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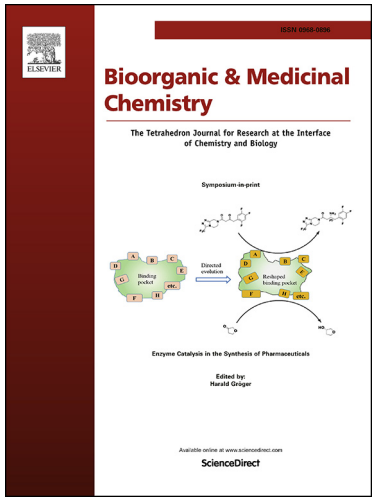

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Structure-based drug design to overcome species differences in kallikrein 7 inhibition of 1,3,6-trisubstituted 1,4-diazepan-7-ones

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ABSTRACT

A series of 1,3,6-trisubstituted 1,4-diazepan-7-ones were prepared as kallikrein 7 (KLK7, stratum corneum chymotryptic enzyme) inhibitors. Previously reported compounds **1**–**3** were potent human KLK7 inhibitors; however, they did not exhibit inhibitory activity against mouse KLK7. Comparison of the human and mouse KLK7 structures reveals the cause of this species differences; therefore, compounds that could inhibit both KLK7s were designed, synthesized, and evaluated. Through this structure-based drug design, compound **22g** was identified as an inhibitor against human and mouse KLK7, and only one of the enantiomers, (–)-**22g**, exhibited potent inhibitory activity. Furthermore, the crystal structure of mouse KLK7 complexed with **22g** enabled the elucidation of structure-activity relationships and justified **22g** as a valuable compound to overcome the species differences.

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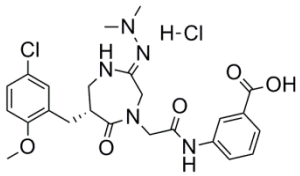
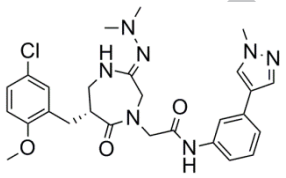
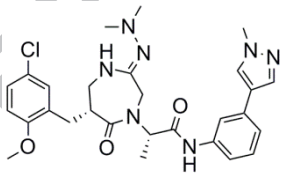
1. Introduction

Tissue kallikreins (KLKs) constitute a family of 15 serine proteases (KLK1–KLK15) that are associated with various physiological and pathological processes.[1,2] KLKs have recently received attention as attractive drug targets.[2,3] The expression of various KLKs were identified in the skin,[4] among which kallikrein 7 (KLK7, stratum corneum chymotryptic enzyme) plays an important role in epidermal desquamation by degrading corneodesmosome proteins.[5-7] The desquamation of corneocytes is an essential process that occurs in normal skin[8] upon degradation of corneodesmosomes by skin-specific proteases.[9] Desquamation is regulated by an appropriate balance of various proteases, including KLK7 and their intrinsic inhibitors such as lympho-epithelial Kazal-type-related inhibitor (LEKTI), which is encoded by serine protease inhibitor Kazal-type 5 (SPINK5), to maintain skin barrier function.[10,11] The stratum corneum (SC), which is composed of corneocytes held together by corneodesmosomes, is responsible for this barrier function.[9,12] A defective epidermal barrier increases moisture loss, and facilitates the penetration of irritants and allergens, thereby increasing the risk of inflammation.[9,12] Atopic dermatitis (AD) is a common and multifactorial chronic allergic skin disease. Skin barrier dysfunction has been implicated in the development of AD,[9, 12, 13] and increased serine protease activities are thought to be associated with impaired skin barrier function.[14] Therefore, the restoration of this barrier function of the skin would promote AD therapy.[15-17] KLK7 is a chymotryptic serine protease abundantly expressed in the skin,[18] and its levels have been reported to significantly increase in AD patients.[19,20] Therefore, we focused our attention on KLK7 inhibitors that would aid AD therapy by restoring the barrier function of the skin.

In previous publications, we reported the discovery and structure-activity relationship study of a 1,3,6-trisubstituted 1,4-diazepan-7-one scaffold as novel human KLK7 inhibitors,[21] and synthesized highly potent and selective human KLK7 inhibitors.[22] However, these compounds showed significantly decreased potency for mouse KLK7 (Table 1). In order to use these inhibitors as tools to elucidate the pathophysiological role of KLK7, it was necessary to break through this species differences by structural modification.

Table 1. Species differences between recombinant human and mouse KLK7 inhibitory activity.

Compound	Structure	human KLK7 IC_{50} (μM)	mouse KLK7	
			IC_{50} (μM)	inhibition ratio @ 30μM (%)
1		1.9	>30	11
2		0.52	>30	−1.2
3		0.058	>30	32

In order to clarify the cause of the species differences in inhibitory activity of our scaffold, we have determined the crystal structure of mouse KLK7. Based on the structural comparison between mouse and human KLK7, we identified the compound substructure that interfered with the binding to mouse KLK7. Moreover, using the crystal structures of mouse and human KLK7 we obtained compounds that overcame the species differences.

2. Results and discussions

2.1. Crystal structure of mouse apo-KLK7

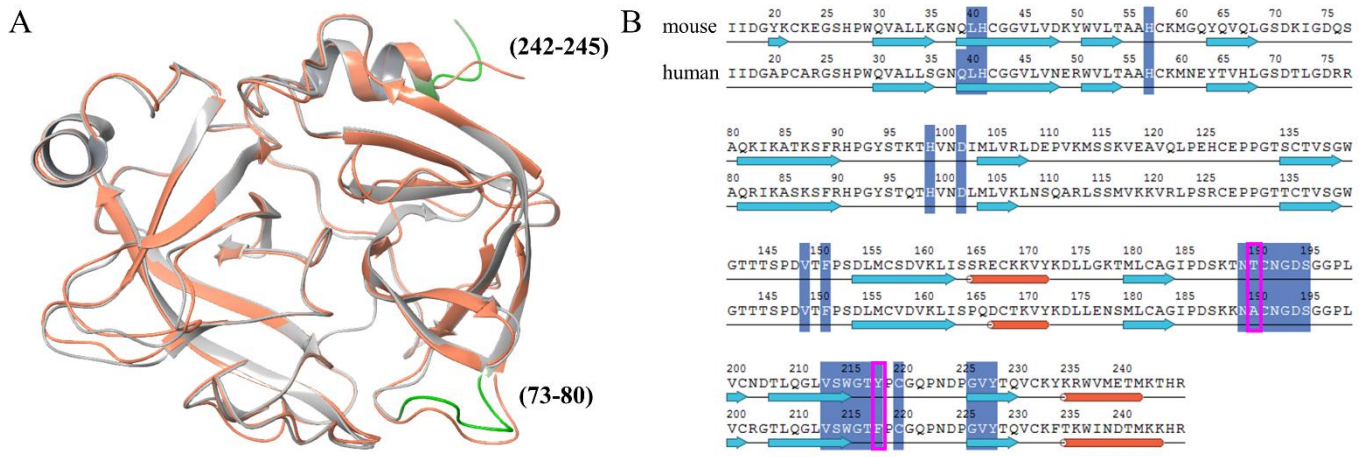


Figure 1. Crystal structure of mouse apo-KLK7 (PDB ID: 5ZFH) and human apo-KLK7 (PDB ID: 3BSQ[23]). (A) Superimposition of mouse and human KLK7 structures. These two structures were overlapped by motion-tree analysis.[24] For human structure, the coordinates of the C chain, which has the smallest magnitude of the top two nodes in the asymmetric unit, were chosen. The residues that belong to two minor clusters in the mouse structure are indicated in green. Mouse and human structures are colored in grey and orange, respectively. (B) Sequence alignment of mouse and human KLK7. The residues located within 5 Å from compound **1** are boxed in blue. Residues 190 and 218, which are not conserved between mouse and human KLK7, are shown within the magenta frame. Sequence alignment was calculated with ClustalW.[25]

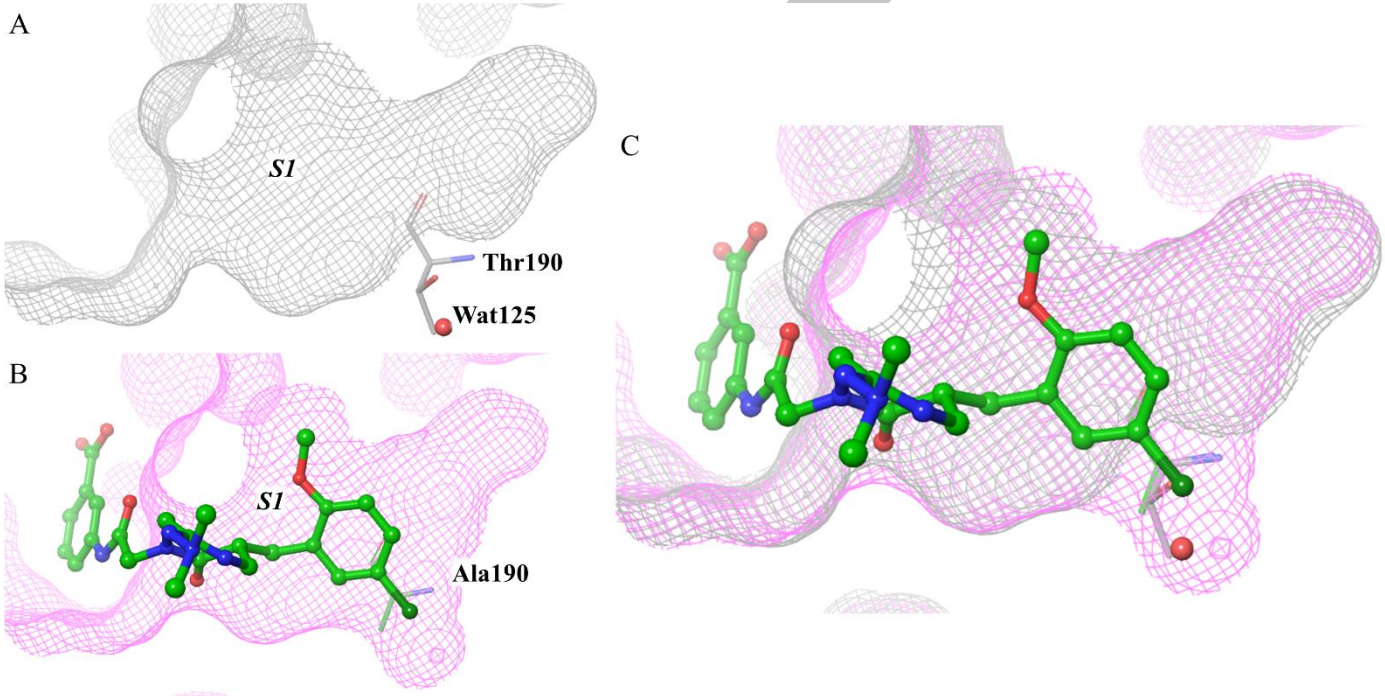


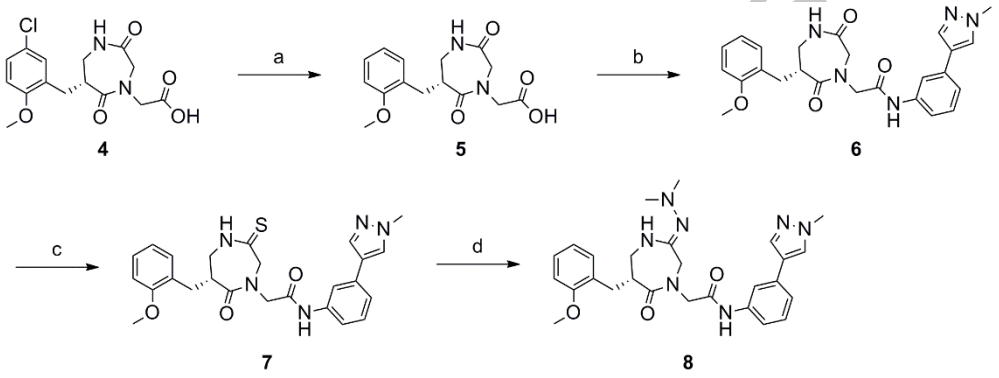
Figure 2. Structural comparison around compound **1**. Surfaces of mouse apo and human complex KLK7 are represented by grey and pink meshes, respectively. Carbon atoms are shown in grey and green in mouse apo and human complex, respectively. Nitrogen and oxygen atoms are colored in blue and red, respectively. (A) Surface structure around active site in mouse KLK7 (PDB ID: 5ZFH). The key residue Thr190 and the surrounding water molecule to determine the shape of the binding pocket are also shown. (B) Surface structure around the binding site of **1** in human complex (PDB ID: 5Y9L[21]). The corresponding residue Ala190 is also shown. Other residues were indicated in previous report.[21] (C) Superimposition of the surface structures of mouse and human KLK7.

We have determined the crystal structure of mouse KLK7 at 1.93 Å resolution (PDB ID: 5ZFH). The crystal contains one monomer in the asymmetric unit, and the main-chain atoms of all residues are ordered. The structures of mouse and human KLK7 are very similar (Fig. 1A). Both are composed of two six-stranded β-barrel structures and six disulfide bonds. The sequence identity between mouse and human KLK7 is 76% (Fig. 1B), and the value of root mean square deviation (r.m.s.d.) of Cα atoms between mouse and human (PDB ID: 3BSQ[23]) is 0.55 Å, which was calculated for all residues except ten Cα atoms in two minor clusters by motion-tree analysis[24] (Figs. S1 and S2). Comparing the mouse and human KLK7 structures, two small regions were found to be slightly different: the C-terminal region (residues 242–245), and residues 73–80. The binding site of compound **1** is far from these regions (9.6 Å of nearest atoms between Ile73 and **1**) as observed in the crystal structure of human KLK7 in complex with compound **1** previously reported by us.[21] Since the structures of mouse apo and human complexes with **1** are also very similar (r.m.s.d = 0.43 Å), the complex structure of human KLK7 with **1** was used for the structural

comparisons around the binding site of **1**. The superimposition of mouse apo and human KLK7 complexed with **1** (PDB ID: 5Y9L[21]) revealed that the bottom of the S1 pocket of the mouse structure narrows around the side chain of Thr190 and a water molecule (Fig. 2). Thr190 in mouse KLK7 is replaced by alanine in human KLK7 (Fig. 1B). Furthermore, the water molecule in mouse KLK7 is captured by the oxygen atom of Thr190 side chain and the oxygen and nitrogen atoms of Val227 main chain with hydrogen-bonding contacts (hydrogen-bonding distances = 2.6, 3.1, and 3.3 Å, respectively). The temperature factor of the water oxygen atom is 34.1 Å^2, which is almost the same as the average value for water molecules (33.8 Å^2). The composite omit electron density map[26] contoured at 1σ clearly shows this water molecule (Fig. S3). The above two results support the existence of a water molecule at this position. By filling the bottom of the S1 pocket, the interaction of **1** appears to be hindered in mouse KLK7. The same interference of interaction might occur in mouse KLK7 for more potent human KLK7 inhibitors **2** and **3** since these compounds bear the same benzyl substituent. To improve the inhibition activity in mouse KLK7 we have modified the 5-chloro-2-methoxy benzyl group of our scaffold and removed the chlorine atom at the *meta* position of **1**.

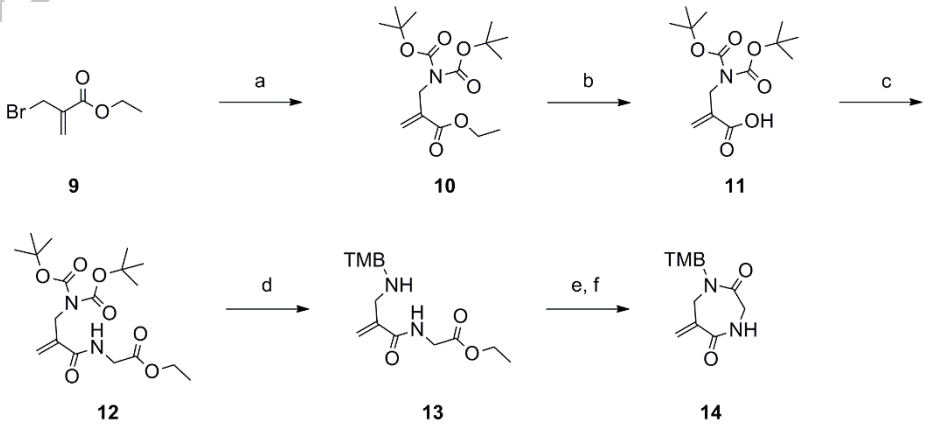
2.2. Chemistry

Considering its potency against human KLK7 and ease of synthesis, the benzyl group of compound **2** was modified. Thus, compound **8**, the dechloro derivative of **2**, was prepared as shown in Scheme 1. Dechlorination of **4**[21] was achieved via hydrogenolysis by Pd-C catalyst. Then, compound **5** was condensed with 3-(1-methyl-1*H*-pyrazol-4-yl)aniline to yield amide derivative **6**, which was converted to thioamide **7**. This thioamidation proceeded at the more reactive and less sterically hindered position. Finally, dimethylamidrazone **8** was obtained by the treatment of **7** with *N*,*N*-dimethylhydrazine in the presence of silver acetate.



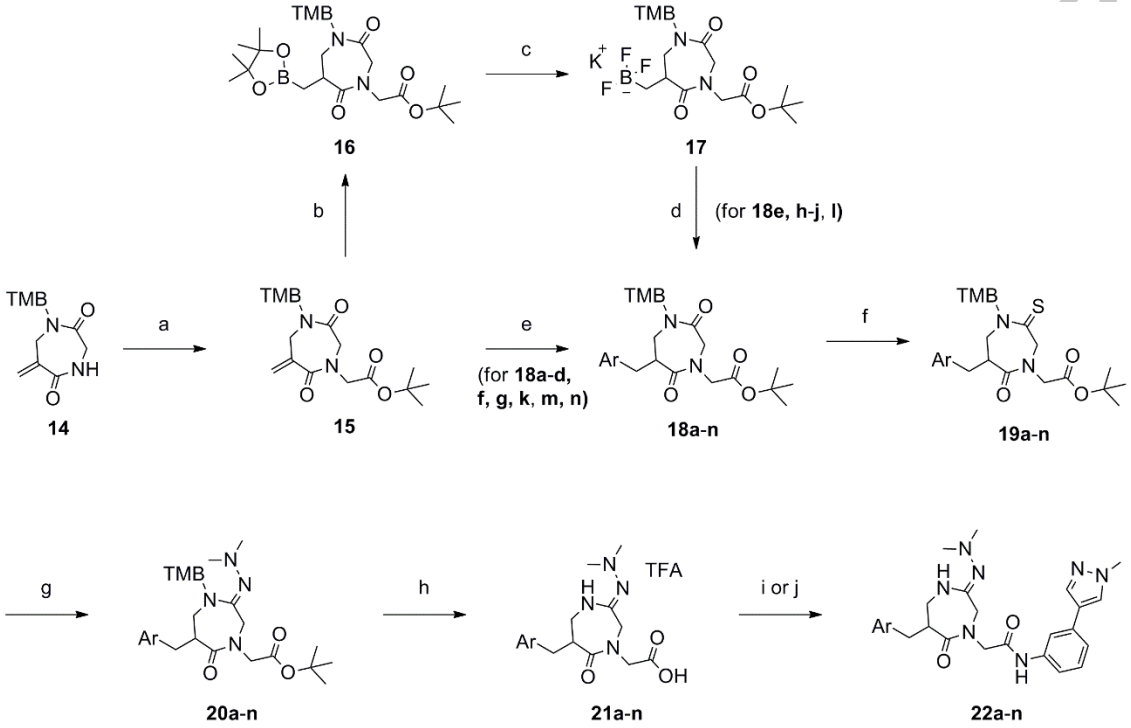
Scheme 1. Reagents and conditions: (a) H_2, 5% Pd-C, Et_3N, MeOH, AcOEt, rt; (b) 3-(1-methyl-1*H*-pyrazol-4-yl)aniline, EDCI·HCl, CH_2Cl_2, rt; (c) Lawesson's reagent, THF, 50 °C; (d) Me_2NNH_2, AgOAc, pyridine, rt.

