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Authors

Chen, Yuanguang

Chen, Lu

Xu, Huashen

et al.

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Structure-directed discovery of potent soluble epoxide hydrolase inhibitors for the treatment of inflammatory diseases

Yuanguang Chen[a], **Lu Chen**[a], **Huashen Xu**[a], **Ruolin Cao**[a], **Christophe Morisseau**[b], **Maoying Zhang**[a], **Yajie Shi**[a], **Bruce D. Hammock**[b], **Jieru Wang**[a], **Junning Zhuang**[a], **Zhongbo Liu**[c], **Guoliang Chen**[a]

[a]Key Laboratory of Structure-Based Drug Design & Discovery of Ministry of Education, School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang 110016, China

[b]Department of Entomology and Nematology and UC Davis Comprehensive Cancer Center, University of California Davis, Davis, CA 95616, USA

[c]School of Pharmacy, Shenyang Pharmaceutical University, Shenyang 110016, China

Abstract

The sEH has been identified as an attractive target for anti-inflammatory drug design in recent years. Picomolar level compound **G1** against sEH was obtained by introducing the hydrophilic group homopiperazine and hydrophobic fragment propionyl onto the structure of lead compound **A**. **G1** showed good microsomal stability, moderate plasma protein binding rate, good oral bioavailability and well tolerated in rats. **G1** has significant analgesic effects on CFA-induced AIA

Corresponding Author: Guoliang Chen - Key Laboratory of Structure-Based Drug Design & Discovery of Ministry of Education, School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang 110016, PR China; Phone: +86-13940195895; chenguoliang@syphu.edu.cn. **Zhongbo Liu** - School of Pharmacy, Shenyang Pharmaceutical University, Shenyang 110016, PR China.; Phone: +86-13032454403; 546265581@qq.com.

Yuanguang Chen - Key Laboratory of Structure-Based Drug Design & Discovery of Ministry of Education, School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang 110016, PR China.

Lu Chen - Key Laboratory of Structure-Based Drug Design & Discovery of Ministry of Education, School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang 110016, PR China.

Huashen Xu - Key Laboratory of Structure-Based Drug Design & Discovery of Ministry of Education, School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang 110016, PR China.

Ruolin Cao - Key Laboratory of Structure-Based Drug Design & Discovery of Ministry of Education, School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang 110016, PR China.

Christophe Morisseau – Department of Entomology and Nematology and UC Davis Comprehensive Cancer Center, University of California Davis, Davis, CA 95616, USA.

Maoying Zhang - Key Laboratory of Structure-Based Drug Design & Discovery of Ministry of Education, School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang 110016, PR China.

Yajie Shi - Key Laboratory of Structure-Based Drug Design & Discovery of Ministry of Education, School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang 110016, PR China.

Bruce D Hammock - Department of Entomology and Nematology and UC Davis Comprehensive Cancer Center, University of California Davis, Davis, CA 95616, USA.

Jieru Wang - Department of Pharmacology, Shenyang Pharmaceutical University, Shenyang 110016, PR China.

Junning Zhuang - Key Laboratory of Structure-Based Drug Design & Discovery of Ministry of Education, School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang 110016, PR China.

mice, ameliorated the pancreatic injury in acute pancreatitis induced by *L*-arginine and reversed pancreatic injury, edema and neutrophil infiltration, increased survival time of C57BL/6 mice in an LPS-induced sepsis model, the expression levels of sEH, COX-2, NOS-2, VCAM, IL-6, MCP-5, and TNF-α were measured by Western blot or ELISA, with varying degrees of decrease. These results suggested **G1** is a drug candidate worthy of further evaluation for the treatment of inflammation-induced diseases such as arthritis, acute pancreatitis and sepsis.

INTRODUCTION

Inflammation is a complex biological protective response to harmful stimuli such as pathogens, damaged cells, irritants etc, it involves immune cells, blood vessels and molecular mediators. But, excessive inflammatory response can cause physical damage, just as the severe COVID-19 patients may die from cytokine storm-induced sepsis and subsequent multiple organ failure(cytokine storm is also called inflammatory storm). Chronic inflammation is associated with many diseases, such as AD, arthritis, cancer etc.

Acute pancreatitis (AP) is an inflammatory disease related to the injury and necrosis of pancreatic exocrine tissue. It has an acute onset, severe condition and poor prognosis, with an incidence rate of 10 / 340000[1, 2]. The prevalence of AP has been increasing in recent years, with a mortality rate of up to 30 %[3]. The disease can cause varying degrees of damage to the lung, kidney, liver, heart, and other important substantive organs, and in severe cases, systemic inflammatory response syndrome (SIRS), multiple organ dysfunction syndrome (MODS), and even death can occur in the early stage of the disease, for which there is no specific treatment method[4–6]. According to research, inflammatory cytokines play an important role in AP progression, and AP progression is driven by an inflammatory cascade initiated by Toll-like receptors (TLRs)-nuclear factor B (NF-κB) activation and cytokine production by glandular follicle cells[7]. More recently, it has been demonstrated that mice with EPHX2 knocked out systemically exhibit attenuated arginine-induced AP, which is due to the potent anti-inflammatory properties of EET and a reduction in endoplasmic reticulum (ER) stress, implying that sEH inhibitors can be used to treat AP[8, 9].

Rheumatoid arthritis (RA) is a chronic inflammatory disease with an unknown etiology that causes multiple, symmetrical joint swelling and pain, abnormal synovial proliferation, cellular invasion, and progressive destruction of cartilage and bone, resulting in further joint destruction, stiffness, deformity, and disability[10]. Inflammation causes pain in many RA patients. Pain is a complex signaling process caused by harmful substances being damaged and the release of inflammatory mediators such as cytokines, ions, bradykinin, prostaglandins, and leukotrienes, which act directly on pain receptors and drive action potentials to produce the sensation of pain[11]. The current clinical use of nonsteroidal anti-inflammatory drugs, glucocorticoids, and other anti-rheumatic drugs and traditional therapies can reduce symptoms and slow the progression of rheumatoid arthritis, but long-term use of these drugs can result in a variety of adverse reactions, including liver and kidney damage, gastrointestinal irritant injury, and so on[12, 13]. As a result, a new treatment for RA with no side effects is desperately needed. Pain is caused by biological or chemical inflammation, and the prostaglandin metabolite PGE2 is an important inflammatory

mediator and pain-causing substance[14]. Many studies have shown that inhibiting sEH activity with small molecules lowers prostaglandin-2 (PGE2) levels, thereby inhibiting inflammation and exerting analgesic effects[15–17]. They are more effective than nonsteroidal anti-inflammatory drugs (NSAIDs) and even improve NSAIDs' anti-inflammatory effects. sEH inhibitors changed the levels of not only EET and diol metabolites, but also some other metabolites in the arachidonic acid cascade of cyclooxygenase (COX) and lipoxygenase (LOX) metabolic pathways[15]. sEH inhibitors have been shown to have therapeutic and protective effects in acute lung injury[18–20], various renal diseases[21], the heart[22, 23], and the blood-brain barrier[24]. Because sEH inhibitors are not addictive in terms of analgesia, they merit further development and investigation[25].

Sepsis is a clinical syndrome characterized by a dysregulated inflammatory response (also known as cytokine storm or inflammatory storm) to infections (including bacteria, fungi, viruses, and parasites) and severe trauma[26, 27]. The COVID-19 pandemic has now become a global crisis, more lethal than any other infectious disease in history. It has had a significant physical and psychological impact on a large portion of the global population, as well as destroyed the global business and social order[28]. According to current research, the pathogenesis of COVID-19 is primarily related to immunopathology[29]. The rapid and massive production of cytokines such as TNF-α, IL-1, IL-6, IL-12, MCP-5, and IL-8 in body fluids following infection is known as cytokine storm, and it is a major cause of acute respiratory distress syndrome and multi-organ failure[30, 31]. Corticosteroids are commonly used in clinic to treat cytokine storm, but they have an immunosuppressive effect and, to some extent, delay the body's clearance of viruses and other pathogens, thereby exacerbating the disease[32, 33]. sEH inhibitors inhibit the production of inflammatory factors and cytokines via the endoplasmic reticulum (ER) stress pathway, allowing the endoplasmic reticulum stress response to evolve toward maintaining the balance of the inflammatory response in vivo, rather than a single cytokine[34]. They show good application prospects in the treatment of sepsis.

Three sEH inhibitors have entered the clinic to date (Figure 1): **AR-9281** (for hypertension and insulin resistance)[35], **GSK-2256294** (for chronic obstructive pulmonary disease)[36], and **EC-5026** (for neuropathic and inflammatory chronic pain)[37], but none of them are currently approved for marketing. Because sEH play an important role in the development and progression of inflammation and pain, we designed and synthesized a series of sEH inhibitors and then tested their sEH inhibitory effects. The result showed that most compounds had good inhibiting activities, especially compound **G1** with picomolar level inhibitory activity. The safety, metabolic stability, pharmacokinetic and therapeutic effects of compound **G1** against rheumatoid arthritis, acute pancreatitis and sepsis were further evaluated. The results showed that compound **G1** is a candidate for the treatment of rheumatoid arthritis, acute pancreatitis and sepsis and sEH inhibitors have a wide range of clinical application prospects.

RESULTS AND DISCUSSION

Rational Design of the sEH Inhibitors.

Based on previous explorations made in our laboratory on sEH inhibitors, we chose compound **A** as the lead compound (Scheme 1)[38, 39]. The memantine part and the introduction of a fluorine atom at the 2-position of the benzene ring retard the metabolism of compound **A**, and it has been demonstrated that the fluorine atom breaks the structural symmetry, lowers the melting point, and increases the solubility of the compound[38, 39]. The protein code of sEH used for docking is 3WKE, and the eutectic molecule is *t*-AUCB. The structure of *t*-AUCB is very similar to that of compound **A**, both of which have urea segment and carboxylic acid segment, so compound **A** was selected as the basis for docking. The molecular docking revealed that compound **A** formed key hydrogen bonds with Try 383 and Try 466, and that the carboxyl group was exposed to the solvent with a large cavity, allowing the introduction of polar functional groups to improve the compound's activity, physicochemical properties, and drug-like properties (Figure 2). Firstly, we introduced the **R** fragment with small amine and substituted amino group onto the 3- or 4-position of piperidine group to get two series of compounds **A1-A5** and **B1-B4**, and found that the activity of the substituent at the 3-position of piperidine was better than that of 4-position, but the inhibitory activity of the substituent at position 4 against rat sEH (MsEH) is better than that against human sEH (HsEH). Then cyclohexylamine was used instead of piperidine, and the **R** segment of polar small molecule amine or alcohol was introduced to obtain **C** series compounds. To increase compound flexibility, we reduced the carbonyl group to methylene to generate compounds **D1-D10**, and their effects against sEH were improved when compared to the **B** series compounds. However, when we reduced the **A** series compounds to get compounds **E1-E4**, the activities were not increased. This result indicated that the substituent on the 3 position of piperidine was optimal, so we introduced the hydrophilic group homopiperazine on the 3 position of piperidine and integrated the **R** fragments of **EC5026** and **TPPU** to obtain compounds **F1-F3**, while reducing the carbonyl group of the **F** series compounds to obtain **G** series compounds. The evaluation of these compounds against sEH revealed that compound **G1** demonstrated strong inhibitory effects against HsEH and MsEH, with IC_{50} values of 0.05 and 0.14 nM, respectively. The result evoked our curiosity in the homopiperazine fragment. We changed the order of piperidine and homopiperazine and introduced **R** fragments of small molecule acids and amines to obtain compounds **H1-H4**, and then reduce the carbonyl group of **H** series compounds and introduce the **R** fragments of small alcohol to obtain **I** series compounds, but bioactivity results showed that no compound had better activity than **G1**, so compound **G1** was chosen for further investigation.

Chemistry.

The technique used to synthesize compounds **A1-A5** was shown on Scheme 2. Intermediate **1** was obtained by commercially available 3-fluoro-4-nitrobenzoic acid with $SOCl_2$ in the presence of methanol. A reduction of intermediate **1** was catalyzed with 5 % Pd-C under H_2 atmosphere to provide intermediate **2**. In the presence of triphosgene and triethylamine, the amino group of memantine was transformed to isocyanate, and then condensed with

intermediate **2** to obtain intermediate **3**. Intermediate **4** was produced immediately after hydrolysis of intermediate **3** in the presence of sodium hydroxide, while intermediate **5** was prepared by condensing intermediate **4** with piperidine-4-carboxylic acid methyl ester under HATU and DIPEA conditions. The hydrolysis of intermediate **5** gave the key intermediate **6**. Intermediate **6** was condensed with diethylamine, ethanolamine, isopropanolamine, 2-(piperazin-1-yl-ethan)-1-ol or 2-aminopropan-1,3-diol in the presence of HATU and DIPEA to give the target compounds **A1-A5**, respectively.

Compounds **B1-B4** were synthesized from intermediate **4** using the technique indicated in Scheme 3. Intermediate **7** was formed by condensing intermediate **4** with ethyl piperidine-3-carboxylate in the presence of HATU and DIPEA. Intermediate **A** was formed by hydrolyzing intermediate **7** with sodium hydroxide. Condensation of key intermediate **A** with diethylamine, ethanolamine, iso-propanolamine, or 2-(piperazin-1-yl-ethan)-1-ol yielded compounds **B1-B4** respectively.

The synthesis of compounds **C1-C8** was similar to the preparation of compounds **A1-A5** depicted on Scheme 2, intermediate **4** was reacted with methyl 4-aminocyclohexane-1-carboxylate in the presence of HATU and DIPEA to give intermediate **8**, and the hydrolysis of intermediate **8** under sodium hydroxide gave intermediate **9**. Similarly intermediate **9** was condensed with *tert*-butyl 4-(2-aminoethyl)piperazine-1-carboxylate, 2-(piperazin-1-yl-ethan)-1-ol, ethanolamine, *iso*-propanolamine, diethanolamine, ammonia, 2-aminopropan-1,3-diol, 2-amino-2-(hydroxymethyl)propan-1,3-diol to give compounds **C1-C8**, respectively (Scheme 4).

Compounds **D1-D9** were prepared *via* the procedure depicted in Scheme 5 from commercially available 3-fluoro-4-nitrobenzoic acid. Intermediate **10** was obtained by reducing 3-fluoro-4-nitrobenzoic acid under borane. Then intermediate **10** underwent nucleophilic substitution reaction with phosphorus tribromide to give intermediate **11**. Intermediate **11** underwent nucleophilic substitution reaction with ethyl piperidine-3-carboxylate at the presence of K_2CO_3 and KI to give intermediate **12**. Reduction of **12** catalyzed with 5 % Pd-C under H_2 atmosphere provided **13**. In the presence of triphosgene and triethylamine, the amino group of memantine was transformed to isocyanate, and then condensed with intermediate **13** to obtain intermediate **14**. Immediately afterwards compound **15** was hydrolyzed in the presence of sodium hydroxide to give the key intermediate **D**. Intermediate **D** was condensed with ammonia, dimethylamine, diethylamine, *iso*-propanolamine, diethanolamine, ethanolamine, 2-aminopropan-1,3-diol, 2-amino-2-(hydroxymethyl)propan-1,3-diol or 2-(piperazin-1-yl-ethan)-1-ol in the presence of HATU and DIPEA to obtain the target compounds **D1-D9**, respectively.

The synthesis of compounds **E1-E5** was similar to the preparation of compounds **A1-A5** depicted on Scheme 2. Intermediate **11** reacted with piperidine-4-carboxylic acid methyl ester in the presence of K_2CO_3 and KI to obtain intermediate **15**, followed immediately by reduction, urea formation and hydrolysis to obtain key intermediate **18**. Similarly intermediate **18** was condensed with ammonia, *iso*-propanolamine, ammonia, 2-aminopropane-1,3-diol, 2-amino-2-(hydroxymethyl)propane-1,3-diol or diethanolamine in

the presence of HATU and DIPEA to give the target compounds **E1-E5**, respectively (Scheme 6).

Compound **A** and *tert*-butyl 1,4-diazepane-1-carboxylate in the presence of the condensing agent HATU and the organic base DIPEA to give intermediate **19**. Intermediate **19** was deprotected under trifluoroacetic acid to give intermediate **20**. The condensation of key intermediate **20** with acetic acid, propionic acid or 2-methylbutyric acid gave compounds **F1-F3** respectively (Scheme 7).

Compound **D** was reacted with *tert*-butyl 1,4-diazepane-1-carboxylate and deprotected Boc group to give intermediate **22**. Then the target compounds were obtained by condensation with propionic acid, 2-methylbutyric acid, acrylic acid, methacrylic acid or cyclopropanecarboxylic acid in the presence of HATU and DIPEA, respectively (Scheme 8).

Compounds **H1-H4** were obtained as shown on Scheme 9. By acylating *tert*-butyl 1,4-diazepane-1-carboxylate with 3-fluoro-4-nitrobenzoic acid to give compound **23**. Reduction of **23** under H_2 atmosphere in presence of 5 % Pd-C gave **24**. In the presence of triphosgene and triethylamine, the amino group of memantine was transformed to isocyanate, and then condensed with compound **24** to obtain compound **25**. Intermediate **26** was obtained by deprotecting Boc in the presence of trifluoroacetic acid, and then condensed with 2-(1-(*tert*-butoxycarbonyl)piperidin-4-yl)acetic acid in the presence of HATU and DIPEA to give intermediate **27**. The key intermediate **28** was obtained by deprotecting Boc in the presence of trifluoroacetic acid. Intermediate **28** was condensed with propionic acid, 2-methylbutyric acid and acrylic acid in the presence of HATU and DIPEA to give the target compounds **H1-H3**, respectively. Intermediate **28** underwent nucleophilic substitution with 2-chloro-*N,N*-dimethylpropan-1-amine in the presence of K_2CO_3 and KI to give compound **H4**(Scheme 9).

Intermediate **11** was condensed with *tert*-butyl 1,4-diazepane-1-carboxylate to give the key intermediate **29**, Intermediates **34** was obtained from intermediate **29** through reduction, urea-forming, removal of Boc group, acylation, and removal of Boc group. Intermediates **34** were condensed with acetic acid, propionic acid or 2-methylbutyric acid in the presence of HATU and DIPEA to give the target compounds **I1-I3** respectively. In the presence of triphosgene and triethylamine, the amino group of intermediate **34** was transformed to isocyanate, and then condensed with methoxyammonium chloride to obtain compound **I4**. Intermediate **34** in the presence of K_2CO_3 and KI with 2-chloroethane-1-ol, 3-chloropropane-1,2-diol, 2-chloro-*N,N*-dimethylethane-1-amine or 2-chloro-*N,N*-diethylethane-1-amine underwent nucleophilic substitution to give compounds **I5-I8** respectively (Scheme 10).

Molecular docking.

The possible binding mode of compound **G1** with the sEH protein active pockets (PDB ID:3WKE) was docked with Discovery Studio 2016 software, as shown in Figure 3. The compound **G1** urea group formed three hydrogen bonds with the catalytic triplet consisting of residues Asp 335 and Tyr 383 of the backbone, one more hydrogen bond than the lead compound **A**. It also filled the cavity around the carboxyl group and bound more tightly to

the protein, which explains the much higher activity of compound **G1** than that of compound **A**.

Structure activity relationships of series derivatives.

Compounds with memantine core are promising sEH inhibitor in our previous research[38]. The activities of target compounds against HsEH and MsEH were evaluated according to the method reported in literature[40] with the lead compounds A and *t*-**TUCB** as control. The results were shown on Table 1–Table 5

The activity of carboxylic acid derivative such as amide at position 4 of piperidine was first investigated, and *N,N*-diethylamide derivative compound **A1** showed potent effects (IC$_{50}$: HsEH = 2.2 nM, MsEH = 0.53 nM). The activities of compound **A1** against HsEH and MsEH were more than twice that of the lead compound **A**, but they still need to be improved compared with *t*-**TUCB** (Table 1). To increase the water solubility and activity of the compounds, 2-aminoethan-1-ol, 1-aminopropan-2-ol, 2-(piperazin-1-yl)ethan-1-ol, or 2-aminopropane-1,3-diol were reacted to yield compounds **A2-A5** to provide additional polar groups respectively. The structure-activity relationship revealed that small molecule amine alcohols significantly improved MsEH activity, with **A3** having the best IC$_{50}$: MsEH = 0.18 nM. Next we changed the position of amide onto the third position of piperidine from the fourth position of piperdine and obtained compound **B1** (IC$_{50}$: HsEH=0.35 nM, MsEH = 0.38 nM). The activity against HsEH is equivalent to that of *t*-**TUCB**, and it is 7 times that of *t*-**TUCB** against MsEH. When we introduced additional polar groups on the structure of compound **A** by the similar way to obtain compounds **B2-B3**, their activities against HsEH remained powerful effects; while 2-(piperazin-1-yl) ethan-1-ol was introduced, its activity against HsEH slightly decreased maybe because the steric hindrance was a little big. The result that compound **B3** (IC$_{50}$: HsEH = 0.28 nM, MsEH = 0.23 nM) is more active than *t*-**TUCB** is encouraging. According to the preliminary structure-activity relationship, the 3 position of piperidine has priority over the 4 position, the introduction of polar groups is beneficial.

We speculate that the poor activity of piperidine at position 4 may be due to the fact that *N* is on the ring, the rigidity of the compound is too strong, and the bond is not easy to rotate. Therefore, we replaced piperidine with cyclohexylamine, and explored the structure-activity relationship. When the introduction of cyclic *tert*- butyl 4-(2-aminoethyl) piperazine-1-carboxylate or 2-(piperazin-1-yl) ethan-1-ol provided compounds **C1** and **C2**, their activities against HsEH obviously decreased. A series of small molecular amine fragments were introduced to obtain compounds **C3-C8**. Among them, **C8** (IC$_{50}$: HsEH = 0.35 nM, MsEH = 0.12 nM) has the best activities (Table 2). The preliminary structure-activity relationship showed that the activities of **B** series compounds were better than these of **C** series compounds in general, and the activity of piperidine fragments was better than that of cyclohexylamine, and the activity of compound C8 with the moiety of 2-amino-2 -(hydroxymethyl) propane-1,3-diol was the best.

In order to improve the potency, we also reduced the carbonyl group on the benzene ring of **B** series compounds to increase the flexibility and rotation of the compounds. Excitingly,

the activities of compound **D** (IC$_{50}$: HsEH = 2.3 nM, MsEH = 0.61 nM) were twice that of compound **A**. We continued to introduce small molecules of amines to obtain amide compounds **D1-D4**. Among them, the activities of compound **D1** (IC$_{50}$: HsEH = 0.14 nM, MsEH = 0.087 nM) were superior to *t*-TUCB, especially the activity against MsEH. When a series of small molecule aminol fragments were introduced, compounds **D5-D10** were obtained. The results showed that the activities of both HsEH and MsEH remained almost similar activities compared to the **B** series compounds. Similarly for the carbonyl reduction of the A series compounds, a series of compounds **E1-E5** were synthesized, with compound **E1** (IC$_{50}$: HsEH = 1.54 nM, MsEH = 0.058 nM) showing the best activities (Table 3). The structure-activity relationship showed that the activity of 3- substituted piperidine is better than that of 4- substituted piperidine, and short-chain small molecular amine with additional polar group was the best substituent.

Next, we further modified the amide fragment at 3 position on the piperidine with the hydrophilic group homopiperazine instead of the small molecule amine and introduced a small molecular acyl fragment on the other *N* atom of homopiperazine according to the design concept of **EC-5026** and **TPPU**. Firstly, acetyl, propionyl or 2-methylbutyryl group were introduced to obtain compound **F1-F3**. The activities of compound **F1-F3** were several times higher than that of lead compound **A** (Table 4). Then we reduced the carbonyl group on the benzene ring to obtain compound **G1-G2**. To our delight, compound **G1** showed very strong activities (IC$_{50}$: HsEH = 0.05 nM, MsEH = 0.14 nM). The activity of **G1** against HsEH is 5.6 times that of *t*-TUCB, and the activity of **G1** against MsEH is 20 times that of *t*-TUCB. Meanwhile, the activity against HsEH of compound **G1** is 2.8 times that against MsEH. In order to increase the binding ability with sEH, we tried to use α,β-unsaturated double bond to form covalent bonds with sEH, and introduce acryl or 2-methylacryl group to obtain compounds **G3** and **G4**. Unfortunately, they didn't work and didn't show high effects. The structure-activity relationship showed that the activity was improved when the carbonyl group was reduced to methylene, and the propionyl group was the best substituent.

Due to the introduction of homopiperazine and the propionyl group, the activity of compound **G1** was significantly improved. This result stimulated our interest in homopiperazine. Based on the structure of compound **G1**, we tried to exchange the position of homopiperazine with piperidine, and substituted piperidine with piperidine acetic acid to synthesize a series of compounds **H1-H4**. Unfortunately, compared with compound **G1**, theirs activities decreased significantly. Then, after carbonyl reduction, acetyl, propionyl, 2-methylbutyryl or methoxycarbonyl were introduced to obtain compounds **I1-I4**, respectively. Compared with **H1-H4**, the activities of compounds **I1-I4** have obviously increased. Compound **I2** (IC$_{50}$: HsEH = 0.35 nM, MsEH = 0.12 nM) has the best activities. Nextly small molecule amines and alcohols were introduced to obtain compounds **I5-I8**. But the activities of compounds **I5-I8** decreased significantly (Table 5). Structure-activity relationship showed that the activity of the compound with reduction of carbonyl group was improved, and propionyl is the best small molecular substituent. The compound **G1** (IC$_{50}$: HsEH = 0.05 nM, MsEH = 0.12 nM) is the best compound, and has a high LLE value (7.553).

Microsomal stability.

The microsomal stability of compound **G1** was evaluated in human and rat and mouse liver microsomes, respectively, which are widely used to determine the extent of possible primary metabolic clearance in the liver (Table 6). Compound **G1** showed satisfactory microsomal stability *in vitro* with half-lives ($t_{1/2}$) of 3.70 h and 3.15 h in human and rat liver microsomes, respectively. Preliminarily, the good metabolic stability of compound **G1** suggests that it has value for further investigation.

Percent plasma protein binding.

Percent plasma protein binding (% PPB) was measured in SD rats by a classical equilibrium dialysis device. Compound **G1** showed a moderate % PPB rate (72.57 %). Since the therapeutic effect of drugs depends on the concentration of free drugs, % PPB of medium intensity (72.57 %) may contribute to the therapeutic effect *in vivo*, thus contributing to its analgesic effect.

Pharmacokinetic study *in vivo*.

To determine the metabolic stability of compound **G1** *in vivo*, the pharmacokinetic profile was therefore determined in Sprague-Dawley (SD) rats by oral (*po*) single dose of 50 mg / kg and intravenous (*iv*) single dose of 10 mg / kg, and the pharmacokinetic parameters are shown in Table 7. After oral administration, blood levels were detected within 8 h. The maximum concentration reached after 0.25 h (2.92 nM); the area under the curve (AUC_{0-8h}) was 6.39 (nM·h); the plasma $t_{1/2}$ for **G1** was 5.55 h. The maximum concentration (3.77 nM) was reached 1.8 min after intravenous injection of compound **G1**; the area under the curve ($AUC_{0-8 h}$) was 6.13 nM·h; the plasma $t_{1/2}$ of **G1** was 5.72 h. **G1** had a moderate bioavailability of 20.85 % laying a good foundation for the next *in vivo* activity exploration.

Safety of compound G1.

To further evaluate whether compound **G1** has potential toxicological effects and adverse events, we conducted acute toxicity tests on mice. After seven consecutive days of oral administration of high dose of **G1** (200 mg / kg), the survival status and body weight of mice were examined and no adverse events were observed, indicating that compound **G1** was well tolerated and no toxicity was observed with continuous oral administration of high dose (200 mg / kg) (Figure 4).

Study of compound G1 against CFA-induced arthritis model in mice.

The mouse model of adjuvant-induced arthritis (AIA) induced by CFA has similar characteristics to human rheumatoid arthritis (RA) in terms of pathogenesis, joint pain, bone destruction and synovial hyperplasia, and is now widely used in the study of human RA pathogenesis and treatment mechanisms[41, 42]. To investigate if compound **G1** is efficacious in Kunming mice with AIA, mice were randomly divided into 3 groups: the model group (CFA), the **G1** group (10 mg / kg, *ip*), and the celecoxib group (10 mg / kg, *ip*). The health status of the animals was assessed by observing changes in their body weight during the experiment (Figure 5A), and the results revealed that no increase in body weight occurred in the mice of the three groups at 24 h of CFA administration, indicating that CFA-induced

RA caused discomfort in the mice, but an increase in body weight was found in the **G1** and **celecoxib** groups at 24 h of drug treatment while no weight gain was observed in the model group. Simultaneously, the pain valves of AIA mice were evaluated using the hot plate technique 10 minutes, 45 minutes, 120 minutes, and 240 minutes after injection (Figure 5B), and a pain valve enhancement rate curve was generated (Figure 5C). When compared to the basic pain valve, all three groups showed a decrease in pain threshold after 24 hours of modeling with CFA. Compound **G1** began to work after 10 minutes of administration, peaked at 2 hours, and remained unchanged for 4 hours. **Celecoxib** did not significantly raise the pain threshold at 2 h and did not have a significant effect until 4 h. Compound **G1** had a faster onset of analgesia than celecoxib and was more effective in terms of the pain threshold improvement rate curve. Finally, the thickness and breadth of mouse's paws were compared before and after modeling. Compound **G1** significantly reduced the swelling rate of mouse paw thickness and was superior to celecoxib, as shown in Figure 5D, and compound **G1** was also superior to celecoxib in terms of swelling rate of mouse paw width (Figure 5E). In conclusion, compound **G1** has significant analgesic and anti-inflammatory effects on CFA-induced AIA mice and is better than celecoxib.

