Regular Article

Design and Synthesis of 2-(1-Alkylaminoalkyl)pyrazolo[1,5-a]pyrimidines as New Respiratory Syncytial Virus Fusion Protein Inhibitors

Toru Yamaguchi-Sasaki,*[,a] Yunoshin Tamura,[b] Yuya Ogata,[a] Takanori Kawaguchi,[a] Jun Kurosaka,[a] Yutaka Sugaya,[c] Kanako Iwakiri,[a] Tsuyoshi Busujima,[a] Ryo Takahashi,[a] Naoko Ueda-Yonemoto,[c] Eiji Tanigawa,[c] Tomoko Abe-Kumasaka,[c] Hiroyuki Sugiyama,[c] and Kosuke Kanuma[a]

[a] *Medicinal Chemistry Laboratories, Taisho Pharmaceutical Co., Ltd.; 1–403 Yoshino-cho, Kita-ku, Saitama 331–9530, Japan:* [b] *Discovery Technologies Laboratories, Taisho Pharmaceutical Co., Ltd.; 1–403 Yoshino-cho, Kita-ku, Saitama 331–9530, Japan: and* [c] *Pharmacology Laboratories, Taisho Pharmaceutical Co., Ltd.; 1–403 Yoshino-cho, Kita-ku, Saitama 331–9530, Japan.*

Received October 10, 2019; accepted December 25, 2019

Respiratory syncytial virus (RSV) is one of the most common causes of lower respiratory tract infections and a significant pathogen for both adults and children. Although two drugs have been approved for the treatment of RSV infections, the low therapeutic index of these drugs have led pharmaceutical companies to develop safe and effective small-molecule anti-RSV drugs. The pyrazolo[1,5-a]pyrimidine series of compounds containing a piperidine ring at the 2-position of the pyrazolo[1,5-a]pyrimidine scaffold are known as candidate RSV fusion (F) protein inhibitor drugs, such as presatovir and P3. The piperidine ring has been revealed to facilitate the formation of an appropriate dihedral angle between the pyrazolo[1,5-a]pyrimidine scaffold and the plane of the amide bond for exertion of anti-RSV activity. A molecular-dynamic study on newly designed compounds with an acyclic chain instead of the piperidine ring proposed and demonstrated a new series of pyrazolo[1,5-a]pyrimidine derivatives, such as 9c with a 1-methyaminopropyl moiety, showing similar dihedral angle distributions to those in presatovir. Compound 9c exhibited potent anti-RSV activity with an EC_{50} value of below 1 nM, which was similar to that of presatovir. A subsequent optimization study on the benzene ring of 9c led to the potent RSV F protein inhibitor 14f with an EC_{50} value of 0.15 nM. The possibility of improving the biological properties of anti-RSV agents by modification at the 7-position of pyrazolo[1,5-a]pyrimidine is also discussed.

Key words respiratory syncytial virus; fusion protein; pyrazolo[1,5-a]pyrimidine; dihedral angle

Introduction

Respiratory syncytial virus (RSV) is one of the leading causes of lower respiratory tract infections, especially in infants and young children.[1)] Primary infection with RSV is always clinically apparent, and manifests with a variety of symptoms, ranging from mild cold-like symptoms to lower respiratory tract illnesses, such as atopic asthma, severe bronchiolitis, and pneumonia.[2–8)] Infants under the age of one developing infection for the first time are likely to develop serious infection. RSV infection also causes lower respiratory tract infection in adults and the elderly with chronic respiratory and heart disease, and is a major reason for hospitalization and cause of death.[9–13)] RSV infection is of particularly serious concern in immunocompromised patients, because in these patients, the infection is difficult to treat and the patients are at imminent risk of pneumonia and death.[14,15)] RSV is distributed worldwide and repeated episodes of RSV infection can occur in humans. It has been suggested that more than a half of infants less than one year of age and most infants less than two years of age are infected with RSV at least once.[16,17)] There were 33.1 million RSV-related episodes of acute lower respiratory tract infection (RSV-ALRI) around the world in 2015, with 3.2 million hospitalizations and 59600 in-hospital deaths.[18)] The RSV infection-related mortality rate is next only to the rate of death from measles and is higher than that from influenza.[19)] There is currently no effective treatment for RSV infection, and management primarily remains supportive, including oxygen supplementation, fluid administration, and respiratory management.[20–23)]

Palivizumab (Synagis®) was initially expected as an effective drug for the treatment of severe respiratory tract inflammation caused by RSV; however, palivizumab was found to not exert significant efficacy, and has been used as a preventive therapy in premature infants and children, who are at a high risk for RSV infection, with underlying diseases of the heart and lungs.[20,21,24–27)] On the other hand, ribavirin is a synthetic guanosine nucleoside analogue and is an antiviral agent with a broad range of antiviral and immunomodulatory effects on DNA or RNA viruses.[28–34)] However, the usefulness of ribavirin is limited by its high cost, by the demonstration of its no benefit for decreasing the rate of hospitalization or mortality, and by its relatively high toxicity for health care workers.[35–37)]

RSV is an RNA virus that belongs to the genus Pneumovirus of the family Paramyxoviridae.[38)] The viral genome of RSV is approximately 12.5 kb in length, consisting of a negative-sense single-stranded RNA. Eleven proteins are encoded in the RSV genome, including fusion (F) protein interacting with nucleolin during viral fusion and attachment (G) protein with a CX3C chemokine motif which interacts with CX3CR1 in the host cells.[39,40)] The F protein forms a trimer on the surface of the virus particle and plays a criti-

* To whom correspondence should be addressed. e-mail: too-yamaguchi@taisho.co.jp

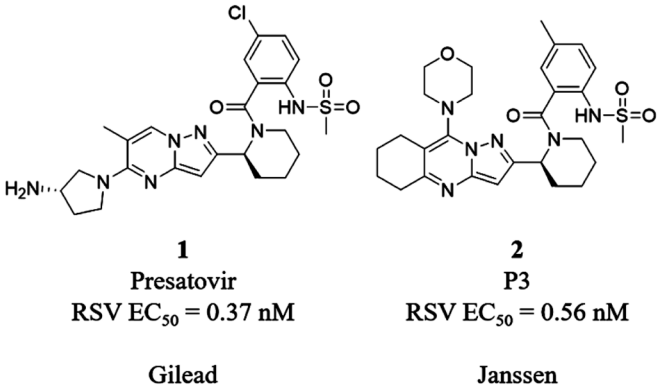


Fig. 1. Structures and Anti-RSV Activities of RSV F Protein Inhibitors with the Pyrazolo[1,5-a]pyrimidine Scaffold

cal role in the fusion of its envelope membrane with the host cell membrane, and entry of the virus genome into the host cell. Palivizumab is a monoclonal antibody that binds to F protein and inhibits infection by blocking virus binding to/fusion with the host cells. Drugs that target several proteins of RSV have been developed; inhibitors of RSV F protein are also being developed as one class of anti-RSV agents. Inhibitors of RSV F protein include several classes of compounds, such as pyrazolo[1,5-a]pyrimidines,[41–44] benzimidazoles,[45–47] and piperazinyl-quinolines.[48] Among the RSV F protein inhibitors with the pyrazolo[1,5-a]pyrimidine scaffold, such as presatovir (GS-5806, **1**) developed by Gilead,[41] and P3 (**2**) reported by Janssen,[44] **1** has advanced the furthest in clinical trials (Fig. 1). The structure–activity relationships (SARs) of **1** and its derivatives have been well-studied and reported.[41] According to one report, the dihedral angle between the pyrazolo[1,5-a]pyrimidine scaffold and the plane of the amide bond on the piperidine ring is a key factor for the anti-RSV activity. Through intensive SAR study of **1** and its derivatives on the 2-position of the pyrazolo[1,5-a]pyrimidine scaffold, it was concluded that the piperidine ring forces the maintenance of an optimal dihedral angle for anti-RSV activity. Therefore, we hypothesized that if an optimal dihedral angle of the pyrazolo[1,5-a]pyrimidine scaffold with the plane of the amide bond can be reproduced in newly designed compounds by introducing an acyclic chain at the C-2 position of the pyrazolo[1,5-a]pyrimidine scaffold in place of the piperidine ring of **1**, potent anti-RSV agents could be obtained. The development of safe and effective small-molecule anti-RSV agents is required for patients, particularly for children and infants. The ring-opening of the piperidine in **1** is a possible idea to obtain compounds with short half-life, which would allow us to manage individual risks of adverse effects.

Computational Chemistry

First, conformational analyses of **1** with a piperidine ring and of the newly designed compounds (**9a**–**d**; **12a, b**) with an acyclic chain at the C-2 position of the pyrazolo[1,5-a]-pyrimidine scaffold was performed by replica-exchange molecular dynamics (REMD).[49] REMD simulations of seven compounds, including **1**, yielded relatively different dihedral angle distributions between the pyrazolo[1,5-a]pyrimidine scaffold and the amide plane (Fig. 2). The filled and lined histograms in all the figures show the dihedral angle distributions of **1** and the compounds with acyclic chains (**9a**–**d**; **12a,**

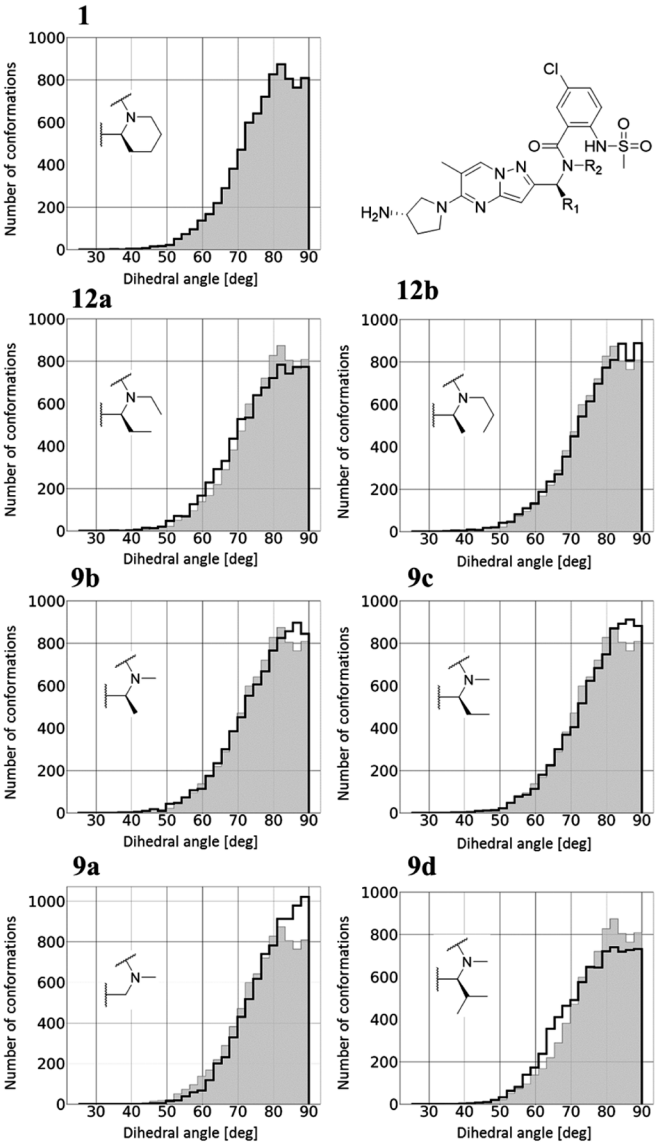
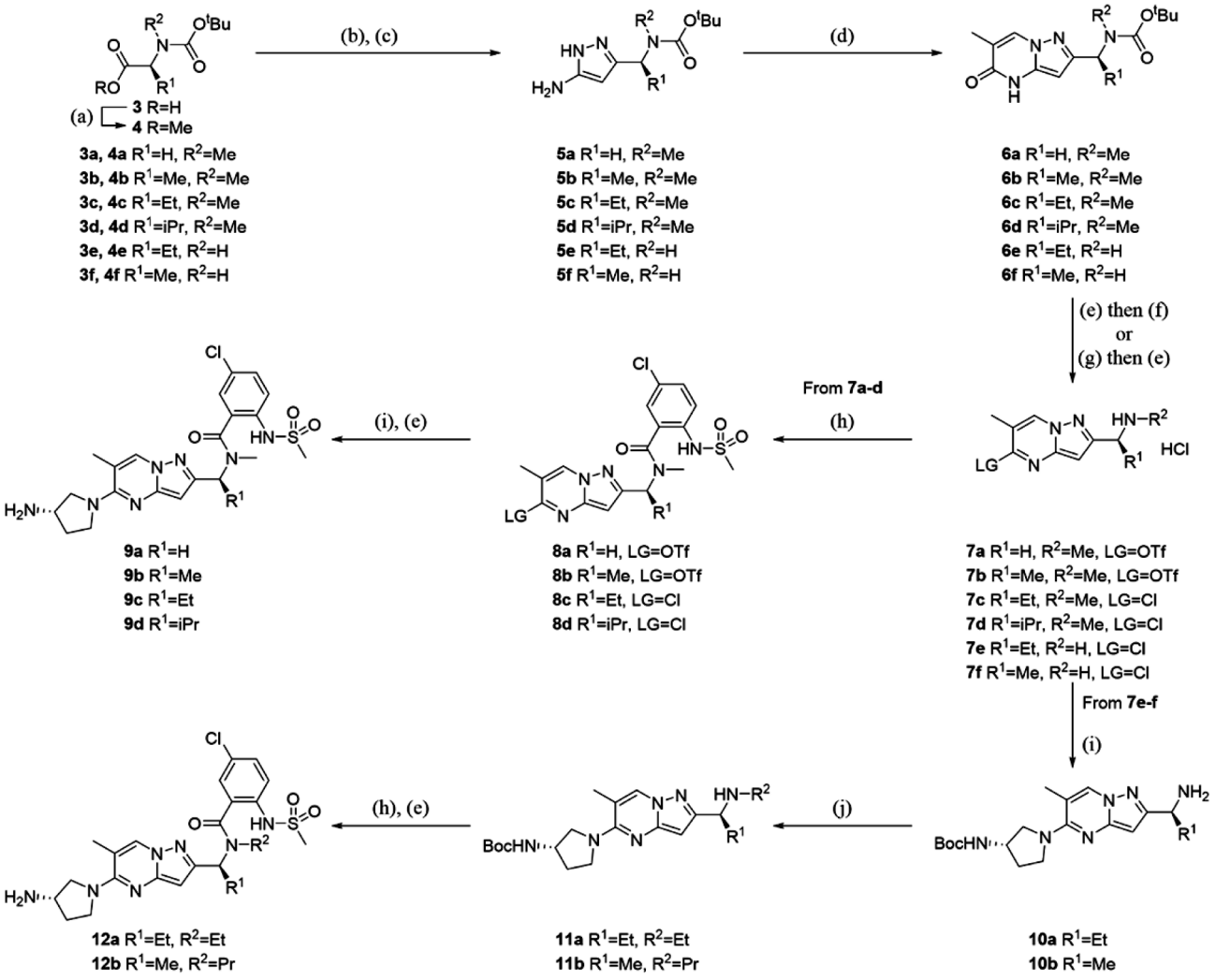


Fig. 2. Dihedral Angle Distributions of Compounds with Substitutions between the C-2 Heteroaryl Ring and the Amide Plane Calculated by REMD

b), respectively. The most frequently observed dihedral angle in **1** was around 82°. Both **12a** and **12b** contain five carbon atoms corresponding to the piperidine moiety of **1**. The most frequently encountered dihedral angle of **12a** was around 82°, while that of **12b** was bimodal at 84° and 89°. In addition, the angle in **9b** and **9c** was 87°. Both **9b** and **9c** contain four or less carbons at the position corresponding to the piperidine moiety of **1**, and the α-position in their pyrazolo[1,5-a]-pyrimidine scaffold was substituted by carbon. That is, these compounds (**9b**–**c**; **12a**, **b**) were not much different from **1** in terms of the most frequently noted dihedral angle or the overall dihedral angle distribution. In contrast, **9a**, without a side chain at the α-position, was determined to have the most stable conformation with a dihedral angle of 89°. The dihedral angle of **9d** with an isopropyl group at the α-position was found to be 82°; however, the overall dihedral angle distribution of **9d** was different from that of **1**.