Compound **14**, a key intermediate for the modification at the 6-position of the 1,4-diazepane core, was prepared as shown in Scheme 2. Di-*tert*-butoxycarbonyl (Boc) derivative **10** was synthesized by substitution of commercially available ethyl 2-(bromomethyl)acrylate **9** with di-*tert*-butyl iminodicarboxylate under basic conditions. The ethyl ester of **10** was hydrolyzed, and the resulting carboxylic acid **11** was coupled with glycine ethyl ester hydrochloride to yield **12**. After Boc deprotection, introduction of 2,4,6-trimethoxybenzyl (TMB) group to the resulting amino group by reductive alkylation gave **13**. Ester hydrolysis of **13**, followed by lactamization afforded exo-olefin compound **14**.



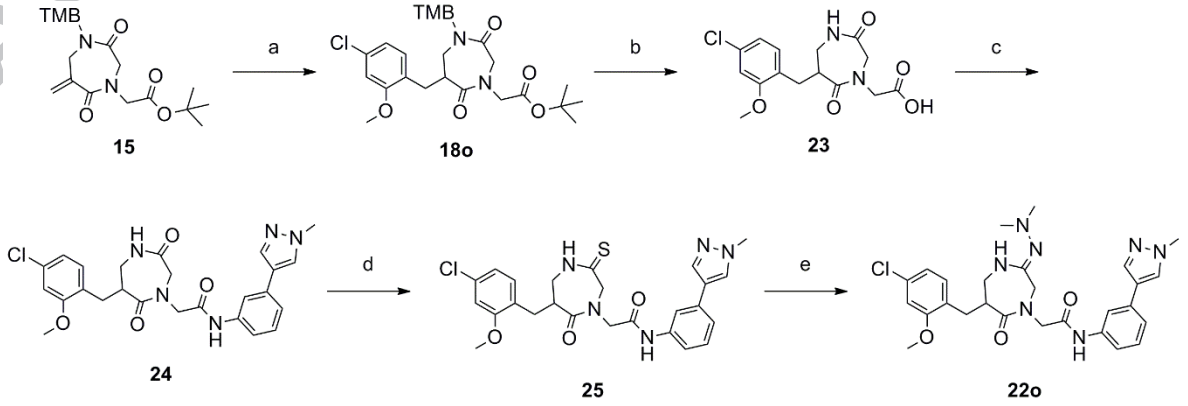
Scheme 2. Reagents and conditions: (a) di-*t*-butyl iminodicarboxylate, K_2CO_3, MeCN, rt; (b) 2 mol/L NaOH aq., MeOH, rt; (c) glycine ethyl ester hydrochloride, HOBt, EDCI·HCl, Et_3N, CH_2Cl_2, rt; (d) $MeSO_3H$, EtOH, 40 °C, then Et_3N, 2,4,6-$(MeO)_3$PhCHO, $NaBH(OAc)_3$, EtOH, 0–40 °C; (e) 4 mol/L NaOH aq. MeOH, rt; (f) HOBt, EDCI·HCl, MeCN, rt.

The synthesis of compounds **22a**–**n** (racemic compounds) containing the modified 6-benzyl group on the 1,4-diazepane core is shown in Scheme 3. Compound **14** was alkylated with *tert*-butyl bromoacetate to afford **15**. Then, compounds **18** were synthesized by the rhodium-catalyzed 1,4-addition reaction[27] of aryl boronic acids to Michael acceptor **15**. Alternatively, **18** were prepared by the 1,4-addition of bis(pinacolato)diboron to compound **15**, and the obtained pinacolboronate **16** was subsequently converted to potassium trifluroroborate **17**. Suzuki-Miyaura cross-coupling of **17** with aryl halides gave the corresponding **18**. The obtained racemic compounds **18a**–**n** were treated with Belleau's reagent[28] to prepare thioamides **19a**–**n**, which were converted to amidorazones **20a**–**n** by reacting with *N*,*N*-dimethylhydrazine in the presence of silver acetate. After the simultaneous removal of *tert*-butyl and TMB groups under acidic conditions, the condensation of 3-(1-methyl-1*H*-pyrazol-4-yl)aniline with the resulting carboxylic acid derivatives **21a**–**n** afforded **22a**–**n**. Optically active compounds (+)-**22g** and (–)-**22g** (not shown in the scheme) were obtained by chromatographic separation using chiral HPLC column (CHIRALPAK ID).



Scheme 3. Reagents and conditions: (a) $BrCH_2CO_2t$-Bu, 60% NaH, NaI, DMF, 0 °C; (b) bis(pinacolato)diboron, CuCl, 1,2-bis(diphenylphosphino)benzene, *t*-BuONa, MeOH, THF, rt; (c) KHF_2, MeCN, H_2O, 0 °C; (d) ArI, or ArBr, $Pd(OAc)_2$, 2-dicyclohexylphosphino-2',6'-di-*i*-propoxy-1,1'-biphenyl, Cs_2CO_3, toluene, H_2O, 80–85 °C; (e) boronic acid derivatives, $[Rh(cod)OH]_2$, dioxane, H_2O, 80–100 °C; (f) Belleau's reagent, THF, rt–50 °C; (g) Me_2NNH_2, AgOAc, pyridine, 0 °C; (h) anisole, TFA, rt–60 °C; (i) 3-(1-methyl-1*H*-pyrazol-4-yl)aniline, EDCI·HCl, CH_2Cl_2, rt; (j) 3-(1-methyl-1*H*-pyrazol-4-yl)aniline, 1-propanephosphonic acid anhydride, $(i\text{-Pr})_2$NEt, AcOEt, CH_2Cl_2, rt.

Compound **22o** was synthesized by an alternative approach shown in Scheme 4. Compound **18o** was prepared analogously to the described method in Scheme 3. Simultaneous removal of TMB and *tert*-butyl groups under acidic conditions gave compound **23**, which was condensed with 3-(1-methyl-1*H*-pyrazol-4-yl)aniline to furnish **24**. The selective thioamidation of compound **24** was accomplished with Lawesson's reagent to give compound **25**, which was converted to compound **22o** by treatment with *N*,*N*-dimethylhydrazine and silver acetate.

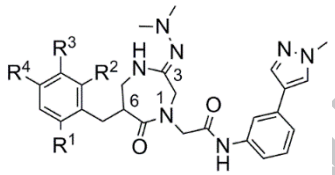


Scheme 4. Reagents and conditions: (a) 4-chloro-2-methoxyphenylboronic acid, $[Rh(cod)OH]_2$, dioxane, H_2O, 80 °C; (b) anisole, TFA, rt; (c) 3-(1-methyl-1*H*-pyrazol-4-yl)aniline, EDCI·HCl, CH_2Cl_2, rt; (d) Lawesson's reagent, THF, 50 °C; (e) Me_2NNH_2, AgOAc, MeOH, THF, rt.

2.3. Structure-activity relationships for human and mouse KLK7

The series of benzyl derivatives synthesized as above were evaluated for the inhibitory activity against human and mouse KLK7 (Table 2). As already mentioned, compound **2** showed potent inhibitory activity against human KLK7, but no inhibitory activity against mouse KLK7 was observed even at 30 μM concentration. The inhibition ratio against mouse KLK7 at 30 μM of dechloro derivative **8** was improved compared to **2**, although its inhibitory potency against human KLK7 slightly decreased. This result suggests that the problem with species differences in the inhibitory activity could be solved by the modification of the substituents on the benzyl group as already indicated by the crystal structure of mouse KLK7. Further investigations of the substituent effect were conducted by using easily prepared racemic compounds. The replacement of the methoxy group of **8** by a cyano group decreased the potency of compound **22a**, instead, the replacement by a chloro group (**22b**) resulted in retention of potency for human KLK7 inhibition and increased potency for mouse KLK7 inhibition. Moving the chloro substituent of **2** to *para* (**22o**) or *ortho* (**22c**) positions decreased the inhibitory activities against human KLK7. However, it is worth emphasizing that compound **22c** showed moderate but equipotent inhibitory activity against both human and mouse KLK7. The comparison between *ortho* substituted **22b** and di-*ortho* substituted **22c** suggests that the di-*ortho* substitution of the benzyl group is effective to overcome the species differences.

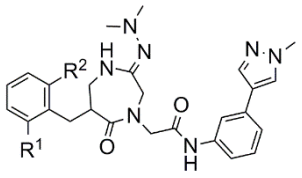
Table 2. Investigation of the benzyl substituents for inhibitory activity against recombinant human and mouse KLK7.



Compound	R^1	R^2	R^3	R^4	6-Config.	human KLK7 IC_{50} (μM)	mouse KLK7 IC_{50} (μM)	mouse KLK7 inhibition ratio @ 30 μM (%)
2	OMe	H	Cl	H	R	0.52	>30	−1.2
8	OMe	H	H	H	R	2.4	>30	41
22a	CN	H	H	H	racemate	17	>30	22
22b	Cl	H	H	H	racemate	2.5	21	59
22o	OMe	H	H	Cl	racemate	7.5	>30	−4.1
22c	OMe	Cl	H	H	racemate	11	14	67

To optimize the effects of the di-*ortho* substitution on the benzyl group, various combinations of di-*ortho* substituents were evaluated (Table 3). Compared to **22c**, compound **22d** bearing methoxy and fluoro groups exhibited slightly higher inhibitory activity for human KLK7, but also showed large differences in the inhibitory activity between human and mouse KLK7. On the other hand, compound **22e** containing methoxy and methyl groups displayed similar potency to **22c** against both KLK7s. Compounds combining a chloro substituent with another group (**22f**–**i**) all, except for larger nitrile substituted derivative **22i**, showed improved potency against both human and mouse KLK7. The fluoro substituted derivative **22f** exhibited species differences between human and mouse KLK7 inhibition, instead, chloro (**22g**) or methyl (**22h**) derivatives showed similar inhibitory potency for both KLK7s. In particular, **22g** was more than tenfold more potent than **22c** against both human and mouse KLK7. A similar tendency was observed in the case of the series containing a methyl group (**22e**, **22h**, and **22j**–**22l**). Fluoro substituted benzyl analogs (**22d**, **22f**, **22j**, **22m**, and **22n**) showed potent human KLK7 inhibition activity, but species differences in the inhibitory activity were observed in all cases. In summary, benzyl di-*ortho* substitution was a useful strategy for overcoming the species differences in inhibitory activity, and di-chloro analog **22g** was the preferred compound not only for its inhibitory activity but also for the absence of species differences.

Table 3. Optimization of the substituents for the inhibitory activity against recombinant human and mouse KLK7.



Compound[a]	R^1	R^2	human KLK7 IC_{50} (μM)	mouse KLK7 IC_{50} (μM)
22c	OMe	Cl	11	14
22d	OMe	F	6.8	>30
22e	OMe	Me	13	19
22f	Cl	F	0.94	6.0
22g	Cl	Cl	0.79	1.2
22h	Cl	Me	1.5	1.6
22i	Cl	CN	11	7.8
22j	Me	F	2.3	7.7
22k	Me	Me	5.2	3.8
22l	Me	CN	11	7.1
22m	F	F	6.9	>30
22n	F	CF_3	2.7	>30

[a]All compounds were racemate.

Table 4. Inhibitory activity against recombinant human and mouse KLK7 of the chiral compounds (+)-**22g** and (–)-**22g**.

Compound	human KLK7 IC_{50} (μM)	mouse KLK7 IC_{50} (μM)
(+)-22g	>30	16
(–)-22g	0.38	0.70

Next, we prepared chiral compounds (+)-**22g** and (–)-**22g** and evaluated their human and mouse KLK7 inhibitory activity to determine their differences in KLK7 inhibitions (Table 4). Compound (–)-**22g** showed more potent inhibitory activity against both human and mouse KLK7 than (+)-**22g**. In our previous report, the configuration of the 6-position of the 1,4-diazepane core affected the activity of KLK7 inhibition; the *R*-enantiomer was more potent than the *S*-enantiomer.[21] Therefore, we assumed that more potent enantiomer (–)-**22g** has *R* configuration.

2.4. Complex structure analysis

In order to visualize the binding mode of **22g,** in particular around the benzyl group, the crystal structure of mouse KLK7 with (±)-**22g** was solved (Fig. 3A). While **22g** was racemate, the crystal structure analysis was carried out assuming that the ligand was (–)-**22g**, which would have *R* configuration based on its potent inhibitory activity as described our previous report.[21]

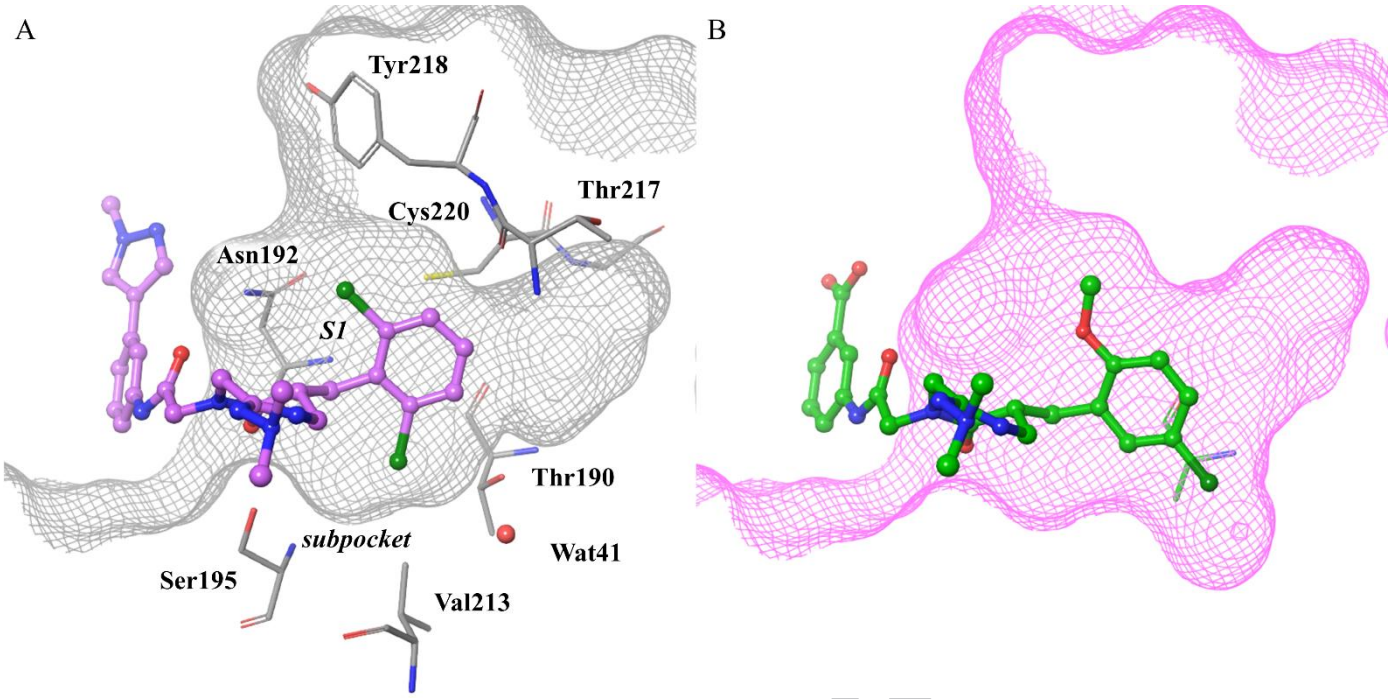


Figure 3. (A) Complex structure of **22g** around the binding site of mouse KLK7 (PDB ID: 5ZFI). The surface of the protease is represented by the gray mesh and **22g** is shown in ball-and-stick form (C: pink, O: red, N: blue, Cl: dark green). The chloro substituent interacts with the subpocket at the S1 site composed by the main chain of Ser195 and side chain of Val213. (B) Complex structure of human KLK7 with **1** (PDB ID: 5Y9L) around the binding site viewed from the same perspective as (A).

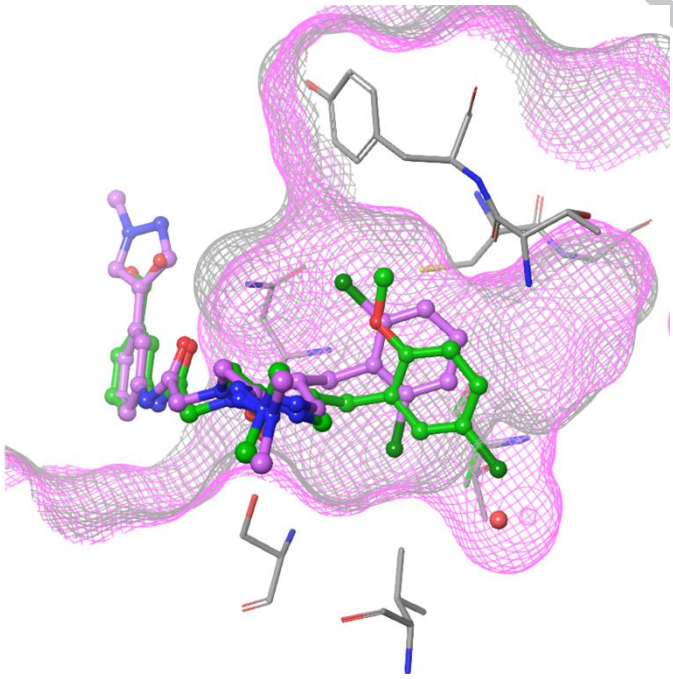


Figure 4. Superimposition of the crystal structure complexes of mouse KLK7 with **22g** (PDB ID: 5ZFI) and of human KLK7 with **1** (PDB ID: 5Y9L). The surface of KLK7 is represented as mesh (gray for mouse and pink for human) and the inhibitors are shown in ball-and stick model (C: pink for **22g**, and green for **1**, O: red, N: blue, Cl: dark green).

