Design, Synthesis, and Biological Evaluation of Novel Pyrrolo[2,3-b] Pyridine Derivatives for Nonalcoholic Fatty Liver Disease

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Nonalcoholic fatty liver disease (NAFLD) is featured with liver fat infiltration and accumulation with no connection with overdrinking. With the rising trends and no FDA-approved drugs, NAFLD attracts global attention. In this study, we successfully synthesized 18 novel SIS3 derivatives and evaluated them for pharmacological activity. F-9 and F-15 had potent weight-losing effects. Importantly, intraperitoneal administration of F-9 at 10 mg·kg⁻¹·day⁻¹ for 5 weeks had the most excellent effects to reduce the weight of the body, liver, and fat, as well as adjusting serum levels of AST, ALT, TC, TG, HDL, and LDL. H&E staining showed that F-9 could suppress fat deposition and increase the adipocyte size in the liver.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is one of the most common chronic liver diseases, and the global prevalence is estimated at 24% [1]. NAFLD is a clinicopathological hepatic steatosis, featured with liver fat infiltration and accumulation with no connection with overdrinking [2]. Many diseases, including obesity, insulin resistance, inflammation, and type 2 diabetes, are closely linked to NAFLD [3–5]. Currently, the pathogenesis of NAFLD developing from “double hit” to “multiple hit,” is still multifactorial and unclear [6, 7]. Researchers mostly pick metabolism, inflammation, and fibrosis as targets to treat this disease [8, 9]. However, there are no FDA-approved drugs for NAFLD so far. A better understanding of the NAFLD pathogenesis has led to the clinical research of various potential drugs for the treatment of NAFLD. These investigational drugs target several aspects of metabolic disruption, oxidative stress, inflammation, and/or inflammatory signaling [10]. Seemingly, because of the complex, multidirectional pathophysiology involved in NAFLD, the perfect animal model representing the complete NAFLD spectrum in a workable time frame does not exist. The investigators should have a clear perception of what they are studying and should choose the best-suited animal model relative to their research goal, taking into account NAFLD comorbidities, grade of fibrosis, and the possible development of hepatocellular carcinoma (HCC) [11].

SIS3 is a selective inhibitor of Smad3 and can significantly inhibit Smad3 phosphorylation, which was first reported in 2006 [12]. Currently, research studies on SIS3 mainly focus on antifibrosis, antiapoptosis, anti-inflammation, and inhibiting cancer progression [13–15]. Wang et al. reported that SIS3 can not only significantly reduce the bodyweight, fat mass, and fasting blood glucose in high-fat diet-induced type 2 diabetes model mice but also improve insulin sensitivity and oral glucose tolerance of high-fat diet-induced type 2 diabetes model mice [16]. Therefore, it may be effective to use SIS3 in the treatment of NAFLD.
In this study, we designed and synthesized 18 novel SIS3 derivatives utilizing the principle of bioelectronic isobaric (Table 1) to test their biological activity in the treatment of NAFLD.

2. Materials and Methods

2.1. Preparation Procedure of the Target Compounds F Series. The novel SIS3 derivatives were prepared following the synthetic route as shown in Scheme 1. The preparation of 2-phenyl-1H-pyrazole [2,3-b] pyridine (1-1) used tetrahydrofuran, benzonitrile and LDA as starting materials. In the second step, amine hydrogen of 1-1 was replaced by methyl. Compound 1–3 was obtained by Vilsmeier–Haack reaction. Then, through Knoevenagel condensation reaction, we got α, β-unsaturated carboxylic acid derivative (1–4). The target compounds F series were obtained by amidation reaction of 1,4 and different amines with EDCI and HOBT. All copies of the spectra for compounds 1-1, 1-2, 1-3, 1-4 and F series are available in the Materials and Methods section.

2.2. Bioassays

2.2.1. In Vivo Efficacy for NAFLD. Male 4-week-old C57BL/6J mice were provided by Beijing Hualian Physiology Research Inc., in China. The mice were fed a normal diet or a high-fat diet (HFD) (60% calories from fat) for 90 days. Then, the HFD mice were randomly divided into 20 groups (model group, vehicle group, and 18 treatment groups) to evaluate the effects of all compounds. After 15 days of continuous administration, the mice’s bodyweight, fasting blood glucose, and intraperitoneal glucose tolerance (IGTT) were measured. According to the results of pharmacological evaluation above, F-9 and F-15 were selected for further in vivo experiments.

