Bioorganic & Medicinal Chemistry 19 (2011) 1881–1894

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Novel and potent calcium-sensing receptor antagonists: Discovery of (5*R*)-*N*-[1-ethyl-1-(4-ethylphenyl)propyl]-2,7,7-trimethyl-5-phenyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidine-3-carboxamide monotosylate (TAK-075) as an orally active bone anabolic agent

Masato Yoshida, Akira Mori, Shinji Morimoto, Etsuo Kotani, Masahiro Oka, Kohei Notoya, Haruhiko Makino, Midori Ono, Mikio Shirasaki, Norio Tada, Hisashi Fujita, Junko Ban, Yukihiro Ikeda, Tomohiro Kawamoto, Mika Goto, Hiroyuki Kimura, Atsuo Baba, Tsuneo Yasuma *

Pharmaceutical Research Division, Takeda Pharmaceutical Company Ltd, 2-17-85, Jusohomachi, Yodogawa-ku, Osaka 532-8686, Japan

ARTICLE INFO

Article history:
Received 6 January 2011
Revised 31 January 2011
Accepted 1 February 2011
Available online 24 February 2011

Keywords:
Orally active
Calcium-sensing receptor antagonists
CaSR
Bone anabolic agents
PTH
TAK-075

ABSTRACT

The calcium-sensing receptor antagonist (CaSR) has been recognized as a promising target of anabolic agents for treating osteoporosis. In the course of developing a new drug candidate for osteoporosis, we found tetrahydropyrazolopyrimidine derivative **1** to be an orally active CaSR antagonist that stimulated transient PTH secretion in rats. However, compound **1** showed poor physical and chemical stability. In order to work out this compound's chemical stability and further understand its in vivo efficacy, we focused on modifying the 2-position of the tetrahydropyrazolopyrimidine. As a result of chemical modification, we discovered (5*R*)-*N*-[1-ethyl-1-(4-ethylphenyl)propyl]-2,7,7-trimethyl-5-phenyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidine-3-carboxamide monotosylate **10m** (TAK-075), which showed improved solubility, chemical stability, and in vivo efficacy. Furthermore, we describe that evaluating the active metabolite is important during repeated treatment with short-acting CaSR antagonists.

1. Introduction

The maintenance of bone mass depends on the balance between bone resorption and formation during bone remodeling.[1] Age-related imbalances between increased bone resorption and decreased formation results in bone loss and osteoporosis. Osteoporosis is a common metabolic bone disease in which bones become fragile, increasing the risk for fractures. Furthermore, osteoporosis affects around 200 million people worldwide and thus represents a substantial financial burden.[2,3] Currently available osteoporosis treatments either aim to inhibit bone resorption through the use of antiresorptive agents (bisphosphonates,[4,5] estradiol, calcitonin, raloxifene,[6] etc.) or promote bone formation with anabolic agents[7–9] (recombinant full-length human parathyroid hormone (PTH) 1–84 (Nycomed) or teriparatide; the recombinant N-terminal PTH 1-34 amino acid fragment (Lilly); etc.).

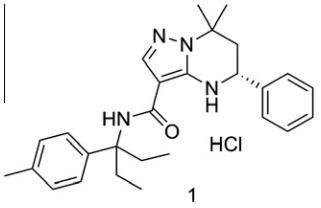
PTH is a hormone released by the parathyroid glands. It is the most important endocrine regulator of human calcium and phosphorus levels. In contrast to the anabolic effects observed after intermittent administration of PTH 1–84 or PTH 1–34, continuous exposure to PTH results in increases in bone turnover with subsequent losses in bone mass.[10,11] The secretion of PTH is strictly controlled by the calcium-sensing receptor (CaSR),[12] which is a G-protein coupled receptor (GPCR) expressed on the surface of parathyroid cells. CaSR senses extracellular levels of the calcium ion and controls homeostasis by releasing PTH.

Recently, it was reported that antagonists of CaSR (calcilytics)[13–16] can increase the endogenous levels of circulating PTH in humans. Therefore, orally active short-acting calcilytics became attractive and promising targets to replace PTH therapies. As part of our CaSR antagonists program,[17,18] we have previously reported that tetrahydropyrazolopyrimidine derivative **1**[18] has potent CaSR antagonistic activity with an IC_{50} of 2.3 nM (Fig. 1). During our discovery of compound **1**, we also learned that solubility and metabolic stability are key factors for rapid and transient PTH release.[18] We demonstrated that salt formation improves the solubility of the tetrahydropyrazolopyrimidine series, resulting in excellent efficacy.

However, our investigation of chemical stability revealed that compound **1** is degraded under the conditions shown in Figure 1. It was assumed that the degradation was derived from the

* Corresponding author. Tel.: +81 6 6300 6117; fax: +81 6 6300 6306.
E-mail address: Yasuma_Tsuneo@takeda.co.jp (T. Yasuma).

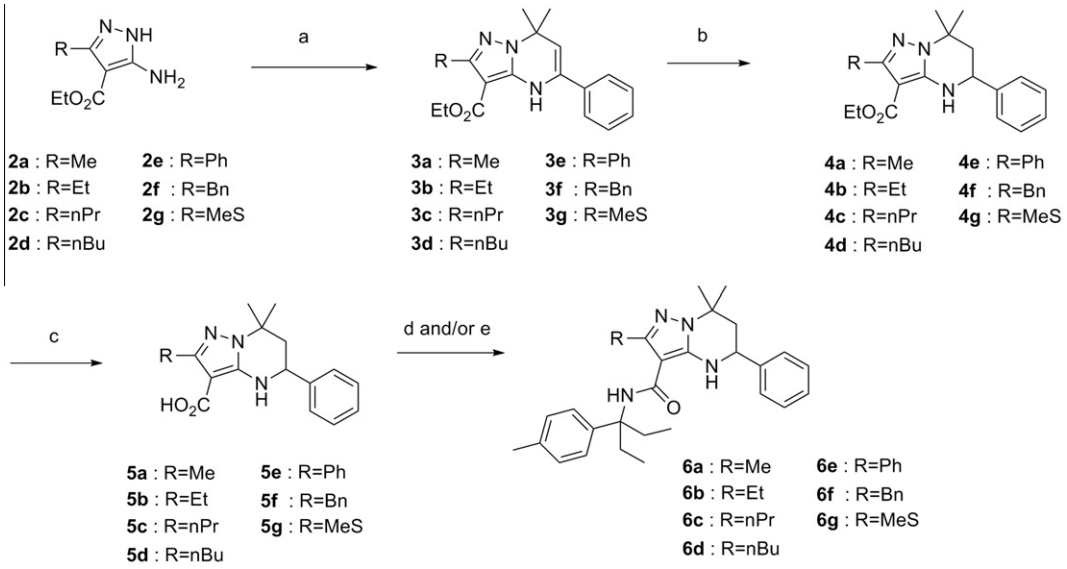
0968-0896/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2011.02.001



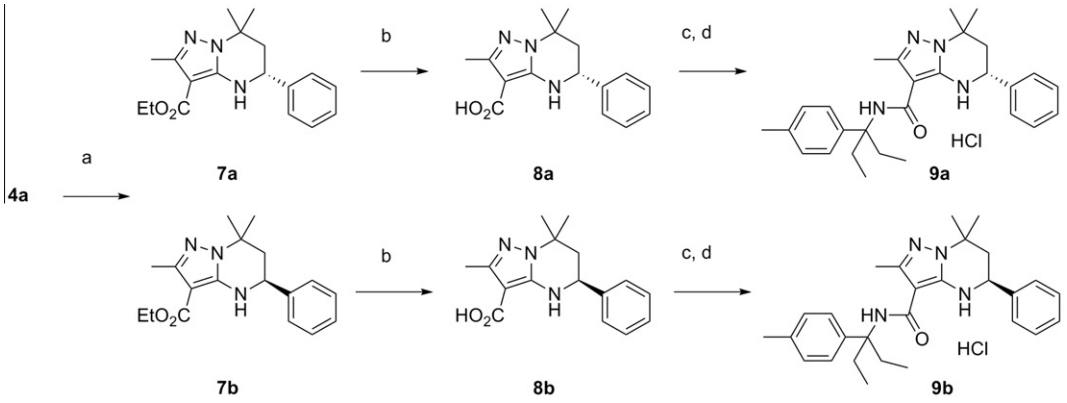
IC50 (nM)[a]	2.3 (0.86-6.4)
Residual ratio (%)[b]	52.1

[a] GTP binding assay,
95% Confidenceintervals are shown in parentheses.
[b] Residual ratio was mesured by HPLC.
Condition : 60℃, 75%RH, exposed to air for 1 week.

Figure 1. Calcilytic compound reported in previous literature.



Scheme 1. Reagents and conditions: (a) $Me_2C{=}CHCOPh$, CF_3CO_2H, 2-methoxyethanol, or $Me_2C{=}CHCOPh$, NaH, DMF; (b) $NaBH_4$, EtOH or $NaBH_4$, THF–MeOH or 10% Pd–C, H_2, THF–MeOH; (c) KOH, H_2O–EtOH; (d) 3-(4-methylphenyl)pentan-3-amine hydrochloride, HATU, iPr_2NEt, DMF or $(COCl)_2$, DMF, toluene then 3-(4-methylphenyl)pentan-3-amine hydrochloride, Et_3N, toluene; (e) 4 N HCl–EtOAc.



Scheme 2. Reagents and conditions: (a) CHIRALCEL OD; (b) KOH, H_2O, EtOH; (c) $SOCl_2$, DMF, toluene, then amine, Et_3N, toluene; (d) 4 N HCl–EtOAc.

liberated hydrochloride from compound **1** due to the weak basicity of tetrahydropyrazolopyrimidine. Since the basicity of tetrahydropyrazolopyrimidine depends on the electron density of the nitrogen atom at 1-position, we considered that introducing an electron-donating substituent at the 2-position of the tetrahydropyrazolopyrimidine ring enhances basicity to form a stable salt. In this report, we describe further efforts to improve chemical stability and in vivo efficacy. Furthermore, the pharmacokinetic profile of active metabolites is also a significant factor for sufficient efficacy during repeated administrations.

2. Chemistry

The synthesis of 2-substituted 7,7-dimethyltetrahydropyrazolopyrimidine derivatives **6a–g** is shown in Scheme 1. Amino pyrazoles[19] **2a–g** were treated with 3-methyl-1-phenylbut-2-en-1-one to afford dihydropyrazolopyrimidines **3a–g**. In this ring formation step, both the acidic condition (trifluoroacetic acid) and basic condition (sodium hydride) were effective. Reduction of the pyrimidine ring with 10% palladium on carbon under a hydrogen atmosphere or sodium tetrahydridoborate ($NaBH_4$)

afforded tetrahydropyrazolopyrimidines **4a–g**, and subsequent hydrolysis afforded carboxylic acids **5a–g**. Finally, condensation reactions were performed using 2-(1*H*-7-azabenzotriazole-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophospate (HATU) as a coupling reagent or through the preparation of the acid chloride of compound **5** to synthesize amide derivatives **6a–g**.

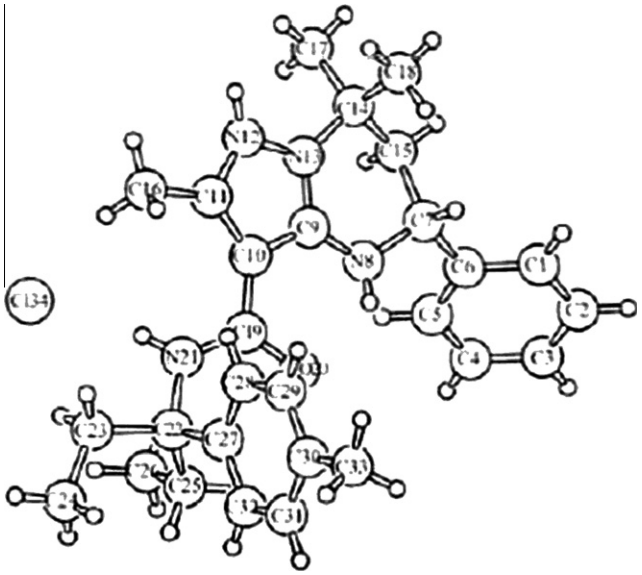
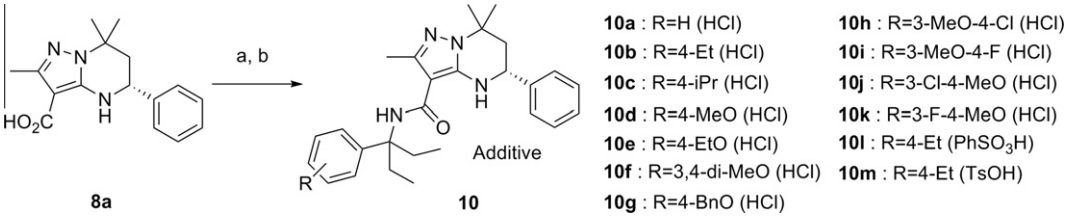


Figure 2. X-ray crystal structure of (*R*)-**9a**.