Both, **22g** in mouse KLK7 and **1** in human KLK7[21] interact with a common binding site; however, the positions of the benzyl group located at the S1 site are different from each other (Figs. 3 and 4). The benzyl group of **1** inserts deeply into the S1 pocket of human KLK7, and this binding pose is defined as type B binding mode in this paper. On the other hand, the benzyl group of **22g** moves to a shallower position in the S1 pocket of mouse KLK7 (this binding pose is defined as type A binding mode in this paper) than in that of human KLK7. In mouse KLK7, one of the chloro substituents of **22g** is located at the subpocket composed of the main chain of Ser195 and side chain of Val213, and makes van der Waals contacts with the side chains of Thr190 and Val213 (Fig. 3A). In addition, one of the *meta* site carbon atoms of the benzyl group in **22g** is positioned within van der Waals distance to the side chain oxygen atom of Thr190, and the other *meta* site carbon atom interacts with the side chain of Cys220 and main chain oxygen atom of Thr217. The other chlorine atom is positioned within van der Waals distances to the side chains of Asn192 and Tyr218. These two residues also form van der Waals contacts with the methoxy group of **1** in human KLK7; Tyr218 is replaced by Phe218 in human KLK7.

Considering Figs. 3B and 4, one of the *ortho* chlorine atoms on the benzyl group might induce steric hindrance in the case of type B binding mode in human KLK7. Therefore, the position of the benzyl group of **22g** in human KLK7 might also change from that of **1**, and the *ortho* chlorine atom could make van der Waals contacts with Val213. The residues that interact with the two chlorine atoms in **22g**, except for Thr190 and Tyr218, are conserved between mouse and human KLK7. Furthermore, in mouse KLK7, compound **22g** interacts with the Cβ atom of Thr190, which is equivalent to the interaction to the methyl group of Ala190 in human KLK7, and also with the phenyl ring of Tyr218, which corresponds to the phenyl ring of Phe218 in human KLK7. To summarize, our results indicate that human KLK7 interacts with **22g** in the same manner as with mouse KLK7. In fact, the inhibitory activities against mouse and human KLK7 are similar (Table 3).

The above binding mode information enables the elucidation of structure-activity relationships. In mouse KLK7, the benzyl group of compound **8** and **22c** would locate in the shallower

position in the S1 pocket similarly to **22g** (type A binding mode), and would not be able to form type B binding mode because of the steric hindrance that arises from the Thr190 residue and water molecule. The methoxy group of **8** might be positioned toward Asn192 and Tyr218, because the methoxy group would be too large to fit in the subpocket composed of Ser195 and Val213. Therefore, it is speculated that there is no interaction with the subpocket, which causes the inhibitory activity of **8** against mouse KLK7 to decrease. Compound **22c**, in which the hydrogen atom at the *ortho* position on the benzyl group of **8** is substituted with a chlorine atom, exhibited increased inhibitory potency against mouse KLK7 because the chlorine atom could interact with the subpocket. On the other hand, the potency of **22c** against human KLK7 was decreased compared with that of **8** because the binding modes of **8** and **22c** to human KLK7 would be different. In human KLK7, the binding mode of **8** might be type B where the methoxy group would interact with Asn192 and Phe218 as observed for **1**. However, the binding mode of **22c** in human KLK7 would be type A because the two large substituents at both *ortho* sites prevent the benzyl group from penetrating into the S1 pocket deeply, allowing the chlorine atom to interact with the conserved subpocket as described above. The binding mode of **22c** would be the same in mouse and human, thereby **22c** exhibited similar inhibitory activity against mouse and human KLK7. In type A binding mode, the methoxy group of **22c** is too large to interact with Asn192 and Phe218. Therefore, **22g**, in which the methoxy group of **22c** is replaced by a chlorine atom, showed ten times more potent inhibitory activity than **22c** against both of human and mouse KLK7. Similarly, other compounds with functional groups at both *ortho* sites (**22e**, **22g**, **22h**, **22i**, **22k** and **22l**) are predicted to exhibit the same binding mode in mouse and human KLK7, and these compounds showed equipotent inhibitory activity against both KLK7s (Table 3 and Fig. 5). In contrast, compounds that displayed different human and mouse KLK7 inhibitory activity (represented by triangles in the Fig. 5) would present different binding modes in each KLK7. These compounds can penetrate deeply into the S1 site of human KLK7 (Type B binding mode) because the *ortho*-substituents of the benzyl group are small (hydrogen or fluorine atom). Exceptionally, compounds **22f**, **22j**, and **22b** would be able to present both, type A and B, binding modes in human KLK7 because they would not only be able to penetrate deeply into the S1 site but also interact with the subpocket via the methyl or chloro substituents on the benzyl group. Therefore, these compounds exhibited more potent inhibitory activity against human KLK7 than against mouse KLK7.

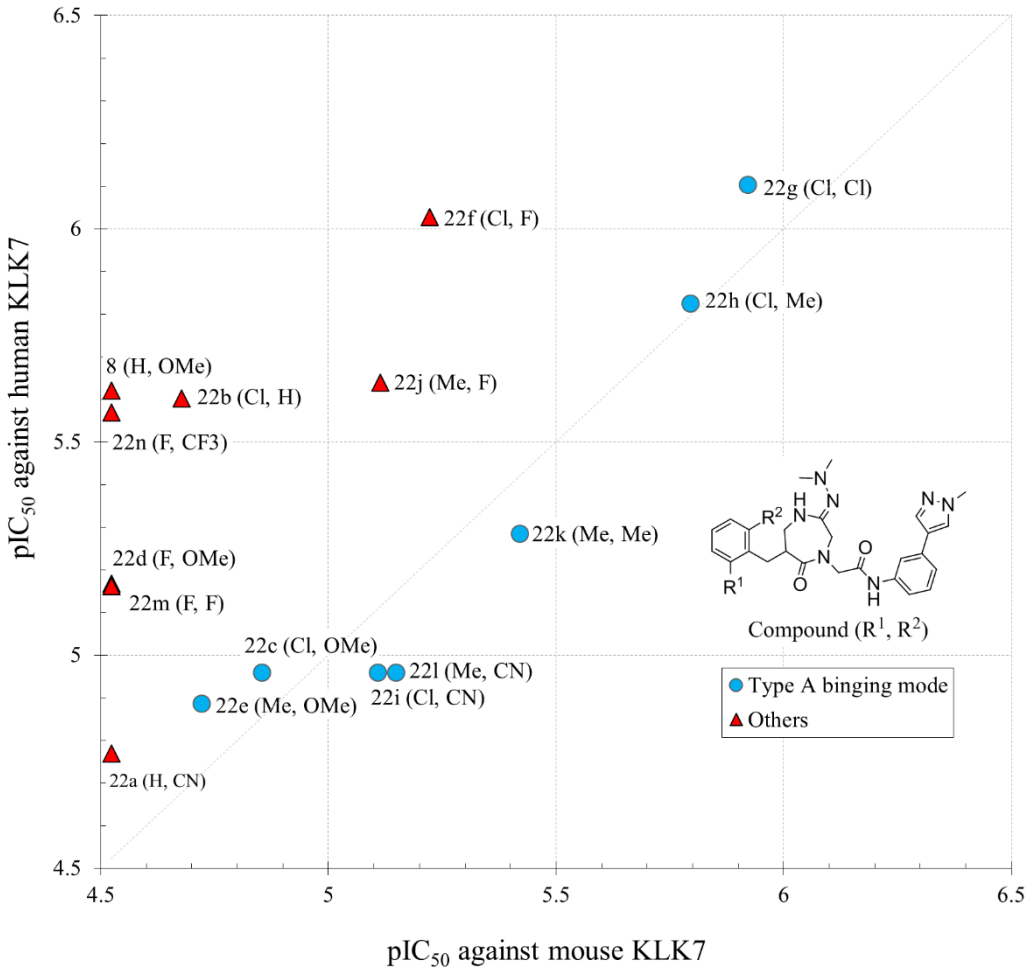


Figure 5. Comparison between human and mouse KLK7 inhibitory activities. R^1 and R^2 are shown in parentheses. R^1 were reassigned by the substituent that would be positioned in the subpocket in mouse KLK7. Circles represent type A binding mode and triangles indicate other binding mode in human KLK7. Inhibitory activity is displayed as pIC_{50} value ($-\log IC_{50}$). In the case of >30 μM of IC_{50} value, pIC_{50} was calculated with IC_{50} as 30 μM.

3. Conclusion

The structural difference between human and mouse KLK7 revealed the cause of the species differences of 1,3,6-trisubstituted 1,4-diazepan-7-one derivatives. To overcome the difference in inhibitory activity between human and mouse KLK7, the substituents on the benzyl group of the 1,4-diazepane scaffold were modified according to structure-based drug design studies. The series of di-*ortho* substituted benzyl derivatives eliminated the species differences, and ultimately, di-*ortho* chlorosubstituted compound **22g** was identified as a potent human and mouse KLK7 inhibitor. Moreover, only one of the optically active isomers of **22g**, (–)-**22g**, exhibited highly potent inhibitory activity against both KLK7s. The elucidation of the crystal structure of mouse KLK7 complexed with **22g** and human KLK7 complexed with **1** reveals the differences in the binding modes and justifies the structure-activity relationships found in human and mouse KLK7, respectively. We found that the binding position of the benzyl group in the S1 site was important for overcoming the species differences and prepared a series of di-*ortho* substituted benzyl compounds that would form the type A binding mode in both KLK7s, caused by similar van der Waals contacts between the substituents on the benzyl group and the residues of both KLK7s. Furthermore, the interaction to the subpocket in the S1 site was essential for the highly potent inhibitory activity.

4. Experimental

4.1. Crystal structure determinations of mouse KLK7

Expression, refolding, and purification of mouse KLK7 were performed as previously reported for human KLK7.[21] Briefly, cDNA encoding mouse KLK7 with DDDDK, enterokinase cutting site at the *N*-terminal side, was inserted into the pQE80 vector (Qiagen). BL21 cells transformed by the plasmid were grown in 2 x YT medium at 37 °C. When A_{660} in the medium reached 0.9, isopropyl β-D-1-thiogalactopyranoside was added to reach a final concentration of 1 mM. After culturing for 5 h, the cells were harvested.

KLK7 was purified using HisTrap HP column (GE healthcare) with 8 M urea. Refolding was performed using the step-wise dialysis method. The eluted KLK7 solution (0.4 mg/ml) in the dialysis membrane (2000 MWCO, Slide-a-Lyzer™, ThermoFisher Scientific) was incubated in a 100-fold volume of 50 mM Tris/HCl (pH 8.0), 100 mM NaCl, and 10 mM dithiothreitol overnight at 25 °C. It was dialyzed at 4 °C by lowering the urea concentration from 4 M to 0 M in buffer conditions (50 mM Tris/HCl (pH 8.0), 100 mM NaCl, 2 mM $CaCl_2$, 0.4 M L-Arg, 5 mM reduced glutathione, and 0.5 mM oxidized glutathione).

After the cleavage of the His-tag of KLK7 by enterokinase of weight ratio of 1/100 (Sigma), refolded KLK7 was purified using a HiTrap heparin HP column (GE healthcare).

Crystallization of KLK7 (10 mg/ml) was performed by the sitting-drop vapor diffusion method. Crystals of apo KLK7 were obtained by the crystallization condition of 20% w/v PEG 8000, 23% w/v PEG 400, 0.1 M $MgCl_2$, and 0.1 M Tris/HCl (pH 8.0). Crystals of the complex with **22g** were obtained by soaking into a crystallization solution a saturated solution of compound for 1.5 h. Crystals were flash-frozen in a cold nitrogen stream around 100 K, and X-ray diffraction data were collected using an R-AXIS IV^{++}imaging-plate area detector mounted on a Rigaku MicroMax-007 rotating-anode source with Cu*K*α radiation (λ = 1.5418 Å, 40 kV, 20 mA). All data were processed and scaled with HKL2000.[29] The crystal structure was solved by the molecular replacement method using the human KLK7 structure (PDB ID: 5Y9L).[21] A molecular-replacement calculation was performed using AMoRe.[30] Model fitting was carried out using Coot[31] and refined with Refmac-5.[32] Secondary structure assignments and 3D structural figures were prepared by Maestro ver. 11.2.[33]

4.2. Chemistry

4.2.1. General

Proton nuclear magnetic resonance spectra (^{1}H NMR) and was recorded on Brucker ARX-400 or Brucker Avance III (400 MHz) spectrometer in the indicated solvent. Chemical shifts (δ) are reported in parts per million relative to the internal standard tetramethylsilane. High-resolution mass spectra (HRMS) and fast atom bombardment (FAB) mass spectra were recorded on JEOL JMS-700 mass spectrometer. Electro-spray ionization (ESI) mass spectra were recorded on Agilent G1956A or Agilent G6120B MSD spectrometer system. Optical rotation were measured by JASCO P-1020. Chemical reagents and solvents were purchased from Aldrich, Tokyo Kasei Kogyo, Wako Pure Chemical Industries, Kanto Kagaku, Nacalai Tesque or other commercial suppliers and were used without purification. Chromatographic purification was performed using Merck Silica Gel 60 (230-400 mesh) or Purif-Pack® SI 30 μm, Purif-Pack® NH 60 μm supplied by Shoko Scientific.

*4.2.2. (R)-2-(6-(2-Methoxybenzyl)-3,7-dioxo-1,4-diazepan-1-yl)acetic acid (**5**)*

To a solution of **4** (1.0 g, 2.93 mmol) in MeOH (15 ml) and AcOEt (15 ml), Et_3N (0.50 ml, 3.6 mmol) and 5% palladium carbon (0.15 g) were added. The mixture was stirred under H_2 atmosphere at rt for 24 h. Then the catalyst was filtered off, and the filtrate was concentrated in vacuo. The residue was diluted with 0.1 M HCl aq., and the product was extracted with $CHCl_3$. The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo to give the title compound as a white solid (335mg, 37%). ^{1}H NMR (400MHz, CD_3OD) δ 7.24 - 7.15 (m, 2H), 6.95 (dd, *J* = 0.6, 8.1 Hz, 1H), 6.87 (dt, *J* = 1.1, 7.4 Hz, 1H), 4.68 (d, *J* = 17.4 Hz, 1H), 4.35 (d, *J* = 17.6 Hz, 1H), 4.07 (d, *J* = 17.4 Hz, 1H), 3.85 (s, 3H), 3.74 (d, *J* = 17.6 Hz, 1H), 3.62 - 3.52 (m, 1H), 3.25 - 3.19 (m, 2H), 3.13 (dd, *J* = 5.0, 14.1 Hz, 1H), 2.67 (dd, *J* = 9.2, 14.0 Hz, 1H); MS (ESI): 307 $(M+H)^+$.

*4.2.3. (R)-2-(6-(2-Methoxybenzyl)-3,7-dioxo-1,4-diazepan-1-yl)-N-(3-(1-methyl-1H-pyrazol-4-yl)phenyl)acetamide (**6**)*

To a solution of **5** (120 mg, 0.39 mmol) and 3-(1-methyl-1*H*-pyrazol-4-yl)aniline (67 mg, 0.39 mmol) in CH_2Cl_2 (10 ml), EDCI·HCl (113 mg, 0.59 mmol) was added. The mixture was stirred at rt overnight and then poured into sat. NH_4Cl aq. The product was extracted with AcOEt, and the organic layer was successively washed with water, brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 99/1 to 90/10) to give the title compound as a yellow solid (161 mg, 89%). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.93 (br. s, 1H), 7.75 (d, *J* = 0.6 Hz, 1H), 7.68 (br. s, 1H), 7.63 (s, 1H), 7.33 - 7.20 (m, 4H), 7.19 - 7.15 (m, 1H), 6.92 - 6.85 (m, 2H), 5.70 (br. s, 1H), 4.61 (d, *J* = 17.3 Hz, 1H), 4.38 (d, *J* = 15.2 Hz, 1H), 4.13 (d, *J* = 15.1 Hz, 1H), 3.95 (d, *J* = 17.3 Hz, 1H), 3.94 (s, 3H), 3.84 (s, 3H), 3.58 - 3.46 (m, 1H), 3.36 - 3.22 (m, 3H), 2.75 (dd, *J* = 9.0, 13.9 Hz, 1H); MS (ESI): 462 $(M+H)^+$.

*4.2.4. (R)-2-(6-(2-Methoxybenzyl)-7-oxo-3-thioxo-1,4-diazepan-1-yl)-N-(3-(1-methyl-1H-pyrazol-4-yl)phenyl)acetamide (**7**)*

To a solution of **6** (160 mg, 0.347 mmol) in THF (5.0 ml) was added Lawesson's reagent (67 mg, 0.166 mmol), and the mixture was stirred at 50 °C for 1 h. Then, water was added to the reaction mixture, and the product was extracted with AcOEt. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by NH silica gel column chromatography ($CHCl_3$/MeOH = 99/1 to 90/10) to give the title compound as a pale yellow solid (90 mg, 54%). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.96 (br. s, 1H), 7.90 (br. s, 1H), 7.75 (d, J = 0.5 Hz, 1H), 7.68 (br. s, 1H), 7.63 (s, 1H), 7.34 - 7.20 (m, 4H), 7.18 - 7.14 (m, 1H), 6.92 - 6.86 (m, 2H), 4.88 (d, J = 17.3 Hz, 1H), 4.56 (d, J = 17.1 Hz, 1H), 4.44 (d, J = 15.3 Hz, 1H), 4.13 (d, J = 15.4 Hz, 1H), 3.94 (s, 3H), 3.85 (s, 3H), 3.62 - 3.51 (m, 1H), 3.50 - 3.31 (m, 2H), 3.27 (dd, J = 5.1, 13.7 Hz, 1H), 2.78 (dd, J = 9.0, 13.7 Hz, 1H); MS (ESI): 478 $(M+H)^+$.

*4.2.5. (R)-2-(3-(2,2-Dimethylhydrazono)-6-(2-methoxybenzyl)-7-oxo-1,4-diazepan-1-yl)-N-(3-(1-methyl-1H-pyrazol-4-yl)phenyl)acetamide (**8**)*

To a solution of **7** (45 mg, 0.094 mmol) and *N*,*N*-dimethylhydrazine (0.012 ml, 0.16 mmol) in pyridine (2.0 ml) was added AgOAc (58.2 mg, 0.35 mmol), and the reaction mixture was stirred at rt for 2 h. Then, water was added to the reaction mixture, and the product was extracted with AcOEt. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by NH silica gel column chromatography ($CHCl_3$/MeOH = 98/2 to 90/10) to give the title compound as a pale yellow solid (28.7 mg, 61%). $[\alpha]_D^{24} = -64°$ (c 0.38, $CHCl_3$); ^{1}H NMR (400MHz, $CDCl_3$) δ 8.25 (br. s, 1H), 7.73 (d, J = 0.8 Hz, 1H), 7.67 (br. s, 1H), 7.60 (s, 1H), 7.29 - 7.15 (m, 5H), 6.91 - 6.85 (m, 2H), 6.27 (br. s, 1H), 4.55 (d, J = 16.1 Hz, 1H), 4.35 (d, J = 15.4 Hz, 1H), 4.18 (d, J = 15.4 Hz, 1H), 3.93 (s, 3H), 3.84 (s, 3H), 3.83 (d, J = 16.1 Hz, 1H), 3.53 - 3.36 (m, 2H), 3.28 - 3.16 (m, 2H), 2.78 (dd, J = 8.9, 13.8 Hz, 1H), 2.32 (s, 6H); MS (ESI): 504 $(M+H)^+$; HRMS (FAB) Calcd for $C_{27}H_{34}N_7O_3^+$: 504.2718; Found: 504.2707.

