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Synthesis and evaluation of 2-azetidinone and 1*H*-pyrrole-2,5-dione derivatives as cholesterol absorption inhibitors for reducing inflammation response and oxidative stress

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Excess lipid accumulation can initiate the development and progression of atherosclerotic lesions, thus eventually leading to cardiovascular disease. Lipid-lowering medication therapy is one of the cornerstones of cardiovascular disease therapy. On the basis of the cholesterol absorption inhibitor ezetimibe, we successfully synthesized seven 2-azetidinone derivatives and eighteen 1*H*-pyrrole-2,5-dione derivatives. Most of the new compounds significantly inhibited cholesterol uptake *in vitro*. In addition, one of the most active inhibitors, compound **14q**, showed no cytotoxicity in L02 and HEK293T cell lines. Further evaluation indicated **14q** considerably inhibited the amount of TNF-α, ROS, MDA, and LDH *in vitro*. Therefore, **14q** might be a novel cholesterol absorption inhibitor.

Keywords: Cholesterol absorption inhibitor • 2-azetidinone • 1H-pyrrole-2,5-dione • Inflammation • Oxidative stress

Introduction

Atherosclerotic coronary artery disease (CAD) is a worldwide health issue and the most common cause of death and morbidity, particularly in developed countries.[1] Coronary atherosclerosis, a lipid-driven inflammatory disease, is the principal cause of CAD.[2] The development of atherosclerosis is closely associated with chronic inflammation and oxidative stress in the arterial plaque. The total blood cholesterol level is primarily regulated by two complementary mechanisms: *de-novo* biosynthesis in the liver and the absorption of dietary cholesterol in the small intestine.[3, 4] Over the past 30 years, 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibitors (statins) have been known to lower the risk of cardiovascular events and overall mortality proportional to suppressing cholesterol biosynthesis and thus have become the predominant lipid-lowering drugs for patients with hyperlipidemia.[5, 6] Nonetheless, severe side effects that result from high-dose statin treatment, such as hepatotoxicity and myopathy, remain a limiting factor for clinical therapy.[7] Therefore, researchers begin to focus on another complementary pathway and have developed cholesterol absorption inhibitors (CAI).

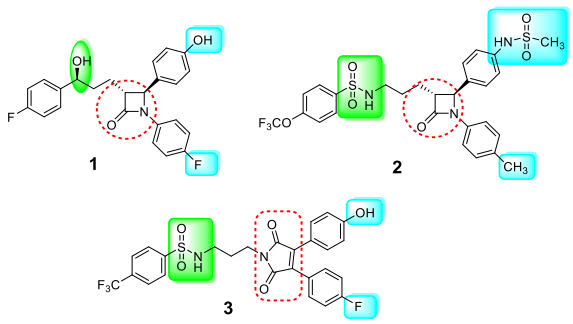


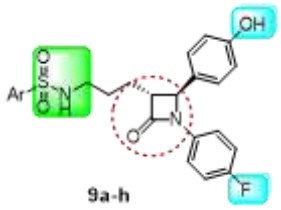
Figure 1. Structure of ezetimibe and its analogs

Ezetimibe (1, **Figure 1**) (trademark name Zetia), the only representative of azetidinone CAI, was approved in late 2002. It contains three para-substituted phenyl rings, a chiral benzylic hydroxyl, and two additional stereogenic centers at the 2-azetidinone scaffold.[8] Ezetimibe targets the Niemann-Pick C1-like 1(NPC1L1) transporter protein on the plasma membrane and decreases LDL-C concentration in plasma by blocking exogenous cholesterol absorption.[9] In addition to its lipid-lowering effect, it has also been shown to directly attenuate platelet activation and the uPAR expression on endothelial cells, thereby providing further evidence of the possible pleiotropic therapeutic relevance of ezetimibe.[10] Recent studies describe that ezetimibe alone indicated the same protective effect on the moderate atherosclerotic lesion compared to atorvastatin; the researchers believe that this anti-atherosclerotic effects of ezetimibe might result from lowering serum cholesterol, decreasing circulatory inflammatory cytokines, and inhibiting macrophage accumulation in lesions.[11, 12] The study of new CAI to improve the anti-hyperlipidemic effect and to give additional choice for the patients has already been acknowledged and recognized.

In our previous study, we reported a series of 2-azetidinone derivatives **2** and 1*H*-pyrrole-2,5-dione derivatives **3** that contain sulfonamide group as potent CAIs.[13] Fortunately, these compounds displayed certain a cholesterol absorption inhibitory activity. Based on the previous research[14], we retained sulfamide group at the C-3 side chain and recovered hydroxyl group and fluorine atom as the para substituent of the C-4 and N-1 aryl group. Then, seven 2-azetidinone derivatives **9a–g** (**Table 1**) were designed and synthesized. We also found 1*H*-pyrrole-2,5-dione derivatives retained moderate inhibitory efficacy. To further investigate the effect of the new molecular scaffold on inhibitory activity, we avoided the introduction of sulfamide group

and applied 1*H*-pyrrole-2,5-dinone to replace 2-azetidinone to obtain compounds **14a–j**. At the same time, we shortened the length of the side chain and completed the synthesis of other compounds **14k–r** (**Table 2**). Afterward, all 25 compounds were evaluated for cholesterol absorption inhibitory potency in Caco-2 cell lines and the nine preferred compounds were measured by cytotoxicity. Finally, the most desired compound was further evaluated for the positive effect on inflammation response and oxidative stress.

Table 1. Structure of 2-azetidinone derivatives **9a–h**



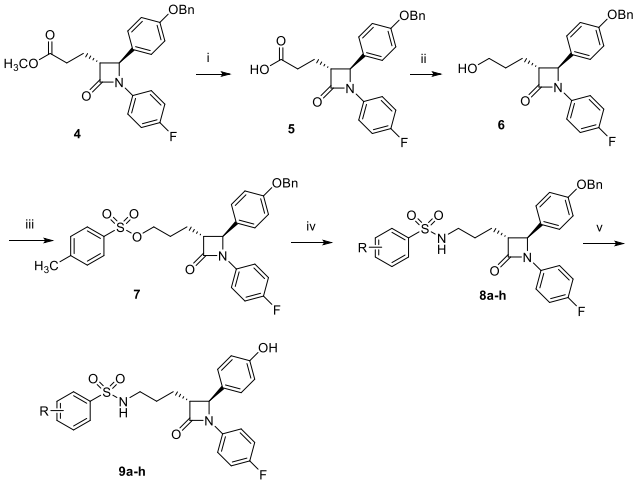
Compound	Ar
9a	Ph
9b	4-CH_3-
9c	4-t-Bu-
9d	4-F-Ph
9e	4-OCF_3-
9f	2-OCF_3-
9g	4-CF_3-Ph

Table 2. Structure of 1*H*-pyrrole-2,5-dinone derivatives **14a–r**

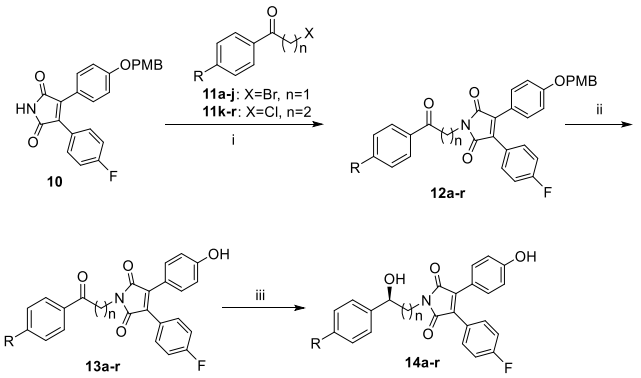
Compound	n	R	Compoun	n	R
14a	1	-H	**14k**	2	-H
14b	1	-CH_3	**14l**	2	-CH_3
14c	1	-C_2H_5	**14m**	2	-C_2H_5
14d	1	-i-Pr	**14n**	2	-i-Pr
14e	1	-t-Bu	**14o**	2	-t-Bu
14f	1	-OH	**14p**	2	-F
14g	1	-OCH_3	**14q**	2	-OH
14h	1	-F	**14r**	2	-OCH_3
14i	1	-CF_3			
14j	1	-OCF_3			

Results and Discussion

The general synthetic strategy for **9a–g** was presented in **Scheme 1**. (3R,4S)-2-azetidinone **4** was prepared as previously described.[13, 15] The intermediate **6** was obtained from the hydrolysis and reduction of **4**. Then, the alcohol 6 was converted into a better leaving group with p-toluenesulfonyl chloride. Intermediate **8a–h** was prepared by reaction of **7** with various substituted aromatic sulfonamides. Finally, deprotection reaction of **8a–g** with 10% Pd/C under H_2 resulted in **9a–g**.



