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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and hypoglycemic activity evaluation of rhein amide derivatives

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Abstract In order to investigate the relationship of the structure and hypoglycemic activity, rhein amide derivatives **2a**–**2e** were synthesized and their hypoglycemic activities were evaluated by glucose consumption in HepG2. Their structures were characterized by ^{1}H-, ^{13}C NMR, IR, mass and elemental analysis. All the compounds exhibited strong hypoglycemic activity in improving glucose consumption in HepG2 cell assays in vitro, which was influenced by the diversity of rhein amide derivatives. The compounds **2a**–**c**, **2f**, and **2g** bearing heterocyclic ring were proved to be more potentially useful in glucose consumption than dimethyldiguanide. Among all the compounds, compound **2f** exhibited the strongest activity on glucose consumption, while compound **2d** showed the weakest activity.

Keywords Rhein derivatives · Glucose consumption · Hypoglycemic activity

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Introduction

Diabetes is a metabolic disease, seriously affecting people's health and quality of life. Type II diabetes, the more prevalent form of diabetes, is characterized by insulin resistance of internal organs and peripheral tissues that results in impaired glucose utilization, and consequently, in abnormally high blood glucose levels between and especially after meals (Jan-Willem *et al*., 2011). It has been estimated that the number of adults affected by diabetes will grow from 135 million in 1995 to 300 million in 2025 worldwide (King *et al*., 1998; Benson *et al*., 2010). Hyperglycemia resulting from uncontrolled glucose regulation, which cause the disorder of fat and protein metabolism and even lead to cardiovascular disease, is widely recognized as the causal link between diabetes and diabetic complications (Anabela and Carlos, 2006; Brownlee, 2001). Therefore, it is of great importance to explore more effective and safer antihyperglycemic drugs, as a pharmacological tool to provide important information regarding treatment for diabetes. Currently, the diabetic drugs are structurally divided into two major classes, sulfonylureas and biguanides. Furthermore, other drugs such as α-glucosidase inhibitors are used as an auxiliary type for diabetes treatment. The development of more effective and safe hypoglycemic agents is still a challenge, due to few species and side effects (McEwen *et al*., 2009; Chao *et al*., 2007). There is a growing trend of using natural products as an alternative treatment of type II diabetes for the safety and diversity (Hnatyszyn *et al*., 2002). China has a long history of using herbs for the treatment of human diseases including diabetes. *Rheum rhaponticum* is one of those herbs which exhibit many pharmacological activities.

Rhein, as quality control marker of *R. rhaponticum*, has good anti-hypoglycemic effect and lipid-lowering activity,

as well as low cytotoxic property (Bettag *et al*., 2008). It has been reported rhein exerts anti-hypoglycemic effect through agitating PPAR-γ (Mu *et al*., 2008). The clinical application of rhein, however, has been limited because of its poor bioavailability and stimulating gastrointestinal disorder. Thus, there is continued interest in the synthesis of new anthraquinone derivatives, either as promising biologically active compounds or as valuable synthons for the preparation of photodynamically active hypericin derivatives.

The modification of rhein had been mainly concentrated on 1,8-hydroxyl and 3-carboxyl derivative of rhein (Owton *et al*., 1995; Baxter and Walmsley, 2005; Pietrangelo and Travagli, 2005). The introduction of acetyl in the 1,8-hydroxy can increase the effect of inhibition of bone and joint inflammation. The introduction of a series of long-chain ester in the 1,8-hydroxy, fluorine atoms on anthraquinone ring, and carboxylic acid in the 3-carboxyl can get better antihypoglycemic or anti-inflammation activity (Kalyoncu *et al*., 2009; Owton *et al*., 1995). Our previous studies have shown that the hypoglycemic activity of rhein derivatives was significantly influenced by the aliphatic chain length. It was found that their activity increased with the aliphatic chain length and then gradually decreased when the number of carbon atoms was higher than eight (Zhu *et al*., 2012). The result suggests that the changes of hydrophobicity or solubility of rhein affect their pharmacological activities. Based on our previous studies, we designed and synthesized a series of rhein homologs by introducing various groups on the carboxylic acid and/or hydroxyl of C4 and C5, and these rhein derivatives were assayed for the activity of promoting glucose consumption in HepG2 cells.