*4.2.6. Ethyl 2-(2-(((2,4,6-trimethoxybenzyl)amino)methyl)acrylamido)acetate (**13**)*

To a solution of ethyl 2-(bromomethyl)acrylate **9** (104.4 g, 0.541 mol) in acetonitrile (900 ml) were added di-*t*-butyl iminodicarboxylate (122.4g 0.564 mol) and K_2CO_3 (156.9 g, 1.14 mol). The mixture was stirred at rt for 2 days and concentrated in vacuo. The residue was diluted with AcOEt and water and the product was extracted. The organic layer was washed with water, dried over anhydrous Na_2SO_4 and concentrated in vacuo to give the crude **10** (182.7 g). To a solution of crude **10** (182.0 g) in MeOH (1000 ml) was added 2 M NaOH aq. (552 ml) slowly, and the mixture was stirred at rt for 1.5 h. The mixture was concentrated in vacuo, and the residue was diluted with $CHCl_3$ and water. After slow addition of sat. $KHSO_4$ aq., the product was extracted with $CHCl_3$. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo to give the crude **11** (171.8 g). To a solution of crude **11** (171.0 g) in CH_2Cl_2 (1200 ml) were added glycine ethyl ester hydrochloride (94.4 g, 0.676 mol), HOBt (94.4 g, 0.698 mol), EDCI·HCl (131.9 g, 0.688 mol), and Et_3N (94.2 ml, 0.676 mol), the reaction was stirred at rt for 4 h. To the mixture were added water and sat. $NaHCO_3$ aq. and the product was extracted with $CHCl_3$. The organic layer was successively washed with water, $KHSO_4$ aq., $NaHCO_3$ aq., and brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo to give the crude **12** (237.5 g). To a solution of crude **12** (118.0 g) in EtOH (1015 ml) was added methanesulfonic acid (60 ml, 0.924 mol), and the mixture was stirred at 40 °C for 17 h. After cooling to 0 °C, to the mixture were added EtOH (252 ml), and Et_3N (129 ml, 0.926 mol). To the resultant mixture were added 2,4,6-trimethoxybenzaldehyde (52.7 g, 0.268 mol), $NaBH(OAc)_3$ (113.3 g, 0.535 mol) at 0 °C. After stirring at 40 °C for 1.5 h, the mixture was concentrated in vacuo. The residue was diluted with $CHCl_3$ and water, and to the resulting mixture was added sat. $NaHCO_3$ aq. slowly. The product was extracted and the organic layer was washed with sat. $NaHCO_3$ aq. and brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 100/0 to 50/50) to give the title compound as a pale yellow solid (50.4 g, 4steps 52%). ^{1}H-NMR (400MHz, $CDCl_3$) δ 9.96 (t, J = 5.3 Hz, 1H) , 6.15 (d, J = 2.0 Hz, 1H), 6.13 (s, 2H), 5.34 - 5.37 (m, 1H), 4.19 (q, J = 7.1 Hz, 2H), 4.09 (d, J = 5.3 Hz, 2H), 3.82 (s, 3H), 3.79 (s, 6H), 3.79 (s, 2H), 3.42 (br. s, 2H), 1.27 (t, J = 7.1 Hz, 3H).

*4.2.7. 6-Methylene-1-(2,4,6-trimethoxybenzyl)-1,4-diazepane-2,5-dione (**14**)*

To a solution of **13** (50.0 g, 0.136 mol) in MeOH (164 ml) was added 4 M NaOH aq. (40.5 ml), and the mixture was stirred at rt for 14 h. After cooling to 0 °C, the mixture was acidified (pH = 4.9) with sat. $KHSO_4$ aq. and concentrated in vacuo. The residue was diluted with MeCN, and the solvent was concentrated in vacuo, and the same procedure was repeated 4 times. Then, MeCN (1000 ml) was added to the residue, and to the resultant mixture were added HOBt (18.4 g, 0.137 mol), EDCI·HCl (26.1 g, 0.136 mol), and the mixture was stirred at rt for 2 days. The mixture was concentrated in vacuo, and the residue was diluted with $CHCl_3$ and water. After slow addition of sat. $NaHCO_3$ aq., the product was extracted. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 96/4 to 95/5) to give the title compound as a pale yellow solid (28.0 g, 64%). ^{1}H-NMR (400MHz, DMSO-d_6) δ 8.02 (t, J = 5.2 Hz, 1H), 6.20 (s, 2H), 5.84 (d, J = 2.4 Hz, 1H), 4.87 (m, 1H), 4.43 (s, 2H), 3.98 (s, 2H), 3.85 (d, J = 5.2 Hz, 2H), 3.78 (s, 3H), 3.72 (s, 6H); MS: 321 $(M+H)^+$.

*4.2.8. tert-Butyl 2-(6-methylene-3,7-dioxo-4-(2,4,6-trimethoxybenzyl)-1,4-diazepan-1-yl)acetate (**15**)*

To a suspension of **14** (10 g, 31.2 mmol) and NaI (6.08 g, 40.6 mmol) in DMF (70 ml), *t*-butyl bromoacetate (6.00 ml, 40.6 mmol) and 60% NaH (1.50 g, 37.5 mmol) were added at 0 °C, and the reaction mixture was stirred for 1 h at the same temperature. After *t*-butyl bromoacetate (1.00 ml, 6.77 mmol) and 60% NaH (0.25 g, 6.25 mmol) were added, the reaction mixture was stirred at 0 °C for 1 h. Then, sat. NH_4Cl aq. was added to the reaction mixture, and the product was extracted with AcOEt. The organic layer was successively washed with water, brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 50/50 to 20/80) to give the title compound as a white solid (12.8 g, 94%). ^{1}H NMR (400MHz, $CDCl_3$) δ 6.10 - 6.07 (m, 1H), 6.08 (s, 2H), 5.01 - 4.98 (m, 1H), 4.65 (s, 2H), 4.27 (s, 2H), 4.19 (s, 2H), 4.01 (s, 2H), 3.81 (s, 3H), 3.76 (s, 6H), 1.47 (s, 9H); MS (ESI): 435 $(M+H)^+$.

*4.2.9. tert-Butyl 2-(3,7-dioxo-6-((4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)methyl)-4-(2,4,6-trimethoxybenzyl)-1,4-diazepan-1-yl)acetate (**16**)*

A mixture of CuCl (10.3 mg, 0.104 mmol), NaO*t*-Bu (30 mg, 0.311mmol) and 1,2-bis(diphenylphosphino)benzene (46 mg, 0.104 mmol) in THF (3 ml) was stirred at rt for 30 min. To the resultant mixture, a solution of bis(pinacolato)diboron (964 mg, 3.80 mmol) in THF (6 ml) was added. After stirring for 15 min at rt, compound **15** (1.50 g, 3.45 mmol) and MeOH (0.28 ml, 6.90

mmol) were added, and the mixture was stirred at rt for 18 h. Then, the reaction mixture was filtered, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 20/80 to 0/100) to give the title compound as a white solid (1.89 g, 97%). ^{1}H NMR (400MHz, $CDCl_3$) δ 6.10 (s, 2H), 4.68 (d, J = 13.7 Hz, 1H), 4.62 (d, J = 13.7 Hz, 1H), 4.44 (d, J = 16.9 Hz, 1H), 4.24 (d, J = 17.3 Hz, 1H), 3.96 (d, J = 16.8 Hz, 1H), 3.95 (d, J = 17.4 Hz, 1H), 3.81 (s, 3H), 3.77 (s, 6H), 3.25 - 3.05 (m, 3H), 1.44 (s, 9H), 1.19 (s, 6H), 1.17 (s, 6H), 1.04 - 0.94 (m, 1H), 0.82 - 0.74 (m, 1H); MS (ESI): 563 $(M+H)^+$.

*4.2.10. Potassium ((4-(2-(tert-butoxy)-2-oxoethyl)-2,5-dioxo-1-(2,4,6-trimethoxybenzyl)-1,4-diazepan-6-yl)methyl)trifluoroborate (**17**)*

To a solution of **16** (1.85 g, 3.29 mmol) in MeCN (30 ml) was added a solution of KHF_2 (0.771 g, 9.87 mmol) in H_2O (10 ml) at 0 °C. After stirring at 0 °C for 2h, solvent was concentrated in vacuo. The residue was diluted with acetone, the insolubles were removed by filtration and filtrate was concentrated in vacuo. The residue was triturated with diethylether, and the precipitate was collected by filtration to give the title compound as a white solid (1.74 g, 98%). ^{1}H NMR (400MHz, DMSO-d_6) δ 6.17 (s, 2H), 4.48 (d, J = 13.4 Hz, 1H), 4.34 (d, J = 13.4 Hz, 1H), 4.09 (d, J = 16.3 Hz, 1H), 4.02 (d, J = 16.9 Hz, 1H), 3.98 (d, J = 16.3 Hz, 1H), 3.88 (d, J = 16.9 Hz, 1H), 3.76 (s, 3H), 3.69 (s, 6H), 3.33 - 3.24 (m, 1H), 2.86 (dd, J = 11.9, 14.3 Hz, 1H), 2.63 - 2.53 (m, 1H), 1.39 (s, 9H), 0.41 - 0.28 (m, 1H), -0.04 - -0.18 (m, 1H); MS (FAB): 503 $(M-K)^-$.

*4.2.11. tert-Butyl 2-(6-(2-cyanobenzyl)-3,7-dioxo-4-(2,4,6-trimethoxybenzyl)-1,4-diazepan-1-yl)acetate (**18a**)*

To a solution of **15** (260 mg, 0.598 mmol) in dioxane/H_2O (10/1) (3 ml) were added 2-cyanophenylboronic acid (172 mg, 1.17 mmol) and hydroxy(1,5-cyclooctadiene)rhodium (I) dimer (27.3 mg, 0.060 mmol), and the mixture was stirred at 80 °C for 2h. The reaction mixture was diluted with AcOEt, and successively washed with sat. $KHSO_4$ aq., sat. $NaHCO_3$ aq., and brine. The organic layer was dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (eluent A: hexane/AcOEt (1/1), eluent B: MeOH; A/B = 95/5 to 90/10) to give the title compound as a pale yellow solid (321 mg, quant.). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.55 (dd, J = 1.1, 7.8 Hz, 1H), 7.45 (dt, J = 1.4, 7.7 Hz, 1H), 7.29 (dt, J = 1.1, 7.5 Hz, 1H), 7.22 - 7.18 (m, 1H), 6.00 (s, 2H), 4.72 (d, J = 13.7 Hz, 1H), 4.44 (d, J = 13.7 Hz, 1H), 4.28 (d, J = 17.3 Hz, 1H), 4.20 (d, J = 16.6 Hz, 1H), 4.14 (d, J = 16.6 Hz, 1H), 4.04 (d, J = 17.3 Hz, 1H), 3.83 (s, 3H), 3.69 (s, 6H), 3.40 - 3.27 (m, 2H), 3.09 - 2.92 (m, 2H), 2.86 (dd, J = 8.4, 14.2 Hz, 1H), 1.46 (s, 9H); MS (ESI): 538 $(M+H)^+$.

*4.2.12. tert-Butyl 2-(6-(2-chlorobenzyl)-3,7-dioxo-4-(2,4,6-trimethoxybenzyl)-1,4-diazepan-1-yl)acetate (**18b**)*

The reaction was carried out according to the procedure for **18a** with 2-chlorophenylboronic acid instead of 2-cyanophenylboronic acid to give the title compound as a pale yellow solid (114 mg, 91%). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.30 - 7.27 (m, 1H), 7.18 - 7.09 (m, 2H), 7.06 - 7.02 (m, 1H), 5.99 (s, 2H), 4.65 (d, J = 13.7 Hz, 1H), 4.47 (d, J = 13.8 Hz, 1H), 4.25 (d, J = 17.3 Hz, 1H), 4.21 (d, J = 16.7 Hz, 1H), 4.16 (d, J = 16.7 Hz, 1H), 4.07 (d, J = 17.3 Hz, 1H), 3.83 (s, 3H), 3.66 (s, 6H), 3.33 - 3.21 (m, 2H), 3.09 - 2.97 (m, 2H), 2.69 (dd, J = 9.3, 14.1 Hz, 1H), 1.46 (s, 9H); MS (ESI): 547 $(M+H)^+$.

*4.2.13. tert-Butyl 2-(6-(2-chloro-6-methoxybenzyl)-3,7-dioxo-4-(2,4,6-trimethoxybenzyl)-1,4-diazepan-1-yl)acetate (**18c**)*

The reaction was carried out according to the procedure for **18a** with 2-chloro-6-methoxyphenylboronic acid instead of 2-cyanophenylboronic acid to give the title compound as a pale yellow solid (134 mg, 98%). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.10 (t, J = 8.2 Hz, 1H), 6.90 (dd, J = 1.0, 8.2 Hz, 1H), 6.68 (dd, J = 0.8, 8.2 Hz, 1H), 5.94 (s, 2H), 4.62 (d, J = 13.7 Hz, 1H), 4.43 (d, J = 13.8 Hz, 1H), 4.24 (d, J = 17.3 Hz, 1H), 4.21 (s, 2H), 4.11 (d, J = 17.3 Hz, 1H), 3.82 (s, 3H), 3.72 (s, 3H), 3.62 (s, 6H), 3.31 (dd, J = 12.2, 14.4 Hz, 1H), 3.16 (dd, J = 3.6, 13.4 Hz, 1H), 3.08 (tt, J = 3.8, 11.5 Hz, 1H), 2.93 (dd, J = 10.9, 13.4 Hz, 1H), 2.82 (dd, J = 4.0, 14.4 Hz, 1H), 1.48 (s, 9H); MS (ESI): 577 $(M+H)^+$.

*4.2.14. tert-Butyl 2-(6-(2-fluoro-6-methoxybenzyl)-3,7-dioxo-4-(2,4,6-trimethoxybenzyl)-1,4-diazepan-1-yl)acetate (**18d**)*

The reaction was carried out according to the procedure for **18a** with 2-fluoro-6-methoxybebzeboronic acid instead of 2-cyanophenylboronic acid to give the title compound as a white solid (112 mg, 87%). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.13 (dt, J = 6.7, 8.3 Hz, 1H), 6.63 - 6.55 (m, 2H), 5.97 (s, 2H), 4.57 (d, J = 13.7 Hz, 1H), 4.50 (d, J = 13.7 Hz, 1H), 4.26 (d, J = 16.6 Hz, 1H), 4.19 (d, J = 17.3 Hz, 1H), 4.14 (d, J = 16.7 Hz, 1H), 4.14 (d, J = 17.4 Hz, 1H), 3.82 (s, 3H), 3.74 (s, 3H), 3.63 (s, 6H), 3.29 - 3.19 (m, 1H), 3.11 - 2.98 (m, 2H), 2.88 (dd, J = 3.7, 14.2 Hz, 1H), 2.71 (dd, J = 11.4, 13.9 Hz, 1H), 1.47 (s, 9H); MS (ESI): 561 $(M+H)^+$.

*4.2.15. tert-Butyl 2-(6-(2-methoxy-6-methylbenzyl)-3,7-dioxo-4-(2,4,6-trimethoxybenzyl)-1,4-diazepan-1-yl)acetate (**18e**)*

A mixture of **17** (200 mg, 0.369 mmol), 2-bromo-1-methoxy-3-methylbenzene (148 mg, 0.737 mmol), $Pd(OAc)_2$ (8.3 mg, 0.037 mmol), 2-dicyclohexylphosphino-2',6'-di-*iso*-propoxy-1,1'-biphenyl (34.4 mg, 0.074 mmol), and Cs_2CO_3 (360 mg, 1.11 mmol) in toluene (7.5 ml) and water (1.5 ml) was stirred at 80 °C for 25 h. Then, water was added to the reaction mixture, and the product was extracted with AcOEt. The organic layer was successively washed with water and brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 40/60 to 10/90) to give the title compound as a white solid (43 mg, 21%). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.06 (t, J = 7.9 Hz, 1H), 6.70 (d, J = 7.5 Hz, 1H), 6.65 (d, J = 8.0 Hz, 1H), 5.93 (s, 2H), 4.62 (d, J = 13.7 Hz, 1H), 4.39 (d, J = 13.7 Hz, 1H), 4.33 (d, J = 17.3 Hz, 1H), 4.30 (d, J = 16.6 Hz, 1H), 4.11 (d, J = 16.4 Hz, 1H), 4.05 (d, J = 17.3 Hz, 1H), 3.82 (s, 3H), 3.72 (s, 3H), 3.58 (s, 6H), 3.36 - 3.25 (m, 1H), 3.03 (dd, J = 3.1, 13.4 Hz, 1H), 2.92 - 2.71 (m, 3H), 2.07 (s, 3H), 1.48 (s, 9H); MS (ESI): 557 $(M+H)^+$.

*4.2.16. tert-Butyl 2-(6-(2-chloro-6-fluorobenzyl)-3,7-dioxo-4-(2,4,6-trimethoxybenzyl)-1,4-diazepan-1-yl)acetate (**18f**)*

The reaction was carried out according to the procedure for **18a** with 2-chloro-6-fluorobenzeneboronic acid instead of 2-cyanophenylboronic acid to give the title compound as a white solid (47 mg, 36%). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.17 - 7.08 (m, 2H), 6.92 - 6.86 (m, 1H), 5.95 (s, 2H), 4.66 (d, J = 13.7 Hz, 1H), 4.45 (d, J = 13.7 Hz, 1H), 4.25 (d, J = 16.7 Hz, 1H), 4.24 (d, J = 17.3 Hz, 1H), 4.18 (d, J = 16.8 Hz, 1H), 4.09 (d, J = 17.3 Hz, 1H), 3.82 (s, 3H), 3.65 (s, 6H), 3.33 (dd, J = 12.5, 14.0 Hz, 1H), 3.26 - 3.18 (m, 1H), 3.09 - 2.99 (m, 1H), 2.93 - 2.79 (m, 2H), 1.47 (s, 9H); MS (ESI): 565 $(M+H)^+$.