These results indicate that the difference in the distributions of the dihedral angles between the pyrazolo[1,5-a]pyrimidine



Reagents and conditions: (a) $TMSCH_2N_2$, toluene, MeOH, r.t., (b)MeCN, NaHMDS, THF, -78°C, (c)NH_2NH_2, AcOH, EtOH, r.t., (d)*E*-ethyl-3-ethoxy-2-methyl acrylate, NaOEt (2.94 M EtOH), EtOH, 90°C, (e)4N HCl in dioxane, 1,4-dioxane, r.t., (f)$POCl_3$, 110°C, (g) Tf_2O, pyridine, $CHCl_3$, r.t., (h) 5-chloro-2-(methylsulfonamido)benzoic acid, HATU, Et_3N, DMF, r.t., (i)(*S*)-*tert*-butyl pyrrolidin-3-ylcarbamate, Et_3N, MeOH, 70°C, (j)iodethane or 1-iodopropane, K_2CO_3, DMF, 80°C

Chart 1. Synthesis of Pyrazolo[1,5-a]pyrimidin-2-yl Derivatives with Modified C-2 Substituents

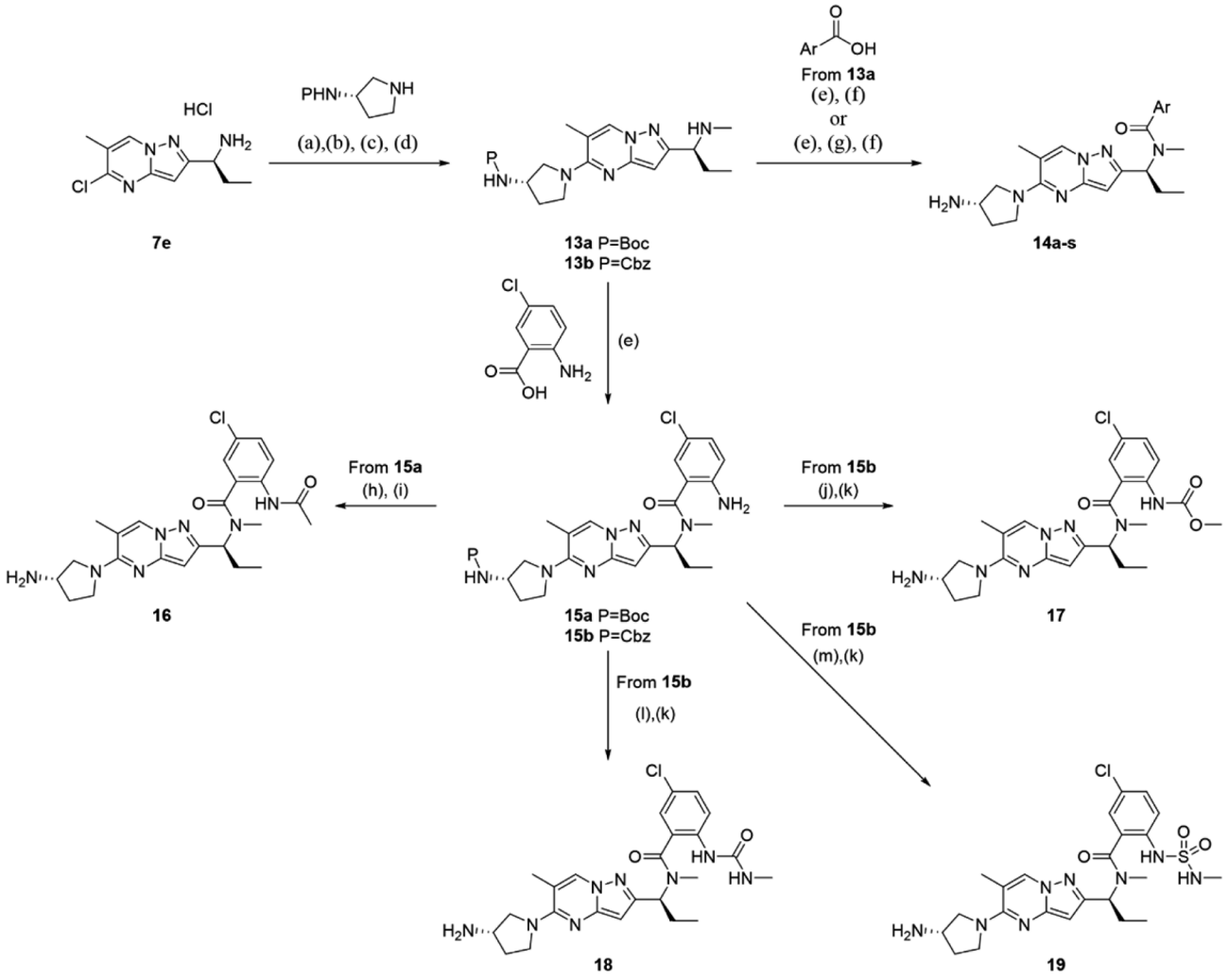
scaffold and the amide plane mainly depended on the substituent at the α-position of the pyrazolo[1,5-a]pyrimidine scaffold. These findings also suggest that it may be possible to design new compounds with appropriate acyclic chains having similar dihedral angle distributions as **1** with a piperidine ring, with anti-RSV activity similar or superior to that of **1**.

The conformations of each compound (**1**; **9a**–**d**; **12a, b**) were sampled using eight independent REMD simulations. The dihedral angles of all compounds between the pyrazolo[1,5-a]pyrimidine scaffold and the amide plane were calculated for each conformation sampled every 10 ps at 310.0 K.

Chemistry

Compounds with an acyclic chain at the C-2 position (**9a**–**d**; **12a**, **b**) were synthesized by the method shown in Chart 1. First, *tert*-butyloxycarbonyl (Boc)-protected amino acids **3a**–**f** with the appropriate substituents at the α-position were converted into methyl esters **4a**–**f**. β-Ketonitriles were synthesized by the nucleophilic addition reactions of acetonitrile anions to **4a**–**f**, followed by treating with hydrazine to obtain aminopyrazoles **5a**–**f**. Compounds **5a**–**f** were treated with ethyl (*E*)-ethoxy-2-methylacrylate in the presence of sodium ethoxide to obtain pyrazolopyrimidones **6a**–**f**. Deprotection of the Boc group of **6a**–**f** and chlorination with phosphorus oxychloride or triflation afforded pyrazolopyrimidines **7a**–**f**. Compounds **7a**–**d** were coupled with 5-chloro-2-(methylsulfonamido)-benzoic acid under standard amidation conditions. The desired products **9a**–**d** were obtained by nucleophilic addition of (*S*)-*tert*-butyl pyrrolidin-3-ylcarbamate under basic conditions and subsequent deprotection of the Boc group. Other desired products **12a,b** were synthesized from **7e**–**f**. After the introduction of (*S*)-*tert*-butyl pyrrolidin-3-ylcarbamate to **7e**–**f** by nucleophilic aromatic substitution, monoalkylation with the corresponding alkyl halides yielded **11a,b**. Finally, **11a,b** were acylated with 5-chloro-2-(methylsulfonamido)benzoic acid, and the Boc group was deprotected to obtain **12a,b**.

The synthesis methods for the desired products (**14a**–**s**,



Reagents and conditions: (a)*tert*-butyl (*S*)-pyrrolidin-3-ylcarbamate or benzyl (*S*)-pyrrolidin-3-ylcarbamate, Et_3N, MeOH, 70°C, (b)Ethyl trifluoroacetate, Et_3N, MeOH, r.t., (c)MeI, Cs_2CO_3, DMF, 65°C, (d)1M NaOH aq., THF, MeOH, r.t. (e)HATU, Et_3N, DMF, r.t., (f)4N HCl in dioxane, 1,4-dioxane, r.t., (g) MsCl, Et_3N, $CHCl_3$, r.t. then NaOEt, EtOH, r.t., (h) AcO_2, pyridine, $CHCl_3$, r.t.(i)TFA, $CHCl_3$, r.t., (j)Methyl chloroformate, pyridine, r.t., (k)H_2, 10% Pd/C, MeOH, r.t., (l)4-nitrophenyl chloroformate, pyridine, $CHCl_3$, r.t. then $MeNH_2$, $CHCl_3$, r.t., (m)chlorosulfonyl isocyanate, 2-chloroethanol, Et_3N, $CHCl_3$, r.t. then $MeNH_2$, Et_3N, $CHCl_3$, r.t.

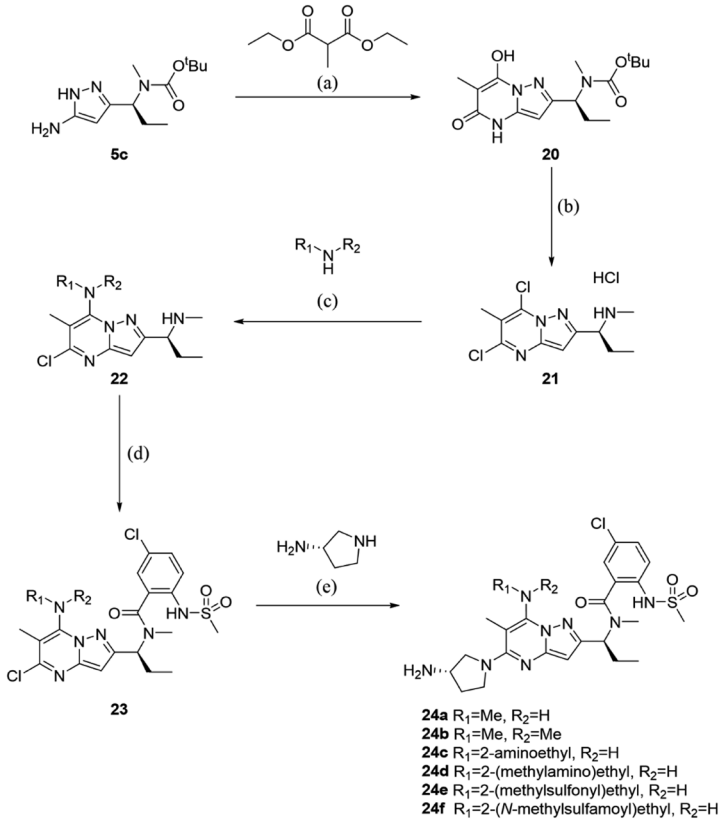
Chart 2. Synthesis of Pyrazolo[1,5-a]pyrimidin-2-yl Derivatives with Modified Aryl Groups

16–**19**) with modified aryl groups are shown in Chart 2. Intermediates **13a,b** were obtained by nucleophilic addition of (*S*)-*tert*-butyl pyrrolidin-3-ylcarbamate or (*S*)-benzyl pyrrolidin-3-ylcarbamate to **7e** under basic conditions, followed by *N*-methylation *via* trifluoroacetylation and deprotection of the trifluoroacetyl group. The desired products **14a**–**s** were obtained from **13a** by acylation with the corresponding carboxylic acids and by deprotecting the Boc group after mesylation, if necessary. Compounds **16**–**19** were synthesized from intermediates **15a,b** obtained by acylation of **13a,b** with 2-amino-5-chlorobenzoic acid. Acylation of **15a** with acetic anhydride and subsequent deprotection of the Boc group yielded **16**. Similarly, **15b** was converted to carbamate, urea, and sulfonylurea, followed by deprotection of the carbobenzoxy (Cbz) group to obtain **17**–**19**.

Subsequently, various substituent groups were introduced into the 7-position of the pyrazolo[1,5-a]pyrimidine scaffold (Chart 3). A dihydropyrazolopyrimidine compound **20** was synthesized by reaction of the 5-aminopyrazole derivative **5c** with diethyl 2-methylmalonate under basic conditions. Deprotection of the Boc group and dichlorination were simultaneously performed by treatment with phosphorus oxychloride to obtain **21**. The intermediate **21** was monoaminated with various amines and then subjected to an amidation reaction with 5-chloro-2-(methylsulfonamido)benzoic acid to obtain **23**. Finally, the desired products **24a**–**f** were synthesized by introducing the (*3S*)-3-aminopyrrolidine moiety using microwaves under basic conditions, followed by deprotection under acidic conditions for compounds possessing a Boc group.

Results and Discussion

The degrees of inhibition of the cytopathic effect (CPE) induced by RSV infection were determined to evaluate the antiviral activities of the synthesized compounds. Table 1 shows the anti-RSV activities of the test compounds (**9a**–**d**; **12a**, **b**). Compounds **9b**, **9c**, **12a**, and **12b** exhibited good anti-RSV activities, with EC_{50} values of less than 4 nM. As described above, the distribution of the dihedral angles between the pyrazolo[1,5-a]pyrimidine scaffold and the plane of the amide bond was similar to that in **1** (Fig. 2). In contrast, the dihedral



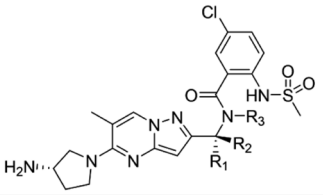
Reagents and conditions: (a) diethyl 2-methylmalonate, NaOEt (2.94 M EtOH), EtOH, 90°C, (b)$POCl_3$, 110°C, (c)$NaHCO_3$, CH_3CN, H_2O, r.t., (d)5-chloro-2-(methylsulfonamido)benzoic acid, HATU, Et_3N, DMF, r.t., (e)Et_3N, DMF, microwave 150°C, then TFA, $CHCl_3$, r.t. (only for compounds possessing Boc group.)

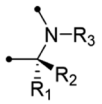
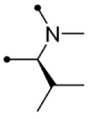
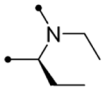
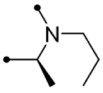
Chart 3. Synthesis of Pyrazolo[1,5-a]pyrimidin-2-yl Derivatives with Modified C-7 Substituents

angle distributions of **9a** and **9d**, which showed markedly decreased anti-RSV activities, were different from that of **1** (Fig. 2). These results indicate that the anti-RSV activities of **9a**–**d** and **12a,b** without a piperidine ring were well correlated with the dihedral angle distribution. Among these compounds, **9c** with the 1-methyaminopropyl moiety showed the most potent anti-RSV activity, with an EC_{50} value in the sub-nanomolar range, which was similar to that of **1**.[41] These findings suggest that the piperidine ring of **1** plays a crucial role in controlling the dihedral angle between the pyrazolo[1,5-a]pyrimidine scaffold and the plane of the amide bond, so as to allow effective interaction of the compound with the RSV F protein. Furthermore, these data suggest that potent anti-RSV activity could be achieved through controlling the dihedral angles of compounds without a piperidine ring. The promising results led us to further design new compounds with the acyclic chain, so as to obtain compounds with anti-RSV activity similar to or better than that of **9c**.

Next, the SAR study was focused on substituents on the benzene ring, which could affect the dihedral angle of the pyrazolo[1,5-a]pyrimidine scaffold and the plane of the amide bond (Table 2). To investigate the presence and position of a chlorine atom at the 5-position of the benzene ring in **9c**, two derivatives with a chlorine atom at different positions **14a,b** were synthesized, as well as a compound without a chlorine atom (**14c**). While the anti-RSV activities of **14a,b** were more than 7-fold less active as compared to the anti-RSV activity of **9c**, **14c** showed comparable activity to that of **9c**. Deletion of the methylsulfonamide group in the benzene ring markedly attenuated the activity (**14d**). Compounds of various shapes and sizes were synthesized (**14e**–**i**) to investigate the steric effect of the substituents at the 5-position in the benzene ring. The chlorine atom (**9c**) was replaceable with other small substituents such as methyl (**14e**), ethyl (**14f**), and methoxy (**14g**) at the 5-position, although longer or bulkier substituents such as propyl (**14h**) and isopropyl (**14i**) reduced the potency by two orders of magnitude. Among compounds with a small substituent at the 5-position in the benzene ring, **14f** exhibited the most potent anti-RSV activity, with an EC_{50} value of 0.15 nM. The dihedral angle distribution of **14f** was calculated and found to be similar to that of presatovir (**1**) (Fig. S1 in Supplementary Materials). To estimate the possibility of replacement of the methylsulfonamide group **9c** at the 2-position of the benzene ring with other groups, seven compounds with no substituent, methyl, or chloro at the 5-position were synthesized (**14j**–**l, 16**–**19**). It was found that methylsulfone substitution (**14j**) drastically reduced the potency by four orders of magnitude as compared to that of **14c** with a methylsulfonamide group in the same position. The antiviral activities were also reduced by 12- and 28-fold, respectively, when the methylsulfonamide group (**14e**) was substituted with methoxy **14k** and trifluoromethyl (**14l**) groups. Conversely, replacement of methylsulfonamide (**9c**) with acetamide (**16**), methoxycarboxamide (**17**), methylureido (**18**), or methylsulfonylureido (**19**) was associated with reduction of the EC_{50} values (0.57–1.8 nM). It appears that substituents having both

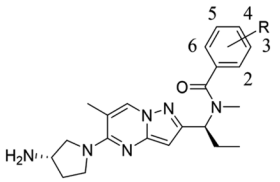
Table 1. RSV Inhibitory Activities of Compounds with an Acyclic Chain at the C-2 Position of the Pyrazolo[1,5-a]pyrimidine Scaffold



Compound		Anti-RSV activity EC_{50} (nM)[a)]
9a		110
9b		2.5
9c		0.58
9d		420
12a		1.8
12b		3.8

a) EC_{50} values of the CPE inhibitory activities of all compounds were evaluated in HEp-2 cells infected with RSV A2.

Table 2. RSV Inhibitory Activities of Compounds with Substitutions on the Benzene Ring



Compound	R	Anti-RSV activity EC_{50} (nM)[a)]
9c	2-$NHSO_2Me$, 5-Cl	0.58
14a	2-$NHSO_2Me$, 3-Cl	4.4
14b	2-$NHSO_2Me$, 4-Cl	13
14c	2-$NHSO_2Me$	0.59
14d	2-H	42
14e	2-$NHSO_2Me$, 5-Me	0.33
14f	2-$NHSO_2Me$, 5-Et	0.15
14g	2-$NHSO_2Me$, 5-OMe	0.43
14h	2-$NHSO_2Me$, 5-Pr	39
14i	2-$NHSO_2Me$, 5-*i*Pr	51
14j	2-SO_2Me	1500
14k	2-OMe, 5-Me	3.8
14l	2-CF_3, 5-Me	9.2
16	2-NHCOMe, 5-Cl	0.84
17	2-$NHCO_2Me$, 5-Cl	0.57
18	2-NHCONHMe, 5-Cl	1.0
19	2-$NHSO_2NHMe$, 5-Cl	1.8

a) EC_{50} values of the CPE inhibitory activities of all compounds were evaluated in HEp-2 cells infected with RSV A2.

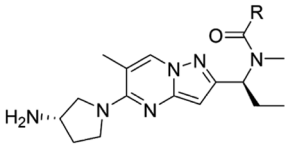
proton-donor and proton-acceptor capabilities at the 2-position of the benzene ring are preferable for obtaining effective antiviral activity. The SAR of substituents on the benzoyl ring obtained in this study showed good consistency with that in the previous report on **1** and its derivatives.[41)] However, little attention has been paid to the replacement of the benzene ring by heterocycles. Therefore, compounds with a variety of five- and six-membered heterocycles (**14m**–**s**) were designed and synthesized to investigate the effect on the anti-RSV activity (Table 3). All heterocycles contained a methylsulfonamide group at the corresponding position of the benzene ring in **14c**, because the methyl sulfonamide group was essential for obtaining potent antiviral activity. Compounds with a six-membered heterocyclic ring, such as pyridine (**14m,n**), and a five-membered heterocyclic ring, such as thiophene (**14o,p**), thiazole (**14q**), and pyrazole (**14r,s**), were synthesized; however, all of the compounds obtained showed a dramatic decrease of the antiviral activity. In addition, nitrogen regioisomers in the pyridine ring of **14m,n**, and compounds with other heterocycles, *e.g.*, pyrazine and pyrimidine, showed remarkable loss of antiviral activity (data not shown).

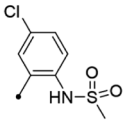
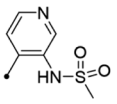
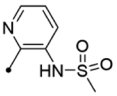
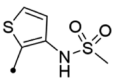
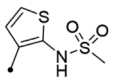
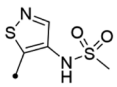
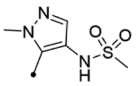
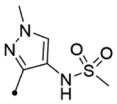
Previously, Gilead and Janssen reported that introduction of a substituent into the 7-position of the pyrazolo[1,5-a]-pyrimidine scaffold had little effect on the antiviral activity in their patent.[42–44)] This result prompted us to investigate the biological properties of **9c** derivatives by introduction of various substituents into the 7-position (Table 4). Compounds with methylamino (**24a**) and dimethylamino (**24b**) groups at the 7-position of pyrazolo[1,5-a]pyrimidine showed a 7.8- and 21-fold reduction in the antiviral efficacy, respectively, as compared to **9c**, suggesting that a secondary amine structure (**24a**) is preferable to a tertiary amine structure (**24b**). Compounds having a cyclic moiety such as oxetane and azetidine *via* substitution of the amino group at the 7-position also showed markedly reduced antiviral activities (data not shown). In contrast, introduction of linear moieties such as ethylenediamine and methyl ethylenediamine (**24c,d**) resulted in a moderate decrease of the antiviral activity, *i.e.*, a 4.2- to 4.7-fold decrease in the activity as compared to **24a**. Interestingly, compounds possessing sulfone (**24e**) and sulfonamide (**24f**) moieties on the side chain at 7-position of the pyrazolo[1,5-a]-pyrimidine scaffold exhibited potent anti-RSV activities, with EC_{50} values of less than 1 nM. These results suggest that sulfone (**24e**) and sulfonamide (**24f**) moieties have some potential to interact with the RSV F-protein. Modification at the 7-position of pyrazolo[1,5-a]pyrimidine could be utilized for tuning the biological and physicochemical properties of the most potent anti-RSV agent **14f** obtained in this study.