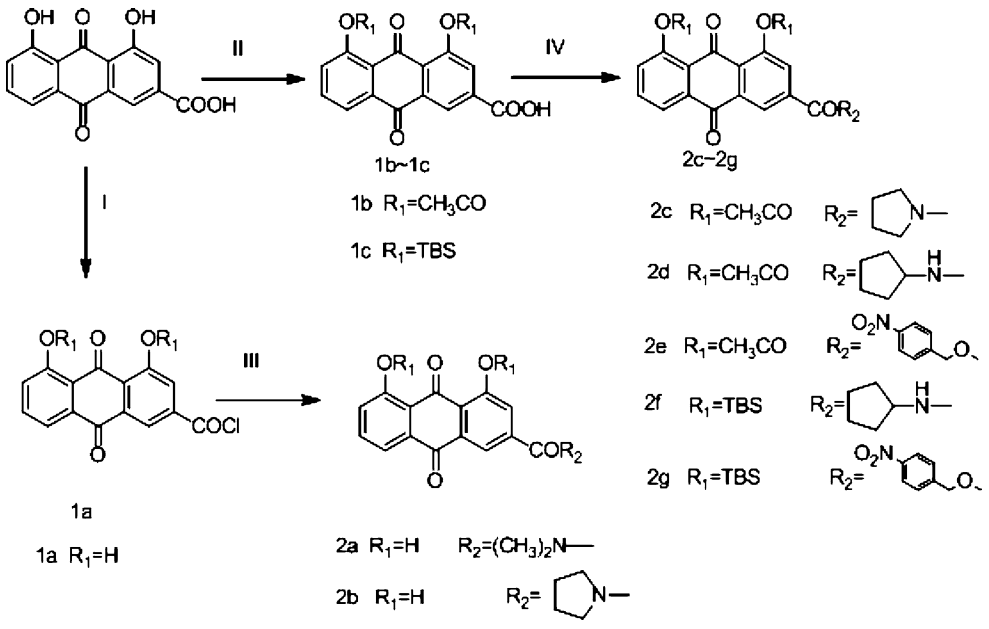
Results and discussion

Chemistry

Our approach to the synthesis of 2-carboxamide-rhein is depicted in Scheme 1. The 4,5-unprotected rhein amides **2a**–**2b** was obtained by the reaction of substituted amines and the highly reactive acyl chloride (**1a**, 2-carbonyl chloride rhein), which was generated by the treatment of $SOCl_2$ with rhein. The approach to 4,5-diprotected rhein amides **2c**–**2g** was achieved by the reaction of substituted amines or alcohols with the anhydride, which was obtained by treatment of compound **1b**–**1c** with oxalyl chloride. The resulting compounds were purified by silica gel chromatography and confirmed by IR, ^{1}H-, ^{13}C NMR, mass spectra (MS), and elemental analysis spectroscopy.

For synthesizing the target compounds **2a**–**2b**, $SOCl_2$ was used to activate the reactions, by generating the active acyl chloride intermediates. On the contrary, the commonly used condensation reagents EDC and dicyclohexyl carbodiimide (DCC) did not efficiently promote the amidation reactions. In the reactions with EDC as the promoter, the TLC indicated that there were many by-products produced. In contrast, the DCC generated compound **3a** as the major product. The possible reaction mechanism of the formation of **3a** is as follows (Scheme 2): at the first stage, rhein reacted with DCC to form the unstable carbodiimide intermediate (**4**), which could be re-arranged to more stable urea (**3a**, Path b).

We optimized the reaction conditions for the synthesis of the target compounds **2c**–**2g** by using ethyl oxalyl chloride to react with compound **1b**–**1c**, to form highly reactive anhydride intermediates, which was easier to react with amines or



Scheme 1 The synthetic routes of rhein amide or rhein ester derivatives; *I* $SOCl_2$, DMF, reflux; *II* Ac_2O, PyH, 80 °C or TBS, Et_3N, CH_2Cl_2, CH_3CN; *III* RNH_2, CH_2Cl_2, Et_3N, room temperature; *IV* RXH, ClCOOEt, Et_3N, room temperature