Scheme 1. Synthesis of compounds **9a–g**: (i) LiOH/H_2O, CH_3OH, r.t., 1 h, 88%; (ii) BH_3·Me_2S, THF, -10 °C, 8 h, 61%; (iii) 4-toluene sulfonyl chloride, Et_3N, CH_2Cl_2, r.t., 6 h, 91%; (iv) substituted aromatic sulfonamide, K_2CO_3, CH_3CN, reflux, 12 h, 51–74%; (v) 10% Pd/C, H_2, CH_3OH, r.t., 60–84%.



Scheme 2. Synthesis of compounds **14a–r**: (i) CH_3CN, K_2CO_3, TBAB, reflux, overnight, 51–95%; (ii) TFA, CH_2Cl_2, 0 °C, 1h, 72–90%; (iii) BH_3·Me_2S, CBS, CH_2Cl_2/ THF= 10/ 1, 6h, 42–77%.

As illustrated in **Scheme 2**, our synthetic approach began with 1*H*-pyrrole-2,5-dinone **10,** which was prepared as described previously.[13] Moreover, **11a–j** was obtained from reaction of various *p*-substituted acetophenone with liquid bromine[16] and **11k–r** was obtained from Friedel–Crafts acylation reaction of various *p*-substituted benzene with 3-chloropropionyl chloride. Treatment of **10** was conducted with **11a–r** under TBAB and K_2CO_3-prepared **12a–r**. Subsequently, deprotection reaction of **12a–r** in acid condition resultled in **13a–r**. N-Cyclohexyl-2-benzothiazolesulfenamide (CBS)-mediated reduction of **13a–r** with borane-dimethylsulfide complex delivered the title compounds **14a–r** with required stereochemistry.

In the present study, we retained adopting the classic Caco-2 cell, which is a useful model for the study of intestinal lipid and lipid–protein metabolism, to preliminarily evaluate the inhibitory potency of compound **9a–g** and **14a–r** at 100 μM concentration.[17, 18] A total-cholesterol assay kit based on COD-PAP method was used to measure the content of cholesterol *in vitro*. As shown in **Figure 2**, most target compounds retained inhibitory activity, and in particular, **9a, 9b, 14e, 14i, 14k, 14l, 14n, 14o,** and **14q** are comparable to ezetimibe. The cholesterol absorption inhibition rates of **9a, 14l** and **14q** respectively reached 78.8%, 82.4%, and 78.6% higher than ezetimibie ($p < 0.05$). The inhibitory potency of **9a–g** suggested the introduction of electron-withdrawing groups into *p*-position of phenyl ring at the C-3 side chain was against inhibitory efficacy. Furthermore, the inhibitory potency exhibited by **14a–r** demonstrated that 1*H*-pyrrole-2,5-dione scaffold enhanced inhibitory activity and deserved further investigation. Furthermore, the overall inhibitory potency of **14k–r** stronger than **14a–j** indicated that when the length of the side chain was three carbons, the efficacy is improved. Notably, when substituents at the *p*-position of phenyl ring were methyl and hydroxyl, compounds showed stronger potency. (e.g., **9b, 14l,** and **14q**)

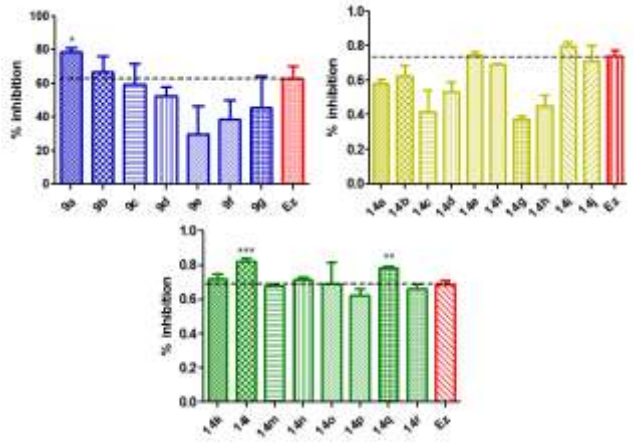


Figure 2, Inhibitory effects of compounds **9a-g** and **14a-r** on cholesterol absorption in the Caco-2 cell. Cholesterol absorption levels are normalized to protein levels. These values represented mean ± SD (n=4). The applied statistical method is t-tests . (*$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus the **Ez** group)

The *in vitro* results indicate that compounds **9a**, **9b**, **14e**, **14i**, **14k**, **14l**, **14n**, **14o,** and **14q** were screened for further evaluation of cytotoxicity. Here, cytotoxicity of compounds was determined by MTT cell proliferation assay in normal human cell lines, L-02 (human normal liver cells) and HEK293T (human embryonic kidney cells). In general, LC_{50} values higher than 100 μM were considered non-toxic.[19] As shown in **Figure 3**, LC_{50} values of ezetimibe were 87.7 μM against HEK293T and 85.6 μM against L02. Fortunately, the LC_{50} values of compound **14q** were higher than 200 μM in both the cell lines, and **14q** displayed far lower cytotoxicity than ezetimibe. In summary, **14q** was selected as the best candidate because of its outstanding potency and lowest cytotoxicity.

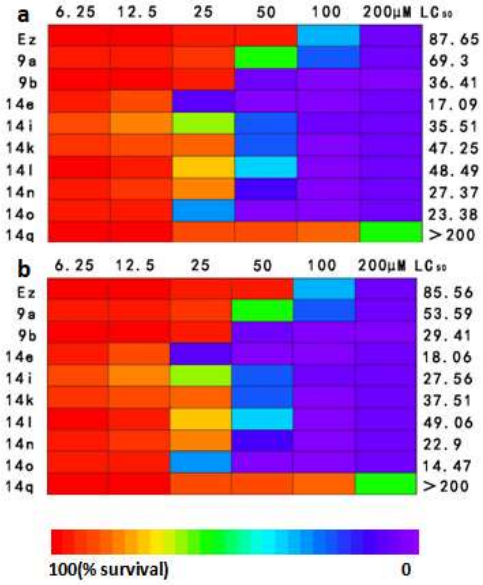


Figure 3, Cytotoxicity assay of compounds **9a–g** and **14a–r** against L02 (a) and HEK293T (b). LC_{50} represented the concentration required to induce cell death by 50%.

During the formation and progression of atherosclerotic plaques, inflammation response, oxidative stress, and endothelial damage appear to play vital roles.[12] Compelling evidence indicates that ezetimibe might attenuate atherosclerosis mediated by lowering cholesterol concentrations, protecting endothelial function, and decreasing inflammatory cytokines.[11] Then, we determined the protein level of inflammatory cytokine TNF-α in Caco-2 cell lines for evaluating the anti-inflammatory activity of compound **14q**. Moreover, studies have shown that oxidative stress, particularly redundant reactive oxygen species (ROS) generation, plays a causal role in atherosclerosis.[21, 22] Excessive production of ROS can oxidize low-density lipoprotein (LDL) and directly damage cell membrane, protein, and DNA, thereby ultimately leading to

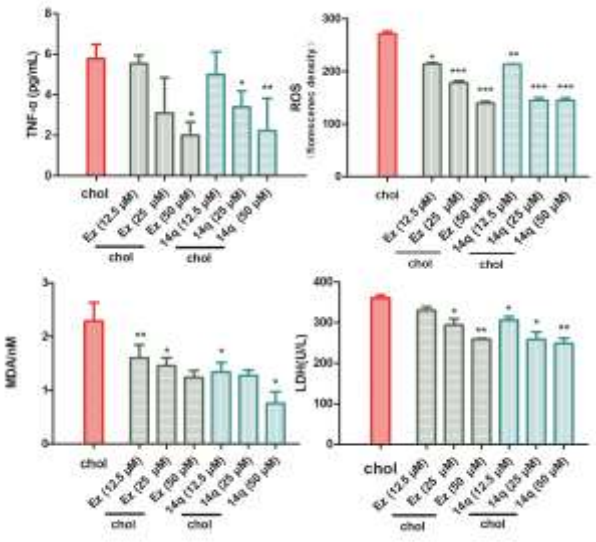


Figure 4. TNF-α, ROS, MDA and LDH content of Caco-2 treated with a cholesterol micellar solution. These values represented mean ± SD. (*$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus the **chol** group)

the liberation of MDA (malonic dialdehyde) and LDH (lactate dehydrogenase).[22,23] To assess further the effect of compound **14q**, we evaluated the amount of ROS, MDA, and LDH in Caco-2 cell line treated with a cholesterol micellar solution. As shown in **Figure 4**, compound **14q** observably reduce TNF-α and ROS by 61.3% and 46.7% at the concentration of 50 μM, which was comparable to ezetimibe. At the same concentration, compound **14q** decreased MDA and LDH by 67.1% and 30.1% and ezetimibe caused the reduction by 45.9% and 29.4%, respectively. Thus, we believe that **14q** could attenuate inflammatory response and oxidative stress in Caco-2 cell line, thereby suggesting that it might revert and cure atherosclerotic lesions.