We randomly divided 30 NAFLD model mice into 6 groups (model group, vehicle group, F-9 low-dose group: 10 mg/kg, F-9 high-dose group: 50 mg/kg, F-15 low-dose group: 10 mg/kg and F-15 high-dose group: 50 mg/kg). The mice’s bodyweight, fasting blood glucose, and intraperitoneal glucose tolerance (IGTT) were measured. According to the results of pharmacological evaluation above, F-9 and F-15 were selected for further in vivo experiments.

2.2.2. Intraperitoneal Glucose Tolerance Test (IGTT). The mice were weighed after 35 days of treatment after fasting for 15 h, the mice were intraperitoneally injected with 20% glucose solution (0.01 mL/g). Also, then, the blood glucose of each mouse was measured and recorded at 0, 30, 60, and 120 min, respectively.

2.2.3. Insulin Tolerance Test (ITT). The mice were fasted for 4 h after IGTT, and then the insulin (0.05 U/mL) was injected intraperitoneally (injection volume was calculated according to the weight of the mice, 0.01 mL/g), and blood glucose was measured and recorded at 0, 30, 60, and 120 min, respectively.

2.2.4. Detection of Blood Biochemical Index. Mice blood samples were collected at the end point of treatment, and serum alanine aminotransferase (ALT), aspartic aminotransferase (AST), total cholesterol (TC), triglycerides (TG), HDL-cholesterol (HDL), LDL-cholesterol (LDL), total protein (TP), and glucose (GLU) were analyzed using Olympus AU5400 automatic chemical system instrument (Olympus).

2.2.5. Hematoxylin-Eosin (H&E) Staining. The liver samples were fixed in formalin and embedded in wax. 3 μm sections were obtained from each paraffin block using a microtome and stained with H&E. The pathological state of liver tissue was observed under a light microscope.

2.2.6. Statistical Analysis. The data were expressed as mean ± SD of three independent experiments. The two-tailed Student’s t-test was used for statistical analysis and statistically significant P values were labeled as follows: *P < 0.05; **P < 0.01; ***P < 0.001.

3. Results and Discussion

3.1. Chemistry

3.1.1. General Experimental. Nonaqueous reactions were performed in oven-dried round bottom flasks under an inert nitrogen atmosphere. Commercial reagents were purchased from Sigma-Aldrich, Alfa Aesar, or TCI Chemicals. Reaction progress was monitored by thin-layer chromatography (TLC) using UV light (254 nm) as a visualizing agent. Flash chromatography was performed on silica gel (200–300 mesh) with EtOAc/P/PE or DCM/MeOH as eluent. 1H NMR spectra were recorded on a Bruker AMX400 (400 MHz) NMR spectrometer. Dimethyl sulfoxide (DMSO) was purchased from Tianjin Kemio Chemical Reagent Co. Ltd., in China. PEG400 were obtained from Sigma-Aldrich, (Merck KGaA, Darmstadt, Germany). Glucose was purchased from Jinshan Chemical Reagent Co. (Chengdu, China). Novolin R (Biosynthetic Human insulin) was prepared by Novo Nordisk A/S, Copenhagen. The remaining chemicals and reagents were supplied from commercial sources.

3.1.2. Preparation of Compound 1-1. To a solution of 3-methylpyridine (5 g) in tetrahydrofuran (100 mL), the lithium diisopropylamide (LDA) solution was added dropwise under nitrogen at 0°C.

After stirring for 0.5 h, benzonitrile (5 g) was added dropwise into the above solution. The reaction mixture was stirred at 0°C for 1.5 h and then was allowed to reach 40°C while stirring for another 4 h. The reaction was quenched with water and extracted with EtOAc. The combined organic layers were dried over Na2SO4 and concentrated to give a crude product. The crude was purified by column
Table 1: SAR for novel SIS3 derivatives.

|   |   |   |   |   |
|---|---|---|---|---|
| F-1 | F-2 | F-3 | F-4 |
| F-5 | F-6 | F-7 | F-8 |
| F-9 | F-10 | F-11 | F-12 |
| F-13 | F-14 | F-15 | F-16 |
| F-17 | F-18 | F-19 | F-20 |
chromatography (PE: EA = 2:1) to yield compound 1-1 (3.5 g, 33.7%). $^1$H NMR (400 MHz, CDCl$_3$): δ 12.68 (s, 1H), 8.31 (dd, $J$ = 4.8, 1.4 Hz, 1H), 7.97 (dd, $J$ = 7.8, 1.3 Hz, 1H), 7.94 to 7.89 (m, 2H), 7.53 (t, $J$ = 7.7 Hz, 1H), 7.40 (t, $J$ = 7.4 Hz, 1H), 7.11 (dd, $J$ = 7.8, 4.8 Hz, 1H), 6.80 (s, 1H).