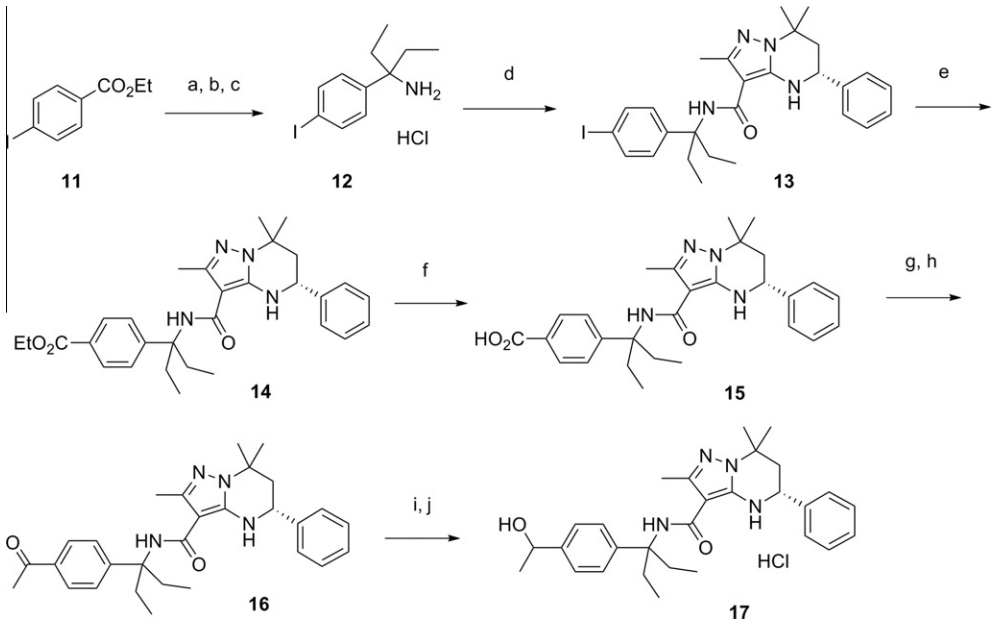
The synthesis of each enantiomer of compound **6a** was accomplished as outlined in Scheme 2. Racemic ethyl ester **4a**, prepared from **2a**, was optically resolved by preparative high performance liquid chromatography (HPLC) using a chiral column [CHIRALPAK OD, hexane/ethanol = 95:5] to afford both enantiomers, **7a** and **7b**, with high enantiomeric purity (>99% ee). These enantiomers were converted to carboxylic acids **8a** and **8b**, respectively, by alkaline hydrolysis with potassium hydroxide (KOH). Finally, carboxylic acids **8a** and **8b** were treated with 3-(4-methylphenyl)pentan-3-amine to afford amide derivatives and were subsequently transformed to hydrochloric acid salts **9a** and **9b**, respectively. The absolute configuration of **9a** was confirmed with X-ray analysis, and its X-ray crystal structure is shown in Figure 2. The absolute configurations of **7a** and **7b** were confirmed to be (*R*)- and (*S*)-forms, respectively.[20]

Furthermore, some optically active alkyl, alkoxy, and thioalkoxy substituted analogues **10a–k** were prepared as shown in Scheme 3. Carboxylic acid **8a**, prepared in Scheme 2, was treated with appropriate amines under the same conditions as described for the synthesis of **6** in Scheme 1. Additionally, in the case of ethyl analogue **10b**, benzenesulfuric acid salt **10l** and *p*-toluenesulfuric acid salt **10m** were prepared to find suitable salt forms.

Active metabolites **15–17** of compound **10m** were synthesized as shown in Scheme 4. Ethyl 4-iodobenzoate **11** was treated with ethyl magnesium bromide to afford 3-(4-iodophenyl)pentan-3-ol. This alcohol, without purification, could undergo a Ritter reaction[21]

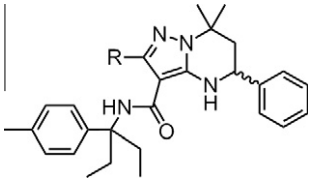


Scheme 3. Reagents and conditions: (a) amine, HATU, iPr$_2$NEt, DMF or SOCl$_2$ DMF, toluene then amine, Et$_3$N, toluene; (b) acid.



Scheme 4. Reagents and conditions: (a) EtMgBr, Et$_2$O; (b) TMSCN, concd H$_2$SO$_4$; (c) 6 N HCl and then 4 N HCl–EtOAc; (d) **8a**, SOCl$_2$ DMF, Et$_3$N, toluene; (e) dppf, Pd (OAc)$_2$, Et$_3$N, CO, EtOH; (f) 1 N NaOH, EtOH; (g) MeONHMe hydrochloride, WSC, HOBt, Et$_3$N, DMF; (h) MeMgBr, THF; (i) NaBH$_4$, EtOH; (j) 4 N HCl–EtOAc.

Table 1
CaSR antagonistic activity, metabolic stability, and solubility data of 2-substituted tetrahydropyrazolopyrimidine



Compd	R	Additive	Form	IC_{50}[a] (nM)	Metabolic stability[b]		Solubility[c]		
					Rat	Human	pH 1.2	pH 6.8	pH 6.8+bile acid
6a	Me	HC1	Racemic	4.1 (2.0–8.4)	213	118	24	0.19	68
6b	Et	HC1	Racemic	6.7 (3.3–14)	191	153	7.8	<0.11	>94
6c	nPr	HC1	Racemic	6.0 (2.9–13)	159	157	3.1	<0.12	>98
6d	*n*Bu	HC1	Racemic	11 (4.7–25)	136	140	0.45	<0.12	>97
6e	Ph	—	Racemic	81 (43–151)	ND	ND	ND	ND	ND
6f	Bn	HC1	Racemic	45 (14–145)	ND	ND	ND	ND	ND
6g	MeS	HC1	Racemic	4.7 (1.4–16)	154	89	<0.11	<0.11	>99
9a	Me	HC1	R-	2.4 (1.1–5.2)	216	124	30	0.38	72
9b	Me	HC1	S-	31 (9.0–106)	ND	ND	ND	ND	ND
1	H	HC1	R-	2.3 (0.86–6.4)	144	99	5.4	0.78	44

ND: no data.
[a] 95% confidence intervals are shown in parentheses.
[b] (μL/min/mg).
[c] (μg/mL).

in the presence of trimethylsilyl cyanide (TMSCN) and sulfuric acid (H_2SO_4) to afford *N*-[1-ethyl-1-(4-methylphenyl)propyl]formamide. The formamide obtained was hydrolyzed with 6 N hydrochloric acid under reflux conditions to provide amine **12**. Carboxylic acid **8a** was treated with thionyl chloride and DMF (cat.) in toluene to form an acyl chloride, which was coupled with amine **12** to afford the corresponding amide **13**. An ester moiety was introduced to the iodo analogue **13** using 1,1′-bis(diphenylphosphino)ferrocene (dppf) and palladium (II) acetate under a carbon monoxide atmosphere. Carboxylic acid **15**, which was the primary metabolite of compound **9a**, was prepared by hydrolysis of **14**, using sodium hydroxide in ethanol. On the other hand, acetyl analogue **16**, which was a metabolite of **10m**, was prepared from carboxylic acid **15** via the Weinreb amide. Finally, hydroxyl analogue **17** was prepared by the reduction of **16** with $NaBH_4$.

3. Results and discussion

The compounds synthesized were evaluated for antagonist activity using a GTP-binding assay, for which membrane fractions were prepared from CaSR-expressing CHO cells. The results are summarized in Tables 1 and 3.

The results obtained for 2-substituted derivatives with regard to in vitro CaSR antagonistic activity, metabolic stability, and solubility are listed in Table 1. Introduction of small alkyl substituents such as methyl **6a**, ethyl **6b**, and *n*-propyl **6c** were tolerated, although compounds with bulky substituents, for example, phenyl group **6e** and benzyl group **6f**, had weak activity. Furthermore, methylthio analogue **6g** also exhibited high potency with an IC_{50} value of 4.4 nM. However, methyl analogue **6a** and ethyl analogue **6b** had more rapid metabolizing profiles compared with compound **1**, while the compounds with other alkyl substituents (**6c** and **6d**) and a methylthio group (**6g**) exhibited similar metabolic stability. Interestingly, the solubility of compound **6a**, with a methyl group at the 2-position, was dramatically improved, especially under the pH 1.2 condition (**6a**: 24 μg/mL, **1**: 5.4 μg/mL).

In our program, improved solubility was reflected as rapid PTH secretion in the in vivo test; therefore, we focused our attention on the activities of the two enantiomers of **6a** with improved solubility, (*R*)-form **9a** and (*S*)-form **9b**, in order to investigate the stereo-

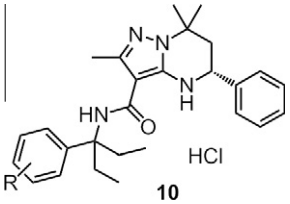
Table 2
Pharmacokinetic parameters for **9a** and **15** in cynomolgus monkey plasma after oral administration of compound **9a**

(a) Pharmacokinetic parameters[a,b]	**9a**	**15**
C_{max} (ng/mL)	20.1	266.8
T_{max} (h)	1.33	4.33
$AUC_{0-24\ h}$ (ng h/mL)	162.9	3680.9
MRT[c] (h)	5.17	10.82

(b) Pharmacokinetic parameters[d,b]	**9a**		
	1st	7th	14th
$C_{1\ h}$ (ng/mL)	32.5	28.2	24.1

[a] Dose: 2.5 mg/kg, po.
[b] Mean (n = 3).
[c] Mean residence time.
[d] Dose: 5 mg/kg, po.

Table 3
CaSR antagonistic activity and metabolic stability data of 2-methyl tetrahydropyrazolopyrimidine



10

Compd	R	IC_{50}[a] (nM)	Metabolic stability[b]	
			Rat	Human
10a	H	2.3 (1.0–5.3)	231	183
10b	4-Et	4.2 (1.2–15)	170	90
10c	4-iPr	1.7 (0.94–3.1)	96	96
10d	4-MeO	2.0 (0.88–4.6)	239	90
10e	4-EtO	3.0 (1.7–5.2)	132	84
10f	3,4-Di-MeO	11 (5.3–22)	180	105
10g	4-BnO	2.2 (1.3–3.6)	48	90
10h	3-MeO-4-Cl	6.1 (4.0–9.1)	189	153
10i	3-MeO-4-F	11 (6.3–19)	218	170
10j	3-Cl-4-MeO	1.7 (0.55–5.5)	136	38
10k	3-F-4-MeO	4.6 (1.9–11)	210	82

[a] 95% confidence intervals are shown in parentheses.
[b] (μL/min/mg).

chemical requirement for inhibitory activities. As expected, a significant difference was observed between the enantiomers, and (*R*)-form **9a** (IC_{50} = 2.4 nM) exhibited approximately 10-fold more potent activity than (*S*)-form **9b** (IC_{50} = 31 nM), as shown in Table 1. To evaluate the in vivo potency of compound **9a** as a CaSR antagonist, compound **9a**, which had identical in vitro activity, more rapid and transient metabolic profile, and superior solubility than compound **1**, was orally administrated to normal rats. Figure 3 shows the effects of PTH secretion after oral administration of 5 mg/kg **1** and oral administration of 2.5 mg/kg **9a** in normal rats. The plasma PTH level increased to 120 pg/mL 30 min after compound **9a** was administered and expeditiously dropped to normal levels after 2 h. The PTH secretion pattern stimulated by compound **9a** was sharp, similar to that stimulated by compound **1**. Judging from the clinical data of teriparatide (PTH 1–34)[9] and our previous data with an OVX rat model,[18] the PTH secretion pattern stimulated by compound **9a** (magnitude and duration) suggests that compound **9a** is effective as a bone anabolic agent for bone formation.

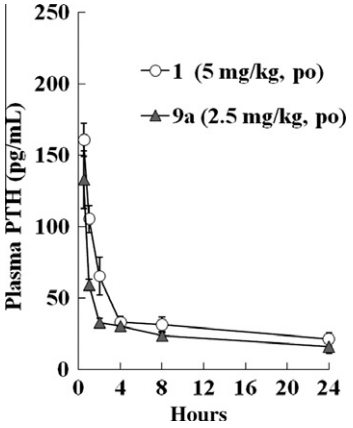


Figure 3. Effects of CaSR antagonists on PTH secretion in normal rats (n = 4).

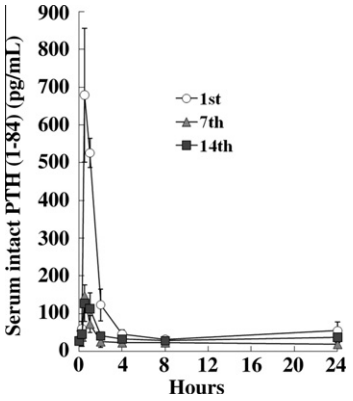
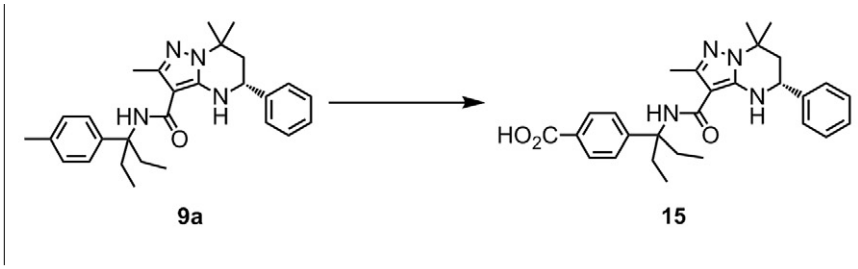


Figure 4. Effects of compound **9a** on PTH secretion in cynomolgus monkeys (n = 3, 5 mg/kg, po, qd).

Next, to investigate the effects of our calcilytic on other species, we attempted to evaluate PTH secretion in cynomolgus monkeys after they were orally administered compound **9a**. As illustrated in Figure 4, repeated administration of compound **9a** (5 mg/kg) for 1 and 2 weeks dramatically reduced PTH secretion, although initial administration of compound **9a** stimulated an ideal PTH secretion pattern, as observed in rats. We speculated that this disappearance of PTH secretion in cynomolgus monkeys was due to an active metabolite of compound **9a**. After identifying the metabolite by comparison with the synthesized molecule, carboxylic acid **15**, with an IC_{50} value of 320 nM, was identified as the primary metabolite of compound **9a** (Fig. 5). Pharmacokinetic parameters for **9a** and **15** in cynomolgus plasma after oral administration of 2.5 mg/kg **9a** are shown in Table 2(a). The C_{max} (266 ng/mL) and AUC_{0-24} (3680.9 ng th/mL) of compound **15** were much higher than those of compound **9a**. Furthermore, the MRT of carboxylic acid **15** was 10.82 h, which is greater than that of compound **9a** (MRT: 5.17 h). We also evaluated the plasma concentration of **9a** after the 1st, 7th, and 14th repeated treatment in cynomolgus (Table 2(b)). In this study, significant reduction of serum concentration of compound **9a** was not observed in contrast to the huge reduction of PTH. These findings indicated the increase levels of carboxylic acid **15** after repeated administration lead to continuous antagonism of CaSR, which might cause downregulation of the receptor and diminish the PTH response.