Scheme 2 The possible reaction mechanism of the formation of **3a**

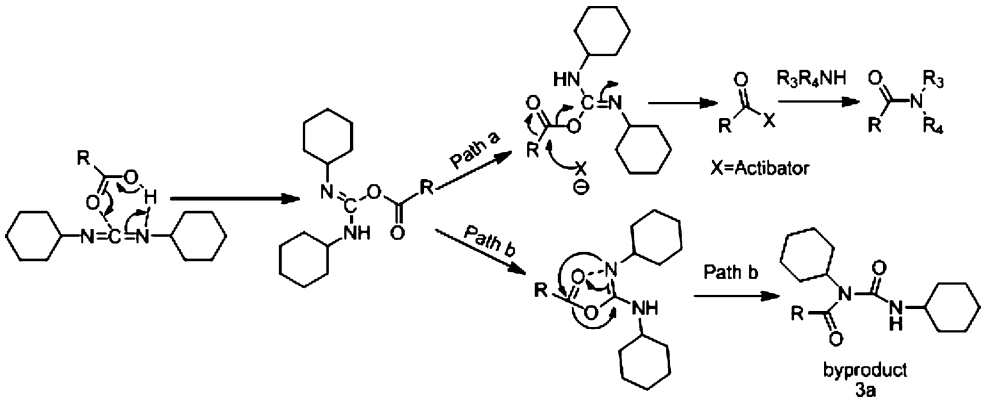


Table 1 The glucose uptake of different rhein derivatives and dimethyldiguanide in HepG2 cell

Group	C (mg L^{-1})	GC (mmol L^{-1})	MTT/A_{490}	GC/MTT	CLgP
Control	10	1.972 ± 0.071	0.574 ± 0.019	3.438 ± 0.139	–
2a	10	2.485 ± 0.083*	0.560 ± 0.042	4.449 ± 0.240**	2.623
2b	10	2.315 ± 0.076*	0.565 ± 0.023	4.116 ± 0.289**	2.949
2c	10	2.541 ± 0.100*	0.591 ± 0.021	4.304 ± 0.316**	1.532
2d	10	2.256 ± 0.098*	0.577 ± 0.017	3.916 ± 0.230*	2.743
2e	10	2.268 ± 0.071*	0.573 ± 0.011	3.958 ± 0.218*	3.941
2f	10	2.578 ± 0.072*	0.565 ± 0.026	4.563 ± 0.123**	8.924
2g	10	2.529 ± 0.090*	0.563 ± 0.017	4.499 ± 0.276**	9.938
Dimethyldiguanide	10	2.250 ± 0.078*	0.574 ± 0.013	3.923 ± 0.171*	−1.633

Data were expressed as mean ± SD ($n = 6$), analyzed by ANOVA

* $P < 0.05$ compared with control group; ** $P < 0.01$ compared with control group

alcohols and gave the desired amides or esters, respectively. The spectral data and other characteristic parameters of these compounds were listed in "Experimental protocols" section.

Hypoglycemic activity in vitro

The glucose consumption of the target compounds **2a**–**2g** in HepG2 cells in vitro was evaluated to investigate the possible antihyperglycemic activity and the preliminary structure–activity relationship. HepG2 cells were used in this study due to their common physiological function to lipid or glucose metabolism with normal hepatic cells (Xu *et al*., 2003).

The glucose consumption of HepG2 cell assay (Table 1) showed that the glucose consumption of all the tested compounds have significant difference compared with vehicle control ($P < 0.05$). All of the 2-substitutional amide-rhein possessed higher glucose consumption ability than the positive control (dimethyldiguanide) at the same concentration, except compound **2d** and **2e**. The result indicated that glucose consumption of **2a**–**2f** has a very significant difference compared to control group ($P < 0.01$). In Table 2, we can find that the glucose consumption in HepG2 cell increased with the concentration of 2-substitutional amide-rhein.

According to the experimental data, the introduction of acetyl ester showed moderate activity on glucose consumption. However, the *N*-substituent amides at C1 position did not significantly affect the hypoglycemic activity, when compared with that induced by *O*-substituents at C1 position. *O*-acetyl group at C4 and C5 position of rhein amide (**2c**) increased glucose consumption, compared with the rhein amide (**2b**). Among all the compounds, compound **2f** and **2g** exhibited the strongest activity on glucose consumption, while compound **2d** showed the weakest activity. The introduction of TBS group of target molecule **2f** and **2g** to rhein derivatives increased CLgP value of the compounds, indicating that TBS group increased their hydrophobicity (Table 1). Furthermore, *O*-TBS at C4, C5 position of rhein amide (**2f** and **2g**) showed higher hypoglycemic activity compared with *O*-acetyl at C4, C5 position (**2d** and **2e**).

