SECTION

Chemistry. ^{1}H NMR spectra were measured with a JEOL EX400 (400 MHz) or GX500 (500 MHz) spectrometer. Chemical shifts are expressed in δ units using tetramethylsilane as the standard (NMR peak description: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad peak). Mass spectra were recorded with a Hitachi M-80 or a JEOL JMS-DX300 spectrometer. Organic solutions were dried over anhydrous MgSO4 during workup. Column chromatography was carried out on silica gel (Kieselgel 60). Unless otherwise noted, all commercial reagents and solvents were used without further purification. The purity of all compounds screened in biological assays was >95% pure as judged by HPLC and/or combustion analysis. HPLC analysis was obtained on an Hitachi LaChrom Elite system, using a TOSOH TSK-gel ODS column (150 mm × 4.6 mm, 5 μm) at 40 °C with a 1.5 mL/min flow rate using acetonitrile and 0.05 M KH_2PO_4 solution as the eluent over 30 min.

6-[2-(4-Fluorophenyl)-2-oxoethyl]pyridazin-3(2*H*)-one (5). To a solution of 3-chloro-6-methylpyridazine (50 g, 389 mmol) and ethyl 4-fluorobenzoate (65 g, 389 mmol) in tetrahydrofuran cooled to 0 °C under a nitrogen atmosphere was added 1 M tetrahydrofuran solution of lithium 1,1,1,3,3,3-hexamethyldisilazane-2-ide (779 mL, 779 mmol), and the mixture was stirred at the same temperature for 3 h. The reaction mixture was poured into aqueous 1 M hydrochloric acid

(1200 mL), and the resulting mixture was stirred at room temperature for 1 h. The resulting precipitates were collected by filtration, air-dried for 16 h, and triturated with hexane (1000 mL). The precipitates were collected by filtration and air-dried for 24 h to give 2-(6-chloropyridazin-3-yl)-1-(4-fluorophenyl)ethanone (74 g, 76%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.84 (2H, s), 7.38−7.44 (2H, m), 7.77−7.80 (1H, m), 7.91−7.96 (1H, m), 8.14−8.19 (2H, m). MS (API-ES) m/z = 273 (M + Na).

2-(6-Chloropyridazin-3-yl)-1-(4-fluorophenyl)ethanone (110 g, 439 mmol) and sodium acetate (45 g, 549 mmol) were dissolved in acetic acid (550 mL), and the mixture was stirred at 120 °C for 3 h. An additional 10 g of sodium acetate was added, and stirring was continued for another 1 h. After the reaction mixture was cooled to 40 °C, water (1300 mL) was added and the mixture was stirred with ice−water bath cooling for 1 h. The resulting precipitates were collected by filtration and then washed with water (580 mL). The isolated precipitates were air-dried at ambient temperature for 1 day. The precipitates were triturated with diisopropyl ether (500 mL) for 1 h and collected by filtration to give the product (83 g, 81%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.42 (2H, s), 6.86 (1H, d, J = 9.72 Hz), 7.35−7.42 (3H, m), 8.08−8.13 (2H, m), 12.91 (1H, s). MS (API-ES) m/z = 255 (M + Na).

6-{[2-(4-Fluorophenyl)-1,3-dioxolan-2-yl]methyl}pyridazin-3(2*H*)-one (6). A mixture of 6-[2-(4-fluorophenyl)-2-oxoethyl]-pyridazin-3(2*H*)-one (49 g, 202 mmol), ethylene glycol (60 mL, 1073 mmol), TsOH·H_2O (3.8 g, 20 mmol), and toluene (450 mL) was refluxed for 6 h with azeotropic removal of water. The reaction mixture was cooled to 50 °C, and 1 M NaOH (21 mL) and water (300 mL) were added. The whole mixture was stirred on an ice bath for 1 h, and the resulting precipitate was collected by filtration. The obtained solid was washed with water (400 mL) and then toluene (200 mL) to give **6** (56 g, 100%) as a gray powder. ^{1}H NMR (400 MHz, DMSO-d_6): δ3.10 (2H, s), 3.67−3.74 (2H, m), 3.89−3.97 (2H, m), 6.76 (1H, d, J = 9.8 Hz), 7.11−7.20 (2H, m), 7.28 (1H, d, J = 9.8 Hz), 7.33−7.40 (2H, m), 12.76 (1H, s). MS (API-ES) m/z = 299 (M + Na).

6-[2-(4-Fluorophenyl)-2-oxoethyl]-2-(2-methylphenyl)-pyridazin-3(2*H*)-one (7). To a mixture of **6** (32 g, 114 mmol), 2-methylphenylboronic acid (34 g, 251 mmol), and pyridine (37 mL, 457 mmol) in DMF (189 mL) was added $Cu(OAc)_2$ (3 g, 17 mmol) at room temperature with stirring. The mixture was stirred for 24 h and partitioned between EtOAc and 5% citric acid. The separated organic layer was filtered through a Celite pad. The aqueous layer was extracted with EtOAc. The combined organic layer were washed with 5% citric acid, 10% K_2CO_3, and brine, dried, and concentrated in vacuo to give an oil. The crude product was purified by column chromatography (*n*-hexane/ethyl acetate = 1/1 to 1/2) to give 6-{[2-(4-fluorophenyl)-1,3-dioxolan-2-yl]methyl}-2-(2-methylphenyl)-pyridazin-3(2*H*)-one (26 g, 62%) as a white solid. ^{1}HNMR (400 MHz, DMSO-d_6): δ1.83 (3H, s), 3.17 (2H, s), 3.71−3.81 (2H, m), 3.95−4.04 (2H, m), 6.95−7.50 (6H, m). MS (API-ES) m/z = 389 (M + Na).

To a solution of 6-{[2-(4-fluorophenyl)-1,3-dioxolan-2-yl]methyl}-2-(2-methylphenyl)pyridazin-3(2*H*)-one(30 g, 82 mmol) in AcOH (120 mL) was added 1 N HCl (12 mL) at room temperature. After the mixture was stirred for 14 h, diisopropyl ether/*n*-hexane (1/1, 240 mL) and water (360 mL) were added to the mixture. The resulting suspension was aged for 30 min at 0 °C. The separated solid was collected and washed with *n*-hexane and water to give **7** (22 g, 84%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.01 (3H, s), 4.51 (2H, s), 7.08 (1H, d, J = 9.6 Hz), 7.20−7.47 (4H, m), 7.53 (1H, d, J = 9.6 Hz), 8.05−8.18 (2H, m). MS (API-ES) m/z = 345 (M + Na).

6-[1-Bromo-2-(4-fluorophenyl)-2-oxoethyl]-2-(2-methylphenyl)pyridazin-3(2*H*)-one (8). To a solution of **7** (20 g, 63 mmol) in AcOH (120 mL) was added pyridine hydrobromide perbromide (26 g, 72 mmol). After being stirred for 1.5 h, the mixture was added to a solution of diisopropyl ether and water. The resulting precipitate was filtered with a funnel, and the filtrate was extracted with EtOAc. The precipitate and the organic phase were combined to dissolve in 5% $Na_2S_2O_4$ (31.5 mL) and water (348 mL). The mixture was stirred for 1 h in an ice-cooled bath. The resulting precipitate was filtered with the funnel and washed with water and diisopropyl ether. The obtained solid was dried under a hood (23 g, 92%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.86 (3H, s), 7.08 (1H, s), 7.13−7.22 (2H, m), 7.26−7.58 (6H, m), 7.79 (1H, d, J = 9.76 Hz), 8.11−8.15 (2H, m). MS (API-ES) m/z = 424 (M + Na).

***N*,1-Dimethylhydrazinecarbothioamide (9a).** To a solution of methylhyrdrazine (14 mL, 274 mmol) in EtOH (55 mL) was added dropwise a solution of methyl isothiocyanate (20 g, 274 mmol) in EtOH (27 mL) at room temperature. A precipitate formed. After being stirred for 6 h, the suspension was stirred at 0 °C, collected, and washed with cold diisopropyl ether. The white solid was dried in vacuo at 45 °C (29 g, 89%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.86 (3H, d, J = 4.5 Hz), 3.42 (3H, s), 4.80 (2H, s), 8.05−8.20 (1H, m).

6-[3-(4-Fluorophenyl)-1-methyl-5-(methylamino)-1*H*-pyrazol-4-yl]-2-(2-methylphenyl)pyridazin-3(2*H*)-one (2a). A mixture of **8** (12 g, 30 mmol) and *N*,1-dimethylhydrazinecarbothioamide **9a** (3.9 g, 33 mmol) in AcOH (96 mL) was stirred for 5 h at 120 °C. After concentration, the mixture was partitioned between EtOAc (200 mL) and water (100 mL). The organic layer was washed with saturated $NaHCO_3$ (60 mL) and brine, dried, and evaporated in vacuo. The residue was purified by column chromatography on silica gel (methanol/chloroform = 1/9) to give **2a** (9.9 g, 85%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.05 (3H, s), 2.66 (3H, d, J = 5.4 Hz), 3.66 (3H, s), 5.53 (1H, q, J = 5.4 Hz), 7.00 (1H, d, J = 9.6 Hz), 7.13−7.49 (9H, m). MS (ESI) m/z = 390 (M + 1).