*4.2.17. tert-Butyl 2-(6-(2,6-dichlorobenzyl)-3,7-dioxo-4-(2,4,6-trimethoxybenzyl)-1,4-diazepan-1-yl)acetate (**18g**)*

The reaction was carried out according to the procedure for **18a** with 2,6-dichlorophenylboronic acid instead of 2-cyanophenylboronic acid to give the title compound as a white solid (116 mg, 87%). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.21 (d, J =

8.7 Hz, 1H), 7.21 (d, *J* = 7.4 Hz, 1H), 7.08 (dd, *J* = 7.4, 8.7 Hz, 1H), 5.93 (s, 2H), 4.70 (d, *J* = 13.7 Hz, 1H), 4.41 (d, *J* = 13.8 Hz, 1H), 4.26 (d, *J* = 17.3 Hz, 1H), 4.23 (s, 2H), 4.09 (d, *J* = 17.3 Hz, 1H), 3.82 (s, 3H), 3.65 (s, 6H), 3.41 (dd, *J* = 11.9, 14.4 Hz, 1H), 3.33 (dd, *J* = 3.5, 13.7 Hz, 1H), 3.19 - 3.10 (m, 1H), 3.04 (dd, *J* = 11.0, 13.7 Hz, 1H), 2.88 (dd, *J* = 3.7, 14.4 Hz, 1H), 1.48 (s, 9H); MS (ESI): 581 $(M+H)^+$.

*4.2.18. tert-Butyl 2-(6-(2-chloro-6-methylbenzyl)-3,7-dioxo-4-(2,4,6-trimethoxybenzyl)-1,4-diazepan-1-yl)acetate (**18h**)*

The reaction was carried out according to the procedure for **18e** with 3-chloro-2-iodotoluene instead of 2-bromo-1-methoxy-3-methylbenzene to give the title compound as a white solid (28 mg, 14%). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.15 (dd, *J* = 0.9, 7.9 Hz, 1H), 7.03 (t, *J* = 7.8 Hz, 1H), 6.99 - 6.96 (m, 1H), 5.93 (s, 2H), 4.70 (d, *J* = 13.7 Hz, 1H), 4.36 (d, *J* = 13.8 Hz, 1H), 4.34 (d, *J* = 17.3 Hz, 1H), 4.32 (d, *J* = 16.4 Hz, 1H), 4.12 (d, *J* = 16.4 Hz, 1H), 4.03 (d, *J* = 17.2 Hz, 1H), 3.82 (s, 3H), 3.61 (s, 6H), 3.48 - 3.37 (m, 1H), 3.26 - 3.15 (m, 1H), 2.95 - 2.83 (m, 3H), 2.13 (s, 3H), 1.48 (s, 9H); MS (ESI): 561 $(M+H)^+$.

*4.2.19. tert-Butyl 2-(6-(2-chloro-6-cyanobenzyl)-3,7-dioxo-4-(2,4,6-trimethoxybenzyl)-1,4-diazepan-1-yl)acetate (**18i**)*

The reaction was carried out according to the procedure for **18e** with 2-bromo-3-chlorobenzonitrile instead of 2-bromo-1-methoxy-3-methylbenzene to give the title compound as a pale yellow solid (16.3 mg, 8%). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.51 (dd, *J* = 1.3, 8.1 Hz, 1H), 7.46 (dd, *J* = 1.3, 7.7 Hz, 1H), 7.29 - 7.23 (m, 1H), 5.93 (s, 2H), 4.78 (d, *J* = 13.7 Hz, 1H), 4.38 (d, *J* = 13.7 Hz, 1H), 4.26 (d, *J* = 16.6 Hz, 1H), 4.26 (d, *J* = 17.3 Hz, 1H), 4.19 (d, *J* = 16.6 Hz, 1H), 4.07 (d, *J* = 17.3 Hz, 1H), 3.83 (s, 3H), 3.67 (s, 6H), 3.54 - 3.43 (m, 2H), 3.13 - 3.04 (m, 1H), 2.99 - 2.88 (m, 2H), 1.47 (s, 9H); MS (ESI): 572 $(M+H)^+$.

*4.2.20. tert-Butyl 2-(6-(2-fluoro-6-methylbenzyl)-3,7-dioxo-4-(2,4,6-trimethoxybenzyl)-1,4-diazepan-1-yl)acetate (**18j**)*

The reaction was carried out according to the procedure for **18e** with 2-bromo-1-fluoro-3-methylbenzene instead of 2-bromo-1-methoxy-3-methylbenzene to give the title compound as a white solid (37 mg, 18%). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.07 (dt, *J* = 5.8, 7.9 Hz, 1H), 6.87 (br. d, *J* = 7.5 Hz, 1H), 6.81 (br. t, *J* = 9.2 Hz, 1H), 5.93 (s, 2H), 4.69 (d, *J* = 13.6 Hz, 1H), 4.37 (d, *J* = 13.6 Hz, 1H), 4.35 (d, *J* = 17.3 Hz, 1H), 4.34 (d, *J* = 16.4 Hz, 1H), 4.08 (d, *J* = 16.3 Hz, 1H), 4.02 (d, *J* = 17.3 Hz, 1H), 3.81 (s, 3H), 3.60 (s, 6H), 3.41 - 3.32 (m, 1H), 3.14 - 3.03 (m, 1H), 2.89 (dd, *J* = 3.3, 14.5 Hz, 1H), 2.77 - 2.66 (m, 2H), 2.12 (s, 3H), 1.47 (s, 9H); MS (ESI): 545 $(M+H)^+$.

*4.2.21. tert-Butyl 2-(6-(2,6-dimethylbenzyl)-3,7-dioxo-4-(2,4,6-trimethoxybenzyl)-1,4-diazepan-1-yl)acetate (**18k**)*

The reaction was carried out according to the procedure for **18a** with 2,6-dimethylphenylboronic acid instead of 2-cyanophenylboronic acid to give the title compound as a white solid (111 mg, 89%). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.00 (dd, *J* = 6.5, 8.3 Hz, 1H), 6.95 - 6.91 (m, 2H), 5.93 (s, 2H), 4.67 (d, *J* = 13.7 Hz, 1H), 4.36 (d, *J* = 17.3 Hz, 1H), 4.35 (d, *J* = 13.7 Hz, 1H), 4.34 (d, *J* = 16.3 Hz, 1H), 4.09 (d, *J* = 16.6 Hz, 1H), 4.04 (d, *J* = 17.3 Hz, 1H), 3.83 (s, 3H), 3.58 (s, 6H), 3.35 (dd, *J* = 12.0, 14.1 Hz, 1H), 3.15 (dd, *J* = 2.6, 13.9 Hz, 1H), 2.87 (dd, *J* = 3.8, 14.4 Hz, 1H), 2.80 - 2.71 (m, 1H), 2.67 (dd, *J* = 11.5, 13.8 Hz, 1H), 2.13 (s, 6H), 1.48 (s, 9H); MS (ESI): 541 $(M+H)^+$.

*4.2.22. tert-Butyl 2-(6-(2-cyano-6-methylbenzyl)-3,7-dioxo-4-(2,4,6-trimethoxybenzyl)-1,4-diazepan-1-yl)acetate (**18l**)*

The reaction was carried out according to the procedure for **18e** with 2-iodo-3-methylbenzonitrile instead of 2-bromo-1-methoxy-3-methylbenzene to give the title compound as a pale yellow solid (25.2 mg, 12%). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.41 (dd, *J* = 1.0, 7.7 Hz, 1H), 7.30 (br. d, *J* = 7.7 Hz, 1H), 7.21 (t, *J* = 7.7 Hz, 1H), 5.88 (s, 2H), 4.81 (d, *J* = 13.7 Hz, 1H), 4.46 (d, *J* = 16.2 Hz, 1H), 4.41 (d, *J* = 17.3 Hz, 1H), 4.27 (d, *J* = 13.7 Hz, 1H), 3.99 (d, *J* = 16.1 Hz, 1H), 3.96 (d, *J* = 17.2 Hz, 1H), 3.81 (s, 3H), 3.60 (s, 6H), 3.58 (dd, *J* = 12.2, 14.7 Hz, 1H), 3.39 (dd, *J* = 3.8, 14.3 Hz, 1H), 2.94 - 2.81 (m, 2H), 2.65 (tt, *J* = 4.1, 11.5 Hz, 1H), 2.17 (s, 3H), 1.48 (s, 9H); MS (ESI): 552 $(M+H)^+$.

*4.2.23. tert-Butyl 2-(6-(2,6-difluorobenzyl)-3,7-dioxo-4-(2,4,6-trimethoxybenzyl)-1,4-diazepan-1-yl)acetate (**18m**)*

The reaction was carried out according to the procedure for **18a** with 2,6-difluorophenylboronic acid instead of 2-cyanophenylboronic acid to give the title compound as a white solid (50 mg, 20%). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.20 - 7.11 (m, 1H), 6.83 - 6.75 (m, 2H), 5.98 (s, 2H), 4.62 (d, *J* = 13.7 Hz, 1H), 4.51 (d, *J* = 13.6 Hz, 1H), 4.28 (d, *J* = 16.8 Hz, 1H), 4.19 (d, *J* = 17.3 Hz, 1H), 4.12 (d, *J* = 17.3 Hz, 1H), 4.12 (d, *J* = 16.8 Hz, 1H), 3.82 (s, 3H), 3.66 (s, 6H), 3.31 - 3.22 (m, 1H), 3.17 - 3.09 (m, 1H), 3.07 - 2.97 (m, 1H), 2.97 - 2.90 (m, 1H), 2.69 (dd, *J* = 10.7, 14.2 Hz, 1H), 1.47 (s, 9H); MS (ESI): 549 $(M+H)^+$.

*4.2.24. tert-Butyl 2-(6-(2-fluoro-6-(trifluoromethyl)benzyl)-3,7-dioxo-4-(2,4,6-trimethoxybenzyl)-1,4-diazepan-1-yl)acetate (**18n**)*

The reaction was carried out according to the procedure for **18a** with 2-fluoro-6-(trifluoromethyl)phenylboronic acid instead of 2-cyanophenylboronic acid to give the title compound as a white solid (35 mg, 21%). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.40 (br. d, *J* = 7.7 Hz, 1H), 7.35 - 7.28 (m, 1H), 7.14 (br. t, *J* = 8.8 Hz, 1H), 5.93 (s, 2H), 4.67 (d, *J* = 13.8 Hz, 1H), 4.42 (d, *J* = 13.7 Hz, 1H), 4.29 (d, *J* = 16.8 Hz, 1H), 4.19 (d, *J* = 17.3 Hz, 1H), 4.14 (d, *J* = 16.9 Hz, 1H), 4.11 (d, *J* = 17.3 Hz, 1H), 3.81 (s, 3H), 3.63 (s, 6H), 3.33 - 3.23 (m, 2H), 3.12 (tt, *J* = 3.7, 11.3 Hz, 1H), 2.91 - 2.81 (m, 2H), 1.48 (s, 9H); MS (ESI): 599 $(M+H)^+$.

*4.2.25. tert-Butyl 2-(6-(2-chlorobenzyl)-7-oxo-3-thioxo-4-(2,4,6-trimethoxybenzyl)-1,4-diazepan-1-yl)acetate (**19b**)*

To a solution of **18b** (106 mg, 0.194 mmol) in THF (2 ml) was added Belleau's reagent (56.3 mg, 0.107 mmol), and the mixture was stirred at rt for 2 h. Then, the solvent was concentrated in vacuo, and the residue was purified by silica gel column chromatography (hexane/AcOEt = 80/20 to 60/40) to give the title compound as white solid (96 mg, 88%). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.28 - 7.23 (m, 1H), 7.17 - 7.08 (m, 2H), 6.96 (dd, *J* = 2.0, 7.3 Hz, 1H), 5.96 (s, 2H), 5.34 (d, *J* = 14.2 Hz, 1H), 4.90 (d, *J* = 16.1 Hz, 1H), 4.67 (d, *J* = 14.2 Hz, 1H), 4.66 (d, *J* = 17.3 Hz, 1H), 4.40 (d, *J* = 16.1 Hz, 1H), 3.90 (d, *J* = 17.3 Hz, 1H), 3.84 (s, 3H), 3.63 (s, 6H), 3.66 - 3.53 (m, 1H), 3.33 - 3.24 (m, 1H), 3.14 (dd, *J* = 4.9, 15.1 Hz, 1H), 2.78 - 2.65 (m, 2H), 1.48 (s, 9H); MS (ESI): 563 $(M+H)^+$.

*4.2.26. tert-Butyl 2-(6-(2-methoxy-6-methylbenzyl)-7-oxo-3-thioxo-4-(2,4,6-trimethoxybenzyl)-1,4-diazepan-1-yl)acetate (**19e**)*

The reaction was carried out according to the procedure for **19b** with **18e** instead of **18b** to give the title compound as a white solid (38 mg, 90%). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.06 (t, *J* = 7.9 Hz, 1H), 6.69 (d, *J* = 7.5 Hz, 1H), 6.62 (d, *J* = 8.0 Hz, 1H), 5.88 (s, 2H), 5.37 (d, *J* = 14.1 Hz, 1H), 5.02 (d, *J* = 15.9 Hz, 1H), 4.72 (d, *J* = 17.3 Hz, 1H), 4.57 (d, *J* = 14.1 Hz, 1H), 4.39 (d, *J* = 15.9 Hz, 1H), 3.87 (d, *J* = 17.3 Hz, 1H), 3.82 (s, 3H), 3.73 - 3.64 (m, 1H), 3.70 (s, 3H), 3.55 (s, 6H), 3.06 - 2.94 (m, 2H), 2.85 (dd, *J* = 11.5, 13.7 Hz, 1H), 2.51 - 2.40 (m, 1H), 2.05 (s, 3H), 1.48 (s, 9H); MS (ESI): 573 $(M+H)^+$.

*4.2.27. tert-Butyl 2-(6-(2-chloro-6-fluorobenzyl)-7-oxo-3-thioxo-4-(2,4,6-trimethoxybenzyl)-1,4-diazepan-1-yl)acetate (**19f**)*

The reaction was carried out according to the procedure for **19b** with **18f** instead of **18b** to give the title compound as a white solid (33 mg, 80%). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.16 - 7.06 (m, 2H), 6.87 (ddd, *J* = 1.5, 7.9, 9.2 Hz, 1H), 5.90 (s, 2H), 5.33 (d, *J* = 14.2 Hz, 1H), 4.98 (d, *J* = 16.2 Hz, 1H), 4.65 (d, *J* = 14.2 Hz, 1H), 4.64 (d, *J* = 17.3 Hz, 1H), 4.45 (d, *J* = 16.2 Hz, 1H), 3.90 (d, *J* = 17.4 Hz, 1H), 3.83 (s, 3H), 3.74 - 3.65 (m, 1H), 3.63 (s, 6H), 3.32 (td, *J* = 3.2, 13.4 Hz, 1H), 3.05 (dd, *J* = 4.9, 15.2 Hz, 1H), 2.84 - 2.76 (m, 1H), 2.76 - 2.66 (m, 1H), 1.48 (s, 9H); MS (ESI): 581 $(M+H)^+$.

*4.2.28. tert-Butyl 2-(6-(2,6-dichlorobenzyl)-7-oxo-3-thioxo-4-(2,4,6-trimethoxybenzyl)-1,4-diazepan-1-yl)acetate (**19g**)*

The reaction was carried out according to the procedure for **19b** with **18g** instead of **18b** to give the title compound as a white solid (110 mg, 97%). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.19 (d, *J* = 8.8 Hz, 1H), 7.19 (d, *J* = 7.3 Hz, 1H), 7.08 (dd, *J* = 7.3, 8.8 Hz, 1H), 5.89 (s, 2H), 5.36 (d, *J* = 14.3 Hz, 1H), 5.02 (d, *J* = 16.2 Hz, 1H), 4.65 (d, *J* = 17.3 Hz, 1H), 4.60 (d, *J* = 14.2 Hz, 1H), 4.46 (d, *J* = 16.1 Hz, 1H), 3.89 (d, *J* = 17.3 Hz, 1H), 3.85 - 3.76 (m, 1H), 3.83 (s, 3H), 3.63 (s, 6H), 3.45 (dd, *J* = 4.1, 13.9 Hz, 1H), 3.06 (dd, *J* = 5.0, 15.1 Hz, 1H), 2.99 (dd, *J* = 11.2, 13.9 Hz, 1H), 2.87 - 2.77 (m, 1H), 1.48 (s, 9H); MS (ESI): 597 $(M+H)^+$.

*4.2.29. tert-Butyl 2-(6-(2-chloro-6-methylbenzyl)-7-oxo-3-thioxo-4-(2,4,6-trimethoxybenzyl)-1,4-diazepan-1-yl)acetate (**19h**)*

The reaction was carried out according to the procedure for **19b** with **18h** instead of **18b** to give the title compound as a white solid (24 mg, 86%). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.14 - 7.10 (m, 1H), 7.03 (t, *J* = 7.7 Hz, 1H), 6.99 - 6.95 (m, 1H), 5.88 (s, 2H), 5.42 (d, *J* = 14.2 Hz, 1H), 5.04 (d, *J* = 16.1 Hz, 1H), 4.71 (d, *J* = 17.4 Hz, 1H), 4.55 (d, *J* = 14.2 Hz, 1H), 4.40 (d, *J* = 16.1 Hz, 1H), 3.86 (d, *J* = 17.3 Hz, 1H), 3.86 - 3.77 (m, 1H), 3.82 (s, 3H), 3.59 (s, 6H), 3.25 (dd, *J* = 3.9, 14.2 Hz, 1H), 3.09 (dd, *J* = 5.3, 15.1 Hz, 1H), 2.91 (dd, *J* = 11.3, 14.2 Hz, 1H), 2.58 - 2.48 (m, 1H), 2.12 (s, 3H), 1.48 (s, 9H); MS (ESI): 577 $(M+H)^+$.

*4.2.30. tert-Butyl 2-(6-(2-fluoro-6-methylbenzyl)-7-oxo-3-thioxo-4-(2,4,6-trimethoxybenzyl)-1,4-diazepan-1-yl)acetate (**19j**)*

The reaction was carried out according to the procedure for **19b** with **18j** instead of **18b** to give the title compound as a white solid (29 mg, 78%). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.07 (dt, *J* = 5.9, 7.8 Hz, 1H), 6.86 (br. d, *J* = 7.4 Hz, 1H), 6.79 (br. t, *J* = 8.9 Hz, 1H), 5.87 (s, 2H), 5.42 (d, *J* = 14.1 Hz, 1H), 5.02 (d, *J* = 15.9 Hz, 1H), 4.72 (d, *J* = 17.3 Hz, 1H), 4.56 (d, *J* = 14.1 Hz, 1H), 4.37 (d, *J* = 16.1 Hz, 1H), 3.86 (d, *J* = 17.3 Hz, 1H), 3.81 (s, 3H), 3.77 - 3.67 (m, 1H), 3.57 (s, 6H), 3.15 - 3.01 (m, 2H), 2.79 - 2.70 (m, 1H), 2.42 - 2.32 (m, 1H), 2.09 (s, 3H), 1.48 (s, 9H); MS (ESI): 561 $(M+H)^+$.