Conclusion

In summary, we established an efficient method for the synthesis of rhein amides using $SOCl_2$ as activator, which afforded the corresponding amides in higher yield than that by the use of condensation reagents DCC and EDC.

Table 2 The glucose uptake of different concentration of rhein derivatives in HepG2 cells

Group	GC (mmol L^{-1})			
	0	1 mg L^{-1}	10 mg L^{-1}	100 mg L^{-1}
Control	1.972 ± 0.071	–	–	–
2a	–	2.444 ± 0.106**	2.485 ± 0.083**	2.598 ± 0.100**
2b	–	2.379 ± 0.052**	2.315 ± 0.076**	2.262 ± 0.009**
2c	–	2.476 ± 0.068**	2.541 ± 0.100**	2.567 ± 0.148**
2d	–	2.151 ± 0.103*	2.256 ± 0.098**	2.390 ± 0.064**
2e	–	2.198 ± 0.042**	2.268 ± 0.098**	2.292 ± 0.089**
2f	–	2.369 ± 0.088**	2.578 ± 0.072**	2.733 ± 0.010**
2g	–	2.576 ± 0.080**	2.529 ± 0.090**	2.506 ± 0.043**
Dimethyldiguanide	–	–	2.250 ± 0.078**	–

Data were expressed as mean ± SD ($n = 6$), analyzed by ANOVA

– Mean not detected

* $P < 0.05$ compared with control group; ** $P < 0.01$ compared with the control group

The novel rhein derivatives exhibited strong ability in improving glucose uptake in HepG2 cells. The introduction of TBS groups in the dihydroxyl groups at anthraquinone ring greatly increased the glucose uptake ability. Compounds **2a–c**, **2f**, and **2g** showed higher activity than dimethyldiguanide in glucose uptake in vitro. However, compounds **2d** and **2e** showed similar abilities as dimethyldiguanide.

Experimental protocols

Chemistry

Rhein (purity of more than 98 %) was purchased from Xi'an Natural Product Company (Shanxi, China). Other reagents were AR grade and purchased from Chongqing Chemical Reagent Company (Chongqing, China). DMF, CH_2Cl_2, Et_3N, and CH_3CN were redistilled and dried using molecular sieves.

Melting points were recorded on a Buchi apparatus and were not corrected. IR spectra and KBr pellets of 400–4,000 cm^{-1} were recorded on a Perkin Elmer 16PC-FT spectrophotometer. ^1H- and ^{13}C NMR spectra were recorded on a Brucker ARX-300 instrument (chemical shifts are expressed as δ values relative to TMS as internal standard). MS and ESI (positive) were recorded on a Thermo Fisher-Ltq-Orbitra spectrometer. Satisfactory micro-analysis was obtained on FlashEA1112 CHN analyzer.

*Procedure for preparation of 4,5-dihydroxy-9,10-dioxo-9,10-dihydro anthracene-2-carboxylic acid **(1b)***

For **1b** preparation, a mixture of 5 mmol rhein and 60 mL Pyridine was added dropwise to 60 mL acetic anhydride under stirring. The resulting mixture was refluxed at 120 °C for 6 h. The resulting yellow solution was poured into ice water, filtered and dried under reduced pressure, and recrystallized from ethyl acetate to obtain pure **1a** as yellow solid, yield 93 %, mp 217.2–218.0 °C, ^1H NMR ($CDCl_3$, 300 MH_Z, ppm) δ: 12.2 (s, 1H, COOH), 8.14 (s, 1H, ArH), 7.85–7.79 (d, $J = 7.4$, 1H, ArH), 7.74–7.72 (d, $J = 7.8$, 2H, ArH), 7.41–7.39 (d, $J = 7.4$, 1H, ArH), 2.50 (s, 6H, CH_3); which is accord with reference.