***N*-(3-Hydroxypropyl)hydrazinecarbothioamide (9b).** To a solution of 3-amino-1-propanol (1.7 mL, 22 mmol) in CH_2Cl_2 (43 mL) were added phenyl chlorothioformate (2 mL, 15 mmol), water (43 mL), and $NaHCO_3$ (3.1 g, 37 mmol) on an ice bath, followed by stirring at room temperature for 24 h. The reaction mixture was extracted with AcOEt, washed with brine, and dried over $MgSO_4$. The crude product was purified by column chromatography to give *O*-phenyl (3-hydroxypropyl)carbamothioate as a colorless oil (2.4 g, 77%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.90 (2H, m), 3.70 (1H, m), 3.83 (4H, m), 7.09 (2H, m), 7.27 (1H, m), 7.39 (2H, m). MS (API-ES) m/z = 234 (M + Na).

To a solution of *O*-phenyl(3-hydroxypropyl)carbamothioate (2.6 g, 12 mmol) in EtOH (20 mL) was added hydrazine hydrate (1.2 mL, 24 mmol) at room temperature, and the mixture was heated at 40 °C. After being stirred for 4 h, the mixture was diluted with water and extracted with diisopropyl ether. The aqueous phase was evaporated to give the desired product (1.8 g, 98%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.63 (2H, m), 3.34 (1H, brs), 3.43 (2H, t, J = 5.6 Hz), 3.50 (2H, q, J = 6.4, 6.8, 6.6 Hz), 4.49 (1H, brs), 7.89 (1H, brs), 8.57 (1H, brs). MS (API-ES) m/z = 172 (M + Na).

6-{3-(4-Fluorophenyl)-5-[(3-hydroxypropyl)amino]-1*H*-pyrazol-4-yl}-2-(2-methylphenyl)pyridazin-3(2*H*)-one (2b). A solution of 6-[1-bromo-2-(4-fluorophenyl)-2-oxoethyl]-2-(2-methylphenyl)pyridazin-3(2*H*)-one (1.0 g, 2.5 mmol) and *N*-(3-hydroxypropyl)hydrazinecarbothioamide (409 mg, 2.74 mmol) in acetic acid (100 mL) was heated at 100 °C. After heating for 1 h, the mixture was diluted with water, extracted with EtOAc, washed with brine, and dried over $MgSO_4$. After removal of solvent, the residue was purified by column chromatography (chloroform/methanol = 10/0 to 7/3) to give **2b** (812 mg, 78%). ^{1}H NMR(400 MHz, $CDCl_3$): δ 1.73 (2H, quint), 2.22 (3H, s), 3.47 (2H, t), 3.61 (2H, t), 6.81 (1H, d), 6.91 (1H, d), 7.13 (2H, t), 7.28−7.46 (6H, m). MS (API-ES) m/z = 442 (M + Na).

6-[2-(4-Fluorophenyl)-4,5,6,7-tetrahydropyrazolo[1,5-*a*]-pyrimidin-3-yl]-2-(2-methylphenyl)pyridazin-3(2*H*)-one (3b). A mixture of **2b** (117 mg, 0.28 mmol), triphenylphosphine (110 mg, 0.42 mmol), and diethyl azodicarboxylate (66 μL, 0.42 mmol) in tetrahydrofuran (5 mL) was stirred at room temperature for 2 h. To the mixture was added water (20 mL). The mixture was extracted with ethyl acetate (30 mL). The extract was concentrated under reduced pressure. To the residue was added 10% hydrochloric acid (25 mL). The mixture was washed with diethyl ether (30 mL × 4). The aqueous layer was neutralized with sodium hydrogen carbonate and extracted with ethyl acetate (30 mL). The extract was concentrated under

reduced pressure. The residue was purified by flash column chromatography (gradient elution, ethyl acetate/hexane = 1/4 to 0/10) to give **3b** as yellow amorphous solid (70 mg, 62%). ^{1}H NMR ($CDCl_3$): δ 2.15−2.20 (2H, m), 2.23 (3H, s), 3.36 (2H, brs), 4.15 (2H, t, J = 6.1 Hz), 5.81 (1H, brs), 6.80 (1H, d, J = 9.9 Hz), 7.02 (1H, d, J = 9.9 Hz), 7.15 (2H, dd, J = 8.7, 8.7 Hz), 7.33−7.38 (4H, m), 7.50 (2H, dd, J = 5.4, 8.7 Hz). MS (API-ES) = 402 (H + M).

6-{3-(4-Fluorophenyl)-5-[(2-hydroxyethyl)amino]-1*H*-pyrazol-4-yl}-2-(2-methylphenyl)pyridazin-3(2*H*)-one (10a). A solution of 6-[1-bromo-2-(4-fluorophenyl)-2-oxoethyl]-2-(2-methylphenyl)pyridazin-3(2*H*)-one (400 mg, 1.0 mmol) and *N*-(2-hydroxyethyl)hydrazinecarbothioamide[34] (168 mg, 1.24 mmol) in acetic acid (1.3 mL) and EtOH (2.7 mL) was heated at 50 °C. After being heated for 30 min, the mixture was diluted with water, extracted with EtOAc, washed with water, saturated $NaHCO_3$, and brine, and dried over Na_2SO_4. After removal of solvent, the crude product was purified by column chromatography on silica gel (chloroform/methanol = 10/0 to 7/3) to give the product (401 mg, 99%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.20 (3H, s), 3.48 (2H, t, J = 5.2, 5.0 Hz), 3.75 (2H, t, J = 5.2, 5.0 Hz), 6.85 (1H, m), 7.10 (2H, m), 7.36 (7H, m). MS (API-ES) m/z = 428(M + Na).

6-[6-(4-Fluorophenyl)-2,3-dihydro-1*H*-imidazo[1,2-*b*]pyrazol-7-yl]-2-(2-methylphenyl)pyridazin-3(2*H*)-one (3a). To a solution of **10a** (420 mg, 1 mmol) in MeCN were added triethylamine (347 μL, 2.5 mmol) and methanesulfonyl chloride (112 μL, 1.5 mmol), followed by heating at 80 °C. To the mixture was added further triethylamine (347 μL, 2.5 mmol) and methanesulfonyl chloride (112 μL, 1.5 mmol). After being heated for 12 h, the mixture was extracted with EtOAc, washed with 5% citric acid, aqueous $NaHCO_3$, and brine, and dried over $MgSO_4$. After removal of the solvent, the residue was purified by column chromatography (chloroform/methanol = 10/0 to 10/1) to give the desired product (42 mg, 10%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.21 (3H, s), 4.28−4.61 (2H, m), 6.94 (1H, d), 7.00−7.13 (3H, m), 7.29−7.38 (4H, m), 7.40−7.49 (2H, m). MS (API-ES) m/z = 386 (M − H). HRMS (M + H)$^+$ found, 388.1576; calcd for $C_{22}H_{19}N_5OF$, 388.1574.

***O*-[3-(Benzyloxy)propyl] Hydrazinecarbothioate (9c).** To a solution of NaH (606 mg, 15 mmol) in THF (2 mL) was slowly added 3-(benzyloxy)propan-1-ol (2.0 mL, 12.6 mmol) in THF (34 mL) at 0 °C, followed by stirring at room temperature for 1 h. To the mixture was then added methanedithione (1.5 mL, 24.2 mmol) at 0 °C, with stirring at room temperature for 4 h, followed by the addition of iodomethane (1.2 mL, 18.9 mmol) at 0 °C. After being stirred at room temperature for 2 h, the mixture was extracted with EtOAc, washed with aqueous NH_4Cl and brine, and dried over $MgSO_4$. After removal of the solvent, the crude product was purified by column chromatography (hexane/EtOAc = 5/1) to give *O*-[3-(benzyloxy)-propyl] *S*-methylcarbonodithioate (2.27 g, 70%). ^{1}H NMR ($CDCl_3$): δ 2.11 (2H, m), 2.53 (3H, s), 3.59 (2H, t, J = 5.6, 6.2 Hz), 4.52 (2H, s, J = 5.6, 6.2 Hz), 4.73 (2H, t). MS (API-ES) m/z = 279 (M + Na).

A mixture of *O*-[3-(benzyloxy)propyl] *S*-methylcarbonodithioate (2.27 g, 8.85 mmol), hydrazine hydrate (4.3 mL, 88.5 mmol), and EtOH (23 mL) was stirred at 0 °C. After being stirred for 1 h, the mixture was diluted with water, extracted with $CHCl_3$/diisopropyl ether (=4/1), washed with brine, and dried over $MgSO_4$. After removal of solvent, the crude product was purified by column chromatography (hexane/EtOAc = 1/1) to give **9c** as an oil (1.97 g, 93%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.91 (2H, m), 3.51 (2H, m), 4.45 (4H, m), 7.32 (5H, m). MS (API-ES) m/z = 263(M + Na).

6-[3-(4-Fluorophenyl)-5-(3-hydroxypropoxy)-1*H*-pyrazol-4-yl]-2-(2-methylphenyl)pyridazin-3(2*H*)-one (10c). A mixture of 6-{5-[3-(benzyloxy)propoxy]-3-(4-fluorophenyl)-1*H*-pyrazol-4-yl}-2-(2-methylphenyl)pyridazin-3(2*H*)-one (2.2 g, 5.5 mmol), **9c** (1.9 g, 7.9 mmol), EtOH (37 mL), and acetic acid (18 mL) was heated at 100 °C. After being stirred for 4 h, the mixture was extracted with EtOAc, washed with aqueous Na_2CO_3 and brine, and dried over $MgSO_4$. After removal of the solvent, the crude was purified by column chromatography (chloroform/methanol = 10/0 to 9/1 and hexane/EtOAc = 2/1 to 0/1) to give 6-{5-[3-(benzyloxy)propoxy]-3-(4-fluorophenyl)-1*H*-pyrazol-4-yl}-2-(2-methylphenyl)pyridazin-3(2*H*)-one (114 mg, 4%). ^{1}H NMR ($CDCl_3$): δ 2.05 (3H, s), 2.14 (2H, m), 3.64 (2H, m), 4.47 (2H, m), 4.51 (2H, s), 6.90−7.10 (5H, m), 7.22−7.37 (7H, m), 7.48 (3H, m). MS (API-ES) m/z = 533(M + Na).