These findings indicated that further structural modifications are required to avoid the production of carboxylic acid metabolite 15, and hence, compounds with alkyl and alkoxy substituents, **10a–k**, were prepared. The CaSR antagonistic activity and



Compd.	IC_{50}(nM)[a]	
9a	2.4	(1.1-5.2)
15	320	(210-470)
10m	0.94	(0.62-1.4)
16	4.8	(3.0-7.7)
17	13	(6.4-24)

[a] 95% Confidenceintervals are shown in parentheses.

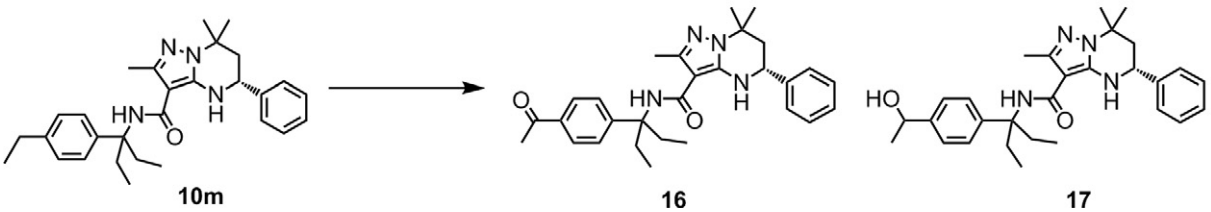


Figure 5. Primary metabolite of compound **9a** and **10m** in rats and monkeys.

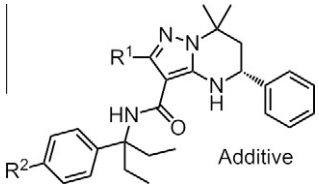
metabolic stability of **10a–k** are shown in Table 3. In the case of alkyl derivatives, 4-*i*-propyl analogue **10c** showed high potency, but its metabolic stability in rats and humans was better than that of **9a**. On the other hand, among the alkoxy derivatives, 3,4-dimethoxy analogue **10f** and 3-methoxy-4-fluoro analogue **10i** showed decreased activity and benzyloxy analogue **10g** (IC_{50} = 2.2 nM) exhibited a stable metabolic profile, contrary to the aim of transient PTH secretion.

Table 4
Effects of CaSR antagonists on PTH secretion in normal rats

Compd	Plasma PTH concentration[a,b,c] (pg/mL)			
	0.5 h	l h	2 h	4 h
10b	94.5 ± 32.2	85.7 ± 30.2	42.0 ± 9.8	26.8 ± 2.1
10e	59.3 ± 4.6	57.0 ± 5.4	24.4 ± 5.8	33.6 ± 0.9
10h	19.1 ± 2.4	19.9 ± 2.0	13.7 ± 0.5	13.3 ± 0.4
10k	21.6 ± 6.0	23.4 ± 2.0	16.8 ± 3.4	12.1 ± 1.1

[a] Mean ± SD (n = 4).
[b] Dose: 2.5 mg/kg, po.
[c] SD rat, ♀, 12 weeks.

Table 5
Chemical stability of tetrahydropyrazolopyrimidine



Compd	R^1	R^2	Additive	Residual ratio[a] (%)
1	H	Me	HC1	52.1
9a	Me	Me	HC1	101.7
10b	Me	Et	HC1	85.5
10l	Me	Et	$PhSO_3H$	98.9
10m	Me	Et	TsOH	99.1

Compounds **1** and **9a**: exposed to air for 1 week.
Compounds **10b**, **10l**, and **10m**: exposed to air for 2 weeks.
[a] Residual ratio was measured by HPLC. Condition: 60 °C, 75% RH, air open.

Table 6
The effects of **10m** (TAK-075) on PTH secretion in cynomolgus monkeys

	Serum intact PTH (l-84) (pg/mL)[a,b]		
	0 h	0.5 h	8 h
1st	81.2 ± 33.5	260.5 ± 112.4	51.6 ± 25.6
28th	42.2 ± 3.4	236.6 ± 181.8	36.2 ± 10.4

[a] Mean ± SD (*n* = 3).
[b] Dose: 10 mg/kg, po, qd.

Therefore, we tested several compounds with potent CaSR antagonistic activity and adequate metabolic stability for their efficacies with regard to in vivo PTH secretion in rats. As shown in Table 4, ethyl analogue **10b** (2.5 mg/kg, po) stimulated an increase in the plasma PTH level to 94.5 pg/mL after 0.5 h, and this level dropped to normal levels after 4 h—a pattern similar to that observed with compound **1** after it was orally administered at a dose of 5 mg/kg.

Therefore, we selected **10b** as the compound for further evaluation and prepared several salt analogues of **10b**. The chemical stability of related analogues is listed in Table 5. Chemical stabilities of all 2-methyl substituted analogues (**9a**, **10b**, **10l**, and **10m**) were improved compared with compound **1**. This result indicated that introduction of a methyl group at the 2-position of the tetrahydropyrazolopyrimidine ring increased the basicity and improved the chemical stability of the hydrochloric salt. Among the ethyl analogues **10b**, **10l**, and **10m**, *p*-toluenesulfonic acid salt **10m** showed the highest stability. Improvement of stability for sulfonic acid derivative **10l** and **10m** was assumed that hydrochloric acid is more volatile and liberated than sulfonic acid.

The results of the in vivo PTH secretion induced by compound **10m** in cynomolgus monkeys are shown in Table 6. To confirm that a reduced PTH response was completely avoided, we evaluated the effects of oral administration of 10 mg/kg compound **10m**. As expected, after the 28th dose of compound **10m**, the PTH response was maintained. The pharmacokinetic parameters for **10m** and primary active metabolites **16** and **17** in rats and cynomolgus monkeys after oral administration are summarized in Table 7. The MRT values of compound **10m** (rat, 3.44 and monkey, 5.99 h), **16** (rat, 5.47 and monkey, 6.36 h), and **17** (rat, 4.82 and monkey, 6.16 h) were shorter than that of carboxylic acid metabolite **15** (rat, 10.82). It was assumed that the absence of carboxylic acid type metabolite **15** allowed repeated treatment in cynomolgus monkeys.

4. Conclusion

A novel structural class of tetrahydropyrazolopyrimidines was optimized as CaSR antagonists and orally active calcilytic agents. For this series of compounds, it was shown that solubility and adequate metabolic stability effectively achieved transient PTH secretion. The introduction of a methyl group at the 2-position of compound **1** improved solubility, chemical stability, and in vivo efficacy. In cynomolgus monkeys with repeated treatment, a significant reduction of PTH secretion which might be caused accumulation of active metabolite was observed. To overcome the accumulation of active metabolite, the phenyl ring at the 3-position was subjected to further chemical modification. Our results revealed that compound **10m**, which possesses a 4-ethyl substituent on the phenyl ring, stimulates PTH secretion in cynomolgus monkeys without significant changes with repeated treatment.

Table 7
Pharmacokinetic parameters[a] for compound **10m**, **16**, and **17** in rat and cynomolgus monkey plasma after oral administration of **10m**

Pharmacokinetic parameters	Rat[b] (dose[d]: 2.5 mg/kg)			Cynomolgus[c] (dose[d]: 5.0 mg/kg)		
	10m	**16**	**17**	**10m**	**16**	**17**
C_{max} (ng/mL)	85.7 ± 17.7	128.2 ± 3.9	116.6 ± 32.9	30.8 ± 17.0	20.3 ± 5.2	20.4 ± 7.0
T_{max} (h)	0.50 ± 0.00	1.33 ± 0.58	0.67 ± 0.29	1.00 ± 0.00	2.33 ± 1.53	3.33 ± 1.15
$AUC_{0-24\ h}$ (ng h/mL)	247.3 ± 63.0	878.8 ± 41.0	805.0 ± 217.4	352.3 ± 174.4	228.5 ± 74.1	241.2 ± 96.0
MRT[e] (h)	3.44 ± 0.26	5.47 ± 0.43	4.82 ± 0.22	5.99 ± 0.47	6.36 ± 0.35	6.16 ± 0.54

[a] Mean ± SD (*n* = 3).
[b] SD, ♀, 12 weeks, non-fasted.
[c] ♂, fed.
[d] Dose: as free base.
[e] Mean residence time.

Compound **10m** also exhibited in vivo efficacy in the OVX rat model of bone loss (manuscript in preparation). On the basis of its excellent in vitro and in vivo efficacy and favorable pharmacokinetic profile, compound **10m** (TAK-075)[22] was selected as a candidate for clinical investigation.

5. Experimental

Melting points were determined on a Yanagimoto micromelting point apparatus or BÜCHI B-545 and uncorrected. ^{1}H NMR spectra of deuteriochloroform ($CDCl_3$) or dimethyl sulfoxide (DMSO-d_6) solution (internal standard tetramethylsilane (TMS), δ 0) were recorded on a Varian Gemini-200, Mercury-300 or Bruker AVANCE-300. Reactions were followed by TLC on Silica Gel 60 F 254 precoated TLC plates (E. Merck) or NH TLC plates (Fuji Silysia Chemical Ltd). Column chromatography was performed with WAKO gel 300 using the indicated eluents. Elemental analysis (C, H, N) were carried out by the Analytical Department of Takeda Chemical Industries.

5.1. 2,7,7-Trimethyl-5-phenyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]-pyrimidine-3-carboxylic acid (5a)

To a solution of **2a** (7.5 g, 44.3 mmol) in DMF (70 mL) was added NaH (1.77 g 60% in oil, 44.3 mmol) at room temperature. After stirring at the same temperature for 30 min, 3-methyl-1-phenylbut-2-en-1-one (5.9 g, 36.8 mmol) was added to the reaction mixture. After stirring at the same temperature for 3 h, the reaction mixture was diluted with EtOH (70 mL) and $NaBH_4$ (6.7 g, 177.1 mol) was added thereto at 0 °C. After stirring at room temperature overnight, the solvent was removed in vacuo. The residue was diluted with EtOAc, washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on SiO_2 with EtOAc–hexane (1:4) to give crystals. A mixture of the crystals and KOH (9.9 g, 176.4 mmol) in EtOH–H_2O (1:1, 300 mL) was stirred at 90 °C overnight, and the solvent was removed in vacuo. The residue was diluted with EtOAc and acidified with 1 N HCl. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo to give **5a** as crystals (4.33 g, 41%), mp 171–172 °C; ^{1}H NMR ($CDCl_3$): δ 1.55 (3H, s), 1.61 (3H, s), 2.05–2.12 (2H, m), 2.37 (3H, s), 4.61 (1H, dd, J = 10.2, 4.5 Hz), 6.09 (1H, s), 7.34–7.41 (5H, m). Anal. Calcd for $C_{16}H_{19}N_3O_2$: C, 67.35; H, 6.71; N, 14.73. Found: C, 67.19; H, 6.74; N, 14.84.

5.2. 2-Ethyl-7,7-dimethyl-5-phenyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid (5b)

A mixture of **2b** (8.0 g, 43.7 mmol), 3-methyl-1-phenylbut-2-en-1-one (7.0 g, 43.7 mmol) and TFA (9.97 g, 87.4 mmol) in 2-methoxyethanol (100 mL) was refluxed overnight. The solvent was concentrated in vacuo. The residue was diluted with EtOAc, washed with aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on SiO_2 with EtOAc–hexane (1:3) to give oil (6.9 g). A mixture of the oil obtained and 10% Pd–C (2.0 g) in THF–MeOH (1:1, 200 mL) was stirred under H_2 atmosphere for 4 h. The inorganic material was filtered off, and the solvent was removed in vacuo to give oil. A mixture of the oil and sodium hydroxide (16.0 g, 0.4 mol) in EtOH–H_2O (1:1, 200 mL) was stirred at 90 °C overnight. The solvent was removed in vacuo. The residue was diluted with EtOAc and acidified with 1 N HCl. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo to give **5b** as crystals (3.42 g, 26%), mp 173–174 °C; ^{1}H NMR ($CDCl_3$): δ 1.23 (3H, t, J = 7.5 Hz), 1.55 (3H, s), 1.61 (3H, s), 2.04–2.11 (2H, m), 2.77 (2H, q, J = 7.5 Hz), 4.60 (1H, dd, J = 10.5, 4.8 Hz), 6.11 (1H, s), 7.33–7.43 (5H, m). Anal. Calcd for $C_{17}H_{21}N_3O_2$: C, 68.20; H, 7.07; N, 14.04. Found: C, 68.08; H, 7.14; N, 14.07.

5.3. Ethyl 7,7-dimethyl-5-phenyl-2-propyl-4,7-dihydropyrazolo[1,5-*a*]pyrimidine-3-carboxylate (3c)

A solution of **2c** (2.62 g, 13.3 mmol) in DMF (10 mL) was added dropwise to a suspension of NaH (1.06 g, 60% in oil, 26.5 mmol) in DMF (17.5 mL) on an ice-bath, and the mixture was stirred at 0 °C for 30 min. 3-Methyl-1-phenylbut-2-en-1-one (2.55 g, 15.9 mmol) was added dropwise to the mixture at 0 °C, and the mixture was stirred at room temperature for 16 h. The mixture was diluted with EtOAc, washed successively with saturated citric acid aqueous solution, H_2O and brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by Purif-Pack (SI 60 μm, SIZE 60) to give **3c** as an oil (1.89 g, 42%). ^{1}H NMR ($CDCl_3$): δ 0.98 (3H, t, J = 7.2 Hz), 1.37 (3H, t, J = 7.2 Hz), 1.62–1.78 (2H, m), 1.70 (6H, s), 2.76 (2H, t, J = 7.5 Hz), 4.29 (2H, q, J = 7.2 Hz), 4.91 (1H, d, J = 2.1 Hz), 7.36–7.45 (3H, m), 7.46–7.54 (2H, m), 7.64 (1H, br s).

5.4. Ethyl 7,7-dimethyl-5-phenyl-2-propyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidine-3-carboxylate (4c)

$NaBH_4$ (468 mg, 12.4 mmol) was added to a solution of **3c** (1.89 g, 5.57 mmol) in EtOH (47 mL) in ice-bath, and the mixture was stirred at room temperature for 19 h, and concentrated in vacuo. The residue was diluted with EtOAc, washed successively with saturated citric acid aqueous solution, H_2O and brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by Purif-Pack (SI 60 um, SIZE 60) to give **4c** as an oil (1.57 g, 83%). ^{1}H NMR ($CDCl_3$): δ 0.97 (3H, t, J = 7 .0 Hz), 1.31 (3H, t, J = 7.0 Hz), 1.58 (6H, d, J = 11.0 Hz), 1.50–1.80 (2H, m), 2.00–2.14 (2H, m), 2.72 (2H, t, J = 5.8 Hz), 4.23 (2H, q, J = 7.2 Hz), 4.61 (1H, dd, J = 9.6, 5.2 Hz), 6.15 (1H, br s), 7.30–7.45 (5H, m).