Conclusion

In summary, we designed and synthesized a new series of 2-(1-alkylaminoalkyl)pyrazolo[1,5-a]pyrimidine derivatives as RSV F protein inhibitors. The pyrazolo[1,5-a]pyrimidine

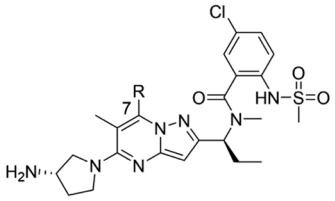
Table 3. RSV Inhibitory Activity of Compounds with Substitution of the Benzene Ring in **14c** by Heterocycles

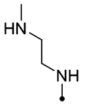
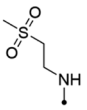
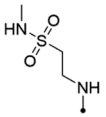


Compound	R	Anti-RSV activity EC_{50} (nM)[a)]
9c		0.58
14m		900
14n		550
14o		53
14p		110
14q		570
14r		880
14s		860

a) EC_{50} values of the CPE inhibitory activities of all compounds were evaluated in HEp-2 cells infected with RSV A2.

Table 4. RSV Inhibitory Activity of Compounds with Substitution at the 7-Position of the Pyrazolo[1,5-a]pyrimidine Scaffold



Compound	R	Anti-RSV activity EC_{50} (nM)[a)]
24a		4.5
24b		12
24c		19
24d		21
24e		0.75
24f		0.86

a) EC_{50} values of the CPE inhibitory activities of all compounds were evaluated in HEp-2 cells infected with RSV A2.

derivatives are well-known candidates as RSV F protein inhibitor drugs, such as presatovir (**1**) and P3 (**2**), which have a piperidine ring at the 2-position of pyrazolo[1,5-a]pyrimidine scaffold. The piperidine ring was considered to be an essential structure for anti-RSV activity, by providing an appropriate dihedral angle between the pyrazolo[1,5-a]pyrimidine scaffold and the plane of the amide bond. Conformational analysis of **1** and newly designed compounds with an acyclic chain in place of the piperidine ring by molecular dynamic study demonstrated that several types of compounds with different dihedral angle distributions showed different levels of anti-RSV activity. Among compounds with an acyclic chain with appropriate dihedral angle distributions, **9c** with 1-methylaminopropyl moiety showed potent anti-RSV activity, with an EC_{50} value in the sub-nanomolar range, which was similar to that of **1**. These results suggest that potent anti-RSV activity could be achieved through controlling the dihedral angle of compounds with an acyclic chain. Subsequent optimization study on the benzene ring located next to the amide bond led to the potent anti-RSV agent **14f** with an EC_{50} value of 0.15 nM, which showed a similar dihedral angle distribution compared to that of **1**. It is considered that introduction of a substituent into the 7-position of the pyrazolo[1,5-a]pyrimidine scaffold has the potential to modify the anti-RSV activity. Compounds **24e** and **24f** with a sulfone and sulfonamide moiety on the side chain at the 7-position of the pyrazolo[1,5-a]pyrimidine scaffold showed potent anti-RSV activities, with EC_{50} values of less than 1 nM, suggesting the possibility that the biological and physicochemical properties of the most potent anti-RSV agent **14f** obtained in this study could be improved further. Currently, further optimization study is in progress.

Experimental

Chemistry All solvents and reagents were purchased from commercial suppliers and used without purification, or were prepared according to published procedures. The ^{1}H-NMR and ^{13}C-NMR spectra of compounds synthesized in this study were recorded on a JNM-ECA600, JNM-ECA500 (JEOL Ltd., Tokyo, Japan), or Avance III HD 400 (Bruker Corp., Billerica, MA, U.S.A.) and the chemical shifts were expressed in δ (:) values, with trimethylsilane as the internal standard (s = singlet, d = doublet, t = triplet, q = quartet,

m = multiplet, and brs = broad singlet). Two sets of NMR signals were observed due to structural variations of the *cis*- and *trans*-amide rotamers. Mass spectra were recorded on a Micromass Platform LC (Micromass Ltd., Manchester, U.K.) or Shimadzu LCMS-2010EV (Shimadzu Corp., Kyoto, Japan). High-resolution (HR) mass spectral data were acquired using an LCMS-IT-TOF equipped with an electrospray ionization (ESI)/atmospheric pressure chemical ionization dual ion source (Shimadzu Corp.). Intermediates and final compounds were purified by preparative HPLC using Agilent 1260 Infinity/Agilent 6130 (Agilent Technologies Inc., Santa Clara, CA, U.S.A.) or a GX-281, UV/VIS-155, 331 PUMP, 332 PUMP, and SOFTA Model 300S ELSD (Gilson Inc., Middleton, WI, U.S.A.), under the following conditions: column, Sunfire prep C18 OBD (5.0 μm, 30 × 50 mm) (Waters Corp., Milford, MA, U.S.A.), YMC-Actus Triart C18 (5.0 μm, 30 × 50 mm) (YMC Co., Ltd., Kyoto, Japan), Xbridge Prep C18 OBD (5.0 μm, 30 × 50 mm) (Waters Corp.), or XSelect CSH C18 (5.0 μm, 30× 50 mm) (Waters Corp.); flow, 50 mL/min; linear gradient, 10–95% acetonitrile in water containing 0.1% formic acid for 7.5–11.5 min; detection wavelength, 254 nm.

***tert*-Butyl [(1*S*)-1-(5-Amino-1*H*-pyrazol-3-yl)ethyl]methylcarbamate (5b)** To a solution of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-alanine (6.0 g, 29.3 mmol) in toluene (18 mL)–methanol (12 mL) was added 2.0 M of trimethylsilyl diazomethane in diethyl ether (22.0 mL, 43.9 mmol) at 0°C, and the mixture was stirred for 1 h at room temperature. Acetic acid was added to the reaction solution until the solution became clear. The reaction mixture was basified with sat. sodium bicarbonate aq. and extracted with chloroform. The organic layer was dried over anhydrous magnesium sulfate and concentrated under reduced pressure to obtain methyl *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-alaninate (6.5 g, quant.) as a colorless oil, which was used for the next reaction without further purification.

A 1.9 M of sodium bis(trimethylsilyl)amide in tetrahydrofuran (68 mL, 88.6 mmol) was added to a solution of acetonitrile (4.6 mL, 88.6 mmol) in tetrahydrofuran (75 mL) at −78°C. After stirring at −50°C for 20 min, methyl *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-alaninate (6.0 g, 29.5 mmol) in tetrahydrofuran (45 mL) at −78°C was added to the mixture. After stirring at −50°C for 1 h, acetic acid (5.2 mL, 91.5 mmol) at −78°C was added to the mixture. The mixture was poured into sat. ammonium chloride aq. and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure to obtain *tert*-butyl (*S*)-(4-cyano-3-oxobutan-2-yl)-methylcarbamate (7.2 g, crude) as a brown oil, which was used for the next reaction without further purification.

To a solution of hydrazine monohydrate (4.1 mL, 84.8 mmol), acetic acid (4.9 mL, 84.8 mmol), and ethanol (20 mL) was added *tert*-butyl (*S*)-(4-cyano-3-oxobutan-2-yl)-methylcarbamate (6.0 g, 28.3 mmol) in ethanol (20 mL) at 0°C, and the mixture was stirred for 2 d at room temperature. Then, the reaction mixture was concentrated under reduced pressure and diluted with chloroform. The organic layer was washed with sat. sodium bicarbonate aq. and brine, dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (0–10% methanol in chloroform) to obtain **5b** (5.8 g, 23.9 mmol, 85%) as a brown gum. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 1.44 (3H, d, J = 7.0 Hz), 1.48 (9H, s), 2.65 (3H, s), 5.36 (1H, brs), 5.55 (1H, brs); MS (ESI/APCI dual) *m/z*: 241 $[M+H]^+$.

Compounds **5a, c–f** were obtained by the same procedure as that described for **5b**.

***tert*-Butyl [(5-Amino-1*H*-pyrazol-3-yl)methyl]methylcarbamate (5a)** Brown gum; ^{1}H-NMR (600 MHz, $CDCl_3$,) δ: 1.41–1.57 (9H, m), 2.75–3.01 (3H, m), 4.26 (2H, brs), 5.59 (1H, s); MS (ESI/APCI dual) *m/z*: 227 $[M+H]^+$.

***tert*-Butyl [(1*S*)-1-(5-Amino-1*H*-pyrazol-3-yl)propyl]methylcarbamate (5c)** Orange amorphous; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.90–1.02 (4H, m), 1.48 (9H, s), 1.74–1.95 (2H, m), 2.63 (3H, s), 3.62 (2H, brs), 4.96–5.14 (1H, m), 5.54 (1H, s); MS (ESI/APCI dual) *m/z*: 255 $[M+H]^+$.

***tert*-Butyl [(1*S*)-1-(5-Amino-1*H*-pyrazol-3-yl)-2-methylpropyl]methylcarbamate (5d)** Colorless amorphous; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.93 (3H, d, J = 15.4 Hz), 0.94 (3H, d, J = 15.4 Hz), 1.46 (9H, brs), 2.22 (1H, brs), 2.67 (3H, s), 3.43–3.77 (2H, m), 4.52–4.63 (1H, m), 5.55 (1H, s); MS (ESI/APCI dual) *m/z*: 269 $[M+H]^+$.

***tert*-Butyl [(1*S*)-1-(5-Amino-1*H*-pyrazol-3-yl)propyl]carbamate (5e)** Pale yellow amorphous; ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 0.95–1.06 (3H, m), 1.43–1.46 (9H, m), 1.63–1.77 (1H, m), 1.83–1.98 (1H, m), 4.35–4.58 (1H, m), 4.61–4.90 (1H, m), 5.49 (1H, m); MS (ESI/APCI dual) *m/z*: 241 $[M+H]^+$; chiral HPLC, 99% *ee* (CHIRALPAK IC-3 5 μm 4.6 × 250 mm; flow, 1 mL/min, 20% ethanol in hexane; detection wavelength, 254 nm), (*S*)-isomer t_R = 6.62 min, (*R*)-isomer t_R = 5.13 min.

***tert*-Butyl [(1*S*)-1-(5-Amino-1*H*-pyrazol-3-yl)ethyl]carbamate (5f)** Pale yellow amorphous; ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 1.43–1.49 (12H, m), 4.71–4.81 (1H, m), 4.85 (1H, brs), 5.49 (1H, s), 5.64 (1H, brs); MS (ESI/APCI dual) *m/z*: 227 $[M+H]^+$.

***tert*-Butyl Methyl[(1*S*)-1-(6-methyl-5-oxo-4,5-dihydropyrazolo[1,5-a]pyrimidin-2-yl)ethyl]carbamate (6b)** To a solution of **5b** (1.7 g, 6.87 mmol) in *N*,*N*-dimethylformamide (10 mL) was added ethyl (*E*)-3-ethoxy-2-methylacrylate (1.6 g, 10.3 mmol) and cesium carbonate (6.7 g, 20.6 mmol). After stirring at 120°C for 8 h, the reaction mixture was poured into water and extracted with chloroform. The organic layer was washed with brine, dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (0–10% methanol in chloroform) to obtain **6b** (1.4 g, 4.41 mmol, 64%) as a brown amorphous. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 1.49 (9H, s), 1.51 (3H, d, J = 7.0 Hz), 2.10 (3H, s), 2.67 (3H, brs), 5.28–5.62 (1H, m), 5.78 (1H, s), 8.01 (1H, s), 10.85 (1H, brs); MS (ESI/APCI dual) *m/z*: 307 $[M+H]^+$.

Compounds **6a, c–f** were obtained by the same procedure as that described for **6b**.

***tert*-Butyl Methyl[(6-methyl-5-oxo-4,5-dihydropyrazolo-[1,5-a]pyrimidin-2-yl)methyl]carbamate (6a)** Pale yellow amorphous; ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 1.39–1.54 (9H, m), 2.09 (3H, s), 2.78–2.95 (3H, m), 4.31–4.51 (2H, m), 5.72–5.90 (1H, m), 7.96 (1H, s), 10.77 (1H, brs); MS (ESI/APCI dual) *m/z*: 291 $(M-H)^-$.

***tert*-Butyl Methyl[(1*S*)-1-(6-methyl-5-oxo-4,5-dihydropyrazolo[1,5-a]pyrimidin-2-yl)propyl]carbamate (6c)** Colorless amorphous; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.92–1.01 (3H, m), 1.49 (9H, s), 1.78–1.93 (1H, m), 1.99–2.13 (4H, m), 2.67 (3H, s), 5.01–5.41 (1H, m), 5.79 (1H, brs), 8.00 (1H, s), 10.81 (1H, brs); MS (ESI/APCI dual) *m/z*: 321 $[M+H]^+$.

***tert*-Butyl Methyl[(1*S*)-2-Methyl-1-(6-methyl-5-oxo-4,5-dihydropyrazolo[1,5-a]pyrimidin-2-yl)propyl]carbamate (6d)** Brown powder; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.91 (3H, d, J = 6.5 Hz), 0.97 (3H, d, J = 6.5 Hz), 1.47 (9H, brs), 2.10 (3H, s), 2.31–2.45 (1H, m), 2.71 (3H, s), 4.64–5.03 (1H, m), 5.71–5.90 (1H, m), 8.01 (1H, s), 10.08–10.27 (1H, m); MS (ESI/APCI dual) m/z: 335 $[M+H]^+$.

***tert*-Butyl [(1*S*)-1-(6-Methyl-5-oxo-4,5-dihydropyrazolo-[1,5-a]pyrimidin-2-yl)propyl]carbamate (6e)** Pale yellow powder; ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 0.88–0.97 (3H, m), 1.39–1.51 (9H, m), 1.78 (1H, dt, J = 13.8, 7.1 Hz), 1.90 (1H, dd, J = 13.6, 7.0 Hz), 2.07–2.14 (3H, m), 4.72 (1H, d, J = 6.2 Hz), 5.06 (1H, d, J = 7.0 Hz), 5.82 (1H, s), 7.98 (1H, d, J = 0.8 Hz), 10.81 (1H, brs); MS (ESI/APCI dual) m/z: 307 $[M+H]^+$.

***tert*-Butyl [(1*S*)-1-(6-Methyl-5-oxo-4,5-dihydropyrazolo-[1,5-a]pyrimidin-2-yl)ethyl]carbamate (6f)** Pale yellow powder; ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 1.45 (9H, s), 1.50 (3H, d, J = 6.6 Hz), 2.09 (3H, s), 4.88 (1H, brs), 5.06 (1H, brs), 5.81 (1H, s), 7.96 (1H, s), 10.23 (1H, brs); MS (ESI/APCI dual) m/z: 293 $[M+H]^+$.

2-{(1*S*)-1-[{5-Chloro-2-[(methanesulfonyl)amino]benzoyl}-(methyl)amino]ethyl}-6-methylpyrazolo[1,5-a]pyrimidin-5-yl Trifluoromethanesulfonate (8b) To a solution of **6b** (717 mg, 2.34 mmol) and pyridine (0.95 mL, 11.7 mmol) in chloroform (8.0 mL) was added trifluoromethanesulfonic anhydride (0.79 mL, 4.68 mmol) at 0°C, and the mixture was stirred for 3.5 h at room temperature. The reaction mixture was poured into sat. ammonium chloride aq. and extracted with chloroform. The organic layer was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (5–100% ethyl acetate in hexane) to obtain 2-{(1*S*)-1-[(*tert*-butoxycarbonyl)(methyl)amino]ethyl}-6-methylpyrazolo[1,5-a]pyrimidin-5-yl trifluoromethanesulfonate (0.87 g, 1.98 mmol, 84%) as a colorless powder.

To a solution of 2-{(1*S*)-1-[(*tert*-butoxycarbonyl)(methyl)-amino]ethyl}-6-methylpyrazolo[1,5-a]pyrimidin-5-yl trifluoromethanesulfonate (0.86 g, 1.96 mmol) in 1,4-dioxane (3.0 mL) was added 4M hydrogen chloride in 1,4-dioxane (10 mL) and the mixture was stirred for 1 h at room temperature. Then, the reaction mixture was concentrated under reduced pressure to obtain **7b** (1.1 g, 2.60 mmol, quant.) as a pale yellow oil. This compound was used for the next reaction without further purification.

To a solution of **7b** (0.81 g, 1.96 mmol) and 5-chloro-2-(methylsulfonamido)benzoic acid (0.59 g, 2.36 mmol) in *N*,*N*-dimethylformamide (10 mL) was added 1-[bis(dimethylamino)-methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (1.1 g, 2.95 mmol) and triethylamine (1.7 mL, 11.8 mmol). After stirring at room temperature for 15 h, the reaction mixture was added to water and extracted with ethyl acetate. The organic layer was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (10–100% ethyl acetate in hexane) to obtain **8b** (0.71 g, 1.24 mmol, 63%) as a colorless amorphous. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 1.68 (1.4H, d, J = 7.4 Hz), 1.72 (1.6H, d, J = 7.4 Hz), 2.39 (1.8H, s), 2.40 (1.2H, s), 2.66 (1.6H, s), 2.80 (1.4H, s), 2.96 (1.8H, s), 3.05 (1.2H, s), 5.00 (0.5H, q, J = 7.4 Hz), 6.31 (0.5H, q, J = 7.4 Hz), 6.45 (0.5H, s), 6.46 (0.5H, s), 7.28–7.34 (1H, m), 7.35–7.42 (1H, m), 7.62 (0.5H, d, J = 8.7 Hz), 7.71 (0.5H, d, J = 8.7 Hz), 8.79 (1H, s), 8.93 (0.5H, s), 9.10 (0.5H, s).

Compound **8a** was obtained by the same procedure as that described for **8b**.