A mixture of 6-{5-[3-(benzyloxy)propoxy]-3-(4-fluorophenyl)-1*H*-pyrazol-4-yl}-2-(2-methylphenyl)pyridazin-3(2*H*)-one (336 mg, 0.66 mmol), Pd−C (101 mg), and EtOH (3.4 mL) was stirred at room temperature for 5 h under H_2 gas. The mixture was filtered with a funnel and the filtrate was evaporated to give **10c** (247 mg, 89%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.89 (2H, m), 1.97 (3H, s), 3.55 (2H, m), 4.31 (2H, m), 4.56 (1H, brs), 7.04−7.37 (8H, m), 7.53 (2H, m). MS (API-ES) m/z = 443(M + Na). Anal. ($C_{23}H_{21}FN_4O_3{\cdot}0.2H_2O$) C, H, N.

6-[2-(4-Fluorophenyl)-6,7-dihydro-5*H*-pyrazolo[5,1-*b*][1,3]oxazin-3-yl]-2-(2-methylphenyl)pyridazin-3(2*H*)-one (3c). To a solution of **10c** (247 mg, 0.59 mmol) in MeCN (2.47 mL) were added triethylamine (197 μL, 1.4 mmol) and methanesulfonyl chloride (55 μL, 0.71 mmol) at 0 °C, and then the mixture was stirred at the same temperature for 30 min. After heating at 80 °C for 3 h, the mixture was extracted with EtOAc, washed with aqueous $NaHCO_3$ and brine, and dried over $MgSO_4$. After removal of the solvent, the residue was purified by column chromatography (chloroform/methanol = 10/0 to 8/2) to give the desired product (52 mg, 22%). ^{1}H NMR ($CDCl_3$): δ 2.13 (3H, s), 2.36 (2H, m), 4.26 (2H, t), 4.42 (2H, t), 6.99 (3H, m), 7.30 (5H, m), 7.52 (2H, m). MS (API-ES) m/z = 425 (M + Na). HRMS (M + H)$^+$ found, 403.1587; calcd for $C_{23}H_{20}N_4O_2F$, 403.1570.

***N*-[(2*R*)-2,3-Dihydroxypropyl]hydrazinecarbothioamide (9d).** (2*R*)-3-Aminopropane-1,2-diol (2.2 g, 24.0 mmol) was dissolved in 2-propanol (84 mL) at 50 °C with stirring. After being stirred for 1 h, the solution was cooled to ambient temperature. *O*-Phenyl hydrazinecarbothioate (3.37 g, 20.0 mmol) and dichloromethane were added to the solution, and the mixture was stirred at ambient temperature for 19 h. The reaction mixture was decanted to separate the solution from undissolved materials attached to the reaction vessel wall. The separated solution was evaporated in vacuo and the residue was washed with diisopropyl ether (50 mL) to give **9d** (4.0 g, 100%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.753 (3H, m), 7.15 (2H, m), 9.29 (1H, brs). MS (API-ES) m/z = 188 (M + Na).

6-[5-{[(2*R*)-2,3-Dihydroxypropyl]amino}-3-(4-fluorophenyl)-1*H*-pyrazol-4-yl]-2-(2-methylphenyl)pyridazin-3(2*H*)-one (10d). To a solution of **9d** (4.0 g, 24 mmol) in acetic acid (35 mL) was added **8** (5.3 g, 13 mmol), and the mixture was stirred at 75 °C for 3 h. After being cooled to ambient temperature, the reaction mixture was diluted with ethyl acetate (100 mL) and water (50 mL). Undissolved materials in the resultant mixture were removed by filtration. To the filtrate were added 50 g of ice and 50 mL of 15 M aqueous sodium hydroxide solution with stirring. The organic phase was separated, and the aqueous phase was extracted with ethyl acetate (50 mL). The organic phase and extracts were combined, washed with water (100 mL) and brine (50 mL), dried over $MgSO_4$, filtered, and evaporated in vacuo to give 7.51 g of crude product. The obtained materials were dissolved in methanol (80 mL) and to the solution was added 3 M aqueous sodium hydroxide (80 mL) for 50 min at ambient temperature. The mixture was concentrated under reduced pressure, and water (100 mL) was added to the residue. The resulting mixture was extracted with ethyl acetate (60 mL), and the combined extracts were successively washed with 1 M aqueous sodium hydroxide (60 mL), water (100 mL), and brine (50 mL), then dried over $MgSO_4$, filtered, and evaporated in vacuo. The residue (6.57 g) was dissolved in ethyl acetate (15 mL), and the mixture was stirred at ambient temperature for 16 h. Precipitates produced were collected by filtration, washed with cooled ethyl acetate (5 mL), and dried under reduced pressure at 40 °C for 1 h to give 3.78 g of **10d**. By use of preparative thin layer chromatography, 44 mg of the product was eluted with 10% methanol in dichloromethane to provide 42 mg of **10d** (4.6 g, 80%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.11 (3H, s), 3.05 (1H, br s), 3.22−3.32 (2H, m), 3.54 (1H, br s), 4.57−4.67 (2H, m), 5.43 (1H, brs), 6.05 (0.3H, brs), 6.92 (1H, d, J = 9.56 Hz), 7.02 (1H, brs), 7.35−7.36 (6H, m), 7.50−7.53 (2H, m), 12.31 (1H, brs). MS (API-ES) m/z = 458 (M + Na).

6-[(6R)-2-(4-Fluorophenyl)-6-hydroxy-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrimidin-3-yl]-2-(2-methylphenyl)-pyridazin-3(2H)-one (3d). To a suspension of **10d** (435 mg, 0.99 mmol) and pyridine (808 μL) in acetonitrile was added the first portion of methanesulfonyl chloride (93 μL, 1.2 mmol) at 0 °C. The mixture was stirred at 0 °C for 30 min, then at ambient temperature for 3 h, followed by stirring at 35 °C for 45 min. During the stirring at ambient temperature, the reaction mixture became a clear solution. The mixture was cooled to 0 °C, and to it was added the second portion of methanesulfonyl chloride (7.7 μL, 0.1 mmol). The resulting mixture was stirred at 0 °C for 45 min, at ambient temperature for 1 h, and at 70 °C for 18 h. After being cooled to ambient temperature, the reaction mixture was partitioned between ethyl acetate (15 mL) and water (22 mL). The aqueous phase was extracted with ethyl acetate (15 mL), and the organic phases were combined and successively washed with brine and then 20% aqueous citric acid (10 mL). The combined citric acid solution was extracted with ethyl acetate (10 mL), and the extract was combined with the organic phase, which was successively washed with 5% aqueous citric acid, brine, 1 M aqueous sodium hydroxide, and brine, then dried over $MgSO_4$, filtered, and evaporated in vacuo. The residue was crystallized from ethyl acetate to give **3d** (124 mg, 30%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.08 (3H, s), 3.13−3.18 (1H, m), 3.24−3.27 (1H, m), 3.89 (1H, dd, J = 12.3 and 2.95 Hz), 4.15 (1H, dd, J = 15.9 and 3.57 Hz), 4.18 (1H, s), 5.34 (1H, d, J = 2.24 Hz), 5.93 (1H, s), 6.92 (1H, d, J = 9.76 Hz), 7.08 (1H, d, J = 9.76 Hz), 7.21−7.25 (2H, m), 7.31−7.37 (4H, m), 7.46−7.49 (2H, m). MS (API-ES) m/z = 440 (M + Na). $[\alpha]_D$ −14.7 (c 0.0044 g/mL, 23.8 °C).

(2S)-4-[2-(Benzyloxy)-3-hydroxypropyl]thiosemicarbazide (9e). To a solution of (2S)-3-aminopropane-1,2-diol (4.00 g, 43.9 mmol) in N,N-dimethylformamide (65 mL) were added triethylamine (12.8 mL, 92.2 mmol) and triphenylchloromethane (25.7 g, 92.2 mmol), and the mixture was stirred at room temperature for 2 days. To the mixture were added triethylamine (1.22 mL, 8.78 mmol) and triphenylchloromethane (2.45 g, 8.78 mmol), and the mixture was stirred at room temperature for 1 day. To the mixture was added water, and the resulting precipitate was extracted with diisopropyl ether. The extracts were combined and washed successively with water, 5% aqueous citric acid solution, saturated aqueous sodium hydrogen carbonate solution, water, and saturated saline, then dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. To the oily residue was added a mixed solvent of diisopropyl ether and hexane, and crystallization was induced by scratching the flask. The crystal was filtered off to give (2S)-1-(tritylamino)-3-(trityloxy)-propan-2-ol as colorless crystals (15.0 g, 59%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.19 (dd, 2H, J = 6, 12 Hz), 2.45 (dd, 1H, J = 5, 12 Hz), 3.02−3.07 (m, 2H), 3.26−3.28 (m, 1H), 3.88 (brs, 1H), 7.17−7.44 (m, 30H).