*Procedure for preparation of 4,5-bis (tert-butyldimethylsilyloxy)-9,10-dioxo-9,10-dihydroanthracene-2-carboxylic acid **(1c)***

A solution of rhein (0.568 g, 2 mmol) in dry CH_2Cl_2 (30 mL) and 2.78 mL triethylamine was treated with *tert*-butyl-chlorodimethylsilane (3.0146 g, 20 mmol) at 0 °C and stirred at reflux temperature for 24 h under nitrogen. After completion of the reaction, the reaction was quenched with 50 mL distilled water and extracted with dichloromethane (3×1.5 dm^3) and the combined extracts were washed with water, dried ($MgSO_4$), filtered, and evaporated to dryness under reduced pressure to give crude product **1c**. The crude product was purified by silica gel column purification using 2:1 (v/v, petroleum ether:ethyl acetate) to obtain pure solid product **1c** as orange solid, yield 82 %, mp >300 °C, ^1H NMR ($CDCl_3$, 300 MH_Z, ppm) δ: 8.56 (s, 1H, ArH), 7.89 (s, 2H, ArH), 7.56–7.54 (t, 1H, ArH), 7.24–7.21 (d, $J = 8.1$, 1H, ArH,), 1.09–1.08 (d, $J = 3.9$, 18, CH_3), 0.36–0.32 (d, $J = 9.6$, 12H, CH_3); IR (KBr) ν: 3422, 2967, 2923, 1678, 1630, 1489, 1432, 1405, 1380, 1286, 1200, 1192 cm^{-1}; MS (ESI, *m/z*): 514 (M + 1).

*Procedure for preparation of 4,5-dihydroxy-N,N-dimethyl-9,10-dihydro anthracene-2-carboxamide **(2a)***

Dry $SOCl_2$ (1 mL) was added to a solution of rhein (0.568 g, 2 mmol) in dry DMF (5 mL). The reaction

mixture was heated under reflux for 45 min under nitrogen. After completion of the reaction, the reaction mixture was cooled to room temperature and diluted with 50 mL water. 2.5 % Na_2CO_3 was added to achieve a pH of 8–9. The aqueous solution was extracted with dichloromethane (3 × 1.5 dm^3) and the combined extracts were washed with water, dried ($MgSO_4$), filtered, and evaporated to dryness under reduced pressure to give crude product **2a**. The crude product was purified by silica gel column purification using 20:1 (v/v, petroleum ether:ethyl acetate) to obtain pure solid product **2a** as brown crystal, yield 64.9 %, mp 219.2–220.0 °C, ^{1}H NMR ($CDCl_3$, 300 MH_Z, ppm) δ: 11.99 (s, 1H, OH), 11.93 (s, 1H, OH), 7.82–7.87 (t, 1H, ArH), 7.73–7.76 (d, J = 7.8, 1H, ArH), 7.64 (s, 1H, ArH), 7.44 (s, 1H, ArH), 7.40–7.42 (d, J = 7.1, 1H, ArH), 3.02 (s, 3H, CH_3), 2.92 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): 191.5, 180.8, 168.7, 162.4, 162.1, 142.4, 136.3, 133.6, 132.1, 124.7, 122.5, 120.1, 118.0, 116.0, 115.4, 38.9, 38.6. Anal. calcd. for **2a**: $C_{17}H_{13}NO_5$, C, 65.59; H, 4.21; N, 4.50. Found: C, 65.25; H, 4.60; N, 4.28; IR (KBr) : 3421, 2956, 2923, 2852, 2360, 1673, 1630, 1480, 1452, 1404, 1350, 1274, 1208, 1182 cm^{-1}; MS (ESI, *m/z*): 312 (M + 1).

