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Research Article

Synthesis, hypoglycemic and aldose reductase inhibition activity of novel ferulic acid derivatives

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Abstract

A novel class of ferulic acid derivatives with urea groups were synthesized and evaluated for hypoglycemic and aldose reductase inhibition activities. Several compounds showed comparable *in vivo* hypoglycemic agents to the commercial drug glibenclamide. Furthermore, of the tested compounds, **7a** and 7**b** displayed the most potent aldose reductase inhibitory activity *in vitro*, with an IC_{50} of 0.55 and 3.88 μM, respectively. Docking simulation was performed to insert compound **7a** and **12a** into the crystal structure of aldose reductase at active site to determine the probable binding model.

Keywords: Ferulic Acid, Aldose reductase inhibitor, Hypoglycemic activity.

1. Introduction

Diabetes mellitus (DM), representing 8.3% of the adult population with equal rates in both women and men, is a fast growing medical problem which critically attacks on metabolic activities of the patients. According to WHO, the number of diabetic patients is expected to increase up to 552 million by the year 2030.[1] It was widely believed that hyperglycaemia due to insulin resistance in liver and peripheral tissues along with other factors such as high caloric intake, sedentary life and styles and lack of exercise, cause to debilite disease.

Patients with diabetes mellitus are faced with the danger of developing long term complications including neuropathy, nephropathy, retinopathy and cataract.[2-5] As the key enzyme of the polyol pathway, aldose reductase (AR) plays important roles in the pathogenesis of a host of diabetic complications such as neuropathy, nephropathy, retinopathy and cataract.

For example, it has been reported that aldose reductase can catalyze an NADPH-dependent reduction of glucose to sorbitol, which in turn is oxidised to fructose by an NADt-dependent sorbitol dehydrogenase in hyperglycaemic conditions. [6-9] Thus inhibition of aldose reductase represents a possible approach to the treatment of some of the secondary diabetic complications. In fact, it have been shown that blockage of AR can have beneficial effect in diabetic complications and aldose reductase inhibitors (ARIs) can reduce tissue sorbitol accumulation in diabetic animals.

An aldose reductase inhibitor (ARI) prevents a spectrum of retinal abnormalities more comprehensively than other types of drugs.[10-14] As a holistic and combinational approach, the use of traditional Chinese medicine (TCM) in the management of DM has its superior advantage.[15] Factually, natural products are always special sources for the discovery of potential drugs with varying biological activity.

Ferulic acid (Figure 1) is an active ingredient in the Chinese traditional medicine, Angelica sinensis, which elicits protective effects against diabetes, cardiovascular disease, cancer, and Alzheimer's disease. [16, 17]

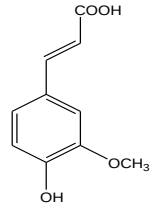
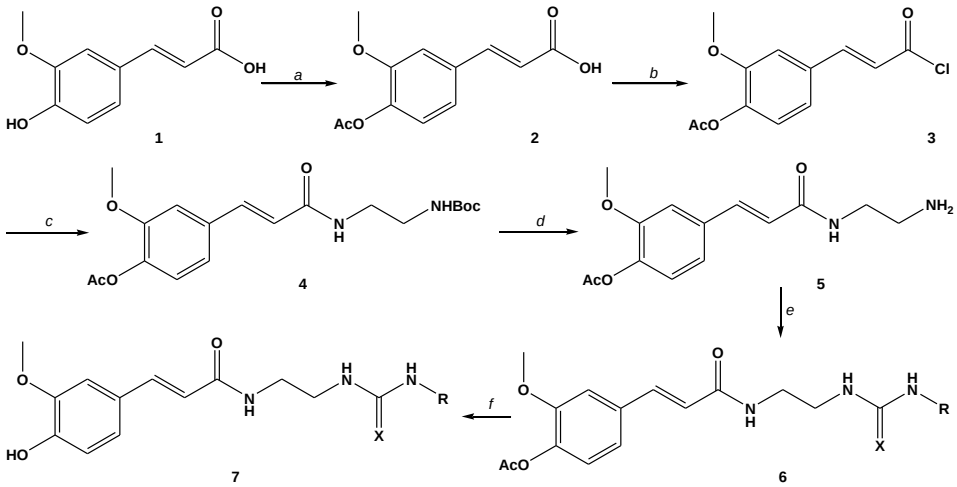


Figure 1: The structure of ferulic acid

Previous studies have investigated the treatment effects of ferulic acid and its combinations with other antioxidants on streptozotocin (STZ) or alloxan-induced diabetes in animals (rats and mice). Experimental studies in diabetic models have demonstrated that FA possesses multiple mechanisms of action associated with anti-hyperglycemic activity. [18] Interest in biological activities of synthetic compounds derived from Ferulic acid has also intensified in recent years. The amide compounds of ferulic acid have been reported to exhibit their stimulatory abilities on insulin secretion in rat pancreatic RIN-5F cells. Furthermore, some styrene ketone derivatives have been identified as a new class of ALR2 inhibitors. [19-21] All of these led us to investigate comparatively the aldose reductase inhibitory activities of ferulic acid derivatives with amide groups. Herein, we have synthesized some novel ferulic acid derivatives with urea groups and evaluated their hypoglycemic and aldose reductase inhibition activities.

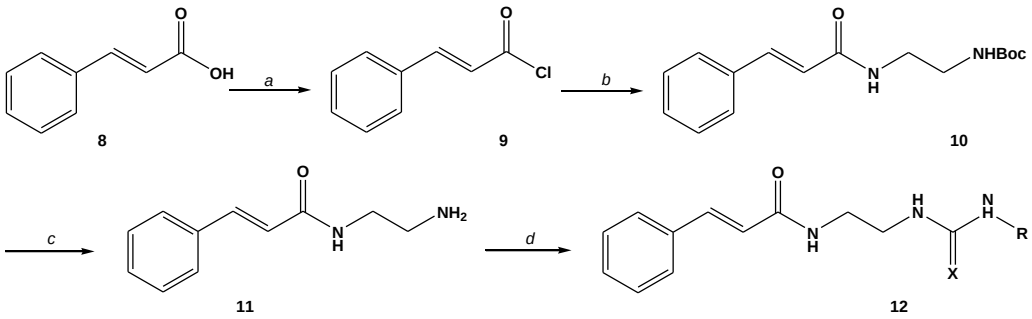


Scheme 1: General synthesis of the ferulic acid derivatives (**7a**–**7f**). Reagents and conditions: (a) C_5H_5N, DMAP, 0 ^oC, 1h; (b) CH_2Cl_2, DMF, 0 ^oC, 1h; (c) CH_2Cl_2, Et_3N, 0 ^oC, 3h; (d) TFA, 25 ^oC, 0.5 h; (e) CH_2Cl_2, DIEA, 0 ^oC, 1h; (f) C_2H_3N, $N_2H_4{\cdot}H_2O$, 6h.