5.5. 7,7-Dimethyl-5-phenyl-2-propyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid (5c)

A mixture of **4c** (1.57 g, 4.60 mmol) and KOH (911 mg, 16.2 mmol) in EtOH–H_2O (1:1, 32 mL) was stirred at 80 °C for 23 h. and then at 100 °C for 3 days. After cooling, the mixture was diluted with saturated citric acid aqueous solution and extracted with EtOAc. The extract was washed successively with H_2O and brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by Purif-Pack (SI 60 μm, SIZE 60) to give **5c** as colorless crystals (849 mg, 59%). Recrystallization from AcOEt–Et_2O gave colorless crystals, mp 156–158 °C. ^{1}H NMR ($CDCl_3$): δ 0.96 (3H, t, J = 7.2 Hz), 1.58 (6H, d, J = 12.0 Hz), 1.50–1.80 (2H, m), 1.95–2.22 (2H, m), 2.73 (2H, dt, J = 7.4, 2.8 Hz), 4.60 (1H, dd, J = 10.6, 4.8 Hz), 6.13 (1H, br s), 7.30–7.48 (5H, m). Anal. Calcd for $C_{18}H_{25}N_3O_2$: C, 68.98; H, 7.40; N, 13.41. Found: C, 68.94; H, 7.46; N, 13.21.

5.6. Ethyl 2-butyl-7,7-dimethyl-5-phenyl-4,7-dihydropyrazolo-[1,5-*a*]pyrimidine-3-carboxylate (3d)

A solution of **2d** (3.83 g, 18.1 mmol) in DMF (15 mL) was added dropwise to a suspension of NaH (1.45 g, 60% in oil, 36.3 mmol) in DMF (24.5 mL) in ice-bath, and the mixture was stirred at 0 °C for 30 min. 3-Methyl-1-phenylbut-2-en-1-one (3.59 g, 22.4 mmol) was added dropwise to the mixture at 0 °C, and the mixture was stirred at room temperature for 16 h. The mixture was diluted with EtOAc, washed successively with saturated citric acid aqueous solution, H_2O and brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by Purif-Pack (SI 60 μm, SIZE 60) to give **3d** as an oil (3.26 g, 49%). ^{1}H NMR ($CDCl_3$):

δ 0.94 (3H, t, J = 6.9 Hz), 1.36 (3H, t, J = 6.9 Hz), 1.32–1.48 (2H, m), 1.60–1.68 (2H, m), 1.70 (6H, s), 2.78 (2H, t, J = 8.1 Hz), 4.29 (2H, q, J = 7.2 Hz), 4.91 (1H, d, J = 2.1 Hz), 7.36–7.45 (3H, m), 7.47–7.54 (2H, m), 7.65 (1H, br s).

5.7. Ethyl 2-butyl-7,7-dimethyl-5-phenyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidine-3-carboxylate (4d)

$NaBH_4$ (775 mg, 20.5 mmol) was added to a solution of **3d** (3.26 g, 9.22 mmol) in EtOH (78 mL) in ice-bath. The mixture was stirred at room temperature for 22 h. and concentrated in vacuo. The residue was diluted with EtOAc, washed successively with saturated citric acid aqueous solution, H_2O and brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by Purif-Pack (SI 60 μm, SIZE 60) to give **4d** as an oil (2.41 g, 74%). ^1H NMR ($CDCl_3$): δ 0.94 (3H, t, J = 7.2 Hz), 1.31 (3H, t, J = 6.9 Hz), 1.36–1.48 (2H, m), 1.58 (6H, d, J = 16.8 Hz), 1.58–1.70 (2H, m), 2.02–2.18 (2H, m), 2.74 (2H, dt, J = 8.4, 3.0 Hz), 4.23 (2H, q, J = 6.3 Hz), 4.61 (1H, dd, J = 10.5, 4.5 Hz), 6.15 (1H, br s), 7.28–7.46 (5H, m).

5.8. 2-Butyl-7,7-dimethyl-5-phenyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid (5d)

A mixture of **4d** (2.41 g, 6.78 mmol) and KOH (1.34 g, 23.9 mmol) in EtOH–H_2O (1:1, 48 mL) was stirred at 80 °C for 17 h. and then at 100 °C for 3 days. After cooling, the mixture was diluted with saturated citric acid aqueous solution and extracted with EtOAc. The extract was washed successively with H_2O and brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by Purif-Pack (SI 60 μm, SIZE 60) to give **5d** as colorless crystals (611 mg, 28%). Recrystallization from AcOEt–Et_2O gave colorless crystals, mp 160–162 °C. ^1H NMR ($CDCl_3$): δ 0.912 (3H, t, J = 7.2 Hz), 1.32–1.45 (2H, m), 1.58 (6H, d, J = 18.0 Hz), 1.58–1.70 (2H, m), 2.00–2.18 (2H, m), 2.75 (2H, dt, J = 8.1, 3.9 Hz), 4.61 (1H, dd, J = 10.8, 4.2 Hz), 6.14 (1H, br s), 7.30–7.46 (5H, m). Anal. Calcd for $C_{19}H_{25}N_3O_2$: C, 69.70; H, 7.70; N, 12.83. Found: C, 69.69; H, 7.67; N, 12.84.

5.9. Ethyl 7,7-dimethyl-2,5-diphenyl-4,7-dihydropyrazolo[1,5-*a*]pyrimidine-3-carboxylate (3e)

A solution of **2e** (2.27 g, 9.82 mmol) in DMF (10 mL) was added dropwise to a suspension of NaH (785 mg, 60% in oil, 19.6 mmol) in DMF (20 mL) in ice-bath, and the mixture was stirred at 0 °C for 10 min. 3-Methyl-1-phenylbut-2-en-1-one (1.73 g, 10.8 mmol) was added dropwise to the mixture at 0 °C, and the mixture was stirred at room temperature for 21 h. The mixture was diluted with EtOAc, washed successively with saturated citric acid aqueous solution, H_2O and brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by Purif-Pack (SI 60 μm, SIZE 60) to give **3e** as an oil (550 mg, 15%). ^1H NMR ($CDCl_3$): δ 1.24 (3H, t, J = 7.2 Hz), 1.77 (6H, s), 4.23 (2H, q, J = 7.2 Hz), 4.98 (1H, d, J = 2.1 Hz), 7.26–7.48 (5H, m), 7.50–7.60 (3H, m), 7.68–7.74 (2H, m), 7.86 (1H, br s).

5.10. Ethyl 7,7-dimethyl-2,5-diphenyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidine-3-carboxylate (4e)

$NaBH_4$ (124 mg, 3.28 mmol) was added to a solution of **3e** (550 mg, 8.78 mmol) in EtOH (12 mL) in ice-bath. The mixture was stirred at room temperature for 24 h, and concentrated in vacuo. The residue was diluted with EtOAc, washed successively with saturated citric acid aqueous solution, H_2O and brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by Purif-Pack (SI 60 μm, SIZE 60) to give **4e** as colorless crystals (374 mg, 68%). Recrystallization from EtOAc–iPr_2O gave colorless crystals, mp 119–121 °C; ^1H NMR ($CDCl_3$): δ 1.16 (3H, t, J = 7.2 Hz), 1.65 (6H, d, J = 16.2 Hz), 2.08–2.24 (2H, m), 4.15 (2H, dq, J = 7.2, 0.9 Hz), 4.69 (1H, dd, J = 10.5, 4.5 Hz), 6.36 (1H, br s), 7.30–7.48 (8H, m), 7.68 (2H, dd, J = 8.1, 1.5 Hz).

5.11. 7,7-Dimethyl-2,5-diphenyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid (5e)

A mixture of **4e** (350 mg, 0.93 mmol) and KOH (164 mg, 2.92 mmol) in EtOH–H_2O (1:1, 7 mL) was stirred at 80 °C for 23 h. After cooling, the mixture was diluted with saturated citric acid aqueous solution and extracted with EtOAc. The extract was washed successively with H_2O and brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by Purif-Pack (SI 60 μm, SIZE 60) to give **5e** as colorless crystals (231 mg, 71%). Recrystallization from EtOAc–Et_2O gave colorless crystals, mp 172–174 °C; ^1H NMR ($CDCl_3$): δ 1.64 (6H, d, J = 16.5 Hz), 2.10–2.24 (2H, m), 4.67 (1H, dd, J = 10.2, 4.2 Hz), 6.36 (1H, br s), 7.30–7.50 (8H, m), 7.68 (2H, dd, J = 8.1, 1.8 Hz). Anal. Calcd for $C_{21}H_{21}N_3O_2 \cdot 1.0C_4H_8O_2$: C, 68.95; H, 6.70; N, 9.65. Found: C, 69.01; H, 6.75; N, 9.68.

5.12. Ethyl 2-benzyl-7,7-dimethyl-5-phenyl-4,7-dihydropyrazolo[1,5-*a*]pyrimidine-3-carboxylate (3f)

A solution of **2f** (4.9 g, 20.0 mmol) in DMF (10 mL) was added dropwise to a suspension of NaH (1.6 g, 60% in oil, 40.0 mmol) in DMF (40 mL) in ice-bath, and the mixture was stirred at 0 °C for 10 min. 3-Methyl-1-phenylbut-2-en-1-one (3.52 g, 22.0 mmol) was added dropwise to the mixture at 0 °C, and the mixture was stirred at room temperature for 21 h. The mixture was diluted with EtOAc, washed successively with saturated citric acid aqueous solution, H_2O and brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by Purif-Pack (SI 60 μm, SIZE 60) to give **3f** as an oil (1.93 g, 25%). ^1H NMR ($CDCl_3$): δ 1.25 (3H, t, J = 7.2 Hz), 1.74 (6H, s), 4.16 (2H, s), 4.20 (2H, q, J = 7.2 Hz), 4.92 (1H, d, J = 2.4 Hz), 7.12–7.34 (5H, m), 7.36–7.44 (3H, m), 7.46–7.52 (2H, m), 7.64 (1H, br s).

5.13. Ethyl 2-benzyl-7,7-dimethyl-5-phenyl-4,7-tetrahydropyrazolo [1,5-*a*]pyrimidine-3-carboxylate (4f)

$NaBH_4$ (419 mg, 11.1 mmol) was added to a solution of **3f** (1.95 g, 5.03 mmol) in EtOH (42 mL) in ice-bath. The mixture was stirred at room temperature for 23 h, and concentrated in vacuo. The residue was diluted with EtOAc, washed successively with saturated citric acid aqueous solution, H_2O and brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by Purif-Pack (SI 60 μm, SIZE 60) to give **4f** as an amorphous solid (1.47 g, 76%). ^1H NMR ($CDCl_3$): δ 1.18 (3H, t, J = 6.9 Hz), 1.62 (6H, d, J = 18.0 Hz), 2.02–2.20 (2H, m), 4.12 (2H, s), 4.13 (2H, q, J = 9.6 Hz), 4.61 (1H, dd, J = 10.2, 4.2 Hz), 6.14 (1H, br s), 7.10–7.44 (10H, m).

5.14. 2-Benzyl-7,7-dimethyl-5-phenyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid (5f)

A mixture of **4f** (1.18 g, 3.03 mmol) and KOH (729 mg, 13.0 mmol) in EtOH–H_2O (1:1, 60 mL) was stirred at 80 °C for 23 h. After cooling, the mixture was diluted with saturated citric acid aqueous solution and extracted with EtOAc. The extract was washed successively with H_2O and brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by Purif-Pack (SI 60 μm, SIZE 60) to give **5f** as colorless crystals (657 mg, 60%). Recrystallization from EtOAc–Et_2O gave colorless

crystals, mp 147–149 °C; ^{1}H NMR ($CDCl_3$): δ 1.60 (6H, d, J = 18.3 Hz), 2.00–2.18 (2H, m), 4.13 (2H, dd, J = 22.5, 14.4 Hz), 4.60 (1H, dd, J = 10.5, 4.5 Hz), 6.08 (1H, br s), 7.11–7.45 (10H, m). Anal. Calcd for $C_{22}H_{23}N_3O_2$: C, 73.11; H, 6.41; N, 11.63. Found: C, 73.19; H, 6.52; N, 11.50.

5.15. 7,7-Dimethyl-2-(methylthio)-5-phenyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid (5g)

A solution of **2g** (11.0 g, 54.7 mmol), 3-methyl-1-phenylbut-2-en-1-one (9.4 g, 58.7 mmol) and TFA (13.4 g, 117 mmol) in 2-methoxyethanol (100 mL) was refluxed overnight, and the solvent was concentrated in vacuo. The residue was diluted with EtOAc, washed with aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on SiO_2 with EtOAc–hexane (1:6) to give oil. $NaBH_4$ (2.55 g, 67.4 mmol) was added to a solution of the oil obtained in MeOH–THF (1:1, 200 mL) at 0 °C. After stirring at room temperature overnight, the solvent was concentrated in vacuo. The residue was diluted with EtOAc, washed with brine, dried over $MgSO_4$, and concentrated in vacuo to give oil. A mixture of the oil and KOH (16.0 g, 285 mmol) in EtOH–H_2O (1:1, 400 mL) was stirred at 90 °C overnight, and the solvent was concentrated in vacuo. The residue was diluted with EtOAc and acidified with 1 N HCl. The organic layer was washed brine, dried over $MgSO_4$, and concentrated in vacuo to give **5g** as crystals (4.95 g, 27%), mp 139–140 °C; ^{1}H NMR ($CDCl_3$): δ 1.55 (3H, s), 1.61 (3H, s), 2.07–2.15 (2H, m), 2.50 (3H, s), 4.57–4.64 (1H, m), 5.67 (1H, s), 6.07 (1H, s), 7.39 (5H, s). Anal. Calcd for $C_{16}H_{19}N_3O_2S$: C, 60.54; H, 6.03; N, 13.24. Found: C, 60.68; H, 6.17; N, 13.11.