Conclusions

In summary, seven 2-azetidinone derivatives and eighteen 1*H*-pyrrole-2,5-dione derivatives were synthesized successfully based on the previous study. Here, we retained sulfamide group at the C-3 side chain and recovered *p*-substituents at the C-4 and N-1 phenyl ring, similar to ezetimibe. Furthermore, we avoided introducing sulfamide group and replaced β-lactam scaffold with 1*H*-pyrrole-2,5-dione to improve anti-hyperlipidmic activity. All novel compounds exhibited significant inhibition of cholesterol uptake in the Caco-2 cell line. Then, the top nine compounds were evaluated for cytotoxicity assay after preliminary activity screening. Compound **14q** was the best candidate for further evaluation *in vitro*. Our in-depth study revealed that compound **14q** could also reduce the amount of TNF-α, ROS, MDA, and LDH in Caco-2 cell line. These results suggested that replacement of 1*H*-pyrrole-2,5-dione as anti-hyperlipidmic moiety contributes to inhibitory potency. Further detailed evaluations for **14q** *in vivo* are underway in our laboratory.

Experimental Section

Chemistry

All commercial reagents and solvents were used without further purification unless expressly stated. Thin-layer chromatography (TLC) was performed on silica gel GF 254 (Tsingtao Haiyang Chemicals, China) and observed under an ultraviolet lamp at 254 nm. Column chromatography was conducted on silica gel (200–300 mesh, Tsingtao Haiyang Chemicals, China). Melting points of the synthesized compounds were determined with capillary apparatus and were not corrected. ^{1}H NMR spectra and ^{13}C NMR of the compounds were measured on Bruker ACF-500Q apparatus at 500 MHz or Bruker AV-300 apparatus at 300 MHz using TMS as the internal standard in $CDCl_3$ or (D_6)DMSO unless otherwise indicated. The IR spectra were obtained with Shimadzu FTIR-8400S spectrophotometer (KBr pellets technique). Mass spectrometry (MS) was performed with Hewlett-Packard 1100 LC/MSD spectrometer. High-resolution-time-of-flight (HR-TOF)-MS were recorded on a BioTOFTM-Q mass spectrometer (Bruker).

General procedure for preparation of **9a–h**

To a solution of (3R,4S)-2-azetidinone **4** (7.5 mmol, 1 eq) in THF (30 mL), 1 M aqueous solution of LiOH (40 mL) was added. The mixture was stirred at room temperature. After 1 h, 1 M HCl was added to the reaction until the pH was adjusted to 5. Then, the mixture was transferred to a separatory funnel, extracted with ethyl acetate, dried over anhydrous Na_2SO_4, and concentrated to afford acid **5** of sufficient purity. 2 M THF solution of $BH_3{\cdot}Me_2S$ (7.32 mL, 2.4 eq) was added to compound **5** (6.1 mmol, 1 eq) in 30 mL anhydrous THF at -10 °C. The reaction was stirred for 8 h at the same temperature and quenched by 1 M HCl (10 mL). **6** was obtained by a silica gel column chromatography using ethyl acetate/n-hexane (1:3). To a solution of **6** (15.8 mmol, 1 eq), TsCl (3.6 g, 18.89 mmol, 1.2 eq) and a catalytic amount of DMAP in CH_2Cl_2 (100 mL), Et_3N (3.19 g, 31.52 mmol, 2 eq) was added in an ice bath. The reaction proceeded overnight and was washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by a silica gel column chromatography using ethyl acetate/n-hexane (1:6) to give **7**. For the synthesis of **8a–h**, K_2CO_3 (0.19 g, 1.38 mmol, 2 eq) and substituted aromatic sulfonamide (1.38 mmol, 2 eq) were added to the solution of **7** (0.69 mmol, 1 eq) in CH_3CN (5 mL). The mixture was stirred at 90 °C for 12 h until complete by TLC. The solvent was concentrated, water (12 mL) added, and the resulting mixture was extracted with CH_2Cl_2. The organic layers were washed with 3 M aqueous solution of NaOH (3 × 4 mL) and water (3 × 4.8 mL), dried over anhydrous Na_2SO_4 and concentrated. The compounds **8a–h** were obtained by silica gel column chromatography using ethyl acetate/n-hexane (1:6). Finally, **8a–h** were dissolved in CH_3OH (15 mL), and the solution was hydrogenated using 10% Pd/C as a catalyst at room temperature under a hydrogen balloon. The reaction was stopped after cessation of H_2 uptake. The catalyst was filtered through a bed of Celite, washed with CH_3OH, and concentrated. The residue was purified by silica gel column chromatography using ethyl acetate/n-hexane (1:3) to afford target compounds **9a–h**.

Specific characterization data of **6, 7, 8e, 9a-h**

(3R,4S)-4-(4-(benzyloxy)phenyl)-1-(4-fluorophenyl)-3-(3-hydroxypropyl)azetidin-2-one (**6**). Yield: 61%. ^{1}H-NMR (500Hz ,$CDCl_3$): 1.72-1.81 (m, 2H, CH_2(6), 1.95-2.04 (m, 2H, CH_2(5)), 3.10-3.13 (m, 1H, H-C(3)), 3.68 (t, 2H, J = 6.0, CH_2(7)), 4.59 (d, 1H, J = 2.0, H-C(4)), 5.05 (s, 2H, -ArCH_2O-), 6.92 (t, 2H, J = 8.5, Ar-H), 6.96 (d, 2H, J = 8.5, Ar-H), 7.23-7.27 (m, 4H, Ar-H), 7.32 (t, 1H, J = 7.0, Ar-H), 7.37-7.42 (m, 4H, Ar-H).

3-((2S,3R)-2-(4-(benzyloxy)phenyl)-1-(4-fluorophenyl)-4-oxoazetidin-3-yl)propyl 4-methylbenzenesulfonate (**7**). Yield: 82%. ^{1}H-NMR (300Hz ,$CDCl_3$): 1.82-1.92 (m, 4H, CH_2(6), CH2(5)), 2.43 (s, 3H, -ArCH_3), 2.99 (dd, 1H, J_1 = 4.8, J_2 = 6.6, H-C(3)), 4.06 (d, 2H, J = 2.1, CH_2(7)), 4.54 (d, 1H, J = 2.1, H-C(4)), 5.05 (s, 2H, -ArCH_2O-), 6.95 (q, 4H, J = 9.0 , Ar-H), 7.22 (dd, 4H, J_1 = 5.7, J_2 = 8.1, Ar-H), 7.30-7.36 (m, 3H, Ar-H), 7.38-7.45 (m, 4H, Ar-H), 7.76 (d, 2H, J = 8.4, Ar-H).

***N*-(3-((2S,3R)-2-(4-(benzyloxy)phenyl)-1-(4-fluorophenyl)-4-oxoazetidin-3-yl)propyl)benzenesulfonamide** (**8e**). Yield: 58%. ^{1}H-NMR (300Hz ,$CDCl_3$): 1.74 (t, 2H, *J* = 6.3, CH_2(6)), 1.90 (t, 2H, *J* = 7.5, CH_2(5)), 2.99-3.05 (m, 1H, H-C(3)), 2.99-3.05 (m, 2H, CH_2(7)), 4.53 (d, 1H, *J* = 2.1, H-C(4)), 4.87 (s, 1H, H-N), 5.05 (s, 2H, -ArCH_2O-), 6.93 (q, 4H, *J* = 8.7, Ar-H), 7.21 (q, 6H, *J* = 8.4, Ar-H), 7.33-7.41 (m, 5H, Ar-H), 7.87 (dd, 2H, J_1 = 4.8, J_2 = 8.7, Ar-H).

