新型苯丙烯酰氨基酸化合物的设计合成及生物活性

高栗繁^{a,b} 张艳春^{a,b} 李家明^{*,a,b} 张 斌^{a,b}
杨 雨^{a,b} 胡孟奇^{a,b}^(a) 安徽中医药大学药学院 合肥 230012)^(b) 安徽省中医药科学院药物化学研究所 合肥 230012)

摘要 依据药效团的拼合策略, 以上市药瑞巴派特为先导化合物, 设计并合成了一系列具有苯丙烯酰氨基酸结构化合物, 其结构均经 ¹H NMR、¹³C NMR 和 MS 确证. 用 Elisa 法测定目标化合物对 LPS 诱导的 RAW264.7 细胞释放 IL-6 和 TNF- α 的抑制活性. 结果显示所设计化合物显示出良好的抑制活性, 其中有 4 个化合物的活性明显优于瑞巴派特.

关键词 IL-6; TNF- α ; 苯丙烯酰氨基酸; 合成

Synthesis and Biological Evaluation of Novel Phenylpropenoyl-amino Acid Derivatives

Gao, Sufan^{a,b} Zhang, Yanchun^{a,b} Li, Jiaming^{*,a,b} Zhang, Bin^{a,b}
Yang, Yu^{a,b} Hu, Mengqi^{a,b}^(a) College of Pharmacy, Anhui University of Chinese Medicine, Hefei 230012)^(b) Department of Medicinal Chemistry, Anhui Academy of Chinese Medicine, Hefei 230012)

Abstract Based on the listed drug rebamipide, a series of novel compounds of phenylpropenoyl-amino acid structures were designed and synthesized according to the pharmacophore-combination strategy. The structures of the target compounds were confirmed by NMR and MS. The inhibitory activities against IL-6 and TNF- α had been investigated. The results demonstrated that all compounds exhibited moderate IL-6 and TNF- α inhibitory activities. In particular, the activities of four compounds were significantly improved than that of rebamipide.

Keywords IL-6; TNF- α ; phenylpropenoyl-amino acid; synthesis

1 Introduction

TNF- α is an important pro-inflammatory cytokine that plays a key role in many physiological immune processes and with a wide range of biological activities.^[1] Over-expression of TNF- α is correlated with many pathological conditions, such as Crohn's disease, diabetes,^[2] atherosclerosis,^[3] rheumatoid arthritis and psoriatic. The TNF- α inhibition has been identified as one of the attractive targets for the design and development of anti-inflammatory agents. Nowadays, TNF- α inhibitors in clinical trials are protein-based drugs (infliximab, adalimumab and golimumab) that have been demonstrated with significant efficacy in the treatment of related diseases. However, proteins used to inhibit TNF- α also present problems, such as poor

oral availability, the high cost and correlated side effects.^[4-9] Therefore the development of small molecule inhibitors of TNF- α and other inflammatory regulatory cytokines might be of great interest to avoid these problems. In spite of the great effort has been made, none of the reliable small molecule inhibitors of this cytokine has been approved.

Amongst the pro-inflammatory cytokines, IL-6 is a pleiotropic cytokine synthesized and released by a variety of cells, which induces a broad range of cellular and physiological responses during the inflammation reaction.^[10] In addition to playing a very important role in rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and Crohn's disease, IL-6 is also involved in brain neurological diseases, such as Alzheimer's disease, Parkinson's disease.

* Corresponding author. E-mail: lijiaming2004@sina.com

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Furthermore, it is significantly over expressed in some metabolic diseases such as obesity and type 2 diabetes. Currently, IL-6-targeted biologics have been marketed with great success and the chemical drugs such as CLX-0921 (Figure 1, 1) and RO-3201195 (Figure 1, 2) have been in clinical trials to treat rheumatoid arthritis, type 2 diabetes, xerophthalmia and other diseases. Till-date, developing the IL-6 inhibitors is the great hope in the mainstream of drug development.

Rebamipide (Figure 1, 3) is a conventional drug, used to treat gastritis and gastric ulcer. It is reported that rebamipide can significantly inhibit TNF- α and IL-6 produced by lipopolysaccharide (LPS)-stimulated mouse peritoneal macrophages.^[11] As a multifunctional compound, rebamipide has been proved for potential pharmacological uses, for example, glycolipid metabolism,^[11] dermatitis,^[12] arthritis,^[13] and xerophthalmia.^[14]

Figure 1 Structures of compounds 1~3

In order to maintain the potential IL-6 and TNF- α inhibitory activities of rebamipide, the structure of rebamipide was modified by the pharmacophore-combination strategy and vinylogy principle. A number of novel phenylpropenoyl-amino acid derivatives of interest bearing various substituted groups on aryl rings were designed and synthesized. Further, in order to investigate the biological activity of structure of the quinolinone, we replaced it with other potentially active amino acid fragment^[15,16] (Figure 2). And the biological activities of inhibiting TNF- α and IL-6 of the target compounds were evaluated.^[17]

2 Results and discussion

2.1 Chemistry

The general synthetic route used to synthesize target compounds **8a~8w** was outlined in Scheme 1. Triethylamine (Et₃N) was taken as an acid scavenger, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDCI) and 1-hydroxybenzotriazole (HOBt) as condensation agent. The carboxylic acid group was activated by EDCI, then a stable active intermediate was formed by HOBt, which reacted with the amino acid ester to obtain intermediates **7a~7w**. The ester

Figure 2 Design ideas of target compounds

was hydrolyzed to give the target compound **8a~8w**. The reaction conditions were mild and the experimental treatment was convenient. All of the target compounds were structurally determined by ¹H NMR, ¹³C NMR and MS.

2.2 Biological activity

In vitro anti-inflammatory activities of synthetic phenylpropenoyl-amino acid derivatives were investigated in LPS-induced RAW 264.7 on the generation of IL-6 and TNF- α . Table 1 demonstrated that rebamipide and its derivatives were screened at concentrations of 100, 50, 10, 5 and 1 $\mu\text{mol}\cdot\text{L}^{-1}$ for their ability to regulate TNF- α and IL-6 production. The results exhibited that all synthetic compounds inhibited the LPS-induced production of IL-6 and TNF- α in a concentration-dependent manner. Among these compounds, **8d**, **8h**, **8j**, **8m**, **8o**, **8q** and **8t** were more potent than rebamipide in inhibiting LPS-induced TNF- α expression. Particularly, compounds **8d**, **8h**, **8j** and **8q** exhibited better IL-6 inhibitory activity than lead compound. Among all synthetic compounds, **8q** was found to be the most potent IL-6 inhibitor ($\text{IC}_{50} = 22.77 \pm 1.53 \mu\text{mol/L}$) and TNF- α inhibitor ($\text{IC}_{50} = 29.55 \pm 2.13 \mu\text{mol/L}$).

The pro-inflammatory mediator NO plays an important role in inflammation-related diseases. We investigated the effect of **8q** on LPS-induced NO production in RAW264.7 cells. As shown in Figure 3, the LPS (200 ng/mL) could markedly stimulate NO releasing. By stimulating of different concentration of **8q** on RAW264.7, the **8q** can inhibit the NO generation induced by LPS in concentration dependent manner. This results preliminary demonstrated that title compound could prevent inflammatory response in RAW264.7 cells LPS-induced.

The data analysis indicated no clear SAR for these compounds, however, the compound with the structure of

Reagents and conditions: (i) SOCl₂, CH₃OH, reflux; (ii) SOCl₂, CH₃OH, reflux; (iii) NaH, DMF, r.t.; (iv) NaOH, CH₃OH, reflux; (v) HOBT, EDCI, Et₃N, DMF, r.t.; (vi) NaOH, CH₃OH, reflux.

Scheme 1 Synthetic route of target compounds

Figure 3 Compound **8q** reduced NO production in RAW264.7 cells

quinolin-2(1H)-one exhibited improved inhibitory activity than other amino acid compounds. It suggested that the structure of quinolin-2(1H)-one might be an appropriate pharmacophore.