*4.2.31. tert-Butyl 2-(6-(2,6-dimethylbenzyl)-7-oxo-3-thioxo-4-(2,4,6-trimethoxybenzyl)-1,4-diazepan-1-yl)acetate (**19k**)*

The reaction was carried out according to the procedure for **19b** with **18k** instead of **18b,** and THF and CH_2Cl_2 was used as a solvent to give the title compound as a white solid (105 mg, 92%). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.02 - 6.97 (m, 1H), 6.93 - 6.89 (m, 2H), 5.88 (s, 2H), 5.42 (d, *J* = 14.1 Hz, 1H), 5.02 (d, *J* = 16.2 Hz, 1H), 4.73 (d, *J* = 17.4 Hz, 1H), 4.53 (d, *J* = 14.1 Hz, 1H), 4.40 (d, *J* = 16.1 Hz, 1H), 3.87 (d, *J* = 17.3 Hz, 1H), 3.83 (s, 3H), 3.73 - 3.64 (m, 1H), 3.56 (s, 6H), 3.20 (dd, *J* = 3.5, 14.2 Hz, 1H), 3.04 (dd, *J* = 5.4, 14.9 Hz, 1H), 2.67 (dd, *J* = 11.4, 14.3 Hz, 1H), 2.52 - 2.41 (m, 1H), 2.11 (s, 6H), 1.48 (s, 9H); MS (ESI): 557 $(M+H)^+$.

*4.2.32. tert-Butyl 2-(6-(2,6-difluorobenzyl)-7-oxo-3-thioxo-4-(2,4,6-trimethoxybenzyl)-1,4-diazepan-1-yl)acetate (**19m**)*

The reaction was carried out according to the procedure for **19b** with **18m** instead of **18b** to give the title compound as a white solid (45 mg, 87%). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.20 - 7.11 (m, 1H), 6.81 - 6.73 (m, 2H), 5.93 (s, 2H), 5.29 (d, *J* = 14.2 Hz, 1H), 4.93 (d, *J* = 16.1 Hz, 1H), 4.72 (d, *J* = 14.2 Hz, 1H), 4.60 (d, *J* = 17.4 Hz, 1H), 4.46 (d, *J* = 16.2 Hz, 1H), 3.93 (d, *J* = 17.3 Hz, 1H), 3.83 (s, 3H), 3.67 - 3.56 (m, 1H), 3.63 (s, 6H), 3.24 - 3.19 (m, 1H), 3.08 (dd, *J* = 4.3, 14.8 Hz, 1H), 2.71 - 2.62 (m, 2H), 1.48 (s, 9H); MS (ESI): 565 $(M+H)^+$.

*4.2.33. tert-Butyl 2-(6-(2-fluoro-6-(trifluoromethyl)benzyl)-7-oxo-3-thioxo-4-(2,4,6-trimethoxybenzyl)-1,4-diazepan-1-yl)acetate (**19n**)*

The reaction was carried out according to the procedure for **19b** with **18n** instead of **18b** to give the title compound as a white solid (31 mg, 94%). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.39 (br. d, *J* = 7.8 Hz, 1H), 7.35 - 7.28 (m, 1H), 7.13 (br. t, *J* = 8.7 Hz, 1H), 5.89 (s, 2H), 5.34 (d, *J* = 14.2 Hz, 1H), 4.96 (d, *J* = 16.3 Hz, 1H), 4.65 (d, *J* = 14.3 Hz, 1H), 4.60 (d, *J* = 17.3 Hz, 1H), 4.48 (d, *J* = 16.3 Hz, 1H), 3.92 (d, *J* = 17.4 Hz, 1H), 3.82 (s, 3H), 3.69 - 3.58 (m, 1H), 3.61 (s, 6H), 3.45 - 3.34 (m, 1H), 3.06 (dd, *J* = 3.8, 15.4 Hz, 1H), 2.84 - 2.72 (m, 2H), 1.48 (s, 9H); MS (ESI): 615 $(M+H)^+$.

*4.2.34. tert-Butyl 2-(6-(2-cyanobenzyl)-3-(2,2-dimethylhydrazono)-7-oxo-4-(2,4,6-trimethoxybenzyl)-1,4-diazepan-1-yl)acetate (**20a**)*

To a solution of **18a** (325 mg, 0.605 mmol) in THF (3 ml) was added Belleau's reagent (320 mg, 0.605 mmol), and the mixture was stirred at rt for 1 h. Then, the reaction mixture was diluted with AcOEt, and was successively washed with sat. $NaHCO_3$ aq., water, and brine. The organic layer was dried over anhydrous Na_2SO_4, and concentrated in vacuo to give crude **19a** as a pale yellow foam (409 mg). To a solution of crude **19a** (409 mg) in Pyridine (5 ml) were added *N*,*N*-dimethylhydrazine (115 µl, 1.49 mmol) and AgOAc (340 mg, 2.04 mmol) at 0 °C, and the mixture was stirred for 30 min. The reaction mixture was diluted with AcOEt and filtered, then filtrate was concentrated in vacuo. The residue was diluted with AcOEt and insolubles were filtered off. The filtrate was concentrated, and the residue was purified by NH silica gel column chromatography (hexane/AcOEt = 70/30 to 35/65) to give the title compound as a pale yellow solid (299 mg, 2 steps 85%). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.53 (dd, *J* = 1.1, 7.7 Hz, 1H), 7.42 (dt, *J* = 1.4, 7.7 Hz, 1H), 7.29 - 7.23 (m, 1H), 7.16 (br. d, *J* = 7.5 Hz, 1H), 5.99 (s, 2H), 4.78 (d, *J* = 15.7 Hz, 1H), 4.64 (d, *J* = 13.3 Hz, 1H), 4.48 (d, *J* = 15.7 Hz, 1H), 4.29 (d, *J* = 17.1 Hz, 1H), 4.28 (d, *J* = 13.4 Hz, 1H), 3.92 (d, *J* = 17.1 Hz, 1H), 3.82 (s, 3H), 3.65 (s, 6H), 3.29 - 3.18 (m, 2H), 2.95 (dd, *J* = 6.0, 14.7 Hz, 1H), 2.86 (dd, *J* = 8.3, 14.2 Hz, 1H), 2.75 - 2.65 (m, 1H), 2.41 (s, 6H), 1.48 (s, 9H); MS (ESI): 580 $(M+H)^+$.

*4.2.35. tert-Butyl 2-(6-(2-chlorobenzyl)-3-(2,2-dimethylhydrazono)-7-oxo-4-(2,4,6-trimethoxybenzyl)-1,4-diazepan-1-yl)acetate (**20b**)*

To a solution of **19b** (92 mg, 0.163 mmol) in pyridine (2 ml) were added AgOAc (82 mg, 0.49 mmol) and *N*,*N*-dimethylhydrazine (25 µl, 0.33 mmol) at 0 °C, and the mixture was stirred for 3h. The reaction mixture was diluted with AcOEt and insolubles were filtered off. Then, filtrate was concentrated in vacuo, and the residue was purified by NH silica gel column chromatography (hexane/AcOEt = 85/15 to 60/40) to give the title compound as a white solid (83 mg, 86%). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.29 - 7.23 (m, 1H), 7.14 - 7.05 (m, 2H),

7.02 - 6.96 (m, 1H), 5.98 (s, 2H), 4.75 (d, *J* = 15.8 Hz, 1H), 4.60 (d, *J* = 13.3 Hz, 1H), 4.55 (d, *J* = 15.8 Hz, 1H), 4.28 (d, *J* = 13.3 Hz, 1H), 4.26 (d, *J* = 17.1 Hz, 1H), 3.95 (d, *J* = 17.1 Hz, 1H), 3.82 (s, 3H), 3.62 (s, 6H), 3.24 - 3.15 (m, 2H), 2.89 (dd, *J* = 5.8, 14.6 Hz, 1H), 2.79 - 2.64 (m, 2H), 2.41 (s, 6H), 1.48 (s, 9H); MS (ESI): 589 $(M+H)^+$.

*4.2.36. tert-Butyl 2-(6-(2-chloro-6-methoxybenzyl)-3-(2,2-dimethylhydrazono)-7-oxo-4-(2,4,6-trimethoxybenzyl)-1,4-diazepan-1-yl)acetate (**20c**)*

The reaction was carried out according to the procedure for **20a** with **18c** instead of **18a** to give the title compound as a pale yellow solid (83.2 mg, 2 steps 62%). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.06 (t, *J* = 8.2 Hz, 1H), 6.88 (dd, *J* = 1.0, 8.2 Hz, 1H), 6.65 (dd, *J* = 0.7, 8.2 Hz, 1H), 5.91 (s, 2H), 4.85 (d, *J* = 15.7 Hz, 1H), 4.65 (d, *J* = 13.3 Hz, 1H), 4.55 (d, *J* = 15.7 Hz, 1H), 4.36 (d, *J* = 17.1 Hz, 1H), 4.18 (d, *J* = 13.2 Hz, 1H), 3.89 (d, *J* = 17.1 Hz, 1H), 3.80 (s, 3H), 3.70 (s, 3H), 3.58 (s, 6H), 3.40 - 3.29 (m, 1H), 3.22 - 3.13 (m, 1H), 2.89 (dd, *J* = 10.7, 13.4 Hz, 1H), 2.76 - 2.64 (m, 2H), 2.41 (s, 6H), 1.49 (s, 9H); MS (ESI): 619 $(M+H)^+$.

*4.2.37. tert-Butyl 2-(3-(2,2-dimethylhydrazono)-6-(2-fluoro-6-methoxybenzyl)-7-oxo-4-(2,4,6-trimethoxybenzyl)-1,4-diazepan-1-yl)acetate (**20d**)*

The reaction was carried out according to the procedure for **20a** with **18d** instead of **18a** to give the title compound as a pale yellow solid (73.3 mg, 2 steps 63%). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.09 (dt, *J*=6.7, 8.3 Hz, 1H), 6.61 - 6.51 (m, 2H), 5.94 (s, 2H), 4.77 (d, *J* = 15.8 Hz, 1H), 4.59 (d, *J* = 13.2 Hz, 1H), 4.58 (d, *J* = 15.8 Hz, 1H), 4.27 (d, *J* = 17.1 Hz, 1H), 4.24 (d, *J* = 13.3 Hz, 1H), 3.95 (d, *J* = 17.1 Hz, 1H), 3.80 (s, 3H), 3.71 (s, 3H), 3.59 (s, 6H), 3.28 - 3.18 (m, 1H), 3.10 - 2.99 (m, 1H), 2.80 - 2.62 (m, 3H), 2.41 (s, 6H), 1.49 (s, 9H); MS (ESI): 603 $(M+H)^+$.

*4.2.38. tert-Butyl 2-(3-(2,2-dimethylhydrazono)-6-(2-methoxy-6-methylbenzyl)-7-oxo-4-(2,4,6-trimethoxybenzyl)-1,4-diazepan-1-yl)acetate (**20e**)*

The reaction was carried out according to the procedure for **20b** with **19e** instead of **19b** to give the title compound as a pale yellow solid (34 mg, 88%). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.03 (t, *J* = 7.9 Hz, 1H), 6.68 (d, *J* = 7.5 Hz, 1H), 6.61 (d, *J* = 8.0 Hz, 1H), 5.89 (s, 2H), 4.92 (d, *J* = 15.7 Hz, 1H), 4.69 (d, *J* = 13.2 Hz, 1H), 4.49 (d, *J* = 15.6 Hz, 1H), 4.45 (d, *J* = 17.1 Hz, 1H), 4.15 (d, *J* = 13.2 Hz, 1H), 3.82 (d, *J* = 17.1 Hz, 1H), 3.80 (s, 3H), 3.69 (s, 3H), 3.52 (s, 6H), 3.35 (dd, *J* = 12.3, 14.9 Hz, 1H), 2.96 (dd, *J* = 4.0, 13.6 Hz, 1H), 2.81 (dd, *J* = 11.2, 13.7 Hz, 1H), 2.70 (dd, *J* = 6.0, 15.1 Hz, 1H), 2.48 - 2.35 (m, 1H), 2.42 (s, 6H), 2.09 (s, 3H), 1.49 (s, 9H); MS (ESI): 599 $(M+H)^+$.

*4.2.39. tert-Butyl 2-(6-(2-chloro-6-fluorobenzyl)-3-(2,2-dimethylhydrazono)-7-oxo-4-(2,4,6-trimethoxybenzyl)-1,4-diazepan-1-yl)acetate (**20f**)*

The reaction was carried out according to the procedure for **20b** with **19f** instead of **19b** to give the title compound as a white solid (25 mg, 83%). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.13 - 7.05 (m, 2H), 6.89 - 6.82 (m, 1H), 5.93 (s, 2H), 4.78 (d, *J* = 15.8 Hz, 1H), 4.63 (d, *J* = 13.2 Hz, 1H), 4.62 (d, *J* = 15.7 Hz, 1H), 4.28 (d, *J* = 17.2 Hz, 1H), 4.24 (d, *J* = 13.3 Hz, 1H), 3.95 (d, *J* = 17.1 Hz, 1H), 3.81 (s, 3H), 3.61 (s, 6H), 3.31 (dd, *J* = 11.0, 14.1 Hz, 1H), 3.21 (td, *J* = 3.3, 12.9 Hz, 1H), 2.85 - 2.68 (m, 3H), 2.42 (s, 6H), 1.49 (s, 9H); MS (ESI): 607 $(M+H)^+$.

*4.2.40. tert-Butyl 2-(6-(2,6-dichlorobenzyl)-3-(2,2-dimethylhydrazono)-7-oxo-4-(2,4,6-trimethoxybenzyl)-1,4-diazepan-1-yl)acetate (**20g**)*

The reaction was carried out according to the procedure for **20b** with **19g** instead of **19b** to give the title compound as a pale yellow solid (92 mg, 88%). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.18 (d, *J* = 8.5 Hz, 1H), 7.18 (d, *J* = 7.4 Hz, 1H), 7.05 (dd, *J* = 7.4, 8.5 Hz, 1H), 5.91 (s, 2H), 4.83 (d, *J* = 15.7 Hz, 1H), 4.68 (d, *J* = 13.3 Hz, 1H), 4.61 (d, *J* = 15.7 Hz, 1H), 4.32 (d, *J* = 17.1 Hz, 1H), 4.20 (d, *J* = 13.2 Hz, 1H), 3.93 (d, *J* = 17.1 Hz, 1H), 3.81 (s, 3H), 3.62 (s, 6H), 3.43 (dd, *J* = 13.1, 15.9 Hz, 1H), 3.35 (dd, *J* = 4.4, 13.8 Hz, 1H), 2.98 (dd, *J* = 10.2, 13.8 Hz, 1H), 2.85 - 2.75 (m, 2H), 2.43 (s, 6H), 1.49 (s, 9H); MS (ESI): 623 $(M+H)^+$.

*4.2.41. tert-Butyl 2-(6-(2-chloro-6-methylbenzyl)-3-(2,2-dimethylhydrazono)-7-oxo-4-(2,4,6-trimethoxybenzyl)-1,4-diazepan-1-yl)acetate (**20h**)*

The reaction was carried out according to the procedure for **20b** with **19h** instead of **19b** to give the title compound as a white solid (17 mg, 71%). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.12 (dd, *J* = 1.0, 7.8 Hz, 1H), 7.00 (t, *J* = 7.7 Hz, 1H), 6.97 - 6.93 (m, 1H), 5.89 (s, 2H), 4.92 (d, *J* = 15.7 Hz, 1H), 4.73 (d, *J* = 13.3 Hz, 1H), 4.52 (d, *J* = 15.6 Hz, 1H), 4.41 (d, *J* = 17.1 Hz, 1H), 4.16 (d, *J* = 13.3 Hz, 1H), 3.84 (d, *J* = 17.1 Hz, 1H), 3.80 (s, 3H), 3.56 (s, 6H), 3.45 (dd, *J* = 12.0, 14.9 Hz, 1H), 3.17 (dd, *J* = 4.1, 14.1 Hz, 1H), 2.87 (dd, *J* = 10.7, 14.1 Hz, 1H), 2.80 (dd, *J* = 5.6, 14.9 Hz, 1H), 2.59 - 2.48 (m, 1H), 2.42 (s, 6H), 2.13 (s, 3H), 1.49 (s, 9H); MS (ESI): 603 $(M+H)^+$.

*4.2.42. tert-Butyl 2-(6-(2-chloro-6-cyanobenzyl)-3-(2,2-dimethylhydrazono)-7-oxo-4-(2,4,6-trimethoxybenzyl)-1,4-diazepan-1-yl)acetate (**20i**)*

The reaction was carried out according to the procedure for **20a** with **18i** instead of **18a** to give the title compound as a colorless oil (15.5 mg, 2 steps 69%). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.48 (dd, *J* = 1.3, 8.0 Hz, 1H), 7.44 (dd, *J* = 1.3, 7.8 Hz, 1H), 7.22 (t, *J* = 7.9 Hz, 1H), 5.92 (s, 2H), 4.81 (d, *J* = 15.4 Hz, 1H), 4.75 (d, *J* = 13.3 Hz, 1H), 4.64 (d, *J* = 15.4 Hz, 1H), 4.27 (d, *J* = 17.1 Hz, 1H), 4.20 (d, *J* = 13.3 Hz, 1H), 3.99 (d, *J* = 17.2 Hz, 1H), 3.82 (s, 3H), 3.64 (s, 6H), 3.50 - 3.41 (m, 2H), 2.98 - 2.85 (m, 2H), 2.83 - 2.73 (m, 1H), 2.43 (s, 6H), 1.48 (s, 9H); MS (ESI): 614 $(M+H)^+$.

*4.2.43. tert-Butyl 2-(3-(2,2-dimethylhydrazono)-6-(2-fluoro-6-methylbenzyl)-7-oxo-4-(2,4,6-trimethoxybenzyl)-1,4-diazepan-1-yl)acetate (**20j**)*

The reaction was carried out according to the procedure for **20b** with **19j** instead of **19b** to give the title compound as a pale yellow solid (20 mg, 71%). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.03 (dt, *J* = 6.0, 7.8 Hz, 1H), 6.85 (br. d, *J* = 7.4 Hz, 1H), 6.78 (br. t, *J* = 8.8 Hz, 1H), 5.89 (s, 2H), 4.92 (d, *J* = 15.7 Hz, 1H), 4.72 (d, *J* = 13.3 Hz, 1H), 4.49 (d, *J* = 15.6 Hz, 1H), 4.42 (d, *J* = 17.1 Hz, 1H), 4.16 (d, *J* = 13.3 Hz, 1H), 3.83 (d, *J* = 17.1 Hz, 1H), 3.80 (s, 3H), 3.54 (s, 6H), 3.40 - 3.31 (m, 1H), 3.02 (td, *J* = 3.4, 13.9 Hz, 1H), 2.83 - 2.66 (m, 2H), 2.47 - 2.31 (m, 1H), 2.42 (s, 6H), 2.11 (s, 3H), 1.49 (s, 9H); MS (ESI): 587 $(M+H)^+$.