5.16. *N*-[1-Ethyl-1-(4-methylphenyl)propyl]-2,7,7-trimethyl-5-phenyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidine-3-carboxamide hydrochloride (6a)

A mixture of **5a** (3.0 g, 10.5 mmol), HATU (4.80 g, 12.6 mmol) and *i*Pr_2NEt (4.08 g, 31.6 mmol) in DMF (50 mL) was stirred at room temperature for 1 h, and then 3-(4-methylphenyl)pentan-3-amine hydrochloride (2.70 g, 12.7 mmol) was added. The whole was stirred at 70 °C overnight, and concentrated in vacuo. The residue was diluted with EtOAc, washed with H_2O and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on SiO_2 with EtOAc–hexane (2:3) to give crystals. The crystals were dissolved in Et_2O and 4 M HCl in EtOAc was added. The crystals were collected by filtration and washed with Et_2O (2.58 g, 51%), mp 161–162 °C; ^{1}H NMR ($CDCl_3$): δ 0.75–0.88 (6H, m), 1.80 (3H, s), 1.95 (3H, s), 2.00–2.30 (6H, m), 2.31 (3H, s), 2.85 (3H, s), 4.56 (1H, br s), 5.66 (1H, br s), 7.17–7.34 (10H, m). Anal. Calcd for $C_{28}H_{37}ClN_4O$: C, 69.91; H, 7.75; N, 11.65. Found: C, 69.84; H, 7.78; N, 11.59.

5.17. 2-Ethyl-*N*-[1-ethyl-1-(4-methylphenyl)propyl]-7,7-dimethyl-5-phenyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]-pyrimidine-3-carboxamide hydrochloride (6b)

A mixture of **5b** (1.0 g, 3.34 mmol), HATU (1.52 g, 4.00 mmol) and *i*Pr_2NEt (1.30 g, 10.1 mmol) in DMF (30 mL) was stirred at room temperature for 1 h, and then, 3-(4-methylphenyl)pentan-3-amine hydrochloride (1.07 g, 5.03 mmol) was added. The whole was stirred at 70 °C overnight, and concentrated in vacuo. The residue was diluted with EtOAc, washed with H_2O and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on SiO_2 with EtOAc–hexane (2:3) to give crystals. The crystals were dissolved in Et_2O and 4 M HCl in EtOAc was added. The crystals were collected by filtration and washed with Et_2O (0.10 g, 6%), mp 182–183 °C; ^{1}H NMR ($CDCl_3$): δ 0.74–0.83 (6H, m), 1.55 (3H, t, J = 7.5 Hz), 1.81 (3H, s), 1.96 (3H, s), 2.00–2.26 (6H, m), 2.31 (3H, s), 3.14–3.32 (2H, m), 4.55 (1H, t, J = 7.5 Hz), 5.72 (1H, s), 7.13–7.20 (4H, m), 7.29–7.37 (6H, m). Anal. Calcd for $C_{29}H_{39}ClN_4O$: C, 70.35; H, 7.94; N, 11.32. Found: C, 69.95; H, 7.99; N, 11.21.

5.18. *N*-[1-Ethyl-1-(4-methylphenyl)propyl]-7,7-dimethyl-5-phenyl-2-propyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidine-3-carboxamide hydrochloride (6c)

Oxalyl chloride (0.17 mL, 2.01 mmol) and a drop of DMF were added dropwise to a solution of **5c** (393 mg, 1.25 mmol) in THF (6 mL), and the mixture was stirred at room temperature for 3 h and concentrated in vacuo. A solution of the residue in toluene (2 mL) was added dropwise to a mixture of 3-(4-methylphenyl)pentan-3-amine hydrochloride (268 mg, 1.26 mmol) and Et_3N (0.53 mL, 3.80 mmol) in toluene (4 mL) at 60 °C, and the mixture was stirred at 60 °C for 1 h. The mixture was diluted with EtOAc, washed successively with H_2O and brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by Purif-Pack (SI 60 μm, SIZE 60) to give colorless crystals (218 mg, 37%). 4 N HCl in EtOAc (0.099 ml, 0.40 mmol) was added dropwise to a solution of the crystals obtained (170 mg, 0.36 mmol) in EtOAc (2 mL) at 0 °C. The mixture was stirred at 0 °C for 1 h. and concentrated in vacuo to give **6c** (121 mg, 66%) as colorless crystals, mp 102–104 °C; ^{1}H NMR ($CDCl_3$): δ 0.78 (6H, dt, J = 17.0, 7.4 Hz), 1.15 (3H, t, J = 7.3 Hz), 1.81 (3H, s) 1.95 (3H, s), 1.98–2.26 (9H, m), 2.31 (3H, s), 3.04–3.29 (2H, m), 4.56 (1H, t, J = 7.3 Hz), 5.70 (1H, s), 7.15 (2H, d, J = 8.0 Hz), 7.18 (2H, d, J = 8.4 Hz), 7.29–7.33 (2H, m), 7.33–7.40 (3H, m). Anal. Calcd for $C_{30}H_{41}ClN_4O{\cdot}0.5C_4H_8O_2$: C, 69.48; H, 8.20; N, 10.13. Found: C, 69.48; H, 8.33; N, 10.25.

5.19. 2-Butyl-*N*-[1-ethyl-1-(4-methylphenyl)propyl]-7,7-dimethyl-5-phenyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]-pyrimidine-3-carboxamide hydrochloride (6d)

Oxalyl chloride (0.13 mL, 1.54 mmol) and a drop of DMF were added dropwise to a solution of **5d** (300 mg, 0.92 mmol) in THF (6 mL). The mixture was stirred at room temperature for 2 h and concentrated in vacuo. A solution of the residue in toluene (2 mL) was added dropwise to a mixture of 3-(4-methylphenyl)pentan-3-amine hydrochloride (195 mg, 0.92 mmol) and Et_3N (0.39 mL, 2.80 mmol) in toluene (4 mL) at 60 °C, and the mixture was stirred at 60 °C for 1.5 h. The mixture was diluted with EtOAc, washed successively with H_2O and brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by Purif-Pack (SI 60 μm, SIZE 60) to give as colorless crystals (148 mg, 33%). 4 N HCl in EtOAc (0.062 mL, 0.25 mmol) was added dropwise to a solution of the crystals obtained (110 mg, 0.23 mmol) in EtOAc (1 mL) at 0 °C, and the mixture was stirred at 0 °C for 1 h. and concentrated in vacuo to give **6d** as colorless crystals (80 mg, 68%). Recrystallization from EtOAc–Et_2O gave colorless crystals, mp 103–105 °C; ^{1}H NMR ($CDCl_3$): δ 0.78 (6H, dt, J = 18.1, 7.3 Hz), 1.00 (3H, t, J = 7.3 Hz), 1.49–1.60 (2H, m), 1.78 (3H, s), 1.82–1.91 (2H, m), 1.92 (3H, s), 1.95–2.26 (7H, m), 2.31 (3H, s), 3.03–3.29 (2H, m), 4.55 (1H, t, J = 6.4 Hz), 5.70 (1H, s), 7.14 (2H, d, J = 8.0 Hz), 7.19 (2H, d, J = 8.4 Hz), 7.29–7.39 (5H, m). Anal. Calcd for $C_{31}H_{43}ClN_4O{\cdot}0.9H_2O$: C, 69.03; H, 8.37; N, 10.39. Found: C, 69.11; H, 8.17; N, 10.18.

5.20. *N*-[1-Ethyl-1-(4-methylphenyl)propyl]-7,7-dimethyl-2,5-diphenyl-4,5,6,7-tetrahydopyrazolo[1,5-*a*]pyrimidine-3-carboxamide (6e)

Oxalyl chloride (0.079 mL, 0.93 mmol) and a drop of DMF were added dropwise to a solution of **5e** (200 mg, 0.58 mmol) in THF

(5 mL). The mixture was stirred at room temperature for 30 min, and concentrated in vacuo. A solution of the residue in toluene (2 mL) was added dropwise to a mixture of 3-(4-methylphenyl)pentan-3-amine hydrochloride (135 mg, 0.63 mmol) and Et_3N (0.24 mL, 1.72 mmol) in toluene (3 mL) at 60 °C, and the mixture was stirred at 60 °C for 1 h. The mixture was diluted with EtOAc, washed successively with H_2O and brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by Purif-Pack (SI 60 μm, SIZE 60) to give **6e** as colorless crystals (207 mg, 71%). Recrystallization from AcOEt–Et_2O gave colorless crystals, mp 204–206 °C. ^{1}H NMR ($CDCl_3$): δ 0.53 (6H, dt, J = 12.0, 7.2 Hz), 1.65 (6H, d, J = 15.0 Hz), 1.68–1.98 (4H, m), 2.02–2.12 (1H, m), 2.23 (1H, t, J = 12.0 Hz), 2.26 (3H, s), 4.61 (1H, dd, J = 11.1, 2.1 Hz), 5.44 (1H, br s), 6.74 (1H, br s), 6.93 (2H, d, J = 7.8 Hz), 7.01 (2H, d, J = 8.4 Hz), 7.24–7.36 (3H, m). 7.40 (2H, d, J = 6.9 Hz), 7.42–7.54 (3H, m), 7.64 (2H, d, J = 8.1 Hz). Anal. Calcd for $C_{33}H_{38}N_4O$: C, 78.23; H, 7.56; N, 11.06. Found: C, 78.14; H, 7.53; N, 10.94.

5.21. 2-Benzyl-*N*-[1-ethyl-1-(4-methylphenyl)propyl]-7,7-dimethyl-5-phenyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]-pyrimidine-3-carboxamide hydrochloride (6f)

Oxalyl chloride (0.095 mL, 1.12 mmol) and a drop of DMF were added dropwise to a solution of **5f** (250 mg, 0.69 mmol) in THF (5 mL). The mixture was stirred at room temperature for 30 min, and concentrated in vacuo. A solution of the residue in toluene (2 mL) was added dropwise to a mixture of 3-(4-methylphenyl)pentan-3-amine hydrochloride (163 mg, 0.77 mmol) and Et_3N (0.29 mL, 2.08 mmol) in toluene (3 mL) at 60 °C, and the mixture was stirred at 60 °C for 1 h. The mixture was diluted with EtOAc, washed successively with H_2O and brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by Purif-Pack (SI 60 μm, SIZE 60) to give colorless crystals (256 mg, 71%). 4 N HCl in EtOAc (0.11 mL, 0.44 mmol) was added dropwise to a solution of the crystals obtained (200 mg, 0.38 mmol) in EtOAc (2 mL) at 0 °C, and the mixture was stirred at 0 °C for 1 h. and concentrated in vacuo to give **6f** as colorless crystals (114 mg, 53%). Recrystallization from MeOH–EtOAc gave colorless crystals, mp 194–196 °C; ^{1}H NMR ($CDCl_3$): δ 0.47 (6H, dt, J = 7.5, 4.8 Hz), 1.40–1.80 (4H, m), 1.92 (6H, d, J = 44.0 Hz), 2.12–2.30 (2H, m), 2.26 (3H, s), 4.55 (1H, dd, J = 10.1, 5.1 Hz), 4.65 (2H, dd, J = 86.1, 16.8 Hz), 5.49 (1H, br s), 6.91 (2H, d, J = 8.4 Hz), 7.13 (2H, d, J = 7.8 Hz), 7.20–7.42 (10H, m). Anal. Calcd for $C_{34}H_{41}ClN_4O$: C, 73.29; H, 7.42; N, 10.06. Found: C, 73.04; H, 7.46; N, 10.13.

5.22. *N*-[1-Ethyl-1-(4-methylphenyl)propyl]-7,7-dimethyl-2-(methylthio)-5-phenyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]-pyrimidine-3-carboxamide hydrochloride (6g)

A mixture of **5g** (1.0 g, 3.15 mmol), HATU (1.44 g, 3.79 mmol), and *i*Pr_2NEt (1.22 g, 9.44 mmol) in DMF (20 mL) was stirred at room temperature for 1 h, and then, 3-(4-methylphenyl)pentan-3-amine hydrochloride (0.80 g, 3.76 mmol) was added thereto. The whole was stirred at 70 °C overnight, and concentrated in vacuo. The residue was diluted with EtOAc, washed with H_2O and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on SiO_2 with EtOAc–hexane (2:3) to give crystals. The crystals were dissolved in Et_2O and 4 N HCl in EtOAc was added thereto. The crystals were collected by filtration and recrystallization from EtOAc–hexane afforded **6g** as prisms (0.58 g, 36%), mp 106–107 °C; ^{1}H NMR ($CDCl_3$): δ 0.72–0.84 (6H, m), 1.85 (3H, s), 1.95 (3H, s), 2.00–2.22 (6H, m), 2.31 (3H, s), 3.04 (3H, s), 4.52–4.60 (1H, m), 7.10–7.36 (11H, m). Anal. Calcd for $C_{28}H_{37}ClN_4OS$: C, 65.54; H, 7.27; N, 10.92. Found: C, 65.47; H, 7.28; N, 10.92.

5.23. Ethyl (5*R*)-2,7,7-trimethyl-5-phenyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidine-3-carboxylate (7a) and Ethyl (5*S*)-2,7,7-trimethyl-5-phenyl-4,5,6,7-tetrahydropyrazolo-[1,5-*a*]-pyramiddine-3-carboxylate (7b)

Compound **4a** (43.2 g, 138 mmol) was separated by CHIRAL Column HPLC (CHIRALCEL OD 50 mm ID × 500 mml, hexane/EtOH 95:5, flow rate 60 mL/min, temperature 30 °C, detection(UV) 254 nm, one shot 600 mg) to give **7a** (20.7 g, retention time big: 99.9% ee) and **7b** (20.6 g, retention time small: 99.9% ee). ^{1}H NMR ($CDCl_3$): δ 1.31 (3H, t, J = 7.5 Hz), 1.55 (3H, s), 1.61 (3H, s), 2.05–2.12 (2H, m), 2.37 (3H, s), 4.23 (2H, q, J = 7.5 Hz), 4.62 (1H, dd, J = 9.9, 4.2 Hz), 6.10 (1H, s), 7.33–7.44 (5H, m).