2-{[{5-Chloro-2-[(methanesulfonyl)amino]benzoyl}(methyl)amino]methyl}-6-methylpyrazolo[1,5-a]pyrimidin-5-yl Trifluoromethanesulfonate (8a) Pale yellow oil; ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 2.36–2.42 (3H, m), 2.83–3.14 (6H, m), 4.58 (0.6H, brs), 5.02 (1.4H, brs), 6.37–6.51 (1H, m), 7.15–7.78 (3H, m), 8.72–8.87 (1H, m), 9.08 (0.7H, s), 10.09 (0.3H, s).

5-Chloro-*N*-[(1*S*)-1-(5-chloro-6-methylpyrazolo[1,5-a]-pyrimidin-2-yl)propyl]-2-[(methanesulfonyl)amino]-*N*-methylbenzamide (8c) To a solution of **6c** (1.6 g, 5.12 mmol) in 1,4-dioxane (15 mL) was added 4M hydrogen chloride in 1,4-dioxane (15 mL) and the mixture was stirred for 0.5 h at room temperature. Then, the reaction mixture was concentrated under reduced pressure to obtain (*S*)-6-methyl-2-[1-(methylamino)propyl]pyrazolo[1,5-a]pyrimidin-5(4*H*)-one hydrochloride (1.3 g, 5.90 mmol, quant.) as a colorless powder, which was used for the next reaction without further purification.

The mixture of (*S*)-6-methyl-2-[1-(methylamino)propyl]-pyrazolo[1,5-a]pyrimidin-5(4*H*)-one hydrochloride (1.3 g, 5.90 mmol) and phosphorus oxychloride (30 mL) was stirred at 100°C for 3 h. Then, the reaction mixture was concentrated under reduced pressure to obtain **7c** (1.4 g, 5.91 mmol, quant.) as black oil, which was used for the next reaction without further purification.

To a solution of **7c** (1.4 g, 5.91 mmol) and 5-chloro-2-(methylsulfonamido)benzoic acid (1.8 g, 7.08 mmol) in *N*,*N*-dimethylformamide (15 mL) was added 1-[bis(dimethylamino)-methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (2.9 g, 7.67 mmol) and triethylamine (6.0 mL, 59.0 mmol). After stirring at room temperature for 2 h, the reaction mixture was added to water and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous magnesium sulfate, filtered, concentrated under reduced pressure. The residue was purified by silica gel column chromatography (20–65% ethyl acetate in hexane) to obtain **8c** (0.88 g, 1.88 mmol, 32%) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ : 1.07–1.20 (3H, m), 1.86–2.35 (4H, m), 2.36–2.44 (3H, m), 2.60 (1H, s), 2.78 (2H, s), 2.96 (1H, s), 3.04 (2H, s), 4.69–4.78 (0.5H, m), 6.03–6.13 (0.5H, m), 6.40–6.55 (1H, m), 7.24–7.32 (2H, m), 7.33–7.43 (1H, m), 7.56–7.77 (1H, m), 8.81 (0.5H, s), 8.92 (0.5H, s), 9.08 (0.5H, s), 10.52 (0.5H, brs); MS (ESI/APCI dual) m/z: 470 $[M+H]^+$.

Compound **8d** was obtained by the same procedure as that described for **8c**.

5-Chloro-*N*-[(1*S*)-1-(5-chloro-6-methylpyrazolo[1,5-a]-pyrimidin-2-yl)-2-methylpropyl]-2-[(methanesulfonyl)-amino]-*N*-methylbenzamide (8d) Colorless amorphous; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.95–1.34 (6H, m), 2.35–2.53 (4H, m), 2.55 (1H, s), 2.73 (2H, s), 2.94–3.03 (3H, m), 4.32–4.44 (0.7H, m), 5.78–5.90 (0.3H, m), 6.52–6.63 (1H, m), 7.17–7.50 (2H, m), 7.59–7.74 (1H, m), 8.36–8.49 (0.3H, m), 8.83 (0.7H, brs), 9.00 (0.3H, brs), 10.51–10.81 (0.7H, m); MS (ESI/APCI dual) m/z: 484 $[M+H]^+$.

***N*-[(1*S*)-1-{5-[(3*S*)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}ethyl]-5-chloro-2-[(methanesulfonyl)amino]-*N*-methylbenzamide Hydrochloride (9b)** To a solution of **8b** (0.70 g, 1.23 mmol) and (*S*)-*tert*-butyl pyrrolidin-3-ylcarbamate (0.46 g, 2.46 mmol) in tetrahydrofuran (10 mL) was added trimethylamine (0.87 mL,

6.16 mmol). After stirring at 80°C for 1 h, the reaction mixture was added to water and extracted with chloroform. The organic layer was dried over ISOLUTE® Phase Separator and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (5–100% ethyl acetate in hexane) to obtain *tert*-butyl [(3*S*)-1-(2-{(1*S*)-1-[{5-chloro-2-[(methanesulfonyl)amino]benzoyl}(methyl)amino]ethyl}-6-methylpyrazolo[1,5-a]pyrimidin-5-yl)pyrrolidin-3-yl]carbamate (0.73 g, 1.21 mmol, 98%) as a colorless amorphous. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 1.45 (9H, s), 1.60 (2H, d, J = 7.0 Hz), 1.64 (1H, d, J = 7.0 Hz), 1.86–1.97 (1H, m), 2.16–2.27 (1H, m), 2.35–2.39 (3H, m), 2.63 (1H, s), 2.81 (2H, s), 2.95 (1H, s), 3.03 (2H, s), 3.50–3.58 (1H, m), 3.69–3.76 (1H, m), 3.77–3.85 (1H, m), 3.90–3.97 (1H, m), 4.24–4.34 (1H, m), 4.67 (1H, br s), 4.88 (0.5H, q, J = 7.0 Hz), 5.97–6.04 (1H, m), 6.23 (0.5H, q, J = 7.0 Hz), 7.27–7.31 (1H, m), 7.33–7.40 (1H, m), 7.61 (0.6H, d, J = 8.7 Hz), 7.69 (0.4H, d, J = 8.7 Hz), 8.36 (1H, s), 8.64 (0.5H, s), 9.40 (0.5H, s); MS (ESI/APCI dual) m/z: 606 $[M+H]^+$.

To a solution of *tert*-butyl [(3*S*)-1-(2-{(1*S*)-1-[{5-chloro-2-[(methanesulfonyl)amino]benzoyl}(methyl)amino]ethyl}-6-methylpyrazolo[1,5-a]pyrimidin-5-yl)pyrrolidin-3-yl]carbamate (0.69 g, 1.14 mmol) in 1,4-dioxane (6.9 mL) was added 4M hydrogen chloride in 1,4-dioxane (6.9 mL) and the mixture was stirred for 5 h at room temperature. Then, the reaction mixture was concentrated under reduced pressure to obtain **9b** (0.62 g, 1.14 mmol, 100%) as a colorless powder. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 1.59 (1.8H, d, J = 7.0 Hz), 1.64 (1.2H, d, J = 7.0 Hz), 1.76–1.86 (1H, m), 2.11–2.22 (1H, m), 2.37 (1.2H, s), 2.38 (1.8H, s), 2.62 (1.2H, s), 2.81 (1.8H, s), 2.95 (1.2H, s), 3.03 (1.8H, s), 3.46 (1H, dd, J = 10.9, 4.3 Hz), 3.66–3.78 (2H, m), 3.88 (2H, dd, J = 10.3, 4.5 Hz), 4.88 (0.6H, q, J = 6.6 Hz), 5.98 (0.6H, s), 5.99 (0.4H, s), 6.22 (0.4H, d, J = 7.0 Hz), 7.22–7.32 (1H, m), 7.32–7.40 (1H, m), 7.61 (0.6H, d, J = 8.7 Hz), 7.68 (0.4H, d, J = 8.7 Hz), 8.34 (0.6H, s), 8.61 (0.4H, s); ^{13}C-NMR (151 MHz, DMSO-d_6) δ: 15.63, 17.66, 29.22, 31.35, 40.05, 40.54, 46.73, 48.73, 52.61, 89.81, 107.96, 126.69, 126.77, 129.67, 129.99, 132.40, 133.49, 134.61, 147.03, 155.29, 155.73, 166.67; HR-MS ESI/APCI dual m/z: 506.1711 $[M+H]^+$ (Calcd for $C_{22}H_{28}ClN_7O_3S$: 506.1736).

Compounds **9a, c, d** were obtained by the same procedure as that described for **9b**.

***N*-({5-[(3*S*)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}methyl)-5-chloro-2-[(methanesulfonyl)amino]-*N*-methylbenzamide Hydrochloride (9a)** Colorless powder; ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 2.02–2.13 (1H, m), 2.19–2.30 (1H, m), 2.35 (3H, s), 2.82 (1.5H, s), 2.93 (1.5H, s), 3.00–3.06 (3H, m), 3.71–3.80 (2H, m), 3.82–3.90 (2H, m), 3.90–3.99 (1H, m), 4.37 (1H, s), 4.75 (1H, s), 6.05 (0.5H, s), 6.10 (0.5H, s), 7.42–7.56 (3H, m), 8.33–8.39 (3H, m), 8.45 (0.5H, s), 8.50 (0.5H, s), 9.27 (0.5H, s), 9.65 (0.5H, s); ^{13}C-NMR (151 MHz, DMSO-d_6) δ: 18.05, 29.64, 37.12, 40.47, 45.00, 47.19, 49.12, 53.04, 90.16, 108.93, 126.41, 127.18, 127.54, 130.17, 133.06, 133.59, 135.07, 147.41, 152.54, 155.67, 167.05; HR-MS ESI/APCI dual m/z: 492.1564 $[M+H]^+$ (Calcd for $C_{21}H_{26}ClN_7O_3S$: 492.1579).

***N*-[(1*S*)-1-{5-[(3*S*)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-5-chloro-2-[(methanesulfonyl)amino]-*N*-methylbenzamide Hydrochloride (9c)** Colorless powder; ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 0.91 (1.5H, br s), 1.03 (1.5H, t, J = 7.4 Hz), 1.82–2.11 (3H, m), 2.12–2.30 (2H, m), 2.35 (1.5H, s), 2.37 (1.5H, s), 2.58 (1.5H, s), 2.72 (1.5H, br s), 2.97–3.06 (3H, m), 3.71–3.82 (2H, m), 3.82–3.90 (2H, m), 3.90–3.98 (1H, m), 4.54 (0.5H, dd, J = 9.9, 5.0 Hz), 5.73 (0.5H, dd, J = 10.3, 5.4 Hz), 6.13 (1H, s), 7.36–7.57 (3H, m), 8.34 (3H, br s), 8.51 (0.5H, s), 9.21 (0.5H, s); ^{13}C-NMR (151 MHz, DMSO-d_6) δ: 10.27, 17.24, 21.98, 28.8, 30.83, 39.63, 40.15, 46.32, 48.31, 52.17, 89.59, 107.62, 126.15, 126.63, 129.23, 129.81, 131.78, 133.55, 134.17, 146.53, 154.51, 154.89, 167.01; HR-MS ESI/APCI dual m/z: 520.1873 $[M+H]^+$ (Calcd for $C_{23}H_{30}ClN_7O_3S$: 520.1892).

***N*-[(1*S*)-1-{5-[(3*S*)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}-2-methylpropyl]-5-chloro-2-[(methanesulfonyl)amino]-*N*-methylbenzamide (9d)** Colorless powder; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.73–1.10 (6H, m), 2.00–2.13 (1H, m), 2.19–2.31 (1H, m), 2.31–2.40 (3H, m), 2.60–2.81 (3H, m), 2.92–3.05 (3H, m), 3.69–3.99 (5H, m), 4.09–4.21 (0.5H, m), 5.42–5.52 (0.5H, m), 6.13 (0.5H, s), 6.26 (0.5H, br s), 7.21–7.61 (3H, m), 8.27–8.40 (3H, m), 8.50 (0.5H, s), 8.99 (0.5H, s); ^{13}C-NMR (151 MHz, DMSO-d_6) δ: 17.53, 19.36, 20.55, 27.56, 29.21, 31.74, 40.75, 46.76, 48.72, 52.59, 57.27, 90.95, 108.27, 126.52, 128.73, 129.58, 130.68, 132.17, 134.60, 135.35, 146.34, 153.92, 155.28, 167.06; HR-MS ESI/APCI dual m/z: 534.2023 $[M+H]^+$ (Calcd for $C_{24}H_{32}ClN_7O_3S$: 534.2049).

***tert*-Butyl [(3*S*)-1-{2-[(1*S*)-1-Aminopropyl]-6-methylpyrazolo[1,5-a]pyrimidin-5-yl}pyrrolidin-3-yl]carbamate (10a)** To a solution of **6e** (5.4 g, 17.6 mmol) in methanol (30 mL) was added 4M hydrogen chloride in 1,4-dioxane (30 mL) and the mixture was stirred for 2 h at room temperature. Then, the reaction mixture was concentrated under reduced pressure to obtain 2-[(1*S*)-1-aminopropyl]-6-methylpyrazolo[1,5-a]-pyrimidin-5(4*H*)-one hydrochloride (4.7 g) as a brown powder, which was used for the next reaction without further purification.

The mixture of 2-[(1*S*)-1-aminopropyl]-6-methylpyrazolo-[1,5-a]pyrimidin-5(4*H*)-one hydrochloride (4.5 g, 18.5 mmol) and phosphorus oxychloride (85 g, 556 mmol) was stirred at 100°C for 2 h. Then, the reaction mixture was concentrated under reduced pressure to obtain (1*S*)-1-(5-chloro-6-methylpyrazolo[1,5-a]pyrimidin-2-yl)propan-1-amine (**7e**) (5.0 g, 19.2 mmol) as a brown amorphous, which was used for the next reaction without further purification.

To a solution of **7e** (4.8 g, 18.4 mmol) in methanol (100 mL) was added (*S*)-*tert*-butyl pyrrolidin-3-ylcarbamate (17.1 g, 91.9 mmol) and trimethylamine (20 mL, 147 mmol). After stirring at 65°C for 4 h, the reaction mixture was poured into water and extracted with chloroform. The organic layer was washed with brine and dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (50–100% ethyl acetate in hexane; 20% methanol in chloroform) to obtain **10a** (4.5 g, 12.0 mmol, 65%) as a brown amorphous. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 0.88–0.99 (3H, m), 1.45 (9H, s), 1.72–1.97 (3H, m), 2.16–2.25 (1H, m), 2.33 (3H, s), 3.53 (1H, dd, J = 10.7, 3.7 Hz), 3.71 (1H, ddd, J = 10.7, 7.8, 5.8 Hz), 3.80 (1H, dt, J = 10.7, 7.4 Hz), 3.88–3.97 (2H, m), 4.30 (1H, br s), 4.71 (1H, br s), 5.91–6.09 (1H, m), 8.02 (1H, s); MS (ESI/APCI dual) m/z: 375 $[M+H]^+$.

Compound **10b** was obtained by the same procedure as that described for **10a**.

***tert*-Butyl [(3*S*)-1-{2-[(1*S*)-1-Aminoethyl]-6-methylpyrazolo[1,5-a]pyrimidin-5-yl}pyrrolidin-3-yl]carbamate (10b)** Pale

yellow powder; ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 1.41–1.50 (12H, m), 1.89–1.95 (1H, m), 2.18–2.24 (1H, m), 2.33 (3H, m), 3.53 (1H, dd, J = 11.4, 3.9 Hz), 3.68–3.74 (1H, m), 3.77–3.83 (1H, m), 3.88–3.95 (1H, m), 4.20 (1H, q, J = 6.9 Hz), 4.30 (1H, brs), 4.69 (1H, brs), 6.02 (1H, s), 8.02 (1H, s); MS (ESI/APCI dual) m/z: 361 $[M+H]^+$.

***N*-[(1*S*)-1-{5-[(3*S*)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-5-chloro-*N*-ethyl-2-[(methanesulfonyl)amino]benzamide Hydrochloride (12a)** To a solution of **10a** (50 mg, 0.134 mmol) in *N*,*N*-dimethylformamide (1.0 mL) was added potassium carbonate (46 mg, 0.334 mmol) and iodoethane (0.011 mL, 0.134 mmol). After stirring at 80°C for 1 h, 5-chloro-2-(methylsulfonamido)benzoic acid (40 mg, 0.160 mmol), 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (66 mg, 0.174 mmol) and trimethylamine (0.093 mL, 0.668 mmol) were added to the reaction mixture. After stirring at room temperature for 16 h, the reaction mixture was purified by reversed-phase preparative HPLC to obtain *tert*-butyl [(3*S*)-1-(2-{(1*S*)-1-[{5-chloro-2-[(methanesulfonyl)amino]benzoyl}(ethyl)amino]propyl}-6-methylpyrazolo[1,5-a]pyrimidin-5-yl)pyrrolidin-3-yl]carbamate (13 mg, 0.021 mmol, 15%) as colorless powder.

To a solution of *tert*-butyl [(3*S*)-1-(2-{(1*S*)-1-[{5-chloro-2-[(methanesulfonyl)amino]benzoyl}(ethyl)amino]propyl}-6-methylpyrazolo[1,5-a]pyrimidin-5-yl)pyrrolidin-3-yl]carbamate (16 mg, 0.025 mmol) in 1,4-dioxane (1.0 mL) was added 4M hydrogen chloride in 1,4-dioxane (1.0 mL) and the mixture was stirred for 1 h at room temperature. Then, the reaction mixture was concentrated under reduced pressure to obtain **12a** (15 mg, 0.026 mmol, 100%) as a colorless powder. ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 0.80–0.98 (6H, m), 1.96–2.30 (4H, m), 2.32–2.40 (3H, m), 2.51–2.64 (3H, m), 2.98–3.05 (2H, m), 3.69–3.97 (5H, m), 4.47–4.55 (1H, m), 6.15–6.21 (1H, m), 7.45–7.56 (3H, m), 8.21 (3H, brs), 8.52 (0.5H, s), 9.20 (0.5H, s); ^{13}C-NMR (151 MHz, DMSO-d_6) δ: 10.85, 13.44, 17.86, 29.22, 36.84, 40.05, 40.80, 46.70, 48.73, 52.57, 58.64, 90.56, 107.98, 126.35, 127.32, 127.70, 129.49, 129.61, 133.63, 134.55, 146.97, 154.69, 155.59, 161.22; HR-MS ESI/APCI dual m/z: 534.2037 $[M+H]^+$ (Calcd for $C_{24}H_{32}ClN_7O_3S$: 534.2049).

Compound **12b** was obtained by the same procedure as that described for **12a**.

