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Potent and selective small molecule NS3 serine protease inhibitors of Hepatitis C virus with dichlorocyclopropylproline as P2 residue

Kevin X. Chen,* Bancha Vibulbhan, Weiying Yang, Kuo-Chi Cheng, Rong Liu, John Pichardo, Nancy Butkiewicz and F. George Njoroge

Schering-Plough Research Institute, 2015 Galloping Hill Road, K-15-3-3545, Kenilworth, NJ 07033, USA

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Abstract—Starting from a pentapeptide Hepatitis C virus NS3 protease inhibitor, a number of α-ketoamide inhibitors based on novel dichlorocyclopropylproline P2 core were synthesized and investigated for their HCV NS3 serine protease activity. The key intermediate 3,4-dichlorocyclopropylproline was obtained through a dichloro carbene insertion to 3,4-dehydroproline. The size of the molecules was reduced significantly through a series of truncations of the initial pentapeptide. By varying P1 side chain in length and size, potency and selectivity were improved. A variety of aliphatic carbamate and urea capping groups were examined. In general, compounds with urea cappings were more potent and selective than their carbamate counterparts. The most potent compound was a *tert*-butyl urea analog. Variations at P3 position were also investigated. Among the three residues incorporated, *tert*-leucine was clearly superior, leading to compounds that had excellent enzyme potency and selectivity. The most potent compound achieved cell-based replicon assay EC_{50} of 40 nM. The most promising compound of all had excellent potency in both enzyme (K_i^* = 9 nM) and replicon assays (EC_{50} = 100 nM). Its bioavailabilities were above 10% in all three animal species (rats, monkeys, and dogs). It has provided a lead for future investigations.

1. Introduction

Hepatitis C virus (HCV) is a human pathogen affecting nearly 170 million, or 3% of the world's population. HCV infection is described as a 'silent epidemic' because only about 20% of patients develop acute clinical hepatitis. In most cases, chronic progress of the infection leads to cirrhosis, liver failure or liver cancer.[1] Currently, the most effective therapies for HCV infection involve treatment with pegylated α-interferon, either alone or in combination with antiviral agent ribavirin.[2] These therapeutics have limited efficacy with sustained response rate of only about 50%. They are also accompanied by considerable side effects in certain patients. More effective antiviral agents for HCV with fewer side effects are in urgent need.

The HCV RNA genome is processed by host and viral proteases into structural and nonstructural (NS) polypeptides.[3] The NS3 protease is located at N-terminal portion of NS3 protein. It is responsible for processing four cleavage sites of the nonstructural region and is essential for viral replication.[4] It has been determined that the HCV NS3 protease belongs to the trypsin or chymotrypsin superfamily of serine proteases.[5] For efficient processing, the protease forms a complex with a small polypeptide co-factor NS4A.[6] The structural data of the protease have revealed a shallow and solvent exposed substrate binding region, where the binding energy is mainly derived from weak lipophilic and electrostatic interactions.[7] Intensive efforts have been focused on NS3 serine protease as a target. Despite tremendous difficulties encountered in the research, a number of novel inhibitors have been reported.[8]

Similar to other proteases, the products from the cleavage of the substrates by HCV NS3 protease have been shown to be competitive inhibitors.[9] The hexapeptide products have been used as leads to develop more potent and smaller HCV inhibitors. Inhibitors with diverse

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* Corresponding author. Tel.: +1 908 740 7556; fax: +1 908 740 7152; e-mail: kevin.chen@spcorp.com

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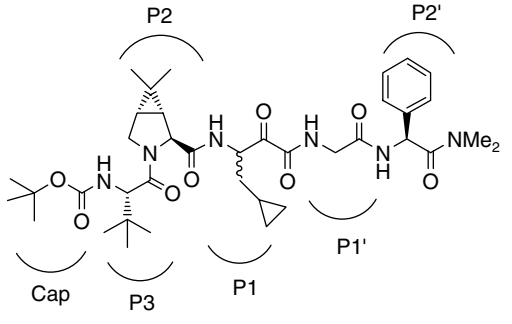
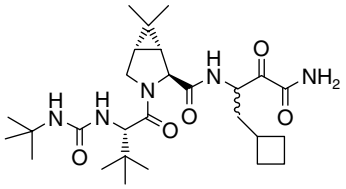
structure types have been discovered.[8] In one class of compounds, a reactive electrophile that can form a covalent bond with active site serine hydroxyl has been introduced. These compounds, exemplified by α-ketoacids and ketoamides, have demonstrated their potential use as hepatitis C therapeutics. VX-950 and SCH-503034, two examples in this class, are in clinical development.[10] In the course of our search for potent HCV inhibitors, we discovered a pentapeptide α-ketoamide **1** (Fig. 1). Compound **1** had excellent potency against NS3 protease (K_i^* = 3.8 nM) and in cell-based replicon assay (EC_{50} = 40 nM).[11] However, its pharmacokinetic (PK) properties were poor: 4% and 1% bioavailability in rats and monkeys, respectively. To improve PK profile of this type of peptic molecules, it was desirable to reduce molecular weight through truncation of the pentapeptide to a smaller tetra- or tripeptide. The result of our early effort was the discovery of the current clinical candidate SCH 503034 (Boceprevir, Fig. 1), which had decent potency and good PK. Parallel to the SAR development using dimethylcyclopropylproline as P2 residue, the analogs with alternative P2 moieties were also investigated. Since the dimethylcyclopropylproline P2 had been demonstrated to be vital for potency,[11] we decided to structurally stay as close to this core as possible. It was envisioned that the two methyl groups at P2 could be replaced by two chloro substituents. The chloro groups resemble the steric bulk of the methyls, but have different electronic characteristics. They could potentially provide improved potency and/or better PK. We believe that the SAR of inhibitors using this new dichloro P2 residue should follow the same pattern as those using dimethyl P2 moiety, because modifications at other sites are similar. Herein, we report our effort and progress in these endeavors.



1 Ki* = 3.8 nM, EC_{50} = 40 nM



SCH 503034 (Boceprevir)

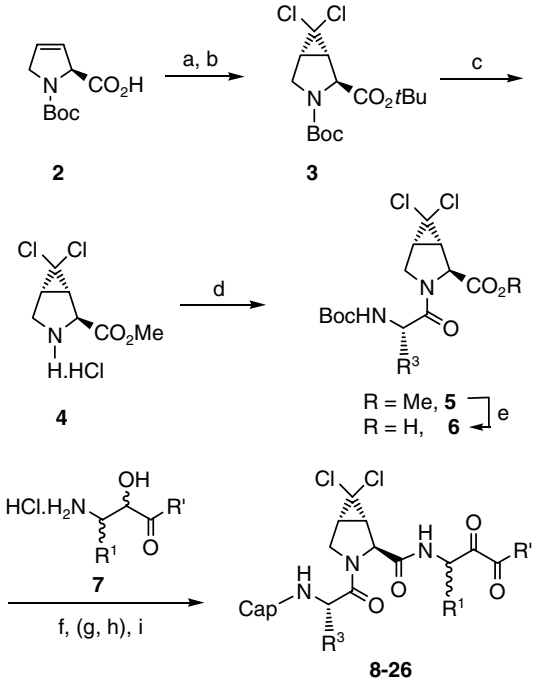
Ki* = 14 nM, EC_{50} = 200 nM

Figure 1. Pentapeptide HCV protease inhibitor **1** and SCH 503034 (Boceprevir).