***N*-(3-((2S,3R)-1-(4-fluorophenyl)-2-(4-hydroxyphenyl)-4-oxoazetidin-3-yl)propyl)benzenesulfonamide** (**9a**). White solid. Yield: 72%. ^{1}H-NMR (500Hz, $CDCl_3$) : 1.66-1.70 (d, 2H, *J* = 6.95, CH_2(6)), 1.83-1.92 (m, 2H, CH_2(5)), 2.96-3.00 (m, 1H, H-C(3)), 2.96-3.00 (m, 2H, CH_2(7)), 4.52 (d, 1H, *J* = 2.05, H-C(4)), 5.11 (t, 1H, *J* = 6.1Hz,H-N), 6.00 (s, 1H, -ArOH), 6.83 (d, 2H, *J* = 8.5, Ar-H), 6.91 (t, 2H, *J* = 8.65, Ar-H), 7.16 (d, 2H, *J* = 8.5, Ar-H), 7.20-7.27 (m, 2H, Ar-H), 7.49 (t, 2H, *J* = 7.5, Ar-H), 7.56 (t, 1H, *J* = 7.4, Ar-H), 7.83 (t, 2H, *J* = 7.3, Ar-H). IR (KBr) cm^{-1}: 3273, 2931, 2865, 1725, 1510, 1447, 1393, 1325, 1155, 1094. HRMS: calcd. m/z $[M+H]^+$ 455.1441, found m/z $[M+H]^+$ 455.1451.

***N*-(3-((2S,3R)-1-(4-fluorophenyl)-2-(4-hydroxyphenyl)-4-oxoazetidin-3-yl)propyl)-4-methylbenzenesulfonamide** (**9b**). White solid. Yield: 78%. ^{1}H-NMR (500Hz , $CDCl_3$) : 1.65-1.71 (m, 2H, CH_2(6)), 1.87-1.92 (m, 2H, CH_2(5)), 2.41 (d, 3H, *J* = 14.3, -ArCH_3), 2.97-3.00 (m, 1H, H-C(3)), 2.97-3.00 (m, 2H, CH_2(7)), 4.52 (d, 1H, *J* = 1.85Hz, H-C(4)), 4.90 (s, 1H, H-N), 6.83 (d, 2H, *J* = 8.4, Ar-H), 6.92 (t, 2H, *J* = 8.6, Ar-H), 7.18 (d, 2H, *J* = 8.45, Ar-H), 7.20-7.27 (m, 2H, Ar-H), 7.28 (d, 2H, *J* = 8.0, Ar-H), 7.71 (d, 2H, *J* = 8.15, Ar-H). IR (KBr) cm^{-1}: 3273, 2925, 2859, 1726, 1510, 1452, 1393, 1324, 1154, 1093.HRMS: calcd. m/z $[M-H]^-$ 467.1077, found m/z $[M-H]^-$ 467.1089.

4-ethyl-*N*-(3-((2S,3R)-1-(4-fluorophenyl)-2-(4-hydroxyphenyl)-4-oxoazetidin-3-yl)propyl)benzenesulfonamide (**9c**). White solid. Yield: 69%. ^{1}H-NMR (500Hz , $CDCl_3$) : 1.20-1.26 (m, 3H, -CH_2CH_3), 1.66 (q, 2H, *J* = 7.0, CH_2(6)), 1.83-1.90 (m, 2H, CH_2(5)), 2.67 (q, 2H, *J* = 7.6, -Ar-CH_2-), 2.93-2.94 (d, 1H, *J* = 7.05, H-C(3)), 2.93-2.94 (m, 2H, CH_2(7)), 4.52 (d, 1H, *J* = 1.75, H-C(4)), 5.22 (t, 1H, *J* = 6.05, H-N), 6.82 (d, 2H, *J* = 8.4, Ar-H), 6.89 (t, 2H, *J* = 8.5, Ar-H), 7.14 (d, 2H, *J* = 8.45, Ar-H), 7.19-7.21 (m, 2H, Ar-H), 7.28 (d, 2H, *J* = 8.15, Ar-H), 7.72 (d, 2H, *J* = 8.2, Ar-H). IR (KBr) cm^{-1}: 3275, 2931, 2872, 1725, 1510, 1449, 1394, 1323, 1154, 1094. HRMS: calcd. m/z $[M+Na]^+$ 505.1568, found m/z $[M+Na]^+$ 505.1576.

4-(tert-butyl)-*N*-(3-((2S,3R)-1-(4-fluorophenyl)-2-(4-hydroxyphenyl)-4-oxoazetidin-3-yl)propyl)benzenesulfonamide (**9d**). White solid. Yield: 84%. ^{1}H-NMR (300Hz , $CDCl_3$) : 1.33 (s, 9H, -$C(CH_3)_3$), 1.71 (t, 2H, *J* = 7.35, CH_2(6)), 1.91 (t, 2H, *J* = 7.95, CH_2(5)), 2.99-3.04 (m, 1H, *J* = 7.35, H-C(3)), 2.99-3.04 (m, 2H, CH_2(7)), 4.54 (d, 1H, *J* = 2.1 , H-C(4)), 4.62 (d, 1H, *J* = 5.7, H-N), 6.81 (d, 2H, *J* = 8.7, Ar-H), 6.82-6.96 (m, 4H, *J* = 8.7, Ar-H), 7.18-7.26 (m, 4H, Ar-H), 7.49 (d, 2H, *J* = 8.7, Ar-H), 7.74 (d, 2H, *J* = 8.7, Ar-H).IR (KBr) cm^{-1}: 3276, 2927, 2868, 1725, 1510, 1450, 1396, 1324, 1157, 1088. HRMS: calcd. m/z $[M+Na]^+$ 511.2061, found m/z $[M+Na]^+$ 511.2069.

4-fluoro-*N*-(3-((2S,3R)-1-(4-fluorophenyl)-2-(4-hydroxyphenyl)-4-oxoazetidin-3-yl)propyl)benzenesulfonamide (**9e**). White solid. Yield: 70%. ^{1}H-NMR (500Hz , $CDCl_3$) : 1.69 (d, 2H, *J* = 5.6 Hz, CH_2(6)), 1.88 (t, 2H, *J* = 7.5 Hz, CH_2(5)), 2.97-3.00 (m, 1H, H-C(3)), 2.97-3.00 (m, 2H, CH_2(7)), 4.52 (d, 1H, *J* = 1.85 Hz, H-C(4)), 5.16 (m, 1H, H-N), 6.83 (d, 2H, *J* = 8.45, Ar-H), 6.91 (t, 2H, *J* = 8.65, Ar-H), 7.15-7.20 (m, 4H, Ar-H), 7.21-7.26 (m, 2H, Ar-H), 7.84 (dd, 2H, J_1 = 5, J_2 = 8.65, Ar-H). IR (KBr) cm^{-1}: 3275, 2917, 2849, 1725, 1510, 1449, 1394, 1328, 1152, 1092. HRMS: calcd. m/z $[M+Na]^+$ 473.1344, found m/z $[M+Na]^+$ 473.1341.

***N*-(3-((2S,3R)-1-(4-fluorophenyl)-2-(4-hydroxyphenyl)-4-oxoazetidin-3-yl)propyl)-4-(trifluoromethoxy)benzenesulfonamide** (**9f**). White solid. Yield:69%. ^{1}H-NMR (500Hz , $CDCl_3$) : 1.69-1.78 (m, 2H, CH_2(6)), 1.91 (q, 2H, *J* = 7.5, CH_2(5)), 3.00-3.04 (m, 1H, H-C(3)), 3.00-3.04 (m, 2H, CH_2(7)), 4.53 (d, 1H, *J* = 1.5,H-C(4)), 5.10 (t, 1H, *J* = 6.0, H-N), 6.85 (d, 2H, *J* = 8.5, Ar-H), 6.92 (t, 2H, *J* = 8.5, Ar-H), 7.19 (d, 2H, *J* = 8.5, Ar-H), 7.22 (q, 2H, *J* = 4.5, Ar-H), 7.31 (d, 2H, *J* = 8.0, Ar-H), 7.89 (d, 2H, *J* = 8.5, Ar-H). IR (KBr) cm^{-1}: 3279, 2927, 2868, 1727, 1511, 1453, 1395, 1334, 1156, 1100. HRMS: calcd. m/z $[M+H]^+$ 537.1107, found m/z $[M+H]^+$ 537.1119.