***N*-[(1*S*)-1-{5-[(3*S*)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}ethyl]-5-chloro-2-[(methanesulfonyl)amino]-*N*-propylbenzamide Hydrochloride (12b)** Colorless powder; ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 0.53 (1H, t, J = 7.2 Hz), 0.77 (2H, t, J = 7.4 Hz), 1.21–1.54 (4H, m), 1.57 (2H, d, J = 7.0 Hz), 1.66 (1H, d, J = 7.0 Hz), 2.00–2.10 (1H, m), 2.20–2.30 (1H, m), 2.34–2.38 (3H, m), 2.85–2.98 (2H, m), 3.04 (3H, s), 3.81–3.96 (5H, m), 4.74–4.82 (0.7H, m), 5.73 (0.3H, brs), 6.13 (1H, s), 7.43–7.58 (3H, m), 8.18 (3H, brs), 8.52 (0.5H, s), 9.34 (0.5H, s); ^{13}C-NMR (151 MHz, DMSO-d_6) δ: 11.49, 17.04, 17.76, 21.44, 29.22, 40.68, 43.95, 46.72, 48.71, 52.59, 52.71, 89.79, 107.84, 125.19, 126.43, 127.48, 129.53, 129.61, 132.18, 134.35, 146.95, 155.45, 156.56, 166.85; HR-MS ESI/APCI dual m/z: 534.2011 $[M+H]^+$ (Calcd for $C_{24}H_{32}ClN_7O_3S$: 534.2049).

***tert*-Butyl [(3*S*)-1-{6-Methyl-2-[(1*S*)-1-(methylamino)propyl]pyrazolo[1,5-a]pyrimidin-5-yl}pyrrolidin-3-yl]carbamate (13a)** To a solution of **10a** (4.3 g, 11.4 mmol) in methanol (48 mL) was added ethyl 2,2,2-trifluoroacetate (2.0 g, 14.8 mmol) and trimethylamine (2.5 mL, 18.2 mmol). After stirring overnight at room temperature, the reaction mixture was concentrated under reduced pressure to obtain *tert*-butyl [(3*S*)-1-{6-methyl-2-[(1*S*)-1-(2,2,2-trifluoroacetamido)propyl]pyrazolo[1,5-a]pyrimidin-5-yl}pyrrolidin-3-yl]carbamate (6.5 g, crude) as a pale yellow powder, which was used for the next reaction without further purification.

To a solution of *tert*-butyl [(3*S*)-1-{6-methyl-2-[(1S)-1-(2,2,2-trifluoroacetamido)propyl]pyrazolo[1,5-a]pyrimidin-5-yl}pyrrolidin-3-yl]carbamate (5.4 g, 11.5 mmol) in *N*,*N*-dimethylformamide (50 mL) was added iodomethane (2.2 mL, 34.4 mmol) and cesium carbonate (15 g, 45.9 mmol). After stirring at 65°C for 2 h, the reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine and dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure to obtain *tert*-butyl [(3*S*)-1-(6-methyl-2-{(1*S*)-1-[methyl(trifluoroacetyl)amino]propyl}pyrazolo[1,5-a]pyrimidin-5-yl)pyrrolidin-3-yl]carbamate (5.60 g, crude) as a pale yellow oil, which was used for the next reaction without further purification.

To a solution of *tert*-butyl [(3*S*)-1-(6-methyl-2-{(1*S*)-1-[methyl(trifluoroacetyl)amino]propyl}pyrazolo[1,5-a]pyrimidin-5-yl)pyrrolidin-3-yl]carbamate (5.5 g, 11.4 mmol) in tetrahydrofuran (40 mL)–methanol (40 mL) was added 1M sodium hydroxide aq. (40 mL). After stirring for 1 h at room temperature, the reaction mixture was poured into water and extracted with chloroform. The organic layer was washed with brine, dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure to obtain **13a** (4.3 g, 11.1 mmol, 98%) as a brown amorphous. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 0.88 (3H, t, J = 7.4 Hz), 1.45 (9H, s), 1.68–1.98 (3H, m), 2.14–2.25 (1H, m), 2.28–2.42 (6H, m), 3.48–3.63 (2H, m), 3.66–3.85 (2H, m), 3.87–3.98 (1H, m), 4.30 (1H, brs), 4.59–4.78 (1H, m), 5.91–6.07 (1H, m), 7.96–8.10 (1H, m); MS (ESI/APCI dual) m/z: 389 $[M+H]^+$.

Compound **13b** was obtained by the same procedure as that described for **13a**.

Benzyl [(3*S*)-1-{6-Methyl-2-[(1*S*)-1-(methylamino)propyl]pyrazolo[1,5-a]pyrimidin-5-yl}pyrrolidin-3-yl]carbamate (13b) Colorless powder; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.82–0.94 (3H, m), 1.70–1.90 (2H, m), 1.90–2.01 (1H, m), 2.17–2.29 (1H, m), 2.32 (3H, s), 2.36 (3H, s), 3.53–3.62 (2H, m), 3.66–3.87 (2H, m), 3.88–4.00 (1H, m), 4.30–4.41 (1H, m), 4.86–4.97 (1H, m), 5.12 (2H, s), 6.03 (1H, s), 7.28–7.41 (5H, m), 8.04 (1H, s); MS (ESI/APCI dual) m/z: 423 $[M+H]^+$.

***N*-[(1*S*)-1-{5-[(3*S*)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-3-chloro-2-[(methanesulfonyl)amino]-*N*-methylbenzamide Hydrochloride (14a)** To a solution of **13a** (10 mg, 0.026 mmol) in *N*,*N*-dimethylformamide (1.0 mL) was added 3-chloro-2-(methylsulfonamido)benzoic acid (6.4 mg, 0.031 mmol), 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (13 mg, 0.034 mmol), and trimethylamine (0.018 mL, 0.13 mmol). After stirring at room temperature for 3 h, the reaction mixture was purified by reversed-phase preparative HPLC to obtain *tert*-butyl [(*S*)-1-(2-{(*S*)-1-[3-chloro-*N*-methyl-2-(methylsulfonamido)benzamido]propyl}-6-methylpyrazolo[1,5-a]pyrimidin-5-yl)pyrrolidin-3-yl]carbamate (12 mg, 0.019 mmol, 72%) as a pale yellow powder.

To a solution of *tert*-butyl [(*S*)-1-(2-{(*S*)-1-[3-chloro-

N-methyl-2-(methylsulfonamido)benzamido]propyl}-6-methylpyrazolo[1,5-a]pyrimidin-5-yl)pyrrolidin-3-yl]carbamate (12 mg, 0.019 mmol) in 1,4-dioxane(1.0 mL) was added 4M hydrogen chloride in 1,4-dioxane (1.0 mL) and the mixture was stirred for 1 h at room temperature. Then, the reaction mixture was concentrated under reduced pressure to obtain **14a** (10 mg, 0.019 mmol, 100%) as a colorless powder. ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 0.79–1.05 (3H, m), 1.84–2.30 (4H, m), 2.33–2.42 (3H, m), 2.43–2.59 (3H, m), 2.98–3.17 (3H, m), 3.68–4.01 (5H, m), 4.37 (0.5H, brs), 5.75 (0.5H, brs), 6.06–6.25 (1H, m), 7.23–7.67 (3H, m), 8.17–8.27 (3H, m), 8.51 (0.5H, s), 9.43 (0.5H, brs); ^{13}C-NMR (151 MHz, DMSO-d_6) δ: 10.43, 17.48, 22.81, 29.28, 31.23, 40.05, 42.69, 46.68, 48.77, 52.55, 90.10, 108.14, 125.89, 128.84, 129.39, 130.20, 130.25, 131.90, 134.53, 146.89, 151.11, 154.89, 166.59; HR-MS ESI/APCI dual *m/z*: 520.1872 $[M+H]^+$ (Calcd for $C_{23}H_{30}ClN_7O_3S$: 520.1892).

Compounds **14c–e, h–l, o, p, r, s** were obtained by the same procedure as that described for **14a**.

***N*-[(1*S*)-1-{5-[(3*S*)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-2-[(methanesulfonyl)-amino]-*N*-methylbenzamide Hydrochloride (14c)** Colorless powder; ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 0.85–1.06 (3H, m), 1.86–2.30 (4H, m), 2.33–2.40 (3H, m), 2.57 (1.5H, s), 2.73 (1.5H, brs), 3.02 (3H, s), 3.76 (2H, brs), 3.82–3.89 (2H, m), 3.90–3.99 (1H, m), 4.55 (0.5H, brs), 5.77 (0.5H, dd, J = 9.7, 5.6 Hz), 6.12 (1H, s), 7.26–7.39 (2H, m), 7.41–7.52 (2H, m), 8.39 (3H, brs), 8.51 (0.5H, s), 9.08 (0.5H, s); ^{13}C-NMR (151 MHz, DMSO-d_6) δ: 10.71, 17.70, 22.43, 29.24, 31.31, 40.47, 46.81, 48.69, 52.63, 58.26, 89.95, 108.04, 125.11, 125.91, 127.07, 127.70, 129.81, 133.33, 134.73, 146.83, 155.15, 155.47, 169.04; HR-MS ESI/APCI dual *m/z*: 486.2257 $[M+H]^+$ (Calcd for $C_{23}H_{31}N_7O_3S$: 486.2282).

***N*-[(1*S*)-1-{5-[(3*S*)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-*N*-methylbenzamide Hydrochloride (14d)** Colorless powder; ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 0.75–0.85 (2H, m), 0.95–1.03 (1H, m), 1.85–2.13 (3H, m), 2.20–2.29 (1H, m), 2.36 (3H, s), 2.65 (1H, s), 2.77 (2H, s), 3.72–3.80 (2H, m), 3.82–3.88 (2H, m), 3.91–3.97 (1H, m), 4.67–4.73 (0.5H, m), 5.72–5.81 (0.5H, m), 6.04 (1H, s), 7.37–7.51 (5H, m), 8.43 (3H, brs), 8.51–8.59 (1H, m); ^{13}C-NMR (151 MHz, DMSO-d_6) δ: 10.51, 17.44, 23.49, 29.22, 40.05, 46.72, 48.73, 52.57, 58.40, 89.61, 108.32, 126.61, 128.38, 129.14, 134.65, 136.92, 146.71, 155.11, 155.35, 171.21; HR-MS ESI/APCI dual *m/z*: 393.2393 $[M+H]^+$ (Calcd for $C_{22}H_{28}N_6O$: 393.2397).

***N*-[(1*S*)-1-{5-[(3*S*)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-2-[(methanesulfonyl)-amino]-*N*,5-dimethylbenzamide Hydrochloride (14e)** Colorless powder; ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 0.86–0.95 (1.5H, m), 0.99–1.06 (1.5H, m), 1.84–2.29 (4H, m), 2.29–2.38 (6H, m), 2.56 (1.5H, s), 2.72 (1.5H, brs), 2.96 (3H, s), 3.70–4.10 (5H, m), 4.51–4.58 (0.5H, m), 5.74–5.80 (0.5H, m), 6.11 (1H, s), 7.14 (1H, s), 7.22–7.29 (1H, m), 7.31–7.38 (1H, m), 8.35 (3H, brs), 8.51 (0.5H, s), 8.97 (0.5H, s); ^{13}C-NMR (151 MHz, DMSO-d_6) δ: 10.68, 17.63, 20.31, 22.38, 29.21, 31.20, 40.04, 40.28, 46.74, 48.68, 52.6, 89.92, 107.99, 125.64, 127.10, 130.22, 130.52, 132.59, 134.60, 135.69, 146.82, 155.14, 155.26, 169.05; HR-MS ESI/APCI dual *m/z*: 500.2414 $[M+H]^+$ (Calcd for $C_{24}H_{33}N_7O_3S$: 500.2438).

***N*-[(1*S*)-1-{5-[(3*S*)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-2-[(methanesulfonyl)-amino]-*N*-methyl-5-propylbenzamide Hydrochloride (14h)** Colorless powder; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.81–1.06 (6H, m), 1.51–1.64 (2H, m), 1.83–2.31 (6H, m), 2.32–2.38 (3H, m), 2.57 (1.5H, s), 2.72 (1.5H, brs), 2.98 (3H, s), 3.45–3.96 (5H, m), 4.48–4.58 (0.5H, m), 5.73–5.81 (0.5H, m), 6.11 (1H, s), 7.14 (1H, s), 7.22–7.31 (1H, m), 7.33–7.41 (1H, m), 8.19–8.35 (3H, m), 8.51 (0.5H, s), 8.95 (0.5H, s); ^{13}C-NMR (151 MHz, DMSO-d_6) δ: 10.72, 13.53, 17.63, 22.40, 23.92, 29.21, 31.22, 36.32, 40.04, 40.34, 46.71, 48.70, 52.56, 89.94, 107.99, 125.54, 126.54, 127.33, 129.56, 130.80, 133.88, 140.55, 146.96, 154.66, 155.58, 169.15; HR-MS ESI/APCI dual *m/z*: 528.2731 $[M+H]^+$ (Calcd for $C_{26}H_{37}N_7O_3S$: 528.2751).

***N*-[(1*S*)-1-{5-[(3*S*)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-2-[(methanesulfonyl)-amino]-*N*-methyl-5-(propan-2-yl)benzamide Hydrochloride (14i)** Pale pink amorphous; ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 0.87–0.96 (1.5H, m), 1.00–1.07 (1.5H, m), 1.13–1.24 (6H, m), 1.84–2.30 (4H, m), 2.34–2.38 (3H, m), 2.56 (1.5H, s), 2.72 (1.5H, brs), 2.86–3.02 (4H, m), 3.70–4.07 (5H, m), 4.47–4.56 (0.5H, m), 5.73–5.81 (0.5H, m), 6.12 (1H, s), 7.13–7.24 (1H, m), 7.29–7.40 (2H, m), 8.36 (3H, brs), 8.51 (0.5H, s), 8.98 (0.5H, s); ^{13}C-NMR (151 MHz, DMSO-d_6) δ: 10.52, 17.65, 22.44, 23.66, 23.72, 29.23, 31.22, 32.71, 40.04, 40.42, 46.72, 48.70, 52.58, 89.98, 108.01, 124.63, 125.26, 125.72, 127.45, 130.82, 132.51, 134.58, 146.94, 155.14, 155.52, 169.19; HR-MS ESI/APCI dual *m/z*: 528.2740 $[M+H]^+$ (Calcd for $C_{26}H_{37}N_7O_3S$: 528.2751).

***N*-[(1*S*)-1-{5-[(3*S*)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-2-(methanesulfonyl)-*N*-methylbenzamide Hydrochloride (14j)** Colorless powder; ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 0.98–1.11 (3H, m), 1.85–1.98 (1H, m), 2.01–2.21 (2H, m), 2.21–2.29 (1H, m), 2.31–2.41 (3H, m), 3.23–3.41 (6H, m), 3.69–4.08 (5H, m), 5.68–5.82 (1H, m), 6.03–6.23 (1H, m), 7.38–7.64 (1H, m), 7.64–7.88 (3H, m), 7.97–8.07 (1H, m), 8.27–8.41 (3H, m), 8.50–8.61 (1H, m); ^{13}C-NMR (151 MHz, DMSO-d_6) δ: 10.76, 17.47, 22.24, 24.39, 29.21, 31.72, 45.01, 46.94, 48.72, 52.74, 90.57, 108.19, 127.55, 129.09, 129.80, 133.60, 134.22, 134.84, 136.83, 137.66, 154.84, 155.04, 169.11; HR-MS ESI/APCI dual *m/z*: 471.2179 $[M+H]^+$ (Calcd for $C_{23}H_{30}N_6O_3S$: 471.2173).

***N*-[(1*S*)-1-{5-[(3*S*)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-2-methoxy-*N*,5-dimethylbenzamide Hydrochloride (14k)** Colorless amorphous; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.72–0.89 (1.5H, m), 0.92–1.05 (1.5H, m), 1.77–1.95 (1H, m), 2.00–2.17 (2H, m), 2.18–2.31 (4H, m), 2.31–2.40 (3H, m), 3.35–3.67 (3H, m), 3.70–4.05 (8H, m), 4.41–4.49 (0.5H, m), 5.74–5.84 (0.5H, m), 5.88–6.05 (1H, m), 6.84–7.08 (2H, m), 7.13–7.23 (1H, m), 8.43–8.59 (4H, m); ^{13}C-NMR (151 MHz, DMSO-d_6) δ: 10.76, 17.47, 19.86, 23.78, 29.21, 30.05, 46.80, 48.74, 52.64, 55.18, 60.10, 89.78, 108.25, 111.31, 126.56, 129.58, 130.24, 130.30, 134.72, 134.84, 138.28, 152.55, 168.37, 171.74; HR-MS ESI/APCI dual *m/z*: 437.2639 $[M+H]_+$ (Calcd for $C_{24}H_{32}N_6O_2$: 437.2660).

***N*-[(1*S*)-1-{5-[(3*S*)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-*N*,5-dimethyl-2-(trifluoromethyl)benzamide Hydrochloride (14l)** Colorless powder; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.72–1.06 (3H, m), 1.75–2.30 (4H, m), 2.31–2.46 (6H, m), 3.70–3.99 (5H, m), 4.37–4.43 (0.5H, m), 5.73–5.81 (0.5H, m), 5.82–6.13 (1H, m), 7.18–7.50 (2H, m), 7.65–7.74 (1H, m), 8.33–8.47 (3H, m),

8.50–8.58 (1H, m); ^{13}C-NMR (151 MHz, DMSO-d_6) δ: 10.81, 17.46, 20.70, 22.29, 29.22, 31.25, 46.89, 48.71, 52.71, 58.60, 89.91, 108.14, 121.93, 122.15, 123.02, 124.84, 126.41, 127.58, 128.46, 129.71, 129.91, 134.71, 134.79, 135.52, 142.39, 143.37, 146.45, 154.81, 155.17, 168.11; HR-MS ESI/APCI dual *m/z*: 475.2402 $[M+H]^+$ (Calcd for $C_{24}H_{29}F_3N_6O$: 475.2428).

***N*-[(1*S*)-1-{5-[(3*S*)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-3-[(methanesulfonyl)amino]-*N*-methylthiophene-2-carboxamide Hydrochloride (14o)** Colorless powder; ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 0.88–0.97 (3H, m), 1.89–2.29 (4H, m), 2.35 (3H, s), 2.81 (3H, brs), 3.06 (3H, brs), 3.69–3.96 (5H, m), 6.09 (1H, s), 7.16 (1H, s), 7.76 (1H, s), 8.23 (3H, s), 8.47 (1H, s), 10.11 (1H, s); ^{13}C-NMR (151 MHz, DMSO-d_6) δ: 10.63, 17.58, 22.95, 29.24, 35.23, 40.29, 46.68, 48.75, 52.55, 58.16, 90.22, 110.98, 119.30, 122.85, 128.06, 128.18, 134.41, 146.89, 154.71, 155.43, 164.07; HR-MS ESI/APCI dual *m/z*: 492.1808 $[M+H]^+$ (Calcd for $C_{21}H_{29}N_7O_3S_2$: 492.1846).