2. Synthesis of HCV protease inhibitors

The general synthesis of the HCV protease inhibitors is summarized in Scheme 1. Preparation of the 3,4-*trans*-dichlorocyclopropylproline P2 core was the key step in the synthesis. Thus, *N*-Boc-3,4-dehydroproline **2** was converted to its corresponding *tert*-butyl ester.[12] The cyclopropanation was achieved through the addition of dichloro carbene[13] onto the dehydroproline to give the desired product **3**. The reaction was conducted in a bi-phasic system of chloroform and 50% aqueous sodium hydroxide. The cyclopropanation occurred exclusively trans to the *tert*-butyl ester moiety as evidenced by proton NMR analysis. However, the C-2 stereocenter of proline was partially racemized under the strongly basic conditions used during the course of the reaction. Removal of the Boc group, cleavage of the *tert*-butyl ester and formation of a methyl ester were accomplished in one pot through refluxing in methanolic hydrochloric acid to provide the key intermediate **4**. Standard coupling (HOOBt, EDC, NMM) between **4** and a *N*-Boc-protected amino acid P3 afforded the dipeptide **5**. The desired compound with the right P2 stereocenter was isolated as the major isomer out of the two diastereomers of **5**. Hydrolysis of the methyl ester provided the carboxylic acid of type **6** which served as the left-hand side section core the inhibitor.

Preparation of the right-hand fragments of the inhibitors (from P1 ketoamide up to P2′) has been described in earlier publications.[14] Coupling of the carboxylic acid



Scheme 1. Reagents and conditions: (a) $(Boc)_2O$, DMAP, CH_3CN; (b) $CHCl_3$, 50% NaOH, 40%; (c) HCl, MeOH, quant.; (d) appropriate *N*-Boc-amino acid, HOOBt, EDC, NMM, DMF/CH_2Cl_2, 50–95%; (e) LiOH, THF/MeOH/water; (f) **6**, HOOBt, EDC, NMM, DMF/CH_2Cl_2, 50–95%; (g) 4 M HCl, dioxane; (h) appropriate isocyanate, saturated $NaHCO_3$/CH_2Cl_2, 40–75%; (i) Dess–Martin periodinane, CH_2Cl_2, 60–90%; or DMSO, Cl_2CHCO_2H, EDC, toluene, 45–70%.

6 with the amine **7** afforded a series of α-hydroxyamide intermediates, which upon oxidation using Dess–Martin periodinane[15] (for secondary amides) or a modified Moffatt protocol[16] (for primary amides) gave rise to the desired final target α-ketoamides **8**–**26**.

3. Results and discussion

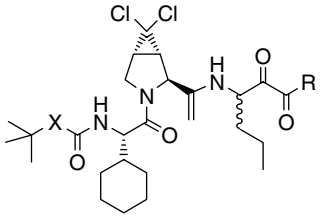
3.1. Enzyme assay and selectivity against HNE

All the inhibitors described above were tested in HCV continuous assay[17] using the NS4A-tethered single chain NS3 serine protease.[18] The K_i^* values in the assay reflected the equilibrium constant determined by the reversible covalent bond formed between the ketone and serine hydroxyl group and by other interactions between the inhibitors and the enzyme.[19] All compounds were measured as a mixture of two diastereomers at P1 α-center. The ratio of the two isomers varied slightly based on specific compound. In cases where single isomers were obtained, racemization at P1 was observed for most of them during in vitro enzyme assay and in vivo pharmacokinetics studies. The selectivity of HCV serine protease inhibitor against other serine proteases was an important consideration since it is relevant to potential side effect of the therapy. In this case, the selectivity was measured against human neutrophil elastase (HNE), which is a serine protease structurally closely related to HCV NS3 serine protease. Our goal was to achieve a selectivity index of one hundred or greater.

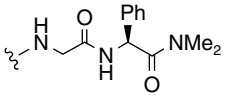
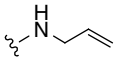
3.2. The P′ side SAR

First, we explored the effect of the truncation on potency at P′ side of the pentapeptide (**8**–**12**). The enzyme assay results for these compounds are presented in Table 1. Similar to compound **1**, inhibitor **8** is a pentapeptide with a glycine-phenylglycine dipeptide piece at the prime side. It was a potent inhibitor of HCV protease with K_i^* of 15 nM. The selectivity (HNE/HCV) was modest at 4. Compound **9** (*tert*-butyl carbamate capped) and compound **10** (*tert*-butyl urea capped), both truncated as allyl tripeptides, demonstrated significantly diminished potency with K_i^* of 90 and 100 nM, respectively. Surprisingly, if the molecule was further shortened to a primary amide such as that in compounds **11** and **12**, the potency was regained. Thus, the two short tripeptides had K_i^* of 36 and 20 nM for *tert*-butyl carbamate and urea capped inhibitors, respectively. In both allyl and primary amide pairs, the *tert*-butyl urea capped inhibitors were more selective than the *tert*-butyl carbamate capped analogs. From X-ray and modeling analysis, the good potency of compounds **1** and **8** came in part from the extra hydrogen-bonding of P2′ amide N–H and hydrophobic interaction between the phenyl group and the backbone Lys136 side chain. The loss of these bindings and the unfavorable steric interaction between the allyl group and the Gln41 side chain in allyl amide analogs **9** and **10** explained their lack of potency. The tripeptide primary amides (**11** and **12**) regained most of the potency probably because they did not have unfavorable steric contacts with Gln41 and a stronger hydrogen bonding between a primary amide N–H and the backbone. These results demonstrated that truncation was feasible and smaller molecule inhibitors with reasonable potency were achievable.