5.24. (5*R*)-2,7,7-Trimethyl-5-phenyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid (8a)

A mixture of **7a** (11.8 g, 37.7 mmol) and KOH (20.7 g, 0.37 mol) in H_2O–EtOH (1:1, 200 mL) was stirred at 90 °C for 16 h, and then concentrated in vacuo. The residue was diluted with H_2O and adjusted at pH 4 with 1 N HCl to give **8a** as crystals (17.8 g, 91%). $[\alpha]_D^{21}$ = 103.65 (c 1.17, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 1.55 (3H, s), 1.61 (3H, s), 2.05–2.12 (2H, m), 2.37 (3H, s), 4.61 (1H, dd, J = 10.2, 4.5 Hz), 6.09 (1H, s), 7.34–7.41 (5H, m). Anal. Calcd for $C_{16}H_{19}N_3O_2$: C, 67.35; H, 6.71; N, 14.73. Found: C, 67.40; H, 6.83; N, 14.63.

5.25. (5*S*)-2,7,7-Trimethyl-5-phenyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid (8b)

A mixture of **7b** (7.0 g, 22.3 mmol) and KOH (5.0 g, 89.1 mmol) in H_2O–EtOH (1:1, 200 mL) was stirred at 90 °C overnight, and then concentrated in vacuo. The residue was diluted with EtOAc and acidified with 1 N HCl. The organic layer was washed brine, dried over $MgSO_4$, and concentrated in vacuo to give **8b** as crystals (5.0 g, 78%). $[\alpha]_D^{21}$ = −109.21 (c 1.25, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 1.55 (3H, s), 1.61 (3H, s), 2.05–2.12 (2H, m), 2.37 (3H, s), 4.61 (1H, dd, J = 10.2, 4.5 Hz), 6.09 (1H, s), 7.34–7.41 (5H, m). Anal. Calcd for $C_{16}H_{19}N_3O_2$: C, 67.35; H, 6.71; N, 14.73. Found: C, 67.19; H, 6.73; N, 14.51.

5.26. (5*R*)-*N*-[1-Ethyl-1-(4-methylphenyl)propyl]-2,7,7-trimethyl-5-phenyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]-pyrimidine-3-carboxamide hydrochloride (9a)

To a mixture of **8a** (19.8 g, 69.4 mmol) and DMF (0.5 mL) in toluene (200 mL) was added $SOCl_2$ (12.4 g, 104 mmol) at room temperature. After stirring at the same temperature for 1 h, the solvent was evaporated off. A mixture of 3-(4-methylphenyl)pentan-3-amine hydrochloride (17.1 g, 80.4 mmol) and Et_3N (20.2 g, 200 mmol) in toluene (200 mL) was stirred at 70 °C for 20 min, and then acid chloride obtained in toluene was added to the reaction mixture at 65 °C. After stirring at the same temperature for 1 h, the reaction mixture was poured into H_2O and extracted with EtOAc. The extract was washed with H_2O, 10% critic acid and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by Purif-Pack (SI 60 μm, SIZE 60) to give an oil. To a solution of the oil obtained in EtOAc was added 4 N HCl in EtOAc (30 mL, 0.12 mol) at 0 °C. The crystals were collected by filtration (26.7 g, 80%), mp 206–207 °C. $[\alpha]_D^{25}$ = −8.1 (c 0.52, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 0.73–0.82 (6H, m), 1.79 (3H, s), 1.94 (3H, s), 1.98–2.25 (6H, m), 2.31 (3H, s), 2.84 (3H, s), 4.55 (1H, t, J = 7.2 Hz), 5.65 (1H, s), 7.12–7.36 (10H, m). Anal. Calcd for $C_{28}H_{37}ClN_4O$: C, 69.91; H, 7.75; N, 11.65. Found: C, 69.66; H, 7.94; N, 11.70.

5.27. (5*S*)-*N*-[1-Ethyl-1-(4-methylphenyl)propyl]-2,7,7-trimethyl-5-phenyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidine-3-carboxamide hydrochloride (9b)

To a mixture of **8b** (0.5 g, 1.75 mmol) and DMF (one drop) in toluene (5 mL) was added $SOCl_2$ (0.42 g, 3.53 mmol) at room temperature. After stirring at the same temperature for 1 h, the solvent was evaporated off. A mixture of 3-(4-methylphenyl)pentan-3-amine hydrochloride (0.49 g, 2.30 mmol) and Et_3N (0.53 g, 5.24 mmol) in toluene (5 mL) was stirred at 70 °C for 1 h, and then acid chloride obtained in toluene was added to the reaction mixture at 70 °C. After stirring at the same temperature for 1 h, the reaction mixture was poured into H_2O and extracted with EtOAc. The extract was washed with H_2O, and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by Purif-Pack (SI 60 μm, SIZE 60) to give an oil. To a solution of the oil obtained in EtOAc was added 4 N HCl in EtOAc (0.5 mL, 2.00 mmol) at 0 °C. Crystallization from EtOAc–Et_2O afforded **9b** (0.56 g, 80%), mp 206–207 °C; ^{1}H NMR ($CDCl_3$): δ 0.73–0.83 (6H, m), 1.80 (3H, s), 1.95 (3H, s), 1.98–2.26 (6H, m), 2.31 (3H, s), 2.85 (3H, s), 4.55 (1H, t, J = 7.2 Hz), 5.69 (1H, s), 7.13–7.37 (10H, m). Anal. Calcd for $C_{28}H_{37}ClN_4O{\cdot}0.25H_2O$: C, 69.26; H, 7.78; N, 11.54. Found: C, 69.13; H, 7.69; N, 11.25.

5.28. (5*R*)-*N*-(1-Ethyl-1-phenylpropyl)-2,7,7-trimethyl-5-phenyl-4,5,6,7-tetrahydropyrazolo-[1,5-*a*]pyrimidine-3-carboxamide hydrochloride (10a)

To a mixture of **8a** (1.0 g, 3.50 mmol) and DMF (one drop) in toluene (10 mL) was added $SOCl_2$ (0.83 g, 7.00 mmol) at room temperature. After stirring at the same temperature for 1 h, the solvent was evaporated off. A mixture of 3-phenylpentan-3-amine hydrochloride (0.77 g, 3.87 mmol) and Et_3N (0.97 g, 9.59 mmol) in toluene (10 mL) was stirred at 70 °C for 1 h, and then the acid chloride obtained in toluene was added to the reaction mixture at 70 °C. After stirring at the same temperature for 3 h, the reaction mixture was poured into H_2O and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on SiO_2 with EtOAc–hexane (1:1) to give an amorphous solid. To a solution of the amorphous solid obtained in Et_2O was added 4 N HCl in EtOAc at 0 °C. The precipitated crystals were collected by filtration and washed with Et_2O (0.85 g, 52%), mp 185–186 °C; ^{1}H NMR ($CDCl_3$): δ 0.74–0.83 (6H, m), 1.80 (3H, s), 1.95 (3H, s), 2.00–2.25 (6H, m), 2.86 (3H, s), 4.53–4.58 (1H, m), 5.68 (1H, s), 7.24–7.36 (11H, m). Anal. Calcd for $C_{27}H_{35}ClN_4O$: C, 69.43; H, 7.55; N, 12.00. Found: C, 69.19; H, 7.82; N, 11.97.

5.29. (5*R*)-*N*-[1-Ethyl-1-(4-ethylphenyl)propyl]-2,7,7-trimethyl-5-phenyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidine-3-carboxamide hydrochloride (10b)

A mixture of **8a** (0.30 g, 1.05 mmol), HATU (0.52 g, 1.37 mmol) and *i*Pr_2NEt (0.47 g, 1.37 mmol) in DMF (5 mL) was stirred at room temperature for 1 h, and then, 3-(4-ethylphenyl)pentan-3-amine hydrochloride (0.31 g, 1.37 mmol) was added thereto. The whole was stirred at 70 °C overnight, and concentrated in vacuo. The residue was diluted with EtOAc, washed with H_2O and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on SiO_2 with EtOAc–hexane (1:1) to give an amorphous solid. The amorphous solid was dissolved in EtOAc and 4 N HCl in EtOAc was added thereto. Crystallization from EtOAc–hexane afforded **10b** as HCl salt (0.27 g, 52%), mp 163–164 °C; ^{1}H NMR ($CDCl_3$): δ 0.3–0.84 (6H, m), 1.22 (3H, t, J = 7.2 Hz), 1.80 (3H, s), 1.95 (3H, s), 2.05–2.20 (6H, m), 2.63 (2H, q, J = 7.2 Hz), 2.85 (3H, s), 4.56 (1H, t, J = 7.0 Hz), 5.68 (1H, s), 7.19–7.34 (10H, m). Anal. Calcd for $C_{28}H_{39}ClN_4O$: C, 70.35; H, 7.94; N, 11.32. Found: C, 70.36; H, 7.91; N, 11.32.

5.30. (5*R*)-*N*-[1-Ethyl-1-(4-isopropylphenyl)propyl]-2,7,7-trimethyl-5-phenyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidine-3-carboxamide hydrochloride (10c)

Compound **10c** was prepared in a manner similar to that described for **10a** as a solid. Yield: 45%. Mp: 134–136 °C (EtOH–Et_2O); ^{1}H NMR ($CDCl_3$): δ 0.72–0.84 (6H, m), 1.23 (6H, d, J = 7.0 Hz), 1.80 (3H, s), 1.95 (3H, s), 2.00–2.28 (6H, m), 2.82–2.94 (4H, m), 4.53–4.61 (1H, m), 5.66 (1H, s), 7.20 (4H, s), 7.25–7.40 (6H, m). Anal. Calcd for $C_{30}H_{41}ClN_4O{\cdot}0.1H_2O$: C, 70.52; H, 8.13; N, 10.97. Found: C, 70.27; H, 8.13; N, 10.95.

5.31. (5*R*)-*N*-[1-Ethyl-1-(4-methoxyphenyl)propyl]-2,7,7-trimethyl-5-phenyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidine-3-carboxamide hydrochloride (10d)

Compound **10d** was prepared in a manner similar to that described for **10a** as a solid. Yield: 51%. Mp: 158–160 °C (EtOAc–Et_2O); ^{1}H NMR ($CDCl_3$): δ 0.74–0.82 (6H, m), 1.80 (3H, s), 1.94 (3H, s), 1.97–2.26 (6H, m), 2.85 (3H, s), 3.78 (3H, s), 4.56 (1H, t, J = 6.9 Hz), 5.68 (1H, s), 6.87 (2H, d, J = 8.7 Hz), 7.21–7.39 (8H, m). Anal. Calcd for $C_{28}H_{37}ClN_4O_4$: C, 67.66; H, 7.50; N, 11.27. Found: C, 67.61; H, 7.64; N, 11.39.

5.32. (5*R*)-*N*-[1-(4-Ethoxyphenyl)-1-ethylpropyl]-2,7,7-trimethyl-5-phenyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidine-3-carboxamide hydrochloride (10e)

Compound **10e** was prepared in a manner similar to that described for **10a** as a solid. Yield: 42%. Mp: 127–129 °C (EtOH–Et_2O); ^{1}H NMR ($CDCl_3$): δ 0.73–0.82 (6H, m), 1.39 (3H, t, J = 6.9 Hz), 1.80 (3H, s), 1.95 (3H, s), 1.97–2.22 (6H, m), 2.84 (3H, s), 4.00 (2H, q, J = 6.9 Hz), 4.56 (1H, t, J = 8.4 Hz), 5.64 (1H, s), 6.86 (2H, d, J = 8.7 Hz), 7.20 (2H, d, J = 8.7 Hz), 7.25–7.37 (6H, m). Anal. Calcd for $C_{29}H_{39}ClN_4O_2{\cdot}0.5H_2O$: C, 66.97; H, 7.75; N, 10.77. Found: C, 66.82; H, 7.73; N, 10.77.

5.33. (5*R*)-*N*-[1-(3,4-Dimethoxyphenyl)-1-ethylpropyl]-2,7,7-trimethyl-5-phenyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]-pyrimidine-3-carboxamide hydrochloride (10f)

Compound **10f** was prepared in a manner similar to that described for **10a** as a solid. Yield: 53%. Mp: 183–184 °C (EtOH–Et_2O); ^{1}H NMR ($CDCl_3$): δ 0.74–0.83 (6H, m), 1.80 (3H, s), 1.94 (3H, s), 1.98–2.26 (6H, m), 2.85 (3H, s), 3.86 (6H, s), 4.56 (1H, t, J = 6.6 Hz), 5.66 (1H, s), 6.81–6.88 (3H, m), 7.29–7.39 (6H, m). Anal. Calcd for $C_{29}H_{39}ClN_4O_3{\cdot}0.1H_2O$: C, 65.85; H, 7.47; N, 10.59. Found: C, 65.58; H, 7.49; N, 10.41.

5.34. (5*R*)-*N*-{1-[4-(Benzyloxy)phenyl]-1-ethylpropyl}-2,7,7-trimethyl-5-phenyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]-pyrimidine-3-carboxamide hydrochloride (10g)

Compound **10g** was prepared in a manner similar to that described for **10a** as a solid. Yield: 32%. Mp: 167–169 °C (EtOH–Et_2O); ^{1}H NMR ($CDCl_3$): δ 0.77–0.80 (6H, m), 1.80 (3H, s), 1.94 (3H, s), 2.00–2.30 (6H, m), 2.84 (3H, s), 4.56 (1H, br s), 5.03 (2H, s), 5.65 (1H, s), 6.95 (2H, d, J = 6.6 Hz), 7.22–7.43 (13H, m). Anal. Calcd for $C_{34}H_{41}ClN_4O_2$: C, 1.25; H, 7.21; N, 9.77. Found: C, 70.97; H, 7.21; N, 9.74.