***N*-(3-((2S,3R)-1-(4-fluorophenyl)-2-(4-hydroxyphenyl)-4-oxoazetidin-3-yl)propyl)-2-(trifluoromethoxy)benzenesulfonamide** (**9g**). White solid. Yield: 60%. ^{1}H-NMR (500Hz, $CDCl_3$) : 1.72 (q, 2H, *J* = 7.0, CH_2(6)), 1.90 (q, 2H, *J* = 7.0, CH_2(5)), 2.99 (d, 1H, H-C(3)), 3.01 (d, 2H, CH_2(7)), 4.54 (d, 1H, *J* = 2.0, H-C(4)), 4.69 (s, 1H, H-N), 6.82 (d, 2H, *J* = 8.5, Ar-H), 6.92 (t, 2H, *J* = 8.5, Ar-H), 7.18-7.26 (m, 4H, Ar-H), 7.49 (d, 2H, *J* = 8.5, Ar-H), 7.74 (d, 2H, *J* = 8.5, Ar-H). IR (KBr) cm^{-1}: 3292, 2924, 2855, 1729, 1511, 1476, 1451, 1395, 1339, 1158, 1101. HRMS: calcd. m/z $[M+H]^+$ 539.1258, found m/z $[M+H]^+$ 539.1268.

***N*-(3-((2S,3R)-1-(4-fluorophenyl)-2-(4-hydroxyphenyl)-4-oxoazetidin-3-yl)propyl)-4-(trifluoromethyl)benzenesulfonamide** (**9h**). White solid. Yield: 75%. ^{1}H-NMR (500Hz ,$CDCl_3$) : 1.69-1.79 (m, 2H, CH_2(6)), 1.93 (q, 2H, *J* = 7.4, CH_2(5)), 3.01-3.05 (m, 1H, H-C(3)), 3.01-3.05 (m, 2H, CH2(7)), 4.53 (d, 1H, *J* = 1.8, H-C(4)), 5.20 (s, 1H, H-N), 6.83 (d, 2H, *J* = 8.4 Hz, Ar-H), 6.93 (t, 2H, *J* = 8.55, Ar-H), 7.18-7.26 (m, 4H, Ar-H), 7.77 (d, 2H, *J* = 8.2, Ar-H), 7.97 (d, 2H, *J* = 8.2, Ar-H). IR (KBr) cm^{-1}: 3280, 2918, 2851, 1725, 1511, 1451, 1404, 1324, 1166, 1106. HRMS: calcd. m/z $[M+H]^+$523.1309, found m/z $[M+H]^+$ 523.1307.

General procedure for preparation of **14a–r**

For example, a mixture of 1H-pyrrole-2,5-dinone **10** (0.99 mmol, 1 eq) and K_2CO_3 (0.2 g, 1.49 mmol, 1.5 eq), TBAB (0.032 g, 0.099 mmol, 0.1 eq), **11a–r** (0.99 mmol, 1 eq) in CH_3CN (10 mL) was stirred and heated to reflux overnight. The solvent was removed, and the residue was diluted with ethyl acetate (30 mL) and washed with water (30 mL × 3). The combined organic layer was dried over anhydrous Na_2SO_4 and evaporated. Intermediate **12a–r** were obtained by a silica gel column chromatography using ethyl acetate/n-hexane (1:6). Trifluoroacetic acid (0.82 g, 7.2 mmol, 10 eq) was added to the solution of **12a–r** (0.72 mmol, 1 eq) in CH_2Cl_2 at 0 °C. After 1 h, 1 M aqueous solution of NaOH was added to the reaction until pH was adjusted to 8–10. The mixture diluted with CH_2Cl_2, washed with water, and dried over anhydrous $NaSO_4$, and concentrated in vacuum. The crude was purified by silica gel column chromatography using ethyl acetate/n-hexane (1:4) to give **13a–r**. To the mixture of dichloromethane (8 mL) and THF (0.8 mL) at 0 °C were added borane dimethylsulfide (1.6 mL, 3.18 mmol, 4 eq) and CBS

(0.08 mL, 0.08 mmol, 0.1 eq). Compounds **13a–r** (0.8 mmol, 1 eq) were added to this reaction mixture; it was stirred at same temperature for 6 h. After completion of the reaction, the reaction mixture was quenched with CH_3OH and the solvent was evaporated. The residue was diluted with ethyl acetate (30 mL), successively washed with hydrochloric acid and brine solution, and dried over anhydrous Na_2SO_4, and concentrated to give the crude product. Then, **14a–r** were purified by a silica gel column chromatography using ethyl acetate/n-hexane (1:4).

Specific characterization data of **12h, 13h, 14a-r**

3-(4-fluorophenyl)-1-(2-(4-fluorophenyl)-2-oxoethyl)-4-(4-((4-methoxybenzyl)oxy)phenyl)-1H-pyrrole-2,5-dione (**12h**). Yellow solid. Yield: 75%. ^{1}H NMR (300 Hz , (D_6)DMSO): 3.76 (s, 3H, -OCH_3), 5.05 (s, 2H, -OCH_3-Ph-), 5.21 (s, 2H, -$COCH_2$N-), 6.96 (d, 2H, *J* = 8.7, Ar-H), 7.07 (d, 2H, *J* = 9.0, Ar-H), 7.26-7.32 (m, 2H, Ar-H), 7.37-7.53 (m, 8H, Ar-H), 8.19 (q, 2H, *J* = 4.7, Ar-H).

3-(4-fluorophenyl)-1-(2-(4-fluorophenyl)-2-oxoethyl)-4-(4-hydroxyphenyl)-1H-pyrrole-2,5-dione (**13h**). Yellow solid. Yield: 96%. ^{1}H NMR (300 Hz , (D_6)DMSO): 5.19 (s, 2H, -$COCH_2$N-), 6.81 (d, 2H, *J* = 2.4, Ar-H), 7.26-7.35 (m, 4H, Ar-H), 7.40-7.52 (m, 4H, Ar-H), 8.19 (q, 2H, *J* = 4.8, Ar-H), 10.05 (s, 1H, Ar-OH).

(R)-3-(4-fluorophenyl)-1-(2-hydroxy-2-phenylethyl)-4-(4-hydroxyphenyl)-1H-pyrrole-2,5-dione (**14a**). Yellow solid. Yield: 66%. M.p. = 198-200 °C. ^{1}H NMR (300 Hz, (D_6)DMSO): 3.53-3.74 (m, 2H, -NCH_2-), 4.86-4.91 (m, 1H, H-C), 5.61 (d, 1H, *J* = 4.5, H-O), 6.79 (d, 2H, *J* = 8.4, Ar-H), 7.25-7.33 (m, 5H, Ar-H), 7.35-7.41 (m, 4H, Ar-H), 7.43-7.45 (m, 2H, Ar-H), 9.99 (s, 1H, Ar-OH). ^{13}C NMR (300 MHz, (D_6)DMSO): 170.35, 170.26, 164.05, 160.76, 159.09, 142.45, 135.79, 132.16, 131.80, 131.69, 131.30, 128.13, 127.34, 125.90, 125.60, 125.55, 118.88, 115.77, 115.51, 115.49, 69.49, 45.70. HRMS: calcd. m/z [M]403.1219, found m/z [M+Na]$^+$426.1112.

(R)-3-(4-fluorophenyl)-1-(2-hydroxy-2-(p-tolyl)ethyl)-4-(4-hydroxyphenyl)-1H-pyrrole-2,5-dione (**14b**). Yellow solid. Yield: 77%. M.p. = 190-191 °C. ^{1}H NMR (300 Hz , (D_6)DMSO): 2.89 (s, 3H, -$ArCH_3$), 3.51-3.71 (m, 2H, -NCH_2-), 4.83-4.86 (m, 1H, H-C), 5.51 (d, 1H, *J* = 2.7, H-O), 6.87 (d, 2H, *J* = 2.4 Hz, Ar-H), 7.16 (d, 2H, *J* = 5.4 Hz, Ar-H), 7.24-7.29 (m, 6H, Ar-H), 7.41-7.44 (m, 2H, Ar-H), 9.96 (s, 1H, Ar-OH). ^{13}C NMR (300 MHz, (D_6)DMSO): 170.36, 170.28, 164.03, 160.75, 159.07, 139.45, 136.37, 135.78, 132.15, 131.79, 131.68, 131.30, 128.67, 125.82, 125.60, 125.55, 118.88, 115.76, 115.50, 69.28, 45.74, 20.64. HRMS: calcd. m/z [M] 417.1381, found m/z [M+Na]$^+$ 440.1273.

