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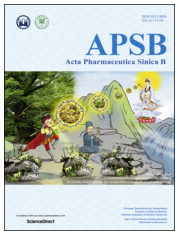

ORIGINAL ARTICLE

Novel benzamido derivatives as PTP1B inhibitors with anti-hyperglycemic and lipid-lowering efficacy

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Abstract Based on a non-competitive and selective PTP1B inhibitor reported by us previously, thirty-nine benzamido derivatives were designed and synthesized as novel PTP1B inhibitors. Among them, twelve compounds exhibited IC_{50} values at micromolar level against human recombinant PTP1B, and most of them exhibited significant selectivity to PTP1B over TC-PTP and CD45. Further evaluation of the most potent compound **27** on high-fat diet (HFD)-induced insulin-resistant (IR) obese mice indicated that **27** could modulate glucose metabolism and ameliorate dyslipidemia simultaneously.

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1. Introduction

As a member of the protein tyrosine phosphatases (PTPs) family, protein tyrosine phosphatase 1B (PTP1B) has been proved to serve as a negative regulator of both insulin and leptin signaling pathways. Therefore, PTP1B has emerged as a potential therapeutic target for type 2 diabetes and obesity[1–5]. In the past two decades, numerous PTP1B inhibitors with a large variety of structural diversity have been discovered. Most of the inhibitors incorporate phosphotyrosine (pTyr) mimetics and bind to the catalytic site of PTP1B[6–10]. However, the highly conserved and polar nature of the PTP1B catalytic site makes it a tremendous challenge for PTP1B inhibitors to achieve selectivity and cell permeability[11,12]. Up to now, only three small-molecule PTP1B inhibitors, ertiprotafib[13], trodusquemine[14] and JTT-551[15], have reached clinical trials (Fig. 1).

To overcome such a dilemma, researchers have endeavored to find alternative binding sites of PTP1B. Alternative binding sites that are less conservative and less polar than the catalytic site could be viable targets to maintain *in vitro* potency, and achieve the desired selectivity and *in vivo* activity simultaneously. A benzbromarone derivative **1** (Fig. 2) was reported as a PTP1B inhibitor in 2004[16]. Instead of interacting with the catalytic site as most known PTP1B inhibitors, compound **1** binds to an allosteric site about 20 Å away from the catalytic site. Unlike the catalytic site, the allosteric site is less polar and less conservative among PTPs. Therefore, developing allosteric inhibitors might be a feasible strategy to obtain selective PTP1B inhibitors with favorable pharmacological properties[17].

As a part of our continuing efforts, we have previously applied an integrated molecular design strategy to the prototype molecule **1**, and have acquired a novel PTP1B inhibitor **2** (Fig. 2)[18]. Compound **2** showed potent inhibition against PTP1B and kinetic analysis indicated that **2** was a non-competitive inhibitor of PTP1B. Docking study suggested that compounds **1** and **2** adopted similar binding modes in the allosteric site of PTP1B. Compound **2** also displayed excellent selectivity to PTP1B over other PTPs and significant *in vivo* insulin sensitizing effects in insulin resistant mice. These results supported the feasibility to develop PTP1B inhibitors with improved selectivity and *in vivo* activity by targeting the allosteric site of PTP1B. To further investigate the structure–activity relationship (SAR) of compound **2**, we report herein the design, synthesis and evaluation of a series of benzamido derivatives as novel PTP1B inhibitors.

2. Results and discussion

2.1. Molecular design

In the evolution from **1** to **2**, the scaffold hopping strategy to replace the benzofuran core of **1** with the phenethyl in **2** was used, which significantly improved the chemical accessibility, and preserved the inhibitory potency as well as the interaction mode of compound **1**[18]. Up to now, only limited number of allosteric PTP1B inhibitors have been reported[16,18,19]. To further expand the structural diversity of allosteric PTP1B inhibitors and explore the SAR of compound **2**, molecular modification of **2** was carried out and reported herein.

The structure of **2** was divided into five parts, namely A, B, C, D and E (Fig. 3). As demonstrated previously[18], the phenyl E ring interacts with Ala189, Leu192 and Phe280, the thiazole ring in part D presents π–π stacking interactions with Phe280, and the ketone oxygen in part B as well as the amide nitrogen in part C forms hydrogen bonds with Asn193 and Glu276, respectively. Accordingly, the phenyl in part E was left intact, while all other parts were subjected to various structural alterations by preserving the pharmacophoric features in **2**. For part A, the 4-methoxyl phenyl was replaced by differently substituted phenyl, cyclohexyl or morpholino. Most of them were suggested as effective moieties for PTP1B inhibition in our previous report[19]. Part B was also subjected to certain adjustment to find more suitable linkers between the two aromatic rings. As for parts C and D, a variety of fragments were introduced in these molecular areas to replace the sulfathiazole moiety, which was present in both **1** and **2** and

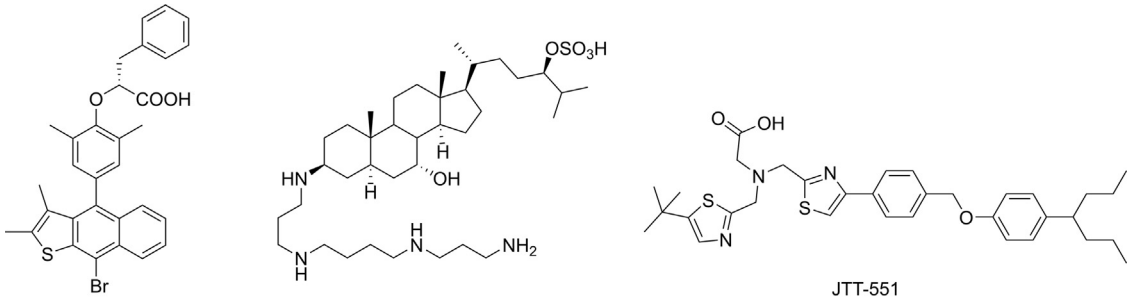


Figure 1 Small-molecule PTP1B inhibitors investigated in clinical trials.

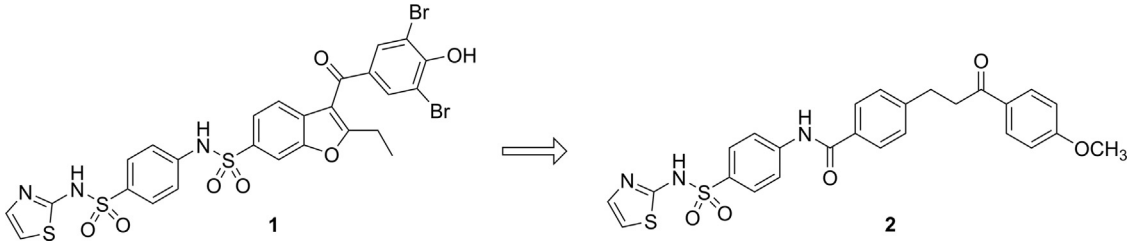


Figure 2 Evolution from the template molecule **1** to the lead compound **2**.

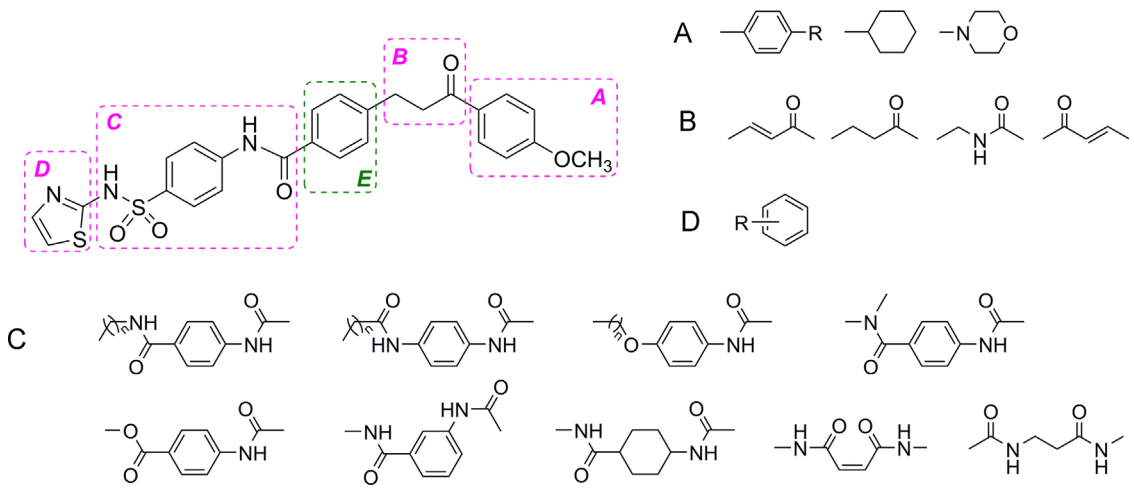
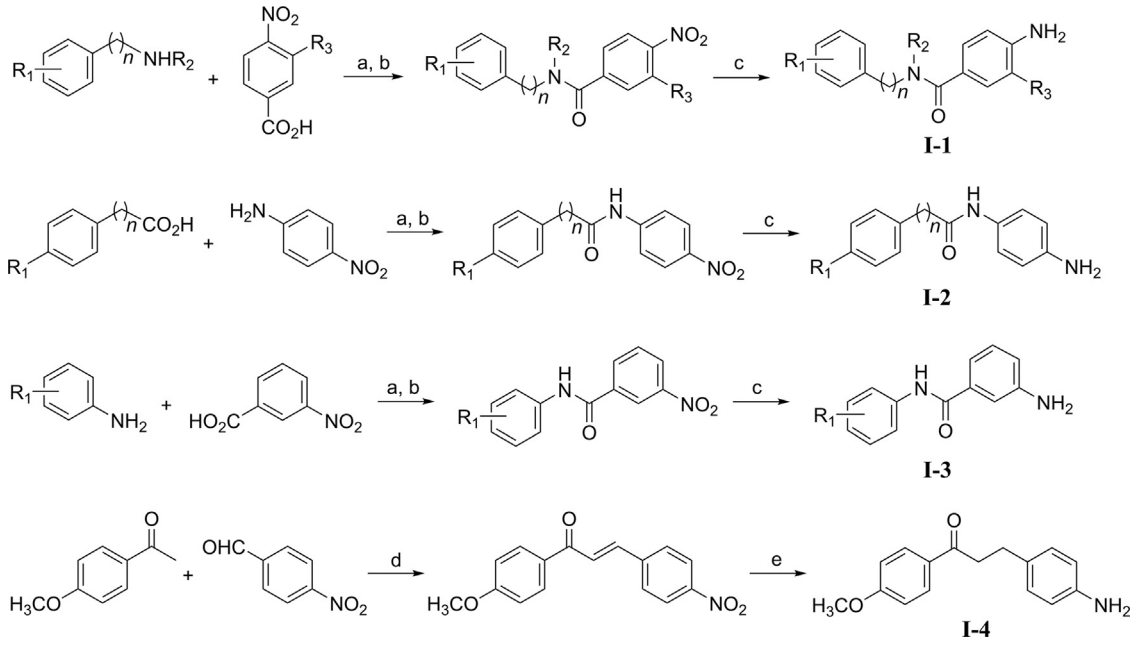


Figure 3 Structural modification and optimization of lead compound **2**.



Scheme 1 Synthesis of intermediates **I-1**–**I-4**. Reagents and conditions: (a) $(COCl)_2$, DMF, CH_2Cl_2, r.t.; (b) Et_3N, CH_2Cl_2, r.t.; (c) H_2, Pd/C, MeOH; (d) NaOH, H_2O/EtOH, r.t.; (e) H_2, Pd/C, diphenyl sulfide (cat), MeOH, r.t.

kept intact in previous molecular evolution. Substituted phenyl was incorporated in part D to retain the π–π interaction between part D and Phe280 of the allosteric site. Various linking moieties were integrated in part C to modulate the conformation of the whole molecule, so as to tune the potency of the inhibitors.