Table 1. P′ and P3-capping variations of the inhibitors



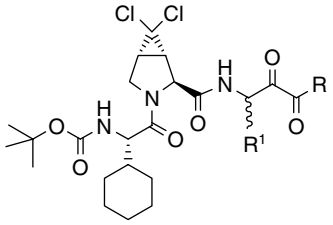
8-12

Compound	X	R	K_i^* (nM)	HNE/HCV
8	O		15	4
9	O		90	1
10	NH		100	4
11	O		36	3
12	NH		20	9

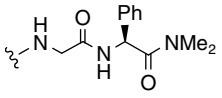
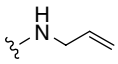
3.3. P1 side chain variations

Modifications were also made at P1 position. The S1 pocket has been shown to be small but lipophilic. It can only accommodate short alkyl straight chains or small ring systems. Thus, the *n*-propyl of norvaline P1 was expanded to cyclopropylmethyl, cyclobutylmethyl, and butenyl groups. Inhibitors with varying number of amino acid residues were prepared (Table 2). For pentapeptide series (**8**, **13**, and **14**), from P1 norvaline (K_i^* = 15 nM) to P1 cyclopropyl alanine (7 nM), a twofold improvement in potency was obtained. A K_i^* of 9 nM was observed for P1 cyclobutylalanine analog **14**. However, the selectivity against elastase improved dramatically from **8**, **13** to **14** (HNE/HCV = 4, 70, 280, respectively) with the bulkier cyclobutylalanine having the best selectivity. The two allyl amide compounds (**9** and **15**) had norvaline and butenyl glycine as P1. The longer butenyl group had certain advantages in potency (44 nM vs 99 nM) and selectivity (6 vs 1). The primary amide series (**11**, **16**–**17**) had similar trend in potency and selectivity to two series discussed above. The larger or longer side chain at P1 gave better potency and selectivity. The analog with cyclopropylalanine P1 had a K_i^* of 11 nM and selectivity of 23, while those for cyclobutylalanine derivative were 17 nM and 200, respectively. These data clearly indicated that cyclopropylalanine was the group that gave best potency at P1 position. On the other hand, cyclobutylalanine P1 analogs were the most selective against elastase. The results also demonstrated that the tripeptide primary amides (**11**, **16**–**17**) were equally potent and selective compared with larger pentapeptide analogs (**8**, **13**–**14**).

Table 2. P1 side chain modifications



8, 9, 11, 13-17

Compound	R^1	R	K_i^* (nM)	HNE/HCV
8			15	4
13			7	70
14			**9**	**280**
9			90	1
15			44	6
11			36	3
16			11	23
17			17	200

3.4. P3 capping modifications

Based on the results from P′ and P1 investigations, we decided to focus our SAR development on primary ketoamides with cyclobutylalanine substituent at P1. The effect of P3 capping groups was explored next. Thus, several alkyl groups were incorporated to P3 nitrogen through either a carbamate or a urea connection (Table 3). Compounds **17**–**19** were carbamate capped analogs. The trifluoro-Boc **18** and norbonyloxycarbonyl **19** capped derivatives (K_i^* of 65 and 60 nM, respectively) were less potent than Boc capped inhibitor **17** (17 nM). They were also less selective than **17**. On the other hand, all three urea capped compounds (**20**–**22**) were quite potent with K_i^* between 6 and 15 nM. The *tert*-butyl urea analog **20** was the most potent of all the inhibitors. Compounds **20** and **21** also had excellent selectivity (HNE/HCV = 460 and 570, respectively). With the improved potency, we clearly achieved our original goal of truncating pentapeptide **1** and maintaining its potency.

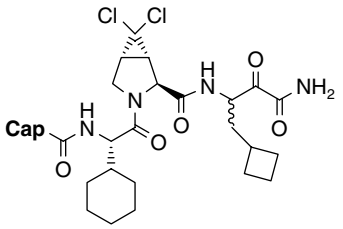
3.5. SAR of P3 amino acid residue

The S3 region of HCV NS3 protease is shallow but highly lipophilic, thus its P3 group binds to the enzyme surface through hydrophobic interactions. From our earlier experience, side chains that had sizable alkyl or aromatic hydrocarbon moieties interacted favorably with S3 pocket.[11] Three amino acid residues were examined at P3 position: cyclohexylglycine, *tert*-leucine, and 2-indanylglycine (Table 4). They were capped either with a Boc or a *tert*-butyl urea group, which, as previously shown, had proved to be the best groups at that position. The results for compounds (**17** and **20**) with cyclohexylglycine P3 were discussed earlier. When *tert*-butylglycine was used as P3 residue, both Boc and *tert*-butyl urea capped compounds (**23**–**24**) had excellent potency (K_i^* of 9 and 7 nM, respectively). Interestingly, both compounds exhibited great selectivity against elastase (HNE/HCV = 2800 and 2400, respectively). The potency and selectivity achieved by these small molecules were remarkable. On the other hand, with the larger aromatic indanylglycine at P3 position, both potency and selectivity were not as good as the other two groups, with K_i^* 4- to 10-fold higher and HNE/HCV 3- to 40-fold lower.

3.6. Cell-based replicon assay potency and pharmacokinetic properties of representative compounds

The potency of inhibitors as measured in cell-based replicon assay[20] has been a very important criterion in selecting the compounds for further advancement. Due to the lack of a good animal model to evaluate HCV NS3 protease, it is the next best assessment of the potential of all these compounds as antiviral agents. A number of the compounds described above were tested in this assay. Results from these studies on selected compounds are listed in Table 5. Compounds **16** and **17** were Boc-capped primary α-ketoamide inhibitors that differed at P1 residue. They had relatively similar potency and PK profiles. They had good K_i^* of 11 and 17 nM, and good replicon EC_{50} of 500 and 300 nM, respectively. Compound **17** had better selectivity than **16** (HNE/HCV, 200 vs 23). They both had good rat PO AUC (2.7 and 2.4 μM h, respectively) and **16** was moderately bioavailable in rats (26%). Unfortunately, they both had poor PK in monkey and dogs with PO AUC's at 0.1 μM h or less and bioavailability of less than 10%. Primary α-ketoamides **19** and **20** had cyclobutylalanine at P1 and differed at P3 capping site. One was norbornyl carbamate (**19**) and the other was *tert*-butyl urea (**20**). The latter was better in enzyme potency (60 nM vs 6 nM) and selectivity (15 vs 460) but had no advantage in replicon potency (200 nM vs 300 nM). Inhibitor **21** had the best replicon potency (EC_{50} = 40 nM) but did not give any rat PO AUC in PK study. The last two compounds (**23** and **24**) were the most interesting of all. With *tert*-leucine as P3 residue, they were both extremely potent with single digit nanomolar potency in enzymatic assay. Both also had excellent selectivity against elastase. In replicon assay, compound **23** was better than **24**, with EC_{50} of 100 nM. Compound **24** is similar in structure to SCH 503034. It was more potent

Table 3. Capping modifications



17-22

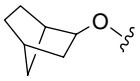
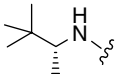
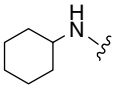
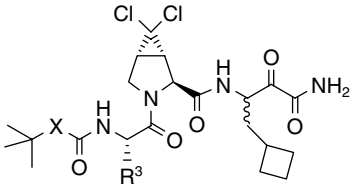
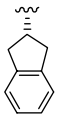
Compound	Cap	K_i^* (nM)	HNE/HCV
17		17	200
18		65	80
19		60	15
20		**6**	**460**
21		10	570
22		15	80

Table 4. SAR at P3 position



17, 20, 23-26

Compound	X	R3	K_i^* (nM)	HNE/HCV
17	O		17	200
20	NH		6	460
23	O		**9**	**2800**
24	NH		**7**	**2400**
25	O		60	70
26	NH		30	170

than SCH 503034 in enzyme assay, but equally potent in replicon assay. It had poor PK in rats. On the other hand, compound **23** demonstrated a superior overall PK profile, with bioavailability above 10% in all three animal species (rat, monkey, and dog). Although the AUC's were moderate, it had an impressive 50% bioavailability in monkeys. Compound **23** had much-improved PK compared to **1**. It was more potent than SCH 503034 in both enzyme and cell-based replicon assays. It has become a lead for future SAR development.