***N*-[(1*S*)-1-{5-[(3*S*)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-2-[(methanesulfonyl)amino]-*N*-methylthiophene-3-carboxamide Hydrochloride (14p)** Colorless powder; ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 0.78–1.02 (3H, m), 1.84–2.33 (4H, m), 2.35 (3H, s), 2.63–2.75 (3H, m), 3.01 (3H, s), 3.68–4.17 (5H, m), 4.61–4.73 (0.5H, m), 5.65–5.78 (0.5H, m), 6.05–6.20 (1H, m), 7.00 (1H, s), 7.39 (1H, s), 8.25 (3H, brs), 8.36–8.55 (1H, m), 9.87 (0.5H, brs), 10.62 (0.5H, brs); ^{13}C-NMR (151 MHz, DMSO-d_6) δ: 10.54, 17.57, 22.86, 29.21, 31.00, 40.04, 40.63, 46.72, 48.70, 52.58, 90.37, 108.01, 120.51, 125.14, 132.95, 134.10, 136.23, 146.72, 152.13, 155.28, 165.59; HR-MS ESI/APCI dual *m/z*: 492.1812 $[M+H]^+$ (Calcd for $C_{21}H_{29}N_7O_3S_2$: 492.1846).

***N*-[(1*S*)-1-{5-[(3*S*)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-4-[(methanesulfonyl)amino]-*N*,1-dimethyl-1*H*-pyrazole-5-carboxamide Hydrochloride (14r)** Colorless powder; ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 0.80–0.94 (1.5H, m), 0.95–1.05 (1.5H, m), 1.84–2.29 (4H, m), 2.33–2.37 (3H, m), 2.63–2.80 (3H, m), 2.81–2.96 (3H, m), 3.70–3.80 (5H, m), 3.81–3.97 (3H, m), 4.61–4.75 (0.5H, m), 5.67–5.75 (0.5H, m), 6.05–6.10 (0.5H, m), 7.47 (1H, s), 8.21–8.30 (3.5H, m), 8.47–8.54 (0.5H, m), 9.07 (0.5h, brs); ^{13}C-NMR (151 MHz, DMSO-d_6) δ: 10.70, 17.43, 24.04, 29.21, 30.09, 37.95, 40.02, 40.36, 46.69, 48.70, 52.54, 90.05, 108.17, 116.35, 119.53, 131.63, 134.50, 146.86, 154.46, 155.36, 161.29; HR-MS ESI/APCI dual *m/z*: 490.2321 $[M+H]^+$ (Calcd for $C_{21}H_{31}N_9O_3S$: 490.2343).

***N*-[(1*S*)-1-{5-[(3*S*)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-4-[(methanesulfonyl)amino]-*N*,1-dimethyl-1*H*-pyrazole-3-carboxamide Hydrochloride (14s)** Colorless powder; ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 0.86–0.95 (3H, m), 1.82–2.28 (4H, m), 2.32 (3H, s), 2.71 (1.5H, s), 2.92 (1.5H, s), 2.96 (3H, s), 3.68–3.76 (2H, m), 3.79–3.94 (6H, m), 5.73–5.78 (0.5H, m), 5.87–5.93 (0.5H, m), 6.03 (0.5H, s), 6.07 (0.5H, s), 7.78–7.82 (1H, m), 8.31 (3H, brs), 8.44 (0.5H, s), 8.49 (0.5H, s), 9.00 (0.5H, s), 9.15 (0.5H, s); ^{13}C-NMR (151 MHz, DMSO-d_6) δ: 10.45, 17.48, 22.43, 29.20, 31.19, 38.93, 40.05, 46.83, 48.73, 52.67, 56.81, 90.12, 108.28, 120.50, 125.79, 134.71, 138.31, 146.35, 155.13, 155.19, 163.51; HR-MS ESI/APCI dual *m/z*: 490.2316 $[M+H]^+$ (Calcd for $C_{21}H_{31}N_9O_3S$: 490.2343).

***N*-[(1*S*)-1-{5-[(3*S*)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-4-chloro-2-[(methanesulfonyl)amino]-*N*-methylbenzamide Hydrochloride (14b)** To a solution of **13a** (50 mg, 0.13 mmol) in *N*,*N*-dimethylformamide (1.0 mL) was added 4-chloroanthranilic acid (27 mg, 0.15 mmol), 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (64 mg, 0.17 mmol) and trimethylamine (0.090 mL, 0.64 mmol). After stirring at room temperature for 3 h, the reaction mixture was purified by reversed-phase preparative HPLC to obtain *tert*-butyl ((*S*)-1-{2-[(*S*)-1-(2-amino-4-chloro-*N*-methylbenzamido)propyl]-6-methylpyrazolo[1,5-a]-pyrimidin-5-yl}pyrrolidin-3-yl)carbamate (52 mg, 0.10 mmol, 75%) as a colorless powder.

To a solution of *tert*-butyl ((*S*)-1-{2-[(*S*)-1-(2-amino-4-chloro-*N*-methylbenzamido)propyl]-6-methylpyrazolo[1,5-a]-pyrimidin-5-yl}pyrrolidin-3-yl)carbamate (52 mg, 0.10 mmol) in chloroform (2.0 mL) was added trimethylamine (0.14 mL, 0.97 mmol) and methanesulfonyl chloride (0.022 mL, 0.29 mmol) at 0°C. After stirring for 10 min at 0°C, the reaction mixture was concentrated under reduced pressure. Ethanol (2.0 mL) and 2.94 mol/L sodium ethoxide in ethanol (0.16 mL, 0.48 mmol) were added to the residue. After stirring at room temperature for 15 min, the reaction mixture was poured into water and extracted with chloroform. The organic layer was dried over ISOLUTE® Phase Separator and concentrated under reduced pressure. The residue was purified by reversed-phase preparative HPLC to obtain *tert*-butyl [(*S*)-1-(2-{(*S*)-1-[4-chloro-*N*-methyl-2-(methylsulfonamido)-benzamido]propyl}-6-methylpyrazolo[1,5-a]pyrimidin-5-yl)-pyrrolidin-3-yl]carbamate (40 mg, 0.065 mmol, 67%) as a colorless powder.

To a solution of *tert*-butyl [(*S*)-1-(2-{(*S*)-1-[4-chloro-*N*-methyl-2-(methylsulfonamido)benzamido]propyl}-6-methylpyrazolo[1,5-a]-pyrimidin-5-yl)pyrrolidin-3-yl]carbamate (40 mg, 0.065 mmol) in 1,4-dioxane (1.0 mL) was added 4M hydrogen chloride in 1,4-dioxane (1.0 mL) and the mixture was stirred for 1 h at room temperature. Then the reaction mixture was concentrated under reduced pressure to obtain **14b** (36 mg, 0.063 mmol, 98%) as a colorless powder. ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 0.86–0.92 (1.5H, m), 1.02 (1.5H, t, J = 7.2 Hz), 1.86–2.30 (4H, m), 2.33–2.38 (3H, m), 2.58 (1.5H, s), 2.73 (1.5H, brs), 3.01–3.12 (3H, m), 3.68–4.15 (5H, m), 4.56 (0.5H, dd, J = 9.9, 4.5 Hz), 5.73 (0.5H, dd, J = 10.1, 5.6 Hz), 6.11 (1H, s), 7.39 (2H, s), 7.49 (1H, d, J = 11.6 Hz), 8.23 (3H, brs), 8.51 (0.5H, s), 9.28 (0.5H, s); ^{13}C-NMR (151 MHz, DMSO-d_6) δ: 10.70, 17.65, 22.40, 29.23, 31.34, 40.05, 40.77, 46.72, 48.70, 52.60, 89.98, 108.03, 124.17, 125.66, 128.83, 129.32, 130.36, 133.88, 134.58, 146.98, 154.96, 155.54, 168.14; HR-MS ESI/APCI dual *m/z*: 520.1872 $[M+H]^+$ (Calcd for $C_{23}H_{30}ClN_7O_3S$: 520.1892).

Compounds **14f, g, m, n, q** were obtained by the same procedure as that described for **14b**.

***N*-[(1*S*)-1-{5-[(3*S*)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-5-ethyl-2-[(methanesulfonyl)amino]-*N*-methylbenzamide Hydrochloride (14f)** Colorless powder; ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 0.86–0.95 (1.5H, m), 1.00–1.06 (1.5H, m), 1.14–1.22 (3H, m), 1.84–2.29 (4H, m), 2.33–2.38 (3H, m), 2.57 (1.5H, s), 2.58–2.66 (2H, m), 2.72 (1.5H, brs), 2.97 (3H, s), 3.71–3.99 (5H, m), 4.50–4.57 (0.5H, m), 5.74–5.80 (0.5H, m), 6.12 (1H, s), 7.16 (1H, brs), 7.26–7.32 (1H, m), 7.34–7.40 (1H, m), 8.35 (3H, brs), 8.51 (0.5H, s), 8.98 (0.5H, s); ^{13}C-NMR (151 MHz, DMSO-d_6) δ: 10.51, 15.41, 17.64, 22.41, 27.37, 29.22, 31.23, 40.05, 40.35, 46.73, 48.69,

52.59, 89.95, 108.00, 125.69, 126.77, 129.02, 130.73, 132.58, 134.57, 141.87, 146.93, 155.15, 155.53, 169.12; HR-MS ESI/APCI dual *m/z*: 514.2559 $[M+H]^+$ (Calcd for $C_{25}H_{35}N_7O_3S$: 514.2595); chiral HPLC, 99% *ee* (CHIRALCEL OZ-3 5 μm 4.6 mm × 150 mm; flow, 1 mL/min, 60% ethanol in hexane; detection wavelength, 254 nm), (*S*)-isomer $t_R = 9.37$ min, (*R*)-isomer $t_R = 7.31$ min.

***N*-[(1*S*)-1-{5-[(3*S*)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-2-[(methanesulfonyl)amino]-5-methoxy-*N*-methylbenzamide Hydrochloride (14g)** Colorless powder; ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 0.85–0.97 (1.5H, m), 0.99–1.06 (1.5H, m), 1.82–2.30 (4H, m), 2.33–2.38 (3H, m), 2.56 (1.5H, s), 2.71 (1.5H, brs), 2.87–2.99 (3H, m), 3.72–3.81 (5H, m), 3.82–3.89 (2H, m), 3.90–3.98 (1H, m), 4.47–4.58 (0.5H, m), 5.73–5.80 (0.5H, m), 6.09–6.16 (1H, m), 6.78–6.92 (1H, m), 6.97–7.06 (1H, m), 7.31–7.39 (1H, m), 8.36 (3H, brs), 8.51 (0.5H, s), 8.96 (0.5H, s); ^{13}C-NMR (151 MHz, DMSO-d_6) δ: 10.73, 17.62, 22.47, 29.22, 31.13, 40.05, 40.21, 46.72, 48.69, 52.59, 55.55, 89.99, 108.04, 111.66, 112.38, 115.00, 125.39, 128.76, 134.55, 135.23, 146.93, 155.33, 157.56, 168.62; HR-MS ESI/APCI dual *m/z*: 516.2378 $[M+H]^+$ (Calcd for $C_{24}H_{33}N_7O_4S$: 516.2387).

***N*-[(1*S*)-1-{5-[(3*S*)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-3-[(methanesulfonyl)amino]-*N*-methylpyridine-4-carboxamide Hydrochloride (14m)** Colorless powder; ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 0.84–0.93 (1.5H, m), 0.97–1.05 (1.5H, m), 1.86–2.30 (4H, m), 2.34–2.39 (3H, m), 2.58 (1.5H, s), 2.75 (1.5H, brs), 3.05–3.12 (3H, m), 3.69–3.99 (5H, m), 4.44–4.49 (0.5H, m), 5.70–5.77 (0.5H, m), 6.12–6.17 (1H, m), 7.44–7.50 (1H, m), 8.27–8.33 (3H, m), 8.34–8.38 (0.5H, m), 8.51–8.53 (0.5H, m), 8.53–8.57 (1H, m), 8.66–8.72 (1H, m); ^{13}C-NMR (151 MHz, DMSO-d_6) δ: 10.72, 17.61, 22.40, 29.21, 31.12, 34.11, 41.21, 46.84, 48.70, 52.62, 90.09, 108.15, 122.60, 123.47, 134.64, 142.44, 144.43, 146.60, 146.84, 154.54, 155.18, 165.99; HR-MS ESI/APCI dual *m/z*: 487.2217 $[M+H]^+$ (Calcd for $C_{22}H_{30}N_8O_3S$: 487.2234).

***N*-[(1*S*)-1-{5-[(3*S*)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-3-[(methanesulfonyl)amino]-*N*-methylpyridine-2-carboxamide Hydrochloride (14n)** Colorless powder; ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 0.88–0.94 (1.5H, m), 0.98–1.06 (1.5H, m), 1.87–2.30 (4H, m), 2.32–2.38 (3H, m), 2.59 (1.5H, s), 2.75 (1.5H, s), 3.06–3.11 (3H, m), 3.65–3.97 (5H, m), 4.55–4.61 (0.5H, m), 5.72–5.80 (0.5H, m), 6.10–6.16 (1H, m), 7.46–7.55 (1H, m), 7.87–7.93 (1H, m), 8.24 (3H, brs), 8.36 (0.5H, s), 8.44–8.49 (1H, m), 8.52 (0.5H, s), 9.39 (0.5H, s), 10.18 (0.5H, s); ^{13}C-NMR (151 MHz, DMSO-d_6) δ: 10.62, 17.77, 22.40, 26.96, 29.19, 30.86, 40.89, 46.86, 48.74, 52.64, 90.09, 108.07, 124.69, 133.52, 134.70, 145.55, 145.88, 146.62, 147.95, 155.14, 155.36, 167.38; HR-MS ESI/APCI dual *m/z*: 487.2200 $[M+H]^+$ (Calcd for $C_{22}H_{30}N_8O_3S$: 487.2234).

***N*-[(1*S*)-1-{5-[(3S)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-4-[(methanesulfonyl)amino]-*N*-methyl-1,2-thiazole-5-carboxamide Hydrochloride (14q)** Colorless powder; ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 0.84–0.91 (1.5H, m), 0.95–1.01 (1.5H, m), 1.88–2.31 (4H, m), 2.35 (3H, s), 2.73 (1.5H, s), 2.78 (1.5H, s), 3.07 (3H, s), 3.68–3.95 (5H, m), 4.98–5.04 (0.5H, m), 5.70–5.76 (0.5H, m), 6.09–6.14 (1H, m), 8.21 (3H, brs), 8.44 (0.5H, s), 8.52 (0.5H, s), 8.81 (0.5H, s), 8.86 (0.5H, s), 9.62 (0.5H, s), 9.93 (0.5H, s); ^{13}C-NMR (151 MHz, DMSO-d_6) δ: 10.63, 17.50, 22.45, 29.22, 31.05, 40.05, 46.72, 48.75, 52.57, 57.66, 90.44, 108.10, 132.16, 134.59, 138.59, 141.04, 146.71, 154.57, 155.31, 163.77; HR-MS ESI/APCI dual *m/z*: 493.1780 $[M+H]^+$ (Calcd for $C_{20}H_{28}N_8O_3S_2$: 493.1799).

***tert*-Butyl [(3*S*)-1-(2-{(1*S*)-1-[(2-Amino-5-chlorobenzoyl)(methyl)amino]propyl}-6-methylpyrazolo[1,5-a]pyrimidin-5-yl)pyrrolidin-3-yl]carbamate (15a)** To a solution of **13a** (50 mg, 0.129 mmol) and 2-amino-5-chloro-benzoic acid (24 mg, 0.142 mmol) in *N*,*N*-dimethylformamide (1.0 mL) was added trimethylamine (0.090 mL, 0.64 mmol) and 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (59 mg, 0.154 mmol). After stirring at room temperature for 1 h, the reaction mixture was purified by reversed-phase preparative HPLC to obtain **15a** (60 mg, 0.117 mmol, 91%) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.87–1.19 (3H, m), 1.46 (9H, s), 1.85–2.30 (4H, m), 2.35 (3H, s), 2.83 (3H, brs), 3.49–3.60 (1H, m), 3.66–3.86 (2H, m), 3.88–3.99 (1H, m), 4.23–4.48 (3H, m), 4.67 (1H, brs), 6.07 (1H, s), 6.58–6.68 (1H, m), 7.04–7.14 (1H, m), 7.22–7.30 (1H, m), 8.04 (1H, s); MS (ESI/APCI dual) *m/z*: 542 $[M+H]^+$.

Compound **15b** was obtained by the same procedure as that described for **15a**.

Benzyl [(3*S*)-1-(2-{(1*S*)-1-[(2-Amino-5-chlorobenzoyl)(methyl)amino]propyl}-6-methylpyrazolo[1,5-a]pyrimidin-5-yl)pyrrolidin-3-yl]carbamate (15b) Colorless amorphous; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.85–1.15 (3H, m), 1.88–2.29 (4H, m), 2.32 (3H, s), 2.70–2.96 (3H, m), 3.52–3.63 (1H, m), 3.67–3.87 (2H, m), 3.89–4.01 (1H, m), 4.28–4.43 (1H, m), 4.89–5.01 (1H, m), 5.12 (2H, s), 6.06 (1H, s), 6.58–6.67 (1H, m), 7.04–7.22 (1.5H, m), 7.29–7.41 (5.5H, m), 8.04 (1H, s); MS (ESI/APCI dual) *m/z*: 576 $[M+H]^+$.

2-Acetamido-*N*-[(1*S*)-1-{5-[(3*S*)-3-aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-5-chloro-*N*-methylbenzamide Hydrochloride (16) To a solution of **15a** (42 mg, 0.081 mmol) in chloroform (3.0 mL) was added acetic anhydride (0.022 mL, 0.24 mmol) and pyridine (0.033 mL, 0.40 mmol). After stirring at room temperature for 4 h, the reaction mixture was concentrated under reduced pressure and purified by reversed-phase preparative HPLC to obtain *tert*-butyl [(3*S*)-1-(2-{(1*S*)-1-[(2-acetamido-5-chlorobenzoyl)(methyl)amino]propyl}-6-methylpyrazolo[1,5-a]pyrimidin-5-yl)pyrrolidin-3-yl]carbamate (26 mg, 0.045 mmol, 60%) as a colorless amorphous.