To a suspension of sodium hydride (55% dispersion in mineral oil, 545 mg, 12.5 mmol) in dimethylsulfoxide (50 mL), from which the mineral oil was removed by washing with petroleum ether, was added (2S)-1-(tritylamino)-3-(trityloxy)propan-2-ol (5.76 g, 10.0 mmol). The mixture was stirred at room temperature for 30 min. To the mixture was added benzyl bromide (1.37 mL, 11.5 mmol), and the mixture was stirred at room temperature for 17 h. To the mixture was added water, and the resulting precipitate was filtered off and washed with hexane to give N-[(2S)-2-benzyloxy-3-(trityloxy)propyl]-N-tritylamine as a colorless powder (4.79 g, 72%). ^{1}H NMR ($CDCl_3$): δ 0.97 (brs, 1H), 2.41 (brt, 2H, J = 9 Hz), 3.26−3.30 (m, 2H), 3.71−3.75 (m, 1H), 4.42 (d, 1H, J = 12 Hz), 4.50 (d, 1H, J = 12 Hz), 7.15−7.42 (m, 35H).

To a solution of N-[(2S)-2-benzyloxy-3-(trityloxy)propyl]-N-tritylamine (1.60 g, 2.40 mmol) in dichloromethane (4 mL) was added trifluoroacetic acid (2 mL), and the mixture was stirred at room temperature for 1 day. To the mixture was added water, and the aqueous solution was washed with diisopropyl ether. The aqueous layer was basified with sodium hydroxide. To the mixture was added salt, and the mixture was extracted with dichloromethane. The extracts were combined and washed with saturated saline, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure to give (2S)-3-amino-2-(benzyloxy)propan-1-ol as brown crystals (330 mg, 76%). ^{1}H NMR ($CDCl_3$): δ 2.95−3.16 (brm, 2H), 3.47−3.50 (m, 1H), 3.77 (dd, 1H, J = 4.5, 11.5 Hz), 3.86 (dd, 1H, J = 4.5, 11.5 Hz), 4.60 (d, 1H, J = 12 Hz), 4.67 (d, 1H, J = 12 Hz), 7.28−7.37 (m, 5H).

To a solution of (2S)-3-amino-2-(benzyloxy)propan-1-ol (730 mg, 4.03 mmol) in dichloromethane (6 mL) was added a solution of sodium carbonate (448 mg, 4.23 mmol) in water (6 mL) and O-phenyl chlorothionoformate (585 μL, 4.23 mmol), and the mixture was stirred at room temperature for 90 min. To the mixture was added diethyl ether, and the mixture was successively washed with 5% aqueous citric acid solution, saturated saline, 5% aqueous sodium carbonate solution, and saturated saline. The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give O-phenyl (2S)-2-(benzyloxy)-3-hydroxypropylthiocarbamate as brown oil (1.35 g, 100%). ^{1}H NMR ($CDCl_3$): δ 2.31 (dd, 1H, J = 5, 8 Hz), 3.69−3.96 (m, 5H), 4.65 (d, 1H, J = 12 Hz), 4.69 (d, 1H, J = 12 Hz), 7.05−7.08 (m, 3H), 7.27 (dd, 2H, J = 7, 7 Hz), 7.33−7.41 (m, 6H).

To a solution of O-phenyl (2S)-2-(benzyloxy)-3-hydroxypropylthiocarbamate (1.35 g, 4.03 mmol) in 2-propanol (4 mL) was added hydrazine monohydrate (1.95 mL, 40.3 mmol), and the mixture was stirred at room temperature for 16 h. To the mixture was added saturated saline, and the mixture was extracted with dichloromethane. The extract was washed with saturated saline, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. To the oily residue was added a mixed solvent of diethyl ether and diisopropyl ether. The supernatant was removed by decanting twice to give the title compound **9e** as a red-brown oil (760 mg, 74%). ^{1}H NMR ($CDCl_3$): δ 3.06 (brs, 1H), 3.64 (d, 2H, J = 6 Hz), 3.70−3.80 (m, 4H), 4.12−4.17 (m, 1H), 4.63 (d, 1H, J = 11 Hz), 4.66 (d, 1H, J = 11 Hz), 7.13 (brs, 1H), 7.29−7.36 (m, 5H), 7.71 (brs, 1H).

(2S)-6-(5-{[2-(Benzyloxy)-3-hydroxypropyl]amino}-3-(4-fluorophenyl)-1H-pyrazol-4-yl)-2-(2-methylphenyl)-3(2H)-pyridazinone (10e). A mixture of (2S)-4-[2-(benzyloxy)-3-hydroxypropyl]-thiosemicarbazide **9e** (251 mg, 0.98 mmol) and **8** (329 mg, 0.82 mmol) in glacial acetic acid (2.5 mL) was stirred at 60−65 °C for 110 min. To the mixture was added ethyl acetate (30 mL), and the resulting insoluble materials were removed by filtration. To the mixture was added water, and the mixture was alkalinized with sodium carbonate. The organic layer was separated and washed successively with 5% aqueous citric acid solution, water, saturated aqueous sodium bicarbonate solution, and saturated saline, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, stepwise gradient from 0% to 10% methanol in chloroform) to give the title compound as a pale yellow amorphous solid (315 mg, yield 73.1%). ^{1}H NMR ($CDCl_3$): δ 2.20 (s, 3H), 3.44−3.61 (m, 5H), 4.50 (s, 2H), 6.01 (brs, 1H), 6.82 (d, 1H, J = 10 Hz), 6.98 (d, 1H, J = 10 Hz), 7.05−7.27 (m, 4H), 7.29−7.36 (m, 7H), 7.45−7.48 (m, 2H).

(2S)-6-[6-(Benzyloxy)-2-(4-fluorophenyl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrimidin-3-yl]-2-(2- methylphenyl)-3(2H)-pyridazinone (11e). To a solution of **10e** (240 mg, 0.46 mmol) in dichloromethane (3 mL) were added triethylamine (95 μL, 0.69 mmol) and methanesulfonyl chloride (46 μL, 0.59 mmol), and the mixture was stirred at room temperature for 55 min. To the mixture were added triethylamine (95 μL, 0.69 mmol) and methanesulfonyl chloride (46 μL, 0.59 mmol), and the mixture was stirred at room temperature for 45 min and refluxed for 3 days. To the mixture was added water, and the mixture was extracted with ethyl acetate. The extracts were combined and successively washed with a 3% aqueous citric acid solution, 5% aqueous sodium carbonate solution, and saturated saline, then dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, stepwise gradient from 0% to 2% methanol in chloroform) to give the title compound as a yellow amorphous solid (205 mg, yield 88%). ^{1}H NMR ($CDCl_3$): δ 2.22 (3H, s), 3.42 (2H, brs), 4.07 (1H, m), 4.23 (2H, m), 4.67 (2H, m), 5.79 (1H, br s), 6.79 (1H, d, J = 8.4 Hz), 7.01 (1H, d, J = 7.6 Hz), 7.14 (2H, m), 7.29−7.38 (9H, m), 7.50 (2H, m). MS (API-ES) m/z = 508 (M + Na).

6-[(6S)-2-(4-Fluorophenyl)-6-hydroxy-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrimidin-3-yl]-2-(2-methylphenyl)-pyridazin-3(2H)-one (3e). A mixture of **11e** (430 mg) and palladium

hydroxide (250 mg, 20% wt on carbon) in EtOH (20 mL) was stirred under a hydrogen atmosphere at 45−50 °C for 6 h. To the reaction mixture was added further palladium hydroxide (50 mg, 20% wt on carbon), and the mixture was stirred under a hydrogen atmosphere at 50 °C for 1 h. After the catalyst was filtered off, the filtrate was concentrated in vacuo. The residue was purified by flash silica gel column chromatography (gradient elution, AcOEt/hexane = 1/2 to 1/1) followed by crystallization from a mixed solvent of diethyl ether and dichloromethane to give the title compound as pale yellow crystals (115 mg, 33%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.08 (3H, s), 3.14−3.18 (1H, m), 3.24−3.28 (1H, m), 3.89 (1H, dd, J = 11.84 and 2.88 Hz), 4.12−4.18 (2H, m), 5.34 (1H, s), 5.93 (1H, s), 6.92 (1H, d, J = 9.80 Hz), 7.08 (1H, d, J = 9.80 Hz), 7.21 (1H, d, J = 1.88 Hz), 7.24 (1H, d, J = 8.88 Hz), 7.31−7.36 (4H, m), 7.46−7.49 (2H, m). MS (ESI) m/z = 418 (M + 1).

Ethyl 3-{[*tert*-Butyl(dimethyl)silyl]oxy}-2-{[(hydrazinocarbothioyl)amino]methyl}propanoate (9f). To a solution of ethyl 2-(hydroxymethyl)acrylate (5 g, 38.4 mmol) and imidazole (2.6 g, 38.4 mmol) in CH_2Cl_2 (40 mL) was added dropwise a solution of *tert*-butyldimethylsilyl chloride (5.8 g, 38.4 mmol) in CH_2Cl_2 (40 mL) with cooling on ice. After being stirred for 2 h at ambient temperature, the mixture was washed with H_2O and brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated to give ethyl 2-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)acrylate as an oil (9.32 g, 99%). ^{1}H NMR (DMSO-d_6): δ 0.06 (6H, s), 0.88 (9H, s), 1.23 (3H, t, J = 7.2 Hz), 4.15 (q, 2H, J = 6.4 Hz), 4.31 (2H, m), 5.83 (1H, m), 6.14 (1H, m). MS (ESI) m/z = 267 (M + Na).