4. Conclusion

In summary, a number of α-ketoamide inhibitors based on dichlorocyclopropylproline P2 core were synthesized and investigated for their HCV NS3 serine protease activity. The size of the molecules was reduced significantly through a series of truncations of the initial lead pentapeptide **1**. The P1 primary ketoamides were found to be potent inhibitors. The P1 residue was modified from norvaline to cyclopropylalanine, and to cyclobutylalanine. Both potency and selectivity were improved as a result of these modifications. A group of aliphatic carbamate and urea capping groups were examined. In general, the urea cappings were more potent than carbamate ones. The most potent compound (**20**) was a *tert*-butyl urea analog. Variations at P3 were also investigated. Among the three residues (cyclohexylglycine, *tert*-leucine, and indanylglycine) incorporated, compounds with *tert*-leucine P3 (**23** and **24**) were clearly superior in potency and selectivity. Selected compounds were evaluated in cell-based replicon assay and in PK studies. Inhibitors **16** and **17** had good PO AUC's in rats, but were poor in monkeys and dogs. Compound **21** was the most potent in replicon with EC_{50} of 40 nM. Compound **23** was the most promising of all the inhibitors, had better PK than compound **1**, and was more potent than SCH 503034. It had excellent potency in both enzyme (K_i^* = 9 nM) and replicon assays (EC_{50} = 100 nM). It also demonstrated good overall PK profile with bioavailability above 10% in all three species (rat, monkey, and dog). This compound is a lead for our future investigations.

5. Experimental

5.1. General methods

Reagents and solvents, including anhydrous THF, dichloromethane, and DMF, were purchased from Aldrich or other commercial sources and were used without further purification. Reactions that were moisture sensitive or using anhydrous solvents were performed under either a nitrogen or an argon atmosphere. Analytical thin layer chromatography (TLC) was performed on precoated silica gel plates obtained from Analtech. Visualization was accomplished with UV light or by staining with basic $KMnO_4$ solution, ethanolic H_2SO_4 or Vaughn's reagent. Compounds were purified by flash chromatography either on a glass column using Merck silica gel 60 (230–400 mesh) or on an ISCO RediSep disposable silica gel column. NMR spectra were recorded at 300, 400 or 500 MHz for ^1H and at 75, 100 or 125 MHz for ^{13}C on a Bruker or Varian spectrometer with $CDCl_3$ or d_6-DMSO

Table 5. Cell-based replicon assay potency and PK of selected compounds

Compound		**16**	**17**	**19**	**20**	**21**	**23**	**24**
K_i^* (nM)		11	17	60	6	10	9	7
EC_{50} (nM, Replicon)		500	300	200	300	**40**	**100**	200
HNE/HCV		23	200	15	460	570	**2800**	2400
Rats	PO AUC (μM h)	2.7[a]	2.4[a]	0.75[a]	0.32[a]	0.0[a]	**0.30**[a]	0.12[f]
	IV AUC (μM h)	10.7[a]	[h]	[h]	[h]	[h]	**2.6**[a]	1.9[g]
	$t_{1/2}$ (h)	4.4	[h]	[h]	[h]	[h]	**5.6**	2.7
	F% (PO)	26%	[h]	[h]	[h]	[h]	**12%**	5%
Monkeys	PO AUC (μM h)	0.13[b]	0.06[d]	[h]	[h]	[h]	**0.77**[b]	[h]
	IV AUC (μM h)	1.2[c]	0.6[e]	[h]	[h]	[h]	**1.2**[c]	[h]
	$t_{1/2}$ (h)	9.6	1.9	[h]	[h]	[h]	**1.1**	[h]
	F% (PO)	8%	4%	[h]	[h]	[h]	**50%**	[h]
Dogs	PO AUC (μM h)	0.07[b]	0.10[d]	[h]	[h]	[h]	**0.61**[b]	[h]
	IV AUC (μM h)	2.6[c]	2.0[e]	[h]	[h]	[h]	**4.5**[c]	[h]
	$t_{1/2}$ (h)	16	4.7	[h]	[h]	[h]	**6.3**	[h]
	F% (PO)	2%	2%	[h]	[h]	[h]	**10%**	[h]

[a] 10 mg/kg.
[b] 3 mg/kg.
[c] 2.2 mg/kg.
[d] 1.5 mg/kg.
[e] 0.6 mg/kg.
[f] 7.5 mg/kg.
[g] 6 mg/kg.
[h] Data not obtained.

as solvent. The chemical shifts are given in ppm, referenced to the internal TMS or deuterated solvent signal.

5.2. General method for coupling of an *N*-Boc amino acid and an amine

To a solution of the *N*-Boc-amino acid (1.0 mmol), amine methyl ester hydrochloride (1.2 mmol), 3-hydroxy-1,2,3-benzotriazin-4(3*H*)-one (HOOBt) (1.2 mmol), and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) (1.3 mmol) in DMF/CH_2Cl_2 (1:1, 100 mL) at −20 °C was added *N*-methylmorpholine (NMM) (4.5 mmol). After stirred at this temperature for 30 min, the mixture was stored in a refrigerator overnight (16 h). Then 5% aqueous H_3PO_4 solution (50 mL), brine (50 mL), and EtOAc (150 mL) were added. After two layers were separated, the organic solution was washed with 5% H_3PO_4 (150 mL), saturated aqueous sodium bicarbonate solution (2× 150 mL), water (150 mL), and brine (150 mL). It was then dried (magnesium sulfate), filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography (0–60%) eluting with acetone/hexanes to give the desired product in 50–95% yield.

5.3. General method for Dess–Martin periodinane oxidation

To a solution of α-hydroxyamide (1.0 mmol) in dichloromethane (50 mL) was added Dess–Martin periodinane (2.0 mmol). The mixture was stirred at rt for 3 h. Saturated aqueous sodium thiosulfate solution (50 mL) was added and the mixture stirred for 5 min. The layers were separated, the aqueous solution was extracted with dichloromethane (2× 50 mL). The combined organic solution was dried (magnesium sulfate), filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography to give the desired α-ketoamide in 60–90% yield.