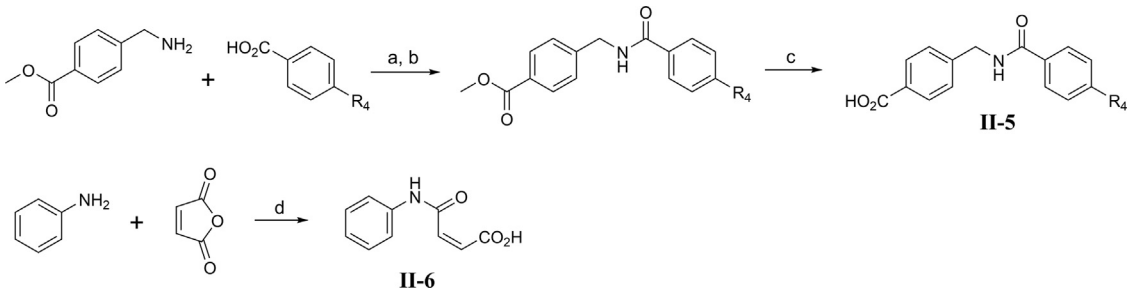
2.2. *Chemistry*

The target compounds were synthesized from appropriate amines and acids by dissecting from the amide bond between parts C and E (Fig. 3). The key amine intermediates (**I-1**–**I-7**) and acid intermediates (**II-1**–**II-7**) were synthesized as shown in Schemes 1 and 2 (**I-1–I-4, II-5–II-6**), prepared according to reported protocols (**II-1**–**II-4**)[18,19], or purchased from commercially available sources (**I-5**–**I-7** and **II-7**). As illustrated in Schemes 1 and 2, **I-1**–**I-3** were prepared by similar procedures involving successive amidation and catalytic hydrogenation. **I-4** was obtained by aldol condensation between 4-nitrobenzaldehyde and 1-(4-methoxyphenyl)-ethanone and subsequent catalytic hydrogenation. Intermediates **II-5** was produced from substituted benzoic acid and methyl 4-(aminomethyl)benzoate by successive amidation and hydrolysis. The reaction between aniline and maleic anhydride in acetic acid provided **II-6**.

The target compounds **3**–**40** were prepared by reacting intermediates **I** with intermediates **II** using various protocols shown in Scheme 3. The synthetic protocol of compound **41** was slightly different. Instead of direct reaction between **I** and **II**, the acid intermediate **II-1** was initially reacted with (1*S*,4*S*)-ethyl 4-aminocyclohexanecarboxylate hydrochloride (**I-7**), which was then followed by hydrolysis and condensation to yield **41**.

2.3. *In vitro evaluation and structure–activity relationships*

The *in vitro* activities of compounds **3**–**41** against human recombinant PTP1B were measured with protocols described



Scheme 2 Synthesis of intermediates **II-5** and **II-6**. Reagents and conditions: (a) $(COCl)_2$, DMF, CH_2Cl_2, r.t.; (b) Et_3N, CH_2Cl_2, r.t.; (c) NaOH, H_2O/MeOH, r.t. then 50 °C; (d) AcOH, r.t.

previously[20], and the results were summarized in Fig. 4 and Table 1. Compound **2**[18] was tested in parallel as the reference compound. Initial evaluation of target compounds against PTP1B was performed under the concentration of 10 μmol/L and most compounds showed significant PTP1B inhibitory activity (Fig. 4).

Thirteen compounds were further tested for their IC_{50} values against PTP1B. Among them, twelve compounds exhibited IC_{50} values comparable to those of the reference compounds **1** and **2** (Table 1).

The data listed in Fig. 3 and Table 1 disclosed some SAR clues of this compound class. The twelve most active compounds with IC_{50} values at micromolar level spanned a range of structural diversity, which suggested that the inhibitor structure was tolerable to certain scaffold variation. In particular, alterations in molecular area B were generally well-accepted (**5** *vs* **11** *vs* **15** *vs* **18**). It is assumed that the aromatic rings in parts D and E could form π–π interaction with Phe280 in the allosteric site, therefore, they were essential for PTP1B inhibition. As expected, removing the phenyl ring in part D led to significantly decreased activity (**5** *vs* **37** and **38**). In contrast, the phenyl rings in parts A and C were supposed to be the skeleton to maintain the appropriate orientation of the pharmacophoric features, and therefore might be dispensable. However, when replacing the phenyl rings in either part A (**16** and **30**) or part C (**39**–**41**) with aliphatic moieties, dramatically lose of inhibition was observed. Such results implied critical roles of aromatic rings in parts A and C. In addition, the substituents on the phenyl ring in part A could have an influence on PTP1B inhibition. In general, methoxy and *N*,*N*-dimethylamino were more favorable than methyl and fluoro (**3** *vs* **4**, **12**/**13** *vs* **14**, **17** *vs* **18**, **26** *vs* **27**). The sulfathiazole moiety in parts C and D, which was unchanged in the previous evolution, was replaced with benzamido extended with an additional phenyl ring. Such a modification at least partially preserved the inhibitory activity against PTP1B (**2** *vs* **5**). When the amide linkage between parts C and D was reversed, the PTP1B inhibitory activity remained at the same level (**18** *vs* **31**). If the amide linkage was further altered to oxygen or methylenoxy, the inhibition against PTP1B was also retained (**5** *vs* **35**). However, *N*-methylation of the amide linkage (**5** *vs* **6**) decreased the inhibitory activity. Notably, *para*-disubsititution of the phenyl ring in part C played a vital role in PTP1B inhibition. Although compound **33** did show moderate activity, *meta*-disubsititution might be less favorable than *para*-disubsititution (**8** *vs* **34**). Replacing the *para*-disubsitituted phenyl ring with vinyl (**39**), ethylene (**40**) or 1,4-cyclohexylene (**41**) led to decrease or even lose of inhibition against PTP1B. Further fluoro-substitution of the phenyl ring in part C caused sharp decrease in PTP1B inhibition (**18** *vs* **19**). In addition, compounds with elongated linkage in part C could still exhibit significant inhibitory activity (**24**, **27**, **28** and **29**). The substituents on the phenyl ring in part D might also affect the activity against PTP1B. Briefly, 4-morpholino was generally an effective moiety for PTP1B inhibition (**8**, **20**), while the effects of methoxy were complicate. *ortho*-Methoxy seemed to be favorable for PTP1B inhibition (**18** *vs* **23**), while introducing *para*-methoxy to ring D might disfavor the inhibitory activity (**9** *vs* **12**, **10** *vs* **13**). In addition, compounds incorporating *para*-methoxy on ring D and elongated linkage in part C generally possessed excellent activity against PTP1B (**27**–**29**). The SAR clues obtained so far reinforce the importance of a global molecular design strategy for developing new PTP1B allosteric inhibitors.

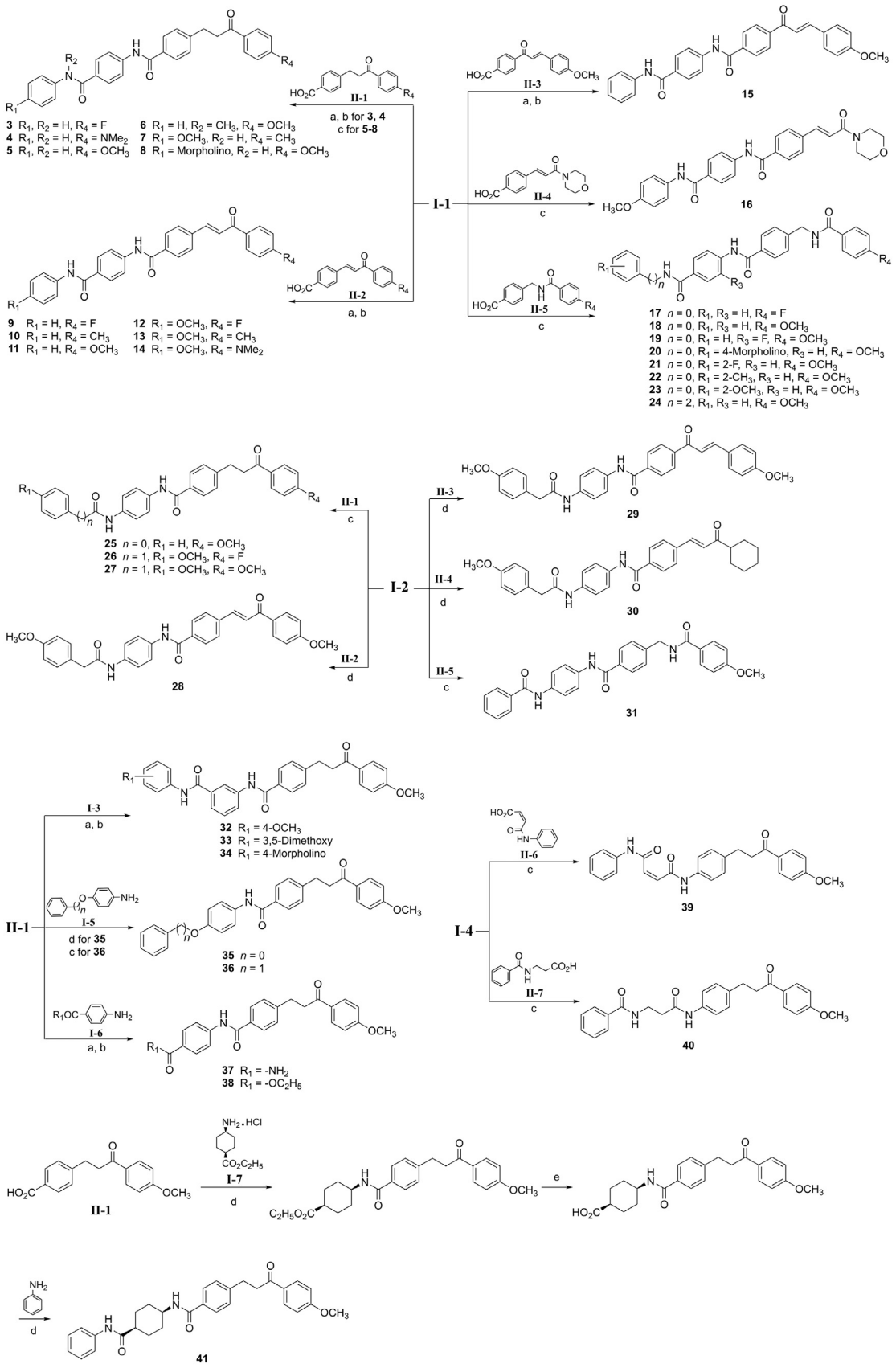
To investigate the selectivity profile of these compounds, nine compounds were further evaluated against TC-PTP (T-cell protein tyrosine phosphatase, the subtype most homologous to PTP1B) and protein tyrosine phosphatase CD45. As shown in Table 2, most compounds showed significant selectivity to PTP1B over TC-PTP and CD45.

2.4. *In vivo evaluation of compound 27*

Compound **27** was further tested for its *in vivo* efficacy in high-fat diet (HFD)-induced insulin-resistant (IR) obese mice as previously described[20]. The IR mice were randomized into four groups with eight mice in each group. Mice in different groups were treated with water (IR), rosiglitazone (10 mg/kg body weight per day, Rosi), metformin (200 mg/kg body weight per day, Met), and **27** (50 mg/kg body weight per day), respectively. Meanwhile, age-matched male C57BL/6 mice fed with the standard chow diet were used as the normal control (Con).

As shown in Fig. 5, IR mice displayed significantly higher fasting blood glucose concentrations (Fig. 5A), impaired glucose tolerance (IGT, Fig. 5C and D) and impaired insulin sensitivity (Fig. 5E and F) as compared with mice in the control group. Similar to rosiglitazone and metformin, compound **27** could decrease fasting blood glucose levels in IR mice (Fig. 5A). In the intraperitoneal glucose tolerance test (IPGTT), blood glucose levels (Fig. 5C) and AUC (area under the blood glucose-time curve) values (Fig. 5D) after glucose loading decreased significantly in all three drugs-treated groups (Rosi, Met and **27**) as compared with those of IR group. Similar results were also observed in the insulin tolerance test (ITT, Fig. 5E and F). Notably, the elevated homeostatic model assessment of insulin resistance (HOMA-IR) index in IR group was reduced by 41.8% in **27**-treated group (Fig. 5B).

As compared with mice in the control group, IR mice were also significantly obese (Fig. 6). Similar to metformin, compound **27**



Scheme 3 Synthesis of target compounds. Reagents and conditions: (a) $(COCl)_2$, DMF, CH_2Cl_2, r.t.; (b) Et_3N, CH_2Cl_2, r.t.; (c) $ClCOOCH_3$, Et_3N, THF, 0 °C then r.t.; (d) HATU, DIEA, CH_2Cl_2, r.t.; (e) NaOH, $MeOH/H_2O$, r.t. then 50 °C.