To a solution of *tert*-butyl [(3*S*)-1-(2-{(1*S*)-1-[(2-acetamido-5-chlorobenzoyl)(methyl)amino]propyl}-6-methylpyrazolo[1,5-a]pyrimidin-5-yl)pyrrolidin-3-yl]carbamate (24 mg, 0.045 mmol) in chloroform (0.50 mL) was added trifluoroacetic acid (0.50 mL). After stirring at room temperature for 1 h, the reaction mixture was poured into sat. sodium bicarbonate aq. and extracted with chloroform. The organic layer was washed with brine, dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure to obtain **16** (25 mg, 0.052 mmol, quant.) as a colorless amorphous. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 0.79–0.99 (1.5H, m), 1.03–1.13 (1.5H, m), 1.74–1.83 (1H, m), 1.89–2.08 (2H, m), 2.10–2.26 (4H, m), 2.34–2.42 (3H, m), 2.73 (1.5H, s), 2.91 (1.5H, s), 3.40–3.47 (1H, m), 3.66–3.79 (2H, m), 3.85–3.94 (2H, m), 4.79–4.88 (0.5H, m), 5.90–5.97 (0.5H, m), 5.98–6.08 (1H, m), 7.23 (0.5H, brs), 7.31–7.37 (1H, m), 8.01 (1H, brs), 8.06–8.21 (1H, m), 8.67 (0.5H, brs); ^{13}C-NMR (151 MHz, DMSO-d_6) δ: 10.73, 17.64,

22.23, 27.51, 31.03, 33.22, 47.49, 50.34, 52.61, 56.97, 89.77, 107.96, 126.35, 127.94, 129.02, 129.33, 133.10, 133.99, 134.15, 147.35, 154.63, 155.61, 167.37, 168.74; HR-MS ESI/APCI dual *m/z*: 484.2200 $[M+H]^+$ (Calcd for $C_{24}H_{30}ClN_7O_2$: 484.2222).

Methyl (2-{[(1*S*)-1-{5-[(3*S*)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl](methyl)-carbamoyl}-4-chlorophenyl)carbamate (17) To a solution of **15b** (60 mg, 0.10 mmol) in pyridine (1.0 mL) was added methyl chloroformate (0.16 mL, 2.1 mmol). After stirring at room temperature for 4 h, the reaction mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (OH, 10–100% ethyl acetate in hexane) to obtain benzyl [(3*S*)-1-(2-{(1*S*)-1-[{5-chloro-2-[(methoxycarbonyl)amino]benzoyl}(methyl)amino]propyl}-6-methylpyrazolo[1,5-a]pyrimidin-5-yl)pyrrolidin-3-yl]carbamate (26 mg, 0.041 mmol, 40%) as a colorless amorphous.

To a solution of benzyl [(3*S*)-1-(2-{(1*S*)-1-[{5-chloro-2-[(methoxycarbonyl)amino]benzoyl}(methyl)amino]propyl}-6-methylpyrazolo[1,5-a]pyrimidin-5-yl)pyrrolidin-3-yl]carbamate (26 mg, 0.041 mmol) in methanol (2.1 mL) was added 10% palladium on activated carbon (13 mg). The reaction was flushed with hydrogen and stirred under hydrogen atmosphere at room temperature for 1 h. The reaction mixture was filtered through membrane filter and concentrated under reduced pressure to obtain **17** (19 mg, 0.037 mmol, 91%) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.79–1.12 (3H, m), 1.83–2.28 (4H, m), 2.28–2.38 (3H, m), 2.67–2.74 (1.5H, m), 2.77–2.86 (1.5H, m), 3.56–3.67 (1H, m), 3.70–3.80 (3H, m), 3.82–4.06 (4H, m), 4.67–4.82 (0.5H, m), 5.72–5.88 (0.5H, m), 5.91–6.07 (1H, m), 7.19–7.24 (0.5H, m), 7.27–7.38 (1H, m), 7.77–8.23 (2H, m), 8.54–8.68 (0.5H, m); ^{13}C-NMR (151 MHz, DMSO-d_6) δ: 10.63, 17.52, 22.23, 29.60, 31.17, 40.05, 46.70, 48.88, 52.07, 52.89, 89.85, 107.94, 126.49, 126.89, 127.52, 128.20, 129.33, 133.51, 134.49, 147.19, 154.33, 154.91, 155.55, 167.57; HR-MS ESI/APCI dual *m/z*: 500.2145 $[M+H]^+$ (Calcd for $C_{24}H_{30}ClN_7O_3$: 500.2171).

***N*-[(1*S*)-1-{5-[(3*S*)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-5-chloro-*N*-methyl-2-[(methylcarbamoyl)amino]benzamide (18)** To a solution of **15b** (50 mg, 0.087 mmol) in chloroform (0.87 mL) was added pyridine (0.028 mL, 0.347 mmol) and 4-nitrophenyl chloroformate (19 mg, 0.095 mmol). After stirring at room temperature for 1 h, the reaction mixture was concentrated under reduced pressure.

To a solution of the residue in chloroform (0.87 mL) was added methylamine (0.071 mL, 0.694 mmol). After stirring at room temperature for 2 h, the reaction mixture was added to water and extracted with chloroform. The organic layer was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (OH, 10–100% ethyl acetate in hexane) to obtain benzyl [(3*S*)-1-(2-{(1*S*)-1-[{5-chloro-2-[(methylcarbamoyl)amino]benzoyl}(methyl)amino]propyl}-6-methylpyrazolo[1,5-a]pyrimidin-5-yl)pyrrolidin-3-yl]carbamate (44 mg, 0.070 mmol, 81%) as a colorless amorphous.

To a solution of benzyl [(3*S*)-1-(2-{(1*S*)-1-[{5-chloro-2-[(methylcarbamoyl)amino]benzoyl}(methyl)amino]propyl}-6-methylpyrazolo[1,5-a]pyrimidin-5-yl)pyrrolidin-3-yl]carbamate (44 mg, 0.070 mmol) in methanol (3.5 mL) was added 10% palladium on activated carbon (22 mg). The reaction was flushed with hydrogen and stirred under hydrogen atmosphere at room temperature for 1 h. After stirring at room temperature for 1 h, the reaction mixture was filtered through a membrane filter and concentrated under reduced pressure to obtain **18** (30 mg, 0.060 mmol, 85%) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.82–1.15 (3H, m), 1.75–1.88 (1H, m), 1.88–2.23 (3H, m), 2.32–2.41 (3H, m), 2.62–2.92 (6H, m), 3.41–3.53 (1H, m), 3.67–3.79 (2H, m), 3.82–3.94 (2H, m), 4.69–4.85 (0.5H, m), 4.93–5.07 (0.5H, m), 5.51–5.64 (0.5H, m), 5.83–5.94 (0.5H, m), 5.95–6.10 (1H, m), 7.10–7.34 (2H, m), 7.97–8.21 (2H, m); ^{13}C-NMR (151 MHz, DMSO-d_6) δ: 10.79, 17.66, 23.45, 26.02, 31.39, 33.24, 47.49, 50.36, 57.01, 58.84, 89.37, 107.94, 123.00, 124.34, 124.83, 125.91, 126.13, 129.00, 134.15, 147.25, 154.47, 155.43, 155.65, 167.99; HR-MS ESI/APCI dual *m/z*: 499.2306 $[M+H]^+$ (Calcd for $C_{24}H_{31}ClN_8O_2$: 499.2331).

***N*-[(1*S*)-1-{5-[(3*S*)-3-Aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-5-chloro-*N*-methyl-2-[(methylsulfamoyl)amino]benzamide (19)** To a solution of chlorosulfonyl isocyanate (0.020 mL, 0.231 mmol) in chloroform (1.2 mL) was added 2-chloroethanol (0.031 mL, 0.462 mmol). After stirring at room temperature for 1 h, the reaction mixture was added trimethylamine (0.032 mL, 0.231 mmol) and **15b** (67 mg, 0.115 mmol). After stirring at room temperature for 6h, the reaction mixture was concentrated under reduced pressure. To a solution of the residue in chloroform (1.2 mL) was added trimethylamine (0.048 mL, 0.346 mmol) and methylamine (0.46 mL, 0.923 mmol). After stirring at 120°C under microwave irradiation for 30 min, the reaction mixture was added to water and extracted with chloroform. The organic layer was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (OH, 10–100% ethyl acetate in hexane) to obtain benzyl [(3*S*)-1-(2-{(1*S*)-1-[{5-chloro-2-[(methylsulfamoyl)amino]benzoyl}(methyl)amino]propyl}-6-methylpyrazolo[1,5-a]pyrimidin-5-yl)pyrrolidin-3-yl]carbamate (23 mg, 0.035 mmol, 30%) as a colorless amorphous.

To a solution of benzyl [(3*S*)-1-(2-{(1*S*)-1-[{5-chloro-2-[(methylsulfamoyl)amino]benzoyl}(methyl)amino]propyl}-6-methylpyrazolo[1,5-a]pyrimidin-5-yl)pyrrolidin-3-yl]carbamate (22 mg, 0.032 mmol) in methanol (1.6 mL) was added 10% palladium on activated carbon (11 mg). The reaction mixture was flushed with hydrogen and stirred under hydrogen atmosphere at room temperature for 1 h. The reaction mixture was filtered through a membrane filter and concentrated under reduced pressure to obtain **19** (16 mg, 0.030 mmol, 92%) as a colorless amorphous. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.00–1.16 (3H, m), 1.77–2.23 (4H, m), 2.34–2.40 (3H, m), 2.60–2.65 (1.5H, m), 2.71 (2H, s), 2.75–2.83 (2.5H, m), 3.39–3.54 (1H, m), 3.67–3.78 (2H, m), 3.82–3.95 (2H, m), 4.60–4.70 (1H, m), 5.92–6.04 (1H, m), 7.22–7.25 (0.5H, m), 7.30–7.39 (1H, m), 7.63–7.74 (1H, m), 8.36 (0.5H, br s); ^{13}C-NMR (151 MHz, DMSO-d_6) δ: 10.63, 17.87, 22.35, 28.17, 31.21, 31.97, 47.21, 49.86, 52.90, 55.67, 89.61, 107.84, 122.29, 126.45, 127.28, 129.51, 130.19, 133.57, 134.35, 147.29, 154.47, 155.69, 167.59; HR-MS ESI/APCI dual *m/z*: 535.1982 $[M+H]^+$ (Calcd for $C_{23}H_{31}ClN_8O_3S$: 535.2001).

***tert*-Butyl [(1*S*)-1-(7-Hydroxy-6-methyl-5-oxo-4,5-dihydropyrazolo[1,5-a]pyrimidin-2-yl)propyl]methylcarbamate (20)** To a solution of **5c** (0.55 g 2.16 mmol) in ethanol (10 mL) was added diethyl 2-methylmalonate (0.55 mL, 3.24 mmol) and 2.94 M sodium ethoxide in ethanol (3.7 mL, 10.81 mmol), and the mixture was stirred at 90°C for 5 h. The reaction mixture was concentrated under reduced pressure.

The residue was acidified with 1 N hydrogen chloride aq. and extracted with chloroform. The organic layer was dried over ISOLUTE® Phase Separator and concentrated under reduced pressure. The residue was added to diethyl ether, precipitated, and collected to obtain **20** (0.62 g, 1.92 mmol, 89%) as a pale yellow powder. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.90–1.01 (3H, m), 1.49 (9H, s), 1.69–1.75 (3H, m), 1.83–1.97 (1H, m), 2.10–2.22 (1H, m), 2.67 (3H, s), 3.58–3.69 (1H, m), 4.98–5.28 (1H, m), 5.73 (1H, brs), 9.16 (1H, brs); MS (ESI/APCI dual) *m/z*: 337 $[M+H]^+$.

(1*S*)-1-(5,7-Dichloro-6-methylpyrazolo[1,5-a]pyrimidin-2-yl)-*N*-methylpropan-1-amine Hydrochloride (21) The mixture of **20** (4.6 g, 13.6 mmol) and phosphorus oxychloride (13 mL, 136.1 mmol) was stirred at 110°C for 3 h, and the reaction mixture was concentrated under reduced pressure. After purifying by silica gel column chromatography (NH, 1–5% methanol in chloroform), 4M hydrogen chloride in AcOEt was added to the residue and the mixture was concentrated under reduced pressure to obtain **21** (1.8 g, 5.77 mmol, 42%) as a colorless powder. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.73–0.87 (3H, m), 1.94–2.20 (2H, m), 2.45 (3H, s), 2.48 (3H, s), 4.34–4.46 (1H, m), 7.05 (1H, s), 9.38 (2H, brs); MS (ESI/APCI dual) *m/z*: 273 $[M+H]^+$.

***N*-[(1*S*)-1-{5-[(3*S*)-3-Aminopyrrolidin-1-yl]-6-methyl-7-(methylamino)pyrazolo[1,5-a]pyrimidin-2-yl}propyl]-5-chloro-2-[(methanesulfonyl)amino]-*N*-methylbenzamide (24a)** To a solution of **21** (0.12 g, 0.388 mmol) in acetonitrile (1.0 mL) and water (1.0 mL) was added 9.8 M methylamine/methanol (0.20 mL, 1.94 mmol) and sodium bicarbonate (0.33 g, 3.88 mmol). After stirring at room temperature for 22 h, the reaction mixture was added to 20% potassium carbonate aq. and extracted with chloroform. The organic layer was dried over ISOLUTE® Phase Separator and concentrated under reduced pressure to obtain 5-chloro-*N*,6-dimethyl-2-[(1*S*)-1-(methylamino)propyl]pyrazolo[1,5-a]pyrimidin-7-amine (0.10 g, 0.372 mmol, 96%) as a colorless powder.

To a solution of 5-chloro-*N*,6-dimethyl-2-[(1*S*)-1-(methylamino)propyl]pyrazolo[1,5-a]pyrimidin-7-amine (97 mg, 0.361 mmol) and 5-chloro-2-(methylsulfonamido)benzoic acid (99 mg, 0.397 mmol) in *N*,*N*-dimethylformamide (1.0 mL) was added trimethylamine (0.25 mL, 1.80 mmol) and 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]-pyridinium 3-oxide hexafluorophosphate (0.17 g, 0.433 mmol). After stirring at room temperature for 1 h, the reaction mixture was purified by reversed-phase preparative HPLC to obtain 5-chloro-*N*-{(1*S*)-1-[5-chloro-6-methyl-7-(methylamino)-pyrazolo[1,5-a]pyrimidin-2-yl]propyl}-2-[(methanesulfonyl)-amino]-*N*-methylbenzamide (0.13 g, 0.904 mmol, 90%) as a colorless powder.

To a solution of 5-chloro-*N*-{(1*S*)-1-[5-chloro-6-methyl-7-(methylamino)pyrazolo[1,5-a]pyrimidin-2-yl]propyl}-2-[(methanesulfonyl)amino]-*N*-methylbenzamide (0.12 mg, 0.252 mmol) in 1-methyl-2-pyrrolidone (1.0 mL) was added trimethylamine (0.35 mL, 2.52 mmol) and (*S*)-pyrrolidin-3-amine (0.11 mL, 1.26 mmol). After stirring at 150°C under microwave irradiation for 30 min, the reaction mixture was purified by reversed-phase preparative HPLC to obtain **24a** (41 mg, 0.30 mmol, 30%) as a pale yellow powder. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.94–1.02 (3H, m), 1.74–2.19 (4H, m), 2.23 (3H, s), 2.80 (3H, s), 2.97 (3H, s), 3.21–3.28 (3H, m), 3.29–3.38 (1H, m), 3.52–3.81 (4H, m), 4.49–4.60 (1H, m), 6.03 (1H, s), 6.07–6.16 (1H, m), 7.32–7.42 (2H, m), 7.52–7.58 (1H, m); ^{13}C-NMR (151 MHz, DMSO-d_6) δ: 10.81, 14.95, 22.85, 27.45, 31.63, 32.72, 40.49, 47.77, 49.96, 52.49, 55.29, 84.19, 90.42, 123.82, 126.23, 127.44, 128.60, 129.12, 133.28, 146.87, 148.18, 154.13, 159.73, 169.12; HR-MS ESI/APCI dual *m/z*: 549.2143 $[M+H]^+$ (Calcd for $C_{24}H_{33}ClN_8O_3S$: 549.2158).

Compounds **24b, e, f** were obtained by the same procedure as that described for **24a**.

***N*-[(1*S*)-1-{5-[(3*S*)-3-Aminopyrrolidin-1-yl]-7-(dimethylamino)-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-5-chloro-2-[(methanesulfonyl)amino]-*N*-methylbenzamide (24b)** Pink powder; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.96–1.04 (3H, m), 1.67–1.77 (1H, m), 1.93–2.06 (2H, m), 2.09–2.20 (4H, m), 2.86–2.95 (6H, m), 3.11–3.19 (6H, m), 3.26–3.34 (1H, m), 3.57–3.70 (4H, m), 3.74–3.85 (0.3H, m), 4.58–4.69 (0.7H, m), 6.05 (1H, s), 7.24–7.33 (1H, m), 7.36–7.41 (1H, m), 7.58–7.63 (1H, m); ^{13}C-NMR (151 MHz, DMSO-d_6) δ: 10.83, 15.57, 22.73, 30.73, 31.87, 40.54, 41.38, 47.59, 50.02, 52.59, 55.49, 58.32, 90.22, 94.88, 124.36, 126.29, 127.12, 128.66, 129.02, 133.45, 148.28, 149.48, 153.91, 159.41, 168.98; HR-MS ESI/APCI dual *m/z*: 563.2274 $[M+H]^+$ (Calcd for $C_{25}H_{35}ClN_8O_3S$: 563.2314).

***N*-[(1*S*)-1-(5-[(3*S*)-3-Aminopyrrolidin-1-yl]-7-{[2-(methanesulfonyl)ethyl]amino}-6-methylpyrazolo[1,5-a]pyrimidin-2-yl)propyl]-5-chloro-2-[(methanesulfonyl)amino]-*N*-methylbenzamide (24e)** Colorless powder; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.99 (3H, brs), 1.40–2.18 (4H, m), 2.24 (3H, brs), 2.81 (3H, brs), 2.97 (3H, brs), 3.01 (3H, brs), 3.21–4.09 (9H, m), 4.53 (1H, brs), 6.06 (2H, brs), 7.21–7.45 (2H, m), 7.49–7.61(1H, m); ^{13}C-NMR (151 MHz, DMSO-d_6) δ: 10.83, 14.57, 22.75, 30.67, 31.57, 38.61, 40.54, 40.96, 47.83, 49.92, 52.47, 54.08, 55.21, 86.26, 90.46, 124.04, 126.29, 127.22, 128.64, 129.00, 133.39, 146.29, 147.31, 154.39, 159.25, 169.08; HR-MS ESI/APCI dual *m/z*: 641.2060 $[M+H]^+$ (Calcd for $C_{26}H_{37}ClN_8O_5S_2$: 641.2090).