A mixture of ethyl 2-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)acrylate (9.3 g, 38.1 mmol) and 1-phenylmethanamine (4.2 mL, 38.1 mmol) in EtOH (100 mL) was stirred for 14 h at 50 °C. After removal of solvent, the crude product was purified by column chromatography on silica gel (hexane/EtOAc = 8/1) to give ethyl 3-(benzylamino)-2-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)propanoate as an oil (5.12 g, 38%). ^{1}H NMR (DMSO-d_6) δ −0.01 (6H, s), 0.81 (9H, s), 1.16 (3H, t, J = 7.0 Hz), 2.16 (1H, brs), 2.53−2.72 (3H, m), 3.65 (2H, s), 3.70−3.78 (2H, m), 3.99−4.09 (2H, m), 7.17−7.33 (5H, m). MS (API-ES) m/z = 352 (M + H).

A mixture of ethyl 3-(benzylamino)-2-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)propanoate (5.12 g, 14.6 mmol) in EtOH (50 mL) was hydrogenized for 2 h under pressure with H_2 gas (3 atm) at 40 °C. After removal of catalyst, the filtrate was concentrated to give ethyl 3-amino-2-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)propanoate as a colorless oil (3.93 g, 100%). ^{1}H NMR (DMSO-d_6): δ 0.01 (3H, s), 0.02 (3H, s), 0.83 (9H, s), 1.18 (3H, t, J = 7.0 Hz), 1.46 (1H, brs), 2.47−2.55 (1H, m), 2.60−2.77 (2H, m), 3.31 (1H, brs), 3.69−3.80 (2H, m), 4.00−4.11 (2H, m). MS (API-ES) m/z = 262 (M + H).

To a mixture of *O*-phenyl carbonochloridothioate (2.0 mL, 14.9 mmol) in toluene (15.6 mL) and saturated $NaHCO_3$ solution (16 mL) was added a solution of 3-amino-2-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)propanoate (3.9 g, 14.9 mmol) in toluene (5 mL) with cooling on ice. After being stirred for 1 h, the organic layer was separated, washed with saturated $NaHCO_3$ solution and brine, and dried over Na_2SO_4. After removal of solvent, the residue was purified by column chromatography on silica gel (hexane/EtOAc = 8/1) to give ethyl 3-{[*tert*-butyl(dimethyl)silyl]oxy}-2-{[(phenoxycarbothioyl)amino]methyl}propanoate as an oil (3.76 g, 63%). ^{1}H NMR (DMSO-d_6): δ 0.04 (6H, s), 0.85 (9H, s), 1.21 (3H, t, J = 7.1 Hz), 2.97−3.04 (1H, m), 3.79−3.90 (4H, m), 4.12 (2H, q, J = 7.1 Hz), 6.73−6.78 (3H, m), 7.12−7.18 (2H, m), 9.31 (1H, s). MS (API-ES) m/z = 398 (M + H).

To a solution of ethyl 3-{[*tert*-butyl(dimethyl)silyl]oxy}-2-{[(phenoxycarbothioyl)amino]methyl}propanoate (3.7 g, 9.31 mmol) in EtOH (50 mL) was added hydrazine hydrate (722 mL, 14.9 mmol) at ambient temperature. The mixture was stirred for 2 h and then for 6 h at 40 °C. After removal of solvent, the residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 95/5) to give the title compound **9f** as an oil (2.33 g, 75%). ^{1}H NMR (DMSO-d_6): δ 0.021 (6H, s), 0.84 (9H, s), 1.19 (3H, t, J = 7.0 Hz), 2.88−2.97 (1H, m), 3.57−3.65 (2H, m), 3.66−3.82 (2H, m), 4.03−4.12 (2H, m), 4.46 (2H, s), 7.87 (1H, brs), 8.73 (1H, s). MS (API-ES) m/z = 336 (M + H).

Ethyl 3-({3-(4-Fluorophenyl)-4-[1-(2-methylphenyl)-6-oxo-1,6-dihydropyridazin-3-yl]-1*H*-pyrazol-5-yl}amino)-2-(hydroxymethyl)propanoate (10f). A mixture of **8** (2.3 g, 5.73 mmol) and **9f** (2.12 g, 6.31 mmol) in EtOH (12 mL) and AcOH (12 mL) was stirred for 14 h at 60 °C. The mixture was diluted with EtOAc, washed with saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was triturated with EtOAc to give the title compound as a yellow solid (1.51 g, 54%). ^{1}H NMR (DMSO-d_6): δ 1.04 (3H, t, J = 7.2 Hz), 2.10 (3H, s), 3.37 (2H, m), 3.54 (2H, m), 3.89 (2H, q, J = 6.8 Hz), 4.82 (1H, m), 5.40 (1H, brs), 6.93 (1H, m), 7.05 (1H, m), 7.26−7.44 (6H, m), 7.52 (2H, m). MS (API-ES) m/z = 514 (M + Na).

Ethyl 2-(4-Fluorophenyl)-3-[1-(2-methylphenyl)-6-oxo-1,6-dihydropyridazin-3-yl]-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidine-6-carboxylate (11f). To a suspension of ethyl 3-({3-(4-fluorophenyl)-4-[1-(2-methylphenyl)-6-oxo-1,6-dihydropyridazin-3-yl]-1*H*-pyrazol-5-yl}amino)-2-(hydroxymethyl)propanoate **10f** (1.45 g, 2.95 mmol) in MeCN (30 mL) were added Et_3N (2.1 μL, 14.8 mmol) and methanesulfonyl chloride (343 μL, 4.42 mmol) at room temperature followed by heating at 100 °C for 6 h. The mixture was cooled to room temperature and evaporated in vacuo. To the residue was added a 10% K_2CO_3 solution. Extraction was with $CHCl_3$, and the sample was washed with water and brine and dried over $MgSO_4$. After removal of solvent, the residue was purified by column chromatography on silica gel (hexane/EtOAc = 4/1) to give ethyl 2-(4-fluorophenyl)-3-[1-(2-methylphenyl)-6-oxo-1,6-dihydropyridazin-3-yl]-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidine-6-carboxylate **11f** as an oil (1.1 g, 79%). ^{1}H NMR (DMSO-d_6): δ 1.19 (3H, t, J = 7.2 Hz), 2.08 (3H, s), 3.36−3.56 (2H, m), 4.12 (2H, m), 4.25 (2H, m), 6.10 (1H, brs), 6.93 (1H, d, J = 9.6 Hz), 7.09 (1H, d, J = 9.6 Hz), 7.23 (2H, m), 7.28−7.41 (4H, m), 7.48 (2H, m). MS (API-ES) m/z = 496 (M + Na).

6-[2-(4-Fluorophenyl)-6-(hydroxymethyl)-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidin-3-yl]-2-(2-methylphenyl)pyridazin-3(2*H*)-one (3f and 3g). A mixture of ethyl 2-(4-fluorophenyl)-3-[1-(2-methylphenyl)-6-oxo-1,6-dihydropyridazin-3-yl]-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidine-6-carboxylate **11f** (10 mg, 0.02 mmol), lithium tetrahydroborate (1 mg, 0.05 mmol), and THF (1 mL) was stirred at room temperature for 2 h. The mixture was quenched with water, extracted with EtOAc, dried over $MgSO_4$, and evaporated in vacuo. The residue was purified by preparative thin layer chromatography to give the title product (7.5 mg, 82%).

The enantiomers of the racemic products were separated by chiral HPLC. HPLC results were obtained on a Hitachi LaChrom Elite system, using a DAICEL Chiralcel OJ-H column (25 cm × 0.46 cm) at 40 °C with a 1.0 mL/min flow rate using acetonitrile and *n*-hexane/EtOH/MeOH/isopropyl alcohol (60:30:10:0.1) solution as the eluent over 30 min. Enantiomer **3f** ($[\alpha]^{26}_D$ +51.3 (c 0.45, $CDCl_3$)) was collected at 11.076 min, and **3g** ($[\alpha]^{26}_D$ −50.4 (c 0.98, $CDCl_3$)) was collected at 14.766 min.

6-[(6*R*)-2-(4-Fluorophenyl)-6-(hydroxymethyl)-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidin-3-yl]-2-(2-methylphenyl)pyridazin-3(2*H*)-one (3f). ^{1}H NMR (DMSO-d_6): δ 2.08 (3H, s), 2.23 (1H, brs), 3.04 (1H, m), 3.46 (2H, m), 3.82 (1H, m), 4.12 (1H, m), 4.85 (1H, m), 5.98 (1H, brs), 6.92 (1H, d, J = 10 Hz), 7.08 (1H, d, J = 10 Hz), 7.23 (2H, m), 7.30−7.40 (4H, m), 7.48 (2H, m). MS (API-ES) m/z = 432 (M + H). $[\alpha]^{26}_D$ +51.3 (c 0.45, $CHCl_3$).