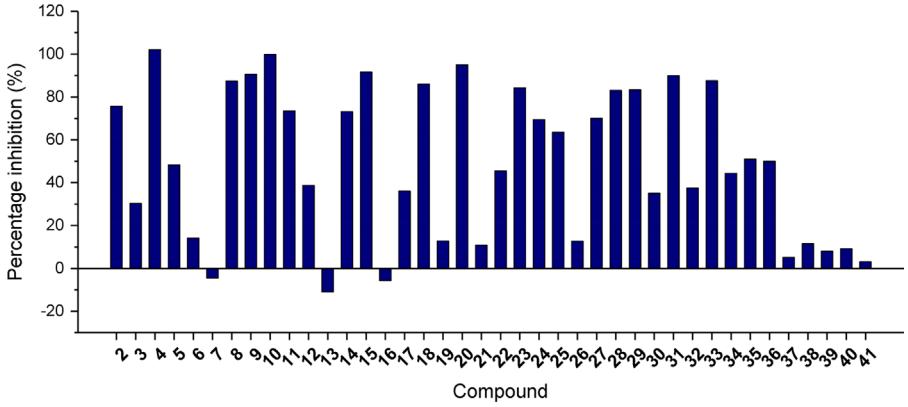


Figure 4 Percentage inhibition of the target compounds against PTP1B (the percentage inhibition was tested under the concentration of 10 μmol/L).

Table 1 IC_{50} values of selected compounds against PTP1B.

Compd.	IC_{50} (μmol/L)	Compd.	IC_{50} (μmol/L)	Compd.	IC_{50} (μmol/L)
1	8[a]	**10**	5.23	**27**	4.19
2	3.20	**15**	7.74	**28**	5.91
4	4.90	**18**	8.16	**29**	4.70
8	7.26	**20**	6.85	**31**	6.22
9	8.81	**23**	4.76	**33**	24.3

[a]Data is taken from Ref.[16].

could reduce the body weight gains of IR mice (Fig. 6A and B). Further evaluation of serum lipid profiles indicated that compound **27** could significantly decrease the serum total cholesterol (TC, Fig. 6C) in IR mice, and noticeable reduction of serum triglyceride (TG) was also observed (Fig. 6D) in the **27**-treated group. These results suggested that inhibitor **27** could modulate glucose metabolism and ameliorate dyslipidemia simultaneously.

3. Conclusions

The bottleneck in developing PTP1B inhibitors into therapeutic drugs is the realization of *in vivo* activity and subtype selectivity.

Table 2 Selective inhibitory activity of compound **27** against PTPs.

Compd.	Inhibition (%)[a]		
	PTP1B	TC-PTP	CD45
4	102.1	84.3	16.2
8	87.5	–34.1	–13.6
18	86.0	–38.0	–16.4
20	95.0	–34.1	2.0
23	84.3	13.0	6.3
27	70.1	28.7	–18.1
28	83.1	–2.0	–4.4
29	83.4	10.7	–8.0
31	90.0	–10.9	24.6

[a]The percentage inhibition was tested under the concentration of 10 μmol/L.

Novel PTP1B inhibitors targeting alternative binding site other than the catalytic site of PTP1B could overcome the afore-mentioned problems. Based on a previously identified non-competitive and selective PTP1B inhibitor **2**, thirty-nine benzamido derivatives were designed and synthesized. Among them, twelve compounds exhibited IC_{50} values against human recombinant PTP1B comparable to that of the lead compound **2**. The SAR of these compounds for PTP1B inhibition was summarized based upon the *in vitro* evaluation results, which implied the importance of a global molecular design strategy for developing this type of PTP1B inhibitors. Preliminary investigation on the selectivity profile of the compounds indicated that most compounds exhibited significant selectivity to PTP1B over TC-PTP and CD45. *In vivo* examination of the most potent compound **27** with HFD-induced insulin-resistant (IR) obese mice identified **27** as a new and orally active inhibitor with both anti-hyperglycemic and lipid-lowering effects. These findings demonstrated the feasibility of developing novel PTP1B inhibitors targeting non-catalytic site, expanded the structural diversity of allosteric PTP1B inhibitors, and also provided new chemical templates for the search of novel PTP1B inhibitors with *in vivo* activity. Further structural optimization following this molecular design is ongoing.

4. Experimental

4.1. Chemistry

All reagents were obtained from local suppliers and were used without further purification. All reactions were monitored by thin-layer chromatography with pre-coated silica gel F254 plates purchased from Merck, Inc. All melting points were determined

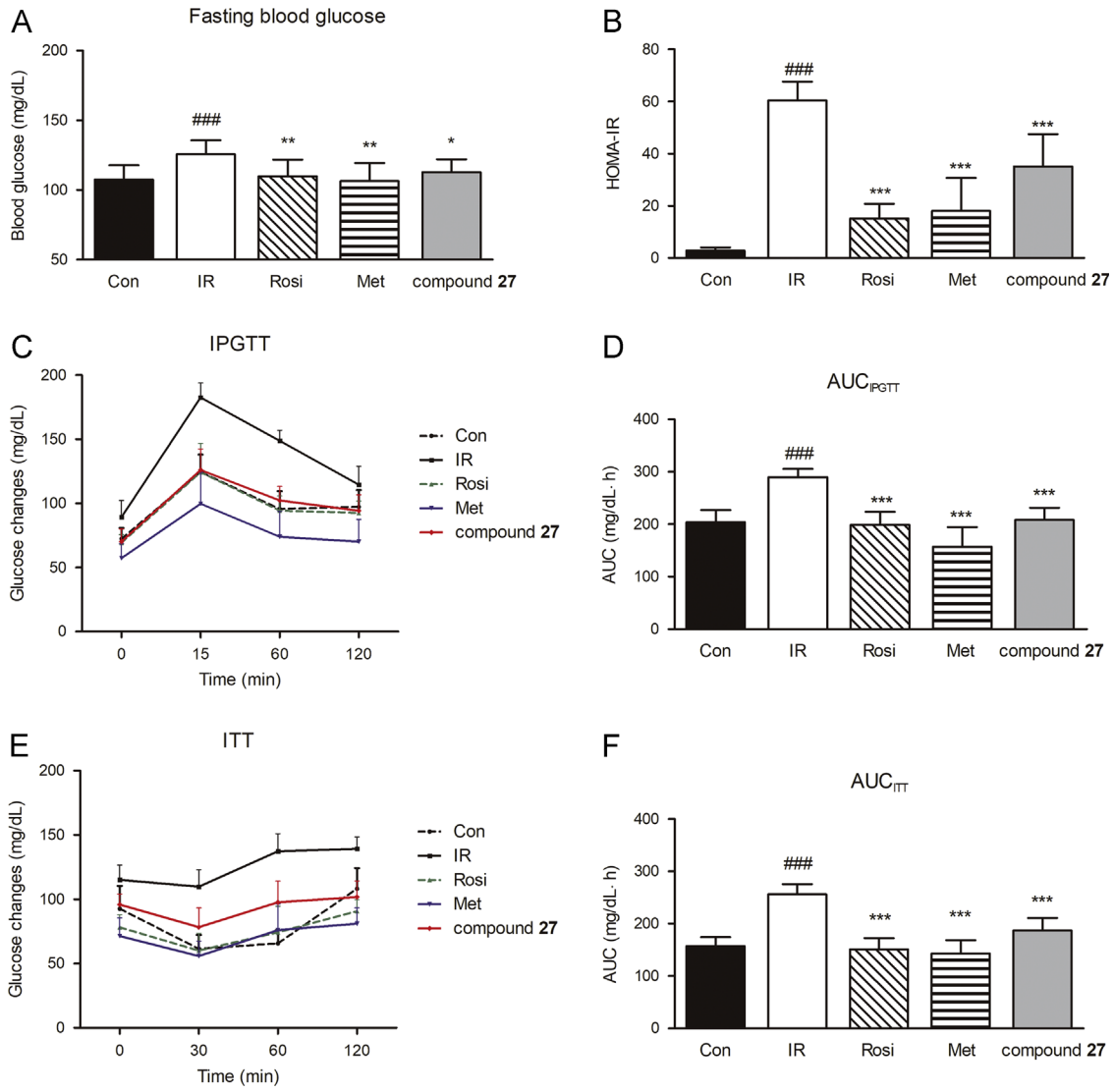


Figure 5 Anti-hyperglycemic efficacy of compound **27** in insulin-resistant (IR) obese mice. (A) Fasting blood glucose levels; (B) HOMA-IR values; (C) Blood glucose changes in the intraperitoneal glucose tolerance test (IPGTT); (D) AUC values in IPGTT; (E) Blood glucose changes in the insulin tolerance test (ITT); (F) AUC values in ITT. Data are expressed as mean ± SD ($n = 8$). $^{###}P < 0.001$ *vs* Con; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ *vs* IR. IR: insulin-resistant obese mice treated with water; Rosi: insulin-resistant obese mice treated with rosiglitazone; Met: insulin-resistant obese mice treated with metformin.

on Yanaco Melting point apparatus and were uncorrected. Mass spectra (MS) were taken in ESI mode on Agilent Technologies LC/MSD TOF instruments. ^{1}H NMR spectra were recorded on Varian Mercury 300 MHz or 400 MHz spectrometer. ^{13}C NMR spectra were recorded on Bruker AVANCEIII 400 or 600 MHz spectrometer.

4.1.1. General procedure for the synthesis of intermediates ***I-1–I-3***

Step 1: To a suspension of aryl acid (1 equiv.) in dried dichloromethane was added 1 drop of dimethyl formamide. A solution of oxalyl chloride (1.2 equiv.) in dichloromethane was added dropwise to the mixture and the reaction mixture was stirred for about 2 h. After removal of the solvent under reduced pressure, the residue was dissolved in dried dichloromethane. To the solution were added amine (1 equiv.) and triethylamine (1 equiv.) dropwise. The mixture was stirred overnight at room temperature and then concentrated. Water was added to the residue and the suspension was stirred for 1 h. After filtration, the solid was dispersed in methanol or acetone, and refluxed for about 2 h. After cooling to room temperature, the suspension was filtered. The filter cake was collected and dried.

Step 2: The solid obtained was dissolved in methanol, and to the solution was added 10% Pd/C (10%, *w*/*w*). The reaction mixture was stirred under hydrogen atmosphere at room temperature for 3–5 h. The reaction was monitored by TLC. Pd/C was removed by filtration, and the filtrate was concentrated under reduced pressure to give corresponding products in the yield of 66–98%.

4.1.2. Preparation of intermediate ***I-4***

4-Nitrobenzaldehyde (1 equiv.) was dissolved in the mixture of aqueous solution of NaOH (2 equiv.) and EtOH, followed by the addition of 1-(4-methoxyphenyl)ethan-1-one (1 equiv.). The

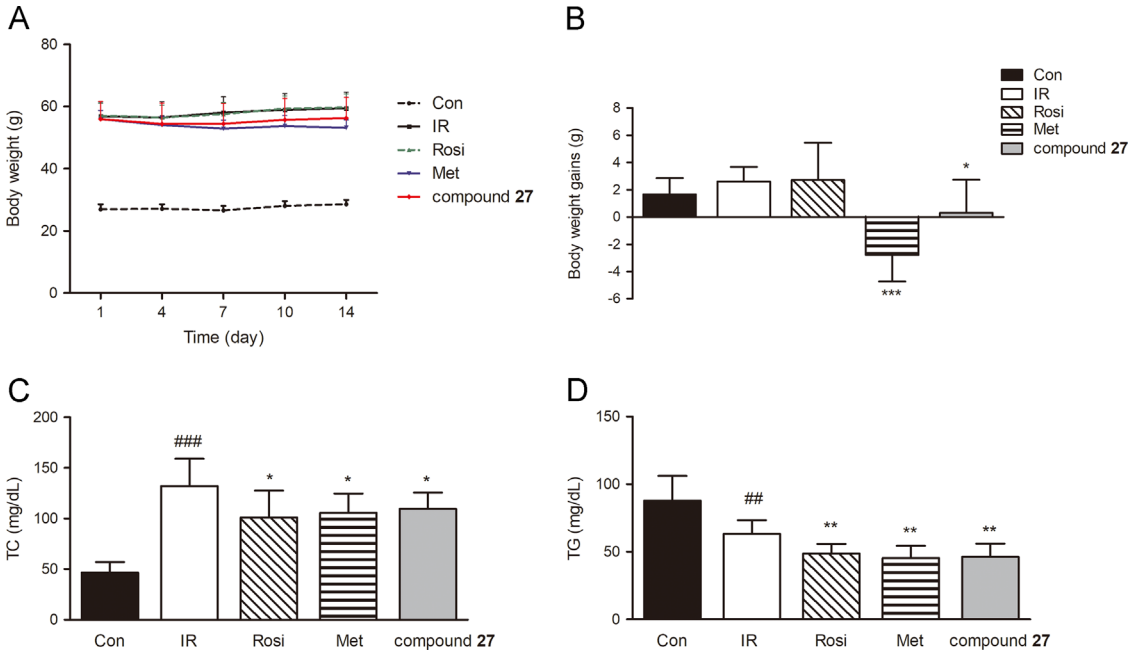


Figure 6 Effects of compound **27** on body weight and lipid profiles in IR mice. (A) Changes in body weight; (B) Body weight gains during the administration period; (C) Serum total cholesterol (TC) levels; (D) Serum triglyceride (TG) levels. Data are expressed as mean ± SD ($n = 8$). $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$ *vs* Con; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ *vs* IR. IR: insulin-resistant obese mice treated with water; Rosi: insulin-resistant obese mice treated with rosiglitazone; Met: insulin-resistant obese mice treated with metformin.

reaction mixture was stirred at room temperature overnight, and precipitation was observed. The precipitate was filtered, washed with water and dried. The dried precipitate was suspended in appropriate amount of methanol, refluxed for about 1 h. After cooling to room temperature, the precipitate was filtered and dried to give a slightly yellow solid.