2. Results and discussion

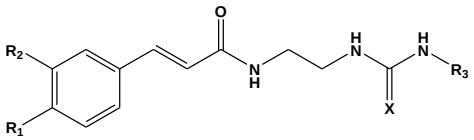
2.1. Chemistry

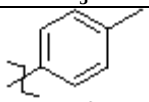
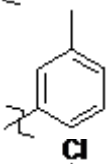
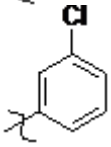
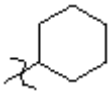
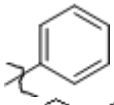
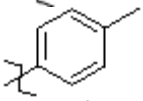
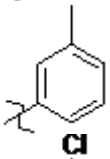
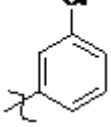
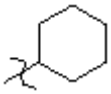
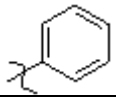
The synthetic route of the ferulic acid derivatives (**7a**–**7f**) are outlined in Scheme 1. Ferulic acid was used as raw materials to get compounds **5** after several steps including phenolic hydroxyl protection, acyl chlorination, amidation and removing the protective group reaction. Then, the obtained compounds react with various isocyanate to get **6**. Finally, the acetyl group was removed to get the targeted compounds **7a**-7**f**. To know whether the unique substituted groups on the benzene ring is vital for the activities of ferulic acid, the corresponding derivatives without any groups on the benzene ring were also synthesized (Scheme 2). The synthetic route was similar with Scheme 1, except that the phenolic hydroxyl need not be protected. All the compounds are reported for the first time (Table 1). The synthetic compounds gave satisfactory elementary analytical and spectroscopic data. ^1H NMR and ESI-MS spectra were consistent with the assigned structures.



Scheme 2: General synthesis of the ferulic acid derivatives (**12a**–**12f**). Reagents and conditions: (a) CH_2Cl_2, DMF, 0 ^oC, 1h; (b) CH_2Cl_2, Et_3N, 0 ^oC, 3h; (c) EtOH, HCl, 40 ^oC, 4h; (d) CH_2Cl_2, DIEA, 0 ^oC, 1h.

Table 1: The chemical structure of ferulic acid



Compounds	R_1	R_2	R_3	X
7a	OH	OCH_3		O
7b	OH	OCH_3		O
7c	OH	OCH_3		O
7d	OH	OCH_3		O
7e	OH	OCH_3		O
7f	OH	OCH_3		S
12a	H	H		O
12b	H	H		O
12c	H	H		O
12d	H	H		O
12e	H	H		O
12f	H	H		S

2.2. ALR2 inhibition

Twelve synthesized compounds were evaluated *in vitro* for their ability to inhibit activity of partially purified rat lens aldose reductase (ALR2). It has been shown that there is an approximately 85% sequence similarity between rat lens and human aldose reductase (ALR2), while the proposed active sites of both enzymes are identical. [22] The IC_{50} values were determined by linear regression analysis of log of the concentration response curve. Sorbinil, a known ALR2 was used as a positive control. Results are presented in Table 2. The result showed that ferulic acid itself was not a good ALR inhibitor, but most of its derivatives (**7a**-**7f**) displayed good activity with IC_{50} ranging from 0.55 to 16.06 µM except compound **7e**. Of all the compounds, **7a** and **7b** were found more effective than the sorbinil, which owned high inhibitory activity with IC_{50} values of 0.55 and

3.88 µM, respectively. With regard to SAR of ferulic acid derivatives we observed that the activity decreased when R2 was aliphatic substituent instead of aromatic substituents (**7a**, **7b**, **7c**, **7f** vs **7d**, **7e**). On the other hand, the data also exhibited the unique substituted groups on the benzene ring of ferulic acid derivatives was very important to the activities, since the compounds **12a**-**12f** showed weak inhibitory activities compared with these derivatives with methoxy and phenolic hydroxyl groups.

Table 2: Aldose reductase (ALR) inhibitory activity data

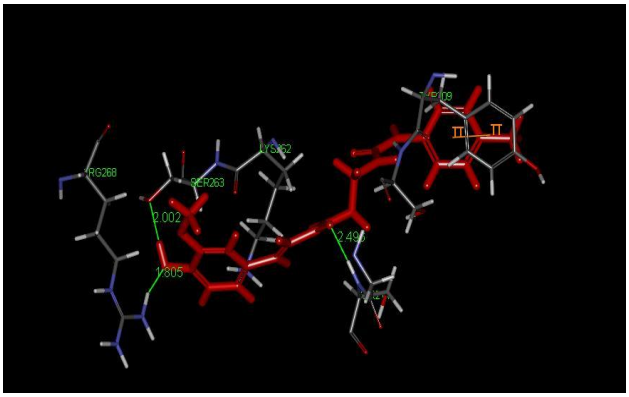
Compounds	IC_{50} (µM)
7a	0.55 ± 0.01
7b	3.88 ± 0.16
7c	9.46 ± 0.68
7d	16.06 ± 0.77
7e	>100
7f	14.13 ± 0.78
12a	11.8 ± 0.81
12b	29.54 ± 0.66
12c	15.53 ± 0.95
12d	>100
12e	>100
12f	22.56 ± 1.33
Ferulic acid	42.78 ± 2.69
Sorbinil	4.34 ± 0.22

[a] IC_{50} values, represent the concentration required to produce 50% enzyme inhibition.

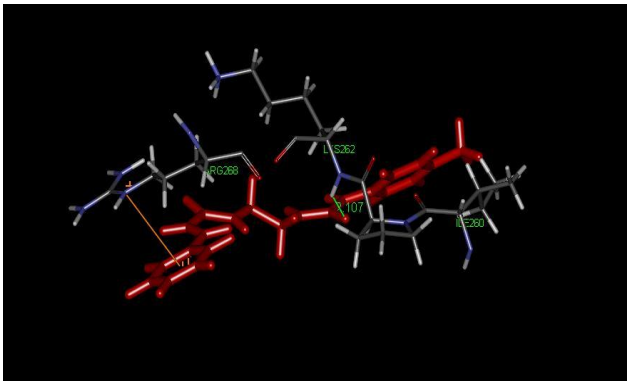
2.3. Docking studies

To gain better understanding on the possible binding modes of the synthesized target compounds on ALR2, we proceeded to examine the interaction of these compounds with ALR2. All docking runs were applied CDOCKER protocol of Discovery Studio 2.5, and the compounds **7a** and **12a** were selected as the representative compounds.