3 Conclusions

The phenylpropenoyl-amino acid derivatives were designed and synthesized, and the inhibition abilities of TNF- α and IL-6 production were evaluated by induced RAW264.7 cells. The results displayed promising activi-

ties of most of the compounds as inhibitor of both cytokines. Compounds **8d**, **8h**, **8j**, **8m**, **8o**, **8q** and **8t** were able to significantly inhibit IL-6, while compounds **8d**, **8h**, **8j** and **8q** presented simultaneously good levels of inhibition of the two cytokines tested. Especially, compound **8q** presented the highest level of inhibition of TNF- α and IL-6. Our results showed the possibility of these new compounds as potential candidates to inhibit the production of the pro-inflammatory cytokines TNF- α and IL-6. The phenylpropenoyl-amino acid moiety can be considered as one of the scaffolds for the design and development of anti-inflammatory agents targeting TNF- α and IL-6. Based on this research, we hope to successfully develop small organic molecules as TNF- α and IL-6 inhibitors for anti-inflammatory in the near future.

4 Experimental section

4.1 Instruments and reagents

Melting points were determined on SGWX-4 melting point apparatus (Precision Science Inc., Shanghai, China), which were uncorrected. NMR spectra were measured at 400 or 300 MHz for ¹H NMR, and 100 or 75 MHz for ¹³C NMR, respectively, on a Bruker DRX-400 (Bruker Inc, CH) in DMSO-*d*₆, using TMS as internal standard. ESI-MS was collected on an LCQ Advantage MAX spectro-

Table 1 Potency of Inhibitors among the LPS induced RAW264.7 cells to secrete TNF- α and IL-6^a

Compd.	IC ₅₀ /($\mu\text{mol}\cdot\text{L}^{-1}$)	
	IL-6	TNF- α
8a	54.03 $\pm$ 4.27 ^{##}	60.72 $\pm$ 6.37 ^{##}
8b	68.1 $\pm$ 3.91 ^{##}	88.86 $\pm$ 9.39 ^{##}
8c	36.45 $\pm$ 3.46	43.12 $\pm$ 4.66 ^{##}
8d	28.23 $\pm$ 2.43 ^{##}	29.64 $\pm$ 2.84 ^{##}
8e	55.98 $\pm$ 5.39 ^{##}	62.63 $\pm$ 5.67 ^{##}
8f	58.62 $\pm$ 7.27 ^{##}	61.06 $\pm$ 6.34 ^{##}
8g	79.04 $\pm$ 8.56 ^{##}	93.22 $\pm$ 8.29 ^{##}
8h	24.98 $\pm$ 3.64 ^{##}	30.59 $\pm$ 2.31 ^{##}
8i	40.85 $\pm$ 3.35 ^{##}	51.3 $\pm$ 4.39 ^{##}
8j	25.17 $\pm$ 2.86 ^{##}	32.79 $\pm$ 3.97 ^{##}
8k	45.11 $\pm$ 5.12 ^{##}	66.87 $\pm$ 7.24 ^{##}
8l	41.83 $\pm$ 3.87 ^{##}	50.98 $\pm$ 4.85
8m	44.84 $\pm$ 3.49 ^{##}	41.90 $\pm$ 3.17 ^{##}
8n	48.83 $\pm$ 4.18 ^{##}	50.04 $\pm$ 4.76
8o	34.31 $\pm$ 2.27	26.34 $\pm$ 1.72 ^{##}
8p	32.26 $\pm$ 1.95	51.39 $\pm$ 4.24 ^{##}
8q	22.77 $\pm$ 1.53 ^{##}	29.55 $\pm$ 2.13 ^{##}
8r	51.79 $\pm$ 3.14 ^{##}	79.32 $\pm$ 4.15 ^{##}
8s	58.04 $\pm$ 3.91 ^{##}	46.68 $\pm$ 4.26
8t	45.55 $\pm$ 4.71 ^{##}	31.88 $\pm$ 2.51 ^{##}
8u	56.31 $\pm$ 3.62 ^{##}	71.81 $\pm$ 3.64 ^{##}
8v	44.17 $\pm$ 2.76 ^{##}	45.21 $\pm$ 2.83
8w	29.01 $\pm$ 1.69	49.22 $\pm$ 1.79
Rebamipide	37.39 $\pm$ 4.36	48.21 $\pm$ 5.06

^a Compared with rebamipide ^{##} $p < 0.01$.

meter (Finnigan Inc. USA). Column chromatography was performed with silica gel (200 mesh, Qingdao Marine Chemical Inc., Qingdao, China). TLC was carried out on glass pre-coated silica gel GF 254 plates (Qingdao Marine Chemical Inc.). Unless otherwise noted, all chemicals were purchased from commercially available sources. If necessary, purification was carried out prior to use.

4.2 General procedures

4.2.1 Synthesis of methyl 2-amino-3-(2-oxo-1,2-dihydroquinolin-4-yl)propanoate hydrochloride (**2**)

In a three-neck flask, 2-amino-3-(2-oxo-1,2-dihydroquinolin-4-yl)propanoic acid (4.64 g, 20.0 mmol), and methanol (20 mL) were added. Thionyl chloride (SOCl₂) (5.6 mL, 77.09 mmol) was added dropwise under ice-cooling. After the addition was complete, The reaction mixture was heated to 70 °C for 4 h with stirring (TLC, ethyl acetate/methanol, $V:V=15:1$) and then evaporated with toluene under reduced pressure to remove excess SOCl₂ to give compound **2** (5.48 g),^[18] yield 97.1%. m.p. 206.9~209.2 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ : 11.96 (s, 1H, ArNHCO), 9.09~8.96 (m, 2H, NH₂), 8.77 (d, $J=5.3$ Hz, 1H, HCl), 7.94~7.80 (m, 1H, ArH), 7.58~7.48 (m, 1H, ArH), 7.42 (d, $J=8.3$ Hz, 1H, ArH), 7.23 (t, $J=7.8$ Hz, 1H, ArH), 6.53 (s, 1H, CH₂C=CHCO), 4.28~4.16 (m, 1H, NH₂CHCOOH), 3.62 (s, 3H, OCH₃), 3.57~3.40 (m, 1H, CH₂CHNH₂), 3.39~3.27 (m, 1H, CH₂CHNH₂); ¹³C NMR (101 MHz, DMSO-*d*₆) δ : 170.32, 169.36,

161.67, 145.22, 139.46, 131.06, 124.59, 123.41, 122.53, 118.77, 116.48, 53.15, 52.00, 32.78; ESI-MS m/z : 247.10 [M+H]⁺.

4.2.2 Synthesis of methyl 5-chloro-1*H*-indole-2-carboxylate (**4j**)

In a three-neck flask, 5-chloro-1*H*-indole-2-carboxylic acid (5 g, 25.6 mmol), and methanol (20 mL) were added. Thionyl chloride (11.74 g, 98.7 mmol) was added dropwise under ice-cooling. After the addition was complete, the reaction mixture was heated to 70 °C for 6 h with stirring (TLC, ethyl acetate/petroleum ether, $V:V=15:1$) and then evaporated under reduced pressure. The residues were dissolved in ethyl acetate (20 mL), then washed with water (20 mL $\times$ 3), saturated sodium bicarbonate (20 mL $\times$ 3) and saturated sodium chloride solution (20 mL $\times$ 3), dried with sodium sulfate after washing separately and evaporated *in vacuo* to afford compound **4j** (5.15 g),^[19] yield 96.3%. m.p. 208.1~210.0 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ : 12.20 (s, 1H, ArNHC=CH), 7.72 (s, 1H, ArH), 7.48 (d, $J=8.8$ Hz, 1H, ArH), 7.26 (d, $J=8.8$ Hz, 1H, ArH), 7.13 (s, 1H, ArCH=), 3.88 (s, 3H, OCH₃); ¹³C NMR (101 MHz, DMSO-*d*₆) δ : 161.90, 136.20, 128.94, 128.12, 125.24, 125.13, 121.54, 114.74, 107.70, 52.42; ESI-MS m/z : 208.1 [M+H]⁺.