The solid was then suspended in methanol, and to the mixture was added 10% Pd/C (10%, *w/w*) and diphenyl sulfide (1%, *w/w*). The reaction mixture was stirred under hydrogen atmosphere at room temperature for 24 h. The reaction was monitored by TLC. After the filtration of Pd/C, the filtrate was removed and concentrated to give **I-4** as a slightly yellow solid in 63% yield.

4.1.3. *Preparation of intermediate **II-5***

To a suspension of 4-substituted benzoic acid (1 equiv.) in dried dichloromethane was added 1 drop of dimethyl formamide. A solution of oxalyl chloride (1.2 equiv.) in dichloromethane was added dropwise to the mixture and the reaction mixture was stirred for about 2 h. The solvent was evaporated. Dried dichloromethane, methyl 4-(aminomethyl)benzoate (1 equiv.) and triethylamine (1 equiv.) were added to the residue. The mixture was stirred overnight at room temperature. After removal of the solvent under reduced pressure, the residue was dissolved to ethyl acetate, and washed with saturated $NaHCO_3$, water, dilute hydrochloric acid, and brine. The organic layer was collected, dried over anhydrous Na_2SO_4, and concentrated to give white solid.

The solid was then dissolved in methanol, and aqueous solution of NaOH (2 equiv.) was added to the solution. The reaction mixture was stirred at room temperature for 5 minutes and then heated to 50 °C for 2 h. The reaction was monitored by TLC. Methanol was removed by evaporation. The residue aqueous solution was buffered to pH 2 with dilute hydrochloric acid and then extracted with dichloromethane. The combined organic layers were dried over anhydrous Na_2SO_4, and concentrated to give corresponding products in 32%–58% yield.

4.1.4. *Preparation of intermediate **II-6***

To a solution of maleic anhydride (1 g, 10.19 mmol) in acetic acid (20 mL) was added aniline (0.948 g, 10.19 mmol). The reaction mixture was stirred overnight and the precipitate was filtrated to give the product as white solid (1.857 g, yield 95%).

4.1.5. *General procedure for the preparation and characterization of compounds **3–40***

Compounds **3–40** were synthesized from intermediates **I** and **II** with the protocols described as follows.

Protocol A: To a suspension of intermediate **II** (1 equiv.) in dried dichloromethane was added 1 drop of dimethyl formamide. A solution of oxalyl chloride (1.2 equiv.) in dichloromethane was added dropwise to the mixture and the reaction mixture was stirred at room temperature. After the completion of reaction, the solvent was evaporated under reduced pressure and the residue was dissolved in dried dichloromethane. To the solution were added corresponding amine **I** (1 equiv.) and triethylamine (1 equiv.) dropwise. The mixture was stirred overnight at room temperature and then concentrated. Water was added to the residue and the suspension was stirred for 1 h. After filtration, the solid was dispersed in methanol and refluxed for about 1 h. After cooling to the room temperature, the suspension was filtered. The filter cake was collected and dried to give the target compound. Compounds **3**, **4**, **9–15**, **32–34**, **37** and **38** were obtained through protocol A.

Protocol B: To a suspension of carboxylic acid (1 equiv.) in THF was sequentially added triethylamine (2.2 equiv.) and a solution of $ClCOOCH_3$ (1.1 equiv.) in THF under ice bath. The resulting mixture was stirred at room temperature for 30 minutes,

and then corresponding amine (1 equiv.) was added in one portion. After stirring at room temperature overnight, the solvent was evaporated. The residue was washed with water and then suspended in methanol or acetone to reflux for 1 h. After cooling to room temperature, the suspension was filtered. The filter cake was collected and dried to give the target compound. Compounds **5**–**8**, **16**–**27**, **31**, **36**, **39** and **40** were obtained through protocol B.

Protocol C: To a flask was added intermediate **I** (1 equiv.), **II** (1 equiv.), 2-(1*H*-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU, 1.2 equiv.), *N*,*N*-diisopropylethylamine (DIEA, 2.5 equiv.) and dichloromethane. After stirring at room temperature overnight, the resulting mixture was concentrated under reduced pressure. The residue was dissolved in ethyl acetate and washed by dilute hydrochloride acid, saturated sodium bicarbonate and brine in order. The organic layer was dried over anhydrous Na_2SO_4 and evaporated. The residue was dispersed in methanol and refluxed for about 1 h. After cooling to room temperature, the suspension was filtered. The filter cake was collected and dried to give the target compound. Compounds **28**–**30**, **35** were obtained through protocol C.

*4.1.5.1. 4-(3-(4-Fluorophenyl)-3-oxopropyl)-N-(4-(phenylcarbamoyl)phenyl)benzamide (**3**).* Yield 30%; white powder; m.p. 263–265 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.42 (s, 1H, CONH), 10.14 (s, 1H, CONH), 8.09 (m, 2H, ArH), 8.00–7.90 (m, 6H, ArH), 7.78 (d, J=7.8 Hz, 2H, ArH), 7.47 (d, J=8.1 Hz, 2 H, ArH), 7.35 (m, 4 H, ArH), 7.09 (m, 1 H, ArH), 3.45 (t, J=7.2 Hz, 2H, CH_2), 3.04 (t, J=7.2 Hz, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 198.0, 166.2, 165.5 (d, $J_{C\text{-}F}$=251 Hz), 165.4, 146.0, 142.8, 139.8, 133.8 (d, $J_{C\text{-}F}$=3 Hz), 132.8, 131.4 (d, $J_{C\text{-}F}$=10 Hz, 2C), 130.0, 129.1 (2C), 129.0 (2C), 128.9 (2C), 128.3 (2C), 124.0, 120.8 (2C), 119.8 (2C), 116.2 (d, $J_{C\text{-}F}$=22 Hz, 2C), 39.4, 29.8; ESI-HR-MS *m/z* 467.1750 $[M+H]^+$ (Calcd. for $C_{29}H_{24}FN_2O_3$, 467.1766).

*4.1.5.2. 4-(3-(4-(Dimethylamino)phenyl)-3-oxopropyl)-N-(4-(phenylcarbamoyl)phenyl)benzamide (**4**).* Yield 35%; yellow powder; m.p. 238–240 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.41 (s, 1H, CONH), 10.13 (s, 1H, CONH), 8.01–7.77 (m, 12H, ArH), 7.35 (t, J = 7.6 Hz, 2H, ArH), 7.18 (m, 1H, ArH), 6.71(d, J = 8.8 Hz, 2H, ArH), 3.26 (m, 2H, CH_2), 3.10–3.08 (m, 8H, CH_2, 2 × NH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 196.6, 166.2, 165.4, 153.7, 146.5, 142.8, 139.8, 132.7, 130.4 (2C), 129.1 (2C), 129.0, 129.0 (2C), 128.9 (2C), 128.7, 128.3 (2C), 124.6, 120.8 (2C), 119.8 (2C), 111.2 (2C), 40.1 (2C), 38.7, 30.3; ESI-HR-MS *m/z* 492.2266 $[M + H]^+$ (Calcd. for $C_{31}H_{30}N_3O_3$, 492.2282).

*4.1.5.3. 4-(3-(4-Methoxyphenyl)-3-oxopropyl)-N-(4-(phenylcarbamoyl)phenyl)benzamide (**5**).* Yield 28%; yellow powder; m.p. 235–238 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.41 (s, 1H, CONH), 10.13 (s, 1H, CONH), 7.99–7.89 (m, 8H, ArH), 7.77 (d, J = 7.8 Hz, 2H, ArH), 7.45 (d, J = 7.8 Hz, 2H, ArH), 7.34 (t, J = 7.5 Hz, 2H, ArH), 7.10–7.02 (m, 3H,ArH), 3.83 (s, 3H, OCH_3), 3.37 (t, J = 7.5 Hz, 2H, CH_2), 3.02 (t, J = 7.5 Hz, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 197.7, 166.2, 165.4, 163.6, 146.2, 142.8, 139.8, 132.7, 130.7 (2C), 130.0, 129.9, 129.1 (2C), 129.0 (2C), 128.9 (2C), 128.3 (2C), 124.0, 120.8 (2C), 119.8 (2C), 114.4 (2C), 56.0, 39.1, 30.0; ESI-HR-MS *m/z* 479.1956 $[M + H]^+$ (Calcd. for $C_{30}H_{26}N_2O_4$ 479.1965).

*4.1.5.4. 4-(4-(3-(4-Methoxyphenyl)-3-oxopropyl)benzamido)-N-methyl-N-phenylbenzamide (**6**).* Yield 51%; white powder; m.p. 181–183 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.11 (s, 1H, CONH), 7.96 (d, J = 8.7 Hz, 2 H, ArH), 7.81 (d, J = 8.4 Hz, 2H, ArH), 7.59 (d, J = 9.0 Hz, 2H, ArH), 7.41 (d, J = 8.1 Hz, 2H, ArH), 7.30–7.13 (m, 7H, ArH), 7.02 (d, J = 9.0 Hz, 2H, ArH), 3.82 (s, 3H, OCH_3), 3.36–3.20 (m, 5H, CH_2, NCH_3), 2.99 (t, J = 7.5 Hz, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 197.7, 169.6, 166.1, 163.6, 146.1, 145.4, 140.78, 132.8, 131.4, 130.7 (2C), 130.0, 129.6 (2C), 129.6 (2C), 128.9 (2C), 128.2 (2C), 127.5 (2C), 126.8, 119.4 (2C), 114.3 (2C), 56.0, 39.1, 38.5, 30.0; ESI-HR-MS *m/z* 493.2114 $[M+H]^+$ (Calcd. for $C_{31}H_{28}N_2O_4$ 493.2122).

*4.1.5.5. N-(4-Methoxyphenyl)-4-(4-(3-oxo-3-(p-tolyl)propyl)benzamido)benzamide (**7**).* Yield 48%; white powder; m.p. 83–85 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.41 (s, 1H, CONH), 10.03 (s, 1H, CONH), 7.99–7.89 (m, 8H, ArH), 7.68 (d, J=9.0 Hz, 2H, ArH), 7.47 (d, J=8.4 Hz, 2H, ArH), 7.33 (d, J=7.8 Hz, 2H, ArH), 6.93 (d, J=9.0 Hz, 2H, ArH), 3.75 (s, 3H, OCH_3), 3.41 (t, J=7.5 Hz, 2H, CH_2), 3.05 (t, J=7.5 Hz, 2H, CH_2), 2.38 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 198.8, 166.7, 164.5, 156.1, 154.0, 147.8, 144.0, 134.5, 132.6, 129.7 (2C), 129.6 (2C), 129.6 (2C), 129.3 (2C), 128.7, 128.5 (2C), 127.8, 122.5 (2C), 118.9 (2C), 114.2 (2C), 55.7, 39.2, 29.9, 21.6; ESI-HR-MS *m/z* 493.2122 $[M+H]^+$ (Calcd. for $C_{31}H_{29}N_2O_4$ 493.2122).

*4.1.5.6. 4-(3-(4-Methoxyphenyl)-3-oxopropyl)-N-(4-((4-morpholinophenyl)carbamoyl)phenyl)benzamide (**8**).* Yield 62%; yellow powder; m.p. 264–267 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.41 (s, 1H, CONH), 10.06 (s, 1H, CONH), 7.99–7.89 (m, 8H, ArH),7.69 (d, 2H, J = 8.7 Hz, ArH), 7.45 (d, 2H, J = 8.1 Hz, ArH), 7.10 (d, 2H, J = 6.6 Hz, ArH), 7.03 (d, 2H, J = 8.7 Hz, ArH), 3.82 (s, 3H, OCH_3), 3.80–3.67 (m, 4H, morpholino-H), 3.37 (t, J = 7.5 Hz, 2 H, CH_2), 3.19-3.13 (m, 4H, morpholino-H), 3.02 (t, J = 7.5 Hz, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 197.7, 166.2, 164.8, 163.6, 147.9, 146.2, 142.5, 132.8, 131.9, 130.8 (2C), 130.2, 130.0, 129.0 (2C), 128.7 (2C), 128.3 (2C), 121.9 (2C), 119.8 (2C), 115.7 (2C), 114.4 (2C), 66.6 (2C), 56.0, 49.4 (2C), 39.1, 30.0; ESI-MS *m/z* 564.3 $[M + H]^+$.