5.4. 6,6-Dichloro-3-aza-bicyclo[3,1,0]hexane-2,3-dicarboxylic acid di-*tert*-butyl ester (3)

The *N*-Boc-3,4-dehydroproline **2** was converted to its *tert*-butyl ester according to a literature procedure.[12] The *tert*-butyl ester (1.0 mmol) was dissolved in chloroform (20 mL). To the mixture was added 50% aqueous sodium hydroxide solution (20 mL). The bi-phasic mixture was vigorously stirred at rt for 18 h. Dichloromethane (50 mL) and water (50 mL) were added and layers were separated. The aqueous solution was extracted with dichloromethane (2× 50 mL). The combined organic solution was dried (magnesium sulfate), filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography to give product **3** (40% yield). Two sets of peaks were observed due to the existence of two rotamers. ^{1}H NMR (500 MHz, d_6-DMSO) δ 4.38 and 4.25 (s, 1H), 3.76–3.75 and 3.72–3.70 (m, 2H), 2.34–2.31 (m, 2H), 1.47 and 1.46 (s, 9H), 1.41 and 1.38 (s, 9H). ^{13}C NMR (125 MHz, d_6-DMSO) δ 169.34, 169.32, 153.20, 152.76, 82.29, 82.21, 80.44, 80.33, 62.58, 61.49, 47.54, 47.46, 37.53, 36.72, 36.58, 34.03, 33.25, 28.26, 28.20, 28.06, 27.92, 24.62. HR-MS Calcd for $C_{15}H_{23}NO_4Cl_2$: 352.1082 $(M+H)^+$. Found: 352.1085.

5.5. 6,6-Dichloro-3-aza-bicyclo[3.1.0]hexane-2-carboxylic acid methyl ester (4)

The *N*-Boc proline *tert*-butyl ester (0.4 mmol) was dissolved in methanol (30 mL) and 1 N aqueous HCl solution (0.6 mL) was added. The resulted mixture was heated to reflux in an oil bath for 18 h before it was

concentrated under reduced pressure to give proline methyl ester hydrochloride **4**. ^{1}H NMR (500 MHz, d_6-DMSO) δ 4.42 (d, J = 1.8 Hz, 1H), 3.8 (ddd, J = 2.8, 9.0, 13.2 Hz, 1H), 3.8 (s, 3H), 3.34 (dd, J = 1.85, 3.77 and 1.8, 3.8, 1 H), 3.12 (d, J = 2.01, 1.60, 1H), 3.06 (d, J = 1.6 and 1.8, 1H), 2.97–2.94 (m, 1H). ^{13}C NMR (125 MHz, d_6-DMSO) δ 167.5, 61.7, 61.4, 53.5, 47.5, 37.8, 35.3. HR-MS Calcd for $C_7H_{10}NO_2$: 210.0090 $(M+H)^+$. Found: 210.0090.

5.6. 3-(2-*tert*-Butoxycarbonylamino-2-cyclohexyl-acetyl)-6,6-dichloro-3-aza-bicyclo[3.1.0]hexane-2-carboxylic acid

(**6**: R^3 is cyclohexyl) ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.96 (d, J = 9.3 Hz, 1H), 4.39 (s, 1H), 4.26 (d, J = 11.3 Hz, 1H), 3.96–3.89 (m, 2H), 2.76–2.74 (m, 1H), 2.68 (d, J = 8.6 Hz, 1H), 2.09 (s, 1H), 2.04 (s, 1H), 1.84 (s, 1H), 1.74–1.58 (m, 4H), 1.33 (s, 9H), 1.17–1.10 (m, 5H), 0.95–0.84 (m, 2H). ^{13}C NMR (125 MHz, d_6-DMSO) δ 170.43, 170.40, 155.4, 153.8, 124.1,77.9, 63.2, 60.7, 56.3, 55.8, 47.7, 38.6, 35.1, 33.6, 31.5, 29.5, 29.2, 29.1, 28.6, 28.3, 28.11, 28.05, 25.9, 25.4, 25.3. HR-MS Calcd for $C_{19}H_{28}N_2O_5Cl_2$: 435.1454 $(M+H)^+$. Found: 435.1460.

5.7. 3-(2-*tert*-Butoxycarbonylamino-3,3-dimethyl-butyryl)-6,6-dichloro-3-aza-bicyclo[3.1.0]hexane-2-carboxylic acid

(**6**: R^3 is *tert*-butyl) ^{1}H NMR (500 MHz, d_6-DMSO) δ 6.69 (d, J = 9.7 Hz, 1H), 4.44 (s, 1 H), 4.17 (d, J = 11.4 Hz, 1H), 4.09 (d, J = 9.7 Hz, 1H), 3.96 (dd, J = 5.1, 11.4 Hz, 1H), 2.75 (dd, J = 5.1, 8.5 Hz, 1H), 2.70 (d, J = 8.6 Hz, 1H), 1.91 (s, 2H), 1.35 (s, 9H), 0.92 (s, 9H); ^{13}C NMR (125 MHz, d_6-DMSO) δ 171.9, 170.3, 169.9, 155.6, 78.1, 63.2, 60.5, 58.2, 48.3, 35.1, 34.3, 33.5, 28.12, 28.10, 28.0, 26.15, 26.10, 21.0, 14.0. HR-MS Calcd for $C_{17}H_{27}N_2O_5Cl_2$: 409.1294 $(M+H)^+$. Found: 409.1297.

5.8. 3-(2-*tert*-Butoxycarbonylamino-2-indan-2-yl-acetyl)-6,6-dichloro-3-aza-bicyclo[3.1.0]hexane-2-carboxylic acid

(**6**: R^3 is 2-indanyl) ^{1}H NMR (500 MHz, d_6-DMSO) δ 7.30 (d, J = 8.5 Hz, 1H), 7.19–7.09 (m, 4H), 4.44 (s, 1H), 4.25 (d, J = 11.0 Hz, 1H), 4.13 (t, J = 9.2 Hz, 1H), 3.89 (dd, J = 5.0, 11.5 Hz, 1H), 2.93 (dd, J = 7.2, 15.4 Hz, 1H), 2.83 (dd, J = 7.2, 15.2 Hz, 1H), 2.76–2.63 (m, 5H), 2.50–2.49 (m, 1H), 1.36 (s, 9H). ^{13}C NMR (125 MHz, d_6-DMSO) δ 170.3, 170.2, 155.4, 142.3, 142.1, 126.13, 126.11, 124.4, 124.06, 78.1, 63.2, 60.7, 54.8, 47.6, 40.9, 35.3, 35.1, 35.0, 33.6, 30.3, 29.2, 29.1, 28.0. HR-MS Calcd for $C_{22}H_{26}N_2O_5Cl_2$: 469.1297 $(M+H)^+$. Found: 469.1310.