4.2.3 Synthesis of compounds **5**

A solution of **4j** (0.71 g, 3.4 mmol) and NaH (0.16 g, 4.0 mmol, 60%) in DMF solvent was stirred for 30 min. To the reaction mixture, the solution of 1-(chloromethyl)-4-methylbenzene (0.56 g, 4.0 mmol) in DMF (5 mL) was added dropwise. The mixture was then stirred at room temperature for 4 h and monitored by TLC (TLC, ethyl acetate/petroleum ether, $V:V=1:5$). The reaction solution was poured into 40 mL of ice water when a large amount of solid was precipitated, and then filtered. The residue was recrystallized with methanol to give **5** directly used in the next reaction.

Methyl 5-chloro-1-(4-methylbenzyl)-1*H*-indole-2-carboxylate (**5o**) as an example. White solid, 0.821 g, yield 76.7%. m.p. 109.4~112.2 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ : 7.79 (s, 1H, ArH), 7.62 (d, $J=9.0$ Hz, 1H, ArH), 7.37~7.28 (m, 2H, ArH, ArCH=), 7.06 (d, $J=7.8$ Hz, 2H, ArH), 6.98~6.88 (m, 2H, ArH), 5.80 (s, 2H, ArCH₂N), 3.83 (s, 3H, OCH₃), 2.21 (s, 3H, ArCH₃); ¹³C NMR (101 MHz, DMSO-*d*₆) δ : 161.83, 137.83, 136.79, 135.51, 129.52, 128.62, 126.93, 126.70, 125.83, 125.73, 121.95, 113.68, 110.52, 52.42, 47.49, 21.05; ESI-MS m/z : 314.2 [M+H]⁺. HRMS calcd for C₁₈H₁₇ClNO₂ [M+H]⁺ 314.0948, found 314.0944.

4.2.4 Synthesis of compounds **6**

To a mixture of **5** (2.5 mmol) in methanol, 10 mL of NaOH (0.3 g, 7.5 mmol) in aqueous methanol was added. The reaction mixture was stirred at room temperature for 2 h and monitored by TLC, then evaporated (TLC, ethyl acetate/petroleum ether, $V:V=1:5$). The residue diluted with 5% HCl (liq.) was slowly added dropwise to pH=4~5, filtrated and dried to give **6** directly used in the next re-

action.

5-Chloro-1-(4-methylbenzyl)-1*H*-indole-2-carboxylic acid (**6o**) as an example. White solid, 0.48 g, yield 62.3%. m.p. 225.9~227.4 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ: 13.22 (s, 1H, COOH), 7.77 (s, 1H, ArH), 7.57 (d, *J*=8.9 Hz, 1H, ArH), 7.33~7.24 (m, 2H, ArH, ArCH=), 7.06 (d, *J*=7.8 Hz, 2H, ArH), 6.92 (d, *J*=7.8 Hz, 2H, ArH), 5.83 (s, 2H, ArCH₂N), 2.21 (s, 3H, ArCH₃); ¹³C NMR (101 MHz, DMSO-*d*₆) δ: 163.10, 137.72, 136.71, 135.73, 129.99, 129.49, 127.02, 126.72, 125.60, 125.30, 121.80, 113.58, 110.24, 47.30, 21.05; ESI-MS *m/z*: 298.02 [M+H]⁺. HRMS calcd for C₁₇H₁₄ClNO₂Na [M+Na]⁺ 322.0611, found 322.0608.

4.2.5 Synthesis of compounds 7

To a solution of **6** (2 mmol), EDC·HCl (0.422 g, 2.2 mmol), HOBt (0.297 g, 2.2 mmol) and **2** (0.564 g, 2 mmol) in DMF (20 mL), was added Et₃N (0.83 mL). The reaction mixture was stirred overnight at room temperature (TLC, ethyl acetate/methanol, *V*:*V*=15:1). The reaction mixture was added 10% citric acid (30 mL) and filtered. The residue was purified via silica using methanol/CH₂Cl₂ (*V*:*V*=1:20) to provide the title compounds **7** directly used in the next reaction.

Methyl 2-(5-chloro-1-(4-methylbenzyl)-1*H*-indole-2-carboxamido)-3-(2-oxo-1,2-dihydroquinolin-4-yl) propanoate (**7o**) as an example. White solid, 0.44 g, yield 41.7%. m.p. 260.2~262.7 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ: 11.75 (s, 1H, ArNHCO), 9.22 (s, 1H, CONH), 7.85~7.79 (m, 1H, ArH), 7.77 (s, 1H, ArH), 7.56 (d, *J*=8.9 Hz, 1H, ArH), 7.55~7.47 (m, 1H, ArH), 7.40~7.30 (m, 1H, ArH), 7.28~7.20 (m, 2H, ArH), 7.13 (s, 1H, ArCH=), 7.01 (d, *J*=7.9 Hz, 2H, ArH), 6.94 (d, *J*=7.8 Hz, 2H, ArH), 6.52 (s, 1H, CH₂C=CHCO), 5.69 (s, 2H, ArCH₂N), 4.86~4.77 (m, 1H, NHCHCOOCH₃), 3.70 (s, 3H, OCH₃), 3.57~3.47 (m, 1H, CH₂CHNH), 3.34~3.22 (m, 1H, CH₂CHNH), 2.20 (s, 3H, ArCH₃); ¹³C NMR (101 MHz, DMSO-*d*₆) δ: 171.94, 162.21, 161.79, 147.46, 139.44, 136.80, 136.77, 135.51, 132.55, 130.82, 129.39, 127.26, 127.23, 125.47, 124.45, 122.38, 122.35, 121.34, 118.94, 116.31, 113.42, 105.96, 52.82, 51.94, 47.08, 32.95, 21.06; ESI-MS *m/z*: 528.14 [M+H]⁺. HRMS calcd for C₃₀H₂₆ClN₃O₄ [M+H]⁺ 528.1690, found 528.1681.

4.2.6 Synthesis of compounds 8

To a mixture of **7o** (0.8 mmol) in methanol, 8 mL of NaOH (0.128 g, 3.2 mmol) in aqueous methanol was added. The reaction mixture was stirred at room temperature for 4 h and then evaporated. The residue diluted with 5% HCl (liq.) was slowly added dropwise to pH=4~5, filtered and dried to give a white solid 2-(5-chloro-1-(4-methylbenzyl)-1*H*-indole-2-carboxamido)-3-(2-oxo-1,2-dihydroquinolin-4-yl)propanoic acid (**8o**) (0.21 g), yield 50.8%. m.p. 295.8~297.4 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ: 13.13 (s, 1H, COOH), 11.72 (s, 1H, ArNHCO), 9.08 (s, 1H, CONH), 7.85 (d, *J*=8.1 Hz, 1H, ArH), 7.77 (s, 1H, ArH), 7.61~7.47 (m, 2H, ArH), 7.34 (d, *J*=8.2 Hz, 1H, ArH), 7.28~7.18 (m, 2H, ArH), 7.13 (s, 1H,

ArCH=), 7.04~6.92 (m, 4H, ArH), 6.52 (s, 1H, CH₂C=CHCO), 5.68 (s, 2H, ArCH₂N), 4.84~4.68 (m, 1H, NHCHCOOH), 3.62~3.47 (m, 1H, CH₂CHNH), 3.31~3.13 (m, 1H, CH₂CHNH), 2.20 (s, 3H, ArCH₃); ¹³C NMR (101 MHz, DMSO-*d*₆) δ: 173.14, 162.17, 161.83, 147.89, 139.43, 136.72, 136.70, 135.49, 132.87, 130.78, 129.37, 127.36, 127.27, 125.39, 124.48, 124.31, 122.40, 122.21, 121.29, 118.99, 116.30, 113.41, 105.73, 52.04, 47.07, 33.30, 21.08; ESI-MS *m/z*: 514.15 [M+H]⁺. HRMS calcd for C₂₉H₂₄ClN₃O₄ [M+H]⁺ 514.1534, found 514.1532.