*4.2.44. tert-Butyl 2-(6-(2,6-dimethylbenzyl)-3-(2,2-dimethylhydrazono)-7-oxo-4-(2,4,6-trimethoxybenzyl)-1,4-diazepan-1-yl)acetate (**20k**)*

The reaction was carried out according to the procedure for **20b** with **19k** instead of **19b** to give the title compound as a white solid (97.7 mg, 89%). ^{1}H NMR (400MHz, $CDCl_3$) δ 6.97 (dd, *J* = 6.3, 8.4 Hz, 1H), 6.93 - 6.88 (m, 2H), 5.88 (s, 2H), 4.95 (d, *J* = 15.6 Hz, 1H), 4.74 (d, *J* = 13.6 Hz, 1H), 4.48 (d, *J* = 15.8 Hz, 1H), 4.46 (d, *J* = 17.1 Hz, 1H), 4.13 (d, *J* = 13.2 Hz, 1H), 3.81 (s, 3H), 3.81 (d, *J* = 17.2 Hz, 1H), 3.52 (s, 6H), 3.40 - 3.31 (m, 1H), 3.16 - 3.10 (m, 1H), 2.77 (dd, *J* = 5.6, 14.7 Hz, 1H),

2.63 (dd, *J* = 11.0, 14.1 Hz, 1H), 2.42 (s, 6H), 2.45 - 2.38 (m, 1H), 2.13 (s, 6H), 1.49 (s, 9H); MS (ESI): 583 $(M+H)^+$.

*4.2.45. tert-Butyl 2-(6-(2-cyano-6-methylbenzyl)-3-(2,2-dimethylhydrazono)-7-oxo-4-(2,4,6-trimethoxybenzyl)-1,4-diazepan-1-yl)acetate (**20l**)*

The reaction was carried out according to the procedure for **20a** with **18l** instead of **18a** to give the title compound as a colorless oil (30.3 mg, 2 steps 73%). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.38 (dd, *J* = 0.8, 7.6 Hz, 1H), 7.27 (br. d, *J* = 7.7 Hz, 1H), 7.18 (t, *J* = 7.7 Hz, 1H), 5.85 (s, 2H), 5.00 (d, *J* = 15.4 Hz, 1H), 4.80 (d, *J* = 13.3 Hz, 1H), 4.46 (d, *J* = 15.4 Hz, 1H), 4.45 (d, *J* = 17.1 Hz, 1H), 4.11 (d, *J* = 13.2 Hz, 1H), 3.81 (d, *J* = 16.8 Hz, 1H), 3.80 (s, 3H), 3.55 (s, 6H), 3.60 - 3.52 (m, 1H), 3.34 (dd, *J* = 4.0, 14.1 Hz, 1H), 2.89 - 2.77 (m, 2H), 2.43 (s, 6H), 2.40 - 2.31 (m, 1H), 2.16 (s, 3H), 1.49 (s, 9H); MS (ESI): 594 $(M+H)^+$.

*4.2.46. tert-Butyl 2-(6-(2,6-difluorobenzyl)-3-(2,2-dimethylhydrazono)-7-oxo-4-(2,4,6-trimethoxybenzyl)-1,4-diazepan-1-yl)acetate (**20m**)*

The reaction was carried out according to the procedure for **20b** with **19m** instead of **19b** to give the title compound as a white solid (40 mg, 85%). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.16 - 7.07 (m, 1H), 6.80 - 6.72 (m, 2H), 5.96 (s, 2H), 4.70 (d, *J* = 15.8 Hz, 1H), 4.65 (d, *J* = 15.9 Hz, 1H), 4.57 (d, *J* = 13.3 Hz, 1H), 4.29 (d, *J* = 13.2 Hz, 1H), 4.20 (d, *J* = 17.1 Hz, 1H), 4.01 (d, *J* = 17.1 Hz, 1H), 3.81 (s, 3H), 3.63 (s, 6H), 3.27 - 3.17 (m, 1H), 3.14 - 3.06 (m, 1H), 2.84 (dd, *J* = 5.4, 13.6 Hz, 1H), 2.77 - 2.61 (m, 2H), 2.41 (s, 6H), 1.48 (s, 9H); MS (ESI): 591 $(M+H)^+$.

*4.2.47. tert-Butyl 2-(3-(2,2-dimethylhydrazono)-6-(2-fluoro-6-(trifluoromethyl)benzyl)-7-oxo-4-(2,4,6-trimethoxybenzyl)-1,4-diazepan-1-yl)acetate (**20n**)*

The reaction was carried out according to the procedure for **20b** with **19n** instead of **19b** to give the title compound as a white solid (26 mg, 83%). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.37 (br. d, *J* = 7.9 Hz, 1H), 7.31 - 7.23 (m, 1H), 7.11 (br. t, *J* = 9.2 Hz, 1H), 5.93 (s, 2H), 4.72 (d, *J* = 15.8 Hz, 1H), 4.67 (d, *J* = 15.9 Hz, 1H), 4.66 (d, *J* = 13.3 Hz, 1H), 4.22 (d, *J* = 13.2 Hz, 1H), 4.22 (d, *J* = 17.2 Hz, 1H), 4.00 (d, *J* = 17.1 Hz, 1H), 3.80 (s, 3H), 3.61 (s, 6H), 3.33 - 3.21 (m, 2H), 2.88 - 2.74 (m, 3H), 2.42 (s, 6H), 1.48 (s, 9H); MS (ESI): 641 $(M+H)^+$.

*4.2.48. 2-(6-(2-Cyanobenzyl)-3-(2,2-dimethylhydrazono)-7-oxo-1,4-diazepan-1-yl)-N-(3-(1-methyl-1H-pyrazol-4-yl)phenyl)acetamide (**22a**)*

Anisole (0.26 ml, 2.38 mmol) and TFA (3 ml, 38.9 mmol) were added to **20a** (274 mg, 0.473 mmol), and the mixture was stirred at rt for 5 h and at 40 °C for 20 h. Then, the mixture was azeotropically concentrated with toluene 3 times, and the residue was triturated with Et_2O. The appeared solid was collected by filtration to give **21a** as a white solid (214 mg). To a mixture of **21a** in CH_2Cl_2 (2 ml) and AcOEt (2 ml) were added *N*,*N*-diisopropylethylamine (0.33 ml, 1.89 mmol), 3-(1-methyl-1*H*-pyrazol-4-yl)aniline (81 mg, 0.468 mmol), and 1.7 mol/l 1-propanephosphonic acid cyclic anhydride in AcOEt (0.83 ml, 1.41 mmol), and the reaction mixture was stirred at rt for 15 min. Then, the reaction mixture was diluted with AcOEt, and successively washed with sat. $NaHCO_3$ aq., water, and brine. The organic layer was dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by NH silica gel column chromatography (eluent A: hexane/EtOAc (1/1), eluent B: MeOH, A/B = 95/5 to 88/12) to give the title compound as a white solid (224 mg, 2 steps 95%). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.96 (br. s, 1H), 7.73 (d, *J* = 0.8 Hz, 1H), 7.67 - 7.62 (m, 2H), 7.61 (s, 1H), 7.53 - 7.44 (m, 2H), 7.35 - 7.17 (m, 4H), 6.30 (br. s, 1H), 4.56 (d, *J* = 16.3 Hz, 1H), 4.30 (d, *J* = 15.6 Hz, 1H), 4.19 (d, *J* = 15.6 Hz, 1H), 3.94 (s, 3H), 3.84 (d, *J* = 16.4 Hz, 1H), 3.64 - 3.47 (m, 3H), 3.42 - 3.33 (m, 1H), 2.99 (dd, *J* = 6.3, 14.1 Hz, 1H), 2.31 (s, 6H); MS (ESI): 499 $(M+H)^+$; HRMS (FAB) Calcd for $C_{27}H_{31}N_8O_2^+$: 499.2564; Found: 499.2543.

*4.2.49. 2-(6-(2-Chlorobenzyl)-3-(2,2-dimethylhydrazono)-7-oxo-1,4-diazepan-1-yl)-N-(3-(1-methyl-1H-pyrazol-4-yl)phenyl)acetamide (**22b**)*

Anisole (0.143 ml, 1.31 mmol) and TFA (1.5 ml, 19.5 mmol) were added to **20b** (77 mg, 0.131 mmol), and the reaction mixture was stirred at 60 °C for 5 h. Then, the mixture was azeotropically concentrated with toluene, and the residue was triturated with Et_2O. The supernatant liquid was removed by decantation, and the residue was collected to give the **21b** as a white solid (55 mg). To a suspension of **21b** in CH_2Cl_2 (2 ml) were added 3-(1-methyl-1*H*-pyrazol-4-yl)aniline (47.3 mg , 0.273 mmol), EDCI·HCl (52.4 mg, 0.273 mmol), and the mixture was stirred at rt for 1 h. To the reaction mixture was added sat. $NaHCO_3$ aq., and the product was extracted with AcOEt. The organic layer was successively washed with water and brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by NH silica gel column chromatography (eluent A: hexane/AcOEt (1/1), eluent B: MeOH, A/B = 98/2 to 92/8) to give the title compound as a white solid (54 mg, 2 steps 81%). ^{1}H NMR (400MHz, $CDCl_3$) δ 8.02 (br. s, 1H), 7.74 (d, *J* = 0.8 Hz, 1H), 7.65 (br. s, 1H), 7.61 (s, 1H), 7.40 - 7.30 (m, 2H), 7.30 - 7.15 (m, 5H), 6.26 (br. s, 1H), 4.57 (d, *J* = 16.3 Hz, 1H), 4.31 (d, *J* = 15.4 Hz, 1H), 4.20 (d, *J* = 15.6 Hz, 1H), 3.94 (s, 3H), 3.83 (d, *J* = 16.3 Hz, 1H), 3.66 - 3.53 (m, 1H), 3.49 - 3.27 (m, 3H), 2.88 (dd, *J* = 7.7, 14.0 Hz, 1H), 2.31 (s, 6H); MS (ESI): 508 $(M+H)^+$; HRMS (FAB) Calcd for $C_{26}H_{31}ClN_7O_2^+$: 508.2222; Found: 508.2222.

*4.2.50. 2-(6-(2-Chloro-6-methoxybenzyl)-3-(2,2-dimethylhydrazono)-7-oxo-1,4-diazepan-1-yl)-N-(3-(1-methyl-1H-pyrazol-4-yl)phenyl)acetamide (**22c**)*

The reaction was carried out according to the procedure for **22a** with **20c** instead of **20a** to give the title compound as a white solid (14.8 mg, 2 steps 21%). ^{1}H NMR (400MHz, $CDCl_3$) δ 8.16 (br. s, 1H), 7.74 (d, *J* = 0.8 Hz, 1H), 7.68 (br. s, 1H), 7.62 (s, 1H), 7.30 - 7.27 (m, 2H), 7.24 - 7.19 (m, 1H), 7.16 (t, *J* = 8.2 Hz, 1H), 7.01 (dd, *J* = 1.1, 8.1 Hz, 1H), 6.79 (dd, *J* = 0.8, 8.3 Hz, 1H), 6.31 (br. s, 1H), 4.55 (d, *J* = 15.9 Hz, 1H), 4.34 (d, *J* = 15.4 Hz, 1H), 4.23 (d, *J* = 15.4 Hz, 1H), 3.93 (s, 3H), 3.90 (br. d, *J* = 15.9 Hz, 1H), 3.84 (s, 3H), 3.55 - 3.45 (m, 1H), 3.35 - 3.25 (m, 3H), 3.16 (dd, *J* = 10.5, 13.8 Hz, 1H), 2.31 (s, 6H); MS (ESI): 538 $(M+H)^+$; HRMS (FAB) Calcd for $C_{27}H_{33}ClN_7O_3^+$: 538.2328; Found: 538.2343.

*4.2.51. 2-(3-(2,2-Dimethylhydrazono)-6-(2-fluoro-6-methoxybenzyl)-7-oxo-1,4-diazepan-1-yl)-N-(3-(1-methyl-1H-pyrazol-4-yl)phenyl)acetamide (**22d**)*

The reaction was carried out according to the procedure for **22a** with **20d** instead of **20a** to give the title compound as a white solid (21.8 mg, 2 steps 36%). ^{1}H NMR (400MHz, $CDCl_3$) δ 8.17 (br. s, 1H), 7.74 (d, *J* = 0.6 Hz, 1H), 7.68 (br. s, 1H), 7.62 (s, 1H), 7.32 - 7.24 (m, 2H), 7.24 - 7.13 (m, 2H), 6.73 - 6.65 (m, 2H), 6.27 (br. s, 1H), 4.55 (d, *J* = 16.1 Hz, 1H), 4.35 (d, *J* = 15.4 Hz, 1H), 4.20 (d, *J* = 15.4 Hz, 1H), 3.93 (s, 3H), 3.87 (d, *J* = 16.1 Hz, 1H), 3.85 (s, 3H), 3.48 - 3.39 (m, 1H), 3.39 - 3.31 (m, 1H), 3.30 - 3.15 (m, 2H), 2.94 (dd, *J* = 10.5, 13.7 Hz, 1H), 2.31 (s, 6H); MS (ESI): 522 $(M+H)^+$; HRMS (FAB) Calcd for $C_{27}H_{33}FN_7O_3^+$: 522.2623; Found: 522.2592.

*4.2.52. 2-(3-(2,2-Dimethylhydrazono)-6-(2-methoxy-6-methylbenzyl)-7-oxo-1,4-diazepan-1-yl)-N-(3-(1-methyl-1H-pyrazol-4-yl)phenyl)acetamide (**22e**)*

The reaction was carried out according to the procedure for **22b** with **20e** instead of **20b** to give the title compound as a white solid (18 mg, 2 steps 66%). ^{1}H NMR (400MHz, $CDCl_3$) δ 8.15 (br. s, 1H), 7.74 (d, *J* = 0.8 Hz, 1H), 7.69 (br. s, 1H), 7.62 (s, 1H), 7.31 - 7.24 (m, 2H), 7.23 - 7.18 (m, 1H), 7.13 (t, *J* = 7.9 Hz, 1H), 6.82 (d, *J* = 7.5 Hz, 1H), 6.75 (d, *J* = 8.2 Hz, 1H), 6.40 (br. s, 1H), 4.55 (d, *J* = 15.8 Hz, 1H), 4.36 (d, *J* = 15.4 Hz, 1H), 4.21 (d, *J* = 15.4 Hz, 1H), 3.93 (s, 3H), 3.88 (d, *J* = 15.6 Hz, 1H), 3.84 (s, 3H), 3.43 - 3.27 (m, 2H), 3.27 - 3.10 (m, 2H), 3.01 (dd, *J* = 10.2, 13.9 Hz, 1H), 2.38 (s, 3H), 2.34 (s, 6H); MS (ESI): 518 $(M+H)^+$; HRMS (FAB) Calcd for $C_{28}H_{36}N_7O_3^+$: 518.2874; Found: 518.2870.

*4.2.53. 2-(6-(2-Chloro-6-fluorobenzyl)-3-(2,2-dimethylhydrazono)-7-oxo-1,4-diazepan-1-yl)-N-(3-(1-methyl-1H-pyrazol-4-yl)phenyl)acetamide (**22f**)*

The reaction was carried out according to the procedure for **22b** with **20f** instead of **20b** to give the title compound as a white solid (12 mg, 2 steps 64%). ^{1}H NMR (400MHz, $CDCl_3$) δ 8.03 (br. s, 1H), 7.74 (d, *J* = 0.8 Hz, 1H), 7.68 (br. s, 1H), 7.62 (s, 1H), 7.32 - 7.25 (m, 2H), 7.23 - 7.15 (m, 3H), 7.03 - 6.96 (m, 1H), 6.29 (br. s, 1H), 4.55 (d, *J* = 16.2 Hz, 1H), 4.32 (d, *J* = 15.4 Hz, 1H), 4.24 (d, *J* = 15.6 Hz, 1H), 3.94 (s, 3H), 3.91 (d, *J* = 16.2 Hz, 1H), 3.59 - 3.48 (m, 1H), 3.40 - 3.32 (m, 3H), 3.11 - 3.03 (m, 1H), 2.30 (s, 6H); MS (ESI): 526 $(M+H)^+$; HRMS (FAB) Calcd for $C_{26}H_{30}ClFN_7O_2^+$: 526.2128; Found: 526.2095.

*4.2.54. 2-(6-(2,6-Dichlorobenzyl)-3-(2,2-dimethylhydrazono)-7-oxo-1,4-diazepan-1-yl)-N-(3-(1-methyl-1H-pyrazol-4-yl)phenyl)acetamide (**22g**)*

The reaction was carried out according to the procedure for **22b** with **20g** instead of **20b** to give the title compound as a white solid (30 mg, 2 steps 88%). ^{1}H NMR (400MHz, $CDCl_3$) δ 8.10 (br. s, 1H), 7.74 (d, *J* = 0.6 Hz, 1H), 7.67 (br. s, 1H), 7.62 (s, 1H), 7.32 (d, *J* = 7.9 Hz, 2H), 7.29 - 7.25 (m, 2H), 7.23 - 7.18 (m, 1H), 7.14 (dd, *J* = 7.7, 8.4 Hz, 1H), 6.31 (br. s, 1H), 4.55 (d, *J* = 16.2 Hz, 1H), 4.33 (d, *J* = 15.4 Hz, 1H), 4.24 (d, *J* = 15.4 Hz, 1H), 3.93 (s, 3H), 3.92 (d, *J* = 16.2 Hz, 1H), 3.68 - 3.59 (m, 1H), 3.51 - 3.41 (m, 2H), 3.36 (td, *J* = 3.9, 13.1 Hz, 1H), 3.28 (dd, *J* = 10.0, 14.3 Hz, 1H), 2.30 (s, 6H); MS (ESI): 542 $(M+H)^+$; HRMS (FAB) Calcd for $C_{26}H_{30}Cl_2N_7O_2^+$: 542.1833; Found: 542.1823.

*4.2.55. (+)-2-(6-(2,6-Dichlorobenzyl)-3-(2,2-dimethylhydrazono)-7-oxo-1,4-diazepan-1-yl)-N-(3-(1-methyl-1H-pyrazol-4-yl)phenyl)acetamide ((+)-**22g**) and (–)-2-(6-(2,6-Dichlorobenzyl)-3-(2,2-dimethylhydrazono)-7-oxo-1,4-diazepan-1-yl)-N-(3-(1-methyl-1H-pyrazol-4-yl)phenyl)acetamide ((–)-**22g**)*

The compound **22g** was separated using CHIRALPAK ID (Daicel Chemical Industries) (movement phase: hexane/EtOH/Et_2NH = 50/50/0.1) to give the (+)-**22g** and (–)-**22g**.

(+)-**22g**: white solid. $[\alpha]_D^{27}$ = +34° (c 0.50, $CHCl_3$); ^{1}H NMR (400MHz, $CDCl_3$) δ 8.30 (br. s, 1H), 7.73 (d, *J* = 0.8 Hz, 1H), 7.66 (br. s, 1H), 7.59 (s, 1H), 7.31 (d, *J* = 8.0 Hz, 2H), 7.28 - 7.22 (m, 2H), 7.22 - 7.16 (m, 1H), 7.14 (dd, *J* = 7.7, 8.4 Hz, 1H), 6.31 (br. s, 1H), 4.57 (d, *J* = 16.2 Hz, 1H), 4.37 (d, *J* = 15.6 Hz, 1H), 4.22 (d, *J* = 15.4 Hz, 1H), 3.92 (s, 3H), 3.89 (d, *J* = 16.2 Hz, 1H), 3.68 - 3.59 (m, 1H), 3.50 - 3.40 (m, 2H), 3.36 (td, *J* = 4.0, 13.2 Hz, 1H), 3.27 (dd, *J* = 10.2, 14.3 Hz, 1H), 2.31 (s, 6H); MS (ESI): 542 $(M+H)^+$; HRMS (FAB) Calcd for $C_{26}H_{30}Cl_2N_7O_2^+$: 542.1833; Found: 542.1862.