(R)-1-(2-(4-ethylphenyl)-2-hydroxyethyl)-3-(4-fluorophenyl)-4-(4-hydroxyphenyl)-1H-pyrrole-2,5-dione (**14c**). Yellow solid. Yield: 65%. M.p. = 137-139 °C. ^{1}H NMR (300 Hz , (D_6)DMSO): 1.16 (t, 3H, *J* = 4.5, -CH2CH_3), 2.58-2.63 (m, 2H, -$ArCH_2$-), 3.52-3.71 (m, 2H, -NCH_2-), 4.83-4.87 (m, 1H, H-C), 5.51 (d, 1H, *J* = 2.7, H-O), 6.78(d, 2H, *J* = 5.1, Ar-H), 7.19(d, 2H, *J* = 1.8, Ar-H), 7.26 (t, 6H, *J* = 4.7, Ar-H), 7.42 (t, 2H, *J* = 4.2, Ar-H), 9.96 (s, 1H, Ar-OH). ^{13}C NMR (300 MHz, (D_6)DMSO): 170.35, 170.27, 164.03, 160.75, 159.07, 142.82, 139.70, 135.78, 132.15, 131.79, 131.67, 131.29, 129.56, 127.49, 125.91, 125.59, 125.55, 118.88, 115.75, 115.49, 113.48, 69.32, 45.71, 27.80, 15.58. HRMS: calcd. m/z [M]431.1536, found m/z [M+Na]$^+$454.1429.

(R)-3-(4-fluorophenyl)-1-(2-hydroxy-2-(4-isopropylphenyl)ethyl)-4-(4-hydroxyphenyl)-1H-pyrrole-2,5-dione (**14d**). Yellow solid. Yield: 72%. M.p. = 161-163 °C. ^{1}H NMR (300 Hz ,(D_6)DMSO): 1.19 (d, 6H, *J* = 4.2 Hz, -CH-(CH_3)$_2$), 2.85-2.90 (m, 1H, -CH-(CH_3)$_2$), 3.52-3.72 (m, 2H, -NCH_2-), 4.83-4.87 (m, 1H, H-C), 5.51 (d, 1H, *J* = 2.7, H-O), 6.87 (d, 2H, *J* = 5.1, Ar-H), 7.21-7.29 (m, 8H, Ar-H), 7.41-7.44 (m, 2H, Ar-H), 9.96 (s, 1H, Ar-OH). ^{13}C NMR (300 MHz, (D_6)DMSO): 170.35, 170.28, 164.03, 160.75, 159.07, 147.50, 139.84, 135.79, 132.16, 131.78, 131.67, 131.52, 131.29, 129.56, 126.01, 125.92, 126.01, 125.92, 125.60, 125.55, 118.88, 115.74, 115.48, 113.49, 69.33, 45.68, 33.08, 23.85, 23.81. HRMS: calcd. m/z [M] 445.1691, found m/z [M+Na]$^+$468.1584.

(R)-1-(2-(4-(tert-butyl)phenyl)-2-hydroxyethyl)-3-(4-fluorophenyl)-4-(4-hydroxyphenyl)-1H-pyrrole-2,5-dione (**14e**). Yellow solid. Yield: 74%. M.p. = 204-205 °C. ^{1}H NMR (300 Hz , (D_6)DMSO): 1.27 (s, 9H, -$C(CH_3)_3$), 3.53-3.73 (m, 2H, -NCH_2-), 4.85 (t, 1H, *J* = 2.7, H-C), 5.50 (d, 1H, *J* = 2.7, H-O), 6.78 (d, 2H, *J* = 5.1, Ar-H), 7.25-7.30 (m, 6H, Ar-H), 7.38 (d, 2H, *J* = 4.8, Ar-H), 7.41-7.44 (m, 2H, Ar-H), 9.96 (s, 1H, -OH). ^{13}C NMR (300 MHz, (D_6)DMSO): 170.36, 170.28, 164.03, 160.75, 159.07, 149.74, 139.42, 135.80, 132.17, 131.78, 131.67, 131.29, 129.56, 125.67, 125.60, 125.56, 124.84, 118.88, 115.74, 115.48, 113.48, 69.27, 45.67, 34.13, 31.09. HRMS: calcd. m/z [M] 459.1853, found m/z [M+Na]$^+$482.1746.

(R)-3-(4-fluorophenyl)-1-(2-hydroxy-2-(4-hydroxyphenyl)ethyl)-4-(4-hydroxyphenyl)-1H-pyrrole-2,5-dione (**14f**). Yellow solid. Yield: 62%. M.p. = 120-123 °C. ^{1}H NMR (300 Hz ,(D_6)DMSO): 3.49-3.69 (m, 2H, -NCH_2-), 4.76-4.79 (m, 1H, H-C), 5.40 (d, 1H, *J* = 2.7, H-O), 6.72-6.78 (m, 4H, Ar-H), 7.15 (d, 2H, *J* = 5.1, Ar-H), 7.23-7.28 (m, 4H, Ar-H), 7.41-7.44 (m, 2H, Ar-H), 9.26 (s, 1H, Ar-OH), 9.97 (s, 1H, Ar-OH). ^{13}C NMR (300 MHz, (D_6)DMSO):170.38, 170.30, 164.04, 160.76, 159.10, 156..63, 135.79, 132.70, 132.14, 131.80, 131.69, 131.29, 127.07, 125.63, 125.58, 118.88, 115.77, 115.52, 115.48, 114.88, 69.15, 45.75. HRMS: calcd. m/z [M] 419.1167, found m/z [M+Na]$^+$442.1059.

(R)-3-(4-fluorophenyl)-1-(2-hydroxy-2-(4-methoxyphenyl)ethyl)-4-(4-hydroxyphenyl)-1H-pyrrole-2,5-dione (**14g**). Yellow solid. Yield: 68%. M.p. = 154-155 °C. ^{1}H NMR (300 Hz , (D_6)DMSO): 3.52-3.71 (m, 2H, -NCH_2-), 3.73 (s, 3H, -OCH_3), 4.81-4.85 (m, 1H, H-C), 5.49 (d, 1H, *J* = 2.7, H-O), 6.78 (d, 2H, *J* = 5.1,Ar-H), 6.91 (d, 2H, *J* = 5.1, Ar-H), 7.27 (t, 6H, *J* = 4.8, Ar-H), 7.41-7.44 (m, 2H, Ar-H), 9.96 (s, 1H, Ar-OH). ^{13}C NMR (300 MHz, (D_6)DMSO):171.47, 171.34, 165.07, 161.74, 159.52, 157.66, 135.84, 133.17, 132.95, 131.91, 131.80, 131.72, 130.59, 129.72, 127.17, 124.88, 124.83, 120.46, 116.00, 115.76, 115.71, 114.09, 72.27, 55.30, 45.91. HRMS: calcd. m/z [M] 433.1327, found m/z [M+Na]$^+$456.1219.

(R)-3-(4-fluorophenyl)-1-(2-(4-fluorophenyl)-2-hydroxyethyl)-4-(4-hydroxyphenyl)-1H-pyrrole-2,5-dione (**14h**). Yellow solid. Yield: 69%. M.p. = 219-221 °C. ^{1}H NMR ,(300 Hz ,(D_6)DMSO): 3.54-3.72 (m, 2H, -NCH_2-), 4.86-4.89 (m, 1H, H-C), 5.66 (d, 1H, *J* = 2.7, H-O), 6.78 (d, 2H, *J* = 5.1, Ar-H), 7.16 (t, 2H, J = 5.1, Ar-H), 7.27 (t, 4H, *J* = 5.1, Ar-H), 7.39-7.44 (m, 4H, Ar-H), 9.97 (s, 1H, Ar-OH). ^{13}C NMR (300 MHz, (D_6)DMSO): 170.31, 170.22, 164.05, 163.03, 160.76, 159.82, 159.10, 138.66, 138.62, 135.78, 132.14, 131.79,

131.67, 127.94, 127.83, 125.57, 125.52, 118.85, 115.76, 115.51, 114.99, 114.71, 113.49, 68.89, 45.59. HRMS: calcd. m/z [M] 421.1125, found m/z [M+Na]$^+$444.1018.