5.9. 1,1- Dimethylethyl [1-cyclohexyl-2-[(1*S*,5*R*)-6,6-dichloro-2(*S*)-[[[1-[2-[[2-[[2-(dimethylamino)-2-oxo-1(*S*)-phenylethyl]amino]-2-oxoethyl]amino]-1,2-dioxoethyl]-butyl]amino]carbonyl]-3-azabicyclo[3.1.0]hexan-3-yl]-2-oxoethyl]carbamate (8)

^{1}H NMR (500 MHz, d_6-DMSO) δ 8.79–8.73 (m, 1H), 8.58–8.51 (m, 1H), 7.36–7.35 (m, 5H), 7.32–7.30 (m 1H), 6.91–6.89 (m, 1H), 5.82 (d, J = 7.8 Hz, 1H), 5.00–4.97 (m, 1H), 4.62 (d, J = 11.3 Hz, 1H), 4.23–4.20 (m, 1H), 3.96–3.76 (m, 5H), 2.92–2.84 (m, 7H), 2.75–2.71 (m, 1H), 1.73–1.42 (m, 9H), 1.34 (d, J = 3.1 Hz, 9H), 1.15–1.08 (m, 3H), 0.91–0.85 (m, 5H). ^{13}C NMR (125 MHz, d_6-DMSO) δ 196.15, 196.00, 169.15, 166.82, 166.77, 155.39, 154.08, 137.42, 137.37, 128.51, 127.77, 127.70, 77.80, 61.19, 56.50, 53.78, 53.36, 52.89, 41.46, 36.50, 36.4, 35.33, 33.50, 31.61, 28.70, 28.45, 28.03, 18.54, 13.48, 13.36. HR-MS Calcd for $C_{37}H_{52}N_6O_8Cl_2$: 779.3302 $(M+H)^+$. Found: 779.3304.

5.10. 1,1- Dimethylethyl [1-cyclohexyl-2-[(1*S*,5*R*)-6,6-dichloro-2(*S*)-[[[1-[1,2-dioxo-2-(2-propenylamino) ethyl]butyl]-amino]carbonyl]-3-azabicyclo[3.1.0]hexan-3-yl]-2-oxoethyl]carbamate (9)

^{1}H NMR (500 MHz, d_6-DMSO) δ 8.92 and 8.88 (t, J = 6.0 and 6.3 Hz, 1H), 8.67 and 8.59 (d, J = 6.8 and 7.7 Hz, 1H), 6.92 (q, J = 4.5 Hz, 1H), 5.82–5.75 (m, 1H), 5.11–5.04 (m, 2H), 4.97–4.93 (m, 1H), 4.61 (d, J = 10.2 Hz, 1H), 4.23–4.20 (m, 1H), 3.96–3.86 (m, 2H), 3.78–3.68 (m, 3H), 2.75–2.70 (m, 1H), 2.42–2.36 (m, 1H), 1.75–1.49 (m, 8H), 1.41–1.29 (m, 10H), 1.14–1.08 (m, 3H), 0.90–0.85 (m, 5H). ^{13}C NMR (125 MHz, d_6-DMSO) δ 197.1, 196.4, 168.9, 160.5, 155.4, 134.8, 134.03, 134.00, 115.6, 115.4, 109.0, 77.84, 77.80, 60.9, 56.5, 56.3, 53.9, 53.5, 40.7, 33.6, 33.5, 31.7, 31.5, 28.0, 25.8, 25.39, 25.35, 25.2, 18.6, 18.5, 13.5, 13.4. HR-MS Calcd for $C_{28}H_{42}N_4O_6Cl_2$: 560.2407 $(M+H)^+$. Found: 560.2416.

5.11. (1*S*,5*R*)-6,6-Dichloro-3-[cyclohexyl[[[(1,1-dimethyl-ethyl)amino]carbonyl]amino]acetyl]-*N*-[1-[1,2-dioxo-2-(2-propenylamino)ethyl]butyl]-3-azabicyclo[3.1.0]hexan-2(*S*)-carboxamide (10)

^{1}H NMR (500 MHz, d_6-DMSO) δ 7.70–7.66 (m, 1H), 7.09 (t, J = 6.1Hz, 1H), 6.98 (t, J = 6.0 Hz, 1H), 5.86–5.77 (m, 2H), 5.24–5.15 (m, 3H), 4.81 (d, J = 7.8 Hz, 1H), 4.49–4.47 (m, 1H), 4.36–4.32 (m, 1H), 3.95–3.87 (m, 3H), 2.72 (dd, J = 8.5, 11.7 Hz, 1H), 2.48–2.42 (m, 1H), 1.90–1.53 (m, 9H), 1.41–1.29 (m, 3H), 1.27 (br s, 9H), 1.23–1.07 (m, 3H), 0.94–0.89 (m, 3H). ^{13}C NMR (125 MHz, d_6-DMSO) δ 196.1, 195.5, 174.3, 174.1, 168.4, 168.2, 158.8, 156.7, 151.8, 132.5, 117.5, 85.6, 61.9, 61.5, 61.3, 56.0, 55.8, 54.2, 50.3, 48.9, 48.7, 41.6, 40.8, 35.2, 35.1, 34.0, 33.3, 33.1, 32.5, 29.5, 29.4, 28.7, 26.1, 25.8, 18.9, 13.6. HR-MS Calcd for $C_{28}H_{44}N_5O_5Cl_2$: 600.2719 $(M+H)^+$. Found: 600.2720.

5.12. 1,1-Dimethylethyl [2-[(1*S*,5*R*)-2(*S*)-[[[1-(2-amino-1,2-dioxoethyl)butyl]amino]carbonyl]-6,6-dichloro-3-azabicyclo[3.1.0]hexan-3-yl]-1(*S*)-cyclohexy;-2-oxoethyl]- carbamate (12)

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.62–7.57 (m, 1H), 5.97–5.83 (m, 1H), 5.31–5.22 (m, 2H), 5.14–5.10 (m, 2H), 4.84 (d, J = 9.33 Hz, 1H), 4.32 (d, J = 11.8 Hz, 1H), 4.20–4.12 (m, 1H), 3.94–3.86 (m, 1H), 2.77 (t, J = 7.72 Hz, 1H), 2.51–2.41 (m, 2H), 1.80–1.45 (m, 11H), 1.39 (br s, 9H), 1.29–1.08 (m, 2H), 0.98–0.88 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 195.40, 172.97, 172.73, 168.45, 168.33, 160.95, 155.50, 85.62, 79.85, 61.91, 61.49, 61.31, 56.97, 56.87, 54.08, 53.99, 48.68, 40.59, 40.61, 34.96, 34.87, 33.93, 33.81, 33.11, 29.53, 28.40, 28.22, 26.04, 25.80, 25.64, 19.00, 18.90, 13.65,

13.56. HR-MS Calcd for $C_{25}H_{38}N_5O_6Cl_2$: 561.2238 $(M+H)^+$. Found: 561.2247.

5.13. 1,1-Dimethylethyl[2-[(1*S*,5*R*)-6,6-dichloro-2(*S*)-[[[1-(cyclopropyl-methyl)-3-[[2-[[2-(dimethylamino)-2-oxo-1-phenylethyl]amino]-2-oxoethyl]amino]-2,3-dioxopropyl]-amino]carbonyl]-3-azabicyclo[3.1.0]-hexan-3-yl]-1-cyclohexyl-2-oxoethyl]carbamate (13)