*Procedure for preparation of N-cyclopentyl-4, 5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxamide (**2b**)*

Dry DMF (0.1 mL) was added to a solution of rhein (0.568 g, 2 mmol) in dry $SOCl_2$ (5 mL). The reaction mixture was heated under reflux for 45 min under nitrogen. After completion of the reaction, the reaction mixture was heated to distill the excrescent $SOCl_2$, cooled to room temperature, and the intermediate product **1a** was obtained as brown liquid. The solution of **1a** and dry dichloromethane (10 mL) was dropwised into a dry solution of triethylamine (4 mL), pyrrolidine (4 mL), and dichloromethane (10 mL) at 0 °C under nitrogen and then stirred at room temperature for 1 h. After completion of the reaction, the reaction mixture was diluted with 50 mL water, and 2.5 % Na_2CO_3 was added to achieve a pH of 8–9. The aqueous solution was extracted with dichloromethane (3 × 1.5 dm^3) and the combined extracts were washed with water, dried ($MgSO_4$), filtered, and evaporated to dryness under reduced pressure to give crude product **2b**. The crude product was purified by silica gel column purification using 4:1 (v/v, petroleum ether:ethyl acetate) to obtain pure solid product **2b** as orange solid, yield 62.9 %, mp 198.5–201.2 °C, ^{1}H NMR (dimethyl sulphoxide [DMSO], 300 MH_Z, ppm) δ: 11.99 (s, 1H, OH), 11.94 (s, 1H, OH), 7.78 (s, 1H, ArH), 7.76 (s, 1H, ArH), 7.49–7.50 (m, 2H, ArH), 7.43–7.46 (d, J = 8.1, 1H, ArH), 3.49–3.54 (m, 2H, CH_2), 3.35–3.43 (t, 6H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): 192.2, 180.7, 166.5, 162.4, 162.3, 145.4, 137.3, 133.6, 133.1, 124.7, 122.5, 120.0, 118.0, 116.0, 115.4, 49.1, 46.2, 26.2, 24.2. Anal. calcd. for **2b**: $C_{19}H_{15}NO_5$, C, 67.65; H, 4.48; N, 4.15. Found: C, 67.21; H, 4.82; N, 3.88; IR (KBr) *v*: 3422, 2971, 2881, 2362, 1669, 1630, 1476, 1452, 1381, 1351, 1288, 1267, 1202, 1158 cm^{-1}; MS (ESI, *m/z*): 338 (M + 1).

*Procedure for preparation of **2c–2e***

For **2c–2e** preparation, a solution of **1b** (0.368 g, 1 mmol) in dry CH_2Cl_2 (20 mL) and 0.42 mL triethylamine was treated with ethyl oxalyl chloride (0.29 mL, 3 mmol) at 0 °C, stirred for 30 min at 0 °C, and then dropwised substitution amine (2 mmol) for 45 min under nitrogen. After completion of the reaction, it was quenched with 30 mL distilled water and extracted with ethyl acetate (3 × 1.5 dm^3) and the combined extracts were washed with water, dried ($MgSO_4$), filtered, and evaporated to dryness under reduced pressure to give crude product **2c–2e**. The crude product was purified by silica gel column purification using 10:1 (v/v, CH_2Cl_2:CH_3OH) to obtain pure solid product **2c–2e** as yellow solid.

*9,10-Dioxo-3-(pyrrolidine-1-carbonyl)-9,10-dihydroanthracene-1,8-diyl diacetate (**2c**)* Deep yellow solid, yield 56.9 %, mp 190.5–192.2 °C, ^{1}H NMR ($CDCl_3$, 300 MH_Z, ppm) δ: 8.33 (s, 1H, ArH), 8.22–8.24 (d, J = 7.5, 1H, ArH), 7.76–7.81 (t, 1H, ArH), 7.59 (s, 1H, ArH), 7.42–7.45 (d, J = 8.1, 1H, ArH), 3.48 (s, 2H, CH_2), 3.68 (s, 2H, CH_2), 2.26 (m, 6H, CH_3), 1. 89–2.05 (m, 4H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): 185.1, 182.4, 169.0, 168.5, 166.0, 156.3, 154.1, 138.5, 135.2, 134.8, 133.5, 130.0, 128.6, 127.6, 121.5, 119.3, 117.4, 49.1, 46.2, 26.2, 24.2, 21.3. Anal. calcd. for **2c**: $C_{23}H_{19}NO_7$, C, 65.55; H, 4.54; N, 3.32. Found: C, 65.01; H, 4.85; N, 3.02; IR (KBr) *v*: 3421, 2971, 2875, 2364, 1868, 1766, 1678, 1626, 1592, 1474, 1370, 1342, 1251, 1204, 1188 cm^{-1}; MS (ESI, *m/z*): 422 (M + 1).