Figure 2 demonstrates the compounds docking into the ATP binding cavity of ALR2 (PDB code: 1Z3N). In the binding mode (a), compound **7a** is nicely bound to the ATP binding site of ALR2 *via* three hydrogen bonds and one π-π stacking interaction. The oxygen and hydrogen atoms of phenolic hydroxyl group formed two hydrogen bonds with the amino group hydrogen of ARG 268 (bond length: 1.805 Å) and hydroxyl of SER 263 (bond length: 2.002 Å). The oxygen atom of urea group carbonyl group formed another hydrogen bond with the hydrogen atoms of amide group and hydroxyl of SER 210 (bond length: 2.230 Å). Furthermore, a benzene ring of compound **7a** stacks against TYR 209 (a, in Figure 2). In contrast with this model, compound **12a** only formed one hydrogen bond and one cation-π interaction as showed depicted in Figure 2 (b), which may explain the much better inhibitory of compound **7a** compared to that of compound **12a**.



(a)



(b)

Figure 2: Compound **7a** (a) and **12a** (b) (red color) is bond into active site of ALR2 (entry 1Z3N in the Protein Data Bank). The blue lines show the hydrogen bonds and the yellow line show the π-π stacking or cation–π interaction.

2.4. Hypoglycemic activity

Newly synthesized compounds were evaluated for hypoglycemic effects at the dose of 10 mg/kg b.w. in glucosefed hypoglycemic normal rats. The marketed sulfonylurea drug glibenclamide was used as positive control. As shown in Table 3, the concentrations of blood glucose was significantly up-regulated in mice fed with fructose solution, while those treated with tested compounds **7a**-**7f** and **12a**-**12f** respectively showed reduced levels of blood glucose. Most derivatives showed better activities than ferulic acid. Commonly, it seems that those compounds with the same substituent group to ferulic acid exhibited relative better efficacy than other derivatives as to blood glucose. Among them, compounds **7a**, **7b** and **7d** showed anti-hyperglycaemic activities comparable to the standard drug glibenclamide. These results are coincident with the data of ALR2 inhibitory activity to a certain extent, exhibiting that the hypoglycemic effects of ferulic acid derivatives owed to their ALR2 inhibitory activity partly.

Table 3: Data of blood glucose concentration

Compounds	Blood glucose (mmol/L)
Normal control	7.34 ± 0.15
Positive control	18.56 ± 0.21
glibenclamide	8.99 ± 0.09
7a	7.33 ± 0.17
7b	6.18 ± 0.08
7c	10.45 ± 0.13
7d	8.35 ± 0.11
7e	12.88 ± 0.19
7f	10.56 ± 0.21
12a	9.97 ± 0.19
12b	10.33 ± 0.10
12c	18.27 ± 0.08
12d	16.69 ± 0.10
12e	10.26 ± 0.13
12f	15.23 ± 0.12
Ferulic acid	15.32 ± 0.55

3. Conclusions

In summary, a series of ferulic acid derivatives with urea groups was first were first prepared and reported. Their hypoglycemic *in vivo* and ALR2 *in vitro* inhibitory activities were evaluated. Among all the compounds, compounds **7a** and **7b** showed comparable hypoglycemic agents to the commercial drug glibenclamide, along with high potent aldose reductase inhibitory activity with IC_{50} values of 0.55 and 3.88 μM. The information of this work might be helpful for the development new hypoglycemic agents.

4. Experimental

4.1. Material and methods

All chemicals and reagents used in current study were of analytical grade. All the ^{1}H NMR spectra were recorded on a Ascend TM 400 (400 MHz) model Spectrometer in DMSO-d_6 or $CDCl_3$ which with tetramethylsilane (TMS) as a intenal standard and chemical shifts were reported in ppm (δ). ESI-MS spectra were recorded on a Shimadzu LC-MS 2020 spectrometer. TLC was performed on the glass backed silica gel sheets (Silica Gel 60 GF254) and visualized in UV light (254 nm). Column chromatography was performed using silica gel (200–300 mesh) eluting with ethyl acetate and petroleum ether.

4.2. General procedure for the synthesis of 2-5

4.2. 1. (E)-3-(4-acetoxy-3-methoxyphenyl) acrylic acid (2)

To a solution of compound **1** (11.64 g, 60 mmol) and DMAP (0.37 g, 3 mmol) in pyridine (45 mL) were dropped acetic anhydride (7.65 g, 75 mmol) at 0 ^{o}C. After stirring for 1 h, the mixture was poured onto ice, the aqueous phase was acidified with hydrochloric acid (2 mol/L) to pH 2 and then extracted with ethyl acetate (750 mL). The combined organic layers were dried over Na_2SO_4, after filtration and removing the solvent *in vacuum*, the residue was treated with light petroleum/ethyl acetate (97/3, v/v, 30 mL) to afford 12.74 g compound **2.** White powders, yield 90%, mp: 192.8-196.3 ^{o}C, ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 12.39 (s, 1H, COOH), 7.58 (d, J = 16.0 Hz, 1H, CH), 7.48 (d, J = 1.6 Hz, 1H, ArH), 7.26 (dd, J = 8.2, 1.7 Hz, 1H, ArH), 7.12 (d, J = 8.1 Hz, 1H, ArH), 6.59 (d, J = 16.0 Hz, 1H, CH), 3.86 (s, 3H, OCH_3), 2.27 (s, 3H, CH_3CO). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 170.99, 169.48, 157.67, 148.61, 138.53, 133.96, 122.45, 119.28, 115.37, 111.16, 54.83, 21.32. MS(ESI): 237.1 ($C_{12}H_{13}O_5$, $[M+H]^+$). Anal. Calcd for $C_{12}H_{12}O_5$: C, 61.01, H, 5.12. Found: C, 61.12, H, 5.15%.

4.2. 2. (E)-4-(3-chloro-3-oxoprop-1-enyl)-2-methoxyphenyl acetate (3)

To a solution of compound **2** (11.8 g, 50 mmol) in CH_2Cl_2 (60 mL) were added DMF (1 mL) and oxalyl chloride (17.58 g, 112 mmol) at 0 ^{o}C. Stirring for 1 hour, the mixture was concentrated *in vacuo* to afford the crude compound **3** as a yellowish solid. The crude product was employed directly in the following step.