Other target compounds **8** were synthesized in the same way.

2-Cinnamamido-3-(2-oxo-1,2-dihydroquinolin-4-yl)propanoic acid (**8a**): White solid, yield 58.3%. m.p. 270.2~271.4 °C; ¹H NMR (DMSO-*d*₆, 300 MHz) δ: 13.04 (s, 1H, COOH), 11.71 (s, 1H, ArNHCO), 8.63 (s, CONH), 7.85 (d, *J*=9.0 Hz, 1H, ArH), 7.57 (d, *J*=9.0 Hz, 2H, ArH), 7.51 (d, *J*=9.0 Hz, 1H, ArH), 7.30~7.47 (m, 5H, ArH), 7.31~7.19 (m, 1H, ArCH=), 6.69 (d, *J*=15.6 Hz, 1H, COCH=), 6.44 (s, 1H, CH₂C=CHCO), 4.60~4.80 (m, 1H, NHCHCOOH), 3.35~3.45 (m, 1H, CH₂CHNH), 3.05~3.25 (m, 1H, CH₂CHNH); ¹³C NMR (DMSO-*d*₆, 75 MHz) δ: 172.6, 165.0, 161.3, 146.9, 139.5, 138.9, 134.6, 130.3, 129.6, 128.9, 127.6, 124.0, 121.8, 121.8, 121.3, 118.4, 115.8, 51.5, 33.5; ESI-MS *m/z*: 361.12 [M-H]⁺. HRMS calcd for C₂₁H₁₈N₂O₄ [M+H]⁺ 363.1345; found 363.1329.

3-(2-Oxo-1,2-dihydroquinolin-4-yl)-2-(3-(*p*-tolyl)acrylamido)propanoic acid (**8b**): White solid, yield 56.7%. m.p. 278.2~279.5 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ: 13.04 (s, 1H, COOH), 11.71 (s, 1H, ArNHCO), 8.58 (s, 1H, CONH), 7.85 (d, *J*=8.2, 1.3 Hz, 1H, ArH), 7.52 (d, *J*=7.7 Hz, 1H, ArH), 7.46 (d, *J*=8.1 Hz, 2H, ArH), 7.41~7.30 (m, 2H, ArH), 7.28~7.17 (m, 3H, ArCH=, ArH), 6.62 (d, *J*=15.8 Hz, 1H, COCH=), 6.43 (s, 1H, CH₂C=CHCO), 4.75~4.66 (m, NHCHCOOH), 3.48~3.38 (m, 1H, CH₂CHNH), 3.16~3.05 (m, 1H, CH₂CHNH), 2.32 (s, 3H, ArCH₃); ¹³C NMR (101 MHz, DMSO-*d*₆) δ: 173.16, 165.67, 161.78, 147.43, 139.99, 139.88, 139.41, 132.37, 130.78, 130.01, 128.06, 124.53, 122.36, 122.26, 120.72, 118.98, 116.29, 52.01, 34.04, 21.42; ESI-MS *m/z*: 375.15 [M-H]⁺. HRMS calcd for C₂₂H₂₀N₂O₄ [M+H]⁺ 377.1501; found 377.1489.

2-(3-(4-Methoxyphenyl)acrylamido)-3-(2-oxo-1,2-dihydroquinolin-4-yl)propanoic acid (**8c**): White solid, yield 62.1%. m.p. 258.8~260.4 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ: 13.03 (s, 1H, COOH), 11.69 (s, 1H, ArNHCO), 8.50 (s, 1H, CONH), 7.84 (d, *J*=8.2 Hz, 1H, ArH), 7.56~7.48 (m, 3H, ArH), 7.38~7.30 (m, 2H, ArH), 7.28~7.20 (m, 1H, ArCH=), 7.00~6.95 (m, 2H, ArH), 6.52 (d, *J*=15.8 Hz, 1H, COCH=), 6.41 (s, 1H, CH₂C=CHCO), 4.60~4.67 (m, 1H, NHCHCOOH), 3.79 (s, 3H, OCH₃), 3.35~3.42 (m, CH₂CHNH), 3.0~3.10 (m, 1H, CH₂CHNH); ¹³C NMR (101 MHz, DMSO-*d*₆) δ: 173.21, 165.80, 161.77, 160.90, 147.46, 139.72, 139.41, 130.77, 129.69, 127.69, 124.55, 122.35, 122.25, 119.26, 118.99, 116.28, 114.86, 55.72, 52.00, 34.07; ESI-MS *m/z*: 391.05

$[M - H]^+$. HRMS calcd for $C_{22}H_{20}N_2O_5$ $[M + H]^+$ 393.1450; found 393.1432.

3-(2-Oxo-1,2-dihydroquinolin-4-yl)-2-(3-(3,4,5-trimethoxyphenyl)acrylamido)propanoic acid (**8d**): Yellow solid, yield 55.3%. m.p. 251.3~253.4 °C; 1H NMR (300 MHz, DMSO- d_6) δ : 13.04 (s, 1H, COOH) 11.70 (s, 1H, ArNHCO), 8.51 (s, CONH), 7.84 (d, $J=9.0$ Hz, 1H, ArH), 7.52 (t, $J=9.0$ Hz, 1H, ArH), 7.29~7.38 (m, 2H, ArH), 7.25 (t, $J=9.0$ Hz, 1H, ArCH=), 6.90 (s, 2H, ArH), 6.63 (d, $J=15.0$ Hz, 1H, COCH=), 6.42 (s, 1H, $CH_2C=CHCO$), 4.60~4.75 (m, 1H, NHCHCOOH), 3.81 (s, 6H, OCH₃), 3.68 (s, 3H, OCH₃), 3.40~3.47 (m, 1H, CH_2CHNH), 3.00~3.17 (m, 1H, CH_2CHNH); ^{13}C NMR (101 MHz, DMSO- d_6) δ : 173.15, 165.63, 161.80, 153.51, 147.44, 140.12, 139.41, 139.18, 130.79, 130.75, 124.51, 122.38, 122.21, 121.18, 118.98, 116.31, 105.45, 60.54, 56.31, 51.97, 40.36, 33.98; ESI-MS m/z : 451.03 $[M - H]^+$. HRMS calcd for $C_{24}H_{24}N_2O_7$ $[M + H]^+$ 453.1662; found 453.1648.

2-(3-(4-Chlorophenyl)acrylamido)-3-(2-oxo-1,2-dihydroquinolin-4-yl)propanoic acid (**8e**): White solid, yield 65.7%. m.p. 268.2~270.8 °C; 1H NMR (300 MHz, DMSO- d_6) δ : 11.72 (s, 1H, ArNHCO), 8.66 (s, CONH), 7.86 (d, $J=8.1$ Hz, 1H, ArH), 7.86 (d, $J=8.4$ Hz, 2H, ArH), 7.45~7.57 (m, 3H, ArH), 7.41 (d, $J=15.6$ Hz, 1H, ArH), 7.35 (d, $J=8.1$ Hz, 1H, ArH), 7.25 (t, $J=7.5$ Hz, 1H, ArCH=), 6.70 (d, $J=15.6$ Hz, 1H, COCH=), 6.44 (s, 1H, $CH_2C=CHCO$), 4.66~4.82 (m, 1H, NHCHCOOH), 3.45~3.54 (m, 1H, CH_2CHNH), 3.06~3.20 (m, 1H, CH_2CHNH); ^{13}C NMR (75 MHz, DMSO- d_6) δ : 172.5, 164.8, 161.3, 146.9, 138.9, 138.2, 134.0, 133.6, 130.3, 129.3, 128.9, 124.0, 122.1, 121.8, 121.7, 118.5, 115.8, 51.5, 33.5; ESI-MS m/z : 395.15 $[M - H]^+$. HRMS calcd for $C_{21}H_{17}ClN_2O_4$ $[M + H]^+$ 397.0955; found 397.0944.