Study of compound G1 against *L*-arginine-induced acute pancreatitis model in mice.

AP is a potentially life-threatening gastrointestinal disease whose incidence has been increasing over the past decades. There are no effective therapeutic drugs in clinic and drug for the treatment of AP are urgent. Given the association of sEH with AP pathogenesis, the sEH inhibitor **G1** at a dose of 3 mg / kg and 8 mg / kg was evaluated in the *L*-arginine-induced acute pancreatitis model in mice and compared with **celecoxib** (5 mg / kg). First, histological analysis of the pancreas was performed to determine whether **G1** treatment reduced the severity of *L*-arginine-induced pancreatitis. Pathological changes were studied on H&E-stained pancreatic sections (Figure 6A).

As expected, the *L*-arginine model group (6.25 ± 0.96) showed pancreatic injury representative of AP, including edema (Figure 6B), inflammatory cell infiltration (Figure 6C), and parenchymal atrophy (Figure 6D). In contrast, both compounds **G1** 8 mg / kg (3.50 ± 0.58) and **G1** 3 mg / kg (4.25 ± 1.5) improved *L*-arginine-induced pancreatic injury in AP. The effect of compound **G1** (8 mg / kg) was better than the effect of 3 mg / kg, and compound **G1** at the dose of 8 mg / kg (3.50 ± 0.58) exhibited more effective reversal of pancreatic injury, edema and neutrophil infiltration than equimolar concentrations of celecoxib 5 mg / kg (3.75 ± 0.96) (Figure 6E).

Study of compound G1 against LPS-induced sepsis model.

The potential therapeutic efficacy of compound **G1** against severe sepsis was investigated. Compound **G1** (5 mg / kg, *ip*) was administered four hours after the sepsis model was induced by a high dosage (30 mg / kg, *ip*) of LPS. As a positive control group, **dexamethasone** (5 mg/kg, *ip*) was also given. **G1** (5 mg / kg, *ip*) and **dexamethasone** (5 mg / kg, *ip*) were given for the second time 12 hours later. The number of mice that survived per hour was recorded, and the specific data are shown in Figure 7A. Because of the higher LPS dose, all of the mice in the dexamethasone group died after 28 hours. The mouse in compound **G1** had a 37.5 % survival rate at this point, and when the period was

extended to 35 hours, all of the mice in the solvent group died, while the animals in the **G1** group had a 12.5 % survival rate. The final results showed that compound **G1** outperformed dexamethasone in terms of extending the life of sick mice. Certain inflammatory related components such as sEH, COX-2, NOS-2, VCAM, IL-6, MCP-5, and TNF-α in blood were examined using Western blot or ELISA. At first, the expression of sEH and COX-2 were detected, and as expected, compound **G1** significantly reduced the expression of sEH compared to the model group, and the similar result was observed in the dexamethasone group. However, COX-2 expression levels in compound **G1** group and dexamethasone group were higher than in the control group. NOS-2 expression and excessive NO production are linked to endotoxic hypotension[43]. Compound **G1** reduced NOS-2 expression, implying that sEH inhibitors can inhibit NOS-2 induction, reducing NO production and endotoxin-induced hypotension. Compared to the solvent group, the LPS group and compound **G1** group had little change in the determination of vascular cell adhesion molecule (VCAM) (Figure 7B). IL-6 is important in the development of acute inflammatory responses, including endothelial and lymphocyte activation, as well as fever induction[44]. We detected a significant reduction in IL-6 levels in both compound **G1** and dexamethasone groups by ELISA, and our findings suggest that compound **G1** reduces IL-6 production, which is consistent with the antipyretic effect of EETs and the action of cyclooxygenase inhibitors[45, 46]. The chemokine MCP-5 is a potent monocyte chemoattractant[47]. Although the effect of compound **G1** lowering MCP-5 was lower than dexamethasone, it demonstrated that the lymphocytes of mice in the LPS group were inhibited but still responded in the presence of sEH inhibitor. TNF-α, initially produced by resident monocytes and macrophages, is considered an early innate inflammatory mediator. Among its many physiological effects is the induction of vascular cell adhesion molecule-1 on endothelial cells, a critical factor for the transmigration of leukocytes from vascular compartments into inflamed tissues. Compound **G1** and the **dexamethasone** considerably lowered TNF-α levels, inhibiting further inflammation and having a therapeutic effect. (Figure 7C).

CONCLUSIONS

As an important factor of controlling CYP metabolic pathway of arachidonic acid, sEH has been identified as a suitable target for several inflammatory diseases. In past decades, a lot of sEH inhibitors have been reported, three out of them, **AR9281**, **GSK2256294** and **EC5026**, have entered clinical trials. In this work, based on a rational structure-based drug design, the picomolar level compound **G1** against sEH (IC$_{50}$: HsEH = 0.05 nM, MsEH = 0.14 nM) was obtained by introducing the hydrophilic group homopiperazine and hydrophobic fragment propionyl of **TPPU** onto the structure of lead compound **A**. Compound **G1** showed good microsomal stability (Human t$_{1/2}$ = 3.70 h, Rat t$_{1/2}$ = 3.15 h), and has moderate plasma protein binding rate(72.57 %) and good oral bioavailability in rats (F % = 20.85 %). All the results pave the foundation for the assessment of *in vivo* efficacy. In a mouse model of arthritis (AIA) induced by Freund's complete adjuvant (CFA), compound **G1** significantly increased the pain valve (70.10 %), reduced foot swelling (thickness: 21.4 %, width: 6.98 %) and outperformed celecoxib (thickness: 17.99 %, width: 6.4 %). Compound **G1** of 8 mg / kg dose ameliorated *L*-arginine-induced acute pancreatic injury (score: 3.50 ± 0.58 vs 6.25 ± 0.96) and reversed pancreatic injury, edema

and neutrophil infiltration. Compound **G1** increased the survival time of animals in the LPS-induced sepsis model and outperformed dexamethasone, while the results of Western blot or ELISA showed that the expression and levels of relevant inflammatory factors were decreased to different degrees. In preliminary safety evaluation experiment, compound **G1** was administered orally at a high dose of 200 mg / kg for seven consecutive days, no adverse events were observed. It showed that compound **G1** has high safety. These results suggests that compound **G1** is a drug candidate worthy of further evaluation for the treatment of inflammation-induced diseases such as arthritis, acute pancreatitis and sepsis.

EXPERIMENTAL SECTION

Chemistry.

All reactions were carried out with magnetic stirring and in dried glassware. The reactions were monitored by thin-layer chromatography (TLC: HG/T2354–92, GF254), and compounds were visualized on TLC with UV light. All chemicals and solvents were purchased from commercial sources and used without purification treatment. Analytical thin-layer chromatography was carried out on 0.20 mm silica gel plates (Haiyang, Qingdao, Shandong, China) with the QF-254 UV indicator. Column chromatography was conducted using Haiyang silica gel 60 (300–400 mesh). Melting points were determined with an X-4 apparatus and were uncorrected. The nuclear magnetic resonance (NMR) spectra were recorded on a Bruker 400 MHz spectrometer in CDCl3 or DMSO-d_6 using tetramethylsilane (TMS) as an internal standard. Peak multiplicity of NMR signals were as follows: s, singlet; brs, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Chemical shift (δ): ppm relative to Me_4Si (internal standard). Coupling constant: J (Hz). High-resolution mass spectra (HRMS) of all target compounds were performed by a Waters Q-TOF Premier spectrometer with acetonitrile and water as solvents. Electrospray ionization mass spectrometry (ESI-MS) analyses were recorded in an Agilent 1100 Series MSD Trap SL (Santa Clara, CA, USA). All final compounds are >95% pure by HPLC analysis.

Methyl 3-fluoro-4-nitrobenzoate(1).—The 3-fluoro-4-nitrobenzoic acid (3.0 g, 16.2 mmol) was dissolved in MeOH (20 mL), and 98% sulfuric acid (0.86 mL, 16.2 mmol) was added. The mixture was heated to reflux 12 h. The reaction solution was cooled to room temperature and concentrated *in vacuum*. The resulting crude residue was dissolved in a sat. $NaHCO_3$(aq) solution and extracted with DCM (20 mL × 2). The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated to afford the **1** (3.06 g, 95%) as a colorless oily substance, **^{1}H NMR** (400 MHz, CDCl$_3$): δ (ppm) 8.13–8.09 (m, 1H), 7.98–7.94 (m, 2H), 3.99 (s, 3H).

Methyl 4-amino-3-fluorobenzoate (2).—To a solution of methyl 3-fluoro-4-nitrobenzoate (3.06 g, 15.40 mmol) in anhydrous EtOH (100 mL) was added 5 % Pd/C (10% w/w). The reaction mixture was stirred with H_2 at 50 °C for 12 h. After the reaction mixture was cooled to rt, the reaction mixture was filtered through a celite pad and concentrated *in vacuum* to give a white solid **2** (2.34 g, 90%), **ESI-MS**: m/z 170.04 [M+H]$^+$.

Methyl 4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzoate (3).

—A solution of memantine (2.98 g, 16.60 mmol) and Et₃N (5.60 g, 55.36 mol) in DCM (30 mL) was added dropwise over 1 h to a thick slurry of triphosgene (2.46 g, 8.3 mmol) in DCM (30 mL) in a cold trap while maintaining the temperature below −65 °C. The cold bath was removed, and the mixture was warmed to rt and stirred for 30 min. The reaction was cooled to −78 °C, and a slurry of methyl 4-amino-3-fluorobenzoate (2.34 g, 13.84 mmol) and Et₃N (5.60 g, 55.36 mol) in DCM (30 mL) was added in small portions over 0.5 h while maintaining the temperature below −65 °C. The reaction was warmed to rt and stirred overnight. The reaction mixture was washed with 10% aq HCl (4 × 50 mL). The organic layer was dried over MgSO₄ and concentrated under reduced pressure to give a crude compound **3** as a viscous yellow oil (4.14 g) which was used in the next step without further purification, **¹H NMR** (400 MHz, CDCl₃): δ (ppm) 8.26 (t, *J*=8.32 Hz, 1H), 7.76 (d, *J*=8.72 Hz, 1H), 7.69–7.66 (m, 1H), 6.84 (s, 1H), 4.93 (s, 1H), 3.88 (s, 3H), 2.16 (s, 1H), 1.85 (s, 2H), 1.66 s, 3H), 1.40–1.29 (m, 4H), 1.16 (s, 2H), 0.85 (s, 6H).

4-(3-((1r,3R,5S,7r)-3,5-Dimethyladamantan-1-yl)ureido)-3-fluorobenzoic acid (4).

—To a solution of methyl 4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzoate **3** (4.14 g, 11.06 mmol), EtOH (30 mL) and water (5 mL) were added NaOH (2.2 g, 55.3 mmol). The resulting mixture was heated to 70 °C for 0.5h. After the reaction was completed, the mixture was cooled to the rt, and the solvent was evaporated *in vacuum*. The residues were poured into water (40 mL), and adjusted pH to 1 using concentrated hydrochloric acid at 0 °C, which was extracted with EtOAc (30 mL × 2). Then, the combined organic layers were respectively washed with water (30 mL × 2) and brine (40 mL). The organic layer was separated, dried with anhydrous MgSO₄, filtered, and concentrated in vacuum produce light yellow oil (3.39 g), which was used without further purification, **ESI-MS**: m/z 361.2 [M+H]⁺.

Methyl-1-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzoyl)piperidine-4-carboxylate (5).

—In a round-bottom flask, the intermediate obtained from the previous step (3.39 g, 6.99 mmol) was dissolved in anhydrous DCM (20 mL). Methyl piperidine-4-carboxylate (1.2 g, 8.39 mmol), HATU (3.19 g, 8.39 mmol), and DIPEA (2.17 g, 16.78 mmol) were added sequentially. The reaction mixture was stirred at room temperature for 2 h. After the reaction was completed, the residues were poured into water (10 mL), which was extracted with DCM (20 mL × 2). Then, the combined organic layers were respectively washed 10% aq HCl (20 mL × 2), 10% aq Na₂CO₃ (20 mL × 2) and brine (20 mL). The organic layer was separated, dried with anhydrous MgSO₄, filtered, and concentrated *in vacuum* produce light yellow oil (2.71 g), which was used without further purification, **¹H NMR** (400 MHz, CDCl₃): δ (ppm) 8.14 (t, *J* = 8.12 Hz, 1H), 7.28 (d, *J* = 2.92 Hz, 1H), 7.07–7.03 (m, 2H), 5.59 (s, 1H), 4.15 (q, *J* = 7.12 Hz), 3.06 (t, 2H, *J* = 10.44 Hz), 2.61–2.54 (m, 1H), 2.13–2.11 (m, 1H), 1.94 (s, 2H), 1.81–1.80 (m, 2H), 1.72 (s, 2H), 1.64–1.57 (m, 4H), 1.37–1.34 (m, 2H), 1.28–1.24 (m, 6H), 1.17–1.10 (m, 2H), 0.83 (s, 6H)..

1-(4-(3-((1r,3R,5S,7r)-3,5-Dimethyladamantan-1-yl)ureido)-3-fluorobenzoyl)piperidine-4-carboxylic acid (6).

—According

to the synthetic process of compound **4**, compound **6**

as a light yellow oil (0.40 g, 29%) was obtained by taking methyl1-(4-(3-((1r,3R,5S,7r)-3,5-dimethyl adamantan-1-yl)ureido)-3-fluorobenzoyl)piperidine-4-carboxylate **5** (2.71 g, 5.58 mmol) as starting material, compound **6**, **ESI-MS**: m/z 472.26 [M+H]+, 494.24 [M+Na]+.

1-(4-(3-((1r,3R,5S,7r)-3,5-Dimethyladamantan-1-yl)ureido)-3-fluorobenzoyl)-N,N-diethylpiperidine-4-carboxamide (A1).—According to the synthetic process

of compound **5**, compound **A1** as a white solid (0.20 g, 60%)

was obtained by taking 1-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzoyl)piperidine-4-carboxylic acid **6** (0.3 g, 0.64 mmol) and diethylamine (55 mg, 0.76 mmol) as starting material, compound **A1**, **m.p.** 113–

114°C. **^{1}H NMR** (400 MHz, CDCl$_3$): δ (ppm) 8.15 (t, *J* = 8.5 Hz, 1H), 7.33 (brs, 1H), 7.07–7.05 (m, 2H), 5.65 (brs, 1H), 4.61 (brs, 1H), 3.93 (brs, 1H), 3.41–3.33 (m, 4H), 3.03–2.95 (m, 2H), 2.75–2.69 (m, 1H), 2.14–2.13 (m, 1H), 1.82 (s, 2H), 1.72 (brs, 3H), 1.64 (s, 5H), 1.39–1.36 (m, 2H), 1.29–1.23 (m, 6H), 1.16–1.09 (m, 5H), 0.82 (s, 6H). **^{13}C NMR** (100 MHz, CDCl$_3$): δ (ppm) 173.03, 169.72, 154.14, 130.26, 130.17, 127.87, 123.27, 120.48, 113.85, 52.82, 50.04, 47.98, 42.72, 41.98, 40.56, 40.47, 38.49, 32.39, 30.17, 30.13, 28.82, 15.06, 13.10. **HRMS** (ESI) calcd for C$_{30}$H$_{44}$FN$_4$O$_3$ [M+H]+: 527.3319, found 527.3405.

1-(4-(3-((1r,3R,5S,7r)-3,5-Dimethyladamantan-1-yl)ureido)-3-fluorobenzoyl)-N-(2-hydroxyethyl)piperidine-4-carboxamide (A2).—According to the synthetic

process of compound **5**, compound **A2** as a white solid (0.21 g,

65%) was obtained by taking 1-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzoyl)piperidine-4-carboxylic acid **6** (0.3 g, 0.64 mmol) and and ethanolamine (46 mg, 0.76 mmol) as starting material, compound **A2**,

m.p. 125–126°C. **^{1}H NMR** (400 MHz, DMSO-*d$_6$*): δ (ppm) 8.30 (s, 1H), 8.20 (t, *J* = 8.32 Hz, 1H), 7.82 (t, *J* = 5.20 Hz, 1H), 7.20 (d, *J* = 11.6 Hz, 1H), 7.09 (d, *J* = 8.44 Hz, 1H), 6.58 (s, 1H), 4.65 (t, *J* = 5.48 Hz, 1H), 4.33 (s, 1H), 3.72 (s, 1H), 3.40–3.37 (m, 1H), 3.10 (q, *J* = 5.84 Hz, 2H), 2.91 (s, 2H), 2.42–2.37 (m, 1H), 2.09 (s, 1H), 1.76 (s, 2H), 1.68 (s, 2H), 1.58 (s, 4H), 1.50–1.48 (m, 2H), 1.35–1.32 (m, 2H), 1.27–1.24 (m, 2H), 1.12 (s, 2H), 0.83 s, 6H). **^{13}C NMR** (100 MHz, DMSO-*d$_6$*): δ 174.32, 168.27, 153.81, 149.77, 123.80, 119.30, 114.38, 114.17, 60.33, 60.33, 52.13, 52.13, 50.71, 47.99, 47.99, 42.76, 42.76, 42.19, 41.85, 32.38, 32.38, 32.38, 32.38, 30.52,30.52, 30.52, 30.03, 30.03, 29.04. **HRMS** (ESI) calcd for C$_{28}$H$_{40}$FN$_4$O$_4$ [M+H]+: 515.2955, found 515.3042.

1-(4-(3-((1r,3R,5S,7r)-3,5-Dimethyladamantan-1-yl)ureido)-3-fluorobenzoyl)-N-(2-hydroxypropyl)piperidine-4-carboxamide (A3).—According to the synthetic

process of compound **5**, compound **A3** as a white solid (0.21 g,

63%) was obtained by taking 1-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzoyl)piperidine-4-carboxylic acid **6** (0.3 g, 0.64 mmol) and and *iso*-propanolamine (57 mg, 0.76 mmol) as starting material, compound **A3**, **m.p.** 115–116°C. **^{1}H NMR** (400 MHz, DMSO-*d$_6$*): δ (ppm) 8.30 (s, 1H), 8.19 (t, *J* = 8.36 Hz, 1H), 7.63 (d, *J* = 7.76 Hz, 1H), 7.20 (dd, *J* = 11.68, *J* = 1.68 Hz), 7.09 (d, 1H, *J* = 8.44 Hz), 6.58 (s, 1H), 4.52 (s, 1H), 3.43 (s, 1H), 2.89 (s, 2H), 2.36–2.30 (m, 1H), 2.09 (s, 1H), 1.78–1.76 (m, 2H), 1.72–1.71 (m, 2H), 1.58 (s, 4H), 1.49–1.46 (m, 2H), 1.35–1.32 (m, 2H), 1.27–1.32 (m, 4H), 1.17–1.15 (m,

4H), 1.21 (s, 3H), 0.83 (s, 6H). **¹³C NMR** (100 MHz, DMSO-d_6): δ 173.42, 168.28, 153.81, 152.18, 149.77, 123.81, 119.30, 114.38, 114.17, 68.63, 52.13, 50.71, 47.99, 47.99, 47.42, 42.76, 42.76, 42.24, 42.24, 34.40, 34.40, 32.38, 32.38, 32.38, 30.73, 30.52, 30.52, 30.52, 30.04, 29.00. **HRMS** (ESI) calcd for $C_{29}H_{42}FN_4O_4$ [M+H]⁺: 529.3112, found 529.3196.

1-((1r,3R,5S,7r)-3,5-Dimethyladamantan-1-yl)-3-(2-fluoro-4-(4-(4-(2-hydroxyethyl)piperazine-1-carbonyl)piperidine-1-carbonyl)phenyl)urea (A4).

—According to the synthetic process of compound **5**, compound **A4** as a white solid (0.22 g, 60%) was obtained by taking 1-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzoyl)piperidine-4-carboxylic acid **6** (0.3 g, 0.64 mmol) and and 2-(piperazin-1-yl)ethan-1-ol (98 mg, 0.76 mmol) as starting material, compound **A4**, **m.p.** 128–129°C. **¹H NMR** (400 MHz, DMSO-d_6): δ (ppm) 8.29 (s, 1H), 8.19 (t, J = 8.24 Hz, 1H), 7.20 (d, J = 11.72 Hz, 1H), 7.08 (d, J = 8.32 Hz, 1H), 6.58 (s, 1H), 4.44 (s, 1H), 3.50–3.49 (m, 4H), 3.43 (s, 2H), 2.92 (s, 2H), 2.40–2.37 (m, 4H), 2.33 (s, 2H), 2.09 (s, 1H), 1.76 (s, 2H), 1.58 (s, 6H), 1.49–1.46 (m, 3H), 1.35–1.32 (m, 2H), 1.27–1.24 (m, 3H), 1.12 (s, 2H), 1.05 (s, 2H), 0.83 (s, 6H). **¹³C NMR** (100 MHz, DMSO-d_6): δ 172.43, 168.26, 153.82, 152.19, 149.78, 123.82, 119.28, 114.42, 114.21, 60.54, 58.93, 58.93, 54.20, 53.43, 52.13, 50.71, 47.99, 47.99, 46.15, 45.28, 42.76, 42.76, 41.60, 37.41, 32.39, 32.39, 32.39, 30.52, 30.52, 30.52, 30.03, 28.88. **HRMS** (ESI) calcd for $C_{32}H_{47}FN_5O_4$ [M+H]⁺: 584.3534, found 584.3618.

1-(4-(3-((1r,3R,5S,7r)-3,5-Dimethyladamantan-1-yl)ureido)-3-fluorobenzoyl)-N-(1-hydroxypropan-2-yl)piperidine-4-carboxamide (A5).

—According to the synthetic process of compound **5**, compound **A5** as a white solid (0.18 g, 54 %) was obtained by taking 1-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzoyl) piperidine-4-carboxylic acid **6** (0.3 g, 0.64 mmol) and and 2-aminopropan-1-ol (57 mg, 0.76 mmol) as starting material, compound **A5**, **m.p.** 116–117°C. **¹H NMR** (400 MHz, DMSO-d_6): δ (ppm) 8.36 (s, 1H), 8.20 (t, J = 8.32 Hz, 1H), 7.63 (d, J = 8.00 Hz, 1H), 7.21 (d, J = 11.68 Hz, 1H), 7.09 (d, J = 8.68 Hz, 1H), 6.67 (s, 1H), 4.62 (t, J = 5.44 Hz, 2H), 3.71–3.66 (m, 1H), 3.40–3.39 (m, 2H), 3.08–3.06 (m, 4H), 2.10 (s, 1H), 1.76 (s, 1H), 1.68 (s, 1H), 1.59 (s, 3H), 1.50–1.48 (m, 2H), 1.41–1.35 (m, 1H), 1.32 (s, 1H), 1.28–1.24 (m, 3H), 1.21–1.18 (m, 5H), 1.12 (s, 2H), 0.83 (s, 6H). **¹³C NMR** (100 MHz, DMSO-d_6): δ 174.24, 168.29, 153.86, 152.19, 149.79, 123.80, 119.35, 114.38, 114.17, 60.59, 60.59, 53.16, 52.13, 50.72, 47.99, 47.99, 45.87, 45.87, 42.77, 42.19, 32.38, 32.38, 32.38, 30.53, 30.53, 30.53, 30.03, 29.03, 8.94. **HRMS** (ESI) calcd for $C_{29}H_{42}FN_4O_5$ [M+H]⁺: 545.3061, found 545.3148.

Ethyl-1-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzoyl) piperidine-3-carboxylate (7).

—According to the synthetic process of compound **5**, compound **7** as a light yellow oil (3.33 g) was obtained by taking 4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzoic acid **4** (3 g, 8.33 mmol) and ethyl piperidine-3-carboxylate (1.57g, 10.0 mmol) as starting material, compound **7**, **ESI-MS**: m/z 500.4 [M+H]⁺, 522.4 [M+Na]⁺.

1-(4-(3-((1r,3R,5S,7r)-3,5-Dimethyladamantan-1-yl)ureido)-3-fluorobenzoyl)piperidine-3-carboxylic acid (A).

—According

to the synthetic process of compound **4**, compound

A as a white solid (2.82 g, 90%) was obtained by taking ethyl 1-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzoyl)piperidine-3-carboxylate **7** (3.33 g, 6.66 mmol) as starting material, compound

A, **m.p.** 154–155 °C. **¹H NMR** (400 MHz, CDCl₃): δ (ppm) 8.11 (t, J = 7.76 Hz, 1H), 7.72–7.63 (m, 1H), 7.03–7.00 (m, 2H), 4.48 (s, 1H), 3.93–3.69 (m, 2H), 3.39–3.33 (m, 2H), 2.50 (s, 1H), 2.12–2.03 (m, 2H), 1.83 (s, 3H), 1.67–1.59 (m, 4H), 1.37–1.26 (m, 4H), 1.17–1.10 (m, 2H), 0.83 (s, 6H). **¹³C NMR** (100 MHz, DMSO-d_6): δ 182.7, 167.8, 167.5, 156.9, 144.4, 130.3, 130.2, 129.8, 127.9, 52.3, 51.5, 50.8, 48.6, 47.3, 46.9, 42.9, 40.9, 32.9, 32.3, 31.9, 30.6, 30.1. **HRMS** (ESI) calcd for $C_{26}H_{35}FN_3O_4$ [M+H]⁺: 472.2533, found 472.2615.

1-(4-(3-((1r,3R,5S,7S)-3,5-Dimethyladamantan-1-yl)ureido)-3-fluorobenzoyl)-N,N-diethylpiperidine-3-carboxamide (B1).—

According to the synthetic process of compound **5**, compound **B1** as a white solid (0.19 g, 58%) was obtained by taking 1-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzoyl)piperidine-3-carboxylic acid **A** (0.3 g, 0.64 mmol) and diethylamine (55 mg, 0.76 mmol) as starting material, compound **B1**, **m.p.** 137–138°C.
¹H NMR (400 MHz, CDCl₃): δ (ppm) 8.20 (t, J = 8.0 Hz, 1H), 7.57–7.54 (m, 1H), 7.07–6.99 (m, 2H), 5.63 (brs, 1H), 4.63 (brs, 1H), 3.81 (s, 1H), 3.48–3.35 (m, 4H), 3.18–2.99 (m, 2H), 2.73–2.56 (m, 1H), 2.13 (s, 1H), 1.91 (s, 2H), 1.84 (s, 3H), 1.63 (s, 4H), 1.38–1.35 (m, 2H), 1.29–1.24 (m, 4H), 1.18–1.10 (m, 7H), 0.83 (s, 6H).
¹³C NMR (100 MHz, CDCl₃): δ (ppm) 172.26, 169.89, 154.13, 136.38, 130.30, 127.80, 123.48, 120.02, 113.97, 52.72, 50.62, 48.01, 42.72, 42.02, 40.61, 40.38, 32.37, 30.13, 14.95, 13.06. **HRMS** (ESI) calcd for $C_{30}H_{44}FN_4O_3$ [M+H]⁺: 527.3319, found 527.3404.

1-(4-(3-((1r,3R,5S,7S)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzoyl)-N-(2-hydroxyethyl)piperidine-3-carboxamide (B2).—

According to the synthetic process of compound **5**, compound **B2** as a white solid (0.20 g, 61.90%) was obtained by taking 1-(4-(3-((1r,3R,5S,7r)-3,5-Dimethyladamantan-1-yl)ureido)-3-fluorobenzoyl) piperidine-3-carboxylic acid **A** (0.3 g, 0.64 mmol) and ethanolamine (46 mg, 0.76 mmol) as starting material, compound **B2**, **m.p.** 128–129°C.
¹H NMR (400 MHz, DMSO-d_6): δ (ppm) 8.30 (s, 1H), 8.20 (t, J = 8.36 Hz, 1H), 7.91 (s, 1H), 7.20 (dd, J = 11.68 Hz, J = 1.56 Hz,), 7.08 (d, J = 8.44 Hz, 1H), 6.58 (s, 1H), 4.66 (s, 1H), 4.34 (s, 1H), 3.62 (s, 1H), 3.36 (s, 2H), 3.09 (s, 2H), 2.89 (s, 2H), 2.36–2.30 (m, 1H), 2.09 (s, 1H), 1.87–1.84 (m, 1H), 1.76 (s, 2H), 1.62–1.55 (m, 5H), 1,35–1.32 (m, 3H), 1.27–1.24 (m, 3H), 1.12 (s, 2H), 0.83 (s, 6H). **¹³C NMR** (100 MHz, DMSO-d_6): δ173.03, 168.33, 153.81, 152.15, 149.75, 123.84, 119.24, 114.44, 114.24, 60.25, 60.25, 52.14, 50.71, 47.99, 47.99, 42.76, 42.76, 41.81, 32.39, 32.39, 32.39, 30.52, 30.52,30.52, 30.04, 30.04, 28.19. **HRMS** (ESI) calcd for $C_{28}H_{40}FN_4O_4$ [M+H]⁺: 515.2955, found 515.3041.