*4.1.5.7. (E)-4-(3-(4-Fluorophenyl)-3-oxoprop-1-en-1-yl)-N-(4-(phenylcarbamoyl)phenyl)benzamide (**9**).* Yield 37%; light yellow powder; m.p. > 300 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.34 (s, 1H, CONH), 10.26 (s, 1H, CONH), 8.30 (dd, J = 8.7, 5.7 Hz, 2H, ArH), 8.12–8.08 (m, 5H, ArH, CH=CHCO), 7.97 (d, J = 9.2 Hz, 2H, ArH), 7.84–7.78 (m, 5H, ArH, CH=CHCO), 7.62–7.51 (m, 3H, ArH), 7.43 (t, J = 9.0 Hz, 2H, ArH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 188.2, 165.8, 165.7 (d, $J_{C\text{-}F}$ = 250 Hz), 164.4, 143.4, 138.0, 136.8, 135.6, 135.5, 135.3, 134.6 (d, $J_{C\text{-}F}$ = 4 Hz), 132.2 (d, $J_{C\text{-}F}$ = 10 Hz, 2C), 132.0, 129.3 (2C), 128.9 (2C), 128.6 (2C), 128.1 (2C), 123.9, 121.2 (2C), 121.1 (2C), 116.4 (d, $J_{C\text{-}F}$ = 21 Hz, 2C); ESI-HR-MS *m/z* 465.1606 $[M + H]^+$ (Calcd. for $C_{29}H_{22}FN_2O_3$ 465.1609).

*4.1.5.8. (E)-4-(3-Oxo-3-(p-tolyl)prop-1-en-1-yl)-N-(4-(phenylcarbamoyl)phenyl)benzamide (**10**).* Yield 70%; light yellow powder; m.p. > 300 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.33 (s, 1H, CONH), 10.26 (s, 1H, CONH), 8.12–8.05 (m, 7H, ArH, CH=CHCO), 7.97 (d, J = 9.2 Hz, 2H, ArH), 7.80 (m, 5H, ArH, CH=CHCO), 7.60–7.51 (m, 3H, ArH), 7.40 (d, J = 10.4

Hz, 2H, ArH), 2.42 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 189.0, 165.8, 165.1, 144.3, 142.9, 138.1, 136.7, 135.6, 135.5, 135.4, 135.3, 132.0, 129.9 (2C), 129.3 (2C), 129.2 (2C), 128.9 (2C), 128.6 (2C), 128.1 (2C), 124.2, 121.2 (2C), 121.1 (2C), 21.7; ESI-HR-MS *m/z* 461.1852 $[M + H]^+$ (Calcd. for $C_{30}H_{25}N_2O_3$ 461.1860).

*4.1.5.9. (E)-4-(3-(4-Methoxyphenyl)-3-oxoprop-1-en-1-yl)-N-(4-(phenylcarbamoyl)phenyl)benzamide (**11**).* Yield 58%; yellow powder; m.p. > 300 °C; ^1H NMR (300 MHz, DMSO-d_6): δ 10.58 (s, 1H, CONH), 10.16 (s, 1H, CONH), 8.21 (d, 2H, J = 8.4 Hz, ArH), 8.12–7.94 (m, 8H, ArH, CH=CHCO), 7.80–7.74(m, 4H, ArH, CH=CHCO), 7.35 (m, 3H), 7.11 (d, 2H, J = 9.0 Hz), 3.88 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 187.8, 165.7, 165.4, 163.9, 142.6, 142.3, 139.8, 138.5, 136.2, 131.6 (2C), 130.8, 130.2, 129.2 (2C), 129.1 (2C), 129.0 (2C), 128.9, 128.8 (2C),124.4, 120.8 (2C), 120.0 (2C), 114.6 (2C), 56.1; ESI-HR-MS *m/z* 477.1804 $[M + H]^+$ (Calcd. for $C_{30}H_{24}N_2O_4$ 477.1809).

*4.1.5.10. (E)-4-(3-(4-Fluorophenyl)-3-oxoprop-1-en-1-yl)-N-(4-((4-methoxyphenyl)carbamoyl)phenyl)benzamide (**12**).* Yield 71%; white powder; m.p. > 300 °C; ^1H NMR (400 MHz, DMSO-d_6): δ 10.57 (s, 1H, CONH), 10.04 (s, 1H, CONH), 8.29 (d, J = 8.4, 6.0 Hz, 2H, ArH), 8.12–7.96 (m, 9H, ArH, CH=CHCO), 7.82 (d, J = 15.6 Hz, 1H,CH=CHCO), 7.68 (d, J = 8.8 Hz, ArH, 2H), 7.42 (d, J = 8.8 Hz, 2H, ArH), 6.93 (d, J = 8.8 Hz, 2H), 3.75 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 188.2, 165.7 (d, $J_{C\text{-}F}$ = 251 Hz), 165.6, 164.9, 155.9, 143.3, 142.4, 138.3, 136.4, 134.6 (d, $J_{C\text{-}F}$ = 2 Hz), 132.8, 132.2 (d, $J_{C\text{-}F}$ = 11 Hz, 2 C), 130.3, 129.4 (2C), 128.8 (2C), 128.8 (2C), 124.1, 122.4 (2C), 120.0 (2C), 116.4 (d, $J_{C\text{-}F}$ = 22 Hz, 2C), 114.2 (2C), 55.6; ESI-HR-MS *m/z* 495.1711 $[M + H]^+$ (Calcd. for $C_{30}H_{24}FN_2O_4$ 495.1715).

*4.1.5.11. (E)-N-(4-Methoxyphenyl)-4-(4-(3-oxo-3-(p-tolyl)prop-1-en-1-yl)benzamido)benzamide (**13**).* Yield 39%; yellow powder; m.p. 237–239 °C; ^1H NMR (400 MHz, DMSO-d_6): δ 10.59 (s, 1H, CONH), 10.05 (s, 1H, CONH), 8.12–7.95 (m, 11H, ArH, CH=CHCO), 7.80 (d, J = 15.6 Hz, 1H, CH=CHCO), 7.69 (d, J = 8.8 Hz, 2H, ArH), 7.40 (d, J = 8.0 Hz, 2H, ArH), 6.93 (d, J = 9.2 Hz, 2 H, ArH), 3.74 (s, 3H, OCH_3), 2.42 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 189.0, 165.6, 164.9, 155.9, 144.3, 142.8, 142.4, 138.4, 136.3, 135.4, 132.8, 130.2, 129.9 (2C), 129.4 (2C), 129.3 (2C), 128.8 (2C), 128.8 (2C), 124.4, 122.4 (2C), 120.0 (2C), 114.2 (2C), 55.6, 21.7; ESI-HR-MS *m/z* 491.1978 $[M + H]^+$ (Calcd. for $C_{31}H_{27}N_2O_4$ 491.1965).

*4.1.5.12. (E)-4-(3-(4-(Dimethylamino)phenyl)-3-oxoprop-1-en-1-yl)-N-(4-((4-methoxyphenyl)carbamoyl)phenyl)benzamide (**14**).* Yield 91%; orange powder; m.p. > 300 °C; ^1H NMR (400 MHz, DMSO-d_6): δ 10.55 (s, 1H, CONH), 10.04 (s, 1H, CONH), 8.09–7.94 (m, 11H, ArH, CH=CHCO), 7.73–7.67 (m, 3H, ArH, CH=CHCO), 6.93 (d, J = 8.0 Hz, 2H, ArH), 6.79 (d, J = 8.4 Hz, 2H, ArH), 3.75 (s, 3H, OCH_3), 3.06 (s, 6H, 2 × NCH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 186.4, 165.7, 165.0, 155.9, 153.9, 142.3, 140.8, 138.9, 135.9, 132.8, 131.3 (2C), 130.3, 129.0 (2C), 128.8 (2C), 128.7 (2C), 125.5, 124.8, 122.4 (2C), 120.0 (2C), 114.2 (2C), 111.4 (2C), 55.6, 40.1 (2C); ESI-HR-MS *m/z* 520.2225 $[M + H]^+$ (Calcd. for $C_{32}H_{30}N_3O_4$ 520.2231).

*4.1.5.13. (E)-4-(3-(4-Methoxyphenyl)acryloyl)-N-(4-(phenylcarbamoyl)phenyl)benzamide (**15**).* Yield 38%; gray powder; m.p. > 300 °C; ^1H NMR (300 MHz, DMSO-d_6): δ 10.70 (s, 1H, CONH), 10.17 (s, 1H, CONH), 8.29 (d, J = 8.4 Hz, 2H, ArH), 8.15 (d, J = 8.1 Hz, 2H, ArH), 8.03–7.95 (m, 4H, ArH), 7.91–7.78 (m, 6H, ArH, COCH=CH, COCH=CH), 7.36 (t, J = 7.8 Hz,2H, ArH), 7.09–7.03 (m, 3H, ArH), 3.84 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 189.1, 165.2, 165.4, 162.1, 145.2, 142.4, 140.7, 139.8, 138.6, 131.5 (2C), 130.4, 129.1 (2C), 129.0 (2C), 128.9 (2C), 128.7 (2C), 127.7, 124.0, 120.8 (2C), 120.1 (2C), 119.9, 114.9 (2C), 55.9; ESI-HR-MS *m/z* 477.1806 $[M + H]^+$ (Calcd. for $C_{30}H_{25}N_2O_4$ 477.1809).

*4.1.5.14. (E)-N-(4-Methoxyphenyl)-4-(4-(3-morpholino-3-oxoprop-1-en-1-yl)benzamido)benzamide (**16**).* Yield 48%; white powder; m.p. > 300 °C; ^1H NMR (300 MHz, DMSO-d_6): δ 10.52 (s, 1H, CONH), 10.04 (s, 1H, CONH), 8.03–7.89 (m, 8H, ArH), 7.68 (d, J = 8.7 Hz, 2H, ArH), 7.59 (d, J = 15.6 Hz, 1H, CH=CHCO), 7.40 (d, J = 15.6 Hz, 1H, CH=CHCO), 6.93 (d, J = 8.4 Hz, 2H, ArH), 3.75–3.61 (m, 11H, OCH_3, 4 × CH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.6, 165.0, 164.8, 155.9, 142.4, 141.0, 138.8, 132.8, 130.3, 129.1, 128.8 (2C), 128.7 (2C), 128.5 (2C), 122.4 (2C), 120.5, 120.0 (2C), 114.2 (2C), 66.9, 66.7, 55.6, 46.1, 42.6; ESI-HR-MS *m/z* 486.2016 $[M + H]^+$ (Calcd. for $C_{28}H_{28}N_3O_5$ 486.2024).

*4.1.5.15. 4-Fluoro-N-(4-((4-(phenylcarbamoyl)phenyl)carbamoyl)benzyl)benzamide (**17**).* Yield 40%; white powder; m.p. > 300 °C; ^1H NMR (300 MHz, DMSO-d_6): δ 10.46 (s, 1H, NH), 10.14 (s, 1H, NH), 9.17 (s, 1H, NH), 8.01–7.92 (m, 8H, ArH), 7.78 (d, J = 7.8 Hz, 2H, ArH), 7.48 (d, J = 8.1Hz, 2H, ArH), 7.37–7.29 (m, 4H, ArH), 7.10 (t, J = 7.2 Hz, 1H, ArH), 4.57 (d, J = 5.7 Hz, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.1, 165.8, 165.4, 164.5 (d, $J_{C\text{-}F}$ = 257 Hz), 144.2, 142.7, 139.8, 133.6, 131.2 (d, $J_{C\text{-}F}$ = 3 Hz), 130.4 (d, $J_{C\text{-}F}$ = 9 Hz, 2C), 130.0, 129.1 (2C), 128.9 (2C), 128.4 (2C), 127.6 (2C), 124.0, 120.8 (2C), 119.9 (2C), 115.8 (d, $J_{C\text{-}F}$ = 22 Hz, 2C), 43.0; ESI-HR-MS *m/z* 468.1703 $[M + H]^+$ (Calcd. for $C_{28}H_{23}FN_3O_3$ 468.1718).