2-(3-(4-Nitrophenyl)acrylamido)-3-(2-oxo-1,2-dihydroquinolin-4-yl)propanoic acid (**8f**): Yellow solid, yield 49.1%. m.p. 280.1~282.1 °C; 1H NMR (300 MHz, DMSO- d_6) δ : 13.12 (s, 1H, COOH), 11.72 (s, 1H, CONH), 8.80 (s, CONH), 8.26 (s, $J=8.7$ Hz, 2H, ArH), 7.75~8.01 (m, 3H, ArH), 7.43~7.65 (m, 2H, ArH), 7.19~7.42 (m, 2H, ArCH=, ArH), 6.88 (d, $J=15.9$ Hz, 1H, COCH=), 6.44 (s, 1H, $CH_2C=CHCO$), 4.61~4.84 (m, 1H, NHCHCOOH), 3.45~3.55 (m, 1H, CH_2CHNH), 3.02~3.25 (m, 1H, CH_2CHNH); ^{13}C NMR (75 MHz, DMSO- d_6) δ : 172.4, 164.3, 161.3, 147.5, 146.8, 141.2, 138.9, 137.2, 130.3, 128.8, 125.5, 124.0, 123.9, 121.8, 121.7, 118.5, 115.8, 51.6, 33.5; ESI-MS m/z : 406.07 $[M - H]^+$. HRMS calcd for $C_{21}H_{17}N_3O_6$ $[M + H]^+$ 408.1196; found 408.1183.

2-(3-(4-Hydroxyphenyl)acrylamido)-3-(2-oxo-1,2-dihydroquinolin-4-yl)propanoic acid (**8g**): White solid, yield 61.8%. m.p. 240.7~242.1 °C; 1H NMR (300 MHz, DMSO- d_6) δ : 11.74 (s, 1H, ArNHCO), 9.97 (s, 1H, OH), 8.53 (s, 1H, CONH), 7.86 (d, $J=8.7$ Hz, 1H, ArH), 7.53 (t, $J=7.2$ Hz, 1H, ArH), 7.43 (d, $J=8.4$ Hz, 2H, ArH), 7.29~7.39 (m, 2H, ArH), 7.25 (t, $J=7.2$ Hz, 1H, ArCH=), 6.83 (d, $J=8.7$ Hz, 2H, ArH), 6.41~6.59 (m,

2H, COCH=, $CH_2C=CHCO$), 4.63~4.82 (m, 1H, NHCHCOOH), 3.44~3.51 (m, 1H, CH_2CHNH), 3.05~3.21 (m, 1H, CH_2CHNH); ^{13}C NMR (75 MHz, DMSO- d_6) δ : 172.7, 165.5, 161.3, 159.0, 147.0, 139.7, 138.9, 130.3, 129.3, 125.6, 124.0, 121.9, 121.7, 118.5, 117.7, 115.7, 51.49, 33.54; ESI-MS m/z : 377.30 $[M - H]^+$. HRMS calcd for $C_{21}H_{18}N_2O_5$ $[M + H]^+$ 379.1294; found 379.1284.

2-(3-(4-Hydroxy-3-methoxyphenyl)acrylamido)-3-(2-oxo-1,2-dihydroquinolin-4-yl)propanoic acid (**8h**): White solid, yield 51.9%. m.p. 241.7~243.7 °C; 1H NMR (300 MHz, DMSO- d_6) δ : 11.70 (s, 1H, ArNHCO), 9.49 (s, 1H, OH), 8.44 (s, 1H, CONH), 7.85 (d, $J=8.4$ Hz, 1H, ArH), 7.52 (t, $J=7.5$ Hz, 1H, ArH), 7.21~7.37 (m, $J=6.3$ Hz, 3H, ArH), 7.13 (s, 1H, ArCH=), 7.00 (d, $J=8.4$ Hz, 1H, ArH), 6.80 (d, $J=8.4$ Hz, 1H, ArH), 6.49 (d, $J=15.9$ Hz, 1H, COCH=), 6.43 (s, 1H, $CH_2C=CHCO$), 4.64~4.75 (m, 1H, NHCHCOOH), 3.81 (s, 3H, OCH₃), 3.44~3.53 (m, 1H, CH_2CHNH), 3.03~3.15 (m, 1H, CH_2CHNH); ^{13}C NMR (75 MHz, DMSO- d_6) δ : 172.73, 165.47, 161.28, 148.41, 147.77, 146.96, 139.89, 138.89, 130.26, 126.10, 124.01, 121.85, 121.80, 121.71, 118.48, 117.98, 115.78, 115.57, 110.61, 55.44, 51.45, 33.55; ESI-MS m/z : 407.05 $[M - H]^+$. HRMS calcd for $C_{22}H_{20}N_2O_6$ $[M + H]^+$ 409.1400; found 409.1390.

2-(1*H*-Indole-2-carboxamido)-3-(2-oxo-1,2-dihydroquinolin-4-yl)propanoic acid (**8i**): White solid, yield 65.6%. m.p. 209.3~211.8 °C; 1H NMR (400 MHz, DMSO- d_6) δ : 13.09 (s, 1H, COOH), 11.67 (s, 1H, ArNHCO), 11.57 (s, 1H, ArNHC=CH), 8.85 (s, 1H, CONH), 7.88 (d, $J=8.2$ Hz, 1H, ArH), 7.64 (d, $J=8.0$ Hz, 1H, ArH), 7.56~7.48 (m, 1H, ArH), 7.43~7.39 (m, 1H, ArH), 7.36~7.29 (m, 1H, ArH), 7.28~7.22 (m, 1H, ArH), 7.21~7.13 (m, 2H, ArH, ArCH=), 7.09~7.01 (m, 1H, ArH), 6.50 (s, 1H, $CH_2C=CHCO$), 4.89~4.75 (m, 1H, NHCHCOOH), 3.65~3.21 (m, 2H, CH_2CHNH); ^{13}C NMR (101 MHz, DMSO- d_6) δ : 173.25, 161.78, 161.64, 147.74, 139.42, 136.96, 131.31, 130.76, 127.39, 124.49, 123.98, 122.37, 122.26, 122.08, 120.25, 118.99, 116.31, 112.76, 103.71, 51.86, 33.51; ESI-MS m/z : 374.02 $[M - H]^+$. HRMS calcd for $C_{21}H_{17}N_3O_4$ $[M + H]^+$ 376.1297; found 376.1284.

2-(5-Chloro-1*H*-indole-2-carboxamido)-3-(2-oxo-1,2-dihydroquinolin-4-yl)propanoic acid (**8j**): White solid, yield 53.6%. m.p. 238.3~240.7 °C; 1H NMR (300 MHz, DMSO- d_6) δ : 11.81 (s, 1H, ArNHC=CH), 11.70 (s, 1H, ArNHCO), 8.98 (s, 1H, CONH), 7.89 (d, $J=8.1$ Hz, 1H, ArH), 7.76 (s, 1H, ArH), 7.52 (t, $J=7.5$ Hz, 1H, ArH), 7.43 (d, $J=8.7$ Hz, 1H, ArH), 7.34 (d, $J=7.2$ Hz, 1H, ArH), 7.16~7.31 (m, 3H, ArH, ArCH=), 6.52 (s, 1H, $CH_2C=CHCO$), 4.71~4.90 (m, 1H, NHCHCOOH), 3.57~3.64 (m, 1H, CH_2CHNH), 3.12~3.40 (m, 1H, CH_2CHNH); ^{13}C NMR (75 MHz, DMSO- d_6) δ : 172.6, 161.3, 160.8, 147.2, 138.9, 134.9, 132.3, 130.3, 127.9, 124.3, 124.0, 123.6, 121.9, 121.7, 120.7, 118.5, 115.8, 113.9, 102.8, 51.4, 33.0; ESI-MS m/z : 408.14 $[M - H]^+$. HRMS calcd for $C_{21}H_{16}ClN_3O_4$ $[M + H]^+$ 410.0908; found 410.0986.