(R)-3-(4-fluorophenyl)-1-(2-hydroxy-2-(4-(trifluoromethoxy)phenyl)ethyl)-4-(4-hydroxyphenyl)-1H-pyrrole-2,5-dione (**14i**). Yellow solid. Yield: 67% M.p. = 132-133 °C. ^{1}H NMR (300 Hz ,(D_6)DMSO): 3.57-3.73 (m, 2H, -NCH_2-), 4.90-4.93 (m, 1H, H-C), 5.74 (d, 1H, *J* = 2.7, H-O), 6.78 (d, 2H, *J* = 5.1 , Ar-H), 7.24-7.27 (m, 4H, Ar-H), 7.34 (d, 2H, *J* = 4.8, Ar-H), 7.41-7.43 (m, 2H, Ar-H),7.50 (d, 2H, *J* = 5.4, Ar-H), 9.96 (s, 1H, Ar-OH). ^{13}C NMR (300 MHz, (D_6)DMSO): 170.28, 170.20, 164.05, 160.77, 159.10, 147.55, 147.53, 141.87, 135.80, 132.17, 131.78, 131.67, 131.28, 129.55, 127.85, 125.55, 125.51, 125.13, 121.74, 120.73, 118.83, 118.34, 115.76, 115.49, 113.49, 68.88, 45.44. HRMS: calcd. m/z [M] 487.1039, found m/z [M+Na]$^+$510.0931.

(R)-3-(4-fluorophenyl)-1-(2-hydroxy-2-(4-(trifluoromethyl)phenyl)ethyl)-4-(4-hydroxyphenyl)-1H-pyrrole-2,5-dione (**14j**). Yellow solid. Yield: 43%. M.p. = 151-153 °C. ^{1}H NMR (300 Hz ,(D_6)DMSO): 3.60-3.74 (m, 2H, -NCH_2-), 4.96-4.99 (m, 1H, H-C), 5.84 (d, 1H, *J* = 2.7, H-O), 6.78 (d, 2H, *J* = 5.1, Ar-H), 7.25-7.29 (m, 4H, Ar-H), 7.41-7.44 (m, 2H, Ar-H), 7.61 (d, 2H, *J* = 2.7, Ar-H), 7.72 (d, 2H, *J* = 5.1, Ar-H), 9.97 (s, 1H, Ar-OH). ^{13}C NMR (300 MHz, (D_6)DMSO): 170.28, 170.20, 164.05, 160.77, 159.11, 147.14, 135.83, 132.19, 131.78, 131.67, 131.29, 129.65, 129.55, 128.62, 128.20, 127.78, 127.36, 126.76, 125.54, 125.50, 125.06, 125.01, 124.96, 122.44, 118.82, 115.76, 115.49, 69.09, 45.40. HRMS: calcd. m/z [M] 471.1092, found m/z [M+Na]$^+$ 494.0984.

(S)-3-(4-fluorophenyl)-1-(3-hydroxy-3-phenylpropyl)-4-(4-hydroxyphenyl)-1H-pyrrole-2,5-dione (**14k**). Yellow solid. Yield: 77%. M.p. = 69-72 °C. ^{1}H NMR (300 Hz , (D_6)DMSO): 1.97 (t, 2H, *J* = 4.2, -CH_2-), 3.56-3.71 (m, 2H, -NCH_2-), 4.62 (q, 1H, *J* = 3.3, H-C), 5.28 (d, 1H, *J* = 2.7, H-O), 6.76 (d, 2H, *J* = 5.1, Ar-H), 7.19-7.26 (m, 5H, Ar-H), 7.27-7.30 (m, 2H, Ar-H), 7.32-7.34 (m, 2H, Ar-H), 7.39-7.42 (m, 2H,Ar-H), 9.93 (s, 1H, Ar-OH). ^{13}C NMR (300 MHz, (D_6)DMSO): 171.46, 171.31, 165.03, 161.70, 157.74, 143.33, 135.81, 133.04, 131.91, 131.80, 131.70, 128.78, 128.51, 127.60, 125.74, 124.87, 120.36, 115.98, 115.80, 115.69, 71.56, 37.11, 35.12. HRMS: calcd. m/z [M] 417.1374, found m/z [M+Na]$^+$ 440.1267.

(S)-3-(4-fluorophenyl)-1-(3-hydroxy-3-(p-tolyl)propyl)-4-(4-hydroxyphenyl)-1H-pyrrole-2,5-dione (**14l**). Yellow solid. Yield: 73%. M.p. = 124-126 °C. ^{1}H NMR (300 Hz , (D_6)DMSO): 1.97(q, 2H, *J* = 4.1, -CH_2-), 2.23 (d, 3H, *J* = 6.6, -$ArCH_3$), 3.58-3.71 (m, 2H, -NCH_2-), 4.57 (q, 1H, *J* = 3.3, H-C), 5.19 (d, 1H, *J* = 2.7, H-O), 6.76 (d, 2H, *J* = 5.1, Ar-H), 7.09 (d, 2H, *J* = 4.5, Ar-H), 7.19-7.26 (m, 6H, Ar-H), 7.38-7.41 (m, 2H, Ar-H), 9.93 (s, 1H, Ar-OH). ^{13}C NMR (300 Hz, (D_6)DMSO): 171.43, 171.27, 165.03, 161.70, 157.65, 140.30, 137.28, 135.77, 133.05, 132.30, 131.90, 131.79, 131.69, 129.74, 129.19, 125.73, 124.88, 124.83, 120.43, 115.97, 115.68, 114.00, 71.51, 36.94, 35.15, 21.05. HRMS: calcd. m/z [M] 431.1537, found m/z [M+Na]$^+$454.1429.

(S)-1-(3-(4-ethylphenyl)-3-hydroxypropyl)-3-(4-fluorophenyl)-4-(4-hydroxyphenyl)-1H-pyrrole-2,5-dione (**14m**). Yellow solid. Yield: 54%. M.p. = 60-62 °C. ^{1}H NMR (300 Hz, (D_6)DMSO): 1.15 (t, 3H, *J* =4.5, -$CH2CH_3$), 1.96 (q, 2H, *J* = 4.3, -CH_2-), 2.50-2.57 (m, 2H, -CH_2CH_3), 3.55-3.71 (m, 2H, -NCH_2-), 4.57 (q, 1H, *J* = 3.3 , H-C), 5.19 (t, 1H, *J* = 2.4, H-O), 6.76 (d, 2H, *J* = 5.1, Ar-H), 7.12 (d, 2H, *J* = 4.8, Ar-H), 7.22-7.26 (m, 6H, Ar-H), 7.39-7.41 (m, 2H, Ar-H), 9.93 (s, 1H, Ar-OH). ^{13}C NMR (300 Hz, (D_6)DMSO) : 171.43, 171.28, 162.03, 161.70, 157.68, 143.66, 140.58, 135.78, 133.06, 132.31, 131.90, 131.79, 131.69, 129.74, 127.99, 125.79, 124.89, 124.84, 120.42, 115.97, 115.79, 115.68, 114.00, 71.52, 36.98, 35.20, 35.06, 28.45, 15.42. HRMS: calcd.m/z [M] 445.1692, found m/z [M+Na]$^+$468.1584.

(S)-3-(4-fluorophenyl)-1-(3-hydroxy-3-(4-isopropylphenyl)propyl)-4-(4-hydroxyphenyl)-1H-pyrrole-2,5-dione (**14n**). Yellow solid. Yield: 87%. M.p. = 78-82 °C. ^{1}H NMR (300 Hz, (D_6)DMSO): 1.16 (d, 6H, *J* = 4.2, -$CH(CH_3)_2$), 1.93-1.98 (m, 2H, -CH_2-), 2.83 (t, 1H, *J* = 4.2, -$CH(CH_3)_2$), 3.56-3.71 (m, 2H, -NCH_2-), 4.57 (q, 1H, *J* = 3.4, H-C), 5.18 (d, 1H, *J* = 2.4, H-O), 6.76 (d, 2H, *J* = 5.1, Ar-H), 7.15 (t, 2H, *J* = 5.7, Ar-H), 7.24 (t, 6H, *J* = 5.1, Ar-H), 7.39-7.42 (m, 2H, Ar-H), 9.93 (s, 1H,Ar-OH). ^{13}C NMR (300 Hz, (D_6)DMSO) : 170.43, 170.34, 164.01, 160.73, 159.01, 146.79, 142.80, 135.73, 132.14, 131.85, 131.74, 131.34, 125.82, 125.76, 125.61, 118.89, 115.67, 115.43, 70.27, 36.96, 35.23, 33.02, 23.85. HRMS: calcd. m/z [M] 459.1847, found m/z [M+Na]$^+$482.1740.