*4.1.5.16. 4-Methoxy-N-(4-((4-(phenylcarbamoyl)phenyl)carbamoyl)benzyl)benzamide (**18**).* Yield 67%; white powder; m.p. > 300 °C; ^1H NMR (300 MHz, DMSO-d_6): δ 10.44 (s, 1H, CONH), 10.13 (s, 1H, CONH), 8.98 (t, 1H, CONH), 7.96–7.87 (m, 8H, ArH), 7.77 (d, J = 7.8 Hz, 2H, ArH), 7.46 (d, J = 7.8 Hz, 2H, ArH), 7.34 (t, 2H, ArH), 7.10–7.00 (m, 3H, ArH), 4.54 (d, J = 5.1 Hz, 2H, CH_2), 3.69 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.3, 166.2, 165.4, 162.1, 144.5, 142.7, 139.8, 133.5, 130.0, 129.6 (2C), 129.1 (2C), 128.9 (2C), 128.3 (2C), 127.6 (2C), 126.9, 124.0, 120.8 (2C), 119.9 (2C), 114.0 (2C), 55.8, 42.9; ESI-MS *m/z* 480.2 $[M + H]^+$.

*4.1.5.17. 3-Fluoro-4-(4-((4-methoxybenzamido)methyl)benzamido)-N-phenylbenzamide (**19**).* Yield 70%; white powder; m.p. 294–296 °C; ^1H NMR (400 MHz, DMSO-d_6): δ 10.27 (s, 1H, CONH), 10.25 (s, 1H, CONH), 8.99 (s, 1H, CONH), 7.96 (d, J = 8.0 Hz, 2H, ArH), 7.97–7.84 (m, 5H, ArH), 7.77 (d, J = 8.0 Hz, 2H, ArH), 7.46 (d, J = 8.0 Hz, 2H, ArH), 7.36 (m, 2H, ArH), 7.11 (t, J = 8.4, 7.2 Hz, 1H, ArH), 7.02 (d, J = 8.8 Hz, 2H, ArH), 4.55 (d, J = 5.6 Hz, 2H, CH_2), 3.81 (s, 3H, OCH_3); ^{13}C NMR (150 MHz, DMSO-d_6): δ 166.2, 165.8, 163.2 (d, $J_{C\text{-}F}$ = 306 Hz), 162.1, 155.8, 144.7, 139.4, 133.1 (d, $J_{C\text{-}F}$ = 7 Hz), 132.6, 129.5

(2C), 129.4, 129.1 (2C), 128.9 (d, $J_{C\text{-}F}$ = 7 Hz), 128.4 (2C), 127.5 (2C), 126.8, 126.6 (d, $J_{C\text{-}F}$ = 8 Hz), 126.3, 124.3 (d, $J_{C\text{-}F}$ = 3 Hz), 124.2, 120.9 (2C), 115.6 (d, $J_{C\text{-}F}$ = 22 Hz), 114.0 (2C), 55.8, 42.8; ESI-HR-MS *m/z* 498.1808 $[M + H]^+$ (Calcd. for $C_{29}H_{25}FO_4N_3$ 498.1824).

*4.1.5.18. 4-Methoxy-N-(4-((4-((4-morpholinophenyl)carbamoyl) phenyl)carbamoyl)benzyl)benzamide (**20**).* Yield 48%; white powder; m.p. > 300 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.42 (s, 1 H, CONH), 9.96 (s, 1H, CONH), 8.98 (t, 1H, CONH), 7.94–7.87 (m, 6H, ArH), 7.63–7.54 (m, 4H, ArH), 7.46(d, 2H, J = 8.1 Hz, ArH), 7.01 (d, 2H, J = 9.0 Hz, ArH), 6.92 (d, 2H, J = 9.0 Hz, ArH), 4.54 (d, 2H, J = 6.0 Hz, CH_2), 3.81 (s, 3H, OCH_3), 3.75–3.69 (m, 4H, morpholino-H), 3.08–3.04 (m, 4H, morpholino-H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.3, 166.1, 164.8, 162.1, 147.9, 144.5, 142.5, 133.6, 131.9,130.2, 129.6 (2C), 128.8 (2C), 128.3 (2C), 127.6 (2C), 126.9, 121.9 (2C), 119.9 (2C), 115.7 (2C), 114.0 (2C), 66.6 (2C), 55.8, 49.4 (2C), 42.9; ESI-HR-MS *m/z* 565.2463 $[M + H]^+$ (Calcd. for $C_{33}H_{32}N_4O_5$ 565.2446).

*4.1.5.19. N-(2-Fluorophenyl)-4-(4-((4-methoxybenzamido) methyl)benzamido)benzamide (**21**).* Yield 55%; white powder; m.p. > 300 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.46 (s, 1H, CONH), 10.01 (s, 1H, CONH), 8.99 (s, 1H, CONH), 8.01–7.89 (m, 8H, ArH), 7.61 (t, J = 7.6 Hz, 1H, ArH), 7.47 (d, J = 7.6 Hz, 2H, ArH), 7.29–7.21 (m, 3H, ArH), 7.02 (d, J = 8.4 Hz, 2H, ArH), 4.55 (d, J = 5.2 Hz, 2H, CH_2), 3.81 (s, 3H, OCH_3);^{13}C NMR (100 MHz, DMSO-d_6): δ 166.3, 166.2, 165.3, 162.1, 156.3 (d, $J_{C\text{-}F}$ = 245 Hz), 144.5, 142.9, 133.5, 129.6 (2C), 129.1 (2C), 129.0, 128.3 (2C), 127.6 (d, $J_{C\text{-}F}$ = 1 Hz), 127.5 (2C), 127.3 (d, $J_{C\text{-}F}$ = 8 Hz), 126.9, 126.3 (d, $J_{C\text{-}F}$ = 13 Hz), 124.7 (d, $J_{C\text{-}F}$ = 3 Hz), 119.9 (2C), 116.3 (d, $J_{C\text{-}F}$ = 19 Hz), 114.0 (2C), 55.8, 42.9; ESI-HR-MS *m/z* 498.1818 $[M + H]^+$ (Calcd. for $C_{29}H_{25}FN_3O_4$ 498.1824).

*4.1.5.20. 4-Methoxy-N-(4-((4-(o-tolylcarbamoyl)phenyl)carbamoyl)benzyl)benzamide (**22**).* Yield 54%; white powder; m.p.: 275–277 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.44 (s, 1H, CONH), 9.78 (s, 1H, CONH), 8.99 (s, 1H, CONH), 8.09 (d, J = 7.5 Hz, 1H, ArH), 7.98–7.88 (m, 7H, ArH), 7.55–7.46 (m, 2H, ArH), 7.35 (d, J = 7.8 Hz, 1H, ArH), 7.26–7.16 (m, 3H, ArH), 7.02 (d, J = 8.1 Hz, 2H, ArH), 4.56 (s, 2H, CH_2), 3.82 (s, 3H, OCH_3), 2.24 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.3, 166.1, 165.2, 162.1, 144.5, 142.6, 137.0, 134.1, 133.6, 130.8, 129.7, 129.6 (2C), 128.9 (2C), 128.3 (2C),127.6, 127.6 (2C), 127.1, 126.9, 126.4, 119.9 (2C), 114.0 (2C), 55.8, 42.9, 18.4; ESI-HR-MS *m/z* 494.2068 $[M + H]^+$ (Calcd. for $C_{30}H_{28}N_3O_4$ 494.2074).

*4.1.5.21. 4-Methoxy-N-(4-((4-((2-methoxyphenyl)carbamoyl)phenyl)carbamoyl)benzyl)benzamide (**23**).* Yield 38%; white powder; m.p.255–257 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.45 (s, 1H, CONH), 9.32 (s, 1H, CONH), 8.99 (s, 1H, CONH), 7.96–7.79 (m, 9H, ArH), 7.47 (d, J = 6.6 Hz, 2H, ArH), 7.17–6.96 (m, 5H, ArH), 4.55 (s, 2H, CH_2), 3.85 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.3, 166.2, 164.8, 162.1, 151.8, 144.5, 142.8, 133.6, 129.6, 129.6 (2C), 128.7 (2C), 128.3 (2C), 127.6 (2C), 127.4, 126.9, 126.0, 124.5, 120.7, 120.0 (2C), 114.0 (2C), 111.8, 56.1, 55.8, 42.9; ESI-HR-MS *m/z* 510.2014 $[M + H]^+$ (Calcd. for $C_{30}H_{28}N_3O_5$ 510.2024).

*4.1.5.22. 4-Methoxy-N-(4-((4-(phenethylcarbamoyl)phenyl)carbamoyl)benzyl)benzamide (**24**).* Yield 43%; white powder; m.p. > 300 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.37 (s, 1H, CONH), 8.97 (t, J = 5.7 Hz, 1H, CONH), 8.47 (t, J = 6.3 Hz,1H, CONH), 7.93–7.79 (m, 8H, ArH), 7.45 (d, J = 7.8 Hz, 2H, ArH), 7.32–7.11 (m, 5H, ArH), 7.01 (d, J = 8.7 Hz, 2H, ArH), 4.53 (d, J = 5.7 Hz, 2H, CH_2), 3.81 (s, 3H, OCH_3), 3.46 (m, 2H, CH_2), 2.83 (t, J = 7.5 Hz, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.7, 166.3, 166.2, 162.1,144.4, 142.2, 140.1, 133.6, 129.9, 129.6 (2C), 129.1 (2C), 128.8 (2C), 128.3 (4C), 127.6, 127.5 (2C), 126.9, 126.6, 119.9 (2C), 114.0 (2C), 55.8, 42.8, 41.3, 35.7; ESI-HR-MS *m/z* 508.2228 $[M + H]^+$ (Calcd. for $C_{31}H_{29}N_3O_4$ 508.2231).

*4.1.5.23. N-(4-Benzamidophenyl)-4-(3-(4-methoxyphenyl)-3-oxopropyl)benzamide (**25**).* Yield 48%; yellow powder; m.p. 255–257 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.23 (s, 1H, CONH), 10.15 (s, 1H, CONH), 8.03–7.95 (m, 4H, ArH), 7.87 (d, J = 7.5 Hz, 2H, ArH), 7.74 (s, 4H, ArH), 7.58–7.52 (m, 3H, ArH), 7.43 (d, 2H, J = 8.1 Hz, ArH), 7.03 (d, 2H, J = 8.4 Hz, ArH), 3.83 (s, 3H, OCH_3), 3.36 (t, J = 7.5 Hz, 2H, CH_2), 3.02 (t, J = 7.5 Hz, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 197.8, 166.8, 165.6, 163.6, 145.8, 135.5, 135.4, 135.3, 133.1, 132.0, 130.7 (2C), 130.0, 128.9 (2C), 128.8 (2C), 128.1 (2C), 128.1 (2C), 121.1 (2C), 121.0 (2C), 114.4 (2C), 56.0, 39.2, 30.0; ESI-HR-MS *m/z* 479.1956 $[M + H]^+$ (Calcd. for $C_{30}H_{26}N_2O_4$ 479.1965).

*4.1.5.24. 4-(3-(4-Fluorophenyl)-3-oxopropyl)-N-(4-(2-(4-methoxyphenyl)acetamido)phenyl)benzamide (**26**).* Yield 81%; brown powder; m.p. 259–260 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.10 (s, 1H, CONH), 10.07 (s, 1H, CONH), 8.08 (dd, J = 5.6, 8.4 Hz, 2H, ArH), 7.86 (d, J = 8.0 Hz, 2H, ArH), 7.67 (d, J = 9.2 Hz, 2H, ArH), 7.55 (d, J = 8.8 Hz, 2H, ArH), 7.42 (d, J = 8.0 Hz, 2H, ArH), 7.34 (t, J = 8.8 Hz, 2H, ArH), 7.25 (d, J = 8.4 Hz, 2H, ArH), 6.88 (d, J = 8.4 Hz, 2H, ArH), 3.73 (s, 3H, OCH_3), 3.54 (s, 2H, CH_2), 3.43 (t, J = 7.6 Hz, CH_2), 3.01 (t, J = 7.6 Hz, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 198.0, 169.6, 165.5, 165.5 (d, $J_{C\text{-}F}$ = 250 Hz), 158.5, 145.5, 135.5, 135.0, 133.8 (d, $J_{C\text{-}F}$ = 3 Hz), 133.1, 131.4 (d, $J_{C\text{-}F}$ = 10 Hz, 2C), 130.5 (2C), 128.8 (2C), 128.5, 128.1 (2C), 121.2 (2C), 119.8 (2C), 116.2 (d, $J_{C\text{-}F}$ = 22 Hz, 2C), 114.2 (2C), 55.5, 42.9, 39.4, 29.7; ESI-HR-MS *m/z* 511.2040 $[M + H]^+$ (Calcd. for $C_{31}H_{28}FN_2O_4$ 511.2028).