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.72–7.66 (m, 2H), 7.62–7.53 (m, 1H), 7.40–7.31 (m, 5H), 5.84 (d, J =7.14 Hz, 1H), 5.38–5.29 (m, 1H), 5.20–5.07 (m, 1H), 4.86 (d, J = 8.79 Hz, 1H), 4.31 (d, J = 11.57 Hz, 1H), 4.22–3.84 (m, 3H), 2.97 (s, 3H), 2.88 (s, 3H), 2.81–2.72 (m, 1H), 2.49–2.41 (m, 1H), 2.31–2.06 (m, 1H), 1.83–1.53 (m, 8H), 1.39 (br s, 9H), 1.24–0.62 (m, 6H), 0.45–0.40 (m, 2H), 0.11–0.01 (m, 2H). ^{13}C NMR (125 MHz, d_6-DMSO) δ 194.9, 194.6, 172.8, 172.5, 168.0, 166.3, 159.2, 155.5, 136.6, 129.0, 128.5, 127.8, 85.6, 79.8, 62.0, 61.4, 61.2, 56.9, 56.8, 55.1, 54.9, 54.1, 48.6, 42.2, 40.6, 36.8, 36.3, 36.0, 35.1, 34.8, 33.9, 33.7, 29.5, 28.2, 26.0, 25.6, 7.2, 5.2, 5.1, 4.5. HR-MS Calcd for $C_{38}H_{52}N_6O_8Cl_2$: 791.3302 $(M+H)^+$. Found: 791.3310.

5.14. 1,1-Dimethylethyl[2-[(1*S*,5*R*)-6,6-dichloro-2(*S*)-[[[1-(cyclobutylmethyl)-3-[[2-[[2-(dimethylamino)-2-oxo-1(*S*)-phenylethyl]amino]-2-oxomethyl]amino]-2,3-dioxopropyl amino]carbonyl]-3-azabicyclo[3.1.0]hexan-3-yl]-1(*S*)-cyclohexyl-2-oxomethyl]carbamate (14)

^{1}H NMR (500 MHz, d_6-DMSO) δ 8.80–8.76 (m, 1H), 8.62–8.55 (m, 2H), 7.36 (s, 5H), 6.92 (t, J = 8.5 Hz, 1H), 5.82 (d, J = 6.9 Hz, 1H), 4.94 (m, 1H), 4.61 (d, J = 10.7 Hz, 1H), 4.23–4.20 (m, 1H), 3.95–3.77 (m, 4H), 2.96 (s, 3H), 2.80 (s, 3H), 2.74–2.71 (m, 1H), 2.49–2.33 (m, 3H), 1.98–1.97 (m, 2H), 1.85–1.61 (m, 12H), 1.34 (s, 9H), 1.09 (m, 2H), 0.865 (m, 2H). ^{13}C NMR (125 MHz, d_6-DMSO) δ 196.5, 196.1, 195.9, 170.13, 170.06, 169.1, 168.8, 168.4, 166.83, 166.80, 160.6, 160.4, 155.39, 155.37, 137.45, 137.40, 128.5, 127.8, 127.7, 77.84, 77.80, 63.40, 63.37, 61.2, 60.9, 56.5, 56.4, 53.0, 52.9, 52.5, 52.4, 48.2, 48.1, 41.6, 41.5, 36.6, 36.5, 36.3, 36.2, 35.3, 33.6, 33.5, 32.1, 28.7, 28.6, 28.4, 28.3, 28.03, 27.97, 27.8, 27.7, 27.2, 25.9, 25.44, 25.36, 25.30, 25.26, 17.80, 17.69. HR-MS Calcd for $C_{39}H_{54}N_6O_8Cl_2$: 805.3458 $(M+H)^+$. Found: 805.3472.

5.15. 2,2,2-Trifluoro-1,1-dimethylethyl[2-[(1*S*,5*R*)-2(*S*)-[[[3-amino-1-(cyclobutylmethyl)-2,3-dioxopropyl]amino]-carbonyl]-6,6-dichloro-3-azabicyclo[3.1.0]hexan-3-yl]-1(*S*)-cyclohexyl-2-oxoethyl]carbamate (18)

^{1}H NMR (500 MHz, d_6-DMSO) δ 8.60 and 8.51 (d, J = 7.5 and 7.2 Hz, 1H), 8.05 (d, J = 15.2 Hz, 1H), 7.81 (d, J = 6.3 Hz, 1H), 7.68–7.63 (m, 1H), 4.95–4.91 (m, 1H), 4.62 (d, J = 6.6 Hz, 1H), 4.20–4.16 (m, 1H), 3.98–3.86 (m, 2H), 2.76–2.75 (m, 1H), 2.36–2.33 (m, 2H), 1.98–1.94 (m, 2H), 1.82–1.48 (m, 17H), 1.09–1.08 (m, 3H), 0.94–0.83 (m, 3H). ^{13}C NMR (125 MHz, d_6-DMSO) δ 207.1, 206.7, 206.5, 179.31, 179.30, 178.7, 178.3, 177.9, 172.3, 173.2, 163.2, 70.9, 70.7, 66.5, 66.4, 62.5, 61.8, 57.90, 57.89, 57.8, 48.1, 47.9, 46.4, 46.2, 46.0, 45.9, 43.2, 43.1, 41.8, 38.1, 38.03, 37.98, 37.5, 37.4,37.0, 36.9, 35.4, 35.04, 35.00, 34.90, 34.86, 29.1, 28.8, 27.4, 26.4. HR-MS Calcd for $C_{27}H_{37}N_4O_6Cl_2F_3$: 641.2112 $(M+H)^+$. Found: 641.2121.

5.16. 6,6-Dichloro-3-[2-cyclohexyl-2-(3-cyclohexyl-ureido)-acetyl]-3-aza-bicyclo[3.1.0]hexane-2-carboxylic acid(2-carbamoyl-1-cyclobutylmethyl-2-oxo-ethyl)-amide (22)

^{1}H NMR (500 MHz, d_6-DMSO) δ 8.56 and 78.42 (d, J = 7.3 and 7.5 Hz, 1H), 8.05–8.01 (m, 1H), 7.80 (s, 1H), 5.87–5.84 (m, 2H), 4.94–4.90 (m, 1H), 4.61–4.59 (m, 1H), 4.16–4.08 (m, 2H), 3.97–3.90 (m, 1H), 2.73–2.71 (m, 1H), 2.43–2.38 (m, 1H), 2.01–1.95 (m, 2H), 1.84–1.37 (m, 17H), 1.27–0.83 (m, 12H); ^{13}C NMR (125 MHz, d_6-DMSO) δ 35.0, 34.0, 33.8, 33.7, 32.6, 32.4, 29.6, 28.8, 28.5, 28.2, 28.0, 26.1, 25.84, 25.82, 24.9, 24.8, 18.4, 18.3. HR-MS Calcd for $C_{29}H_{43}N_5O_5Cl_2$: 612.2714 $(M+H)^+$. Found: 612.2720.