*3-(Cyclopentylcarbamoyl)-9,10-dihydroanthracene-1,8-diyl diacetate (**2d**)* Light yellow crystal, yield 53.0 %, mp 263.5–265.0 °C, ^{1}H NMR ($CDCl_3$, 300 MH_Z, ppm) δ: 8.40 (s, 1H, ArH), 8.25–8.23 (d, J = 7.5, 1H, ArH), 7.91 (s, 1H, ArH), 7.82–7.70 (t, 1H, ArH), 7.45–7.43 (d, J = 7.8, 1H, ArH), 6.31–6.29 (d, J = 6.3, 1H, NH), 4.46–4.39 (m, 1H, CH), 2.46 (s, 6H, CH_3), 1.75–1.53 (t, 8H,CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): 185.1, 182.4, 168.9, 168.6, 165.9, 156.3, 154.2, 138.6, 135.1, 134.9, 133.6, 129.7, 128.5, 127.5, 121.3, 119.3, 117.4, 53.0, 24.1, 21.2, 18.7. Anal. calcd. for **2d**: $C_{24}H_{21}NO_7$, C, 66.20; H, 4.86; N, 3.22. Found: C, 65.91; H, 5.01; N, 3.02; IR (KBr) *v*: 3421, 2958, 2873, 2360, 1771, 1677, 1632, 1594, 1543, 1448, 1377, 1349, 1298, 1261, 1184, 1116 cm^{-1}; MS (ESI, *m/z*): 436 (M + 1).

*3-((4-Nitrobenzyloxy)carbonyl)-9,10-dioxo-9,10-dihydroanthracene-1,8-diyl diacetate (**2e**)* Deep yellow crystal,

yield 78.3 %, mp 225.2–227.5 °C, ^{1}H NMR ($CDCl_3$, 300 MH_Z, ppm) δ: 8.86 (s, 1H, ArH), 8.30–8.27 (t, 3H, ArH), 8.06 (s, 1H. ArH), 7.84–7.79 (t, 1H, ArH), 7.65–7.63 (d, $J = 8.1$, 2H, ArH), 7.47–7.44 (d, $J = 7.8$, 1H, ArH), 5.51 (s, 2H, CH_2), 2.46 (s, 6H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): 185.2, 181.6, 168.9, 168.7, 165.9, 156.1, 155.9, 148.1, 142.5, 135.3, 135.0, 133.9, 129.2, 128.6, 128.4, 128.2, 125.8, 124.2, 124.1, 121.0, 120.3, 66.0, 21.1. Anal. calcd. for **2e**: $C_{26}H_{17}NO_{10}$, C, 62.03; H, 3.40; N, 2.78. Found: C, 61.82; H, 3.81; N, 2.46; IR (KBr) *v*: 3428, 3082, 2813, 2724, 1777, 1756, 1723, 1681, 1668, 1631, 1593, 1522, 1447, 1372, 1353, 1331, 1281, 1255, 1220, 1156 cm^{-1}; MS (ESI, *m/z*): 502 (M − 1).

*Procedure for preparation of **2f–2g***

For **2f–2g** preparation, a solution of **1c** (0.512 g, 1 mmol) in dry THF (20 mL) and 0.42 mL triethylamine was treated with ethyl oxalyl chloride (0.29 mL, 3 mmol) at 0 °C, stirred for 30 min at 0 °C, and then dropwised substitution amine (2 mmol) for 45 min at room temperature under nitrogen. After completion of the reaction, it was quenched with 30 mL distilled water and extracted with ethyl acetate (3×1.5 dm^3) and the combined extracts were washed with water, dried ($MgSO_4$), filtered, and evaporated to dryness under reduced pressure to give crude product **2f–2g**. The crude product was purified by silica gel column purification using 2:1 (v/v, petroleum ether:ethyl acetate) to obtain pure solid product **2f–2g** as yellow solid.