5.35. (5*R*)-*N*-[1-(4-Chloro-3-methoxyphenyl)-1-ethylpropyl]-2,7,7-trimethyl-5-phenyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]-pyrimidine-3-carboxamide hydrochloride (10h)

Compound **10h** was prepared in a manner similar to that described for **10a** as a solid. Yield: 8%. Mp: 167–169 °C (AcOEt–Et_2O); ^1H NMR ($CDCl_3$): δ 0.802 (6H, dt, J = 11.4, 7.2 Hz), 1.84 (6H, d, J = 41.4 Hz), 1.86–2.30 (6H, m), 2.82 (3H, s), 3.88 (3H, s), 4.56 (1H, t, J = 6.6 Hz), 5.65 (1H, br s), 6.80–6.90 (2H, m), 7.13 (1H, br s), 7.22–7.40 (6H, m). Anal. Calcd for $C_{27}H_{37}Cl_2N_5O{\cdot}0.4H_2O$: C, 61.69; H, 7.25; N, 13.32. Found: C, 61.79; H, 7.28; N, 13.04.

5.36. (5*R*)-*N*-[1-Ethyl-1-(4-fluoro-3-methoxyphenyl)propyl]-2,7,7-trimethyl-5-phenyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidine-3-carboxamide (10i)

Compound **10i** was prepared in a manner similar to that described for **10a** as a solid. Yield: 45%. Mp: 167–169 °C (MeOH–Et_2O); ^1H NMR ($CDCl_3$): δ 0.796 (6H, dt, J = 10.8, 7.5 Hz), 1.82 (6H, d, J = 39.6 Hz), 1.92–2.30 (6H, m), 2.78 (3H, s), 3.88 (3H, s), 4.56 (1H, t, J = 6.6 Hz), 5.63 (1H, br s), 6.82–6.94 (2H, m), 7.03 (1H, dd, J = 11.1, 8.7 Hz), 7.09 (1H, br s), 7.28–7.40 (5H, m). Anal. Calcd for $C_{28}H_{36}ClFN_4O_2$: C, 65.29; H, 7.04; N, 10.88. Found: C, 65.26; H, 7.05; N, 10.77.

5.37. (5*R*)-*N*-[1-(3-Chloro-4-methoxyphenyl)-1-ethylpropyl]-2,7,7-trimethyl-5-phenyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]-pyrimidine-3-carboxamide hydrochloride (10j)

Compound **10j** was prepared in a manner similar to that described for **10a** as a solid. Yield: 34%. Mp: 178–180 °C (*i*-PrOH-Et_2O); ^1H NMR ($CDCl_3$): δ 0.75–0.83 (6H, m), 1.80 (3H, s), 1.95 (3H, s), 1.91–2.25 (6H, m), 2.86 (3H, s), 3.87 (3H, s), 4.56 (1H, t, J = 6.3 Hz), 5.69 (1H, s), 6.89 (1H, d, J = 8.7 Hz), 7.15–7.26 (2H, m), 7.29–7.39 (6H, m). Anal. Calcd for $C_{28}H_{36}Cl_2N_4O_2{\cdot}0.25H_2O$: C, 62.74; H, 6.86; N, 10.45. Found: C, 62.68; H, 6.99; N, 10.42.

5.38. (5*R*)-*N*-[1-Ethyl-1-(3-fluoro-4-methoxyphenyl)propyl]-2,7,7-trimethyl-5-phenyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidine-3-carboxamide hydrochloride (10k)

To a mixture of **8a** (0.35 g, 1.23 mmol) and DMF (one drop) in toluene (3 mL) was added $SOCl_2$ (0.29 g, 2.44 mmol) at room temperature. After stirring at the same temperature for 1 h, the solvent was evaporated off. A mixture of 3-(3-fluoro-4-methoxyphenyl)pentan-3-amine hydrochloride (0.40 g, 1.62 mmol) and Et_3N (0.37 g, 3.66 mmol) in toluene (3 mL) was stirred at 70 °C for 1 h, and then, the acid chloride obtained in toluene was added to the reaction mixture at 70 °C. After stirring at the same temperature for 3 h, the reaction mixture was poured into H_2O and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on SiO_2 with EtOAc–hexane (1:1) to give an amorphous solid. To a solution of the amorphous solid obtained in EtOAc (1 mL) was added 4 N HCl in EtOAc (0.4 mL, 1.60 mmol) at 0 °C. The solvent was evaporated off. Crystallization from EtOAc–Et_2O and recrystallization from EtOAc–Et_2O afforded **10k** as HCl salt (0.10 g, 16%), mp 180–182 °C. ^1H NMR ($CDCl_3$): δ 0.80 (6H, s), 1.81 (3H, s), 1.95 (3H, s), 2.00–2.40 (6H, m), 2.88 (3H, s), 3.86 (3H, s), 4.57 (1H, br s), 5.65 (1H, br s), 6.80–7.40 (9H, m). Anal. Calcd for $C_{28}H_{36}ClFN_4O_2{\cdot}0.25H_2O$: C, 64.73; H, 7.08; N, 10.78. Found: C, 64.55; H, 7.04; N, 10.77.

5.39. (5*R*)-*N*-[1-Ethyl-1-(4-ethylphenyl)propyl]-2,7,7-trimethyl-5-phenyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidine-3-carboxamide benzenesulfonate (10l)

Free base of compound **10b** (0.90 g, 1.96 mmol) and benzenesulfonic acid monohydrate (0.38 g, 2.16 mmol) were dissolved in EtOH (4 mL). Crystallization from EtOH–Et_2O and recrystallization from EtOH–Et_2O afforded **10l** as benzenesulfonate (1.15 g, 96%), mp 165–166 °C. ^1H NMR ($CDCl_3$): δ 0.73–0.81 (6H, m), 1.22 (3H, t, J = 7.8 Hz), 1.72 (3H, s), 1.80 (3H, s), 2.04–2.22 (6H, m), 2.63 (2H, q, J = 7.8 Hz), 2.80 (3H, s), 4.57 (1H, t, J = 8.7 Hz), 5.68 (1H, s), 7.18–7.34 (13H, m). Anal. Calcd for $C_{35}H_{44}N_4O_4S$: C, 68.15; H, 7.19; N, 9.08. Found: C, 68.07; H, 7.19; N, 9.13.

5.40. (5*R*)-*N*-[1-Ethyl-1-(4-ethylphenyl)propyl]-2,7,7-trimethyl-5-phenyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidine-3-carboxamide *p*-toluenesulfonate (10m)

Free base of compound **10b** (120 mg, 0.26 mmol) and *p*-toluenesulfonic acid monohydrate (50 mg, 0.29 mmol) were dissolved in EtOH (2 mL). Crystallization from EtOH–Et_2O and recrystallization from EtOH–Et_2O afforded **10m** as *p*-toluenesulfonate (130 mg, 79%), mp 160–161 °C. ^1H NMR ($CDCl_3$): δ 0.73–0.81 (6H, m), 1.22 (3H, t, J = 7.8 Hz), 1.72 (3H, s), 1.80 (3H, s), 2.04–2.22 (6H, m), 2.37 (3H, s), 2.62 (2H, q, J = 7.8 Hz), 2.80 (3H, s), 4.56 (1H, t, J = 7.5 Hz), 5.69 (1H, s), 7.18–7.34 (12H, m), 7.85 (2H, d, J = 8.1 Hz). Anal. Calcd for $C_{36}H_{46}N_4O_4S$: C, 68.54; H, 7.35; N, 8.88. Found: C, 68.54; H, 7.48; N, 8.92.

5.41. 3-(4-Iodophenyl)pentan-3-amine hydrochloride (12)

To a solution of ethyl 4-iodobenzoate **11** (31.4 g, 114 mmol) in Et_2O (200 mL) was added EtMgBr (95 mL, 3 M in Et_2O solution, 285 mmol) at 0 °C. After stirring at room temperature for 3 h, the reaction mixture was quenched with 1 N HCl. Organic layer separated was washed with brine, dried over $MgSO_4$, and concentrated in vacuo to give as oil. To a mixture of the oil obtained and TMSCN (20.3 g, 205 mmol) was added concd H_2SO_4 (30.0 g) at −10 °C, and the whole was stirred at room temperature for 2 day. The reaction mixture was quenched with 28% NH_3 solution and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The mixture of residue obtained and 6 N HCl (100 mL) were refluxed for 3 h. The reaction mixture was bacidified with 8 N NaOH and extracted with Et_2O. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was diluted with EtOAc and 4 N HCl in EtOAc was added thereto. The precipitated crystals were collected by filtration and washed with Et_2O (8.25 g, 23%). ^1H NMR (DMSO-d_6): δ 0.73 (6H, t, J = 7.5 Hz), 1.87–2.04 (4H, m), 7.24 (2H, d, J = 8.7 Hz), 7.81 (2H, d, J = 8.7 Hz), 8.58 (2H, s).

5.42. (5*R*)-*N*-[1-Ethyl-1-(4-iodophenyl)propyl]-2,7,7-trimethyl-5-phenyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidine-3-carboxamide (13)

To a mixture of **8a** (6.0 g, 21.0 mmol) and DMF (three drops) in toluene (60 mL) was added $SOCl_2$ (3.75 g, 31.5 mmol) at room temperature. After stirring at the same temperature for 1 h, the solvent was evaporated off. A mixture of **12** (8.21 g, 25.2 mmol) and Et_3N (4.67 g, 46.2 mmol) in toluene (60 mL) was stirred at 70 °C for 1 h, and then, the acid chloride obtained in toluene was added to the reaction mixture at 70 °C. After stirring at the same temperature for 3 h, the reaction mixture was poured into H_2O and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on SiO_2 with EtOAc–hexane (1:2) to give **13** as an

oil (6.26 g, 54%); ^{1}H NMR ($CDCl_3$): δ 0.73–0.82 (6H, m), 1.54 (3H, s), 1.61 (3H, s), 1.93–2.21 (6H, m), 2.50 (3H, s), 4.52 (1H, dd, J = 11.7, 3.0 Hz), 5.58 (1H, s), 6.55 (1H, s), 7.10 (2H, d, J = 8.4 Hz), 7.27–7.36 (5H, m), 7.61 (2H, d, J = 8.4 Hz).

5.43. Ethyl 4-[1-ethyl-1-({[(5*R*)-2,7,7-trimethyl-5-phenyl-4,5,6,7-tetrahydropyrazolo-[1,5-*a*]-pyrimidin-3-yl]-carbonyl}amino)-propyl]benzoate (14)

A mixture of **13** (6.2 g, 11.1 mmol), dppf (0.33 g, 0.60 mmol), $Pd(OAc)_2$ (0.14 g, 0.60 mmol) and Et_3N (2.25 g, 22.3 mmol) in EtOH (120 mL) was stirred at 90 °C under 1 atom CO atmosphere for 24 h. The inorganic products were removed by filtration, and filtrate was concentrated in vacuo. The residue was diluted with EtOAc, washed with $NaHCO_3$ solution and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on SiO_2 with EtOAc–hexane (1:2) to give **14** as an amorphous solid (3.50 g, 63%). ^{1}H NMR ($CDCl_3$): δ 0.74–0.83 (6H, m), 1.33 (3H, J = 6.9 Hz), 1.54 (3H, s), 1.61 (3H, s), 1.98–2.23 (6H, m), 2.51 (3H, s), 4.33 (2H, q, J = 6.9 Hz), 4.51 (1H, dd, J = 11.4, 3.0 Hz), 5.63 (1H, s), 6.53 (1H, s), 7.24–7.34 (5H, m), 7.41 (2H, d, J = 8.1 Hz), 7.52 (1H, s), 7.98 (2H, d, J = 8.1 Hz).

5.44. 4-[1-Ethyl-1-({[(5*R*)-2,7,7-trimethyl-5-phenyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]-pyrimidin-3-yl]carbonyl}amino)-propyl]benzoic acid (15)

A mixture of **14** (3.5 g, 6.96 mmol) and 1 N NaOH (30 mL, 30.0 mmol) in EtOH (50 mL) was stirred at 60 °C for 12 h, and the solvent was concentrated in vacuo. The residue was diluted with EtOAc and acidified with 1 N HCl. The organic layer was washed brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on SiO_2 with EtOAc–hexane (4:1) to give **15** as crystals (2.70 g, 82%), mp 234–236 °C. ^{1}H NMR ($CDCl_3$): δ 0.76–0.88 (6H, m), 1.56 (3H, s), 1.62 (3H, s), 1.97–2.25 (6H, m), 2.53 (3H, s), 4.53 (1H, dd, J = 11.7, 3.0 Hz), 5.67 (1H, s), 6.56 (1H, s), 7.24–7.36 (5H, m), 7.45 (2H, d, J = 8.4 Hz), 8.01 (2H, d, J = 8.4 Hz). Anal. Calcd for $C_{28}H_{34}N_4O_2{\cdot}0.25H_2O$: C, 70.19; H, 7.26; N, 11.69. Found: C, 70.25; H, 7.16; N, 11.49.

5.45. (5*R*)-*N*-[1-(4-Acetylphenyl)-1-ethylpropyl]-2,7,7-trimethyl-5-phenyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]-pyrimidine-3-carboxamide (16)

A mixture of **15** (2.65 g, 5.58 mmol), WSC (1.60 g, 8.38 mmol), HOBt (1.28 g, 8.38 mmol), MeONHMe hydrochloride (0.82 g, 8.38 mmol) and Et_3N (1.13 g, 11.2 mmol) in DMF (20 mL) was stirred at 60 °C for 3 h. The reaction mixture was poured into H_2O and extracted with EtOAc. The extract was washed brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on SiO_2 with EtOAc–hexane (3:1) to give an oil (2.5 g, 87%). ^{1}H NMR ($CDCl_3$): δ 0.75–0.88 (6H, m), 1.55 (3H, s), 1.61 (3H, s), 1.99–2.25 (6H, m), 2.51 (3H, s), 3.34 (3H, s), 3.56 (3H, s), 4.54 (1H, dd, J = 11.1, 3.0 Hz), 5.63 (1H, s), 6.55 (1H, s), 7.24–7.39 (7H, m), 7.65 (2H, d, J = 8.7 Hz). To a solution of the oil obtained (2.5 g) in THF was added MeMgBr (50 mL, 1 M in THF solution, 50.0 mmol) at 0 °C. After stirring at room temperature for 1 h, the reaction mixture was quenched with 1 N HCl and extracted with EtOAc. The extract was washed brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on SiO_2 with EtOAc–hexane (1:2) to give crystals. Recrystallization from EtOAc–hexane afforded **16** as prisms (1.21 g, 53%), mp 144–145 °C; ^{1}H NMR ($CDCl_3$): δ 0.75–0.84 (6H, m), 1.55 (3H, s), 1.61 (3H, s), 1.96–2.28 (6H, m), 2.52 (3H, s), 2.56 (3H, s), 4.51 (1H, dd, J = 11.4, 3.0 Hz), 5.64 (1H, s), 6.52 (1H, s), 7.24–7.31 (5H, m), 7.44 (2H, d, J = 8.4 Hz), 7.80 (2H, d, J = 8.4 Hz). Anal. Calcd for $C_{29}H_{36}N_4O_2$: C, 73.70; H, 7.68; N, 11.85. Found: C, 73.46; H, 7.72; N, 11.72.