1-(4-(3-((1r,3R,5S,7S)-3,5-Dimethyladamantan-1-yl)ureido)-3-fluorobenzoyl)-N-(2-hydroxypropyl)piperidine-3-carboxamide (B3).—

According to the synthetic process of compound **5**, compound **B3** as a white solid (0.22 g, 66%) was obtained by taking 1-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzoyl)piperidine-3-carboxylic acid **A** (0.3 g, 0.64 mmol) and *iso*-propanolamine

(57 mg, 0.76 mmol) as starting material, compound **B3, m.p.**141–142 °C.
1H NMR (400 MHz, CDCl$_3$): δ (ppm) 8.20 (s, 1H), 7.61 (s, 1H),
7.05–7.03 (m, 2H), 5.85 (br, 1H), 4.05 (br, 1H), 3.86 (brs, 1H), 3.55 (brs, 4H), 3.38–
3.35 (m, 1H), 3.29–3.20 (m, 1H), 3.2–2.95 (m, 1H), 2.81 (brs, 1H), 2.48 (brs, 1H), 2.13 (brs, 1H), 2.06–2.02 (m, 1H), 1.88–1.84 (m, 1H), 1.81 (s, 2H), 1.62 (s, 5H), 1.44–1.43 (m, 1H), 137–1.34 (m, 2H), 1.30–1.24 (m, 3H), 1.44–1.11 (m, 5H), 0.84 (m, 6H). **13C NMR** (100 MHz, CDCl$_3$): δ (ppm) 173.03, 169.72, 154.14, 130.26, 130.17, 127.87, 123.27, 120.48, 113.85, 52.82, 50.04, 47.98, 42.72, 41.98, 40.56, 40.47, 38.49, 32.39, 30.17, 30.13, 28.82, 15.06, 13.10. **HRMS** (ESI) calcd for C$_{29}$H$_{42}$FN$_4$O$_4$ [M+H]$^+$: 529.3112, found 529.3198.

1-((1r,3R,5S,7r)-3,5-Dimethyladamantan-1-yl)-3-(2-fluoro-4-(3-(4-(2-hydroxyethyl) piperazine-1-carbonyl)piperidine-1-carbonyl)phenyl)urea (B4).

—According to the synthetic process of compound **5**, compound
B4 as a white solid (0.22 g, 54.5%) was obtained by taking 1-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzoyl)piperidine-3-carboxylic acid **A**
(0.3 g, 0.64 mmol) and 2-(piperazin-1-yl)ethan-1-ol (98 mg, 0.76 mmol) as starting material,
compound **B4, m.p.** 141–145°C. **1H NMR** (400 MHz, DMSO-d_6): δ (ppm) 8.30 (s, 1H),
8.20 (t, J = 8.36 Hz, 1H), 7.24 (d, J=11.80 Hz, 1H), 7.11 (d, J= 8.52 Hz, 1H), 6.58 (s, 1H),
4.43 (s, 1H), 4.32 (s, 1H), 3.49 (s, 6H), 3.05 (s, 1H), 2.79 (s, 2H), 2.38 (s, 5H), 2.09 (s, 1H),
1.82–1.76 (m, 3H), 1.58 (s, 8H), 1.35–1.32 (m, 2H), 1.27–1.24 (m, 3H), 1.18–1.12 (m, 3H),
0.83 (s, 6H). **13C NMR** (100 MHz, DMSO-d_6): δ 168.44, 153.81, 152.16, 149.76, 123.75,
119.34, 114.31, 114.11, 60.44, 58.79, 54.04, 53.29, 52.13, 52.13, 50.71, 47.98, 47.98,
45.19, 42.76, 42.76, 41.39, 38.29, 32.38, 32.38, 32.38, 32.38, 30.52, 30.52, 30.52, 30.04,
30.04, 27.98. **HRMS** (ESI) calcd for C$_{32}$H$_{47}$FN$_5$O$_4$ [M+H]$^+$: 584.3534, found 584.3619.

Methyl-4-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzamido)cyclohexane-1-carboxylate (8).

—According to
the synthetic process of compound **5**, compound
8 as a light yellow oil (3.54 g) was obtained by taking 4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzoic acid **4** (3 g, 8.33 mmol)
and methyl 4-aminocyclohexane-1-carboxylate (1.57g, 10.0 mmol) as starting material,
compound **8**, **1H NMR** (400 MHz, DMSO-d_6): δ (ppm) 8.35 (s, 1H), 8.23 (t, J = 8.52
Hz, 1H), 8.11 (d, J = 7.76 Hz, 1H), 7.66–7.59 (m, 2H), 6.60 (s, 1H), 3.76–3.68 (m, 1H), 3.61
(s, 3H), 2.11–2.10 (m, 1H), 1.97–1.94 (m, 2H), 1.89–1.86 (m, 2H), 1.76 (s, 2H), 1.59 (s,
4H), 1.45–1.41 (m, 2H), 1.36–1.33 (m, 3H), 1.28–1.24 (m, 4H), 1.13 (s, 2H), 0.84 (s, 6H).

4-(4-(3-((1r,3R,5S,7r)-3,5-Dimethyladamantan-1-yl)ureido)-3-fluorobenzamido) cyclohexane-1-carboxylic acid (9).

—According to the synthetic process
of compound **4**, compound **9** as a light yellow oil (3.10 g) was
obtained by taking methyl-4-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzamido)cyclohexane-1-carboxylate **8** (3.54 g, 7.09 mmol) as starting material,
compound **9**, which was used without further purification, **1H NMR** (400 MHz, DMSO-d_6):
δ (ppm) 8.35 (s, 1H), 8.23 (t, J = 8.52 Hz, 1H), 8.11 (d, J = 7.76 Hz, 1H), 7.67–7.59 (m,
2H), 6.60 (s, 1H), 3.76–3.68 (m, 1H), 3.61 (s, 3H), 2.16–2.07 (m, 1H), 1.96–1.94 (m, 2H),

1.88–1.86 (m, 2H), 1.76 (s, 2H), 1.59 (s, 4H), 1.41–1.33 (m, 5H), 1.28–1.24 (m, 4H), 1.12 (s, 2H), 0.83 (s, 6H).

Tert-butyl-4-(2-(4-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzamido)cyclohexane-1-carboxamido)ethyl)piperazine-1-carboxylate (C1).

—According to the synthetic process of compound **5**, compound **C1** as a white solid (0.26 g, 60.0%) was obtained by taking 4-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzamido)cyclohexane-1-carboxylic acid **9** (0.3 g, 0.64 mmol) and *tert*-butyl 4-(2-aminoethyl)piperazine-1-carboxylate (0.17 g, 0.76 mmol) as starting material, compound **C1**, **m.p.** 131–132°C. ^{1}**H NMR** (400 MHz, DMSO-d_6) δ 8.36 (s, 1H), 8.23 (t, J = 8.52 Hz, 1H), 8.09 (d, J = 7.88 Hz, 1H), 7.68–7.59 (m, 3H), 6.62 (s, 1H), 3.75–3.66 (m, 1H), 3.29 (s, 3H), 3.16 (s, 2H), 2.33 (s, 5H), 2.10–2.06 (m, 2H), 1.87–1.85 (m, 2H), 1.76 (s, 4H), 1.59 (s, 4H), 1.47–1.45 (m, 1H), 1.39 (s, 10H), 1.34–1.32 (m, 4H), 1.28 (s, 2H), 1.25–1.23 (m, 2H), 1.12 (s, 2H), 0.83 (s, 6H). ^{13}**C NMR** (100 MHz, DMSO-d_6): δ 175.21, 164.27, 154.25, 153.69, 152.09, 149.70, 124.04, 118.47, 114.22, 114.01, 79.21, 57.44, 52.91, 52.16, 52.16, 50.70, 48.38, 47.98, 47.98, 46.12, 43.72, 43.72, 42.76, 42.76, 36.45, 32.39, 32.39, 31.98, 31.98, 30.51, 30.51, 30.04, 30.04, 28.83, 28.83, 28.52, 28.52, 28.52. **HRMS** (ESI) calcd for $C_{38}H_{58}FN_6O_5$ [M+H]$^+$: 697.4374, found 697.4460.

4-(3-((1r,3R,5S,7r)-3,5-Dimethyladamantan-1-yl)ureido)-3-fluoro-N-(4-(4-(2-hydroxyethyl)piperidine-1-carbonyl)cyclohexyl)benzamide (C2).

—According to the synthetic process of compound **5**, compound **C2** as a white solid (0.22 g, 58 %) was obtained by taking 4-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzamido)cyclohexane-1-carboxylic acid **9** (0.3 g, 0.64 mmol) and 2-(piperidin-4-yl)ethan-1-ol (98 mg, 0.76 mmol) as starting material, compound **C2**, **m.p.** 126–127 °C. ^{1}**H NMR** (400 MHz, DMSO-d_6) δ 8.35 (s, 1H), 8.23 (t, J = 8.4 Hz, 1H), 8.13 (d, J = 7.56 Hz, 1H), 7.66–7.60 (m, 2H), 6.61 (s, 1H), 4.44 (s, 1H), 3.71 (s, 1H), 3.53–3.44 (m, 6H), 2.42–2.41 (m, 4H), 2.35 (s, 2H), 2.10 (s, 1H), 1.87–1.85 (m, 2H), 1.76–1.69 (m, 4H), 1.59 (s, 4H), 1.46 (s, 1H), 1.43 (s, 1H), 1.40 (s, 1H), 1.35 (s, 1H), 1.32 (s, 2H), 1.28–1.23 (m, 4H), 1.12 (s, 2H), 0.83 (s, 6H). ^{13}**C NMR** (100 MHz, DMSO-d_6): δ 173.30, 164.38, 153.69, 152.07, 149.68, 124.15, 118.46, 114.26, 114.05, 60.54, 58.90, 54.26, 53.45, 52.16, 52.16, 50.70, 48.70, 47.98, 47.98, 45.29, 42.75, 42.75, 41.50, 38.71, 32.39, 32.39, 31.83, 31.83, 30.51, 30.51, 30.39, 30.39, 28.68. **HRMS** (ESI) calcd for $C_{34}H_{50}FN_4O_4$ [M+H]$^+$: 597.3738, found 597.3775.

4-(3-((1r,3R,5S,7r)-3,5-Dimethyladamantan-1-yl)ureido)-3-fluoro-N-(4-((2-hydroxyethyl)carbamoyl)cyclohexyl)benzamide (C3).

—According to the synthetic process of compound **5**, compound **C3** as a white solid (0.20 g, 61 %) was obtained by taking 4-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzamido) cyclohexane-1-carboxylic acid **9** (0.3 g, 0.64 mmol) and ethanolamine (46 mg, 0.76 mmol) as starting material, compound **C3**, **m.p.** 117–118 °C. ^{1}**H NMR** (400 MHz, DMSO-d_6) δ 8.38 (s, 1H), 8.23 (t, J = 8.40 Hz, 1H), 8.09 (d, J = 7.8 Hz, 1H), 7.73 (t, J = 5.32 Hz, 1H), 7.66–7.59 (m, 2H), 6.64 (s, 1H), 4.64 (s, 1H), 3.71–3.69 (m, 1H), 3.24 (s, 1H), 3.12–3.08 (m, 2H), 3.01–3.00 (m, 1H), 2.09 (s, 2H), 1.87–1.84 (m, 2H), 1.76 (s, 4H), 1.59 (s, 4H), 1.47–1.41 (m, 2H), 1.37–1.32 (m, 4H), 1.28–1.25

(m, 2H), 1.12 (s, 2H), 0.83 (s, 6H). **^{13}C NMR** (100 MHz, DMSO-d_6): δ 175.40, 164.27, 153.71, 152.09, 149.70, 124.07, 118.50, 114.21, 114.01, 60.44, 60.44, 52.16, 52.16, 50.70, 48.36, 47.98, 47.98, 46.01, 43.70, 42.75, 41.80, 32.39, 32.39, 32.00, 30.51, 30.51, 30.04, 28.86, 28.86. **HRMS** (ESI) calcd for $C_{29}H_{42}FN_4O_4$ [M+H]$^+$: 529.3112, found 529.3198.

4-(3-((1r,3R,5S,7r)-3,5-Dimethyladamantan-1-yl)ureido)-3-fluoro-N-(4-((2-hydroxypropyl)carbamoyl)cyclohexyl)benzamide (C4).—According to the

synthetic process of compound **5**, compound **C4** as a white
solid (0.20 g, 59 %) was obtained by taking 4-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzamido) cyclohexane-1-carboxylic acid
9 (0.3 g, 0.64 mmol) and *iso*-propanolamine (57 mg, 0.76 mmol)
as starting material, compound **C4**, **m.p.** 123–124 °C. **^{1}H NMR** (400 MHz, DMSO-d_6)
δ 8.35 (d, J = 2.96 Hz, 1H), 8.23 (t, J = 8.52 Hz, 1H), 8.09 (d, J = 7.92 Hz, 1H), 7.69–7.59 (m, 3H), 6.60 (s, 1H), 4.63 (s, 1H), 3.74–3.67 (m, 1H), 3.64–3.59 (m, 1H), 2.98 (t, J = 5.88 Hz, 2H), 2.13–2.09 (m, 2H), 1.87–1.84 (m, 2H), 1.76 (s, 3H), 1.59 (s, 3H), 1.48–1.42 (m, 2H), 1.35–1.34 (m, 1H), 1.32 (s, 2H), 1.28 (s, 2H), 1.23 (s, 2H), 1.17 (s, 1H), 1.12 (s, 2H), 1.00 (d, J = 6.2 Hz, 3H), 0.83 (s, 6H). **^{13}C NMR** (100 MHz, DMSO-d_6): δ 175.40, 164.27, 153.69, 152.10, 149.71, 124.04, 118.50, 114.22, 114.01, 65.75, 65.75, 52.17, 52.17, 50.71, 48.38, 47.99, 47.99, 46.67, 43.68, 42.76, 32.39, 32.39, 32.00, 30.51, 30.51, 30.05, 28.93, 28.84, 21.51. **HRMS** (ESI) calcd for $C_{30}H_{44}FN_4O_4$ [M+H]$^+$: 543.3268, found 543.3353.

N-(4-(Bis(2-hydroxyethyl)carbamoyl)cyclohexyl)-4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzamide (C5).—According to

the synthetic process of compound **5**, compound **C5**
as a white solid (0.22 g, 62 %) was obtained by taking 4-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzamido) cyclohexane-1-carboxylic acid
9 (0.3 g, 0.64 mmol) and 2,2'-azanediylbis(ethan-1-ol) (80 mg, 0.76 mmol) as starting
material, compound **C5**, **m.p.** 104–105 °C. **^{1}H NMR** (400 MHz, DMSO-d_6) δ 8.38 (d, J = 2.72 Hz, 1H), 8.23 (t, J = 8.52 Hz, 1H), 8.11 (d, J = 7.68 Hz, 1H), 7.66–7.59 (m, 2H), 6.63 (s, 1H), 4.88 (s, 1H), 4.67 (s, 1H), 3.71–3.69 (m, 1H), 3.52–3.51 (m, 2H), 3.44–3.43 (m, 4H), 3.34–3.32 (m, 1H), 2.61–2.55 (m, 1H), 2.10 (s, 1H), 1.88–1.85 (m, 2H), 1.76–1.70 (m, 4H), 1.59 (s, 4H), 1.48–1.44 (m, 2H), 1.41–1.38 (m, 2H), 1.35–1.32 (m, 3H), 1.28–1.25 (m, 2H), 1.12 (s, 2H), 0.83 (s, 6H). **^{13}C NMR** (100 MHz, DMSO-d_6): δ 175.61, 164.37, 153.70, 152.09, 149.69, 124.09, 118.51, 114.24, 114.03, 59.81, 59.40, 52.16, 50.89, 50.70, 48.76, 48.66, 47.98, 47.98, 42.76, 39.12, 32.39, 32.39, 32.39, 31.89, 30.51, 30.51, 30.51, 30.04, 28.91, 28.91. **HRMS** (ESI) calcd for $C_{31}H_{46}FN_4O_5$ [M+H]$^+$: 573.3374, found 573.3458.

N-(4-Carbamoylcyclohexyl)-4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzamide (C6).—According to the synthetic process

of compound **5**, compound **C6** as a white solid (0.17 g,
62 %) was obtained by taking 4-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzamido)cyclohexane-1-carboxylic acid **9** (0.3 g, 0.64 mmol) and ammonium
hydroxide (88 mg, 0.76 mmol) as starting material, compound **C6**, **m.p.** 107–108°C. **^{1}H
NMR** (400 MHz, DMSO-d_6) δ 8.53 (s, 1H), 8.22 (t, J = 8.12 Hz, 1H), 8.15 (d, J = 7.64 Hz, 1H), 7.68–7.60 (m, 2H), 7.26 (s, 1H), 6.88 (s, 1H), 6.70 (s, 1H), 3.69 (s, 1H), 3.05 (q, J =

6.84 Hz, 4H), 2.69 (s, 1H), 2.09 (s, 1H), 1.77 (s, 3H), 1.59 (s, 3H), 1.45–1.35 (m, 4H), 1.32 (s, 2H), 1.27 (s, 2H), 1.12 (s, 2H), 0.83 (s, 6H). **^{13}C NMR** (100 MHz, DMSO-d_6): δ 177.47, 164.29, 153.82, 152.11, 149.71, 124.07, 118.57, 114.21, 114.02, 52.15, 50.72, 48.41, 47.99, 45.76, 45.76, 43.46, 42.77, 38.71, 32.38, 32.38, 32.00, 30.53, 30.53, 30.03, 28.79, 8.89, 8.89. **HRMS** (ESI) calcd for $C_{27}H_{38}FN_4O_3$ [M+H]$^+$: 485.2850, found 485.3297.

N-(4-((1,3-Dihydroxypropan-2-yl)carbamoyl)cyclohexyl)-4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzamide (C7).—According to the

synthetic process of compound **5**, compound **C7** as a white solid (0.20 g, 56 %) was obtained by taking 4-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzamido) cyclohexane-1-carboxylic acid **9** (0.3 g, 0.64 mmol) and 2-aminopropane-1,3-diol (69 mg, 0.76 mmol) as starting material, compound **C7**, **m.p.** 110–111 °C. **^{1}H NMR** (400 MHz, DMSO-d_6) δ 8.38 (s, 1H), 8.22 (t, J = 8.48 Hz, 1H), 8.09 (d, J = 7.88 Hz, 1H), 7.65–7.59 (m, 2H), 7.42 (d, J = 8.08 Hz, 1H), 6.64 (s, 1H), 4.62 (s, 2H), 3.71–3.66 (m, 2H), 3.39–3.38 (m, 4H), 2.10 (s, 1H), 1.86–1.84 (m, 2H), 1.76 (s, 3H), 1.59 (s, 4H), 1.47–1.41 (m, 2H), 1.37–1.32 (m, 4H), 1.28 (s, 2H), 1.25 (s, 2H), 1.12 (s, 2H), 0.83 (s, 6H). **^{13}C NMR** (100 MHz, DMSO-d_6): δ 175.33, 164.23, 153.72, 152.10, 149.71, 124.05, 118.52, 114.21, 114.01, 60.67, 60.67, 53.04, 52.16, 52.16, 50.70, 48.37, 47.98, 47.98, 43.66, 42.76, 32.39, 32.39, 32.39, 32.00, 30.52, 30.52, 30.04, 28.92, 28.92. **HRMS** (ESI) calcd for $C_{30}H_{44}FN_4O_5$ [M+H]$^+$: 559.3217, found 559.3304.

N-(4-((1,3-Dihydroxy-2-(hydroxymethyl)propan-2-yl)carbamoyl)cyclohexyl)-4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzamide (C8).—According to

the synthetic process of compound **5**, compound **C8** as a white solid (0.22 g, 60 %) was obtained by taking 4-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzamido)cyclohexane-1-carboxylic acid **9** (0.3 g, 0.64 mmol) and 2-amino-2-(hydroxymethyl)propane-1,3-diol (92 mg, 0.76 mmol) as starting material, compound **C8**, **m.p.** 121–122 °C. **^{1}H NMR** (400 MHz, DMSO-d_6) δ 8.53 (s, 1H), 8.22 (t, J = 8.40 Hz, 1H), 8.14 (d, J = 7.80 Hz, 1H), 7.67–7.60 (m, 2H), 7.29 (s, 1H), 6.89 (s, 1H), 4.88 (s, 3H), 3.70 (s, 1H), 3.52 (s, 5H), 3.17 (d, J = 5.16 Hz, 1H), 2.26 (s, 1H), 2.09 (s, 1H), 1.85–1.77 (m, 6H), 1.59 (s, 3H), 1.46–1.40 (m, 2H), 1.36–1.32 (m, 4H), 1.27–1.25 (m, 3H), 1.12 (s, 2H), 0.83 (s, 6H). **^{13}C NMR** (100 MHz, DMSO-d_6): δ 177.09, 164.28, 153.84, 152.11, 149.72, 124.08, 118.59, 114.21, 114.00, 62.62, 61.27, 61.27, 52.15, 52.15, 50.73, 49.04, 48.35, 47.99, 47.99, 43.86, 42.77, 38.71, 32.38, 32.38, 32.38, 31.89, 30.53, 30.53, 30.53, 30.03, 28.98. **HRMS** (ESI) calcd for $C_{31}H_{46}FN_4O_6$ [M+H]$^+$: 589.3323, found 589.3411.

(3-Fluoro-4-nitrophenyl)methanol (10).—To a solution of 3-fluoro-4-nitrobenzoic acid

(10 g, 54.05 mmol), THF (60 mL) was added BH$_3$ (2.2 g, 55.3 mmol) slowly dropwise at 0 °C. Transfer to room temperature and continue the reaction for 12h. After the reaction was completed, the reaction solution was lowered to 0°C and MeOH (100 mL) was slowly added. The reaction solution is concentrated under vacuum to produce a light yellow oil **10** (7.86 g), which is used without further purification, **ESI-MS**: m/z 194.1[M+H]$^+$.

4-(Bromomethyl)-2-fluoro-1-nitrobenzene (11).—To a solution of (3-fluoro-4-nitrophenyl)methanol **10** (7.86 g, 45.96 mmol), DCM (50 mL) was added PBr$_3$ (14.93 g, 55.15 mmol) slowly dropwise at 0 °C. Transfer to room temperature and continue the reaction for 12h. After the reaction was completed, the reaction mixture was washed with sat. NaHCO$_3$ (3 × 30 mL). The combined organic layer was dried over MgSO4 and concentrated under reduced pressure to give a crude compound **11** as a viscous yellow oil (8.57 g) which was used in the next step without further purification, **ESI-MS**: m/z 233.2[M+H]$^+$.

Ethyl-1-(3-fluoro-4-nitrobenzyl)piperidine-3-carboxylate (12).—To a solution of 4-(bromomethyl)-2-fluoro-1-nitrobenzene **11** (8.57 g, 36.77 mmol), acetonitrile (40 mL) were added ethyl piperidine-3-carboxylate (6.93 g, 44.12 mmol), K$_2$CO$_3$ (7.6 g, 55.2 mmol), KI (1.2 g, 7.35 mmol). The resulting mixture was heated to 70 °C for 8 h. After the reaction was completed, the mixture was cooled to the rt, and the solvent was evaporated in vacuum. The residues were poured into water (30 mL), which was extracted with EtOAc (40 mL × 2). Then, the combined organic layers were respectively washed with water (30 mL × 2) and brine (30 mL). The organic layer was separated, dried with anhydrous MgSO$_4$, filtered, and concentrated in vacuum produce light yellow oil **12** (7.98 g), which was used without further purification, **ESI-MS**: m/z 311.1 [M+H]$^+$.

Ethyl-1-(4-amino-3-fluorobenzyl)piperidine-3-carboxylate (13).—According to the synthetic process of compound **2**, compound **13** as a light yellow oil (6.48 g) was obtained by taking ethyl 1-(3-fluoro-4-nitrobenzyl)piperidine-3-carboxylate **12** (7.98 g, 25.73 mmol) as starting material, compound **13**, which was used without further purification, **ESI-MS**: m/z 281.1 [M+H]$^+$.

Ethyl-1-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzyl) piperidine-3-carboxylate (14).—According to the synthetic process of compound **3**, compound **14** as a light yellow oil (7.86 g) was obtained by taking ethyl 1-(4-amino-3-fluorobenzyl)piperidine-3-carboxylate **13** (6.48 g, 23.13 mmol) as starting material, compound **14**, which was used without further purification, **ESI-MS**: m/z 486.33 [M+H]$^+$.

1-(4-(3-((1r,3R,5S,7r)-3,5-Dimethyladamantan-1-yl)ureido)-3-fluorobenzyl) piperidine-3-carboxylic acid (D).—According to the synthetic process of compound **4**, compound **D** as a white solid (5.92 g, 70%) was obtained by taking ethyl-1-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzyl)piperidine-3-carboxylate **14** (7.86 g, 16.20 mmol) as starting material, compound **D, m.p.** 211–212 °C. **^{1}H NMR** (400 MHz, DMSO-d_6): δ (ppm) 11.06 (s, 1H), 8.38 (s, 1H), 8.20 (t, J = 8.42 Hz, 1H), 7.52 (d, J = 12.08 Hz, 1H), 7.22 (d, J = 8.24 Hz, 1H), 6.72 (s, 1H), 4.20 (s, 2H), 3.35–3.24 (m, 4H), 2.96–2.94 (m, 3H), 2.09–1.76 (m, 6H), 1.61–1.55 (m, 3H), 1.35–1.24 (m, 4H), 1.11 (s, 2H), 0.83 (s, 6H). **^{13}C NMR** (100 MHz, DMSO-d_6): δ 173.7, 154.1, 152.6, 129.7, 127.4, 119.8, 117.6, 59.2, 52.8, 52.4, 52.1, 51.5, 50.8, 48.5, 48.2, 48.1, 46.3, 42.9, 42.8, 41.9, 32.4, 30.5, 30.1, 25.7, 22.2. **HRMS** (ESI) calcd for C$_{26}$H$_{37}$FN$_3$O$_3$ [M+H]$^+$: 458.2741, found 458.2823.

1-(4-(3-((1r,3R,5S,7r)-3,5-Dimethyladamantan-1-yl)ureido)-3-fluorobenzyl)piperidine –3-carboxamide (D1).—According to the synthetic process of compound **5**, compound **D1** as a white solid (0.18 g, 60 %) was obtained by taking 1-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzyl)piperidine-3-carboxylic acid **D** (0.3 g, 0.66 mmol) and aqueous ammonia (92 mg, 0.79 mmol) as starting material, compound **D1**, **m.p.** 87–88°C. **^{1}H NMR** (400 MHz, CDCl$_3$): δ (ppm) 8.27–8.17 (m, 1H), 7.81–7.56 (m, 1H), 7.04–7.00 (m, 2H), 3.83–3.46 (m, 8H), 2.43–2.35 (m, 2H), 2.12–2.11 (m, 1H), 2.01 (s, 1H), 1.79 (s, 2H), 1.62–1.61 (m, 5H), 1.37–1.33 (m, 2H), 1.29–1.26 (m, 3H), 1.18–1.10 (m, 5H), 0.83 (s, 6H). **^{13}C NMR** (100 MHz, DMSO-d_6): δ (ppm) 173.7, 170.9, 154.1, 153.9, 130.2, 128.1, 122.7, 120.0, 113.4, 52.7, 52.6, 50.8, 50.6, 48.0, 46.9, 46.1, 42.7, 40.6, 40.6, 32.4, 30.2, 30.1, 29.7, 28.3, 26.8, 26.2, 9.6, 9.5. **HRMS** (ESI) calcd for C$_{26}$H$_{38}$FN$_4$O$_2$ [M+H]$^+$: 457.2901, found 457.2982.

1-(4-(3-((1r,3R,5S,7r)-3,5-Dimethyladamantan-1-yl)ureido)-3-fluorobenzyl)-N,N-dimethylpiperidine-3-carboxamide (D2).—According to the synthetic process of compound **5**, compound **D2** as a white solid (0.18 g, 58 %) was obtained by taking 1-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzyl)piperidine-3-carboxylic acid **D** (0.3 g, 0.66 mmol) and dimethylamine (36 mg, 0.79 mmol) as starting material, compound **D2**, **m.p.** 111–112°C. **^{1}H NMR** (400 MHz, CDCl$_3$): δ (ppm) 7.93 (t, J = 8.92Hz, 1H),7.13–7.12 (m, 1H), 6.91–6.85 (m, 2H), 5.53 (s, 1H), 3.39–3.27 (m, 2H), 2.94 (s, 3H), 2.83 (s, 3H), 2.79–2.72 (m, 3H), 2.07–2.02 (m, 2H), 1.89–1.85 (m, 1H), 1.76–1.70 (m, 3H), 1.62–1.54 (m, 6H), 1.42–1.39 (m, 1H), 1.30–1.27 (m, 2H), 1.21–1.18 (m, 2H), 1.10–1.02 (m, 2H), 0.75 (s, 6H). **^{13}C NMR** (100 MHz, DMSO-d_6): δ (ppm) 174.5, 154.5, 153.4, 151.1, 124.9, 120.9, 115.2, 115.1, 65.6, 62.5, 55.6, 53.6, 52.7, 50.7, 48.1, 42.8, 40.7, 39.6, 37.2, 35.6, 32.4, 30.5, 30.2, 30.2, 29.7, 27.3, 24.9, 13.7. **HRMS** (ESI) calcd for C$_{28}$H$_{42}$FN$_4$O$_2$ [M+H]$^+$: 485.3214, found 485.3297.