4.2. 2. (E)-4-(3-(2-(tert-butoxycarbonyl) ethylamino)-3-oxoprop-1-enyl)-2-methoxyph-enyl acetate (4)

A solution of compound **3** (10.18 g, 40 mmol) in CH_2Cl_2 (40 mL) was dropped into a mixture of N-Boc-ethylenediamine (6.40 g, 40 mmol) and Et_3N (4.85 g, 48 mmol) in CH_2Cl_2 (60 mL) at 0 ^{o}C. After 3 h, the mixture was poured into 5% aq. $NaHCO_3$ (to hydrolyse any unreacted compound **3**) and extracted with CH_2Cl_2. After the solvent was evaporated, the product was purified by column chromatography using petroleum ether and ethyl acetate (1:1) to afford 12.25 g of compound **4**. White powders, yield 81%, mp: 120.6–124.5 ^{o}C, ^{1}H NMR (400 MHz, $CDCl_3$, *ä* ppm): 7.56 (d, J = 15.6 Hz, 1H, CH), 7.13-6.99 (m, 3H, ArH), 6.45 (s, 1H, NH), 6.33 (d, J = 15.6 Hz, 1H, CH), 4.96 (s, 1H, NH), 3.85 (s, 3H, OCH_3), 3.57-3.43 (m, 2H, CH_2), 3.35 (s, 2H, CH_2), 2.32 (s, 3H, CH_3CO), 1.44 (s, 9H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 170.99, 166.48, 157.67, 155.44, 148.61, 138.53, 133.96, 122.45, 119.28, 115.37, 111.16, 79.23, 54.83, 39.12, 37.55, 20.37, 11.36 (3C). MS(ESI): 379.2 ($C_{19}H_{27}N_2O_6$, $[M+H]^+$). Anal. Calcd for $C_{19}H_{26}N_2O_6$: C, 60.30, H, 6.93, N, 7.40. Found: C, 60.25, H, 6.91, N, 7.45%.

(E)-4-(3-(2-aminoethylamino)-3-oxoprop-1-enyl)-2-methoxyphenyl acetate (5)

4 g (11.6 mmol) of compound **4** was dissolved in trifluoroacetic acid (TFA) (40 mL) and the mixture was stirred at room temperature for 30 min. TFA was then removed *in vacuum*. Compound 5 was obtained as brownness oils, yield 91%, which could be used without any further purification and the product was employed directly in the following reaction.

4.3. General procedure for the synthesis of 6a-6f

To a solution of compound **5** (5 mmol) and *N*, *N*-Diisopropylethylamine (10 mmol) in CH_2Cl_2 (25 mL) was added various isocyanate (5 mmol) at 0 °C dropwise. After the addition was completed, the resulting mixture was allowed to raise to room temperature and stirred 1 hours before being poured into water (75 mL). The mixture was extracted with CH_2Cl_2 (150 mL). The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated to give the crude product, which was purified by column chromatography using petroleum ether and ethyl acetate (2:3) to afford compound **6**.

(E)-2-methoxy-4-(3-oxo-3-(2-(3-p-tolylureido) ethylamino) prop-1-enyl)phenyl acetate (6a)

White powders, yield 74%, mp: 188.8–194.4 °C, ^{1}H NMR (400 MHz, DMSO-d_6, ä ppm): 8.41 (s, 1H, NH), 8.19 (s, 1H, NH), 7.43 (d, *J* = 15.8 Hz, 1H, CH), 7.32 (s, 1H, NH), 7.27 (d, *J* = 8.2 Hz, 2H, ArH), 7.14 (m, 2H, ArH), 7.01 (d, *J* = 8.2 Hz, 2H, ArH), 6.63 (d, *J* = 15.8 Hz, 1H, CH), 6.14 (m, 1H, ArH), 3.81 (s, 3H, CH_3O), 3.28 (m, 2H, CH2), 3.20 (m, 2H, CH_2), 2.26 (s, 3H, CH_3CO), 2.21 (s, 3H, $ArCH_3$). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 170.63, 165.49, 156.22, 148.61, 139.57, 133.82 (2C), 129.77, 122.45 (3C), 121.72 (3C), 119.33, 115.37, 112.15, 54.89, 39.02, 37.55, 24.55, 20.33. MS (ESI): 412.2 ($C_{22}H_{26}N_3O_5$, $[M+H]^+$). Anal. Calcd for $C_{22}H_{25}N_3O_5$: C, 64.22, H, 6.12, N, 10.21. Found: C, 64.24, H, 6.13, N, 10.27%.

(E)-2-methoxy-4-(3-oxo-3-(2-(3-m-tolylureido) ethylamino) prop-1-enyl) phenyl acetate (6b)

White powders, yield 76%, mp: 173.6–177.7 °C, ^{1}H NMR (400 MHz, DMSO-d_6, ä ppm): 8.45 (s, 1H, NH), 8.19 (d, *J* = 5.5 Hz, 1H, ArH), 7.43 (d, *J* = 15.8 Hz, 1H, CH), 7.32 (s, 1H, NH), 7.23 (s, 1H, NH), 7.12 (m, 4H, ArH), 6.70 (d, *J* = 7.3 Hz, 1H, ArH), 6.63 (d, *J* = 15.8 Hz, 1H, CH), 6.19 (m, 1H, ArH), 3.81 (s, 3H, CH_3O), 3.27 (m, 2H, CH_2), 3.20 (m, 2H, CH_2), 2.26 (s, 3H, CH_3CO), 2.23 (s, 3H, $ArCH_3$). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 171.21, 165.76, 155.43, 148.65, 139.78, 133.87 (2C), 127.08, 123.49 (4C), 120.92 (2C), 118.55, 115.33, 112.11, 54.86, 39.88, 37.76, 24.09, 21.45. MS (ESI): 412.2 ($C_{22}H_{26}N_3O_5$, $[M+H]^+$). Anal. Calcd for $C_{22}H_{25}N_3O_5$: C, 64.22, H, 6.12, N, 10.21. Found: C, 64.16, H, 6.17, N, 10.25%.

(E)-4-(3-(2-(3-(3-chlorophenyl) ureido) ethylamino)-3-oxoprop-1-enyl)-2-methoxyphenyl acetate (6c)

White powders, yield 83%, mp: 162.3–168.2 °C, ^{1}H NMR (400 MHz, DMSO-d_6, ä ppm): 8.83 (s, 1H, NH), 8.22 (m, 1H, ArH), 7.68 (s, 1H, NH), 7.43 (d, *J* = 15.8 Hz, 1H, CH), 7.32 (s, 1H, NH), 7.18 (m, 4H, ArH), 6.93 (m, 1H, ArH), 6.63 (d, *J* = 15.8 Hz, 1H, CH), 6.36 (m, 1H, ArH), 3.81 (s, 3H, CH_3O), 3.28 (m, 2H, CH_2), 3.221 (m, 2H, CH_2), 2.26 (s, 3H, CH_3CO). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 170.22, 166.47, 155.21, 148.69, 139.95, 137.03 (2C), 133.87 (2C), 127.68 (2C), 123.41, 121.27 (2C), 119.82, 115.61, 112.17, 55.72, 39.39, 37.75, 24.16. MS (ESI): 430.1 ($C_{21}H_{21}ClN_3O_5$, $[M-H]^-$). Anal. Calcd for $C_{21}H_{22}ClN_3O_5$: C, 58.40, H, 5.13, Cl, 8.21, N, 9.73. Found: C, 58.43, H, 5.15, Cl, 8.18, N, 9.70%.