6-[(6*S*)-2-(4-Fluorophenyl)-6-(hydroxymethyl)-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidin-3-yl]-2-(2-methylphenyl)pyridazin-3(2*H*)-one (3g). ^{1}H NMR (DMSO-d_6): δ 2.08 (3H, s), 2.22 (1H, brs), 3.03 (1H, m), 3.45 (2H, m), 3.81 (1H, m), 4.12 (1H, m), 4.84 (1H, m), 5.99 (1H, brs), 6.93 (1H, d, J = 10 Hz), 7.09 (1H, d, J = 10 Hz), 7.23 (2H, m), 7.30−7.40 (4H, m), 7.48 (2H, m). MS (API-ES) m/z = 432 (M + H). $[\alpha]^{26}_D$ −50.4 (c 0.98, $CHCl_3$).

6-{2-(4-Fluorophenyl)-6-[(4-methylpiperazin-1-yl)methyl]-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidin-3-yl}-2-(2-methylphenyl)pyridazin-3(2*H*)-one Dihydrochloride (3h). A mixture of 6-[2-(4-fluorophenyl)-6-(iodomethyl)-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidin-3-yl]-2-(2-methylphenyl)pyridazin-3(2*H*)-one (200 mg, 0.37 mmol) and 1-methylpiperazine (800 μL, 7.27 mmol) was stirred at 60 °C for 12 h. The reaction

mixture was quenched with H_2O, and the products were extracted with AcOEt. The extract was dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography ($CHCl_3$/MeOH = 95/5 to 90/10), and the fractions were combined and evaporated. The residue was dissolved in AcOEt, and to the mixture was added 4 M HCl–AcOEt. The precipitate was filtered and crystallized from EtOH–AcOEt to give the title compound as a yellow powder (47 mg, 22%). ^{1}H NMR (DMSO-d_6): δ 2.09 (3H, s), 2.60–2.71 (1H, m), 2.81 (3H, s), 3.04–4.39 (14H, m), 6.95 (1H, d, J = 9.8 Hz), 7.10 (1H, d, J = 9.8 Hz), 7.21–7.28 (2H, m), 7.32–7.39 (4H, m), 7.46–7.52 (2H, m). MS (API-ES) m/z = 514 (M + H).

***N*-[(2*R*)-1-{[*tert*-Butyl(diphenyl)silyl]oxy}-4-hydroxybutan-2-yl]-hydrazinecarbothioamide (9i).** To a solution of (2*R*)-4-{[*tert*-butyl(dimethyl)silyl]oxy}-2-(dibenzylamino)butan-1-ol (1.47 g, 3.7 mmol) in DMF (29.4 mL) were added imidazole (601 mg, 8.8 mmol) and *tert*-butyl(chloro)diphenylsilane (1.15 mL, 4.4 mmol) at room temperature, and the mixture was stirred for 15 h. The mixture was partitioned between ethyl acetate and water. The organic layer was separated, successively washed with water and brine, dried over $MgSO_4$, and evaporated under reduced pressure to give the crude product. The crude product was purified by column chromatography on silica gel (hexane/EtOAc = 100/0 to 95/5) to give (6*R*)-*N*,*N*-dibenzyl-2,2,10,10,11,11-hexamethyl-3,3-diphenyl-4,9-dioxa-3,10-disiladodecan-6-amine (1.97 g, 84%). ^{1}H NMR (400 MHz, $CDCl_3$): δ −0.04 (6H, s), 0.82 (9H, s), 1.07 (9H, s), 1.63–1.72 (1H, m), 1.80–1.89 (1H, m), 2.87–2.94 (1H, m), 3.50–3.58 (1H, m), 3.60–3.82 (7H, m), 7.16–7.21 (2H, m), 7.23–7.28 (4H, m), 7.31–7.44 (10H, m), 7.64–7.69 (4H, m). MS (API-ES) m/z = 639 (M + H).

To a solution of (6*R*)-*N*,*N*-dibenzyl-2,2,10,10,11,11-hexamethyl-3,3-diphenyl-4,9-dioxa-3,10-disiladodecan-6-amine (1.97 g, 3.0 mmol) in dioxane (19.7 mL) was added 4 N HCl (3.94 mL) at room temperature, and the mixture was stirred for 1 h. The mixture was partitioned between ethyl acetate and water. The organic layer was separated, successively washed with $NaHCO_3$ solution and brine, dried over $MgSO_4$, and evaporated under reduced pressure to give the crude product. The crude product was purified by column chromatography on silica gel (hexane/EtOAc = 9/1 to 4/1) to give (3*R*)-4-{[*tert*-butyl(diphenyl)silyl]oxy}-3-(dibenzylamino)butan-1-ol (1.53 g, 95%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.10 (9H, s), 1.47–1.56 (1H, m), 1.86–1.99 (1H, m), 3.02–3.11 (1H, m), 3.56 (2H, d, J = 13 Hz), 3.69–3.80 (2H, m), 3.83–3.88 (2H, m), 3.93 (2H, d, J = 13 Hz), 7.20–7.33 (10H, m), 7.38–7.47 (6H, m), 7.66–7.71 (4H, m). MS (API-ES) m/z = 524 (M + H).

To a solution of (3*R*)-4-{[*tert*-butyl(diphenyl)silyl]oxy}-3-(dibenzylamino)butan-1-ol (1.57 g, 3.0 mmol) in ethanol (24 mL) were added ammonium formate (2.84 g, 45.0 mmol) and palladium on carbon (628 mg, 10% wt on carbon) at room temperature, and the mixture was refluxed for 4 h. After cooling to room temperature, the mixture was filtered through a funnel. The filtrate was evaporated under reduced pressure to give (3*R*)-3-amino-4-{[*tert*-butyl(diphenyl)-silyl]oxy}butan-1-ol (1 g, 97%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.06 (9H, s), 1.53–1.60 (2H, m), 3.01–3.05 (1H, m), 3.44 (1H, dd, J = 6, 10 Hz), 3.56 (1H, dd, J = 4, 10 Hz), 3.79–3.82 (2H, m), 7.34–7.46 (6H, m), 7.62–7.68 (4H, m). MS (API-ES) m/z = 344 (M + H).

To a solution of (3*R*)-3-amino-4-{[*tert*-butyl(diphenyl)silyl]oxy}-butan-1-ol (1.0 g, 2.9 mmol) in CH_2Cl_2/H_2O (1/1, 30 mL) were added *O*-phenyl carbonochloridothioate (471 μL, 3.5 mmol) and $NaHCO_3$ (489.1 mg, 5.8 mmol) at 0 °C followed by stirring at room temperature for 30 min. The mixture was partitioned between ethyl acetate and water. The organic layer was separated, successively washed with water and brine, dried over $MgSO_4$, and evaporated under reduced pressure to give 1.42 g of *O*-phenyl [(2*R*)-1-{[*tert*-butyl(diphenyl)silyl]oxy}-4-hydroxybutan-2-yl]carbamothioate. MS (ESI) m/z = 502 (M + Na). To a solution of the above product (1.42 g, 3.0 mmol) in isopropyl alcohol (8.0 mL) was added hydrazine hydrate (1.4 mL, 29.6 mmol) at room temperature, and the mixture was stirred for 2 h. The mixture was partitioned between ethyl acetate and water. The organic layer was separated, washed successively with 1 N NaOH solution and brine, dried over $MgSO_4$, and evaporated under reduced pressure to give the crude product, which was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 99/1 to 95/5) to give the title compound **9i** (630 mg, 51%). ^{1}H NMR (DMSO-d_6): δ 1.01 (9H, s), 1.73–1.90 (2H, m), 2.07 (3H, s), 3.44–3.49 (2H, m), 3.63 (1H, dd, J = 9.5, 5.5 Hz), 3.74 (1H, dd, J = 10, 3.5 Hz), 4.45–4.49 (4H, m), 7.42–7.47 (6H, m), 7.60–7.65 (4H, m), 7.86 (1H, brs), 8.73 (1H, brs). MS (ESI) m/z = 440 (M + Na).

6-[5-{[(2*R*)-1-{[*tert*-Butyl(diphenyl)silyl]oxy}-4-hydroxybutan-2-yl]amino}-3-(4-fluorophenyl)-1*H*-pyrazol-4-yl]-2-(2-methylphenyl)pyridazin-3(2*H*)-one (13i). To a solution of **8** (630 mg, 1.6 mmol) in EtOH/acetic acid (1/1, 8.4 mL) was added **9i** (655.8 mg, 1.6 mmol) at room temperature, and the mixture was stirred at 50 °C for 2 h. The mixture was quenched with $NaHCO_3$ solution, and extraction was with ethyl acetate. The organic layer was separated, successively washed with $NaHCO_3$ solution and brine, dried over $MgSO_4$, and evaporated under reduced pressure to give the crude product. The crude product was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 100/0 to 90/10) to give the title compound (850 mg, 79%). ^{1}H NMR (DMSO-d_6): δ 0.80 (9H, s), 1.52–1.62 (1H, m), 1.70–1.83 (1H, m), 2.07 (3H, s), 3.46–3.86 (5H, m), 4.46 (1H, brs), 5.53 (1H, brs), 6.92 (1H, d, J = 10 Hz), 7.03 (1H, d, J = 10 Hz), 7.24–7.56 (18H, m), 12.27 (1H, brs). MS (API-ES) m/z = 688 (M + H).