*4,5-Bis(tert-butyldimethylsilyloxy)-N-cyclopentyl-9,10-dioxo-9,10-dihydroanthracene-2-carboxamide (**2f**)* Yellow crystal, yield 48.5 %, mp 202.0–204.5 °C, ^{1}H NMR ($CDCl_3$, 300 MH_Z, ppm) δ: 8.00 (s, 1H, ArH), 7.89–7.86 (d, $J = 7.5$, 1H, ArH), 7.79 (s, 1H, ArH), 7.58–7.53 (t, 1H, ArH), 7.24–7.21 (d, $J = 8.1$, 1H, ArH), 6.32–6.30 (d, $J = 6.3$, 1H, NH), 4.50–4.33 (m, 1H, CH), 1.62 (s, 8H, CH_2), 1.08 (s, 18H, CH_3), 0.340–0.314 (d, 12H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): 184.1, 181.4, 164.9, 156.5, 155.9, 138.9, 135.1, 134.9, 133.6, 128.4, 127.7, 127.2, 120.3, 119.2, 116.4, 52.2, 33.3, 26.1, 25.9, 24.1, 18.8. Anal. calcd. for **2f**: $C_{32}H_{45}NO_5Si_2$, C, 66.28; H, 7.82; N, 2.42. Found: C, 65.92; H, 7.65; N, 2.11; IR (KBr) *v*: 3421, 3306, 2954, 2930, 2657, 2361, 1677, 1632, 1588, 1558, 1454, 1417, 1386, 1347, 1315, 1284, 1256, 1227, 1123 cm^{-1}; MS (ESI, *m/z*): 581 (M + 1).

*4-Nitrobenzyl-4,5-bis(tert-butyldimethylsilyloxy)-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate (**2g**)* Orange crystal, yield 42.5 %, mp 198.5–200.5 °C, ^{1}H NMR ($CDCl_3$, 300 MH_Z, ppm) δ: 8.50–8.41 (t, 1H, ArH), 8.30–8.27 (t, 2H, ArH), 8.01–7.96 (t, 1H. ArH), 7.90–7.86 (t, 1H, ArH), 7.78–7.72 (t, 1H, ArH), 7.65–7.63 (d, $J = 7.2$, 2H, ArH), 7.56–7.54 (d, $J = 6.9$, 1H, ArH), 5.50 (s, 2H, CH_2), 1.36–1.26 (m, 6H, CH_3), 1.07 (s, 9H, CH_3), 0.85 (s, 9H, CH_3), 0.33–0.32 (d, $J = 5.1$, 6H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): 183.5, 181.4, 164.6, 156.1, 155.9, 147.9, 142.8, 135.4, 134.9, 133.8, 129.2, 128.6, 128.5, 128.2, 125.8, 124.1, 123.9, 120.7, 120.3, 65.9, 30.9, 26.0. Anal. calcd. for **2g**: $C_{34}H_{41}NO_8Si_2$, C, 63.03; H, 6.38; N, 2.16; O, 19.76; Si, 8.67. Found: C, 62.76; H, 6.00; N, 1.98; IR (KBr) *v*: 3422, 2958, 2928, 2856, 1733, 1679, 1631, 1603, 1528, 1478, 1453, 1417, 1381, 1345, 1279, 1238, 1164 cm^{-1}; MS (ESI, *m/z*): 649 (M + 1).

Pharmacology

HepG2 cells were provided by the Cell Bank of the Institute of Fundamental Medicine, Chinese Academy of Medical Science (Beijing, China). Fetal bovine serum (FBS), RPMI 1640 medium, and blood glucose diagnosis kits were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). MTT and trypsin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Dimethyldiguanide hydrochloride was supplied by Nanjin Langze Institute (Nanjin, China). Final concentrations of DMSO for dissolving drugs in medium were below 0.1 % (v/v). MTT and rhein derivatives were diluted with PBS buffer and filtered for sterilization. Cells with 10 % fetal calf serum were cultured in RPMI 1640 medium at 37 °C in a humidified incubator in 5 % CO_2 atmosphere.

Glucose consumption activity was analyzed by measuring the consumption glucose in HepG2 cell. The HepG2 cell in 48-well plates was pre-incubated with RPMI 1640 medium containing 1, 10, 100 mg/L **2a–2g** and 10 mg/L dimethyldiguanide 250 μL at 37 °C for 24 h, each repeated three times. Glucose consumption was measured at 505 nm using a glucose enzymatic kit on an automatic biochemical analyzer. The media was ended to MTT for 4 h. The absorbance (OD) retained by the cell lysates was determined at 490 nm by Multiskan Spectrum.

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