1-(4-(3-((1r,3R,5S,7r)-3,5-Dimethyladamantan-1-yl)ureido)-3-fluorobenzyl)-N,N-diethylpiperidine-3-carboxamide (D3).—According to the synthetic process of compound **5**, compound **D3** as a white solid (0.20 g, 61 %) was obtained by taking 1-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzyl)piperidine-3-carboxylic acid **D** (0.3 g, 0.66 mmol) and diethylamine (58 mg, 0.79 mmol) as starting material, compound **D3**, **m.p.** 159–160°C. **^{1}H NMR** (400 MHz, DMSO-d_6): δ (ppm) 8.27–8.22 (m, 2H), 7.42–7.14 (m, 2H), 6.55 (s, 1H), 4.31–4.24 (m, 1H), 3.27–3.17 (m, 6H), 2.91 (s, 3H), 2.09 (s, 1H), 1.75 (s, 5H), 1.57 (s, 4H), 1.41 (s, 2H), 1.40–1.25 (m, 5H), 1.12–1.09 (m, 5H), 1.00–0.98 (m, 3H), 0.83 (s, 6H).**^{13}C NMR** (100 MHz, DMSO-d_6): δ (ppm) 172.5, 153.8, 152.0, 150.4, 129.9, 127.5, 119.8, 117.3, 59.3, 54.0, 52.2, 52.1, 52.1, 52.0, 50.7, 48.0, 42.7, 42.3, 41.8, 32.5, 32.3, 30.5, 30.0, 28.9, 26.8, 23.0, 22.9. **HRMS** (ESI) calcd for C$_{30}$H$_{46}$FN$_4$O$_2$ [M+H]$^+$: 513.3527, found 513.3611.

1-(4-(3-((1r,3R,5S,7r)-3,5-Dimethyladamantan-1-yl)ureido)-3-fluorobenzyl)-N-(2-hydroxypropyl)piperidine-3-carboxamide (D4).—According to the synthetic process of compound **5**, compound **D4** as a white solid (0.20 g, 61 %) was obtained by taking 1-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzyl)piperidine-3-carboxylic acid **D** (0.3 g, 0.66 mmol) and *iso*-propanolamine (59

mg, 0.79 mmol) as starting material, compound **D4**, **m.p.** 91–92°C. **¹H NMR** (400 MHz, CDCl₃): δ (ppm) 7.98 (t, *J* = 7.36Hz, 1H), 7.01–6.94 (m, 3H), 5.29 (s, 1H), 3.91–3.85 (m, 1H), 3.50–3.26 (m, 4H), 3.10–3.07 (m, 3H), 2.81 (s, 2H), 2.51 (s, 1H), 2.30 (s, 2H), 2.14 (s, 1H), 1.84 (s, 3H), 1.15–1.58 (m, 7H), 1.27–1.22 (m, 4H), 1.16–1.14 (m, 4H), 0.84 (s, 6H).**¹³C NMR** (100 MHz, DMSO-*d₆*): δ (ppm) 176.5, 154.3, 153.6, 151.2, 125.3, 121.3, 115.6, 115.4, 67.4, 67.3, 62.3, 54.2, 53.9, 52.9, 50.6, 48.1, 47.3, 47.0, 46.9, 42.7, 40.7, 32.4, 30.2, 30.1, 26.7, 22.5, 20.9, 8.8, 8.6. **HRMS** (ESI) calcd for C₂₉H₄₄FN₄O₃ [M+H]⁺: 515.3319, found 515.3404.

1-(4-(3-((1r,3R,5S,7r)-3,5-Dimethyladamantan-1-yl)ureido)-3-fluorobenzyl)-N,N-bis(2-hydroxyethyl)piperidine-3-carboxamide (D5).—According to the synthetic process of compound **5**, compound **D5** as a white solid (0.21 g, 59 %) was obtained by taking 1-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzyl)piperidine-3-carboxylic acid **D** (0.3 g, 0.66 mmol) and diethanolamine (83 mg, 0.79 mmol) as starting material, compound **D5**, **m.p.** 123–124°C. **¹H NMR** (400 MHz, CDCl₃): δ (ppm) 8.08 (t, *J* = 8.36Hz, 1H),6.95–6.90 (m, 2H), 5.67 (s, 1H), 3.73–3.62 (m, 2H), 3.45–3.43 (m, 1H), 3.14 (q, *J* = 8.00Hz, 2H), 2.86 (s, 2H), 2.45 (s, 1H), 2.14 (s, 2H), 1.98–1.97 (m, 3H), 1.89–1.83 (m, 3H), 1.65 (s, 4H), 1.57 (s, 2H), 1.42–1.33 (m, 5H), 1.30–1.24 (m, 5H), 1.15–1.14 (m, 2H), 0.75 (s, 6H). **¹³C NMR** (100 MHz, DMSO-*d₆*): δ (ppm) 174.8, 154.5, 154.4, 153.5, 151.1, 125.3, 120.9, 115.1, 125.3, 120.9, 115.1, 69.7, 62.5, 60.4, 54.3, 54.1, 52.8, 50.6, 48.1, 47.3, 42.7, 40.7, 33.8, 32.4, 30.9, 30.2, 26.7, 22.2, 21.1, 14.2, 8.9. **HRMS** (ESI) calcd for C₃₀H₄₆FN₄O₄ [M+H]⁺: 545.3425, found 545.3147.

1-(4-(3-((1r,3R,5S,7r)-3,5-Dimethyladamantan-1-yl)ureido)-3-fluorobenzyl)-N-(2-hydroxyethyl)piperidine-3-carboxamide (D6).—According to the synthetic process of compound **5**, compound **D6** as a white solid (0.20 g, 62 %) was obtained by taking 1-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzyl)piperidine-3-carboxylic acid **D** (0.3 g, 0.66 mmol) and ethanolamine (48 mg, 0.79 mmol) as starting material, compound **D6**, **m.p.** 94–95°C. **¹H NMR** (400 MHz, CDCl₃): δ (ppm) 8.00 (t, *J* = 8.33 Hz, 1H), 7.08 (s, 1H), 7.00–6.95 (m, 2H), 5.46 (s, 1H), 3.64–3.62 (m, 2H), 3.51–3.45 (m, 1H), 3.37–3.35 (m, 3H), 3.16–3.09 (m, 2H), 2.79 (s, 1H), 2.49 (s, 1H), 2.29 (s, 2H), 2.14 (s, 1H), 1.83 (s, 2H), 1.65–1.62 (m, 6H), 1.42–1.26 (m, 8H), 1.14 (s, 4H), 0.84 (s, 6H). **¹³C NMR** (100 MHz, DMSO-*d₆*): δ (ppm) 176.4, 154.3, 151.2, 127.3, 125.3, 121.1, 115.5, 115.3, 62.3, 62.1, 54.1, 52.9, 50.6, 48.1, 47.2, 42.7, 42.2, 40.7, 32.4, 30.2, 30.1, 26.7, 22.6, 22.5, 14.1, 8.9. **HRMS** (ESI) calcd for C₂₈H₄₂FN₄O₃ [M+H]⁺: 501.3163, found 501.3246.

N-(1,3-Dihydroxypropan-2-yl)-1-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzyl)piperidine-3-carboxamide (D7).—According to the synthetic process of compound **5**, compound **D7** as a white solid (0.20 g, 59 %) was obtained by taking 1-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzyl) piperidine-3-carboxylic acid **D** (0.3 g, 0.66 mmol) and 2-aminopropane-1,3-diol (72 mg, 0.79 mmol) as starting material, compound **D7**, **m.p.** 113–114°C. **¹H NMR** (400 MHz, CDCl₃): δ (ppm) 7.87–7.86 (m, 1H), 7.45 (s, 1H), 7.04–7.01 (m, 1H), 6.85–6.83 (m, 1H), 5.88 (s, 1H), 3.87 (s, 1H), 3.67–3.30 (m,

7H), 3.01–2.70 (m, 2H), 2.43 (s, 1H), 2.03 (s, 1H), 1.73 (s, 3H), 1.55(s, 7H), 1.28–1.77 (m, 7H), 1.05 (s, 3H), 0.74 (s, 6H). **^{13}C NMR** (100 MHz, DMSO-d_6): δ (ppm) 166.5, 150.0, 148.9, 146.5, 122.8, 120.7, 116.0, 110.9, 57.2, 57.0, 55.7, 49.4, 47.9, 47.6, 45.9, 43.4, 41.9, 37.9, 35.8, 27.6, 27.2, 25.4, 24.9, 24.6, 22.1, 17.9, 17.6, 16.3, 9.4, 9.3. **HRMS** (ESI) calcd for $C_{29}H_{44}FN_4O_4$ [M+H]$^+$: 531.3268, found 531.3340.

N-(1,3-Dihydroxy-2-(hydroxymethyl)propan-2-yl)-1-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzyl)piperidine-3-carboxamide (D8).—According to the synthetic process of compound **5**, compound **D8** as a white solid (0.22 g, 61 %) was obtained by taking 1-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzyl)piperidine-3-carboxylic acid **D** (0.3 g, 0.66 mmol) and 2-amino-2-(hydroxymethyl)propane-1,3-diol (96 mg, 0.79 mmol) as starting material, compound **D8**, **m.p.** 111–112°C. **^{1}H NMR** (400 MHz, CDCl$_3$): δ (ppm) 7.88 (t, J = 8.16Hz, 1H), 7.16–7.09 (m, 2H), 6.96–6.94 (m, 1H), 5.50 (s, 1H), 4.63 (s, 2H), 3.67–3.60 (m, 4H), 3.46–3.35 (m, 2H), 3.12 (q, J = 7.28Hz, 2H), 2.81–2.78 (m, 2H), 2.50 (s, 1H), 2.29 (s, 1H), 2.13 (s, 1H), 1.81 (s, 3H), 1.63 (s, 6H), 1.37–1.26 (m, 7H), 1.17–1.10 (m, 2H), 0.84 (s, 6H). **^{13}C NMR** (100 MHz, DMSO-d_6): δ (ppm) 175.4, 154.1, 152.5, 150.9, 127.7, 125.3, 119.9, 115.6, 62.6, 61.9, 61.1, 60.6, 55.6, 53.3, 52.0, 50.7, 48.1, 46.2, 42.7, 32.4, 32.4, 32.3, 30.6, 30.5, 30.0, 27.7, 23.8. **HRMS** (ESI) calcd for $C_{30}H_{46}FN_4O_5$ [M+H]$^+$: 561.3374, found 561.3447.

1-((1r,3R,5S,7r)-3,5-Dimethyladamantan-1-yl)-3-(2-fluoro-4-((3-(4-(2-hydroxyethyl) piperazine-1-carbonyl)piperidin-1-yl)methyl)phenyl)urea (D9).—According to the synthetic process of compound **5**, compound **D9** as a white solid (0.21 g, 56 %) was obtained by taking 1-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzyl)piperidine-3-carboxylic acid **D** (0.3 g, 0.66 mmol) and 2-(piperazin-1-yl-ethan)-1-ol (96 mg, 0.79 mmol) as starting material, compound **D9**, **m.p.** 116–117°C. **^{1}H NMR** (400 MHz, CDCl$_3$): δ (ppm) 7.99 (t, J = 8.24 Hz, 1H), 7.01–6.98 (m, 2H), 6.80 (s, 1H), 5.12 (s, 1H), 3.65–3.62 (m, 3H), 3.49–3.47 (m, 5H), 3.65–3.62 (m, 3H), 3.49–3.47 (m, 5H), 2.89–2.78 (m, 4H), 2.55 (t, J = 5.32Hz, 2H), 2.50–2.40 (m, 4H), 2.15–2.14 (m, 3H), 1.84–1.73 (m, 4H), 1.66 (s, 4H), 1.36–1.33 (m, 2H), 1.31–1.25 (m, 4H), 1.16–1.15 (m, 2H), 0.84 (s, 6H). **^{13}C NMR** (100 MHz, DMSO-d_6): δ (ppm) 172.6, 154.0, 153.5, 151.1, 125.1, 120.9, 115.4, 115.2, 62.3, 59.2, 57.8, 55.6, 53.7, 53.2, 52.9, 52.6, 50.6, 48.2, 46.8, 45.5, 42.7, 41.6, 40.7, 32.7, 30.2, 30.1, 29.7, 27.3, 24.7, 22.7, 9.2. **HRMS** (ESI) calcd for $C_{32}H_{49}FN_5O_3$ [M+H]$^+$: 570.3741, found 570.3812.

Ethyl-1-(3-fluoro-4-nitrobenzyl)piperidine-4-carboxylate (15).—According to the synthetic process of compound **12**, compound **15** as a light yellow oil (4.79 g) was obtained by taking 4-(bromomethyl)-2-fluoro-1-nitrobenzene **11** (5 g, 21.46 mmol) and ethyl piperidine-4-carboxylate (4.04 g, 25.76 mmol) as starting material, compound **15**, which was used without further purification, **ESI-MS**: m/z 311.12 [M+H]$^+$.

Ethyl-1-(4-amino-3-fluorobenzyl)piperidine-4-carboxylate (16).—According to the synthetic process of compound **2**, compound **16** as a light yellow oil (3.94 g) was obtained by taking ethyl 1-(3-fluoro-4-nitrobenzyl)piperidine-4-carboxylate **15** (4.79 g, 15.44 mmol) as starting material, compound **16**, which was used without further purification, **^{1}H NMR**

(400 MHz, CDCl₃): δ (ppm) 7.10 (dd, J = 11.28 Hz, J = 1.76 Hz, 1H), δ 7.03 (dd, J = 8.08 Hz, J = 1.4 Hz, 1H), 6.76 (t, J = 8.48 Hz, 1H), 4.28 (s, 0.5H), 4.13 (q, J = 7.08 Hz, 2H), 4.02 (s, 0.5H),3.51 (s, 2H), 3.19 (t, J = 10.8 Hz, 1H), 3.04 (td, J = 12.44 Hz, J = 1.96 Hz, 1H),2.52 (m, 1H), 2.17 (m, 1H), 1.74 (m, 2H), 1.53 (m,1H), 1.24 (t, J = 7.08 Hz, 4H).

Ethyl-1-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzyl) piperidine-4-carboxylate (17).—According to the synthetic process of compound **3**, compound **17** as a light yellow oil (4.64 g) was obtained by taking thyl-1-(4-amino-3-fluorobenzyl)piperidine-4-carboxylate (**16**) (3.94 g, 14.06 mmol) as starting material, compound **17**, which was used without further purification, **ESI-MS**: m/z 486.3 [M+H]⁺, 508.32 [M+Na]⁺.

1-(4-(3-((1r,3R,5S,7r)-3,5-Dimethyladamantan-1-yl)ureido)-3-fluorobenzyl) piperidine-4-carboxylic acid (18).—According to the synthetic process of compound **4**, compound **18** as a light yellow oil (3.85 g) was obtained by taking thyl 1-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzyl)piperidine-4-carboxylate **17** (4.64 g, 9.56 mmol) as starting material, compound **18**, which was used without further purification, **ESI-MS**: m/z 458.3 [M+H]⁺.

1-(4-(3-((1r,7r)-3,5-Dimethyladamantan-1-yl)ureido)-3-fluorobenzyl)piperidine-4-carboxamide (E1).—According to the synthetic process of compound **5**, compound **E1** as a white solid (0.20 g, 66 %) was obtained by taking 1-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzyl)piperidine-4-carboxylic acid **18** (0.3 g, 0.66 mmol) and aqueous ammonia (92 mg, 0.79 mmol) as starting material, compound **E1**, **m.p.**117–118°C. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 8.10 (s, 1H), 7.25–7.01 (m, 2H), 6.77 (s, 1H), 6.48 (s, 1H), 3.34 (s, 2H), 3.09 (s, 1H), 2.90 (s, 2H), 2.51 (s, 2H), 2.10 (s, 3H), 1.76–1.58 (m, 8H), 1.40–1.12 (m, 8H), 0.83 (s, 6H). **¹³C NMR** (100 MHz, DMSO-d_6): δ (ppm) 176.5, 161.4, 154.0, 152.4, 150.8, 125.8, 119.9, 115.9, 52.4, 52.0, 50.2, 48.2, 48.1, 46.2, 43.8, 42.9, 42.7, 42.2, 32.4, 32.3, 30.7, 30.5, 30.0, 28.7, 28.0. **HRMS** (ESI) calcd for $C_{26}H_{38}FN_4O_2$ [M+H]⁺: 457.2901, found 457.2971.

1-(4-(3-((1r,7r)-3,5-Dimethyladamantan-1-yl)ureido)-3-fluorobenzyl)-N-(2-hydroxypropyl)piperidine-4-carboxamide (E2).—According to the synthetic process of compound **5**, compound **E2** as a white solid (0.19 g, 57 %) was obtained by taking 1-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzyl)piperidine-4-carboxylic acid **18** (0.3 g, 0.66 mmol) and *iso*-propanolamine (59 mg, 0.79 mmol) as starting material, compound **E2**, **m.p.** 99–100°C. **¹H NMR** (400 MHz, CDCl₃): δ (ppm) 7.88 (t, J = 8.24Hz, 1H),7.02–6.93 (m, 2H), 6.84 (s, 1H), 6.28 (s, 1H), 5.12 (s, 1H), 3.92–3.88 (m, 1H), 3.41 (s, 3H), 3.14–2.96 (m, 3H), 2.88–2.86 (m, 2H), 2.14–1.96 (m, 4H), 1.83–1.72 (m, 5H), 1.68–1.62 (m, 4H), 1.38–1.26 (m, 4H), 1.18–1.15 (m, 4H), 0.86 (s, 6H). **¹³C NMR** (100 MHz, DMSO-d_6): δ (ppm) 176.5, 154.4, 151.5, 126.5, 124.8, 121.3, 115.3, 115.1, 67.4, 62.0, 52.9, 52.8, 50.6, 48.2, 46.9, 43.0, 42.7, 40.7, 32.4, 30.2, 30.1, 28.7, 20.9, 9.6. **HRMS** (ESI) calcd for $C_{29}H_{44}FN_4O_3$ [M+H]⁺: 515.3319, found 515.3394.

N-(1,3-Dihydroxypropan-2-yl)-1-(4-(3-((1r,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzyl)piperidine-4-carboxamide (E3).

—According to the synthetic process of compound **5**, compound **E3** as a white solid (0.19 g, 54 %) was obtained by taking 1-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzyl)piperidine-4-carboxylic acid **18** (0.3 g, 0.66 mmol) and 2-aminopropane-1,3-diol (72 mg, 0.79 mmol) as starting material, compound **E3**, **m.p.** 150–151°C. **^{1}H NMR** (400 MHz, DMSO-d_6): δ (ppm) 8.61–8.60 (m, 1H), 8.39 (dd, J = 8.36 Hz, J = 1.12 Hz, 1H), 8.36 (s, 1H), 8.12 (t, J = 8.44 Hz, 1H), 7.67 (d, J = 7.88 Hz, 1H), 7.41–7.38 (m, 2H), 7.15 (d, J = 8.64Hz, 1H), 6.75 (s, 1H), 4.66 (s, 2H), 3.95 (s, 2H), 3.68–3.64 (m, 1H), 3.39–3.38 (m, 6H), 2.09 (s, 1H), 1.80–1.76 (m, 5H), 1.61–1.54 (m, 4H), 1.36–1.27 (m, 4H), 1.11 (s, 3H), 0.83 (s, 6H). **^{13}C NMR** (100 MHz, DMSO-d_6): δ (ppm) 173.4, 154.0, 152.0, 150.4, 129.1, 128.0, 119.7, 118.1, 61.4, 60.9, 58.9, 58.2, 54.8, 53.6, 51.0, 50.7, 49.0, 48.2, 42.7, 32.5, 32.2, 30.6, 29.5, 28.8, 26.2, 25.6, 22.8, 22.5. **HRMS** (ESI) calcd for $C_{29}H_{44}FN_4O_4$ [M+H]$^+$: 531.3268, found 531.3337.

N-(1,3-Dihydroxy-2-(hydroxymethyl)propan-2-yl)-1-(4-(3-((1r,7r)-3,5-dimethyl adamantan-1-yl)ureido)-3-fluorobenzyl)piperidine-4-carboxamide (E4).

—According to the synthetic process of compound **5**, compound **E4** as a white solid (0.20 g, 55 %) was obtained by taking 1-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzyl)piperidine-4-carboxylic acid **18** (0.3 g, 0.66 mmol) and 2-amino-2-(hydroxymethyl)propane-1,3-diol (96 mg, 0.79 mmol) as starting material, compound **E4**, **m.p.** 117–118°C. **^{1}H NMR** (400 MHz, DMSO-d_6): δ (ppm) 8.06–8.01 (m, 2H), 7.08–7.04 (m, 2H), 6.97–6.95 (m, 1H), 6.45 (s, 1H), 4.75 (t, J = 5.60Hz, 2H), 3.51–3.50 (m, 5H), 3.41–3.33 (m, 4H), 2.87–2.79 (m, 1H), 2.22 (s, 1H), 2.09–2.08 (m, 1H), 1.97–1.84 (m, 2H), 1.77–1.74 (m, 2H), 1.65–1.51 (m, 7H), 1.34–1.24 (m, 6H), 1.15–1.11 (m, 2H), 0.83 (s, 6H). **^{13}C NMR** (100 MHz, DMSO-d_6): δ (ppm) 173.8, 154.1, 152.5, 150.9, 132.1, 125.2, 119.9, 115.4, 72.7, 61.9, 60.7, 60.6, 60.5, 55.7, 53.3, 52.9, 52.0, 50.7, 48.1, 46.2, 42.8, 42.7, 42.6, 32.3, 30.5, 30.0, 29.5, 27.7, 24.2, 22.5. **HRMS** (ESI) calcd for $C_{30}H_{46}FN_4O_5$ [M+H]$^+$: 561.3374, found 561.3447.

1-(4-(3-((1r,7r)-3,5-Dimethyladamantan-1-yl)ureido)-3-fluorobenzyl)-N,N-bis(2-hydroxyethyl)piperidine-4-carboxamide (E5).

—According to the synthetic process of compound **5**, compound **E4** as a white solid (0.22 g, 61 %) was obtained by taking 1-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzyl)piperidine-4-carboxylic acid **18** (0.3 g, 0.66 mmol) and diethanolamine (83 mg, 0.79 mmol) as starting material, compound **E5**, **m.p.** 104–105°C. **^{1}H NMR** (400 MHz, CDCl$_3$): δ (ppm) 7.88 (t, J = 8.44 Hz, 1H), 7.03–6.94 (m, 2H), 6.61 (s, 1H), 4.92 (s, 1H), 3.83–3.77 (m, 3H), 3.54–3.52 (m, 2H), 3.44 (s, 1H), 2.89–2.81 (m, 3H), 2.57 (s, 1H), 2.15 (s, 1H), 2.03–1.97 (m, 2H), 1.84 (s, 2H), 1.68–1.66 (m, 4H), 1.43 (s, 2H), 1.37–1.21 (m, 8H), 1.20–1.15 (m, 5H), 0.84 (s, 6H). **^{13}C NMR** (100 MHz, DMSO-d_6): δ (ppm) 177.7, 154.0, 124.9, 121.1, 115.4, 115.2, 61.7, 60.8, 53.0, 52.6, 51.7, 50.6, 50.3, 48.2, 46.6, 42.7, 40.7, 38.8, 32.5, 31.6, 30.2, 30.1, 29.7, 28.7, 26.9, 22.6, 14.1, 10.1. **HRMS** (ESI) calcd for $C_{30}H_{46}FN_4O_4$ [M+H]$^+$: 545.3425, found 545.3499.

Tert-butyl-4-(1-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzoyl)piperidine-3-carbonyl)-1,4-diazepane-1-carboxylate (19).—

According to the synthetic process of compound **5**, compound
19 as a light yellow oil (3.33 g) was obtained by taking 1-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzoyl)piperidine-3-carboxylic acid **A** (3 g, 6.37 mmol) and *tert*-butyl 1,4-diazepane-1-carboxylate (1.53 g, 7.64 mmol) as starting material, compound **19**, which was used without further purification, **¹H NMR** (400 MHz, CDCl₃): δ (ppm) 8.18–8.15 (m, 1H), 7.36–7.35 (m, 1H), 7.06 (s, 2H), 4.54 (s, 1H), 3.76–3.41 (m, 9H), 3.11–3.01 (m, 1H), 3.84–2.97 (m, 1H), 2.14 (t, *J* = 2.76 Hz, 1H), 1.91–1.72 (m, 7H), 1.64 (s, 4H), 1.45–1.36 (m, 1H), 1.33–1.23 (m, 4H), 1.19–1.15 (m, 2H), 0.85 (s, 6H).

1-(4-(3-(1,4-Diazepane-1-carbonyl)piperidine-1-carbonyl)-2-fluorophenyl)-3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)urea (20).—

To a solution of *tert*-butyl 4-(1-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzoyl)piperidine-3-carbonyl)-1,4-diazepane-1-carboxylate **19** (3.33 g, 5.10 mmol), DCM (10 mL) were added TFA (5.82 g, 51 mmol) slowly dropwise at 0 °C. Transfer to room temperature and continue the reaction for 12h. After the reaction was completed, the reaction solution is concentrated under vacuum to produce a light yellow oil **20** (7.86 g), which is used without further purification, **ESI-MS**: m/z 554.44 [M+H]⁺.

1-(4-(3-(4-Acetyl-1,4-diazepane-1-carbonyl)piperidine-1-carbonyl)-2-fluorophenyl)-3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)urea (F1).—

According to the synthetic process of compound **5**, compound **F1** as a white solid (0.21 g, 66 %) was obtained by taking 1-(4-(3-(1,4-diazepane-1-carbonyl)piperidine-1-carbonyl)-2-fluorophenyl)-3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)urea **20** (0.3 g, 0.56 mmol) and acetic acid (40 mg, 0.67 mmol) as starting material, compound **F1**, **m.p.** 136–137°C. **¹H NMR** (400 MHz, CDCl₃): δ (ppm) 8.26–8.20 (m, 1H), 7.60 (s, 1H), 7.08 (s, 2H), 5.92 (s, 1H), 4.58 (s, 1H), 3.74–3.47 (m, 9H), 3.14–2.60 (m, 3H), 2.13–2.05 (m, 4H), 1.87–1.80 (m, 6H), 1.63 (s, 4H), 1.42–1.35 (m, 5H), 1.29–1.24 (m, 3H), 1.18–1.11(m, 2H), 0.83 (s, 6H). **¹³C NMR** (100 MHz, DMSO-*d₆*): δ (ppm) 175.8, 172.6, 168.5, 153.8, 151.7, 150.2, 130.3, 128.8, 123.8, 119.2, 52.1, 50.7, 48.2, 47.9, 46.7, 46.6, 46.2, 46.1, 45.1, 44.2, 42.7, 36.6, 36.5, 36.4, 32.4, 30.5, 30.0, 27.4, 27.3, 27.2, 18.1, 12.3, 12.2. **HRMS** (ESI) calcd for C₃₃H₄₇FN₅O₄ [M+H]⁺: 596.3534, found 596.3600.

1-((1r,3R,5S,7r)-3,5-Dimethyladamantan-1-yl)-3-(2-fluoro-4-(3-(4-propionyl-1,4-diazepane-1-carbonyl)piperidine-1-carbonyl)phenyl)urea (F2).—

According to the synthetic process of compound **5**, compound **F2** as a white solid (0.21 g, 63 %) was obtained by taking 1-(4-(3-(1,4-diazepane-1-carbonyl)piperidine-1-carbonyl)-2-fluorophenyl)-3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)urea **20** (0.3 g, 0.56 mmol) and propionic acid (50 mg, 0.67 mmol) as starting material, compound **F2**, **m.p.** 96–98°C. **¹H NMR** (400 MHz, CDCl₃): δ (ppm) 8.30 (s, 1H), 8.16–8.13 (m, 1H), 7.59 (s, 1H), 6.99 (s, 2H), 5.88 (s, 1H), 4.48 (s, 1H), 3.71–3.60 (m, 7H), 3.07–3.04 (m, 3H), 2.69 (s, 2H), 2.25 (s, 3H), 2.05 (s, 1H), 1.74 (s, 6H), 1.56 (s, 4H), 1.22–1.18 (m, 3H), 1.06–1.01 (m, 5H), 0.76 (s, 6H). **¹³C NMR** (100 MHz, DMSO-*d₆*): δ (ppm) 173.9, 169.7, 168.4, 154.0, 152.2, 150.6, 130.4, 130.3, 120.1, 124.8, 54.7, 52.8, 50.6, 47.9,

47.4, 47.3, 42.8, 42.7, 40.6, 32.4, 30.2, 30.1, 29.7, 27.7, 26.5, 26.1, 25.9, 18.5, 17.1, 12.2, 9.6, 9.5, 9.4. **HRMS** (ESI) calcd for $C_{34}H_{49}FN_5O_4$ [M+H]$^+$: 610.3690, found 610.3767.