6-[(5*R*)-2-(4-Fluorophenyl)-5-(hydroxymethyl)-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidin-3-yl]-2-(2-methylphenyl)-pyridazin-3(2*H*)-one (3i). To a solution of **13i** (850 mg, 1.2 mmol) in acetonitrile (17 mL) were added methanesulfonyl chloride (143.5 μL, 1.8 mmol) and triethylamine (516.7 μL, 3.7 mmol), and the mixture was stirred at 80 °C for 2 h. The mixture was partitioned between ethyl acetate and water. The organic layer was separated, successively washed with 10% citric acid solution and brine, dried over $MgSO_4$, and evaporated under reduced pressure to give the crude product, which was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 99/1 to 95/5) to give 6-[(5*R*)-5-({[*tert*-butyl-(diphenyl)silyl]oxy}methyl)-2-(4-fluorophenyl)-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidin-3-yl]-2-(2-methylphenyl)-pyridazin-3(2*H*)-one (495.6 mg, 60%). ^{1}H NMR (DMSO-d_6): δ 0.90 (9H, s), 1.97–2.00 (1H, m), 2.03 (3H, s), 2.15–2.23 (1H, m), 3.52–3.68 (3H, m), 4.00–4.05 (2H, m), 6.03 (1H, brs), 6.92 (1H, d, J = 10 Hz), 7.07 (1H, d, J = 10 Hz), 7.19–7.57 (18H, m). MS (ESI) m/z = 692 (M + Na).

To a solution of 6-[(5*R*)-5-({[*tert*-butyl(diphenyl)silyl]oxy}-methyl)-2-(4-fluorophenyl)-4,5,6,7-tetrahydropyrazolo[1,5-*a*]-pyrimidin-3-yl]-2-(2-methylphenyl)pyridazin-3(2*H*)-one (450 mg, 0.67 mmol) in THF (4.5 mL) was added a solution of 1 M tetrabutylammonium fluoride in THF (0.67 mL, 0.67 mmol) at room temperature. After being stirred for 30 min, the mixture was partitioned between EtOAc and H_2O. The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 98/2 to 95/5). The obtained oil was crystallized from ^{i}PrOH–Hex to give the title compound (269 mg, 93%). ^{1}H NMR (DMSO-d_6): δ 1.71–1.84 (1H, m), 2.05–2.09 (1H, m), 2.11 (3H, s), 3.26–3.33 (1H, m), 3.40 (1H, m), 3.48–3.52 (1H, m), 3.98–4.14 (2H, m), 4.89 (1H, t, 5 Hz), 6.11 (1H, brs), 6.92 (1H, d, J = 10 Hz), 7.03 (1H, d, J = 10 Hz), 7.26 (2H, t, J = 9 Hz), 7.31–7.37 (4H, m), 7.49 (2H, dd, J = 9, 5 Hz). MS (ESI) m/z = 454 (M + Na).

***N*-[(2*S*)-1-{[*tert*-Butyl(diphenyl)silyl]oxy}-4-hydroxybutan-2-yl]-hydrazinecarbothioamide (9j).** (6*S*)-*N*,*N*-Dibenzyl-2,2,10,10,11,11-hexamethyl-3,3-diphenyl-4,9-dioxa-3,10-disiladodecan-6-amine was synthesized from (2*S*)-4-{[*tert*-butyl(dimethyl)silyl]oxy}-2-(dibenzylamino)butan-1-ol according to the procedure for the *R*-isomer ((6*R*)-*N*,*N*-dibenzyl-2,2,10,10,11,11-hexamethyl-3,3-diphenyl-4,9-dioxa-3,10-disiladodecan-6-amine) (99%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.03 (6H, s), 0.85 (9H, s), 1.05 (9H, s), 1.63–1.73 (1H, m), 1.81–1.90 (1H, m), 2.86–2.94 (1H, m), 3.51–3.58 (1H, m), 3.59–3.82 (7H, m), 7.18–7.23 (2H, m), 7.25–7.30 (4H, m), 7.31–7.46 (10H, m), 7.65–7.68 (4H, m). MS (API-ES) m/z = 639 (M + H).

(3*S*)-4-{[*tert*-Butyl(diphenyl)silyl]oxy}-3-(dibenzylamino)butan-1-ol was synthesized from (6*S*)-*N*,*N*-dibenzyl-2,2,10,10,11,11-hexamethyl-3,

3-diphenyl-4,9-dioxa-3,10-disiladodecan-6-amine according to the procedure for the *R*-isomer ((3*R*)-4-{[*tert*-butyl(diphenyl)silyl]oxy}-3-(dibenzylamino)butan-1-ol) (98%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.12 (9H, s), 1.47−1.55 (1H, m), 1.88−2.00 (1H, m), 3.01−3.12 (1H, m), 3.58 (2H, d, *J* = 13 Hz), 3.68−3.80 (2H, m), 3.83−3.89 (2H, m), 3.95 (2H, d, *J* = 13 Hz), 7.25−7.33 (10H, m), 7.35−7.46 (6H, m), 7.66−7.73 (4H, m). MS (API-ES) *m*/*z* = 524 (M + H).

(3*S*)-4-{[*tert*-Butyl(diphenyl)silyl]oxy}-3-(dibenzylamino)butan-1-ol was synthesized from (3*S*)-4-{[*tert*-butyl(diphenyl)silyl]oxy}-3-(dibenzylamino)butan-1-ol according to the procedure for the *R*-isomer ((3*S*)-4-{[*tert*-butyl(diphenyl)silyl]oxy}-3-(dibenzylamino)-butan-1-ol) (100%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.05 (9H, s), 1.55−1.60 (2H, m), 3.01−3.06 (1H, m), 3.44 (1H, dd, *J* = 6, 10 Hz), 3.56 (1H, dd, *J* = 4, 10 Hz), 3.78−3.83 (2H, m), 7.33−7.46 (6H, m), 7.63−7.68 (4H, m). MS (API-ES) *m*/*z* = 344 (M + H).

N-[(2*S*)-1-{[*tert*-Butyl(diphenyl)silyl]oxy}-4-hydroxybutan-2-yl]-hydrazinecarbothioamide (**9j**) was synthesized from (3*S*)-3-amino-4-{[*tert*-butyl(diphenyl)silyl]oxy}butan-1-ol according to the procedure for the *R*-isomer (**9i**). ^{1}H NMR (DMSO-d_6): δ 1.01 (9H, s), 1.74−1.91 (2H, m), 2.07 (3H, s), 3.45−3.48 (2H, m), 3.64 (1H, dd, *J* = 9.5, 5.5 Hz), 3.74 (1H, dd, *J* = 10, 3.5 Hz), 4.46−4.48 (4H, m), 7.41−7.49 (6H, m), 7.61−7.66 (4H, m), 7.89 (1H, brs), 8.74 (1H, brs). MS (ESI) *m*/*z* = 440 (M + Na).

6-[5-{[(2*S*)-1-{[*tert*-Butyl(diphenyl)silyl]oxy}-4-hydroxybutan-2-yl]amino}-3-(4-fluorophenyl)-1*H*-pyrazol-4-yl]-2-(2-methylphenyl)pyridazin-3(2*H*)-one (13j). 6-[5-{[(2*S*)-1-{[*tert*-Butyl(diphenyl)silyl]oxy}-4-hydroxybutan-2-yl]amino}-3-(4-fluorophenyl)-1*H*-pyrazol-4-yl]-2-(2-methylphenyl)pyridazin-3(2*H*)-one (**13j**) was synthesized from *N*-[(2*S*)-1-{[*tert*-butyl(diphenyl)silyl]-oxy}-4-hydroxybutan-2-yl]hydrazinecarbothioamide (**11j**) according to the procedure for the *R*-isomer (**13i**) (77%). ^{1}HNMR (DMSO-d_6): δ 0.90 (9H, s), 1.53−1.62 (1H, m), 1.70−1.83 (1H, m), 2.07 (3H, s), 3.44−3.85 (5H, m), 4.46 (1H, brs), 5.52 (1H, brs), 6.93 (1H, d, *J* = 10 Hz), 7.00 (1H, d, *J* = 10 Hz), 7.23−7.56 (18H, m), 12.27 (1H, brs). MS (ESI) *m*/*z* = 688 (M + H).

6-[(5*S*)-2-(4-Fluorophenyl)-5-(hydroxymethyl)-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidin-3-yl]-2-(2-methylphenyl)-pyridazin-3(2*H*)-one (3j). 6-[(5*S*)-5-({[*tert*-Butyl(diphenyl)silyl]-oxy}methyl)-2-(4-fluorophenyl)-4,5,6,7-tetrahydropyrazolo[1,5-*a*]-pyrimidin-3-yl]-2-(2-methylphenyl)pyridazin-3(2*H*)-one was synthesized from **12j** according to the procedure for the *R*-isomer (6-[(5*R*)-5-({[*tert*-butyl(diphenyl)silyl]oxy}methyl)-2-(4-fluorophenyl)-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidin-3-yl]-2-(2-methylphenyl)-pyridazin-3(2*H*)-one) (76%). ^{1}HNMR (DMSO-d6): δ 0.91 (9H, s), 1.97−2.05 (1H, m), 2.03 (3H, s), 2.14−2.24 (1H, m), 3.53−3.66 (3H, m), 4.01−4.05 (2H, m), 6.04 (1H, brs), 6.92 (1H, d, *J* = 10 Hz), 7.09 (1H, d, *J* = 10 Hz), 7.20−7.57 (18H, m). MS (ESI) *m*/*z* = 692 (M + Na).