2-(6-Chloro-1*H*-indole-2-carboxamido)-3-(2-oxo-1,2-di-

hydroquinolin-4-yl)propanoic acid (**8k**): White solid, yield 70.2%. m.p. 235.7 ~ 237.8 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ: 13.08 (s, 1H, COOH), 11.78 (s, 1H, ArNHC=CH), 11.68 (s, 1H, ArNHCO), 8.95 (s, 1H, CONH), 7.88 (d, *J*=7.8 Hz, 1H, ArH), 7.75 (s, 1H, ArH), 7.51 (t, *J*=7.5 Hz, 1H, ArH), 7.42 (d, *J*=8.4 Hz, 1H, ArH), 7.32 (d, *J*=8.1 Hz, 1H, ArH), 7.15 ~ 7.24 (m, 3H, ArH, ArCH=), 6.49 (s, 1H, CH₂C=CHCO), 4.73 ~ 4.90 (m, 1H, NHCHCOOH), 3.47 ~ 3.62 (m, 1H, CH₂CHNH), 3.16 ~ 3.32 (m, 1H, CH₂CHNH); ¹³C NMR (75 MHz, DMSO-*d*₆) δ: 172.6, 161.3, 160.8, 147.2, 138.9, 134.9, 132.3, 130.2, 127.9, 124.2, 124.0, 123.6, 121.8, 121.7, 120.7, 118.5, 115.8, 113.9, 102.8, 51.4, 33.0; ESI-MS *m/z*: 407.94 [M+H]⁺. HRMS calcd for C₂₁H₁₆ClN₃O₄ [M+H]⁺ 410.0908; found 410.0896.

2-(5-Chloro-1-(4-fluorobenzyl)-1*H*-indole-2-carboxamido)-3-(2-oxo-1,2-dihydroquinolin-4-yl)propanoic acid (**8l**): White solid, yield 46.7%. m.p. 277.6 ~ 278.9 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ: 11.72 (s, 1H, ArNHCO), 9.11 (s, 1H, CONH), 7.85 (d, *J*=8.2 Hz, 1H, ArH), 7.78 (s, 1H, ArH), 7.60 (d, *J*=9.0 Hz, 1H, ArH), 7.56 ~ 7.48 (m, 1H, ArH), 7.33 (d, *J*=8.2 Hz, 1H, ArH), 7.29 ~ 7.21 (m, 2H, ArH), 7.18 ~ 7.08 (m, 3H, ArH, ArCH=), 7.08 ~ 7.00 (m, 2H, ArH), 6.51 (s, 1H, CH₂C=CHCO), 5.80 ~ 5.64 (m, 2H, ArCH₂N), 4.81 ~ 4.70 (m, 1H, NHCHCOOH), 3.59 ~ 3.48 (m, 1H, CH₂CHNH), 3.28 ~ 3.18 (m, 1H, CH₂CHNH); ¹³C NMR (101 MHz, DMSO-*d*₆) δ: 172.97, 162.92, 162.13, 161.83, 160.51, 147.84, 139.41, 136.68, 134.70 (*J*_{C-F}=3.15 Hz), 132.65, 130.80, 129.41 (*J*_{C-F}=8.24 Hz), 127.29, 125.54, 124.50, 124.46, 122.30 (*J*_{C-F}=25.47 Hz), 120.18 (*J*_{C-F}=240.3 Hz), 116.32, 115.70, 115.49, 113.30, 105.94, 51.88, 46.63, 33.20; ESI-MS *m/z*: 518.16 [M+H]⁺. HRMS calcd for C₂₈H₂₁ClF₃N₃O₄ [M+H]⁺ 518.1283; found 518.1284.

2-(5-Chloro-1-(4-chlorobenzyl)-1*H*-indole-2-carboxamido)-3-(2-oxo-1,2-dihydroquinolin-4-yl)propanoic acid (**8m**): White solid, yield 52.3%. m.p. 286.5 ~ 286.8 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ: 13.04 (s, 1H, COOH), 11.72 (s, 1H, ArNHCO), 9.11 (s, 1H, CONH), 7.91 ~ 7.75 (m, 2H, ArH), 7.61 ~ 7.46 (m, 2H, ArH), 7.33 (d, *J*=8.3 Hz, 1H, ArH), 7.30 ~ 7.21 (m, 4H, ArH), 7.17 (s, 1H, ArCH=), 7.11 ~ 7.05 (m, 2H, ArH), 6.51 (s, 1H, CH₂C=CHCO), 5.72 (s, 2H, ArCH₂N), 4.82 ~ 4.68 (m, 1H, NHCHCOOH), 3.56 ~ 3.47 (m, 1H, CH₂CHNH), 3.29 ~ 3.19 (m, 1H, CH₂CHNH); ¹³C NMR (101 MHz, DMSO-*d*₆) δ: 173.06, 162.06, 161.83, 147.87, 139.42, 137.55, 136.70, 132.60, 132.23, 130.80, 129.19, 128.83, 127.27, 125.59, 124.55, 124.46, 122.41, 122.16, 121.43, 118.97, 116.31, 113.28, 105.98, 51.95, 46.73, 33.23; ESI-MS *m/z*: 533.92 [M+H]⁺. HRMS calcd for C₂₈H₂₁Cl₂N₃O₄ [M+H]⁺ 534.0987; found 535.0987.

(1-(4-Bromobenzyl)-5-chloro-1*H*-indole-2-carboxamido)-3-(2-oxo-1,2-dihydroquinolin-4-yl)propanoic acid (**8n**): White solid, yield 49.0%. m.p. 294.7 ~ 296.3 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ: 11.72 (s, 1H, ArNHCO), 9.11 (s, 1H, CONH), 7.84 (d, *J*=7.9 Hz, 1H, ArH), 7.80 (s, 1H, ArH), 7.56 (d, *J*=8.9 Hz, 1H, ArH), 7.54 ~ 7.49 (m, 1H,

ArH), 7.45 ~ 7.39 (m, 2H, ArH), 7.33 (d, *J*=8.3 Hz, 1H, ArH), 7.28 ~ 7.22 (m, 2H, ArH), 7.17 (s, 1H, ArCH=), 7.05 ~ 6.99 (m, 2H, ArH), 6.51 (s, 1H, CH₂C=CHCO), 5.71 (s, 2H, ArCH₂N), 4.80 ~ 4.70 (m, 1H, NHCHCOOH), 3.58 ~ 3.47 (m, 1H, CH₂CHNH), 3.29 ~ 3.18 (m, 1H, CH₂CHNH); ¹³C NMR (101 MHz, DMSO-*d*₆) δ: 173.04, 162.06, 161.82, 147.85, 139.42, 137.98, 136.70, 132.58, 131.75, 130.80, 129.53, 127.27, 125.59, 124.56, 124.46, 122.41, 122.15, 121.43, 120.76, 118.97, 116.30, 113.28, 105.98, 51.93, 46.80, 33.20; ESI-MS *m/z*: 577.96 [M+H]⁺. HRMS calcd for C₂₈H₂₁BrClN₃O₄ [M+H]⁺ 578.0482; found 578.0497.