(E)-4-(3-(2-(3-cyclohexylureido) ethylamino)-3-oxoprop-1-enyl)-2-methoxyphenyl acetate (6d)

White powders, yield 79%, mp: 183.8–188.9 °C, ^{1}H NMR (400 MHz, DMSO-d_6, ä ppm): 8.14 (m, 1H, ArH), 7.41 (d, *J* = 15.8 Hz, 1H, CH), 7.31 (s, 1H, NH), 7.13 (m, 2H, ArH), 6.61 (d, *J* = 15.8 Hz, 1H, CH), 5.81 (m, 2H, NH), 3.81 (s, 3H, CH_3O), 3.21 (m, 2H, CH_2), 3.09 (m, 2H, CH_2), 2.26 (s, 3H, CH_3O), 1.76-1.68 (m, 2H, CH_2), 1.62-1.58 (m, 2H, CH_2), 1.51 (m, 1H, CH), 1.29-1.25 (m, 3H, CH_2), 1.18-1.01 (m, 3H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 169.09, 163.33, 156.36, 151.33, 146.11, 138.33, 137.03, 133.87, 128.55, 123.98, 122.36, 55.77, 51.26, 40.37, 38.25, 33.78 (2C), 28.21, 24.16, 22.39 (2C). MS (ESI): 404.2 ($C_{21}H_{30}N_3O_5$, $[M+H]^+$). Anal. Calcd for $C_{21}H_{29}N_3O_5$: C, 62.51, H, 7.24, N, 10.41. Found: C, 62.59, H, 7.22, N, 10.33%.

(E)-4-(3-(2-(3-butylureido) ethylamino)-3-oxoprop-1-enyl)-2-methoxyphenyl acetate (6e)

White powders, yield 82%, mp: 173.0–177.8 °C, ^{1}H NMR (400 MHz, DMSO-d_6, ä ppm): 8.14 (m, 1H, ArH), 7.42 (d, *J* = 15.8 Hz, 1H, CH), 7.32 (s, 1H, NH), 7.14 (m, 2H, ArH), 6.61 (d, *J* = 15.8 Hz, 1H, CH), 5.89 (m, 2H, NH), 3.81 (s, 3H, CH_3O), 3.17 (m, 2H, CH_2), 3.11 (m, 2H, CH_2), 2.96 (m, 2H, CH_2), 1.33-1.22 (m, 4H, CH_2), 0.86 (t, *J* = 7.2 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 170.23, 161.67, 155.39, 151.56, 146.31, 137.96 (2C), 133.88, 128.03, 123.91, 121.69, 55.91, 43.66, 40.31, 38.67, 24.76, 22.58, 19.73, 15.88. MS (ESI): 378.2 ($C_{19}H_{28}N_3O_5$, $[M+H]^+$). Anal. Calcd for $C_{19}H_{27}N_3O_5$: C, 60.46, H, 7.21, N, 11.13. Found: C, 60.36, H, 7.28, N, 11.05%.

(E)-2-methoxy-4-(3-oxo-3-(2-(3-phenylthioureido) ethylamino)prop-1-enyl)phenyl acetate (6f)

White powders, yield 85%, mp: 174.1–179.9 °C, ^{1}H NMR (400 MHz, DMSO-d_6, ä ppm): 9.61 (s, 1H, NH), 8.25 (s, 1H, NH), 7.80 (s, 1H, NH), 7.46 (s, 1H), 7.44-7.37 (m, 3H, ArH and CH), 7.35-7.25 (m, 3H, ArH), 7.17-7.07 (m, 3H, ArH), 6.63 (d, *J* = 15.8 Hz, 1H, CH), 3.82 (s, 3H, CH_3O), 3.61 (m, 2H, CH_2), 3.40 (m, 2H, CH_2), 2.26 (s, 3H, CH_3O). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 181.23, 169.23, 160.11, 155.76, 150.39, 146.55 (2C), 138.09 (2C), 127.43 (2C), 124.93 (3C), 121.71, 119.12 (2C), 54.32, 41.08, 38.72, 23.67. MS (ESI): 414.1 ($C_{21}H_{24}N_3O_4S$, $[M+H]^+$). Anal. Calcd for $C_{21}H_{23}N_3O_4S$: C, 61.00, H, 5.61, N, 10.16, O, 15.48, S, 7.75. Found: C, 61.13, H, 5.66, N, 10.11, S, 7.79%.

4.4. General procedure for the synthesis of 7a-7f

To a solution of compound **6** in acetonitrile was added hydrazine monohydrate. The mixture was stirred for 6 h at room temperature. The organic portion was extracted with ethyl acetate, washed with water and saturated aqueous NaCl, and dried over Na_2SO_4. The filtrate was concentrated to give the crude product, which was purified by column chromatography using petroleum ether and ethyl acetate (1:2) to afford compounds **7**.

(E)-1-(2-(3-(4-hydroxy-3-methoxyphenyl) acrylamido) ethyl)-3-p-tolylurea (7a)

White powders, yield 75%, mp: 163.4–170.6 °C, ^{1}H NMR (400 MHz, DMSO-d_6, *ä* ppm): 9.42 (s, 1H, OH), 8.41 (s, 1H, NH), 8.06 (m, 1H, ArH), 7.30 (m, 3H, ArH and CH), 7.12 (s, 1H, NH), 7.00 (m, 3H, ArH and NH), 6.79 (m, 1H, ArH), 6.44 (d, J = 15.7 Hz, 1H, CH), 6.14 (m, 1H, ArH), 3.80 (s, 3H, CH_3O), 3.26 (m, 2H, CH_2), 3.18 (m, 2H, CH_2), 2.21 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 166.32, 155.13, 151.73, 144.16 (2C), 134.55, 129.34, 125.76 (3C), 120.98, 119.01 (3C), 114.32, 111.58, 56.27, 39.87, 36.21, 24.33. MS (ESI): 370.2 ($C_{20}H_{24}N_3O_4$, $[M+H]^+$). Anal. Calcd for $C_{20}H_{23}N_3O_4$: C, 65.03, H, 6.28, N, 11.37. Found: C, 65.27, H, 6.29, N, 11.27%.