1-((1r,3R,5S,7r)-3,5-Dimethyladamantan-1-yl)-3-(2-fluoro-4-(3-(4-(2-methylbutanoyl) –1,4-diazepane-1-carbonyl)piperidine-1-carbonyl)phenyl)urea (F3).—

According to the synthetic process of compound **5**, compound **F3** as a white solid (0.23 g, 65 %) was obtained by taking 1-(4-(3-(1,4-diazepane-1-carbonyl)piperidine-1-carbonyl)-2-fluorophenyl)-3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)urea **20** (0.3 g, 0.56 mmol) and 2-methylbutanoic acid (68 mg, 0.67 mmol) as starting material, compound **F3**, **m.p.** 149–150°C. **1H NMR** (400 MHz, CDCl$_3$): δ (ppm) 8.26–8.20 (m, 1H), 7.58 (s, 1H), 7.24–7.08 (m, 2H), 5.90 (s, 1H), 4.59 (s, 1H), 3.82–3.37 (m, 10H), 3.05–2.55 (m, 4H), 2.13–2.05(m, 2H), 1.80–1.62 (m, 11H), 1.37–1.24 (m, 6H), 1.14–1.06 (m, 5H), 0.86 (s, 2H), 0.83 (s, 6H). **13C NMR** (100 MHz, DMSO-d_6): δ (ppm) 175.8, 172.6, 168.5, 153.8, 121.7, 150.2, 130.4, 128.8, 123.8, 119.2, 52.1, 50.7, 48.2, 47.9, 47.8, 46.8, 46.7, 46.2, 46.1, 45.1, 44.2, 42.7, 36.7, 36.6, 36.5, 36.4, 32.4, 30.5, 30.0, 28.7, 27.4, 27.3, 27.2, 27.2, 18.0, 17.7. **HRMS** (ESI) calcd for $C_{36}H_{53}FN_5O_4$ [M+H]$^+$: 638.4003, found 638.4076.

Tert-butyl-4-(1-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzyl)piperidine-3-carbonyl)-1,4-diazepane-1-carboxylate (21).—

According to the synthetic process of compound **5**, compound **21** as a light yellow oil (3.27 g) was obtained by taking 1-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzyl)piperidine-3-carboxylic acid **D1** (3.0 g, 6.56 mmol) and *tert*-butyl 1,4-diazepane-1-carboxylate (1.57 g, 7.87 mmol) as starting material, compound **21**, which was used without further purification, **ESI-MS**: m/z 640.53 [M+H]$^+$.

1-(4-((3-(1,4-Diazepane-1-carbonyl)piperidin-1-yl)methyl)-2-fluorophenyl)-3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)urea (22).—

According to the synthetic process of compound **20**, compound **22** as a light yellow oil (1.59 g) was obtained by taking *tert*-butyl-4-(1-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzyl)piperidine-3-carbonyl)-1,4 diazepane-1-carboxylate **21** (3.27 g, 5.12 mmol) as starting material, compound **22**, which was used without further purification, **ESI-MS**: m/z 540.41 [M+H]$^+$.

1-((1r,3R,5S,7r)-3,5-Dimethyladamantan-1-yl)-3-(2-fluoro-4-((3-(4-propionyl-1,4-diazepane-1-carbonyl)piperidin-1-yl)methyl)phenyl)urea (G1).—

According to the synthetic process of compound **5**, compound **G1** as a white solid (0.22 g, 68 %) was obtained by taking 1-(4-((3-(1,4-diazepane-1-carbonyl)piperidin-1-yl)methyl)-2-fluorophenyl)-3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)urea **22** (0.3 g, 0.56 mmol) and acetic acid (40 mg, 0.67 mmol) as starting material, compound **G1**, **m.p.** 99–100°C. **1H NMR** (400 MHz, CDCl$_3$): δ (ppm) 8.02–7.97 (m, 1H), 7.02–7.69 (m, 2H), 6.85 (br, 1H), 5.20 (m, 1H), 3.84–3.73 (m, 1H), 3.69–3.58 (m, 2H), 3.56–3.49 (m, 4H), 3.47–3.33 (m, 4H), 2.90–2.75 (m, 3H), 2.37–2.38 (m, 2H), 2.15–2.14 (m, 2H), 1.98–1.92 (m, 1H), 1.84 (s, 4H), 1.74–1.69 (m, 2H), 1.66 (s, 4H), 1.64–1.59 (m, 1H), 1.55–1.46 (m, 1H), 1.40–1.37 (m, 2H), 1.33–1.24 (m, 3H), 1.20–1.13 (m, 3H), 1.12–1.08 (m, 2H), 0.85 (s, 6H). **13C NMR** (100 MHz, CDCl$_3$): δ (ppm) 174.19, 173.76, 173.59, 173.16, 154.02, 125.08, 120.93, 115.33,

115.14, 62.39, 56.09, 55.92, 53.42, 52.95, 50.66, 48.56, 47.47, 47.38, 46.92, 46.21, 44.53, 44.29, 42.74, 40.78, 39.47, 32.45, 30.11, 27.84, 27.67, 27.08, 26.54, 25.99, 24.75, 9.57, 9.41. **HRMS** (ESI) calcd for $C_{34}H_{51}FN_5O_3$ [M+H]$^+$: 596.3898, found 596.4743.

1-((1r,3R,5S,7r)-3,5-Dimethyladamantan-1-yl)-3-(2-fluoro-4-((3-(4-(2-methylbutanoyl) –1,4-diazepane-1-carbonyl)piperidin-1-yl)methyl)phenyl)urea (G2).

—According to the synthetic process of compound **5**, compound **G2** as a white solid (0.21 g, 60 %) was obtained by taking 1-(4-((3-(1,4-diazepane-1-carbonyl)piperidin-1-yl)methyl)-2-fluorophenyl)-3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)urea **22** (0.3 g, 0.56 mmol) and 2-methylbutanoic acid (78 mg, 0.67 mmol) as starting material, compound **G2**, **m.p.** 108–110°C. **¹H NMR** (400 MHz, CDCl₃): δ (ppm) 8.04–7.95 (m, 1H), 7.02–7.95 (m, 2H), 6.83 (br, 1H), 5.18 (m, 1H), 3.87–3.74 (m, 1H), 3.66–3.55 (m, 4H), 3.53–3.46 (m, 4H), 3.40–3.38 (m, 2H), 2.84–2.73 (m, 3H), 2.55–2.51 (m, 1H), 2.15 (s, 2H), 2.00–1.84 (m, 6H), 1.71–1.62 (m, 7H), 1.58–1.53 (m, 2H), 1.43–1.37 (m, 3H), 1.31 (s, 1H), 1.26–1.24 (m, 1H), 1.19–1.15 (m, 2H), 1.12–1.05 (m, 2H), 0.88–0.83 (m, 9H). **¹³C NMR** (100 MHz, CDCl₃): δ (ppm) 176.63, 176.48, 174.21, 174.12, 154.05, 125.02, 120.92, 115.30, 115.11, 62.45, 56.20, 56.10, 55.99, 53.51, 52.95, 50.66, 48.22, 42.75, 40.78, 37.34, 32.46, 30.23, 30.11, 28.04, 27.88, 27.68, 27.42, 27.35, 27.26, 27.13, 27.01, 24.81, 17.90, 17.46, 10.04. **HRMS** (ESI) calcd for $C_{36}H_{55}FN_5O_3$ [M+H]$^+$: 624.4211, found 624.4286.

1-(4-((3-(4-acryloyl-1,4-Diazepane-1-carbonyl)piperidin-1-yl)methyl)-2-fluorophenyl) –3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)urea (G3).

—According to the synthetic process of compound **5**, compound **G3** as a white solid (0.21 g, 63 %) was obtained by taking 1-(4-((3-(1,4-diazepane-1-carbonyl)piperidin-1-yl)methyl)-2-fluorophenyl)-3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)urea **22** (0.3 g, 0.56 mmol) and acrylic acid (55 mg, 0.67 mmol) as starting material, compound **G3**, **m.p.** 62–65°C. **¹H NMR** (400 MHz, DMSO-d_6): δ (ppm) 8.93–8.85 (m, 2H), 8.17 (s, 2H), 7.20–7.09 (m, 2H), 6.52 (s, 1H), 6.51–5.59 (m, 1H), 3.65–3.59 (m, 8H), 3.53–3.51 (m, 6H), 2.09 (s, 1H), 1.75–1.60 (m, 6H), 1.58 (s, 5H), 1.34–1.32 (m, 5H), 1.28–1.23 (m, 3H), 0.86 (s, 6H). **¹³C NMR** (100 MHz, DMSO-d_6): δ (ppm) 168.5, 165.7, 165.3, 153.9, 150.5, 128.9, 128.3, 128.0, 119.8, 52.9, 52.7, 52.6, 52.2, 52.1, 50.7, 48.2, 44.8, 44.3, 42.7, 32.4, 30.5, 30.0, 27.7, 27.3, 25.6, 25.2, 24.8. **HRMS** (ESI) calcd for $C_{34}H_{49}FN_5O_3$ [M+H]$^+$: 594.3741, found 594.3817.

1-((1r,3R,5S,7r)-3,5-Dimethyladamantan-1-yl)-3-(2-fluoro-4-((3-(4-methacryloyl-1,4-diazepane-1-carbonyl)piperidin-1-yl)methyl)phenyl)urea (G4).

—According to the synthetic process of compound **5**, compound **G4** as a white solid (0.25 g, 65 %) was obtained by taking 1-(4-((3-(1,4-diazepane-1-carbonyl)piperidin-1-yl)methyl)-2-fluorophenyl)-3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)urea **22** (0.3 g, 0.56 mmol) and methacrylic acid (65 mg, 0.67 mmol) as starting material, compound **G4**, **m.p.** 79–81°C. **¹H NMR** (400 MHz, DMSO-d_6): δ (ppm) 7.99 (s, 1H), 7.19–7.02 (m, 2H), 5.15 (s, 1H), 4.96–4.93 (s, 1H), 3.60–3.49 (m, 7H), 3.13–3.11 (m, 4H), 2.15–2.08 (m, 1H), 1.93–1.90 (m, 2H), 1.84 (m, 2H), 1.77–1.76 (m, 2H), 1.66 (m, 3H), 1.31–1.25 (m, 14H), 1.17–1.15 (m, 2H), 0.85 (s, 6H), 0.80–0.77 (m, 2H). **¹³C NMR** (100 MHz, DMSO-d_6): δ (ppm) 171.9, 171.4, 153.9, 152.1, 150.5, 141.0, 119.8, 115.1, 114.5, 60.1, 52.1, 50.7, 49.4, 48.5,

48.0, 46.7, 46.4, 45.6, 44.9, 43.9, 43.3, 42.7, 32.5, 32.4, 32.2, 30.5, 30.0, 28.2, 28.0, 23.0, 20.7, 20.6, 19.1. **HRMS** (ESI) calcd for $C_{35}H_{51}FN_5O_3$ [M+H]$^+$: 608.3898, found 608.3969.

1-(4-((3-(4-(Cyclopropanecarbonyl)-1,4-diazepane-1-carbonyl)piperidin-1-yl)methyl) –2-fluorophenyl)-3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)urea (G5).—According to the synthetic process of compound **5**, compound **G5** as a white solid (0.20 g, 61 %) was obtained by taking 1-(4-((3-(1,4-diazepane-1-carbonyl)piperidin-1-yl)methyl)-2-fluorophenyl)-3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)urea **22** (0.3 g, 0.56 mmol) and cyclopropanecarboxylic acid (65 mg, 0.67 mmol) as starting material, compound **G5, m.p.** 106–108°C. ^{1}**H NMR** (400 MHz, DMSO-d_6): δ (ppm) 8.00–7.96 (m, 1H), 7.05–6.98 (m, 2H), 6.74 (s, 1H), 5.04 (s, 1H), 3.76–3.48 (m, 12H), 2.87–2.78 (m, 3H), 2.16–2.14 (m, 1H), 1.93–1.90 (m, 1H), 1.83–1.73 (m, 4H), 1.72–1.66 (m, 8H), 1.40–1.37 (m, 2H), 1.28–1.26 (m, 2H), 1.20–1.12 (m, 2H), 0.96–0.91 (m, 2H), 0.85 (s, 6H), 0.77–0.75 (m, 2H). ^{13}**C NMR** (100 MHz, DMSO-d_6): δ (ppm) 169.6, 168.8, 168.4, 167.7, 149.5, 149.1, 120.5, 116.4, 110.7, 49.3, 48.7, 48.1, 45.9, 43.6, 43.1, 42.7, 42.5, 40.3, 39.7, 37.9, 37.4, 36.1, 28.0, 27.7, 25.6, 25.1, 23.7, 23.7, 23.0, 22.4, 21.9, 6.4, 4.4, 3.0, 2.8. **HRMS** (ESI) calcd for $C_{35}H_{51}FN_5O_3$ [M+H]$^+$: 608.3898, found 608.3972.

Tert-butyl 4-(3-fluoro-4-nitrobenzoyl)-1,4-diazepane-1-carboxylate (23).—According to the synthetic process of compound **5**, compound **23** as a light yellow oil (7.94 g) was obtained by taking 3-fluoro-4-nitrobenzoic acid (5 g, 27.03 mmol) and *tert*-butyl 1,4-diazepane-1-carboxylate (6.49 g, 32.44 mmol) as starting material, compound **23**, which was used without further purification, **ESI-MS**: m/z 390.16 [M+Na]$^+$.

Tert-butyl 4-(4-amino-3-fluorobenzoyl)-1,4-diazepane-1-carboxylate (24).—According to the synthetic process of compound **2**, compound **24** as a light yellow oil (6.20 g) was obtained by taking *tert*-butyl 4-(3-fluoro-4-nitrobenzoyl)-1,4-diazepane-1-carboxylate **23** (7.94 g, 21.63 mmol) as starting material, compound **24**, which was used without further purification, **ESI-MS**: m/z 360.1 [M+Na]$^+$.

Tert-butyl-4-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzoyl) –1,4-diazepane-1-carboxylate (25).—According to the synthetic process of compound **3**, compound **25** as a light yellow oil (6.78 g) was obtained by taking *tert*-butyl 4-(4-amino-3-fluorobenzoyl)-1,4-diazepane-1-carboxylate **24** (6.20 g, 18.40 mmol) as starting material, compound **25**, which was used without further purification, ^{1}**H NMR** (400 MHz, CDCl$_3$): δ (ppm) 8.13 (t, *J* = 7.8 Hz, 1H), 7.09–6.95 (m, 2H), 6.88–6.78 (m, 2H), 3.76–3.43 (m, 10H), 2.15 (t, *J* = 2.9 Hz, 1H), 1.96 (s, 1H), 1.83–1.82 (m, 2H), 1.64 (s, 5H), 1.47 (s, 7H), 1.39 (s, 2H), 1.31–1.26 (m, 2H), 1.19–1.12 (m, 2H), 0.85 (s, 6H).

1-(4-(1,4-Diazepane-1-carbonyl)-2-fluorophenyl)-3-((1r,3R,5S,7r)-3,5-dimethy ladamantan-1-yl)urea (26).—According to the synthetic process of compound **20**, compound **26** as a light yellow oil (4.98 g) was obtained by taking *tert*-butyl-4-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzoyl)-1,4-diazepane-1-carboxylate **25** (6.78 g, 12.51 mmol) as starting material, compound **26**, which was used without further purification, ^{1}**H NMR** (400 MHz, CDCl$_3$):

δ (ppm) 8.25–8.21 (m, 1H), 7.80–7.61 (m, 1H), 7.05–7.01 (m, 2H), 5.99 (s, 1H), 3.82–3.47 (m, 7H), 2.71–2.01 (m, 5H), 1.79 (s, 2H), 1.12–1.61 (m, 4H), 1.37–1.24 (m, 5H), 1.17–1.09 (m, 2H), 0.83 (s, 6H).

Tert-butyl-4-(2-(4-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzoyl)-1,4-diazepan-1-yl)-2-oxoethyl)piperidine-1-carboxylate (27).—

According to the synthetic process of compound **5**, compound **27** as a light yellow oil (4.98 g) was obtained by taking 1-(4-(1,4-diazepane-1-carbonyl)-2-fluorophenyl)-3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)urea **26** (4.98 g, 11.27 mmol) and 2-(1-(*tert*-butoxycarbonyl)piperidin-4-yl)acetic acid (3.29 g, 13.52 mmol) as starting material, compound **27**, which was used without further purification, **¹H NMR** (400 MHz, CDCl₃): δ (ppm) 8.23–8.16 (m, 1H), 7.16–7.15 (m, 1H), 7.06–7.00 (m, 2H), 4.08 (t, *J* = 11.48 Hz, 2H), 3.81–3.45 (m, 10H), 2.75–2.67 (m, 2H), 2.30–2.24 (m, 3H), 2.15–2.14 (m, 1H), 2.04–2.00 (m, 3H), 1.82 (s, 2H), 1.72–1.64 (m, 7H), 1.45–1.44 (m, 11H), 1.39–1.25 (m, 5H), 1.16–1.11 (m, 4H), 0.85 (s, 6H).

1-((1r,3R,5S,7r)-3,5-Dimethyladamantan-1-yl)-3-(2-fluoro-4-(4-(2-(piperidin-4-yl)acetyl)-1,4-diazepane-1-carbonyl)phenyl)urea (28).—

According to the synthetic process of compound **20**, compound **28** as a light yellow oil (5.82 g) was obtained by taking ert-butyl-4-(2-(4-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzoyl)-1,4-diazepan-1-yl)-2-oxoethyl)piperidine-1-carboxylate **27** (6.46 g, 9.69 mmol) as starting material, compound **28**, which was used without further purification, **ESI-MS**: m/z 568.44 [M+H]⁺.

1-((1r,3R,5S,7r)-3,5-Dimethyladamantan-1-yl)-3-(2-fluoro-4-(4-(2-(1-propionyl piperidin-4-yl)acetyl)-1,4-diazepane-1-carbonyl)phenyl)urea (H1).—

According to the synthetic process of compound **5**, compound **H1** as a white solid (0.20 g, 61 %) was obtained by taking 1-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)-3-(2-fluoro-4-(4-(2-(piperidin-4-yl)acetyl)-1,4-diazepane-1-carbonyl)phenyl)urea **28** (0.3 g, 0.53 mmol) and propionic acid (65 mg, 0.67 mmol) as starting material, compound **H1**, **m.p.** 101–102°C. **¹H NMR** (400 MHz, CDCl₃): δ (ppm) 8.27–8.22 (m, 1H), 7.68–7.55 (m, 1H), 7.04–7.02 (m, 2H), 4.60 (s, 1H), 3.72–3.46 (m, 9H), 3.02 (s, 1H), 2.58 (s, 1H), 2.37–2.25 (m, 4H), 2.13–1.99 (m, 4H), 1.88 (s, 4H), 1.63 (s, 5H), 1.38–1.26 (m, 4H), 1.14–1.11 (m, 7H), 0.84 (s, 6H). **¹³C NMR** (100 MHz, DMSO-*d₆*): δ (ppm) 169.9, 169.8, 168.1, 153.0, 129.4, 129.3, 127.1, 121.7, 119.1, 112.5, 59.4, 52.8, 51.8, 49.6, 47.1, 45.6, 41.7, 41.0, 40.8, 39.6, 32.0, 31.9, 31.7, 31.7, 31.4, 30.9, 29.2, 29.1, 20.4, 20.0, 17.6, 16.4, 13.2, 10.9. **HRMS** (ESI) calcd for $C_{35}H_{51}FN_5O_4$ [M+H]⁺: 624.3847, found 624.3921.

1-((1r,3R,5S,7r)-3,5-Dimethyladamantan-1-yl)-3-(2-fluoro-4-(4-(2-(1-(2-methyl butanoyl)piperidin-4-yl)acetyl)-1,4-diazepane-1-carbonyl)phenyl)urea (H2).—

According to the synthetic process of compound **5**, compound **H2** as a white solid (0.20 g, 60 %) was obtained by taking 1-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)-3-(2-fluoro-4-(4-(2-(piperidin-4-yl)acetyl)-1,4-diazepane-1-carbonyl)phenyl)urea **28** (0.3 g, 0.53 mmol) and 2-methylbutanoic acid (68 mg, 0.67 mmol) as starting material, compound **H2**, **m.p.** 106–107°C. **¹H NMR** (400 MHz, CDCl₃): δ (ppm) 8.26–8.21 (m, 1H), 7.57–7.42 (m,

1H), 7.05–7.00 (m, 2H), 4.63 (s, 1H), 3.73–3.46 (m, 9H), 3.02 (s, 1H), 2.66–2.59 (m, 2H), 2.30–2.27 (m, 2H), 2.14 (s, 2H), 1.99 (s, 1H), 1.82 (s, 4H), 1.71–1.64 (m, 5H), 1.40–1.36 (m, 3H), 1.30–1.19 (m, 3H), 1.19–1.10 (m, 3H), 1.10–1.07 (m, 4H), 0.90–0.87 (m, 3H), 0.83 (s, 6H). **^{13}C NMR** (100 MHz, DMSO-d_6): δ (ppm) 175.1, 171.2, 171.0, 170.8, 153.9, 130.2, 128.3, 122.8, 120.1, 133.6, 60.4, 52.8, 50.7, 48.1, 45.7, 42.7, 42.11, 40.7, 39.6, 39.1, 36.9, 33.3, 33.2, 32.4, 30.2, 30.1, 27.1, 21.1, 17.3, 14.2, 10.7. **HRMS** (ESI) calcd for $C_{37}H_{55}FN_5O_4$ [M+H]$^+$: 652.4160, found 652.4235.

1-((1r,3R,5S,7r)-3,5-Dimethyladamantan-1-yl)-3-(4-(4-(2-(1-methacryloylpiperidin-4-yl)acetyl)-1,4-diazepane-1-carbonyl)phenyl)urea (H3).—According to the synthetic process of compound **5**, compound **H3** as a white solid (0.18 g, 56 %) was obtained by taking 1-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)-3-(2-fluoro-4-(4-(2-(piperidin-4-yl)acetyl)-1,4-diazepane-1-carbonyl)phenyl)urea **28** (0.3 g, 0.53 mmol) and methacrylic acid (58 mg, 0.67 mmol) as starting material, compound **H3**, **m.p.** 95–96°C. **^{1}H NMR** (400 MHz, CDCl$_3$): δ (ppm) 8.27–8.22 (m, 1H), 7.68–7.56 (m, 1H), 7.04–7.02 (m, 2H), 4.60 (s, 1H), 3.72–3.46 (m, 9H), 3.62 (s, 1H), 2.58 (s, 1H), 2.37–2.25 (m, 4H), 2.13–1.99 (m, 4H), 1.81 (s, 4H),1.63 (s, 5H), 1.38–1.24 (m, 4H), 1.14–1.11 (m, 7H), 0.84 (s, 6H). **^{13}C NMR** (100 MHz, DMSO-d_6): δ (ppm) 171.2, 171.0, 170.9, 154.1, 130.4, 130.3, 128.1, 122.7, 120.1, 113.5, 60.4, 52.7, 50.6, 48.1, 46.4, 45.7, 43.9, 42.7, 41.9, 40.6, 39.6, 39.1, 33.2, 33.1, 32.4, 32.0, 30.2, 30.1, 29.7, 29.3, 26.6, 21.1, 14.2, 9.7. **HRMS** (ESI) calcd for $C_{36}H_{52}N_5O_4$ [M+H]$^+$: 618.3941, found 618.3425.

1-((1r,3R,5S,7r)-3,5-Dimethyladamantan-1-yl)-3-(4-(4-(2-(1-(1-(dimethylamino) propan-2-yl)piperidin-4-yl)acetyl)-1,4-diazepane-1-carbonyl)-2-fluorophenyl)urea (H4).—According to the synthetic process of compound **12**, compound **H4** as a white solid (0.16 g, 46 %) was obtained by taking 1-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)-3-(2-fluoro-4-(4-(2-(piperidin-4-yl)acetyl)-1,4-diazepane-1-carbonyl)phenyl) urea **28** (0.3 g, 0.53 mmol) and 2-chloro-*N*,*N*-dimethylpropan-1-amine (81 mg, 0.67 mmol) as starting material, compound **H4**, **m.p.** 112–113°C. **^{1}H NMR** (400 MHz, CDCl$_3$): δ (ppm) 8.28–8.20 (m, 1H), 7.76–7.57 (m, 1H), 7.05–7.02 (m, 2H), 5.97–5.84 (m, 1H), 3.81–3.40 (m, 9H), 2.88–2.80 (m, 3H), 2.47–2.43 (m, 1H), 2.31–2.25 (m, 7H), 2.25–2.23 (m, 2H), 2.13–2.12 (m, 2H), 2.04–1.91 (m, 3H), 1.80 (s, 2H), 1.67–1.62 (m, 6H), 1.37–1.26 (m, 7H), 1.81–1.11 (m, 2H), 1.01–0.99 (m, 4H), 0.84 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 174.3, 168.3, 153.9, 151.8, 150.2, 130.3, 123.8, 119.4, 114.2, 73.6, 61.5, 60.6, 58.2, 53.2, 52.1, 50.7, 47.9, 46.6, 45.9, 42.7, 42.2, 32.4, 32.3, 30.5, 30.0, 29.4, 29.1, 19.2. **HRMS** (ESI) calcd for $C_{37}H_{58}FN_6O_3$ [M+H]$^+$: 653.4476, found 653.4549.

Tert-butyl 4-(3-fluoro-4-nitrobenzyl)-1,4-diazepane-1-carboxylate (29).—
According to the synthetic process of compound **12**, compound **29** as a light yellow oil (6.29 g) was obtained by taking 4-(bromomethyl)-2-fluoro-1-nitrobenzene (5.0 g, 21.46 mmol) and *tert*-butyl 1,4-diazepane-1-carboxylate (5.15 g, 25.76 mmol) as starting material, compound **29**, which was used without further purification, **ESI-MS**: m/z 376.17 [M+Na]$^+$.

Tert-butyl 4-(4-amino-3-fluorobenzyl)-1,4-diazepane-1-carboxylate (30).—
According to the synthetic process of compound **2**, compound **30** as a light yellow oil (5.24 g) was obtained by taking *tert*-butyl 4-(3-fluoro-4-nitrobenzyl)-1,4-diazepane-1-carboxylate **29** (6.29 g, 17.82 mmol) as starting material, compound **30**, which was used without further purification, **ESI-MS**: m/z 324.20 [M+H]$^+$.

Tert-butyl-4-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzyl) –1,4-diazepane-1-carboxylate (31).—According to
the synthetic process of compound **3**, compound **31**
as a light yellow oil (5.82 g) was obtained by taking used *tert*-butyl-4-(4-amino-3-fluorobenzyl)-1,4-diazepane-1-carboxylate **30** (5.24 g, 16.22 mmol) as starting material, compound **31**, which was used without further purification, **ESI-MS**: m/z 529.3 [M+H]$^+$.

1-(4-((1,4-Diazepan-1-yl)methyl)-2-fluorophenyl)-3-((1r,3R,5S,7r)-3,5-dimethyl adamantan-1-yl)urea (32).—According to the synthetic process of compound
20, compound **32** as a light yellow oil (4.15 g) was obtained
by taking used *tert*-butyl-4-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzyl)-1,4-diazepane-1-carboxylate **31** (5.82 g, 11.0 mmol) as starting material,
compound **32**, which was used without further purification, **ESI-MS**: m/z 529.45 [M+H]$^+$.

Tert-butyl-4-(2-(4-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzyl)-1,4-diazepan-1-yl)-2-oxoethyl)piperidine-1-carboxylate (33).—
According to the synthetic process of compound **5**, compound **33** as
a light yellow oil (4.94 g) was obtained by taking used 1-(4-((1,4-diazepan-1-yl)methyl)-2-fluorophenyl)-3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)urea **32** (4.15 g, 9.70 mmol) and 2-(1-(*tert*-butoxycarbonyl)piperidin-4-yl)acetic acid (2.72 g, 11.64 mmol) as starting material, compound **33**, which was used without further purification, **ESI-MS**: m/z 654.5 [M+H]$^+$.

1-((1r,3R,5S,7r)-3,5-Dimethyladamantan-1-yl)-3-(2-fluoro-4-((4-(2-(piperidin-4-yl) acetyl)-1,4-diazepan-1-yl)methyl)phenyl)urea (34).—According to the synthetic
process of compound **20**, compound **34** as a light yellow oil (3.77 g) was
obtained by taking used *tert*-butyl-4-(2-(4-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzyl)-1,4-diazepan-1-yl)-2-oxoethyl)piperidine-1-carboxylate **33** (4.94 g, 7.57 mmol) as starting material, compound **34**, which was used without further purification, **ESI-MS**: m/z 554.43 [M+H]$^+$.