2-(5-Chloro-1-(4-cyanobenzyl)-1*H*-indole-2-carboxamido)-3-(2-oxo-1,2-dihydroquinolin-4-yl)propanoic acid (**8p**): White solid, yield 48.8%. m.p. 284.4 ~ 286.1 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ: 13.16 (s, 1H, COOH), 11.72 (s, 1H, ArNHCO), 9.11 (s, 1H, CONH), 7.89 ~ 7.78 (m, 2H, ArH), 7.74 ~ 7.66 (m, 2H, ArH), 7.58 ~ 7.48 (m, 2H, ArH), 7.35 ~ 7.29 (m, 1H, ArH), 7.28 ~ 7.18 (m, 3H, ArH), 7.18 ~ 7.08 (m, 2H, ArH, ArCH=), 6.49 (s, 1H, CH₂C=CHCO), 5.84 (s, 2H, ArCH₂N), 4.80 ~ 4.66 (m, 1H, NHCHCOOH), 3.56 ~ 3.48 (m, 1H, CH₂CHNH), 3.27 ~ 3.19 (m, 1H, CH₂CHNH); ¹³C NMR (101 MHz, DMSO-*d*₆) δ: 172.99, 161.90, 161.81, 147.85, 144.37, 139.40, 136.79, 132.84, 132.49, 130.80, 127.89, 127.27, 125.73, 124.73, 124.45, 122.40, 122.17, 121.53, 119.18, 118.96, 116.30, 113.13, 110.36, 106.10, 51.92, 47.27, 33.24; ESI-MS *m/z*: 524.92 [M+H]⁺. HRMS calcd for C₂₉H₂₁ClN₄O₄ [M+H]⁺ 525.1330; found 525.1331.

2-(5-Chloro-1-(4-(trifluoromethyl)benzyl)-1*H*-indole-2-carboxamido)-3-(2-oxo-1,2-dihydroquinolin-4-yl)propanoic acid (**8q**): White solid, yield 54.1%. m.p. 287.6 ~ 289.1 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ: 13.09 (s, 1H, COOH), 11.72 (s, 1H, ArNHCO), 9.12 (s, CONH), 7.91 ~ 7.79 (m, 2H, ArH), 7.60 (d, *J*=8.2 Hz, 2H, ArH), 7.56 (d, *J*=8.9 Hz, 1H, ArH), 7.54 ~ 7.48 (m, 1H, ArH), 7.32 (d, *J*=8.3 Hz, 1H, ArH), 7.28 ~ 7.20 (m, 5H, ArH, ArCH=), 6.51 (s, 1H, CH₂C=CHCO), 5.84 (s, 2H, ArCH₂N), 4.79 ~ 4.68 (m, 1H, NHCHCOOH), 3.60 ~ 3.48 (m, 1H, CH₂CHNH), 3.32 ~ 3.21 (m, 1H, CH₂CHNH); ¹³C NMR (101 MHz, DMSO-*d*₆) δ: 173.03, 162.00, 161.82, 147.84, 143.40, 139.41, 136.77, 132.58, 130.79, 128.16 (*J*_{C-F}=31.82 Hz), 127.82, 127.28, 125.80 (*J*_{C-F}=3.82 Hz), 125.68, 124.69, 124.62 (*J*_{C-F}=272.78 Hz), 124.46, 122.39, 122.14, 121.50, 118.97, 116.29, 113.19, 106.06, 51.96, 47.11, 33.19; ESI-MS *m/z*: 567.81 [M+H]⁺. HRMS calcd for C₂₉H₂₁ClF₃N₃O₄ [M+H]⁺ 568.1251; found 568.1257.

(5-Chloro-1-(4-methoxybenzyl)-1*H*-indole-2-carboxamido)-3-(2-oxo-1,2-dihydroquinolin-4-yl)propanoic acid (**8r**): White solid, yield 46.8%. m.p. 270.2 ~ 271.4 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ: 13.17 (s, 1H, COOH), 11.72 (s, 1H, ArNHCO), 9.06 (s, CONH), 7.86 (d, *J*=8.2 Hz, 1H, ArH), 7.75 (s, 1H, ArH), 7.60 (d, *J*=8.9 Hz, 1H, ArH), 7.55 ~ 7.49 (m, 1H, ArH), 7.33 (d, *J*=8.3 Hz, 1H, ArH), 7.28 ~ 7.20 (m, 2H, ArH), 7.10 (s, 1H, ArCH=), 7.08 ~ 7.02 (m, 2H, ArH), 6.80 ~ 6.73 (m, 2H, ArH), 6.53 (s, 1H, CH₂C=CHCO), 5.65 (s, 2H, ArCH₂N), 4.82 ~ 4.70

(m, 1H, NHCHCOOH), 3.66 (s, 3H, OCH₃), 3.57~3.49 (m, 1H, CH₂CHNH), 3.28~3.19 (m, 1H, CH₂CHNH); ¹³C NMR (101 MHz, DMSO-*d*₆) δ: 173.18, 162.24, 161.88, 158.81, 147.99, 139.41, 136.62, 132.86, 130.79, 130.44, 128.91, 127.30, 125.38, 124.50, 124.27, 122.43, 122.15, 121.26, 119.03, 116.32, 114.18, 113.43, 105.76, 55.41, 52.06, 46.70, 33.34; ESI-MS *m/z*: 529.85 [M+H]⁺. HRMS calcd for C₂₉H₂₄ClN₃O₅ [M+H]⁺ 530.1483; found 530.1482.

2-(5-Chlorobenzofuran-2-carboxamido)-3-(2-oxo-1,2-dihydroquinolin-4-yl)propanoic acid (**8s**): White solid, yield 64.8%. m.p. 186.6~188.2 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ: 13.16 (s, 1H, COOH), 11.69 (s, 1H, ArNHCO), 9.21 (s, 1H, CONH), 7.89 (s, 1H, ArH), 7.84 (d, *J*=8.2 Hz, 1H, ArH), 7.72 (d, *J*=8.8 Hz, 1H, ArH), 7.55 (s, 1H, ArH), 7.54~7.47 (m, 2H, ArH), 7.32 (d, *J*=8.3 Hz, 1H, ArH), 7.28~7.20 (m, 1H, ArCH=), 6.46 (s, 1H, CH₂C=CHCO), 4.83~4.73 (m, 1H, NHCHCOOH), 3.57~3.47 (m, 1H, CH₂CHNH), 3.33~3.25 (m, 1H, CH₂CHNH); ¹³C NMR (101 MHz, DMSO-*d*₆) δ: 172.75, 161.78, 158.32, 153.19, 150.10, 147.71, 139.40, 130.80, 129.04, 128.57, 127.50, 124.46, 122.76, 122.39, 122.11, 118.94, 116.31, 114.02, 110.17, 51.85, 33.08; ESI-MS *m/z*: 410.82 [M+H]⁺. HRMS calcd for C₂₁H₁₅ClN₂O₅ [M+H]⁺ 411.0748; found 411.0731.

Chloro-1*H*-indole-2-carbonyl)phenylalanine (**8t**): White solid, yield 60.3%. m.p. 266.1~267.5 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ: 12.87 (s, 1H, COOH), 11.75 (s, 1H, ArNHC=CH), 8.85 (s, 1H, CONH), 7.73 (s, 1H, ArH), 7.40 (d, *J*=8.7 Hz, 1H, ArH), 7.35~7.31 (m, 2H, ArH), 7.26 (d, *J*=8.4 Hz, 2H, ArH), 7.22~7.18 (m, 2H, ArH), 7.18~7.14 (m, 1H, ArCH=), 4.71~4.60 (m, 1H, NHCHCOOH), 3.27~3.18 (m, 1H, CH₂CHNH), 3.12~3.01 (m, 1H, CH₂CHNH); ¹³C NMR (101 MHz, DMSO-*d*₆) δ: 173.25, 161.78, 161.64, 147.74, 139.42, 136.96, 131.31, 130.76, 127.39, 124.49, 123.98, 122.37, 122.26, 122.08, 120.25, 118.99, 116.31, 112.76, 103.71, 51.86, 33.51; ESI-MS *m/z*: 343.14 [M+H]⁺. HRMS calcd for C₁₈H₁₅ClN₂O₃ [M+H]⁺ 343.0849; found 343.0844.