(E)-1-(2-(3-(4-hydroxy-3-methoxyphenyl) acrylamido) ethyl)-3-m-tolylurea (7b)

White powders, yield 80%, mp: 165.5–169.1 °C, ^{1}H NMR (400 MHz, DMSO-d_6, *ä* ppm): 9.42 (s, 1H, OH), 8.44 (s, 1H, NH), 8.06 (m, 1H, ArH), 7.33 (d, J = 15.7 Hz, 1H, CH), 7.22 (s, 1H, NH), 7.17 (m, 1H, ArH), 7.09 (m, 2H, ArH and NH), 6.99 (m, 1H, ArH), 6.79 (m, 1H, ArH), 6.70 (m, 1H, ArH), 6.44 (d, J = 15.7 Hz, 1H, CH), 6.18 (m, 1H, ArH), 3.80 (s, 3H, CH_3O), 3.26 (m, 2H, CH_2), 3.18 (m, 2H, CH_2), 2.23 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 167.62, 154.23, 150.66, 143.65, 136.93, 128.91 (2C), 126.77 (2C), 121.06 (2C), 118.65 (3C), 113.77, 110.87, 55.33, 38.07, 36.18, 24.07. MS (ESI): 470.2 ($C_{20}H_{24}N_3O_4$, $[M+H]^+$). Anal. Calcd for $C_{20}H_{23}N_3O_4$: C, 65.03, H, 6.28, N, 11.37. Found: C, 65.13, H, 6.23, N, 11.41%.

(E)-1-(3-chlorophenyl)-3-(2-(3-(4-hydroxy-3-methoxyphenyl) acrylamido) ethyl) urea (7c)

White powders, yield 81%, mp: 172.5–176.0 °C, ^{1}H NMR (400 MHz, DMSO-d_6, *ä* ppm): 9.41 (s, 1H, OH), 8.79 (s, 1H, NH), 8.05 (s, 1H, NH), 7.67 (s, 1H, NH), 7.33 (d, J = 15.6 Hz, 1H, CH), 7.21 (m, 2H, ArH), 7.12-6.99 (m, 2H, ArH), 6.92 (m, 1H, ArH), 6.79 (m, 1H, ArH), 6.44 (d, J = 15.8 Hz, 1H, CH), 6.30 (m, 1H, ArH), 3.80 (s, 3H, CH_3O), 3.26 (m, 2H, CH_2), 3.21 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 165.78, 155.37, 149.66, 138.12 (2C), 137.03 (3C), 131.12 (2C), 126.43, 121.44 (2C), 119.22, 116.77, 112.69, 56.55, 40.07, 37.82. MS (ESI): 388.1 ($C_{19}H_{19}ClN_3O_4$, $[M-H]^-$). Anal. Calcd for $C_{19}H_{20}ClN_3O_4$: C, 58.54, H, 5.17, Cl, 9.09, N, 10.78, O, 16.42. Found: C, 58.44, H, 5.23, Cl, 9.19, N, 10.38%.

(E)-1-cyclohexyl-3-(2-(3-(4-hydroxy-3-methoxyphenyl) acrylamido) ethyl) urea (7d)

White powders, yield 79%, mp: 88.7–96.0 °C, ^{1}H NMR (400 MHz, DMSO-d_6, *ä* ppm): 9.41 (s, 1H, OH), 8.00 (s, 1H, NH), 7.31 (d, J = 16.0 Hz, 1H, CH), 7.11 (s, 1H, NH), 6.98 (m, 1H, ArH), 6.79 (m, 1H, ArH), 6.42 (d, J = 16.0 Hz, 1H, CH), 5.80 (m, 1H, ArH and NH), 3.80 (s, 3H, CH_3O), 3.17 (m, 2H, CH_2), 3.09 (m, 2H, CH_2), 1.76-1.68 (m, 2H, CH_2), 1.62-1.58 (m, 2H, CH_2), 1.51 (m, 1H, CH), 1.29-1.25 (m, 3H, CH_2), 1.18-1.01 (m, 3H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 161.56, 155.55, 153.72, 148.98, 138.88, 137.04, 130.42, 126.77, 123.98, 118.71, 55.77, 50.06, 41.87, 38.11, 34.42 (2C), 28.67, 23.46 (2C). MS (ESI): 362.2 ($C_{19}H_{28}N_3O_4$, $[M+H]^+$). Anal. Calcd for $C_{19}H_{27}N_3O_4$: C, 63.14, H, 7.53, N, 11.63. Found: C, 63.18, H, 7.55, N, 11.58%.

(E)-1-butyl-3-(2-(3-(4-hydroxy-3-methoxyphenyl) acrylamido) ethyl) urea (7e)

White powders, yield 72%, mp: 136.5–139.9 °C, ^{1}H NMR (400 MHz, DMSO-d_6, *ä* ppm): 9.40 (s, 1H, OH), 7.98 (m, 1H, NH), 7.31 (d, J = 15.7 Hz, 1H, CH), 7.12 (s, 1H, NH), 6.98 (d, J = 8.1 Hz, 1H, ArH), 6.79 (d, J = 8.1 Hz, 1H, ArH), 6.42 (d, J = 15.7 Hz, 1H, CH), 5.87 (m, 2H, ArH and NH), 3.80 (s, 3H, CH_3O), 3.17 (m, 2H, CH_2), 3.10 (m, 2H, CH_2), 2.97 (m, 2H, CH_2), 1.33 (m, 2H, CH_2), 1.26 (m, 2H, CH_2), 0.86 (t, J = 7.1 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 160.55, 154.01, 150.33, 145.71, 138.67, 130.86, 127.32, 123.77, 119.79 (2C), 55.08, 43.43, 40.01, 38.97, 22.58, 20.11, 15.12. MS (ESI): 336.2 ($C_{17}H_{26}N_3O_4$, $[M+H]^+$). Anal. Calcd for $C_{19}H_{25}N_3O_4$: C, 60.88, H, 7.51, N, 12.53. Found: C, 60.69, H, 7.54, N, 12.60%.

(E)-1-(2-(3-(4-hydroxy-3-methoxyphenyl) acrylamido) ethyl)-3-phenylthiourea (7f)

White powders, yield 71%, mp: 164.1–167.8 °C, ^{1}H NMR (400 MHz, DMSO-d_6, *ä* ppm): 9.71 (s, 1H, OH), 9.44 (s, 1H, NH), 8.13 (s, 1H, NH), 7.89 (s, 1H, NH), 7.43-7.32 (m, 5H, ArH and CH), 7.17-7.04 (m, 2H, ArH), 7.00 (m, 1H, ArH), 6.80 (m, 1H, ArH), 6.45 (d, J = 15.7 Hz, 1H, CH), 3.80 (s, 3H, CH_3O), 3.59 (m, 2H, CH_2), 3.38 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 181.78, 170.67, 163.25, 151.31, 146.31, 138.76 (2C), 129.49 (2C), 126.66, 123.03 (2C), 121.78, 119.18 (3C), 55.33, 42.08, 37.98. MS (ESI): 372.1 ($C_{19}H_{22}N_3O_3S$, $[M+H]^+$). Anal. Calcd for $C_{19}H_{21}N_3O_3S$: C, 61.44, H, 5.70, N, 11.31, S, 8.63. Found: C, 61.34, H, 5.74, N, 11.39, S, 8.53%.