1-(4-((4-(2-(1-Acetylpiperidin-4-yl)acetyl)-1,4-diazepan-1-yl)methyl)-2-fluorophenyl)-3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)urea (I1).—According
to the synthetic process of compound **5**, compound **I1** as a white
solid (0.20 g, 63 %) was obtained by taking 1-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)-3-(2-fluoro-4-((4-(2-(piperidin-4-yl)acetyl)-1,4-diazepan-1-yl)methyl)phenyl)urea **34** (0.3 g, 0.54 mmol) and acetic acid (39 mg, 0.65 mmol) as starting material, compound **I1**, **m.p.** 92–95°C. ^{1}**H NMR** (400 MHz, CDCl$_3$): δ (ppm) 8.05–8.00 (m, 1H), 7.11 (s, 1H), 7.03–6.99 (m, 2H), 6.95–6.93 (m, 1H), 5.47 (s, 1H), 4.63–4.57 (m, 1H), 3.81–3.78 (m,

1H), 3.72–3.65(m, 2H), 3.56–3.49 (m, 3H), 3.46–3.43 (m, 1H), 3.11–3.05 (m, 1H), 2.67 (s, 1H), 2.59–2.57 (m, 4H), 2.26–2.23 (m, 1H), 2.18 (s, 1H), 2.15–2.14 (m, 1H), 2.09 (s, 3H), 1.84 (s, 4H), 1.67 (s, 5H), 1.39–1.36 (m, 2H), 1.31–1.26 (m, 3H), 1.19–1.11 (m, 4H), 0.85 (s, 6H). **13C NMR** (100 MHz, CDCl$_3$): δ 171.12, 171.04, 169.01, 168.98, 154.25, 154.22, 151.15, 124.58, 120.72, 61.94, 61.58, 55.54, 55.34, 55.14, 53.68, 52.83, 52.81, 50.66, 48.19, 46.74, 46.70, 44.49, 42.75, 41.91, 41.86, 40.75, 39.51, 33.10, 32.80, 32.43, 31.98, 30.21, 30.14, 21.50. **HRMS** (ESI) calcd for C$_{34}$H$_{51}$FN$_5$O$_3$ [M+H]$^+$: 596.3898, found 596.3972.

1-((1r,3R,5S,7r)-3,5-Dimethyladamantan-1-yl)-3-(2-fluoro-4-((4-(2-(1-propionyl piperidin-4-yl)acetyl)-1,4-diazepan-1-yl)methyl)phenyl)urea (I2).—According to the synthetic process of compound **5**, compound **I2** as a white solid (0.19 g, 59 %) was obtained by taking 1-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)-3-(2-fluoro-4-((4-(2-(piperidin-4-yl)acetyl)-1,4-diazepan-1-yl)methyl)phenyl)urea **34** (0.3 g, 0.54 mmol) and propionic acid (48 mg, 0.65 mmol) as starting material, compound **I2**, **m.p.** 86–89°C. **1H NMR** (400 MHz, CDCl$_3$): δ (ppm) 8.04–7.99 (m, 1H), 7.10–7.09 (s, 1H), 7.01–6.93 (m, 3H), 5.45–5.41 (m, 1H), 4.65–4.58 (m, 1H), 3.85–3.82 (d, *J* = 12.56 Hz, 1H), 3.71–3.43(m, 6H), 3.07–3.01 (m, 1H), 2.66 (s, 1H), 2.60–2.56 (m, 4H), 2.35 (q, *J* = 7.48 Hz, 2H), 2.26–2.14 (m, 4H), 1.85–1.84 (m, 4H), 1.67 (s, 5H), 1.39–1.36 (m, 2H), 1.30–1.27 (m, 3H), 1.16–1.12 (m, 6H), 0.85 (s, 6H). **13C NMR** (100 MHz, CDCl$_3$): δ 172.36, 172.32, 171.17, 171.10, 154.26, 154.23, 124.57, 120.86, 120.77, 61.93, 61.59, 55.55, 55.41, 55.07, 52.83, 52.81, 50.67, 48.18, 45.81, 45.76, 42.75, 40.75, 39.56, 39.54, 33.24, 33.21, 32.89, 32.43, 32.05, 30.22, 30.14, 26.65, 26.64, 9.72, 9.68. **HRMS** (ESI) calcd for C$_{35}$H$_{53}$FN$_5$O$_3$ [M+H]$^+$: 610.4054, found 610.4096.

1-((1r,3R,5S,7r)-3,5-Dimethyladamantan-1-yl)-3-(2-fluoro-4-((4-(2-(1-(2-methyl butanoyl)piperidin-4-yl)acetyl)-1,4-diazepan-1-yl)methyl)phenyl)urea (I3).—According to the synthetic process of compound **5**, compound **I3** as a white solid (0.19 g, 57 %) was obtained by taking 1-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)-3-(2-fluoro-4-((4-(2-(piperidin-4-yl)acetyl)-1,4-diazepan-1-yl)methyl)phenyl)urea **34** (0.3 g, 0.54 mmol) and 2-methylbutanoic acid (66 mg, 0.65 mmol) as starting material, compound **I3**, **m.p.** 96–100°C. **1H NMR** (400 MHz, CDCl$_3$): δ (ppm) 7.99 (t, *J* = 8.36 Hz, 1H), 7.17 (d, *J* = 7.48 Hz, 1H), 7.06 (s, 1H), 6.99 (s, 1H), 6.96–6.93 (m, 1H), 5.50–5.48 (m, 1H), 4.68–4.61 (m, 1H), 3.97–3.83 (d, *J* = 12.76 Hz, 1H), 3.75–3.44 (m, 6H), 3.08–3.02 (m, 1H), 2.65–2.55 (m, 6H), 2.27–2.14 (m, 4H), 1.84 (s, 5H), 1.66 (s, 5H), 1.39–1.36 (m, 3H), 1.30–1.26 (m, 3H), 1.16–1.14 (m, 2H), 1.11–1.08 (m, 4H), 0.89–0.87 (m, 3H), 0.84 (s, 6H). **13C NMR** (100 MHz, CDCl$_3$): δ 175.04, 171.22, 171.14, 154.31, 154.31, 124.56, 121.00, 120.87, 114.94, 114.75, 61.91, 61.59, 55.53, 55.43, 52.81, 52.78, 50.68, 48.20, 47.17, 45.81, 45.35, 44.49, 42.76, 42.18, 40.74, 39.56, 36.95, 33.37, 33.27, 30.22, 30.13, 28.46, 27.51, 27.06, 17.39, 17.20, 12.01, 11.91. **HRMS** (ESI) calcd for C$_{37}$H$_{57}$FN$_5$O$_3$ [M+H]$^+$: 638.4367, found 638.4437.

Methyl-4-(2-(4-(4-(3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)ureido)-3-fluorobenzyl)-1,4-diazepan-1-yl)-2-oxoethyl)piperidine-1-carboxylate (I4).—According to the synthetic process of compound **5**, compound **I4** as a white solid (0.20 g, 60 %) was obtained by taking 1-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-

yl)-3-(2-fluoro-4-((4-(2-(piperidin-4-yl)acetyl)-1,4-diazepan-1-yl)methyl)phenyl)urea **34** (0.3 g, 0.54 mmol) and methyl carbonic acid (49 mg, 0.65

mmol) as starting material, compound **I4**, **m.p.** 121–122°C. **¹H NMR** (400 MHz, CDCl₃): δ (ppm) 8.05–8.00 (m, 1H), 7.24–7.20 (m, 1H), 7.00–6.93 (m, 2H), 5.61–5.60 (m, 1H), 4.13–4.12 (m, 2H), 3.68 (s, 3H), 3.64–3.59 (m, 2H), 3.53–3.51 (m, 3H), 3.46 (s, 1H), 2.78 (s, 2H), 2.64–2.60 (m, 2H), 2.56–2.55 (m, 2H), 2.25–2.19 (m, 2H), 2.13–2.12 (m, 1H),1.83–1.78 (m, 4H), 1.74–1.72 (m, 2H), 1.65 (s, 4H), 1.38–1.35 (m, 2H), 1.29–1.26 (m, 3H), 1.15–1.14 (m, 3H), 0.85 (s, 6H). **¹³C NMR** (100 MHz, DMSO-d_6): 171.32, 171.26, 156.04, 154.13, 124.56, 120.86, 114.84, 61.85, 61.85, 55.63, 55.58, 55.02, 53.77, 52.88, 52.56, 50.64, 48.19, 48.19, 47.15, 44.50, 44.11, 42.73, 40.76, 39.70, 39.66, 33.04, 32.15, 32.15, 30.20, 30.12, 30.12, 27.48. **HRMS** (ESI) calcd for C₃₄H₅₁FN₅O₄ [M+H]⁺: 612.3847, found 612.3920.

1-((1r,3R,5S,7r)-3,5-Dimethyladamantan-1-yl)-3-(2-fluoro-4-((4-(2-(1-(2-hydroxyethyl)piperidin-4-yl)acetyl)-1,4-diazepan-1-yl)methyl)phenyl)urea (I5).

—According to the synthetic process of compound **12**, compound **I5** as a white solid (0.13 g, 42 %) was obtained by taking 1-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)-3-(2-fluoro-4-((4-(2-(piperidin-4-yl)acetyl)-1,4-diazepan-1-yl)methyl)phenyl)urea **34** (0.3 g, 0.54 mmol) and 2-chloroethan-1-ol (52 mg, 0.65 mmol) as starting material, compound **I5**, **m.p.** 101–102°C. **¹H NMR** (400 MHz, DMSO-d_6): δ (ppm) 8.14 (s, 1H), 8.04–8.00 (m, 1H), 7.08–7.04 (m, 1H Hz), 6.96–6.94 (m, 1H),6.57 (s, 1H), 5.34 (s, 1H), 4.48 (s, 1H), 3.52–3.46 (m, 10 H), 2.90–2.88 (m, 2H), 2.59 (s, 1H), 2.44 (s, 3H), 2.21–2.17 (m, 2H), 2.08–2.05 (m, 3H), 1.75 (s, 4H), 1.65–1.62 (m, 4H), 1.57 (s, 4H), 1.34–1.31 (m, 4H), 1.11 (s, 2H), 0.82 (s, 6H). **¹³C NMR** (100 MHz, DMSO-d_6): δ 170.97, 154.17, 153.00, 150.61, 124.67, 120.04, 115.09, 114.90, 63.05, 60.72, 60.62, 58.61, 56.03, 55.22, 54.52, 54.01, 53.79, 52.48, 51.99, 50.75, 48.09, 47.75, 46.73, 44.99, 44.39, 42.80, 32.66, 32.37, 31.90, 30.55, 30.05, 28.50, 27.42, 7.66. **HRMS** (ESI) calcd for C₃₄H₅₃FN₅O₃ [M+H]⁺: 598.4054, found 598.4125.

1-(4-((4-(2-(1-(2,3-Dihydroxypropyl)piperidin-4-yl)acetyl)-1,4-diazepan-1-yl)methyl)-2-fluorophenyl)-3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)urea (I6).

—According to the synthetic process of compound **12**, compound **I6** as a white solid (0.13 g, 38 %) was obtained by taking 1-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)-3-(2-fluoro-4-((4-(2-(piperidin-4-yl)acetyl)-1,4-diazepan-1-yl)methyl)phenyl)urea **34** (0.3 g, 0.54 mmol) and 3-chloropropane-1,2-diol (71 mg, 0.65 mmol) as starting material, compound **I6**, **m.p.** 104–105°C. **¹H NMR** (400 MHz, DMSO-d_6): δ (ppm) 8.14 (s, 1H), 8.03–7.99 (m, 1H), 7.09–7.04 (m, 1H Hz), 6.98–6.94 (m, 1H),6.57 (s, 1H), 5.33 (s, 4H), 5.01 (s, 1H), 3.67 (s, 1H), 3.52–3.43 (m, 10 H), 2.99 (s, 3H), 2.60–2.59 (m, 2H), 2.21–2.19 (m, 4H), 2.09 (s, 1H), 1.75 (s, 5H), 1.68 (s, 5H), 1.57 (s, 3H), 1.34 (s, 1H), 1.31 (s, 1H), 1.11 (s, 2H), 0.82 (s, 6H). **¹³C NMR** (100 MHz, DMSO-d_6): δ 170.90, 154.18, 127.62, 124.68, 120.08, 65.23, 63.07, 56.03, 55.20, 54.51, 53.83, 52.50, 52.50, 50.76, 48.10, 48.10, 47.74, 46.71, 44.41, 42.80, 42.80, 32.38, 32.38, 30.55, 30.55, 30.05, 28.48, 27.39, 7.67, 7.67. **HRMS** (ESI) calcd for C₃₅H₅₅FN₅O₄ [M+H]⁺: 628.4160, found 628.4230.

1-((1r,3R,5S,7r)-3,5-Dimethyladamantan-1-yl)-3-(4-((4-(2-(1-(2-(dimethylamino) ethyl)piperidin-4-yl)acetyl)-1,4-diazepan-1-yl)methyl)-2-fluorophenyl)urea (I7).

—According to the synthetic process of compound **12**, compound **I7** as a white solid

(0.13 g, 39 %) was obtained by taking 1-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)-3-(2-fluoro-4-((4-(2-(piperidin-4-yl)acetyl)-1,4-diazepan-1-yl)methyl)phenyl)urea **34** (0.3 g, 0.54 mmol) and 2-chloro-*N,N*-dimethylethan-1-amine (69 mg, 0.65 mmol) as starting material, compound **I7**, **m.p.**108–109°C. **^{1}H NMR** (400 MHz, CDCl$_3$): δ (ppm) 8.08–8.01 (m, 1H), 7.01–6.96 (m, 1H), 6.94–6.91 (m, 1H), 5.62 (s, 2H), 3.62–3.61 (m, 5H), 3.54 (s, 1H), 3.51–3.48 (m, 2H), 3.44–3.42 (m, 1H), 3.08–3.06 (m, 2H), 2.72 (s, 1H), 2.66 (s, 1H), 2.61 (s, 3H), 2.57–2.55 (m, 1H), 2.43 (s, 2H), 2.37 (s, 2H), 2.32 (s, 1H), 2.25–2.23 (m, 2H), 2.20–2.18 (m, 1H), 2.13 (s, 1H), 1.95 (s, 1H), 1.88 (s, 2H), 1.80–1.77 (m, 4H), 1.70 (s, 3H), 1.39 (s, 1H), 1.36 (s, 1H), 1.25 (s, 4H), 1.16 (s, 1H), 1.13 (s, 1H), 0.84 (s, 6H). **^{13}C NMR** (100 MHz, CDCl$_3$): δ 171.33, 154.79, 154.73, 124.48, 120.86, 114.79, 114.61, 63.43, 62.07, 61.59, 53.95, 53.01, 53.01, 52.75, 50.74, 48.07, 47.26, 45.30, 45.15, 44.34, 42.83, 40.70, 39.37, 32.51, 32.51, 32.40, 31.62, 31.53, 30.20, 29.69, 28.69, 27.92, 8.13. **HRMS** (ESI) calcd for C$_{36}$H$_{58}$FN$_6$O$_2$ [M+H]$^+$: 625.4527, found 625.4597.

1-(4-((4-(2-(1-(2-(Diethylamino)ethyl)piperidin-4-yl)acetyl)-1,4-diazepan-1-yl)methyl) –2-fluorophenyl)-3-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)urea

(I8).—According to the synthetic process of compound **12**, compound **I8** as a white solid (0.11 g, 32 %) was obtained by taking 1-((1r,3R,5S,7r)-3,5-dimethyladamantan-1-yl)-3-(2-fluoro-4-((4-(2-(piperidin-4-yl)acetyl)-1,4-diazepan-1-yl)methyl)phenyl)urea **34** (0.3 g, 0.54 mmol) and 2-chloro-*N,N*-diethylethan-1-amine (88 mg, 0.65 mmol) as starting material, compound **I8**, **m.p.**111–112°C. **^{1}H NMR** (400 MHz, CDCl$_3$): δ (ppm) 8.06–7.98 (m, 1H), 6.94–6.89 (m, 1H), 6.83–6.77 (m, 1H), 5.57 (s, 2H), 4.55 (s, 3H), 3.57–3.55 (m, 5H), 3.46–3.43(m, 3H), 3.35 (s, 1H), 3.10–3.07 (m, 1H), 3.01–2.98 (m, 2H), 2.95–2.93 (m, 2H), 2.86–2.84 (m, 2H), 2.80–2.78 (m, 2H), 2.63 (s, 1H), 2.55 (s, 2H), 2.51 (s, 1H), 2.23–2.15 (m, 2H), 2.05 (s, 1H), 1.91 (s, 1H), 1.82 (s, 1H), 1.74 (s, 3H), 1.65 (s, 2H), 1.32–1.29 (m, 2H), 1.24 (s, 1H), 1.22–1.21 (m, 3H), 1.18–1.15 (m, 5H), 1.09 (s, 1H), 1.05 (s, 1H), 0.77 (s, 6H). **^{13}C NMR** (100 MHz, CDCl$_3$): δ 171.29,171.10, 154.99, 154.90, 124.63, 63.47, 61.60, 55.76, 55.44, 55.13, 54.78, 53.82, 53.04, 53.04, 52.69, 50.78, 48.31, 48.02, 48.02, 47.09, 44.09, 42.87, 40.68, 39.19, 32.38, 32.38, 32.07, 31.46, 31.19, 30.23,30.23, 29.68, 8.15. **HRMS** (ESI) calcd for C$_{38}$H$_{62}$FN$_6$O$_2$ [M+H]$^+$: 653.4840, found 653.4910.

Biological activity assays *in vitro*.

All HsEH and MsEH IC$_{50}$ values is the concentration of a compound that reduces the sEH activity by 50%. The IC$_{50}$ values were determined using a fluorescent-based assay [(3-phenyloxiranyl) acetic acid cyano(6-methoxynaphthalen-2-yl)methyl ester (PHOME) as the substrate][31].The fluorescent assay was used with purified recombinant human, or mouse sEH proteins. The enzymes were incubated at 37 °C with the inhibitors ([I]$_{final}$ = 0.4 – 100,000 nM) for 10 min in 25 mM Tris HCl buffer (pH = 7.4) containing 0.1 mg/mL of BSA and 1% of DMSO. The substrate (PHOME) was then added ([S]$_{final}$ = 50 mM). Activity was assessed by measuring the formation of the fluorescent 6-methoxynaphthaldehyde product (lex = 330 nm, lem = 465 nm) on a SpectraMax M2 (molecular devices). Results were obtained by regression analysis from a linear region of the curve.

Microsomal stability.

Human and SD rat hepatic microsomes were purchased from Research Institute for Liver Diseases (Shanghai, China) Co., Ltd. The incubation mixture consisted of microsomal protein in PBS buffer (100 mM, pH = 7.4) and 100 μg / mL **G1** in a final volume of 100 mL. The concentration of human hepatic microsomal protein was 0.5 mg/mL (0.53 mg / mL of SD rat). A 0.83 mg/mL NADPH solution was prepared and added to the PBS buffer. After the addition of the NADPH-generating system, the resulting mixture was incubated at 37 °C for 0, 10, 30, 60 min. The reaction was terminated by the addition of methanol 300 μL containing diphenhydramine (0.02 ng / mL). The mixture was vortexed for 1 min, centrifuged at $21,528 \times g$ for 10 min at 4 °C. After centrifugation, 200 μL of the supernatant was transferred to 96-well plate, and 400 mL of purified water was added, and the solution was mixing with shaker at 500 rpm for 5 min, and the final concentration of compound **G1** was analyzed by HPLC.

Pharmacokinetics study *in vivo*.

SPF-grade healthy Sprague-Dawley (3 females, 3 males, 8 weeks old), weighing 180 ± 20 g, were provided by Liaoning Changsheng Biotechnology Co., Ltd (Liaoning, China), license No. SCXK (Liao) 2015–0001. SD rats were housed under controlled environmental conditions at 22 – 24 °C, 50 – 60 % relative humidity, natural circadian rhythm, free access to water and food, and acclimatized for one week. The experiments were approved by the Animal Management and Use Committee of Shenyang Pharmaceutical University, and the experiments conformed to the relevant experimental animal research guidelines of the Ethics Committee. Six SD rats were randomly divided into two groups (half male and half female). Weigh before each dose to adjust the required volume. Three SD rats were given 10 mg / kg of compound **G1** by tail vein injection, and the other three SD rats were given 50 mg / kg of compound **G1** by gavage. Blood samples (*iv* groups) were collected at different times (0 min, 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, 6 h, 8 h). Blood samples (*po* groups) were collected at different times (0 min, 10 min, 30 min, 1h, 2h, 4h, 6h, 8h). Blood was collected from the orbital vein on an Eppendorf tube (1.5 mL) containing EDTA and then centrifuged at 4800 rpm for 10 minutes. Each plasma sample (50 μL) was mixed with 150 μL tolbutamide in acetonitrile (5 ng / mL) and centrifuged at 13000 rpm for 10 min. Afterwards, 50 μL of supernatant was reconstituted with 200 μL of acetonitrile and the final concentration of compound **G1** was analyzed by HPLC.

Percentage plasma protein binding (% PPB).

The detailed experimental procedure followed the procedures reported of our previously work[31]. The experimental assay was calibrated using warfarin of known % PPB: rat plasma (99.5 %).

In Vivo Efficacy.

All mice used for pharmacological experiments were C57BL/6 (males, body weight 20 – 22 g) and Kunming (female, weight 20–22 g) provided by Liaoning Changsheng Biotechnology Co., Ltd (Liaoning, China), License No. SCXK (Liao) 2015–0001. Mice were kept under controlled environmental conditions at 22 – 24 °C, 50 – 60 % relative humidity, natural

circadian rhythm, free access to water and food, and acclimatized for one week. The experiments were approved by the Animal Management and Use Committee of Shenyang Pharmaceutical University, and the experiments conformed to the relevant experimental animal research guidelines of the Ethics Committee.

Evaluation of the efficacy of compound G1 in the AIA model.

Twenty-four Kunming mice (female, body weight 20 –22 g) were randomly divided into three groups, namely CFA, CFA + **G1** and CFA + **Celecoxib** groups. The mice were fasted for 6 – 8 h and freely drinking water before the experiment. Each mouse was weighed and the basal pain threshold (s) was measured by XR1700 hot plate meter and the thickness and width of each mouse's paw was measured by micrometer, and then CFA 25μL / 25 g was injected right in the middle of the paw, and the mice showed obvious redness and swelling after modeling, and the behavior was significantly reduced, indicating successful modeling. The mice were weighed again 24 h after modeling and the pain threshold (s) was measured. Compound **G1** and celecoxib were dissolved in 10 % DMSO, 5 % Tween 80 and 85 % 0.9 % saline and injected intraperitoneally at the dose of **G1** and **celecoxib** (10 mg / kg, *ip*), and the same volume of 0.9 % saline was given to the CFA group. The pain threshold (s) of each mouse was measured at 10 min, 45 min, 120 min, and 240 min after drug administration, and the increase in pain threshold (%) = (pain threshold measured after drug administration (s) - pain threshold after CFA modeling (s) / pain threshold after CFA modeling $\times$ 100 %, and the thickness and width of the paw of each mouse were also measured by micrometer at 1 h, 3 h, 6 h, and 20 h after drug administration. The same volume of 0.9 % saline was given to the CFA group. The thickness and width of the paw of each mouse were measured by micrometer at 24h and 28h. Edema (%) = (m / thickness or width of the paw of the mouse before CFA modeling) $\times$ 100 % (m = thickness or width of the paw measured after drug administration - thickness or width of the paw of the mouse before CFA modeling).

Evaluation of the efficacy of compound G1 in the AP model.

Twenty Kunming mice (females, body weight 20 – 22 g) were randomly divided into five groups: solvent group, model group (*L*-Arg), **G1**(3 mg / kg) group, **G1**(8 mg / kg) group, and **celecoxib** group. The mice were fasted for 6–8 h and freely drinking water before the experiment. Each mouse was weighed and injected intraperitoneally with 20 % *L*-arginine (2 g / kg, *ip*) except for the solvent group, which was re-injected with 20% *L*-arginine (2 g / kg, *ip*) after an interval of 1 h. After 14 h of modeling, **G1**(3 mg / kg, *ip*) group, **G1**(8 mg / kg, *ip*) and **celecoxib** (5 mg / kg, *ip*) were dissolved in 10 % DMSO, 5 % Tween 80 and 85 % 0.9 % saline. **G1** and **celecoxib** (5 mg / kg, *ip*) were injected intraperitoneally, and the solvent group, and the modeling group (*L*-Arg) were injected intraperitoneally with an equal volume of 0.9 % saline. At 10 h of drug treatment, each group of mice was executed and the pancreatic tissue was removed and fixed in 10% formalin for the next step of sectioning. Basic sectioning process: embedding sections, dewaxing sections to water, staining, dehydration, transparency, sealing, slide scanning.

Evaluation of the efficacy of compound G1 in LPS-induced sepsis model.

Fifty C57BL/6 mice (males, body weight 20 – 22 g) were randomly divided into four groups: solvent group (12 mice), model group (LPS, 12 mice), **G1** group (14 mice) and **dexamethasone** group (12 mice). The mice were fasted for 6–8 h and freely drinking water before the experiment. After each mouse was weighed, except for the solvent group Which each mouse was injected intraperitoneally with LPS (30 mg / kg), and 4 h later compound **G1** and **dexamethasone** were dissolved with 10 % DMSO, 5 % Tween 80 and 85 % 0.9 % saline and injected intraperitoneally at the dose of **G1** and dexamethasone (5 mg / kg), and the solvent group, and the LPS group were injected intraperitoneally with an equal volume of 0.9 % saline. Six mice from each group were executed at 12 h of drug treatment to obtain blood for Western blot and ELISA assays. Immediately afterwards, mice were injected intraperitoneally second time at the dose of compound **G1** (5 mg / kg) and **dexamethasone** (5 mg / kg) and observed for death per hour.

Western blot. Total protein extraction and western blot were performed according to our previous study. The plasma of experimental animals was centrifuged at 5000rmp for 5min. The protein concentration determined with BCA Protein Assay Kit (Fdbiio science cat: FD2001). The proteins were mixed with loading buffer and denatured at 100 °C for 10 min. Then the lysates were separated using an SDS-PAGE gel, and the proteins were transferred onto 0.45-μm polyvinylidene difluoride (PVDF) membranes. The membranes were blocked with 5 % fat-free milk and were incubated overnight at 4 °C with mouse anti-COX-2 antibody (1: 2000, SANTA CRUZ Biothechnology, cat: sc-19999, USA), mouse anti-NOS-2 antibody (SANTA CRUZ Biothechnology, cat: sc-7271, USA), mouse anti-sEH antibody (1: 2000, SANTA CRUZ Biothechnology, cat: sc-166961, USA), mouse anti-VCAM-1 antibody (1: 2000, SANTA CRUZ Biothechnology, cat: sc13160, China), mouse anti-GAPDH antibody (1: 4000, Zhongshanjinqiao, cat: TA-08, China). Subsequently, the membranes were washed three times with TBST and were incubated for 1 h at room temperature with anti-mouse (COX-2, NOS2, sEH, VCAM-1, GAPDH, zhongshanjinqiao, cat: ZB-2305) horseradish peroxidase-conjugated secondary antibodies. Film was used to develop protein in darkroom.The expression of proteins in the plasma was respectively normalized to GAPDH as a loading control.

Enzyme-linked immunosorbent assay (ELISA).

The protein level of monocyte chemoattractant protein-5 (MCP-5), (Boster, Cat: EK1128, China), tumor necrosis factor α (TNF-α), (Multi Sciences, Cat: 70-EK282 / 4–96, China), interleukin-6 (IL-6), (Multi Sciences, Cat: EK206/3, China) in serum was measured using an ELISA kit according to the manufacturer's instructions. The concentrations of the MCP-5, TNF-α, IL-6 were quantified by reference to a standard curve.

Molecular Docking.