5.17. {1-[2-(2-Carbamoyl-1-cyclobutylmethyl-2-oxo-ethyl-carbamoyl)-6,6-dichloro-3-aza-bicyclo[3.1.0]hexane-3-carbonyl]-2,2-dimethyl-propyl}-carbamic acid *tert*-butyl ester (23)

^{1}H NMR (500 MHz, d_6-DMSO) δ 8.57 and 8.51 (d, J = 7.4 and 7.3 Hz, 1H), 8.04 and 7.79 (d, J = 11.9 and 18.9 Hz, 1H), 5.93 (s, 1H), 5.87 (q, J = 4.46 Hz, 1H), 4.97–4.86 (m, 1H), 4.64 (d, J = 3.2 Hz, 1H), 4.23–4.14 (m, 2H), 3.96–3.92 (m, 1H), 2.74–2.70 (m, 1H), 2.40 (dd, J = 8.7, 8.5 Hz, 1H), 2.01–1.93 (m, 2H), 1.84–1.72 (m, 2H), 1.67–1.56 (m, 3H), 1.24 (br s, 1H), 1.17 (d, J = 1.9 Hz, 9H), 1.10 (s, 1H) 0.87 (d, J = 6.9 Hz, 9H); ^{13}C NMR (125 MHz, d_6-DMSO) δ 198.4, 197.7, 171.9, 171.4, 169.7, 169.3, 163.7, 163.5, 157.93, 157.90, 64.2, 61.7, 61.6, 57.44, 57.40, 53.2, 49.8, 49.6, 37.5, 37.3, 37.1, 35.2, 34.6, 34.4, 33.1, 33.0, 32.1, 30.0, 28.9, 28.7, 28.32, 28.30, 27.1, 23.0. HR-MS Calcd for $C_{25}H_{38}N_4O_6Cl_2$: 561.2240 $(M+H)^+$. Found: 561.2247.

5.18. 3-[2-(3-*tert*-Butyl-ureido)-3,3-dimethyl-butyryl]-6,6-dichloro-3-aza-bicyclo[3.1.0]hexane-2-carboxylic acid(2-carbamoyl-1-cyclobutylmethyl-2-oxo-ethyl)-amide (24)

^{1}H NMR (500 MHz, d_6-DMSO) δ 8.60 (d, J = 7.1 Hz, 1H), 8.06 (s, 1H), 7.81 (s, 1H), 6.63 (d, J = 9.8 Hz, 1H), 4.97–4.93 (m, 1H), 4.65 (s, 1H), 4.14 (d, J = 11.4 Hz, 1H), 4.05 (d, J = 9.7 Hz, 1H), 3.95 (dd, J = 5.3, 11.0 Hz, 1H), 2.74 (dd, J = 8.6, 8.8 Hz, 1H), 2.41 (d, J = 8.9 Hz, 1H), 2.00–1.92 (m, 2H), 1.84–1.70 (m, 3H), 1.66–1.55 (m, 3H), 1.41 (br s, 1H), 1.35 (s, 9H), 1.24 (br s, 1H), 0.90 (s, 9H). ^{13}C NMR (125 MHz, d_6-DMSO) δ 198.4, 170.5, 169.7, 163.5, 156.6, 79.0, 64.3, 61.8, 59.3, 53.2, 49.7, 37.5, 37.3, 35.1, 34.6, 33.0, 32.1, 29.0, 28.9, 28.7, 28.2, 27.1, 23.0, 18.7, 18.6, 14.8. HR-MS Calcd for $C_{25}H_{39}N_5O_5Cl_2$: 560.2339 $(M+H)^+$. Found: 560.2407.

5.19. {2-[2-(2-Carbamoyl-1-cyclobutylmethyl-2-oxo-ethyl-carbamoyl)-6,6-dichloro-3-aza-bicyclo[3.1.0]hex-3-yl]-1-indan-2-yl-2-oxo-ethyl}-carbamic acid *tert*-butyl ester (25)

^{1}H NMR (500 MHz, d_6-DMSO) δ 8.62 and 8.49 (d, J = 7.3 and 7.5 Hz, 1H) 8.07 (d, J = 13.8 Hz, 1H), 7.83

(d, $J = 8.0$ Hz, 1H) 7.29–7.25 (m, 1H), 7.18–7.09 (m, 4H), 5.00–4.95 (m, 1H), 4.64 (d, $J = 13.9$ Hz, 1H), 4.21–4.08 (m, 1H), 3.95–3.86 (m, 1H), 3.36 (s, 1H), 2.94–2.64 (m, 7H), 2.47–2.38 (m, 2H), 2.03–1.97 (m, 1H), 1.88–1.59 (m, 6H), 1.37–1.36 (m, 9H); ^{13}C NMR (125 MHz, d_6-DMSO) δ 207.1 206.5, 179.6, 178.38, 178.00, 172.3, 172.2, 165.1, 165.0, 152.0, 151.9, 151.8, 135.73, 135.70, 134.0, 133.7, 87.71, 87.66, 71.0, 70.7, 64.7, 64.4, 62.1, 61.8, 57.8, 50.5, 50.4, 46.3, 46.7, 46.0, 45.8, 45.7, 44.91, 44.86, 44.66, 44.56, 43.2, 43.1, 41.8, 37.6, 37.5, 37.4, 36.9, 35.9, 27.40, 27.36. HR-MS Calcd for $C_{30}H_{38}N_4O_6Cl_2$: 621.2235 $(M+H)^+$. Found: 621.2247.

5.20. 3-[2-(3-*tert*-Butyl-ureido)-2-indan-2-yl-acetyl]-6,6-dichloro-3-aza-bicyclo[3.1.0]hexane-2-carboxylic acid(2-carbamoyl-1-cyclobutylmethyl-2-oxo-ethyl)-amide (26)

^{1}H NMR (500 MHz, d_6-DMSO) δ 8.62 and 8.49 (d, $J = 7.3$ and 7.5 Hz, 1H) 8.07 (d, $J = 13.8$ Hz, 1H), 7.83 (s, 1H) 7.18–7.09 (m, 4H), 6.07–6.04 (m, 1H), 5.83 (s, 1H), 5.00–4.95 (m, 1H), 4.64 (d, $J = 13.9$ Hz, 1H), 4.31–4.28 (m, 1H), 4.15–4.12 (m, 1H), 3.95–3.86 (m, 1H), 2.94–2.64 (m, 7H), 2.47–2.38 (m, 2H), 2.03–1.97 (m, 1H), 1.88–1.59 (m, 6H), 1.37–1.36 (m, 9H); ^{13}C NMR (125 MHz, d_6-DMSO) δ 207.1 206.5, 180.4, 180.3, 178.4, 178.0, 172.3, 166.4, 152.0, 151.8, 135.7, 134.0, 133.7, 72.9, 70.9, 70.5, 62.6, 62.1, 61.8, 58.62, 58.60, 57.7, 57.5, 51.3, 51.1, 46.2, 46.0, 45.7, 44.7, 44.6, 44.2, 44.1, 43.1, 43.0, 41.8, 39.1, 38.7, 37.42, 37.40, 37.0, 36.9, 27.37. HR-MS Calcd for $C_{30}H_{39}N_5O_5Cl_2$: 620.2400 $(M+H)^+$. Found: 620.2407.

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