*4.1.5.25. 4-(3-(4-Methoxyphenyl)-3-oxopropyl)-N-(4-(2-(4-methoxyphenyl)acetamido)phenyl)benzamide (**27**).* Yield 50%; white powder; m.p. 241–242 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.09 (s, 1H, CONH), 10.06 (s, 1H, CONH), 7.97 (d, J = 8.8 Hz, 2H, ArH), 7.85 (d, J = 8.0 Hz, 2H, ArH), 7.67 (d, J = 8.8 Hz, 2H, ArH), 7.54 (d, J =8.8 Hz, 2H, ArH), 7.41 (d, J = 8.0 Hz, 2H, ArH), 7.24 (d, J = 8.4 Hz, 2H, ArH), 7.02 (d, J = 8.8 Hz, 2H, ArH), 6.88 (d, J = 8.4 Hz, 2H, ArH), 3.83 (s, 3H, OCH_3), 3.72 (s, 3H, OCH_3), 3.53 (s, 2H, CH_2), 3.35 (t, J = 8.2 Hz, 2 H, CH_2), 3.00 (t, J = 8.2 Hz, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 197.7, 169.6, 165.6, 163.6, 158.5, 145.7, 135.5, 135.0, 133.1, 130.7 (2C), 130.6 (2C), 130.0, 128.9 (2C), 128.5, 128.1 (2C), 121.2 (2C), 119.8 (2C), 114.3 (2C), 114.2 (2C), 56.0, 55.5, 42.9, 39.1, 30.0; ESI-HR-MS *m/z* 523.2221 $[M + H]^+$ (Calcd. for $C_{32}H_{30}N_2O_5$ 523.2228).

*4.1.5.26. (E)-4-(3-(4-Methoxyphenyl)-3-oxoprop-1-en-1-yl)-N-(4-(2-(4-methoxyphenyl)acetamido)phenyl)benzamide (**28**).* Yield 72%; brown powder; m.p. 292–294 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.27 (s, 1H, CONH), 10.09 (s, 1H,CONH), 8.20 (d, J = 8.8 Hz, 2H, ArH), 8.09–8.02 (m, 5H, ArH, CH=CHCO), 7.76 (d, J = 15.2 Hz, 1H, CH=CHCO), 7.70 (d, J = 8.4 Hz, 2H, ArH), 7.58 (d, J = 8.8 Hz, 2H, ArH), 7.26 (d, J = 8.4 Hz, 2H, ArH), 7.10 (d, J = 8.8 Hz, 2H, ArH), 6.89 (d, J = 8.4 Hz, 2H, ArH), 3.87 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3), 3.55 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 187.8, 169.7, 165.0, 163.9, 158.5, 142.4, 138.1, 136.6,135.7, 134.8, 131.5 (2C), 130.8, 130.6 (2C), 129.2 (2C), 128.6 (2C), 128.4, 124.1, 121.4 (2C), 119.8 (2C), 114.6 (2C), 114.2 (2C), 56.1, 55.5, 42.9; ESI-HR-MS m/z 521.2090 $[M + H]^+$ (Calcd. for $C_{32}H_{29}N_2O_5$ 521.2071).

*4.1.5.27. (E)-N-(4-(2-(4-Methoxyphenyl)acetamido)phenyl)-4-(3-(4-methoxyphenyl)acryloyl)benzamide (**29**).* Yield 80%; yellow powder; m.p. 284–286 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.38 (s, 1H, CONH), 10.11 (s, 1H, CONH), 8.25 (d, J = 8.0 Hz, 2 H, ArH), 8.10 (d, J = 8.0 Hz, 2H, ArH), 7.89–7.83 (m, 3H, ArH, COCH=CH), 7.77 (d, J = 15.6 Hz, 1H, COCH=CH), 7.71 (d, J = 8.8 Hz, 2H, ArH), 7.58 (d, J = 8.4 Hz, 2H, ArH), 7.25 (d, J = 8.4 Hz, 2H, ArH), 7.03 (d, J = 8.4 Hz, 2H, ArH), 6.89 (d, J = 8.4 Hz, 2H, ArH), 3.83 (s, 3 H, OCH_3), 3.73 (s, 3H, OCH_3), 3.55 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 189.1, 169.7, 165.0, 162.0, 158.5, 145.2, 140.4, 139.0, 135.8, 134.7, 131.5 (2C), 130.6 (2C), 128.9 (2C), 128.5 (2C), 128.4, 127.7, 121.4 (2C), 119.9, 119.8 (2C), 114.9 (2C), 114.2 (2C), 44.9, 55.5, 42.9; ESI-HR-MS m/z 521.2090 $[M + H]^+$ (Calcd. for $C_{32}H_{29}N_2O_5$ 521.2071).

*4.1.5.28. (E)-4-(3-Cyclohexyl-3-oxoprop-1-en-1-yl)-N-(4-(2-(4-methoxyphenyl)acetamido)phenyl)benzamide (**30**).* Yield 83%; light yellow powder; m.p. 285–286 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.25 (s, 1H, CONH), 10.09 (s, 1H, CONH), 7.99 (d, J = 8.4 Hz, 2H, ArH), 7.88 (d, J = 8.0 Hz, 2H, ArH), 7.69 (d, J = 8.8 Hz, 2H, ArH), 7.63 (d, J = 16.0 Hz, 1H, CH=CHCO), 7.57 (d, J = 8.8 Hz, 2H, ArH), 7.25 (d, J = 8.4 Hz, 2H, ArH), 7.15 (d, J = 16.0 Hz, 1H, CH=CHCO), 6.89 (d, J = 8.4 Hz, 2H, ArH), 3.73 (s, 3H, OCH_3), 3.54 (s, 2H, CH_2), 2.79-2.75 (m, 1H, CH), 1.86-1.64 (m, 5H, cyclohexyl-H), 1.38-1.16 (m, 5H, cyclohexyl-H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 202.8, 169.7, 165.0, 158.5, 140.9, 137.9, 136.5, 135.7, 134.8, 130.5 (2 C), 128.8 (2C), 128.6 (2C), 128.4, 127.1, 121.3 (2C), 119.8 (2C), 114.2 (2C), 55.5, 48.6, 42.9, 28.6 (2C), 26.0, 25.6 (2C); ESI-HR-MS m/z 497.2446 $[M + H]^+$ (Calcd. for $C_{31}H_{33}N_2O_4$ 497.2435).

*4.1.5.29. N-(4-Benzamidophenyl)-4-((4-methoxybenzamido) methyl)benzamide (**31**).* Yield 53%; white powder; m.p. > 300 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.23(s, 1H, CONH), 10.17 (s, 1H, CONH), 8.97 (t, J = 6.0 Hz, 1H, CONH), 7.96–7.87 (m, 6H, ArH), 7.73 (s, 4H, ArH), 7.58–7.50 (m, 3H, ArH), 7.43 (d, J = 8.4 Hz, 2H, ArH), 7.01 (d, J = 8.7 Hz, 2H, ArH), 4.53 (d, J = 6.0 Hz, 2H, CH_2), 3.81 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.3, 165.8, 165.6, 162.1, 144.1, 135.5, 135.4, 133.9, 132.0, 129.9, 129.6 (2C), 128.8 (2C), 128.2 (2C), 128.1 (2C), 127.6, 127.5 (2C), 126.9 (2C), 121.1 (2C), 114.0 (2C), 55.8, 42.8; ESI-HR-MS m/z 480.1915 $[M + H]^+$ (Calcd. for $C_{29}H_{25}N_3O_4$ 480.1918).

*4.1.5.30. N-(4-Methoxyphenyl)-3-(4-(3-(4-methoxyphenyl)-3-oxopropyl)benzamido)benzamide (**32**).* Yield 48%; yellow powder; m.p. 230–233 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.34 (s, 1H, CONH), 10.14 (s, 1H, CONH), 8.28 (s, 1H, ArH), 8.06 7.90 (m, 5H, ArH), 7.69–7.64 (m, 3H, ArH), 7.51 7.43 (m, 3H, ArH), 7.03 (d, 2H, J = 8.7 Hz, ArH), 6.93 (d, 2H, J = 9.0 Hz), 3.83 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 3.37 (t, J = 7.5 Hz, 2H, CH_2), 3.01 (t, J = 7.5 Hz, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6):O δ 197.8, 166.0, 165.6, 163.6, 156.0, 146.1, 139.9, 136.2, 132.7, 132.7, 130.8 (2C), 130.0, 129.0, 128.9 (2C), 128.2 (2C), 123.6, 122.9, 122.4 (2C), 120.3, 114.4 (2C), 114.2 (2C), 56.0, 55.7, 39.1, 30.0; ESI-HR-MS m/z 509.2063 $[M + H]^+$ (Calcd. for $C_{31}H_{28}N_2O_5$ 509.2071).

*4.1.5.31. N-(3,5-Dimethoxyphenyl)-3-(4-(3-(4-methoxyphenyl)-3-oxopropyl)benzamido)benzamide (**33**).* Yield 47%; white powder; m.p. 179–182 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.35 (s, 1H, CONH), 10.18 (s, 1H, CONH), 8.28 (s, 1H, ArH), 8.01–7.89 (m, 5H, ArH), 7.64 (d, J = 7.5 Hz, 1H, ArH), 7.52–7.42 (m, 3H, ArH), 7.08–7.01 (m, 4H, ArH), 6.26 (t, J = 2.1 Hz, 1H, ArH), 3.83 (s, 3H, OCH_3), 3.73 (s, 6H, 2 × OCH_3), 3.37 (t, J =7.5 Hz, 2H, CH_2), 3.01 (t, J = 7.5 Hz, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 197.8, 166.1, 166.0, 163.6, 160.9 (2C), 146.1, 141.3, 139.9, 136.1, 132.7, 130.7 (2C), 130.0, 129.1, 128.9 (2C), 128.2 (2C), 123.7, 123.0, 120.3, 114.4 (2C), 99.0 (2C), 96.1, 56.0, 55.6, 39.1, 30.0; ESI-HR-MS m/z 539.2168 $[M + H]^+$ (Calcd. for $C_{32}H_{30}N_2O_6$ 539.2177).

*4.1.5.32. 3-(4-(3-(4-Methoxyphenyl)-3-oxopropyl)benzamido)-N-(4-morpholinophenyl)benzamide (**34**).* Yield 50%; gray powder; m.p. 215–217 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.34 (s, 1H, CONH), 10.08 (s, 1H, CONH), 8.28 (s, 1H, ArH), 7.98–7.90 (m, 5H, ArH), 7.65–7.60 (m, 3H, ArH), 7.47–7.42 (m, 3H, ArH), 7.03 (d, 2H, J =7.2 Hz, ArH), 6.94 (d, 2H, J = 7.5 Hz, ArH), 3.83 (s, 3H, OCH_3), 3.76-3.70 (m, 4H, morpholino-H), 3.36 (t, 2H, CH_2), 3.08-3.00 (m, 6H,CH_2, morpholino-H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 197.8, 166.1, 165.6, 163.6, 148.0, 146.1, 139.9, 136.3, 132.8, 132.0, 130.8 (2C), 130.0, 129.1, 129.0 (2C), 128.3 (2C), 123.6, 122.9, 122.0 (2C), 120.4, 115.9 (2C), 114.4 (2C), 66.6 (2C), 56.0, 49.4 (2C), 39.2, 30.0; ESI-HR-MS m/z 564.2477 $[M + H]^+$ (Calcd. for $C_{34}H_{33}N_3O_5$ 564.2493).

*4.1.5.33. 4-(3-(4-Methoxyphenyl)-3-oxopropyl)-N-(4-phenoxyphenyl)benzamide (**35**).* Yield 39%; white powder; m.p. 163–165 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.18 (s, 1H, CONH), 7.97 (d, J = 9.0 Hz, 2H, ArH), 7.86 (d, J = 8.1 Hz, 2H, ArH), 7.77 (d, J = 9.0 Hz, 2H, ArH), 7.44–7.34 (m, 4H, ArH), 7.12–6.96 (m, 7H, ArH), 3.83 (s, 3H, OCH_3), 3.36 (t, J = 7.5 Hz, 2H, CH_2), 3.00 (t, J = 7.5 Hz, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 197.8, 165.7, 163.6, 157.8, 152.5, 145.8, 135.6, 133.0, 130.7 (2C), 130.5 (2C), 130.0, 128.9 (2C), 128.1 (2C), 123.5, 122.5 (2C), 119.8 (2C), 118.4 (2C), 114.4 (2C), 56.0, 39.2, 30.0; ESI-HR-MS m/z 452.1845 $[M + H]^+$ (Calcd. for $C_{29}H_{25}NO_4$ 452.1856).