5.46. (5*R*)-*N*-{1-Ethyl-1-[4-(1-hydroxyethyl)phenyl]propyl}-2,7,7-trimethyl-5-phenyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]-pyrimidine-3-carboxamide (17)

To a solution of **16** (0.80 g, 1.69 mmol) in EtOH was added $NaBH_4$ (0.26 g, 6.77 mmol) at room temperature. After stirring at the same temperature for 4 h, the solvent was evaporated off. The residue was diluted with EtOAc, washed with 1 N HCl and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on SiO_2 with EtOAc–hexane (1:1) to give an oil. The oil was diluted with EtOAc, and 4 N HCl in EtOAc (0.5 mL, 2.0 mmol) was added thereto. Crystallization from EtOAc–Et_2O and recrystallization from EtOH–Et_2O afforded **17** as prisms (0.61 g, 71%), mp 182–184 °C; ^{1}H NMR ($CDCl_3$): δ 0.75–0.83 (6H, m), 1.49 (3H, d, J = 6.6 Hz), 1.80 (3H, s), 1.94 (3H, s), 2.00–2.30 (6H, m), 2.85 (3H, s), 4.55 (1H, t, J = 6.0 Hz), 4.88 (1H, q, J = 6.6 Hz), 5.67 (1H, s), 7.22–7.36 (10H, m). Anal. Calcd for $C_{29}H_{39}Cl\ N_4O_2$: C, 68.15; H, 7.69; N, 10.96. Found: C, 67.84; H, 7.70; N, 10.84.

5.47. GTPγS binding assay

The GTPγS binding activity was measured as follows. The CaR-expressing cell membrane was incubated with test compounds for 10 min. The assays were carried out at room temperature for an hour in a reaction solution mixture containing 20 mM HEPES (pH.7.4), 100 mM NaCl, 1 mM $MgCl_2$, 167 μg/mL DTT, 5 μM guanosine 5′-diphosphate, 0.4 nM [35S]-guanosine 5′-(γ-thio) triphosphate ([35S]-GTPγS) and 6 mM $CaCl_2$. The mixture was filtrated through a GF/C filter. After washing fourth with 300 μL of phosphate-buffered saline, radioactivity of the filter was measured using a Top-count scintillation counter. Data from GTPγS binding assays for antagonists were analyzed with the use of the Prism program (GraphPad Software, Inc). IC_{50} values were determined through nonlinear regression analysis performed with Prism.

5.48. Measurement of in vivo PTH secretion

To estimate in vivo PTH secretion of these compounds in rat, plasma intact PTH levels were assayed using a rat Enzyme-Linked Immunosorbent assay (ELISA) kit (Rat Bioactive Intact PTH ELISA kit, Immutopics, Inc.) after oral administration. In case of monkey, plasma intact PTH levels were measured using an immunoradiometric assay human kit (Allegro intact PTH, Kyowa Medex Co., Ltd).

5.49. Metabolic stability assay

Metabolic stability assay Hepatic microsomes from rats and humans were purchased from Xenotech, LLC (Lenexa, KS). An incubation mixture with a final volume of 0.1 mL consisted of microsomal protein in 50 mmol/L KH_2PO_4–K_2HPO_4 phosphate buffer (pH 7.4) and 1 lmol/L test compound. The concentration of hepatic microsomal protein was 0.2 mg/mL. An NADPH-generating system containing 50 mmol/L $MgCl_2$, 50 mmol/L glucose-6-phosphate, 5 mmol/L beta-NADP+ and 15 unit/mL glucose-6-phosphate dehydrogenase was prepared and added to the incubation mixture with a 10% volume of the reaction mixture. After the addition of the NADPH-generating system, the mixture was incubated at 37 °C for 0 and 20 min. The reaction was terminated by the addition of acetonitrile equivalent to the volume of the reaction mixture. All incubations were made in duplicate. Test compound in the reaction mixture was measured by HPLC system equipped with a UV

detector. For metabolic stability determinations, chromatograms were analyzed for parent compound disappearance from the reaction mixtures.

5.50. Solubility determination

Small volumes of the compound DMSO solutions were added to the aqueous buffer solution (pH's 1.2, 6.8, and 6.8+bile acid). After incubation, precipitates were separated by filtration. The solubility was determined by HPLC analysis of each filtrate.

5.51. Chemical stability determination

The compounds were stored under 75% RH at 60 °C for 2 weeks. The residual percentage of the compound was analyzed by HPLC, after dissolved with CH_3CN.

5.52. Plasma concentration in monkeys (compound 9a)

Compound **9a** was administered orally to fed cynomolgus monkeys (male, $n = 3$) at a dose of 2.5 or 5.0 mg/kg in 0.5% methylcellulose suspension. At 0.25, 0.5, 1, 2, 4, 8, and 24 h after oral administration, blood samples were collected, and then immediately centrifuged to obtain the plasma fraction. The plasma samples were deproteinized with acetonitrile containing an internal standard. After centrifugation, the supernatant obtained was diluted with 0.01 mol/L ammonium formate (pH 3.0) and centrifuged again. The compound concentration in the supernatant was measured by high performance liquid chromatography/triple quadrupole mass spectrometry system, API3000 (Applied Biosystem, CA, USA), equipped with a binary solvent manager (LC-10AD$_{vp}$), sample organizer/manager (SIL-HT$_c$) and column oven (CTO-10AC) from SHIMADZU (Kyoto, Japan). The mass spectrometer was equipped with a turbo ionspray source and operated in positive ion mode. The HPLC conditions were as follows: column, Inertsil C8–3 (2.1 × 33 mm) from GL Sciences (Tokyo, Japan); mobile phase, (A) 0.01 mol/L ammonium formate (pH 3.0)/(B) acetonitrile = 8.5:1.5; gradient condition, 0–1.4 min: 15% B; 1.4–1.5 min: 15–91.5% B; 1.5–5.5 min: 91.5% B; 5.5–8.0 min: 91.5–15% B; 8.0–9.0 min: 15% B; flow rate, 0.2 mL/min; column temperature, 40 °C.

5.53. Plasma concentration in rats and monkeys (compound 10m)

Compound **10m** was administered orally to non-fasted Crl: CD(SD) rats (female, 12 weeks old, $n = 3$) and fed cynomolgus monkeys (male, $n = 3$) orally at a dose of 2.5 and 5 mg/kg, respectively (0.5% methylcellulose suspension). At 0.25, 0.5, 1, 2, 4, 8, and 24 h after oral administration, blood samples were collected, and then immediately centrifuged to obtain the plasma fraction. The plasma samples were deproteinized with acetonitrile containing an internal standard. After centrifugation, the supernatant obtained was diluted with 0.01 mol/L ammonium formate (pH 3.0) and centrifuged again. The compound concentration in the supernatant was measured by high performance liquid chromatography/triple quadrupole mass spectrometry system, API4000 (Applied Biosystem, CA, USA), equipped with a binary solvent manager (LC-10AD$_{vp}$), sample organizer/manager (SIL-HT$_c$) and column oven (CTO-10AC) from SHIMADZU (Kyoto, Japan). The mass spectrometer was equipped with a turbo ionspray source and operated in positive ion mode. The HPLC conditions were as follows: column, Inertsil ODS-3 (2.1 × 50 mm) from GL Sciences (Tokyo, Japan); mobile phase, (A) 0.01 mol/L ammonium formate (pH 3.0)/(B) acetonitrile = 8.5:1.5; gradient condition, 0–0.5 min: 15–95% B; 0.5–3.0 min: 95% B; 3.0–3.1 min: 95–15% B; 3.1–5.0 min: 15% B; flow rate, 0.25 mL/min; column temperature, 40 °C.

Acknowledgments

We thank Dr. S. Ohkawa, Dr, Y. Ikeura, Mr. H. Maezaki for their encouragement and helpful advice. We thank Ms. K. Higashikawa for X-ray crystallographic analysis, and Dr. T. Yamano and Mr. S. Yamamoto for optical resolution, Dr. M. Yamaguchi for obtaining rat and monkey pharmacokinetic study, Mr. Y. Suzuki for measurement of chemical stability, Dr. T. Okuda for measurement of metabolic stability. We thank Mr. T. Kobayashi and Mr. N. Tsuruta for in vivo assays.

Supplementary data

Supplementary data (X-ray crystallographic data for (*R*)-**9a**) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.02.001.

References and notes

1. Ettinger, M. P. *Arch. Intern. Med.* **2003**, *163*, 2237.
2. Tarantino, U.; Cannata, G.; Lecce, D.; Celi, M.; Cerocchi, I.; Iundusi, R. *Aging Clin. Exp. Res.* **2007**, *19*, 7.
3. Cummings, S. R.; Melton, L. J. *Lancet* **2002**, *359*, 1761.
4. Black, D. M.; Cummings, S. R.; Karpf, D. B.; Cauley, J. A.; Thompson, D. E.; Nevitt, M. C.; Bauer, D. C.; Genant, H. K.; Haskell, W. L.; Marcus, R.; Ott, S. M.; Torner, J. C.; Quandt, S. A.; Reiss, T. F.; Ensrud, K. E. *Lancet* **1996**, *348*, 1535.
5. Reginster, J.; Minne, H. W.; Sorensen, O. H.; Hooper, M.; Roux, C.; Brandi, M. L.; Lund, B.; Ethgen, D.; Pack, S.; Roumagnac, I.; Eastell, R. *Osteoporos. Int.* **2000**, *11*, 83.
6. Ettinger, B.; Black, D. M.; Mitlak, B. H.; Knickerbocker, R. K.; Nickelsen, T.; Genant, H. K.; Christiansen, C.; Delmas, P. D.; Zanchetta, J. R.; Stakkestad, J.; Glüer, C. C.; Krueger, K.; Cohen, F. J.; Eckert, S.; Ensrud, K. E.; Avioli, L. V.; Lips, P.; Cummings, S. R. *JAMA* **1999**, *282*, 637.
7. Pleiner-Duxneuner, J.; Zwettler, E.; Paschalis, E.; Roschger, P.; Nell-Duxneuner, V.; Klaushofer, K. *Calcif. Tissue Int.* **2009**, *84*, 159.
8. Dobnig, H. *Expert Opin. Pharmacother.* **2004**, *5*, 1153.
9. Neer, R. M.; Arnaud, C. D.; Zanchetta, J. R.; Prince, R.; Gaich, G. A.; Reginster, J. Y.; Hodsman, A. B.; Eriksen, E. F.; Ish-Shalom, S.; Genant, H. K.; Wang, O.; Mitlak, B. H. *N. Eng. J. Med.* **2001**, *344*, 1434.
10. Lotinun, S.; Sibonga, J. D.; Turner, R. T. *Endocrine* **2002**, *17*, 29.
11. Frolik, C. A.; Black, E. C.; Cain, R. L.; Satterwhite, J. H.; Brown-Augsburger, P. L.; Sato, M.; Hock, J. M. *Bone* **2003**, *33*, 372.
12. Chattopadhyay, N. *Int. J. Biochem. Cell Biol.* **2000**, *32*, 789.
13. Fox, J.; Miller, M. A.; Stroup, G. B.; Nemeth, E. F.; Miller, S. C. *Bone* **1997**, *21*, 163.
14. Gowen, M.; Stroup, G. B.; Dodd, R. A.; James, I. E.; Votta, B. J.; Smith, B. R.; Bhatnagar, P. K.; Lago, A. M.; Callahan, J. F.; DelMar, E. G.; Miller, M. A.; Nemeth, E. F.; Fox, J. *J. Clin. Invest.* **2000**, *105*, 1595.
15. Kumar, S.; Matheny, C. J.; Hoffman, S. J.; Marquis, R. W.; Schultz, M.; Liang, X.; Vasko, J. A.; Stroup, G. B.; Vaden, V. R.; Haley, H.; Fox, J.; Delmar, E. G.; Nemeth, E. F.; Lago, A. M.; Callahan, J. F.; Bhatnagar, P.; Huffman, W. F.; Gowen, M.; Yi, B.; Danoff, T. M.; Fitzpatrick, L. A. *Bone* **2010**, *46*, 534.
16. Carter, P. H.; Liu, R. Q.; Foster, W. R.; Tamasi, J. A.; Tebben, A. J.; Favata, M.; Staal, A.; Cvijic, M. E.; French, M. H.; Dell, V.; Apanovitch, D.; Lei, M.; Zhao, Q.; Cunningham, M.; Decicco, C. P.; Trzaskos, J. M.; Feyen, J. H. *Proc. Natl. Acad. Sci.* **2007**, *104*, 6846.
17. Yoshida, M.; Mori, A.; Inaba, A.; Oka, M.; Makino, H.; Yamaguchi, M.; Fujita, H.; Kawamoto, T.; Goto, M.; Kimura, H.; Baba, A.; Yasuma, T. *Bioorg. Med. Chem.* **2010**, *18*, 8501.
18. Yoshida, M.; Mori, A.; Kotani, E.; Oka, M.; Makino, H.; Fujita, H.; Ban, J.; Ikeda, Y.; Kawamoto, T.; Goto, M.; Kimura, H.; Kawase, M.; Baba, A.; Yasuma. *J. Med. Chem.*, ASAP, doi:10.1021/jm101452x.
19. Huppatz, J. L. *Aust. J. Chem.* **1985**, *38*, 221.
20. X-ray crystallographic data for compound **9a** has been deposited with the Cambridge Crystallographic Data Center as CCDC 800243. The crystallographic data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).
21. Chen, H. G.; Goel, O. P.; Kesten, S.; Knobelsdorf, J. *Tetrahedron Lett.* **1996**, *37*, 8129.
22. The practical synthesis of TAK-075 will be reported in due course.