(6-Chloro-1*H*-indole-2-carbonyl)tyrosine (**8u**): White solid, yield 57.9%. m.p. 270.2~271.4 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ: 12.79 (s, 1H, COOH), 11.74 (s, 1H, ArNHC=CH), 9.19 (s, 1H, CONH), 8.76 (d, *J*=8.3 Hz, 1H, ArH), 7.73 (s, 1H, ArH), 7.44~7.40 (m, 1H, ArH), 7.22~7.16 (m, 2H, ArH), 7.13~7.09 (m, 2H, ArH, ArCH=), 6.67~6.61 (m, 2H, ArH, ArOH), 4.63~4.50 (m, 1H, NHCHCOOH), 3.12~3.05 (m, 1H, CH₂CHNH), 2.99~2.90 (m, 1H, CH₂CHNH); ¹³C NMR (101 MHz, DMSO-*d*₆) δ: 173.72, 161.16, 156.29, 135.28, 133.07, 130.46, 128.49, 124.67, 123.97, 121.14, 115.44, 114.34, 103.16, 54.71, 36.04; ESI-MS *m/z*: 358.97 [M+H]⁺. HRMS calcd for C₁₈H₁₅ClN₂O₄ [M+H]⁺ 359.0799; found 359.0786.

(5-Chloro-1*H*-indole-2-carbonyl)tryptophan (**8v**): White solid, yield 66.3%. m.p. 270.2~271.4 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ: 12.80 (s, 1H, COOH), 11.75 (s, 1H, ArNHC=CH), 10.83 (s, 1H, ArNHCH=C), 8.78 (s, 1H,

CONH), 7.72 (s, 1H, ArH), 7.62 (d, *J*=7.8 Hz, 1H, ArH), 7.41 (d, *J*=8.7 Hz, 1H, ArH), 7.35~7.30 (m, 1H, ArH), 7.22 (d, *J*=2.2 Hz, 2H, ArH), 7.18 (d, *J*=8.7 Hz, 1H, ArH), 7.10~7.03 (m, 1H, ArNHCH=C), 7.02~6.96 (m, 1H, ArCH=), 4.73~4.65 (m, 1H, NHCHCOOH), 3.34~3.29 (m, 1H, CH₂CHNH), 3.26~3.17 (m, 1H, CH₂CHNH); ¹³C NMR (101 MHz, DMSO-*d*₆) δ: 173.97, 161.20, 136.52, 135.27, 133.12, 128.49, 127.52, 124.67, 124.08, 123.97, 121.41, 121.11, 118.87, 118.59, 114.34, 111.89, 110.75, 103.19, 53.77, 27.19; ESI-MS *m/z*: 381.82 [M+H]⁺. HRMS calcd for C₂₀H₁₆ClN₃O₃ [M+H]⁺ 382.0958; found 382.0953.

(5-Chloro-1*H*-indole-2-carbonyl)histidine (**8w**): White solid, yield 61.1%. m.p. 270.2~271.4 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ: 11.86 (s, 1H, ArNHC=CH), 8.78 (d, *J*=7.8 Hz, 1H, ArH), 7.71 (d, *J*=2.1 Hz, 1H, NHCH=C), 7.58 (s, 1H, ArH), 7.43 (d, *J*=8.7 Hz, 1H, CHNHCH), 7.18 (d, *J*=8.7 Hz, 1H, ArH), 7.14 (d, *J*=2.1 Hz, 1H, NHCH=N), 6.85 (s, 1H, ArCH=), 4.66~4.56 (m, 1H, NHCHCOOH), 3.16~3.00 (m, 2H, CH₂CHNH); ¹³C NMR (101 MHz, DMSO-*d*₆) δ: 173.72, 160.93, 135.30, 135.10, 133.92, 133.39, 128.49, 124.63, 123.90, 121.05, 117.52, 114.35, 103.05, 53.40, 29.00; ESI-MS *m/z*: 332.98 [M+H]⁺. HRMS calcd for C₁₅H₁₃ClN₄O₃ [M+H]⁺ 333.0754; found 333.0742.

4.3 Cell culture

RAW264.7 cells (ATCC, Rockville, MD, USA) were maintained in RPMI 1640 medium supplemented with 100 U/mL of penicillin, 100 μg/mL of streptomycin, and 10% FBS. Cells were grown at 37 °C and 5% CO₂ in humidified air.

4.4 Assay for TNF-α and IL-6 inhibition

TNF-α and IL-6 productions were measured by Enzyme-Linked Immuno-Sorbent Assay (ELISA). In brief, RAW264.7 macrophage cells (1 × 10⁵ cells/well) were plated in 96-well plates, until the 90% cells fusion, then cells were starved by being cultured in serum free medium for another 2 h to eliminate FBS influence. The cells were then treated with compounds for 2 h before exposure to 200 ng/ml LPS for 8 h. The culture medium was used to assay the cytokine production with mouse ELISA kit (TNF-α, IL-6; BOSTER Biological Technology co.ltd, China) according to manufacturer instructions.

4.5 Assay for NO inhibition

NO production was quantified by nitrite accumulation in the culture medium using the Griess reaction. Briefly, RAW264.7 cells were pretreated with compounds for 2 h, and then stimulated with or without LPS (200 ng/ml) for 8 h. At the indicated time points, the cells were harvested and washed, and cell lysates were prepared. The lysates were mixed with Griess reagent for 30 min measured at 540 nm. The cells treated with diluent were used as negative controls for the background levels of nitrite production, while sodium nitrite at different concentrations was used as the positive controls for the establishment of a

standard curve.

Supporting Information The ^1H NMR, ^{13}C NMR and MS spectra of compounds. The Supporting Information is available free of charge *via* the Internet at <http://sioc-journal.cn>.

References

- [1] Balkwill F. *Nat. Rev. Cancer*. **1992**, 4(2), 121.
- [2] Hotamisligil, G. S.; Arner, P.; Caro, J. F. *J. Clin. Invest.* **1995**, 95(5), 2409.
- [3] Rus H. G.; Niculescu, F.; Vlaicu, R. *Atherosclerosis* **1991**, 89(2~3), 247.
- [4] Guirado, A.; López Sánchez, J. I.; Ruiz-Alcaraz, A. J. *Eur. J. Med. Chem.* **2012**, 54(50), 87.
- [5] Hauwermeiren, F. V.; Vandenbroucke, R. E.; Libert, C. *Cytokine Growth Factor. Rev.* **2011**, 22(5), 311.
- [6] Gómez-Reino, J. J.; Carmona, L.; Valverde, V. R. *Arthritis Rheum-US* **2003**, 48(8), 2122.
- [7] Wei, T.; Lu, Y.; Zhang, Y. *Science* **2011**, 332(6028), 478.
- [8] Koike, T.; Harigai, M.; Inokuma, S. *Ann. Rheum. Dis.* **2011**, 70(12), 2148.
- [9] Desai, S. B.; Furst, D. E. *Best Pract. Res., Clin. Rheumatol.* **2006**, 20(4), 757.
- [10] Bandgarab, B. P.; Korbadi, B. L.; Nile, S. H. *Eur. J. Med. Chem.* **2010**, 45(6), 2629.
- [11] La, M. I.; Jun, K. I.; Jhun KR 20140016848, **2014**.
- [12] Li, W. W.; Zhao, Y. P.; Xu, X. L. *FEBS J.* **2015**, 282(12), 2317.
- [13] Moon, S. J.; Park, J. S.; Woo, Y. J. *Arthritis Rheumatol.* **2014**, 66(4), 874.
- [14] Cho, E. H.; Choi, S. J.; Lee, S. W. EP2987491, **2016**.
- [15] Li, X. M.; Zhao, M.; Tang, Y. R. *Eur. J. Med. Chem.* **2008**, 43(1), 8.
- [16] Leite, A. C. L.; Barbosa, F. F.; Moreira, D. R. M. *Med. Chem. Res.* **2013**, 23(4), 1701.
- [17] Papadakis, K. A.; Targan, S. R. *Annu. Rev. Med.* **2000**, 51(1), 289.
- [18] Uchida, M.; Tabusa, F.; Komatsu, M.; Morita, S.; Kanbe, T.; Nakagawa, K. *Chem. Pharm. Bull.* **1986**, 34, 4821.
- [19] Bashiardes, G.; Safir, I.; Barbot, F. *Synlett* **2007**, 1707.

(Li, L.; Fan, Y.)