*4.1.5.34. N-(4-(Benzyloxy)phenyl)-4-(3-(4-methoxyphenyl)-3-oxopropyl)benzamide (**36**).* Yield 47%; yellow powder; m.p. 177–179 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.02 (s, 1H, CONH), 7.97(d, J =8.7 Hz, 2H, ArH), 7.84 (d, J = 8.4 Hz, 2H, ArH), 7.65 (d, J = 9.0 Hz, 2H, ArH), 7.45–7.31 (m, 7H, ArH), 7.02 (d, J = 8.7 Hz, 2H, ArH), 6.99 (d, J = 8.7 Hz, 2H, ArH), 5.08 (s, 2H,

OCH_2), 3.83 (s, 3H, OCH_3), 3.35 (t, J = 7.5 Hz, 2H, CH_2), 3.00 (t, J = 7.5 Hz, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 197.8, 165.4, 163.6, 155.0, 145.7, 137.7, 133.1, 133.0, 130.7 (2C), 130.0, 128.9 (2C), 128.8 (2C), 128.3, 128.2 (2C), 128.0 (2C), 122.3 (2C), 115.2 (2C), 114.4 (2C), 69.8, 56.0, 39.2, 30.0; ESI-HR-MS *m/z* 466.2005 $[M + H]^+$ (Calcd. for $C_{30}H_{27}NO_4$ 466.2013).

*4.1.5.35. N-(4-Carbamoylphenyl)-4-(3-(4-methoxyphenyl)-3-oxopropyl)benzamide (**37**).* Yield 68%; white powder; m.p. 261–263 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.34 (s, 1H, CONH), 7.97 (d, J = 9.0 Hz, 2H, ArH), 7.89–7.85 (m, 6H, ArH, ArH), 7.44 (d, J = 8.1 Hz, 2H, ArH), 7.25 (s, 2H, $CONH_2$), 7.02 (d, J = 8.7 Hz, 2 Hz, ArH), 3.82(s, 3H, OCH_3), 3.36(t, J = 7.2 Hz, 2H, CH_2), 3.01 (t, J = 7.2 Hz, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 197.7, 168.9, 166.1, 163.6, 146.1, 142.4, 132.8, 130.7 (2C), 130.0, 129.5, 128.9 (2C), 128.7 (2C), 128.3 (2C), 119.7 (2C), 114.3 (2C), 56.0, 39.1, 30.0; ESI-HR-MS *m/z* 403.1639 $[M + H]^+$ (Calcd. for $C_{24}H_{22}N_2O_4$ 403.1652).

*4.1.5.36. Ethyl 4-(4-(3-(4-methoxyphenyl)-3-oxopropyl)benzamido)benzoate (**38**).* Yield 69%; white powder; m.p. 166–167 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.58 (bs, 1H, CONH), 7.97–7.88 (m, 8H, ArH), 7.44 (d, J = 7.8 Hz, 2H, ArH), 7.02 (d, J = 8.7 Hz, 2H, ArH), 4.29 (q, J = 7.2 Hz, 2 H, $\underline{CH_2}CH_3$), 3.82 (s, 3H, OCH_3), 3.36 (t, J = 7.5 Hz, 2H, CH_2), 3.00 (t, J = 7.5 Hz, 2H, CH_2), 1.31 (t, J = 7.2 Hz, 3H, $CH_2\underline{CH_3}$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 197.7, 166.3, 165.8, 163.6, 146.3, 144.2, 132.6, 130.7 (2C), 130.5 (2C), 130.0, 128.9 (2C), 128.3 (2C), 124.9, 120.0 (2C), 114.4 (2C), 60.9, 56.0, 39.1, 30.0, 14.7; ESI-HR-MS *m/z* 432.1793 $[M + H]^+$ (Calcd. for $C_{26}H_{25}N_2O_5$ 432.1806).

*4.1.5.37. N^1-(4-(3-(4-Methoxyphenyl)-3-oxopropyl)phenyl)-N4-phenylmaleamide (**39**).* Yield 18%; yellow powder; m.p. 154–155 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.54 (s, 1H, CONH), 10.45 (s, 1H, CONH), 7.95 (d, 2H, J = 8.7 Hz, ArH), 7.61 (d, J = 7.5 Hz, 2H, ArH), 7.51 (d, J = 8.1 Hz, 2H, ArH), 7.31 (t, 2H, ArH),7.20 (d, J = 8.7 Hz, 2H, ArH), 7.08–7.00 (m, 3H, ArH), 6.41 (s, 2H, 2 × CH), 3.82 (s, 3H, OCH_3), 3.26 (t, J = 7.5 Hz, 2H, CH_2), 2.86 (t, J = 7.5 Hz, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 198.0, 164.0, 163.7, 163.5, 139.3, 137.2, 137.1, 132.4, 132.1, 130.7 (2C), 130.1, 129.2 (2C), 129.1 (2C), 124.0, 119.9 (2C), 119.8 (2C), 114.3 (2C), 59.0, 39.7, 29.7; ESI-HR-MS *m/z* 429.1797 $[M + H]^+$ (Calcd. for $C_{26}H_{24}N_2O_4$ 429.1809).

*4.1.5.38. N-(3-((4-(3-(4-Methoxyphenyl)-3-oxopropyl)phenyl)amino)-3-oxopropyl)benzamide (**40**).* Yield 62%; yellow powder; m.p. 150–153 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.87 (s, 1H, CONH), 8.57 (t, J = 6.6 Hz,1H, CONH), 7.94 (d, J = 8.7 Hz,2H, ArH), 7.82 (d, J = 6.9 Hz, 2H, ArH), 7.50–7.41(m, 5H, ArH), 7.17 (d, J =8.4 Hz, 2H, ArH), 7.01 (d, J = 8.7 Hz, 2H), 3.82 (s, 3H, OCH_3), 3.55 (m, 2H, CH_2), 3.25 (t, J = 7.5 Hz, 2H, CH_2), 2.85 (t, J = 7.5 Hz, 2H, CH_2), 2.59 (t, J = 7.2 Hz, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 198.0, 169.7, 166.8, 163.5, 137.6, 136.5, 135.0, 131.6, 130.7(2C), 130.1, 129.0(2C), 128.7(2C), 127.6(2C), 119.6(2C), 114.3(2C), 56.0, 39.7, 36.8, 36.4, 29.6; ESI-HR-MS *m/z* 431.1954 $[M + H]^+$ (Calcd. for $C_{26}H_{26}N_2O_4$ 431.1965).

*4.1.6. Preparation of 4-(3-(4-methoxyphenyl)-3-oxopropyl)-N-((1S,4S)-4-(phenylcarbamoyl) cyclohexyl) benzamide (**41**)*

Step 1: 4-(3-(4-Methoxyphenyl)-3-oxopropyl)benzoic acid (1 equiv.), (1 *S*,4 *S*)-ethyl 4-aminocyclohexane carboxylate hydrochloride (1 equiv.), HATU (1.2 equiv.) and DIEA (4 equiv.) were dissolved in dichloromethane, the reaction mixture was stirred overnight at room temperature. After removal of the solvent under reduced pressure, the residue was dissolved in ethyl acetate and sequentially washed by dilute hydrochloride acid, saturated sodium bicarbonate and brine. The organic layer was dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate 1:1) to yield white solid.

Step 2: The solid (1 equiv.) was then dissolved in methanol, to the solution was added aqueous solution of NaOH (2 equiv.). The mixture was stirred at 50 °C for 2 h. The reaction was monitored by TLC. Methanol was removed by evaporation. The residue aqueous solution was adjusted to pH 2 with dilute hydrochloric acid and precipitation was observed. The precipitate was filtered, washed with water and dried to give white solid.

Step 3: The solid obtained in step 2 (1 equiv.) was dissolved in dichloromethane, and to the solution were added aniline (1 equiv.), HATU (1.2 equiv.) and DIEA (2.5 equiv.). The reaction mixture was stirred overnight at room temperature and concentrated. The residue was dissolved in ethyl acetate and sequentially washed by dilute hydrochloride acid, saturated sodium bicarbonate and brine. The organic layer was dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate 3:1) to yield compound **41** as slightly yellow solid.

The overall yield of the three steps is 35%. m.p. 100–102 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.74 (s, 1H, $CO\underline{NH}C_6H_5$), 8.08 (d, J = 6.6 Hz, 1H, $CO\underline{NH}CH$), 7.95 (d, J = 8.7 Hz, 2H, ArH), 7.76 (d, J = 8.4 Hz, 2H, ArH), 7.61 (d, J = 7.5 Hz, 2H, ArH), 7.34–7.24 (m, 4H, ArH), 7.03–6.98 (m, 3H, ArH), 3.82 (s, 3H, OCH_3), 3.62–3.58 (m, 1H, $N\underline{H}CH$), 3.31 (t, J = 7.5 Hz, 2H, CH_2), 3.16–3.12 (m, 1H, $CO\underline{CH}$), 2.96 (t, J = 7.5 Hz, 2H, CH_2), 1.63–1.57 (m, 4H, cyclohexyl-H), 1.27–1.22 (m, 4H, cyclohexyl-H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 197.8, 174.3, 166.4, 163.6, 145.0, 140.0, 133.1, 130.7 (2C), 130.0, 129.1 (2C), 128.5 (2C), 128.0 (2C), 123.3, 119.6 (2C), 114.3 (2C), 56.0, 54.1, 46.5, 39.2, 29.9, 29.2, 25.7, 18.5, 17.2; ESI-HR-MS *m/z* 485.2428 $[M + H]^+$ (Calcd. for $C_{30}H_{32}N_2O_4$ 485.2435).

4.2. *In vitro assay for PTP1B inhibition*

Recombinant human GST-PTP1B protein was overexpressed by hGST-PTP1B-BL21 *E. coli* and purified by GST affinity chromatography. The reagent pNPP was used as a substrate for the measurement of PTP1B activity. The compounds were pre-incubated with the enzyme at room temperature for 5 minutes. Assay was performed in the final volume of 100 μL in the active system containing 50 mmol/L HEPES, 5 mmol/L DTT, 150 mmol/L NaCl, 2 mmol/L EDTA, and 2 mmol/L pNPP (pH 7.0). The system was incubated at 30 °C for 10 minutes, and the reaction was stopped by addition of 50 μL 3 mol/L NaOH. Then, the absorbance was determined at 405 nm wavelength. The similar system without GST-PTP1B protein was used as blank. The IC_{50} value was calculated by nonlinear regression with GraphPad Prism 5.0.

4.3. *In vivo assays for anti-hyperglycemic and lipid-lowering efficacy*

The glucose level was determined using a glucose-oxidase assay kit (Sigma). The triglyceride and cholesterol levels were determined according to the kit instruction.

4.3.1. *Animal model and treatment*

Four-week old male C57BL/6 mice were obtained from the Animal Center of Institute of Laboratory Animal Sciences, Chinese Academy of Medical Sciences & Peking Union Medical College. The animals were housed at 21–23 °C and a humidity level of 40%–60%. They were exposed to a 12-h lighting cycle and allowed ad libitum access to water and the appointed chows. After 8 weeks feeding with high-fat diet (HFD) (50% fat, 36% carbohydrate, and 14% protein in energy), the model mice were randomly divided into four groups with eight mice in each group. Mice in different groups were treated with water (IR), rosiglitazone (10 mg/kg body weight per day, Rosi), metformin (200 mg/kg body weight per day, Met), and **27** (50 mg/kg body weight per day), respectively. Meanwhile, Aged-matched male C57BL/6 mice fed with the standard chow diet (1022, Beijing HFK Bioscience Co., Ltd., China, containing 12% fat, 62% carbohydrate, and 26% protein in energy) were used as normal control (Con). All experimental procedures were approved by the Animal Care Committee of the Peking Union Medical College and Chinese Academy of Medical Sciences (Beijing, China).

4.3.2. *Estimating glucose metabolism and insulin sensitivity*

In IPGTT, the mice were fasted for 2 h and then injected with glucose 1 g/kg body weight intraperitoneally; blood samples for the glucose determination were collected from the tails at 0, 15, 60, and 120 min after glucose loading. The area under the glucose-time curve (AUC) was calculated.

In ITT, the mice were fasted for 2 h, and injected with insulin (0.28 mU/kg) subcutaneously; then, the blood samples for the determination of glucose levels were collected from the tails at 0, 30, 60, and 120 minutes after the insulin loading. The values of AUC were calculated.

The HOMA-IR index was calculated according to the following Eq. (1):

$$\text{HOMA} - \text{IR index} = (\text{FPI} \times \text{FPG})/22.5 \quad (1)$$

where FPI is fasting plasma insulin concentration (mU/L) and FPG is fasting plasma glucose (mmol/L)[21].

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