(S)-1-(3-(4-(tert-butyl)phenyl)-3-hydroxypropyl)-3-(4-fluorophenyl)-4-(4-hydroxyphenyl)-1H-pyrrole-2,5-dione (**14o**). Yellow solid. Yield: 81%. M.p. = 89-91 °C. ^{1}H NMR (300 Hz, (D_6)DMSO): 1.24 (s, 9H, -$C(CH_3)_3$), 1.96 (d, 2H, *J* = 4.2, -CH_2-), 3.57-3.71 (m, 2H, -NCH_2-), 4.57 (q, 1H, *J* = 3.3 , H-C), 5.18 (d, 1H, *J* = 2.7, H-O), 6.76 (d, 2H, *J* = 4.8, Ar-H), 7.23-7.26 (m, 6H, Ar-H), 7.32 (d, 2H, *J* = 4.8, Ar-H), 7.40-7.43 (m, 2H, Ar-H), 9.93 (s, 1H, Ar-OH). ^{13}C NMR (300 Hz, (D_6)DMSO): 170.42, 170.33, 164.00, 160.72, 159.01, 149.04, 142.37, 135.70, 132.11, 131.85, 131.73, 131.34, 129.56, 125.50, 124.63, 118.88, 115.65, 115.41, 115.37, 113.47, 70.21, 36.93, 35.25, 34.05, 31.12. HRMS: calcd. m/z [M] 473.2003, found m/z [M+Na]$^+$496.1896.

(S)-3-(4-fluorophenyl)-1-(3-(4-fluorophenyl)-3-hydroxypropyl)-4-(4-hydroxyphenyl)-1H-pyrrole-2,5-dione (**14p**). Yield: 59%. M.p. Yellow solid. ^{1}H NMR (300 Hz, (D_6)DMSO): 1.94-1.98 (m, 2H, -CH_2-), 3.55-3.71 (m, 2H, -NCH_2-), 4.63 (q, 1H, *J* = 3.3, H-C), 5.33 (d, 1H, *J* = 2.7, H-O), 6.76 (d, 2H, *J* = 5.1, Ar-H), 7.10 (t, 2H, *J* = 5.4, Ar-H), 7.24 (t, 4H, *J* = 5.4, Ar-H), 7.35-7.39 (m, 2H, Ar-H), 7.41-7.42 (m, 2H, Ar-H), 9.93 (s, 1H, Ar-OH) ^{13}C NMR (300 Hz, (D_6)DMSO) : 170.37, 170.27, 163.98, 162.62, 160.70, 159.42, 158.98, 141.52, 141.49, 135.74, 132.15, 131.81, 131.70, 131.30, 127.64, 127.53, 125.57, 125.53, 118.86, 115.64, 115.41, 113.36, 114.71, 114.43, 69.62, 36.94, 34.97. HRMS: calcd. m/z [M] 435.1284, found m/z [M+Na]$^+$458.1177.

(S)-3-(4-fluorophenyl)-1-(3-hydroxy-3-(4-hydroxyphenyl)propyl)-4-(4-hydroxyphenyl)-1H-pyrrole-2,5-dione (**14q**). Yellow solid. Yield: 72%. M.p. = 112-115 °C. ^{1}H NMR (300 Hz, (D_6)DMSO): 1.90-1.94 (m, 2H, -CH_2-), 3.52-3.62 (m, 2H, -NCH_2-), 4.87 (t, 1H, *J* = 3.0, H-C), 5.06 (d, 1H, *J* = 2.7, H-O), 6.67 (d, 2H, *J* = 5.4, Ar-H), 6.75 (t, 2H, *J* = 4.2, Ar-H), 7.11 (d, 2H, *J* = 2.1, Ar-H), 7.23-7.26 (m, 4H, Ar-H), 7.39-7.42 (m, 2H, Ar-H), 9.15 (s, 1H, Ar-OH), 9.93 (s, 1H, Ar-OH). ^{13}C NMR (300 Hz, (D_6)DMSO) δ 170.39, 170.29, 163.97, 160.39, 158.95, 156.12, 135.77, 135.57, 132.21, 131.84, 131.73, 131.32, 127.31, 126.86, 125.57, 118.90, 115.62, 115.40, 115.34, 114.63,

70.15, 36.95, 35.19. HRMS: calcd. m/z [M] 433.1332, found m/z [M-H]$^-$ 432.1260.

(S)-3-(4-fluorophenyl)-1-(3-hydroxy-3-(4-methoxyphenyl)propyl)-4-(4-hydroxyphenyl)-1H-pyrrole-2,5-dione (**14r**). Yellow solid. Yield: 93%. M.p. = 72-75 °C. ^{1}H NMR (300 Hz, (D_6)DMSO): 1.94-1.98 (m, 2H, -CH_2-), 3.55-3.61 (m, 2H, -NCH_2-), 3.68 (t, 3H, *J* = 4.5, -OCH_3), 4.55 (q, 1H, *J* = 3.4 Hz, H-C), 5.15 (d, 1H, *J* = 2.4, H-O), 6.76 (d, 2H, *J* = 5.1, Ar-H), 6.83 (d, 2H, *J* = 5.1, Ar-H), 7.21-7.26 (m, 6H, Ar-H), 7.38-7.41 (m, 2H, Ar-H), 9.93 (s, 1H, Ar-OH). ^{13}C NMR (300 Hz, (D_6)DMSO) : 170.39, 170.29, 164.01, 160.73, 158.99, 158.08, 137.27,135.71, 132.13, 131.86, 131.74, 131.35, 129.59, 126.94, 125.60, 125.56, 118.90, 115.67, 115.43, 115.38, 113.51, 113.30, 70.05, 54.89, 36.80, 35.10. HRMS: calcd. m/z [M] 447.1484, found m/z [M+Na]$^+$470.1376.

Methods of in vitro cholesterol absorption inhibition assay

The preparation of cholesterol micellar solution was modified from Field et al. DMEM culture media containing cholesterol (2.5 mM), sodium taurocholate (3 mM) and monoolein (30 μM) was mixed and sonicated at 37 ℃ for 1 h. Finally, the micelle was passed through a 0.22 μm filter and kept at 37°C until use.

Caco-2 cells were seeded in 6-well plates in DMEM containing 20% fetal bovine serum (FBS), 1% penicillin-streptomycin, 1% non-essential-amino acids and incubated for 48 h before treatment. Cells were washed twice with PBS and incubated in medium (1.5 mL/well) containing tested compounds (100 μM). Following the incubation for 2 h, cells were washed twice with PBS, added with cholesterol micellar solutions (1 mL/well) and incubated for another 2 h. Cells were washed twice with PBS again, and then lysed with RIPA lysis buffer (200 μL/well). The lysate was transferred to centrifuge tubes for centrifugation. BCA protein assay kit was used to quantify the protein concentration of lysate. T-CHO assay kit was used to quantify the total cholesterol concentration of lysate. The lysate 25 μL was seeded in 96-well and incubated in 200 μL enzyme-containing reagents for 10 min. Then, a microplate reader was used to measure the OD value of each well at 510 m.

Methods of cytotoxicity assay

Cytotoxicity assay was completed using the MTT cell proliferation assay. Human cell lines, L-02 (normal human liver cells, 8000 cells/well) and HEK293T (human embryonic kidney cells, 8000 cells/well) were seeded in 96-well microtiter plates in a 100 μL culture medium. The cells were allowed to settle and attached for 12 h. Then, both of the cell lines were exposed to different concentrations of tested compounds in twofold serial dilutions that range from 200 μM to 12.5 μM and incubated for a further 48 h. Cell viability was measured by MTT assay. The results are expressed as LC_{50}. The absorption was measured on a microplate reader at 490 nm.

Methods of in vitro anti-inflammatory and anti-oxidative activity assay

A reactive oxygen species assay kit were obtained from Beyotime (Shanghai, China). Tumor necrosis factor alpha assay kit (Cat.EH009-48) was purchased from Excell Co. Following the kit instructions, cell suspensions or cell lysates are collected, an appropriate amount of working reagent is added, and detection is performed under specific operating parameters.

Supplementary Material

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/MS-number.

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Author Contribution Statement

Yineng Xia and Lijuan Zhu performed the experiments, analyzed the data, and wrote the paper. Xinrui Yuan provided many constructive suggestions on the experiments. Yubin Wang conceived and designed the experiments.

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Entry for the Table of Contents

Synthesis and evaluation of 2-azetidinone and 1*H*-pyrrole-2,5-dione derivatives as cholesterol absorption inhibitors for reducing inflammation response and oxidative stress.

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