4.5. General procedure for the synthesis of 9-11

Cinnamoyl chloride (9)

To a solution of compound **8** (2.00 g, 13.5 mmol) in CH_2Cl_2 (20.00 mL) were added DMF (0.25 mL) and oxalyl chloride (2.40 mL, 28.0 mmol) at 0 °C. Stirring for 1

hour, the mixture was concentrated *in vacuo* to afford the crude 3-phenylacryloyl chloride as a yellow solid. The crude product was employed directly in the following step.

(E)-tert-butyl 2-cinnamamidoethylcarbamate (10)

A solution of compound **2** (1.67 g, 10.0 mmol) in CH_2Cl_2 (10.0 mL) was dropped into a mixture of N-Boc-ethylenediamine (1.60 g, 10.0 mmol) and Et_3N (1.21 g, 12.0 mmol) in CH_2Cl_2 (15.0 mL) at 0 ^oC. Cooling was accomplished by an ice-bath. After 3 h, the mixture was poured into 5% aq. $NaHCO_3$ (to hydrolyse any unreacted **2**) and extracted with CH_2Cl_2. After the solvent was evaporated, the product was purified by column chromatography using petroleum ether and ethyl acetate (1:1) to afford 2.43 g of compound **3**. Yellowish solid, yield 83%, mp: 130.1-133.5 ^oC, ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 7.61 (d, J = 15.6 Hz, 1H, CH), 7.49 (m, 1H, ArH), 7.42-7.31 (m, 3H, ArH), 6.50 (m, 1H, ArH), 6.41 (d, J = 15.6 Hz, 1H, CH), 3.51 (m, 2H, CH_2), 3.35 (m, 2H, CH_2), 1.44 (s, 9H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 167.46, 155.36, 144.69, 135.88, 132.12 (2C), 126.66, 121.22 (2C), 119.53, 75.87, 42.15, 37.32, 26.69 (3C). MS (ESI): 291.2 ($C_{16}H_{23}N_2O_3$, $[M+H]^+$). Anal. Calcd. for $C_{16}H_{22}N_2O_3$: C, 66.18, H, 7.64, N, 9.65. Found: C, 66.23, H, 7.66, N, 9.58%.

N-(2-aminoethyl)cinnamamide (11)

To a solution of compound **3** (2.70 g, 9.3 mmol) in EtOH (10.0 mL) was added aq. 1N HCl (32.0 mL) and the mixture warmed to 40 ^oC for 4 h. Afterwards, the solution was washed with EtOAc and the organic phase discarded. The aq. phase was first saturated with NaCl and then aq. NaOH was added to get a strong basic solution, which was extracted with EtOAc. After separating the organic phase, drying with Na_2SO_4 and evaporating resulted in 1.48 g of compound **4**. Viscous white oil, yield 84%, which could be used without any further purification and the product, was employed directly in the following reaction.

Compounds 12

To a solution of compound **11** (5.0 mmol) and N,N-Diisopropylethylamine (1.6 mL, 10.0 mmol) in CH_2Cl_2 (50.0 mL) was added various isocyanate (5.0 mmol) at 0 ^oC dropwise. Cooling was accomplished by an ice-bath. After the addition was completed, the resulting mixture was allowed to rise to room temperature and stirred 1 hour before being poured into water (75.0 mL). The mixture was extracted with CH_2Cl_2 (150.0 mL). The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated to give the crude product, which was purified by column chromatography using petroleum ether and ethyl acetate (1:4) to afford compound **12**.

4.6. General procedure for the synthesis of 12a-12f

(E)-1-(2-cinnamamidoethyl)-3-p-tolylurea (12a)

White powders, yield 83%, mp: 179.4-181.5 ^oC, ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 8.42 (s, 1H, NH), 8.23 (s, 1H, NH), 7.56 (m, 2H, ArH), 7.47-7.69 (m, 4H, ArH and CH), 7.27 (m, 2H, ArH), 7.01 (m, 2H, ArH), 6.63 (d, J = 15.8 Hz, 1H, CH), 6.16 (s, 1H, NH), 3.26 (m, 2H, CH_2), 3.21 (m, 2H, CH_2), 2.21 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 167.78, 153.16, 144.45, 135.09 (2C), 131.78 (4C), 128.55, 125.68, 121.21 (4C), 119.32, 41.45, 38.98, 24.33. MS (ESI): 324.2 ($C_{19}H_{22}N_3O_2$, $[M+H]^+$). Anal. Calcd for $C_{19}H_{21}N_3O_2$: C, 70.57, H, 6.55, N, 12.99. Found: C, 70.61, H, 6.57, N, 12.95%.

(E)-1-(2-cinnamamidoethyl)-3-m-tolylurea (12b)

White powders, yield 75%, mp: 185.5–189.9 ^oC, ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 8.50 (s, 1H, NH), 8.24 (s, 1H, NH), 7.56 (m, 2H, ArH), 7.50-7.31 (m, 4H, ArH and CH), 7.23 (m, 1H, ArH), 7.17 (m, 1H, ArH), 7.08 (m, 1H, ArH), 6.70 (m, 1H, ArH), 6.63 (d, J = 15.8 Hz, 1H, CH), 6.24 (s, 1H, NH), 3.28 (m, 2H, CH_2), 3.19 (m, 2H, CH_2), 2.23 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 167.11, 153.35, 144.37, 136.01 (3C), 132.76 (3C), 128.59 (2C), 125.67, 122.33 (3C), 119.30, 40.75, 38.71, 24.97. MS (ESI): 324.2 ($C_{19}H_{22}N_3O_2$, $[M+Na]^+$). Anal. Calcd for $C_{19}H_{21}N_3O_2$: C, 70.57, H, 6.55, N, 12.99. Found: C, 70.60, H, 6.52, N, 12.85%.