6-[(5*S*)-2-(4-Fluorophenyl)-5-(hydroxymethyl)-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidin-3-yl]-2-(2-methylphenyl)-pyridazin-3(2*H*)-one (**3j**) was synthesized from 6-[(5*S*)-5-({[*tert*-butyl(diphenyl)silyl]oxy}methyl)-2-(4-fluorophenyl)-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidin-3-yl]-2-(2-methylphenyl)-pyridazin-3(2*H*)-one according to the procedure for the *R*-isomer (**3i**) (91%). Chiral HPLC AS-H, 99.9% (15.82 min), Hex/EtOH/MeOH/Et_2NH = 60/30/10/0.1, flow = 0.5 mL/min, 254 nm). ^{1}HNMR (DMSO-d_6): δ 1.73−1.86 (1H, m), 2.06−2.08 (1H, m), 2.11 (3H, s), 3.25−3.33 (1H, m), 3.42 (1H, m), 3.49−3.53 (1H, m), 4.01−4.13 (2H, m), 4.89 (1H, t, 5 Hz), 6.12 (1H, brs), 6.92 (1H, d, *J* = 10 Hz), 7.03 (1H, d, *J* = 10 Hz), 7.25 (2H, t, *J* = 9 Hz), 7.30−7.36 (4H, m), 7.48 (2H, dd, *J* = 9, 5 Hz). MS (ESI) *m*/*z* = 454 (M + Na).

6-[2-(4-Fluorophenyl)-6-methylene-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidin-3-yl]-2-(2-methylphenyl)-pyridazin-3(2*H*)-one (14). A mixture of 6-[2-(4-fluorophenyl)-6-(iodomethyl)-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidin-3-yl]-2-(2-methylphenyl)pyridazin-3(2*H*)-one **12** (1.43 g, 2.64 mmol) and 28% NaOMe (612 mg, 3.17 mmol) in MeOH (14.3 mL) was refluxed for 12 h. The mixture was partitioned between ethyl acetate and water. The organic layer was separated, washed with 5% citric acid and brine, dried over $MgSO_4$, and evaporated under reduced pressure to give the title product (1.0 g, 92%). ^{1}H NMR ($CDCl_3$): δ 2.22 (3H, s), 3.90 (2H, s), 4.81 (2H, s), 5.31 (2H, d), 5.97 (1H, brs), 6.82 (1H, d, *J* = 8.8 Hz), 7.02 (1H, d, *J* = 10 Hz), 7.17 (2H, m), 7.30−7.42 (4H, m), 7.53 (2H, m). MS (ESI) *m*/*z* = 436 (M + Na).

6-[2-(4-Fluorophenyl)-6-(hydroxymethyl)pyrazolo[1,5-*a*]-pyrimidin-3-yl]-2-(2-methylphenyl)pyridazin-3(2*H*)-one (3k). A mixture of **14** (150 mg, 0.36 mmol), OsO4 (46 mg, 0.18 mmol), *N*-methylmorpholine *N*-oxide (55.3 mg, 0.47 mmol), H_2O (0.6 mL), acetone (0.6 mL), and MeCN (0.6 mL) was stirred for 3 weeks and then filtered through a Celite pad. The filtrate was evaporated and the residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 10/0 to 98/2) to give the title compound (37.0 mg, 24%). ^{1}H NMR ($CDCl_3$): δ 2.14 (3H, s), 4.83 (2H, s), 7.04 (2H, t), 7.14 (3H, m), 7.30 (2H, m), 7.70 (2H, dd), 7.92 (1H, d), 8.58 (1H, s), 8.71 (1H, s). MS (API-ES) *m*/*z* = 450 (M + Na).

Biological Methods. Inhibition of TNF-α Production in THP-1 Cells. THP-1 cells, from a human monocytic cell line, were maintained in RPMI 1640 (Sigma R8758) supplemented with penicillin (50 U/mL), streptomycin (50 μg/mL), and 10% fetal bovine serum (Moregate BioTech) at 37 °C and 5% CO_2 in a humidified incubator. Initial stock solutions of test compounds were made in DMSO. All cells, reagents, and test compounds were diluted in culture media. THP-1 cells (1×10^5 cells/well final) and lipopolysaccharide (LPS, 10 μg/mL final, Sigma L-4005, from *E. coli* serotype 055:B5) were added to 96-well polypropylene culture plates (Sumilon, MS-8196F5; sterile) containing test compound or 0.1% DMSO vehicle. The cell mixture was incubated for 20 h in a humidified incubator at 37 °C, 5% CO_2. The culture supernatants were harvested, and TNF-α levels from LPS-stimulated cells in the presence of 100 nM test compound were calculated and compared with those from control cells stimulated in the presence of 0.1% DMSO.

Inhibition of Hind Paw Swelling in Adjuvant-Induced Arthritis in Rats. Arthritis was induced by injection of 0.5 mg of *Mycobacterium tuberculosis* (Difco Laboratories, Detroit, MI) in 50 μL of liquid paraffin into the right hind footpad of female Lewis rats aged 7 weeks (day 0). Normal untreated rats were used as negative controls. Animals were randomized and grouped ($n \geq 5$) for drug treatment based on an increase in left hind paw volume and body weight on day 15. Drugs were suspended in vehicle (0.5% methylcellulose) and orally administered once a day from day 15 to day 24. The volume of the left hind paw was measured on day 25 by a water displacement method using a plethysmometer for rats (MK-550; Muromachi Kikai Co., Ltd., Tokyo, Japan).

In Vivo Assays. LPS-Induced TNFα in the Rat. Four hours prior to LPS administration, rats were dosed orally with the compounds suspended in 0.5% methylcellulose/0.1% Tween-80. LPS (10 μg/animal) was administered intraperitoneally, and 1.0 or 1.5 h later, rats were anesthetized and retroorbital blood was collected in heparin tubes. Plasma was separated by centrifugation and diluted one-fifth prior to assaying in a standard rat TNF-α ELISA.

Kinase Isoform Selectivity Assays. The human p38α and p38β kinase activities of **3f** were evaluated using a Z′-LyteTM kinase assay system (Life Technologies, Madison, FL, U.S.). JNK2 kinase activity was examined by following an in-house protocol. Preactivated recombinant human JNK2α2/SAPK1a (40 ng/well; Upstate Biotechnology, Lake Placid, NY, U.S.) was diluted in 1.5× assay dilution buffer I (1× ADB-I, 20 mM MOPS, pH 7.2, 25 mM β-glycerol phosphate, 5 mM EGTA, 1 mM sodium orthovanadate, 1 mM DTT), and then 20 μL of 1.5× ADB-I (containing recombinant JNK2) was mixed with 20 μL of Mg/ATP/1× ADBI buffer (37.5 mM $MgCl_2$, 2 μM ATP diluted with 1× ADB-I) and 10 μL of **3f** at final concentrations of 10−1000 nM in 96-well MaxiSorp Immuno Plates (Nalge Nunc International, Rochester, NY, U.S.) precoated with recombinant human ATF-2 (0.1 μg/well, Upstate Biotechnology). After 30 min of incubation at 30 °C, the plate was washed three times with phosphate buffered saline containing Tween 0.05% and then incubated with 1:1000 diluted anti-phospho-ATF-2 (Thr71) (Cell Signaling Technology, Danvers, MA, U.S.) antibody for 60 min at room temperature. The plate was then washed as described above and reacted with 1:5000 diluted anti-rabbit IgG-conjugated HRP

(Zymed Laboratories, South San Francisco, CA, U.S.) for 30 min at room temperature. After the plate was washed, TMB substrate (BD Biosciences Pharmingen, San Diego, CA, U.S.) was added, and the plate was incubated for 20 min in the dark. The reaction was stopped by adding 50 μL of 2 N H_2SO_4, and sample absorbance was measured with a microplate reader at 450 nm.

Pharmacokinetic Studies. **2a**, **3d**, **3e**, **3f**, **3g**, or **3j** was dissolved in PEG400 and administered to Sprague–Dawley rats at a dose of 1 mg/kg. Blood was collected and centrifuged to obtain plasma, and the plasma concentrations of each compound were determined by LC/MS/MS.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Combustion analysis data and information on docking study. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

TNFα, tumor necrosis factor α; MAPK, mitogen-activated protein kinase; LPS, lipopolysaccharide; COPD, chronic obstructive pulmonary disease; IBD, inflammatory bowel disease; CYP, cytochrome; °C, degree Celsius; ClogP, calculated log *P*; SAR, structure–activity relationship; IC_{50}, half-maximum inhibitory concentration; ED_{50}, 50% effective dose; *F*, % oral bioavailability; AUC, area under the curve; $t_{1/2}$, half-life; PAMPA, parallel artificial membrane permeability assay; po, oral administration; iv, intravenous; PK, pharmacokinetics; Ac, acetyl; Bn, benzyl; Et, ethyl; Me, methyl; Ts, *p*-toluenesulfonyl; Ms, methanesulfonyl; ph, phenyl; DEAD, diethyl azodicarboxylate; DMF, dimethylformamide; TBAF, tetrabutylammonium fluoride; HPLC, high-performance liquid chromatography; MS, mass spectrometry; HRMS, high-resolution mass spectrometry; NMR, nuclear magnetic resonance; ppm, parts per million; *J*, coupling constant; RA, rheumatoid arthritis; $LiBH_4$, lithium borohydride; OsO_4, osmium tetroxide; LiHMDS, lithium bis(trimethylsilyl)amide; $PyBr_3$, pyridinium tribromide; NMM, *N*-methylmorpholine; DIPEA, *N*,*N*-diisopropylethylamine; CL_{int}, intrinsic clearance; CL_{tot}, total body clearance; V_{ss}, volume of distribution at steady state; AIA, adjuvant-induced arthritis; MC, methylcellulose; ED_{30}, 30% effective dose

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