***N*-[(1*S*)-1-(5-[(3*S*)-3-Aminopyrrolidin-1-yl]-6-methyl-7-{[2-(methylsulfamoyl)ethyl]amino}pyrazolo[1,5-a]pyrimidin-2-yl)propyl]-5-chloro-2-[(methanesulfonyl)amino]-*N*-methylbenzamide (24f)** Colorless powder; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.98 (3H, t, $J=7.0$ Hz), 1.70–1.82 (1H, m), 1.94–2.06 (2H, m), 2.08–2.19 (4H, m), 2.21 (3H, brs), 2.82 (6H, brs), 2.97 (3H, s), 3.26–3.80 (7H, m), 3.98 (2H, q, $J=6.6$ Hz), 4.53 (1H, t, $J=7.2$ Hz), 4.82 (1H, brs), 6.05 (1H, s), 6.21 (1H, brs), 7.32–7.44 (2H, m), 7.57 (1H, d, $J=8.7$ Hz); ^{13}C-NMR (151 MHz, DMSO-d_6) δ: 10.83, 14.61, 22.75, 27.57, 28.52, 30.67, 31.53, 40.54, 47.83, 49.84, 49.92, 52.51, 55.19, 85.94, 90.48, 124.06, 126.29, 127.22, 128.64, 129.02, 133.39, 146.45, 147.29, 154.39, 159.31, 169.08; HR-MS ESI/APCI dual *m/z*: 656.2170 $[M+H]^+$ (Calcd for $C_{26}H_{38}ClN_9O_5S_2$: 656.2199).

***N*-[(1*S*)-1-{7-[(2-Aminoethyl)amino]-5-[(3*S*)-3-aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-5-chloro-2-[(methanesulfonyl)amino]-*N*-methylbenzamide (24c)** To a solution of **21** (120 mg, 0.38 mmol) in acetonitrile (1.0 mL) and water (1.0 mL) was added 1-Boc-ethylenediamine (0.30 mL, 1.91 mmol) and sodium bicarbonate (0.32 g, 3.81 mmol). After stirring at room temperature for 20 h, the reaction mixture was poured into sat. sodium bicarbonate aq. and extracted with chloroform. The organic layer was dried over ISOLUTE® Phase Separator and concentrated under reduced pressure. The residue was purified by reversed-phase preparative HPLC to obtain *tert*-

butyl [2-({5-chloro-6-methyl-2-[(1*S*)-1-(methylamino)propyl]-pyrazolo[1,5-a]pyrimidin-7-yl}amino)ethyl]carbamate (40 mg, 0.10 mmol, 27%) as a colorless amorphous.

To a solution of *tert*-butyl [2-({5-chloro-6-methyl-2-[(1*S*)-1-(methylamino)propyl]pyrazolo[1,5-a]pyrimidin-7-yl}-amino)ethyl]carbamate (40 mg, 0.10 mmol) and 5-chloro-2-(methylsulfonamido)benzoic acid (30 mg, 0.12 mmol) in *N*,*N*-dimethylformamide (1.0 mL) was added trimethylamine (0.070 mL, 0.50 mmol) and 1-[bis(dimethylamino)-methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (50 mg, 0.13 mmol). After stirring at room temperature for 2 h, the reaction mixture was purified by reversed-phase preparative HPLC to obtain *tert*-butyl {2-[(5-chloro-2-{(1*S*)-1-[{5-chloro-2-[(methanesulfonyl)amino]-benzoyl}(methyl)amino]propyl}-6-methylpyrazolo[1,5-a]-pyrimidin-7-yl)amino]ethyl}carbamate (33 mg, 0.052 mmol, 52%) as a colorless amorphous.

To a solution of *tert*-butyl {2-[(5-chloro-2-{(1*S*)-1-[{5-chloro-2-[(methanesulfonyl)amino]benzoyl}(methyl)-amino]propyl}-6-methylpyrazolo[1,5-a]pyrimidin-7-yl)amino]-ethyl}carbamate (33 mg, 0.052 mmol) in 1-methyl-2-pyrrolidone (1.0 mL) was added trimethylamine (0.12 mL, 0.83 mmol) and (*S*)-pyrrolidin-3-amine (0.037 mL, 0.42 mmol). After stirring at 150°C under microwave irradiation for 30 min, the reaction mixture was purified by reversed-phase preparative HPLC to obtain *tert*-butyl {2-[(5-[(3*S*)-3-aminopyrrolidin-1-yl]-2-{(1*S*)-1-[{5-chloro-2-[(methanesulfonyl)amino]benzoyl}(methyl)-amino]propyl}-6-methylpyrazolo[1,5-a]pyrimidin-7-yl)amino]-ethyl}carbamate (14 mg, 0.021 mmol, 40%) as a colorless powder.

To a solution of *tert*-butyl {2-[(5-[(3*S*)-3-aminopyrrolidin-1-yl]-2-{(1*S*)-1-[{5-chloro-2-[(methanesulfonyl)amino]-benzoyl}(methyl)amino]propyl}-6-methylpyrazolo[1,5-a]-pyrimidin-7-yl)amino]ethyl}carbamate (14 mg, 0.021 mmol) in chloroform (1.0 mL) was added trifluoroacetic acid (1.0 mL). After stirring at room temperature for 1 h, the reaction mixture was poured into sat. sodium bicarbonate aq. and extracted with chloroform. The organic layer was dried over ISOLUTE® Phase Separator and concentrated under reduced pressure to obtain **24c** (11 mg, 0.019 mmol, 91%) as a colorless powder. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.94–1.03 (3H, m), 1.65–2.27 (7H, m), 2.82 (3H, s), 2.91–3.03 (5H, m), 3.22–3.31 (1H, m), 3.51–3.83 (7H, m), 4.52–4.63 (1H, m), 6.06 (1H, s), 6.08–6.17 (1H, m), 7.32–7.43 (2H, m), 7.52–7.60 (1H, m); ^{13}C-NMR (151 MHz, DMSO-d_6) δ: 10.73, 13.99, 22.37, 30.22, 32.90, 40.05, 41.14, 43.59, 47.95, 50.38, 51.41, 56.97, 88.43, 90.58, 118.61, 120.78, 125.71, 126.07, 128.38, 131.26, 132.44, 146.51, 154.07, 158.99, 170.57; HR-MS ESI/APCI dual *m*/*z*: 578.2381 $[M+H]^+$ (Calcd for $C_{25}H_{36}ClN_9O_3S$: 578.2423).

Compound **24d** was obtained by the same procedure as that described for **24c**.

***N*-[(1*S*)-1-(5-[(3*S*)-3-Aminopyrrolidin-1-yl]-6-methyl-7-{[2-(methylamino)ethyl]amino}pyrazolo[1,5-a]pyrimidin-2-yl)propyl]-5-chloro-2-[(methanesulfonyl)amino]-*N*-methylbenzamide (24d)** Colorless powder; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.94–1.02 (3H, m), 1.70–1.79 (1H, m), 1.94–2.24 (6H, m), 2.48 (3H, s), 2.83 (3H, s), 2.86–2.99 (5H, m), 3.22–3.31 (1H, m), 3.53–3.81 (7H, m), 4.52–4.65 (1H, m), 6.02–6.12 (2H, m), 7.32–7.43 (2H, m), 7.53–7.59 (1H, m); ^{13}C-NMR (151 MHz, DMSO-d_6) δ: 10.75, 14.19, 22.61, 30.47, 32.01, 34.41, 40.05, 42.56, 47.85, 50.06, 50.93, 51.85, 55.87, 79.14, 90.54, 122.11, 125.99, 127.20, 128.50, 128.80, 132.00, 146.75, 147.45, 154.23, 159.09, 170.04; HR-MS ESI/APCI dual *m*/*z*: 592.2546 $[M+H]^+$ (Calcd for $C_{26}H_{38}ClN_9O_3S$: 592.2580).

Computational Chemistry

The conformations of the compounds were sampled using REMD simulation[49] run on GROMACS 5.0.4.[50] For each compound, eight independent REMD simulations were performed under NVT conditions for 10 ns each, to sample sufficient conformational space. Temperatures of the replicas were set at 310.0, 366.5, 433.2, 512.1, 605.4, 715.6, 845.9, and 1000 K. GAFF forcefiled[51] and GBSA model[52] were applied to the compounds and the solvent, respectively. The dihedral angle between the pyrazolo[1,5-a]pyrimidine ring and the amide plane was calculated for each conformation sampled every 10 ps at 310.0 K.

Cell and Virus HEp-2 cells were purchased from DS Pharma Biomedical Co., Ltd. (Osaka, Japan) and cultured in minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS), 50 μg/mL gentamicin and 600 μg/mL L-glutamine. RSV A2 (ATCC VR-1540) were purchased from American Type Culture Collection (Manassas, VA, U.S.A.).

Antiviral Assay HEp-2 cells were cultured overnight in 96-well plates, each test compound diluted with MEM was added, and the medium was supplemented with 2% FBS, 100 units/mL penicillin, 100 μg/mL streptomycin and 300 μg/mL L-glutamine. The cells were then infected with RSV A2. After incubation at 37°C in the presence of 5% CO_2 for 4 d, the RSV-induced CPE was determined by adding XTT reagent. The concentration of the test compound required to inhibit the CPE by 50% (EC_{50}) was calculated by the least squares method. The EC_{50} values were determined in duplicate with at least five concentrations ($n = 1$–3).

Acknowledgments The authors thank Dr. Taiji Asami for carefully reading the manuscript and making useful suggestions to improve it, Dr. Takashi Yoshizumi, Mr. Susumu Yamanobe and Dr. Tomohiro Sugimoto for their helpful discussions, and Mr. Akira Higuchi for his assistance with the analytical and spectrum studies.

Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

References

1) Lozano R., Naghavi M., Foreman K., *et al.*, *Lancet*, **380**, 2095–2128 (2012).
2) Sigurs N., Bjarnason R., Sigurbergsson F., Kjellman B., *Am. J. Respir. Crit. Care Med.*, **161**, 1501–1507 (2000).
3) Sigurs N., Aljassim F., Kjellman B., Robinson P. D., Sigurbergsson F., Bjarnason R., Gustafsson P. M., *Thorax*, **65**, 1045–1052 (2010).
4) Sigurs N., Gustafsson P. M., Bjarnason R., Lundberg F., Schmidt S., Sigurbergsson F., Kjellman B., *Am. J. Respir. Crit. Care Med.*, **171**, 137–141 (2005).
5) Stein R. T., Sherrill D., Morgan W. J., Holberg C. J., Halonen M., Taussig L. M., Wright A. L., Martinez F. D., *Lancet*, **354**, 541–545 (1999).
6) Bont L., Steijn M., van Aalderen W. M., Kimpen J. L., *Pediatr. In-*

fect. Dis. J., **23**, 414–417 (2004).
7) Henderson J., Hilliard T. N., Sherriff A., Stalker D., Al Shammari N., Thomas H. M., *Pediatr. Allergy Immunol.*, **16**, 386–392 (2005).
8) Nair H., Nokes D. J., Gessner B. D., *et al.*, *Lancet*, **375**, 1545–1555 (2010).
9) Fleming D. M., Cross K. W., *Lancet*, **342**, 1507–1510 (1993).
10) Griffin M. R., Coffey C. S., Neuzil K. M., Mitchel E. F. Jr., Wright P. F., Edwards K. M., *Arch. Intern. Med.*, **162**, 1229–1236 (2002).
11) Nicholson K. G., *Epidemiol. Infect.*, **116**, 51–63 (1996).
12) Neuzil K. M., Maynard C., Griffin M. R., Heagerty P., *Clin. Infect. Dis.*, **37**, 201–207 (2003).
13) Thompson W. W., Shay D. K., Weintraub E., Brammer L., Cox N., Anderson L. J., Fukuda K., *JAMA*, **289**, 179–186 (2003).
14) Resch B., Manzoni P., Lanari M., *Paediatr. Respir. Rev.*, **10**, 148–153 (2009).
15) Hall C. B., Powell K. R., MacDonald N. E., Gala C. L., Menegus M. E., Suffin S. C., Cohen H. J., *N. Engl. J. Med.*, **315**, 77–81 (1986).
16) Glezen W. P., Taber L. H., Frank A. L., Kasel J. A., *Am. J. Dis. Child.*, **140**, 543–546 (1986).
17) Committee on Infectious Diseases, *Pediatrics*, **124**, 1694–1701 (2009).
18) Shi T., McAllister D. A., O'Brien K. L., *et al.*, *Lancet*, **390**, 946–958 (2017).
19) Weiss R. A., McMichael A. J., *Nat. Med.*, **10** (Suppl.), S70–S76 (2004).
20) Smith D. K., Seales S., Budzik C., *Am. Fam. Physician*, **95**, 94–99 (2017).
21) Kou M., Hwang V., Ramkellawan N., *Emerg. Med. Clin. North Am.*, **36**, 275–286 (2018).
22) Offer I., Ashkenazi S., Livni G., Shalit I., *Isr. Med. Assoc. J.*, **2**, 108–110 (2000).
23) Livni G., Rachmel A., Marom D., Yaari A., Tirosh N., Ashkenazi S., *Pediatr. Infect. Dis. J.*, **29**, 71–73 (2010).
24) Meissner H. C., *N. Engl. J. Med.*, **374**, 62–72 (2016).
25) Impact-RSV study group, *Pediatrics*, **102**, 531–537 (1998).
26) Wu P., Escobar G. J., Gebretsadik T., Carroll K. N., Li S. X., Walsh E. M., Mitchel E. F., Sloan C., Dupont W. D., Yu C., Horner J. R., Hartert T. V., *Am. J. Epidemiol.*, **187**, 1490–1500 (2018).
27) Obando-Pacheco P., Justicia-Grande A. J., Rivero-Calle I., Rodríguez-Tenreiro C., Sly P., Ramilo O., Mejías A., Baraldi E., Papadopoulos N. G., Nair H., Nunes M. C., Kragten-Tabatabaie L., Heikkinen T., Greenough A., Stein R. T., Manzoni P., Bont L., Martinón-Torres F., *J. Infect. Dis.*, **217**, 1356–1364 (2018).
28) Revankar G. R., Robins R. K., *Ann. N. Y. Acad. Sci.*, **255** (1 Chemistry, Bi), 166–176 (1975).
29) Sidwell R. W., Huffman J. H., Khare G. P., Allen L. B., Witkowski J. T., Robins R. K., *Science*, **177**, 705–706 (1972).
30) Hall C. B., Walsh E. E., Hruska J. F., Betts R. F., Hall W. J., *JAMA*, **249**, 2666–2670 (1983).
31) Streeter D. G., Witkowski J. T., Khare G. P., Sidwell R. W., Bauer R. J., Robins R. K., Simon L. N., *Proc. Natl. Acad. Sci. U.S.A.*, **70**, 1174–1178 (1973).
32) Ohmit S. E., Moler F. W., Monto A. S., Khan A. S., *J. Clin. Epidemiol.*, **49**, 963–967 (1996).
33) Graci J. D., Cameron C. E., *Rev. Med. Virol.*, **16**, 37–48 (2006).
34) Hultgren C., Milich D. R., Weiland O., Sällberg M., *J. Gen. Virol.*, **79**, 2381–2391 (1998).
35) Smith D. W., Frankel L. R., Mathers L. H., Tang A. T., Ariagno R. L., Prober C. G., *N. Engl. J. Med.*, **325**, 24–29 (1991).
36) Englund J. A., Piedra P. A., Ahn Y. M., Gilbert B. E., Hiatt P., *J. Pediatr.*, **125**, 635–641 (1994).
37) Meert K. L., Sarnaik A. P., Gelmini M. J., Lieh-Lai M. W., *Crit. Care Med.*, **22**, 566–572 (1994).
38) Blount R. E. Jr., Morris J. A., Savage R. E., *Proc. Soc. Exp. Biol. Med.*, **92**, 544–549 (1956).
39) Tayyari F., Marchant D., Moraes T. J., Duan W., Mastrangelo P., Hegele R. G., *Nat. Med.*, **17**, 1132–1135 (2011).
40) Tripp R. A., Jones L. P., Haynes L. M., Zheng H., Murphy P. M., Anderson L. J., *Nat. Immunol.*, **2**, 732–738 (2001).
41) Mackman R. L., Sangi M., Sperandio D., *et al.*, *J. Med. Chem.*, **58**, 1630–1643 (2015).
42) Babaoglu K., Eisenberg E. J., Machkman R. L., Sangi M., Siegel D., Yang H., WO Patent WO/2011/163518, December 29 2011.
43) Tahri A., Vendeville S. M. H., Jonckers T. H. M., Raboisson P. J. M., Demin S. D., Hu L., Cooymans L. P., WO Patent WO/2016/091774, June 16, 2016.
44) Tahri A., Vendeville S. M. H., Jonckers T. H. M., Raboisson P. J. M., Demin S. D., Hu L., WO Patent WO/2016/091791, June 16, 2016.
45) Shi W., Jiang Z., He H., Xiao F., Lin F., Sun Y., Hou L., Shen L., Han L., Zeng M., Lai K., Gu Z., Chen X., Zhao T., Guo L., Yang C., Li J., Chen S., *ACS Med. Chem. Lett.*, **9**, 94–97 (2018).
46) Feng S., Hong D., Wang B., Zheng X., Miao K., Wang L., Yun H., Gao L., Zhao S., Shen H. C., *ACS Med. Chem. Lett.*, **6**, 359–362 (2015).
47) Feng S., Li C., Chen D., Zheng X., Yun H., Gao L., Shen H. C., *Eur. J. Med. Chem.*, **138**, 1147–1157 (2017).
48) Zheng X., Wang L., Wang B., Miao K., Xiang K., Feng S., Gao L., Shen H. C., Yun H., *ACS Med. Chem. Lett.*, **7**, 558–562 (2016).
49) Sugita Y., Okamoto Y., *Chem. Phys. Lett.*, **314**, 141–151 (1999).
50) Abraham M. J., Murtola T., Schulz R., Páll S., Smith J. C., Hess B., Lindahl E., *SoftwareX*, **1–2**, 19–25 (2015).
51) Wang J., Wolf R. M., Caldwell J. W., Kollman P. A., Case D. A., *J. Comput. Chem.*, **25**, 1157–1174 (2004).
52) Onufriev A., Case D. A., Bashford D., *J. Comput. Chem.*, **23**, 1297–1304 (2002).