(–)-**22g**: white solid. $[\alpha]_D^{27}$ = −34° (c 0.50, $CHCl_3$); ^{1}H NMR (400MHz, $CDCl_3$) δ 8.26 (br. s, 1H), 7.73 (d, *J*=0.8 Hz, 1H), 7.66 (br. s, 1H), 7.60 (s, 1H), 7.32 (d, *J* = 8.0 Hz, 2H), 7.28 - 7.23 (m, 2H), 7.22 - 7.17 (m, 1H), 7.14 (dd, *J* = 7.7, 8.4 Hz, 1H), 6.31 (br. s, 1H), 4.56 (d, *J* = 16.2 Hz, 1H), 4.36 (d, *J* = 15.6 Hz, 1H), 4.22 (d, *J* = 15.6 Hz, 1H), 3.92 (s, 3H), 3.90 (d, *J* = 16.2 Hz, 1H), 3.68 - 3.59 (m, 1H), 3.50 - 3.40 (m, 2H), 3.36 (td, *J* = 3.9, 13.1 Hz, 1H), 3.27 (dd, *J* = 10.0, 14.3 Hz, 1H), 2.31 (s, 6H); MS (ESI): 542 $(M+H)^+$; HRMS (FAB) Calcd for $C_{26}H_{30}Cl_2N_7O_2^+$: 542.1833; Found: 542.1842.

*4.2.56. 2-(6-(2-Chloro-6-methylbenzyl)-3-(2,2-dimethylhydrazono)-7-oxo-1,4-diazepan-1-yl)-N-(3-(1-methyl-1H-pyrazol-4-yl)phenyl)acetamide (**22h**)*

The reaction was carried out according to the procedure for **22b** with **20h** instead of **20b** to give the title compound as a white solid (9 mg, 2 steps 63%). ^{1}H NMR (400MHz, $CDCl_3$) δ 8.02 (br. s, 1H), 7.74 (d, *J* = 0.6 Hz, 1H), 7.67 (br. s, 1H), 7.61 (s, 1H), 7.32 - 7.18 (m, 4H), 7.11 - 7.06 (m, 2H), 6.31 (br. s, 1H), 4.54 (d, *J* = 16.1 Hz, 1H), 4.32 (d, *J* = 15.6 Hz, 1H), 4.24 (d, *J* = 15.6 Hz, 1H), 3.94 (s, 3H), 3.91 (d, *J* = 16.1 Hz, 1H), 3.59 - 3.49 (m, 1H), 3.45 - 3.31 (m, 3H), 3.08 (dd, *J* = 9.5, 14.4 Hz, 1H), 2.41 (s, 3H), 2.30 (s, 6H); MS (ESI): 522 $(M+H)^+$; HRMS (FAB) Calcd for $C_{27}H_{33}ClN_7O_2^+$: 522.2379; Found: 522.2352.

*4.2.57. 2-(6-(2-Chloro-6-cyanobenzyl)-3-(2,2-dimethylhydrazono)-7-oxo-1,4-diazepan-1-yl)-N-(3-(1-methyl-1H-pyrazol-4-yl)phenyl)acetamide (**22i**)*

The reaction was carried out according to the procedure for **22a** with **20i** instead of **20a** to give the title compound as a white solid (10.2 mg, 2 steps 83%). ^{1}H NMR (400MHz, $CDCl_3$) δ 8.00 (br. s, 1H), 7.74 (d, *J* = 0.8 Hz, 1H), 7.65 (br. s, 1H), 7.62 (s, 1H), 7.63 - 7.60 (m, 1H), 7.58 (dd, *J* = 1.3, 7.8 Hz, 1H), 7.31 (t, *J* = 8.0 Hz, 1H), 7.29 - 7.23 (m, 2H), 7.23 - 7.18 (m, 1H), 6.36 (br. s, 1H), 4.54 (d, *J* = 16.3 Hz, 1H), 4.35 (d, *J* = 15.6 Hz, 1H), 4.21 (d, *J* = 15.6 Hz, 1H), 3.94 (s, 3H), 3.94 (d, *J* = 16.2 Hz, 1H), 3.71 - 3.57 (m, 2H), 3.55 - 3.40 (m, 2H), 3.18 (dd, *J* = 8.0, 13.9 Hz, 1H), 2.31 (s, 6H); MS (ESI): 533 $(M+H)^+$; HRMS (FAB) Calcd for $C_{27}H_{30}ClN_8O_2^+$: 533.2175; Found: 533.2194.

*4.2.58. 2-(3-(2,2-Dimethylhydrazono)-6-(2-fluoro-6-methylbenzyl)-7-oxo-1,4-diazepan-1-yl)-N-(3-(1-methyl-1H-pyrazol-4-yl)phenyl)acetamide (**22j**)*

The reaction was carried out according to the procedure for **22b** with **20j** instead of **20b** to give the title compound as a white solid (10 mg, 2 steps 63%). ^{1}H NMR (400MHz, $CDCl_3$) δ 8.00 (br. s, 1H), 7.74 (d, *J* = 0.8 Hz, 1H), 7.68 (br. s, 1H), 7.62 (s, 1H), 7.32 - 7.18 (m, 3H), 7.12 (dt, *J* = 5.8, 7.9 Hz, 1H), 6.98 (br. d, *J* = 7.7 Hz, 1H), 6.89 (br. t, *J* = 9.0 Hz, 1H), 6.30 (br. s, 1H), 4.54 (d, *J* = 16.1 Hz, 1H), 4.33 (d, *J* = 15.6 Hz, 1H), 4.22 (d, *J* = 15.6 Hz, 1H), 3.94 (s, 3H), 3.90 (d, *J* = 16.1 Hz, 1H), 3.44 - 3.27 (m, 3H), 3.25 - 3.18 (m, 1H), 2.94 (dd, *J* = 8.6, 13.9 Hz, 1H), 2.39 (s, 3H), 2.31 (s, 6H); MS (ESI): 506 $(M+H)^+$; HRMS (FAB) Calcd for $C_{27}H_{33}FN_7O_2^+$: 506.2674; Found: 506.2695.

*4.2.59. 2-(6-(2,6-Dimethylbenzyl)-3-(2,2-dimethylhydrazono)-7-oxo-1,4-diazepan-1-yl)-N-(3-(1-methyl-1H-pyrazol-4-yl)phenyl)acetamide (**22k**)*

The reaction was carried out according to the procedure for **22b** with **20k** instead of **20b** to give the title compound as a white solid (82.3 mg, 2 steps 98%). ^{1}H NMR (400MHz, $CDCl_3$) δ 8.49 (br. s, 1H), 7.72 (d, *J* = 0.8 Hz, 1H), 7.67 (br. s, 1H), 7.57 (s, 1H), 7.26 - 7.21 (m, 2H), 7.20 - 7.14 (m, 1H), 7.08 - 7.00 (m, 3H), 6.27 (br. s, 1H), 4.49 (d, *J* = 16.3 Hz, 1H), 4.36 (d, *J* = 15.7 Hz, 1H), 4.27 (d, *J* = 15.7 Hz, 1H), 3.93 (d, *J* = 16.3 Hz, 1H), 3.91 (s, 3H), 3.43 - 3.22 (m, 4H), 2.96 - 2.87 (m, 1H), 2.33 (s,

6H), 2.30 (s, 6H); MS (ESI): 502 $(M+H)^+$; HRMS (FAB) Calcd for $C_{28}H_{36}N_7O_2^+$: 502.2925; Found: 502.2945.

*4.2.60. 2-(6-(2-Cyano-6-methylbenzyl)-3-(2,2-dimethylhydrazono)-7-oxo-1,4-diazepan-1-yl)-N-(3-(1-methyl-1H-pyrazol-4-yl)phenyl)acetamide (**22l**)*

The reaction was carried out according to the procedure for **22a** with **20l** instead of **20a** to give the title compound as a white solid (22.6 mg, 2 steps 90%). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.97 (br. s, 1H), 7.74 (d, *J* = 0.8 Hz, 1H), 7.67 (br. s, 1H), 7.62 (s, 1H), 7.51 (br. d, *J* = 7.7 Hz, 1H), 7.41 (br. d, *J* = 7.3 Hz, 1H), 7.30 - 7.23 (m, 3H), 7.23 - 7.18 (m, 1H), 6.38 (br. s, 1H), 4.54 (d, *J* = 16.2 Hz, 1H), 4.36 (d, *J* = 15.7 Hz, 1H), 4.21 (d, *J* = 15.7 Hz, 1H), 3.94 (s, 3H), 3.95 (d, *J* = 16.1 Hz, 1H), 3.56 - 3.43 (m, 4H), 3.10 - 3.02 (m, 1H), 2.44 (s, 3H), 2.32 (s, 6H); MS (ESI): 513 $(M+H)^+$; HRMS (FAB) Calcd for $C_{28}H_{33}N_8O_2^+$: 513.2721; Found: 513.2725.

*4.2.61. 2-(6-(2,6-Difluorobenzyl)-3-(2,2-dimethylhydrazono)-7-oxo-1,4-diazepan-1-yl)-N-(3-(1-methyl-1H-pyrazol-4-yl)phenyl)acetamide (**22m**)*

The reaction was carried out according to the procedure for **22b** with **20m** instead of **20b** to give the title compound as a white solid (34 mg, 2 steps quant.). ^{1}H NMR (400MHz, $CDCl_3$) δ 8.29 (br. s, 1H), 7.73 (d, *J* = 0.5 Hz, 1H), 7.66 (br. s, 1H), 7.59 (s, 1H), 7.29 - 7.15 (m, 4H), 6.93 - 6.84 (m, 2H), 6.30 (br. s, 1H), 4.57 (d, *J* = 16.2 Hz, 1H), 4.36 (d, *J* = 15.6 Hz, 1H), 4.21 (d, *J* = 15.6 Hz, 1H), 3.92 (s, 3H), 3.86 (d, *J* = 16.3 Hz, 1H), 3.53 - 3.43 (m, 1H), 3.42 - 3.21 (m, 3H), 2.91 (dd, *J* = 9.6, 14.2 Hz, 1H), 2.31 (s, 6H); MS (ESI): 510 $(M+H)^+$; HRMS (FAB) Calcd for $C_{26}H_{30}F_2N_7O_2^+$: 510.2424; Found: 510.2391.

*4.2.62. 2-(3-(2,2-Dimethylhydrazono)-6-(2-fluoro-6-(trifluoromethyl)benzyl)-7-oxo-1,4-diazepan-1-yl)-N-(3-(1-methyl-1H-pyrazol-4-yl)phenyl)acetamide (**22n**)*

The reaction was carried out according to the procedure for **22b** with **20n** instead of **20b** to give the title compound as a white solid (16 mg, 2 steps 78%). ^{1}H NMR (400MHz, $CDCl_3$) δ 8.03 (br. s, 1H), 7.74 (d, *J* = 0.8 Hz, 1H), 7.67 (br. s, 1H), 7.62 (s, 1H), 7.50 (br. d, *J* = 7.9 Hz, 1H), 7.41 - 7.33 (m, 1H), 7.30 - 7.19 (m, 4H), 6.28 (br. s, 1H), 4.54 (d, *J* = 16.2 Hz, 1H), 4.33 (d, *J* = 15.4 Hz, 1H), 4.22 (d, *J* = 15.4 Hz, 1H), 3.94 (s, 3H), 3.90 (d, *J* = 16.4 Hz, 1H), 3.60 - 3.50 (m, 1H), 3.47 - 3.38 (m, 1H), 3.38 - 3.32 (m, 2H), 3.08 (dd, *J* = 9.6, 14.7 Hz, 1H), 2.30 (s, 6H); MS (ESI): 560 $(M+H)^+$; HRMS (FAB) Calcd for $C_{27}H_{30}F_4N_7O_2^+$: 560.2392; Found: 560.2365.

*4.2.63. tert-Butyl 2-(6-(4-chloro-2-methoxybenzyl)-3,7-dioxo-4-(2,4,6-trimethoxybenzyl)-1,4-diazepan-1-yl)acetate (**18o**)*

The reaction was carried out according to the procedure for **18a** with 4-chloro-2-methoxyphenylboronic acid instead of 2-cyanophenylboronic acid to give the title compound (270 mg, quant.). ^{1}H NMR (400MHz, $CDCl_3$) δ 6.83 (d, *J* = 8.0 Hz, 1H), 6.78 (dd, *J* = 1.9, 7.9 Hz, 1H), 6.74 (d, *J* = 1.9 Hz, 1H), 6.00 (s, 2H), 4.64 (d, *J* = 13.7 Hz, 1H), 4.42 (d, *J* = 13.7 Hz, 1H), 4.26 (d, *J* = 17.3 Hz, 1H), 4.20 (d, *J* = 16.7 Hz, 1H), 4.15 (d, *J* = 16.6 Hz, 1H), 4.06 (d, *J* = 17.3 Hz, 1H), 3.84 (s, 3H), 3.73 (s, 3H), 3.65 (s, 6H), 3.23 - 3.08 (m, 2H), 2.97 - 2.87 (m, 2H), 2.50 (dd, *J* = 9.8, 13.8 Hz, 1H), 1.46 (s, 9H).

*4.2.64. 2-(6-(4-Chloro-2-methoxybenzyl)-3,7-dioxo-1,4-diazepan-1-yl)-N-(3-(1-methyl-1H-pyrazol-4-yl)phenyl)acetamide (**24**)*

The reaction was carried out according to the procedure for **22b** with **18o** instead of **20b** to give the title compound (190 mg, 2 steps 83%).^{1}H NMR (400MHz, $CDCl_3$) δ 7.95 (br. s, 1H), 7.74 (d, *J* = 0.6 Hz, 1H), 7.68 (br. s, 1H), 7.61 (s, 1H), 7.31 - 7.25 (m, 1H), 7.24 - 7.18 (m, 2H), 7.09 (d, *J* = 7.9 Hz, 1H), 6.89 - 6.84 (m, 2H), 5.83 (br. s., 1H), 4.60 (d, *J* = 17.4 Hz, 1H), 4.35 (d, *J* = 15.3 Hz, 1H), 4.15 (d, *J* = 15.3 Hz, 1H), 3.94 (d, *J* = 17.1 Hz, 1H), 3.93 (s, 3H), 3.83 (s, 3H), 3.53 - 3.44 (m, 1H), 3.34 - 3.27 (m, 2H), 3.19 (dd, *J* = 5.6, 13.9 Hz, 1H), 2.70 (dd, *J* = 8.3, 14.0 Hz, 1H).

*4.2.65. 2-(6-(4-Chloro-2-methoxybenzyl)-7-oxo-3-thioxo-1,4-diazepan-1-yl)-N-(3-(1-methyl-1H-pyrazol-4-yl)phenyl)acetamide (**25**)*

The reaction was carried out according to the procedure for **19b** with **24** instead of **18b** and Lawesson's reagent instead of Belleau's reagent to give the title compound (184 mg, quant.). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.97 (br. s, 1H), 7.86 (br. s, 1H), 7.75 (d, *J* = 0.4 Hz, 1H), 7.68 (br. s, 1H), 7.62 (s, 1H), 7.32 - 7.20 (m, 3H), 7.09 (d, *J* = 8.0 Hz, 1H), 6.89 - 6.85 (m, 2H), 4.87 (d, *J* = 17.3 Hz, 1H), 4.56 (d, *J* = 17.4 Hz, 1H), 4.41 (d, *J* = 15.2 Hz, 1H), 4.14 (d, *J* = 15.3 Hz, 1H), 3.94 (s, 3H), 3.85 (s, 3H), 3.59 - 3.49 (m, 1H), 3.47 - 3.32 (m, 2H), 3.20 (dd, *J* = 5.5, 13.7 Hz, 1H), 2.72 (dd, *J* = 8.5, 13.9 Hz, 1H); MS (ESI): 512 $(M+H)^+$.

*4.2.66. 2-(6-(4-Chloro-2-methoxybenzyl)-3-(2,2-dimethylhydrazono)-7-oxo-1,4-diazepan-1-yl)-N-(3-(1-methyl-1H-pyrazol-4-yl)phenyl)acetamide (**22o**)*

The reaction was carried out according to the procedure for **20b** with **25** instead of **19b** and pyridine instead of MeOH/THF (1/10) to give the title compound as white solid (13.7 mg, 43%). ^{1}H NMR (400MHz, CDCl3) δ 8.06 (br. s., 1H), 7.74 (d, *J* = 0.6 Hz, 1H), 7.69 (br. s, 1H), 7.61 (s, 1H), 7.31 - 7.25 (m, 1H), 7.23 - 7.18 (m, 2H), 7.13 (d, *J* = 7.9 Hz, 1H), 6.89 - 6.84 (m, 2H), 6.27 (br. s, 1H), 4.54 (d, *J* = 16.1 Hz, 1H), 4.32 (d, *J* = 15.4 Hz, 1H), 4.18 (d, *J* = 15.4 Hz, 1H), 3.94 (s, 3H), 3.84 (s, 3H), 3.84 (d, *J* = 16.4 Hz, 1H), 3.49 - 3.35 (m, 2H), 3.25 - 3.15 (m, 2H), 2.74 (dd, *J* = 8.3, 13.7 Hz, 1H), 2.31 (s, 6H); MS (ESI): 538 $(M+H)^+$; HRMS (FAB) Calcd for $C_{27}H_{33}ClN_7O_3^+$: 538.2328; Found: 538.2343.

4.3. Enzymatic assay

Recombinant human KLK7 (R&D systems) was activated with thermolysin for 2 h at 37 °C. Recombinant mouse KLK7 for crystallization before activation was activated with enterokinase for 30 min at 37 °C. Test compounds were incubated with activated enzyme (1 µg/mL) in reaction buffer (50 mM Tris-HCl (pH 8.5), 0.15 M NaCl, 0.1% BSA) for 10 min at room temperature, and 10 µM of substrate (MOCAc-Arg-Pro-Lys-Pro-Val-Glu-Nva-Trp-Arg-Lys(Dnp)-NH_2, Peptide Institute) was added. After 30 min of incubation at room temperature, the reaction was stopped by adding 30% acetic acid, and fluorescence (Ex. 340/30 nm, Em. 400/30 nm) was measured.

Supplementary Material

Supplementary data associated with this article can be found, in the online version at:

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Graphical Abstract

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Structure-based drug design to overcome species differences in kallikrein 7 inhibition of 1,3,6-trisubstituted 1,4-diazepan-7-ones

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