The X-ray crystal structures of sEH (PDB ID: 3WKE) was retrieved from the Protein Data Bank. The protein structures were prepared using the Protein Preparation Wizard module. Hydrogen atoms were added, water molecules in the entire system were removed, followed by energy minimization and optimization by the Discovery Studio 2016 software. Grids of sEH was generated using receptor-ligand interactions, following the standard procedure

recommended by Discovery Studio 2016 software. The small molecules was employed to prepare the compounds for molecular docking. To prepare ligand structures, hydrogens were added, and 3D geometries, ionization, and tautomeric states were generated. Finally, the ligand structures were minimized using the full minimization. The conformational ensembles were docked flexibly using libdock. Only poses with low energy conformations and good hydrogen-bond geometries were considered.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

ABBREVIATIONS USED

sEH	soluble epoxide hydrolase
WB	Western Blot
ELISA	enzyme linked immunosorbent assay
COX-2	cyclooxygenase-2
NOS-2	nitric oxide synthase-2
VCAM	vascular cell adhesion molecule
IL-6	interleukin-6
MCP-5	monocyte chemotactic protein-5
TNF-α	tumor necrosis factor-α
NF-κB	nuclear factor B
EET	epoxyeicosatrienoic acids
EPHX2	epoxide hydrolase 2
ER	endoplasmic reticulum
HATU	2-(7-Azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate
DIPEA	*N,N*-diisopropylethylamine
NADPH	nicotinamide adenine dinucleotide phosphate
TFA	trifluoroacetic acid

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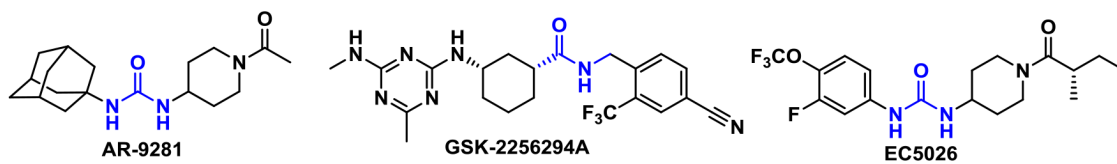
Figure 1.
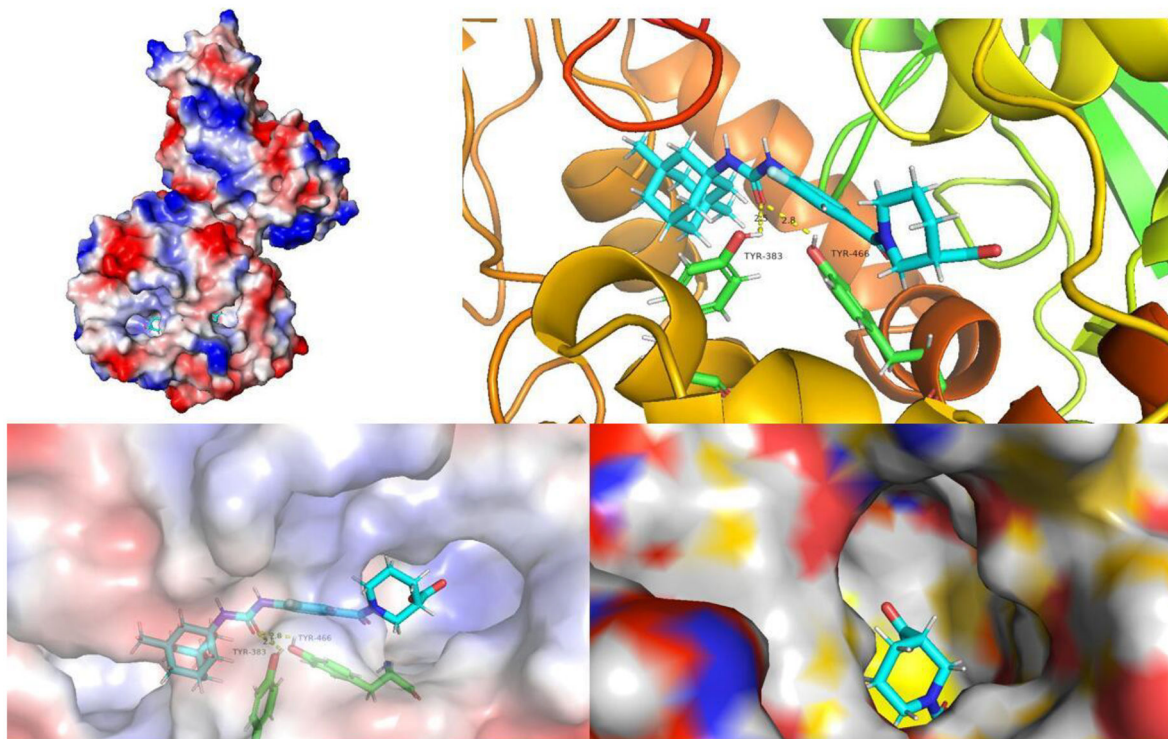Chemical structures of sEH inhibitors that moved into clinical trials.

Figure 2.
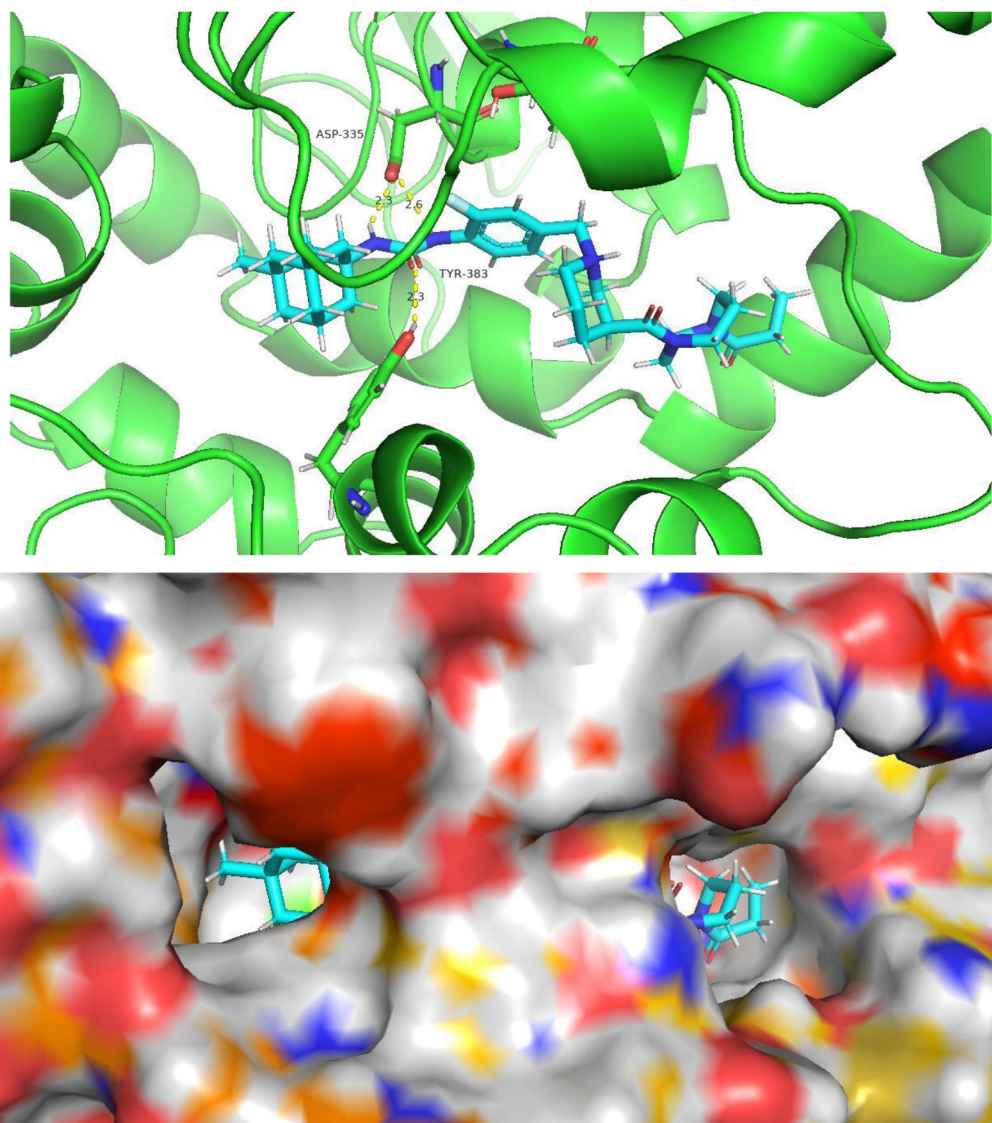Docked pose of compound **A** in blue bound to sEH (PDB ID: 3WKE).

Figure 3.
Molecular simulation of compounds **G1** bound to sEH (PDB ID: 3WKE)
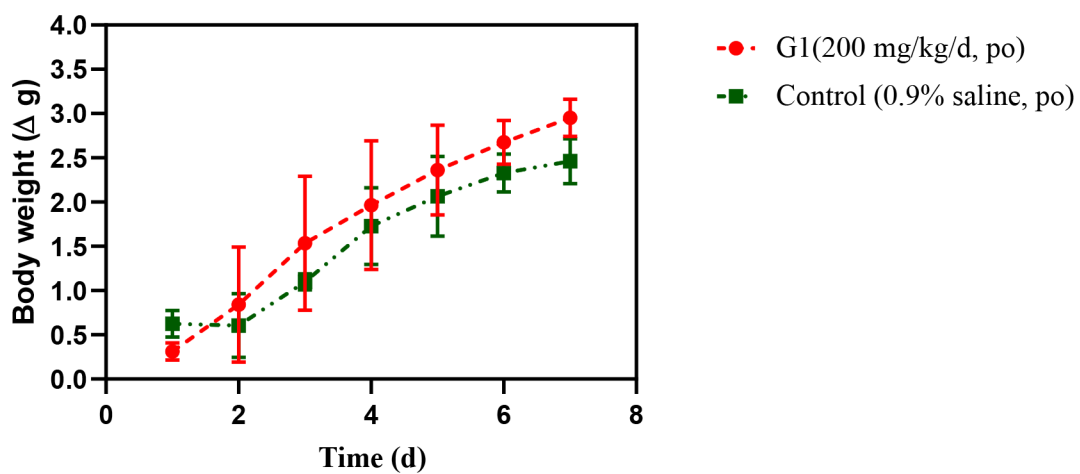
Figure 4.
Effect of compound **G1** on body weight of mice. **G1** was prepared in 0.5 % CMC-Na solution and administered orally at a dose of 200 mg / kg for seven consecutive days.
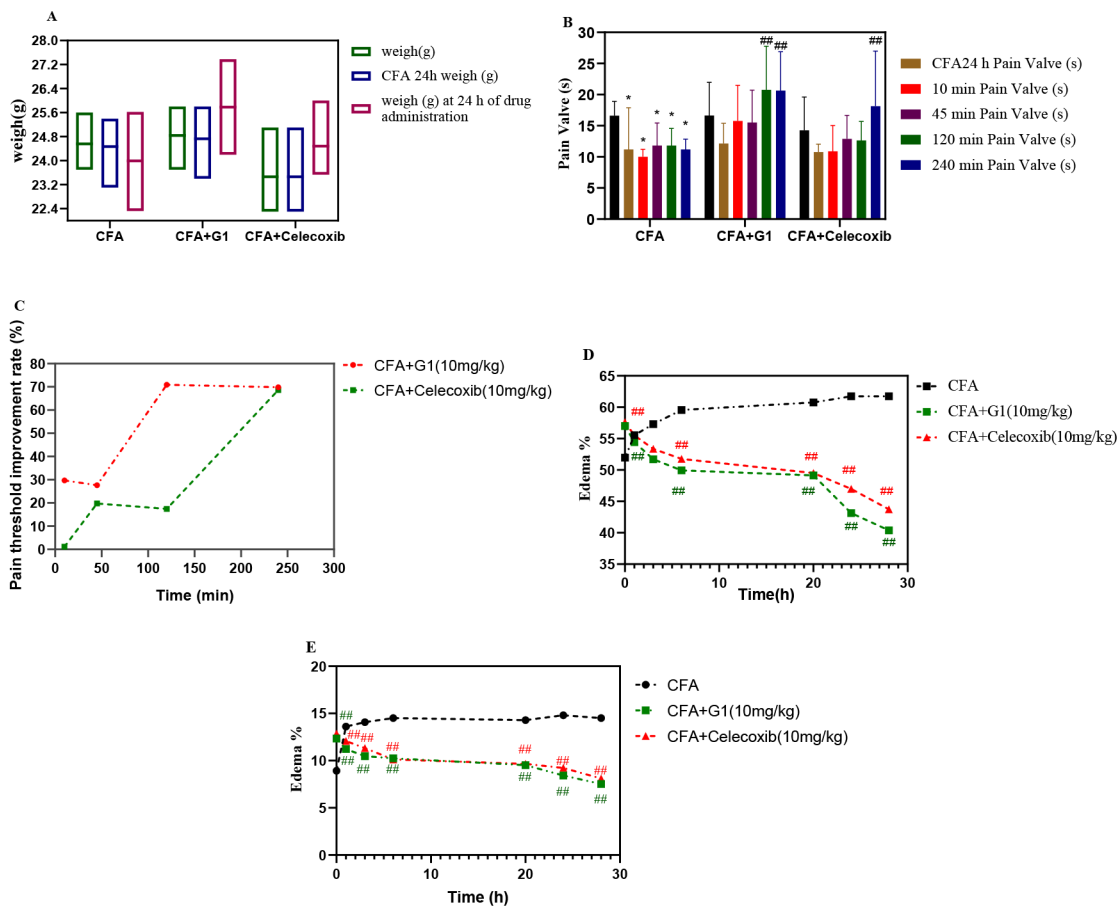
Figure 5.

Evaluation of compound **G1** for the treatment of (CFA)-induced arthritis in a mouse model (AIA); (**A**) Body weight changes of mice in CFA group, CFA + **G1** group and CFA + **Celecoxib** group; (**B**) Changes in pain thresholds of mice in CFA group, CFA + **G1** group and CFA + **Celecoxib** group; (**C**) Curves of pain threshold improvement in mice in CFA + **G1** group and CFA + **Celecoxib** group ; (**D**) Curves of paw thickness swelling rate of mice in CFA group, CFA + **G1** group and CFA + **Celecoxib** group; (**E**) Curves of paw width swelling rate of mice in CFA group, CFA + **G1** group and CFA + **Celecoxib** group; **G1** (10 mg / kg, *ip*) and **Celecoxib** (10 mg / kg, *ip*) were administered to mice 24 h and 44 h after induction of CFA by AAI (25 μL / 25g). (n = 8 per group); Significance: $^{##}P < 0.01$ and $^{#}P < 0.05$ compared with the Mod group; $**P < 0.01$ and $*P < 0.05$ compared with the Con group.
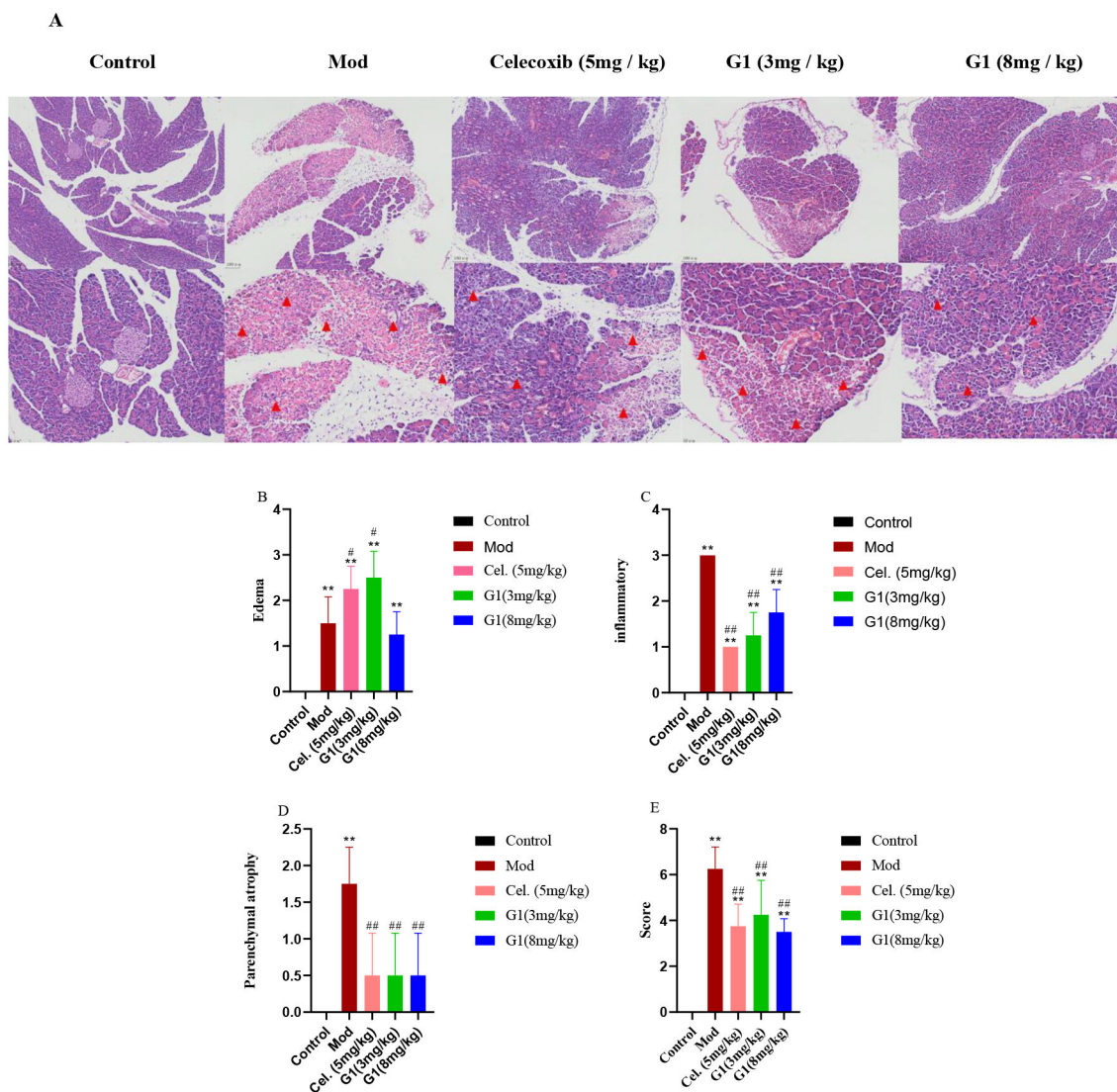
Figure 6.

Results of the histologic analysis of pancreas from mice treated with control, Model, Celecoxib and compound **G1**. (**A**) Representative H&E-stained sections of the pancreas from the *in vivo* efficacy study. Arrows indicate inflammatory cells and edema. Bold arrows indicate intracellular vacuoles (the red arrows on the image represent edema and inflammatory cells). (**B-E**) Histologic scoring of pancreatic tissues. (**B**), edema. (**C**), inflammatory cells (mononuclear and polymorphonuclear). (**D**), parenchymal atrophy. (**E**), total scoring (edema, mononuclear and polymorphonuclear and parenchymal atrophy). (n = 4 per group); Significance: $*p < 0.05$, and $**p < 0.01$ *vs* control. $\#p < 0.05$ and $\#\#p < 0.01$ *vs* Mod.
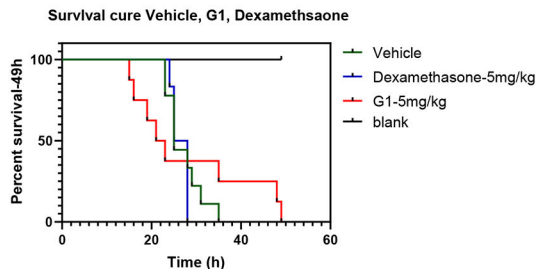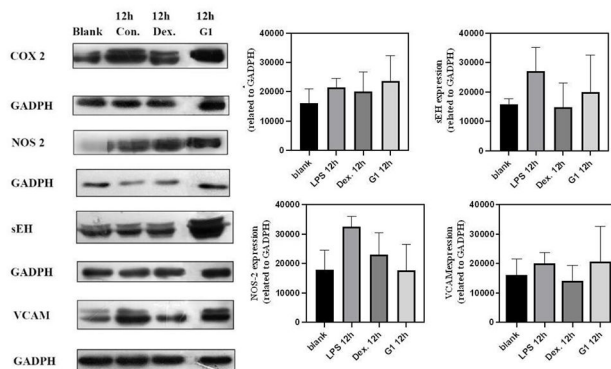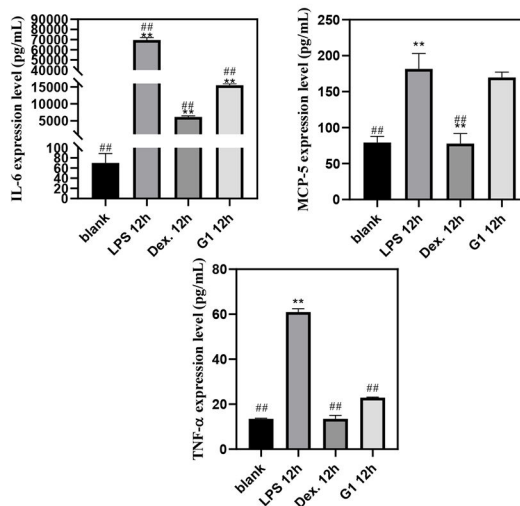
Figure 7.
Prophylactic and therapeutic treatment of **G1** prevents death from LPS administration in
mice. **G1** (5 mg / kg, *ip*) and **Dexamethasome** (5 mg / kg, *ip*) were administered to mice 4 h
and 12 h after induction of ALI by a lethal dose of LPS (30 mg / kg, *ip*). The mortality of the
mice was monitored per hour, (**A**) the percent survival rate was expressed as a Kaplan-Meier
survival curve (n = 12–14 per group); (**B**) The expression of inflammatory factors of COX-2,
sEH, NOS-2 and VCAM in mouse plasma was detected by Western blot; (**C**) Measurement
of plasma IL-6, MCP-5 and TNF-α inflammatory factors in mice by ELISA. Significance:

$^{\#\#}P < 0.01$ and $^{\#}P < 0.05$ compared with the LPS group; $^{**}P < 0.01$ and $^{*}P < 0.05$ compared with the blank group.
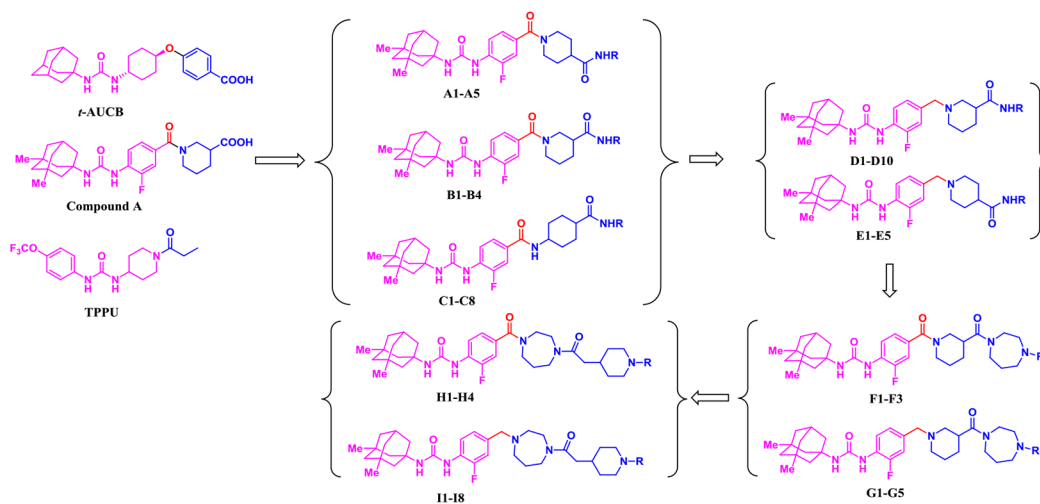
Scheme 1.
Design strategy of target compounds based on lead compound **A**
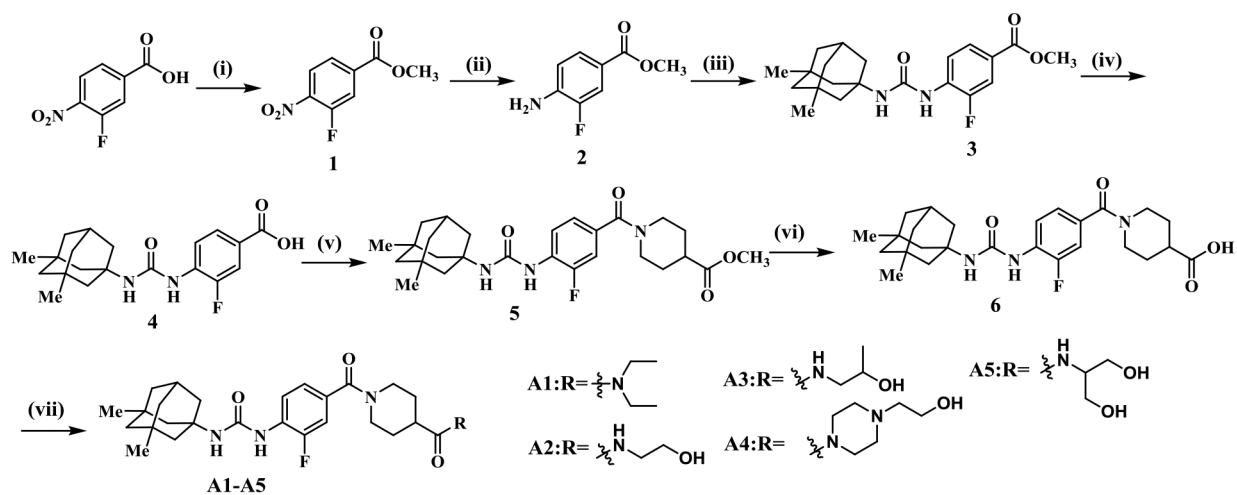
Scheme 2.

Synthetic route to compounds **A1-A5**. Reactions and conditions: (i) $SOCl_2$, MeOH, rt; (ii) H_2 (g), 5 % Pd-C, EtOH, 60 °C, 12 h; (iii) (1) memantine, triphosgene, Et_3N, DCM, −78 °C; (2) Et_3N, DCM, 0 °C, 2 h; (vi) NaOH, THF/H_2O, 60 °C, 1 h; (v)methyl piperidine-4-carboxylate, HATU, DIPEA, DCM, rt 2 h; (vi) NaOH, THF / H_2O, 60 °C, 1 h; (vii) amines, HATU, DIPEA, DCM, rt.
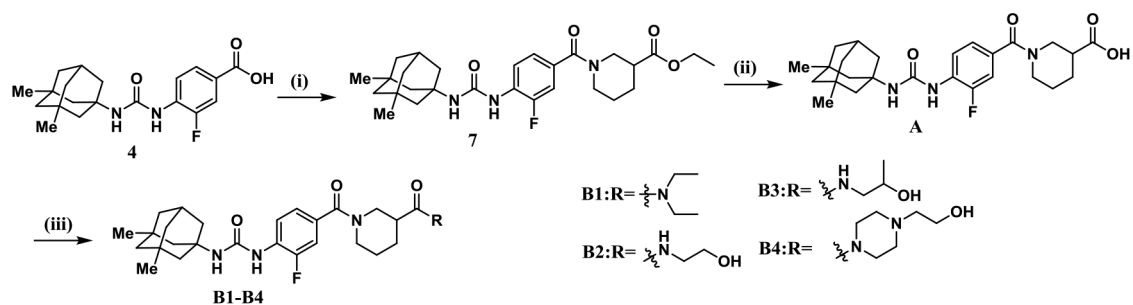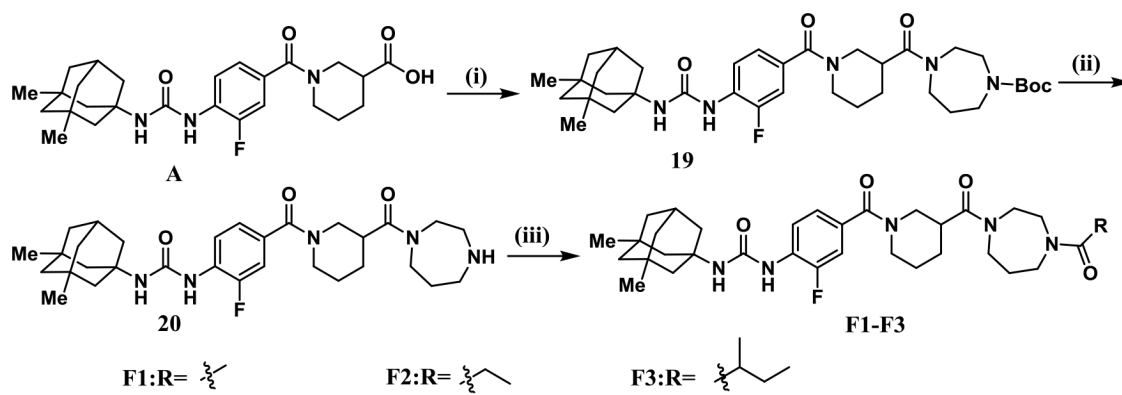
Scheme 3.