(E)-1-(3-chlorophenyl)-3-(2-cinnamamidoethyl) urea (12c)

White powders, yield 83%, mp: 175.5–180.4 ^oC, ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 9.20 (s, 1H, NH), 8.32 (s, 1H, NH), 7.67 (m, 1H, ArH), 7.56 (m, 2H, ArH), 7.44-7.34 (m, 5H, ArH and CH), 7.22 (m, 1H, ArH), 6.91 (m, 1H, ArH), 6.66 (d, J = 15.8 Hz, 1H), 6.60 (s, 1H, NH), 3.28 (m, 2H, CH_2), 3.21 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 168.33, 156.42, 144.37, 136.23 (2C), 134.27, 132.76 (2C), 130.77, 128.71 (2C), 125.60, 122.37 (2C), 119.19 (2C), 40.75, 38.71. MS (ESI): 342.1 ($C_{18}H_{17}ClN_3O_2$, $[M-H]^-$). Anal. Calcd for $C_{18}H_{18}ClN_3O_2$: C, 62.88, H, 5.28, Cl, 10.31, N, 12.22. Found: C, 62.94, H, 5.27, Cl, 10.26, N, 12.28%.

(E)-1-(2-cinnamamidoethyl)-3-cyclohexylurea (12d)

White powders, yield 85%, mp: 193.8–198.4 ^oC, ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 9.20 (s, 1H, NH), 8.32 (s, 1H, NH), 8.18 (m, 1H, ArH), 7.56 (m, 2H, ArH), 7.49-7.23 (m, 3H, ArH and CH), 6.61 (d, J = 15.8 Hz, 1H, CH), 5.83 (s, 1H, NH), 3.21 (m, 2H, CH_2), 3.10 (m, 2H, CH_2), 1.73 (d, J = 12.1 Hz, 2H, Cyclohexane-H), 1.66-1.57 (m, 2H, Cyclohexane-H), 1.51 (d, J = 12.0 Hz, 1H, Cyclohexane-H), 1.39-0.94 (m, 6H, Cyclohexane-H). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 162.33, 154.12, 150.12, 146.12, 139.89, 131.01, 129.76, 126.33, 123.11, 117.41, 50.06, 41.85, 38.19, 34.56 (2C), 28.61, 23.59 (2C). MS (ESI): 316.2 ($C_{18}H_{26}N_3O_2$, $[M+H]^+$). Anal. Calcd for

$C_{18}H_{25}N_3O_2$: C, 68.54, H, 7.99, N, 13.32. Found: C, 68.51, H, 7.96, N, 13.37%.

(E)-1-butyl-3-(2-cinnamamidoethyl) urea (12e)

White powders, yield 78%, mp: 170.1-173.8 °C, ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 9.20 (s, 1H, NH), 8.32 (s, 1H, NH), 8.16 (m, 1H, ArH), 7.56 (m, 2H, ArH), 7.48-7.31 (m, 3H, ArH and CH), 6.61 (d, J = 15.8 Hz, 1H, CH), 5.89 (s, 1H, NH), 3.19 (m, 2H, CH_2), 3.10 (m, 2H, CH_2), 2.97 (m, 2H, CH_2), 1.42-1.08 (m, 4H, CH_2), 0.85 (t, J = 7.2 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 161.35, 154.77, 150.56, 145.78, 138.61, 130.02, 128.66, 124.31 (2C), 118.68, 50.78, 40.89, 38.66, 28.67, 19.55, 15.31. MS (ESI): 290.2 ($C_{16}H_{24}N_3O_2$, $[M+H]^+$). Anal. Calcd for $C_{16}H_{23}N_3O_2$: C, 66.41, H, 8.01, N, 14.52. Found: C, 66.35, H, 8.03, N, 14.48%.

(E)-1-(2-cinnamamidoethyl)-3-phenylthiourea (12f)

White powders, yield 93%, mp: 169.5-170.9 °C, ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 9.61 (s, 1H, NH), 8.28 (s, 1H, NH), 7.79 (s, 1H, NH), 7.55 (m, 2H, ArH), 7.49-7.34 (m, 6H, ArH and CH), 7.30 (m, 2H, ArH), 7.10 (m, 1H, ArH), 6.61 (d, J = 15.8 Hz, 1H, CH), 3.59 (m, 2H, CH_2), 3.39 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 180.33, 170.21, 162.67, 151.77, 146.33, 136.79 (3C), 128.47, 126.08, 123.55, 121.06 (2C), 119.69 (3C), 41.15, 38.36. MS (ESI): 325.1 ($C_{18}H_{20}N_3OS$, $[M+Na]^+$). Anal. Calcd for $C_{18}H_{19}N_3OS$: C, 66.43, H, 5.88, N, 12.91, S, 9.85. Found: C, 66.33, H, 5.91, N, 12.93, S, 9.89%.

4.7. Animals

150 ICR mice (20-22 g), purchased from Comparative Medicine Centre of Yangzhou University, were housed in a temperature and humidity controlled animal facility with a set of 12 h light-dark cycle. Mice were provided with water and food pellets *ad libitum*. All mice were allowed to acclimatize to the laboratory environment for a week in advance.

4.8. Experimental protocol

Mice were divided into 15 groups (n=10) randomly as follows: control groups, glibenclamide (5 mg/kg) group, a (10 mg/kg) group, b (10 mg/kg) group, c (10 mg/kg) group, d (10 mg/kg) group, e (10 mg/kg) group, f (10 mg/kg) group, g (10 mg/kg) group, h (10 mg/kg) group, i (10 mg/kg) group, j (10 mg/kg) group, k (10 mg/kg) group and l (10 mg/kg) group. Every mouse was subjected to 30% fructose water for 4 weeks, while those in the control group were given normal saline instead. From the fifth week on, mice were administered with every group correspondingly for 2 weeks. In the last 24 hours, mice were placed in metabolic cages to collect urine. Subsequently, blood samples were collected from the orbit and centrifuged at 4500 rpm for 15 min. Blood glucose was determined using glucose kit and ELISA kit according to the manufacturer's protocol.

4.9. Aldose reductase inhibition assay

All the metabolites were dissolved in DMSO to obtain a stock solution of 2mM, and appropriate dilutions were made before the enzyme assay. The supernatant fluid of rat lens homogenate was used as the crude enzyme. The incubation mixture contained 135-mM Na, K-phosphate buffer (pH 7.0), 100 mM Li_2SO_4, 0.03 mM NADPH, 1 mM DL-glyceraldehyde as a substrate, and 100 μL of enzyme fraction, with or without 25 μL of sample solution, in a total volume of 0.5 mL. The reaction was initiated by the addition of NADPH at 30 °C. After 30 min, the reaction was stopped by the addition of 150 μL of 0.5 M HCl. Then, 0.5 mL of 6 M NaOH containing 10 mM imidazole was added, and the solution was heated at 60 °C for 10 min to convert NADP to a fluorescent product. The values of IC_{50} were calculated from the least-squares regression line of the logarithmic concentrations plotted against the remaining activity.

4.10. Docking experiments

Molecular docking of compound 5a into the three-dimensional X-ray structure of ALR2 (PDB code: 1Z3N) was carried out using Ligand Fit Dock protocol of Discovery Studio 2.5.

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