Synthetic route to compounds **B1-B4**. Reactions and conditions: (i) ethyl piperidine-3-carboxylate, HATU, DIPEA, DCM, rt; (ii) NaOH, THF / H_2O, 60 °C, 1 h; (iii) amines, HATU, DIPEA, DCM, rt.
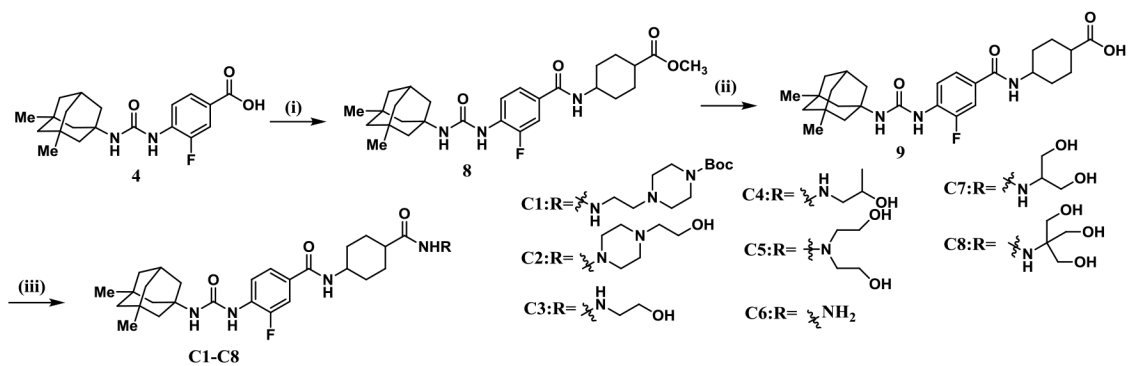
Scheme 4.
Synthetic route to compounds **C1-C8**. Reactions and conditions: (i) methyl 4-aminocyclohexane-1-carboxylate, HATU, DIPEA, DCM, rt, (ii) NaOH, THF / H$_2$O, 60 °C, 1 h; (iii) amines, HATU, DIPEA, DCM, rt, 2h.
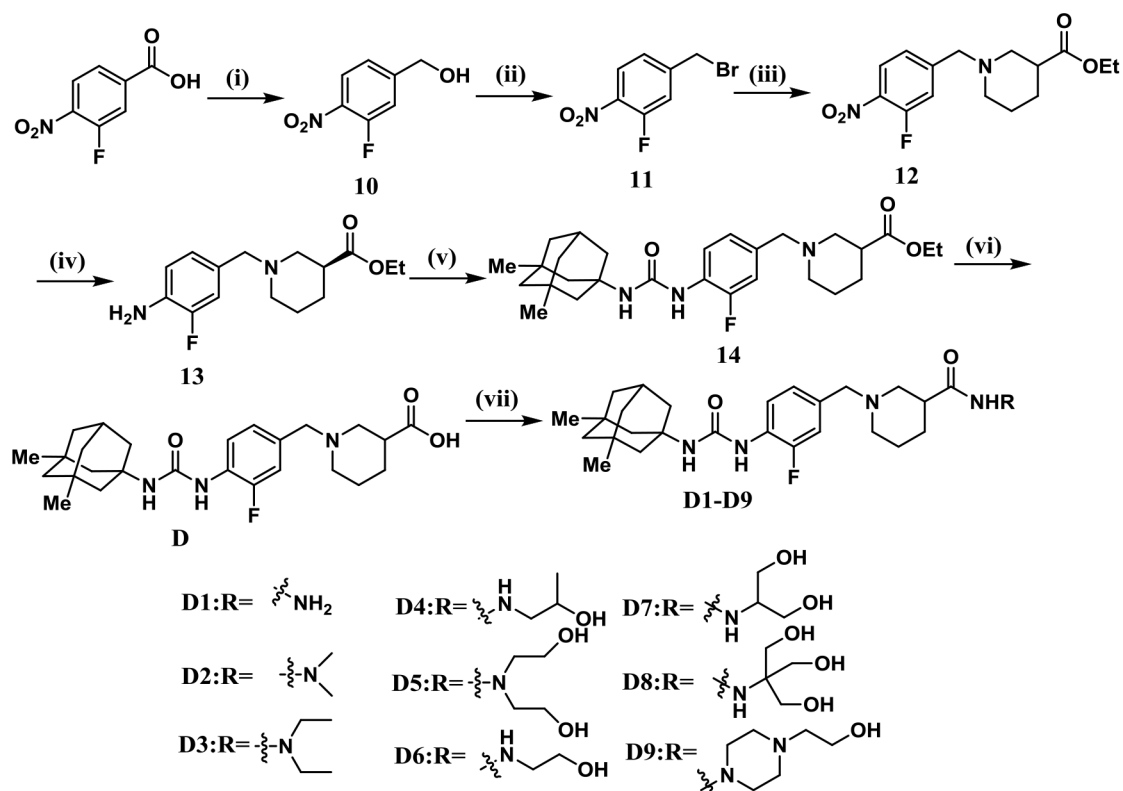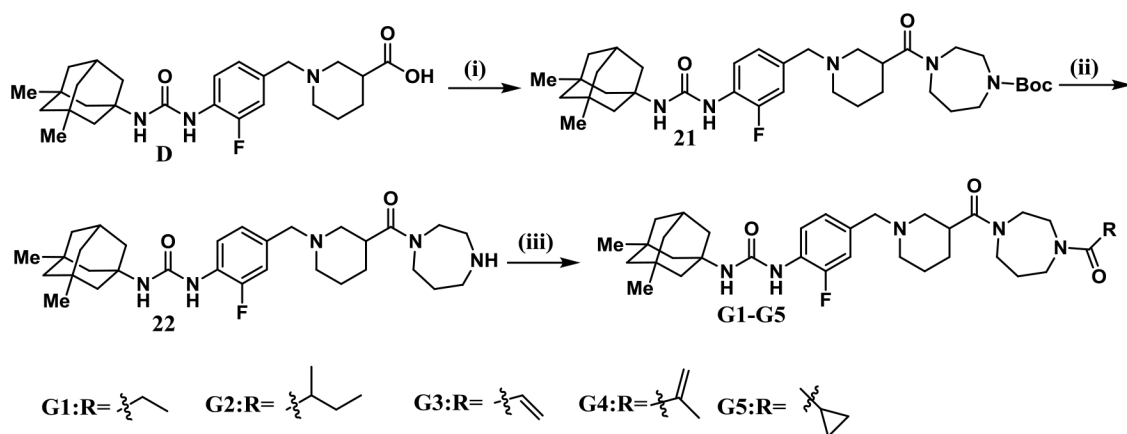
Scheme 5.
Synthetic route to compounds **D1-D10**. Reactions and conditions: (i) Borane-tetrahydrofuran complex, THF, 0 °C, 2h; (ii) phosphorus tribromide, DCM, rt, 8 h; (iii) ethyl piperidine-3-carboxylate, K_2CO_3, ACN, 50 °C, 4h; (iv) H_2 (g), 5 % Pd-C, EtOH, 60 °C, 12 h; (v) (1) memantine, triphosgene, Et_3N, DCM, −78 °C; (2) Et_3N, DCM, 0 °C, 2 h; (vi) NaOH, THF / H_2O, 60 °C, 1 h; (vii) amines, HATU, DIPEA, DCM, rt, 2h.
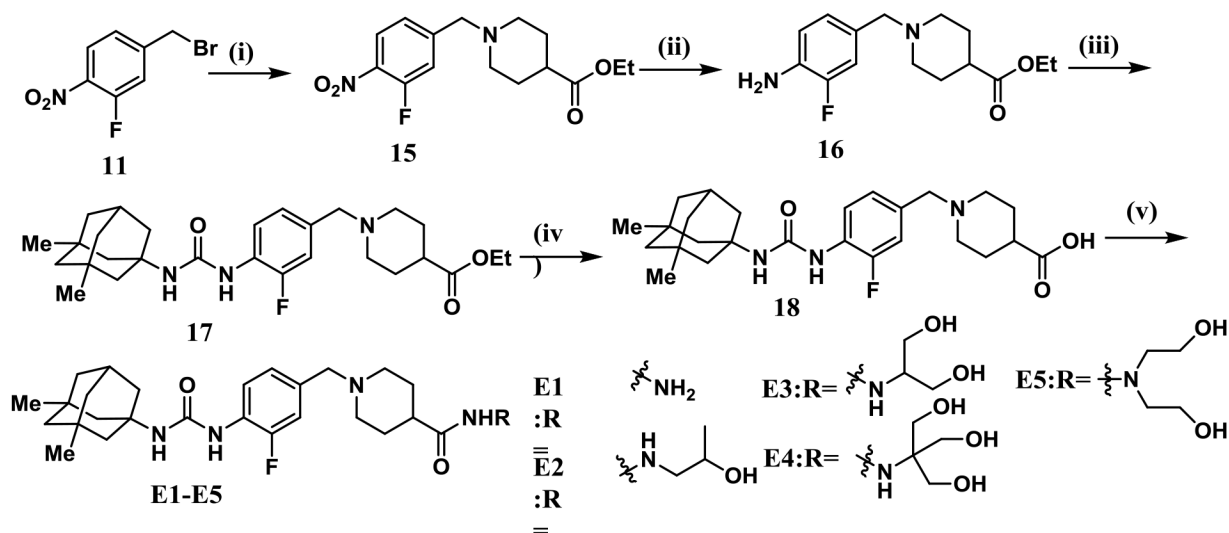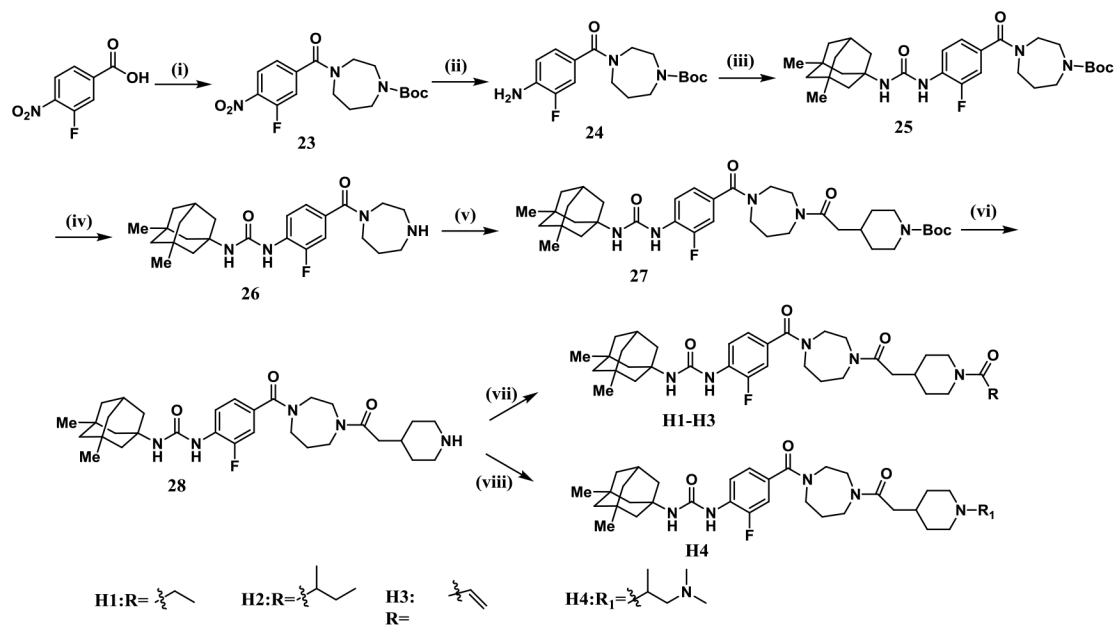
Scheme 6.
Synthetic route to compounds **E1-E5**. Reactions and conditions: (i) ethyl piperidine-3-carboxylate, K_2CO_3, ACN, 50 °C, 4h; (ii) H_2 (g), 5 % Pd-C, EtOH, 60 °C, 12 h; (iii) (1) memantine, triphosgene, Et_3N, DCM, −78 °C; (2) Et_3N, DCM, 0 °C, 2 h; (iv) NaOH, THF / H_2O, 60 °C, 1 h; (v) amines, HATU, DIPEA, DCM, rt, 2h.

Scheme 7.

Synthetic route to compounds **F1-F3**. Reactions and conditions: (i) *tert*-butyl 1,4-diazepane-1-carboxylate, HATU, DIPEA, DCM, rt, 2h. (ii) TFA, DCM, rt, 2 h; (iii) acids, HATU, DIPEA, DCM, rt, 2h.
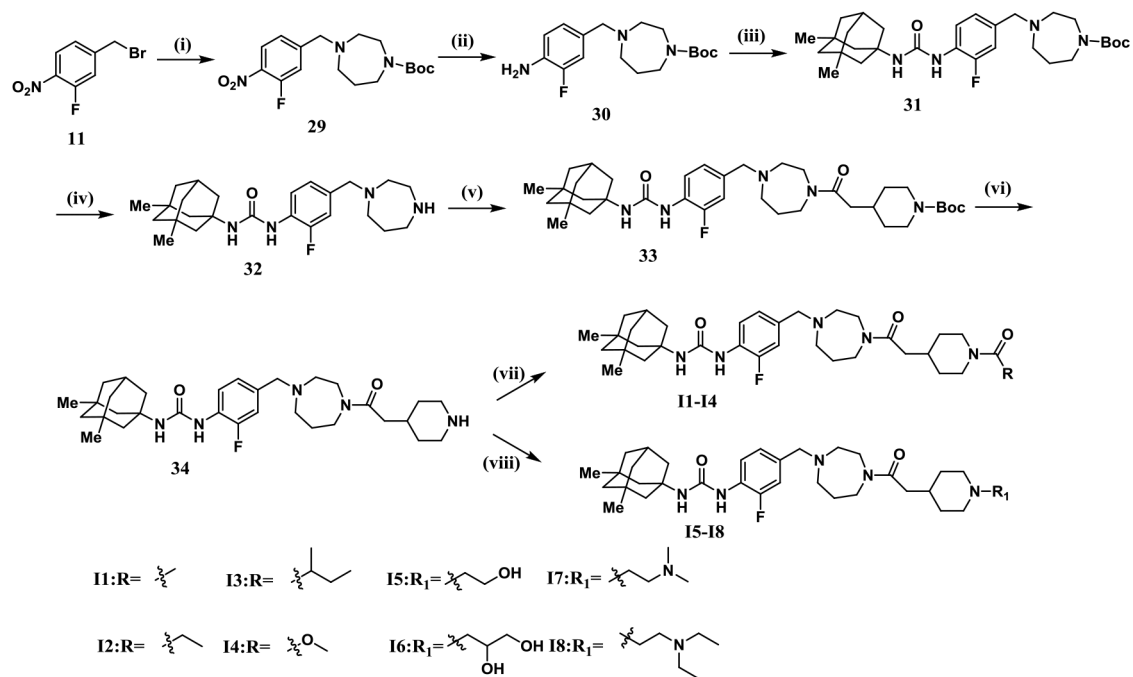
Scheme 8.
Synthetic route to compounds **F1-F3**. Reactions and conditions: (i) *tert*-butyl 1,4-diazepane-1-carboxylate, HATU, DIPEA, DCM, rt, 2h. (ii) TFA, DCM, rt, 2 h; (iii) acids, HATU, DIPEA, DCM, rt, 2h.

Scheme 9.

Synthetic route to compounds **H1-H4**. Reactions and conditions: (i) *tert*-butyl 1,4-diazepane-1-carboxylate, HATU, DIPEA, DCM, rt, 2h; (ii) H_2 (g), 5 % Pd-C, EtOH, 60 °C, 12 h; (iii) (1) memantine, triphosgene, Et_3N, DCM, −78 °C, 2h; (iv) TFA, DCM, rt, 2 h; (v) *tert*-butyl 1,4-diazepane-1-carboxylate, HATU, DIPEA, DCM, rt, 2h; (vi) TFA, DCM, rt, 2 h; (vii) acids, HATU, DIPEA, DCM, rt, 2h; (viii) 2-chloro-*N,N*-dimethylpropan-1-amine, K_2CO_3, ACN, 50 °C, 4h.

Scheme 10.

Synthetic route to compounds **I1-I8**. Reactions and conditions: (i) *tert*-butyl 1,4-diazepane-1-carboxylate, HATU, DIPEA, DCM, rt, 2h; (ii) H$_2$ (g), 5 % Pd-C, EtOH, 60 °C, 12 h; (iii) (1) memantine, triphosgene, Et$_3$N, DCM, −78 °C, 2h; (iv) TFA, DCM, rt, 2 h; (v) *tert*-butyl 1,4-diazepane-1-carboxylate, HATU, DIPEA, DCM, rt, 2h; (vi) TFA, DCM, rt, 2 h; (vii) acids, HATU, DIPEA, DCM, rt, 2h; (viii) chlorides, K$_2$CO$_3$, ACN, 50 °C, 4h.

Table 1

IC$_{50}$ values of **A1-A5** and **B1-B4** against Human sEH (HsEH) and Murine sEH (MsEH)[a].
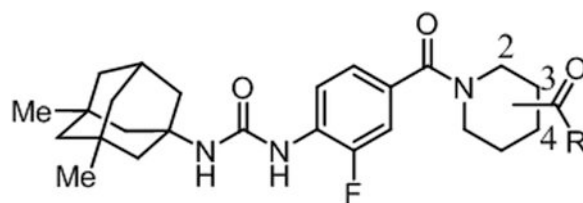
Compd.	R	Subst.	HsEH (nM)	MsEH (nM)	cLogP[b]	LLE[c]
A1		4	2.2	0.53	3.70	4.954
A2		4	3.43	0.26	2.262	6.203
A3		4	4.5	0.18	2.639	5.708
A4		4	12.5	0.32	2.639	6.264
A5		4	7.1	0.55	2.639	5.510
B1		3	0.35	0.38	3.839	5.617
B2		3	0.35	0.15	2.397	7.069
B3		3	0.28	0.23	2.774	6.779
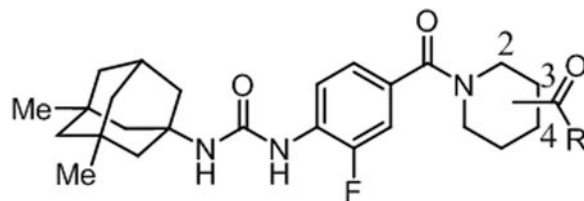
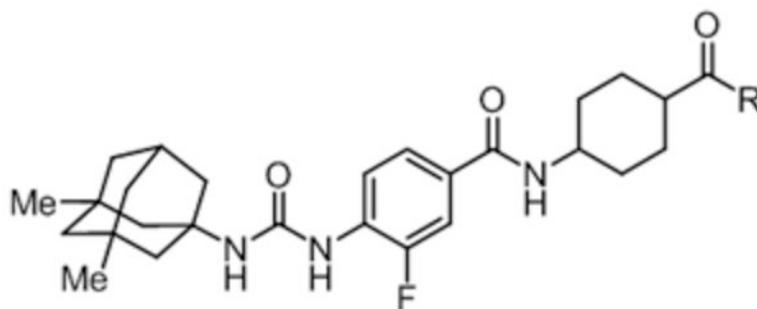| Compd.	R	Subst.	HsEH (nM)	MsEH (nM)	cLogP[b]	LLE[c]
B4		3	0.84	0.15	2.560	6.516
A	-	3	4.6	1.3	3.358	4.979
t-TUCB	-		0.28	2.8	5.489	4.064

[a]IC$_{50}$ values were recorded on recombinant human sEH (HsEH) and murine sEH (MsEH) using PHOME as substrate at 50 mM concentration.

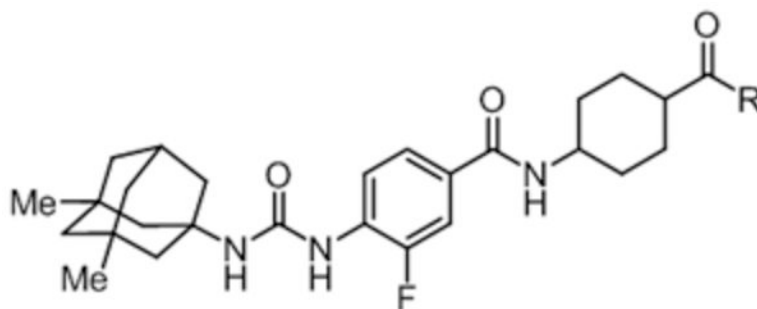[b]The cLogP values were predicted by BIOVIA Discovery Studio 2016.

[c]Ligand lipophilicity efficiency (LLE) = pIC$_{50}$-cLogP.
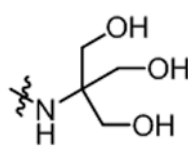
Table 2

IC$_{50}$ values of **C1-C8** against Human sEH(HsEH) and Murine sEH (MsEH)[a].

Compd.	R	HsEH (nM)	MsEH (nM)	cLogP[b]	LLE[c]
C1		183	2.0	4.466	2.272
C2		16	0.53	4.141	3.655
C3		0.8	0.18	2.997	6.100
C4		1.54	0.44	3.374	5.438
C5		2.94	0.23	2.663	5.869
C6		0.84	0.35	3.330	5.746
C7		0.91	0.55	2.486	6.555

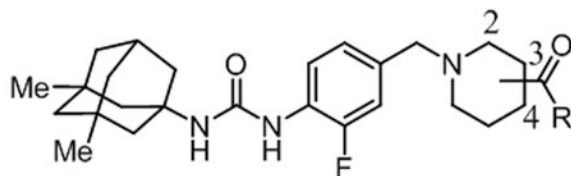| Compd.	R	HsEH (nM)	MsEH (nM)	cLogP[b]	LLE[c]
C8		0.35	0.12	1.803	7.653
A	-	4.6	1.3	3.358	4.979
t-TUCB	-	0.28	2.8	5.489	4.064

[a] IC_{50} values were recorded on recombinant human sEH (HsEH) and murine sEH (MsEH) using PHOME as substrate at 50 mM concentration.

[b] The cLogP values were predicted by BIOVIA Discovery Studio 2016.

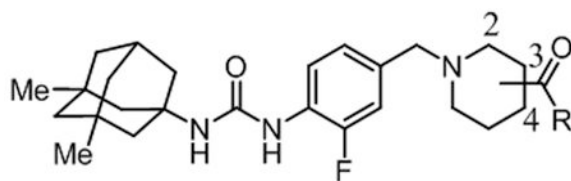[c] Ligand lipophilicity efficiency (LLE) = pIC_{50}-cLogP.

Table 3

IC$_{50}$ values of **D1-D9** and **E1-E5** against Human sEH (HsEH) and Murine sEH (MsEH)[a].

Compd.	R	Subst.	HsEH (nM)	MsEH (nM)	cLogP[b]	LLE[c]
D	-OH	3	2.3	0.61	0.982	7.656
D1		3	0.14	0.087	3.390	6.464
D2		3	0.7	0.12	3.801	5.354
D3		3	0.35	0.56	4.499	4.957
D4		3	0.28	0.58	3.434	6.119
D5		3	0.28	0.29	2.723	6.879
D6		3	0.63	0.18	3.056	6.145
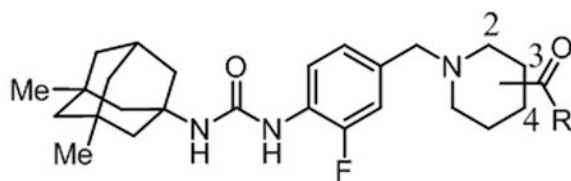
Compd.	R	Subst.	HsEH (nM)	MsEH (nM)	cLogP[b]	LLE[c]
D7		3	0.28	0.35	2.545	7.008
D8		3	0.14	0.32	1.862	7.992
D9		3	0.98	0.55	3.219	5.790
E1		4	1.54	0.058	3.255	5.557
E2		4	6.0	0.47	4.955	3.267
E3		4	31.6	2.25	2.410	5.090
E4		4	6.51	0.12	1.727	6.459
E5		4	6.72	0.55	2.588	5.585

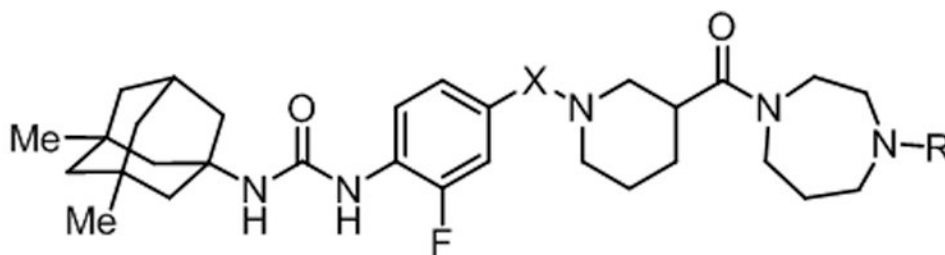| Compd.	R	Subst.	HsEH (nM)	MsEH (nM)	cLogP[b]	LLE[c]
A	-	-	4.6	1.3	3.358	4.979
t-TUCB	-	-	0.28	2.8	5.489	4.064

[a]IC$_{50}$ values were recorded on recombinant human sEH (HsEH) and murine sEH (MsEH) using PHOME as substrate at 50 mM concentration.

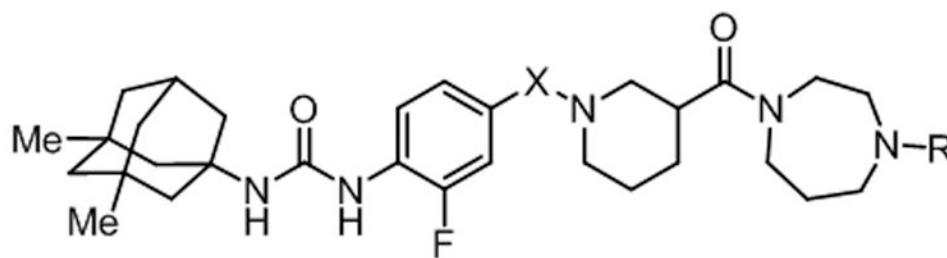[b]The cLogP values were predicted by BIOVIA Discovery Studio 2016.

[c]Ligand lipophilicity efficiency (LLE) = pIC$_{50}$-cLogP.

Table 4

IC$_{50}$ values of **F1-F3** and **G1-G5** against Human sEH (HsEH) and Murine sEH (MsEH)[a].

Compd.	X	R	HsEH (nM)	MsEH (nM)	cLogP[b]	LLE[c]
F1			0.6	0.26	2.421	6.801
F2			0.56	0.58	3.088	6.164
F3			1.4	0.9	4.007	4.847
G1			0.05	0.14	3.748	7.553
G2			0.25	0.62	4.616	4.986

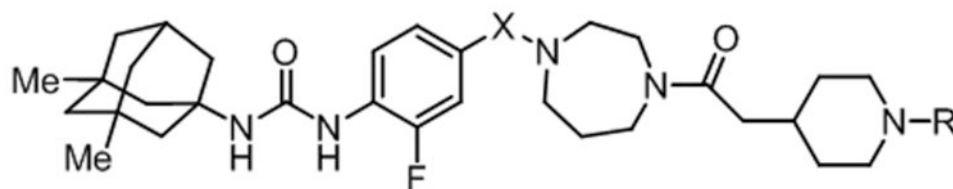| Compd.	X	R	HsEH (nM)	MsEH (nM)	cLogP[b]	LLE[c]
G3			0.42	1.1	3.78	5.598
G4			0.9	1.3	4.225	4.821
G5			0.42	0.8	3.839	4.498
A	-		4.6	1.3	3.358	4.979
t-TUCB	-		0.28	2.8	5.489	4.064

[a] IC_{50} values were recorded on recombinant human sEH (HsEH) and murine sEH (MsEH) using PHOME as substrate at 50 mM concentration.

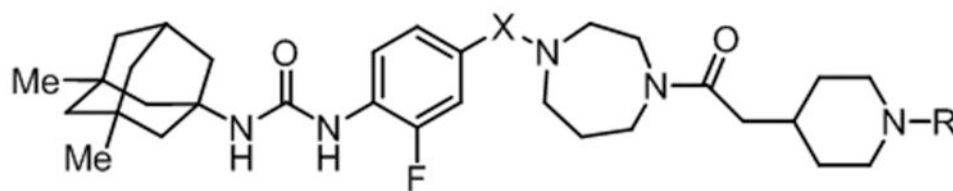[b] The cLogP values were predicted by BIOVIA Discovery Studio 2016.

[c] Ligand lipophilicity efficiency (LLE) = pIC_{50}-cLogP.

Table 5

IC$_{50}$ values of **H1-H4** and **I1-I8** against Human sEH(HsEH) and Murine sEH (MsEH)[a].

Compd.	X	R	HsEH (nM)	MsEH (nM)	cLogP[b]	LLE[c]
H1			0.9	0.39	3.198	5.848
H2			1.0	0.35	4.117	4.883
H3			1.0	0.13	3.470	5.530
H4			19.6	0.8	3.788	3.920

Compd.	X	R	HsEH (nM)	MsEH (nM)	cLogP[b]	LLE[c]
I1			0.49	0.11	3.191	6.119
I2			0.33	0.07	3.858	5.623
I3			0.77	0.16	4.777	4.337
I4			1.1	0.41	3.834	5.125
I5			4.69	0.61	3.392	4.937
I6			58.1	1.25	2.881	5.986
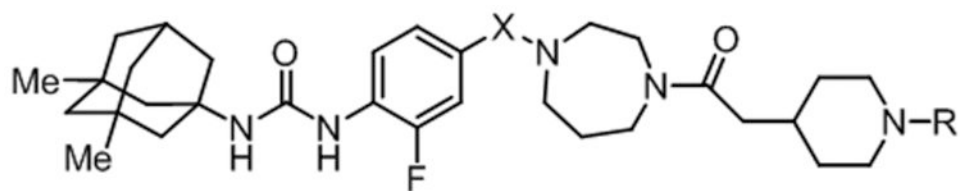
Compd.	X	R	HsEH (nM)	MsEH (nM)	cLogP[b]	LLE[c]
I7			2.59	0.67	4.07	4.517
I8			2.1	1.37	4.768	3.910
A	-		4.	1.3	3.358	4.979
t-TUCB	-		0.28	2.8	5.489	4.064

[a]IC$_{50}$ values were recorded on recombinant human sEH (HsEH) and murine sEH (MsEH) using PHOME as substrate at 50 mM concentration.

[b]The cLogP values were predicted by BIOVIA Discovery Studio 2016.

[c]Ligand lipophilicity efficiency (LLE) = pIC$_{50}$-cLogP.

Table 6

Mean of concentrations of **G1** in microsomal buffer at different time (µg / mL).

Time(h)	0	10	30	60	$t_{1/2}$(h)
Human	100	81.12	74.76	68.78	3.7
Rat	100	75.04	66.63	62.01	3.15

Table 7

Pharmacokinetics of **G1** in rats following intravenous and oral administration (n = 3).

Parameter	G1 (n = 3)	G1 (n = 3)
	iv (10 mg / kg)	*po* (50 mg / kg)
T_{max}(h)	0.03	0.25
C_{max}(nM)	3.77	2.92
$t_{1/2}$(h)	5.72	5.55
CL(L / h / kg)	0.29	1.70
V_Z(L / kg)	2.39	13.12
$AUC_{(0-8)}$ (nM·h)	6.13	6.39
$AUC_{(0-\infty)}$ (nM·h)	11.47	10.15
F (%)		20.85