3.1.3. Preparation of Compound 1-2. To the solution of compound 1-1 (3.5 g) in DMF (100 mL) was added NaH (1 g) under nitrogen. After stirring at 0 °C for 10 min, CH$_3$I was slowly added dropwise and stirred at room temperature overnight. The reaction was quenched with water and extracted with EtOAc. The combined organic layers were dried over Na$_2$SO$_4$. The crude product was purified by column chromatography to give a pale-yellow product (2.8 g, 75%).

3.1.4. Preparation of Compound 1-3. To the solution of compound 1-2 (2 g) in DMF (100 mL), POCl$_3$ (2.4 mL) was slowly added under nitrogen and stirred at 0 °C for 3 h. After the reaction was completed, the pH was adjusted to higher than 14 with a 1N NaOH solution. The reaction was washed with water and extracted with EtOAc. The combined organic layers were dried over Na$_2$SO$_4$. The solvent was removed via rotary evaporation. The crude product was purified by column chromatography to obtain the corresponding amine (0.6eq) was added. The reaction was stirred for 0.5 h at room temperature, and then the corresponding amine (0.6eq) was added. The reaction was washed with water and extracted with EtOAc. The combined organic layers were dried over Na$_2$SO$_4$ and removed via rotary evaporation. The crude product was purified by column chromatography to obtain corresponding products.

3.1.5. Preparation of Compound 1-4. To the solution of compound 1-3 (2.8 g) in pyridine (30 mL) were added hexahydropyridine (7 mL) and malonic acid (1.5 g). Then, the mixture was stirred under reflux for 4 h. After the reaction was completed, the pH was adjusted to 6 with 1N HCl solution. The reaction was washed with water and extracted with EtOAc. The combined organic layers were dried over Na$_2$SO$_4$ and removed via rotary evaporation, and the crude product was purified by column chromatography to obtain compound 1-4 (2.8 g, 87.5%). $^1$H NMR (400 MHz, DMSO-$d_6$): δ 10.17 (s, 1H), 8.52–8.41 (m, 2H), 7.70–7.61 (m, 3H), 7.60–7.54 (m, 2H), 7.48 (d, $J$ = 16.0 Hz, 1H), 7.35 (dd, $J$ = 7.9, 4.8 Hz, 1H), 6.45 (d, $J$ = 16.0 Hz, 1H), 3.71 (s, 3H).

3.1.6. General Procedure for Compounds F Series. To the solution of compound 1-4 (1eq) in DMF were added EDCI (3eq), HOBT (1eq), and triethylamine (3eq). The mixture was stirred for 0.5 h at room temperature, and then the corresponding amine (0.6eq) was added. The reaction was washed with water and extracted with EtOAc. The combined organic layers were dried over Na$_2$SO$_4$ and removed via rotary evaporation. The crude product was purified by column chromatography to obtain the corresponding products.

F-1: $^1$H NMR (400 MHz, CDCl$_3$) δ 8.43 (d, 1H), 8.25 (s, 1H), 7.77 (d, 1H), 7.54 (q, 4H), 7.44 (d, 2H), 7.24 (s, 2H), 7.15 (d, 1H), 6.51 (d, 1H), 3.76 (s, 3H), 2.88 (dd, 4H), 2.07 (m, 2H).
F-2: $^1$H NMR (400 MHz, CDCl$_3$) δ 8.42 (d, 1H), 8.22 (s, 1H), 7.77 (d, 1H), 7.54-7.42 (m, 6H), 7.33 (s, 1H), 7.20 (m, 1H), 7.12 (d, 2H), 6.52 (d, 1H), 3.75 (s, 3H), 2.31 (s, 3H).
F-3: $^1$H NMR (400 MHz, CDCl$_3$) δ 8.43 (d, 1H), 8.24 (d, 1H), 7.78 (d, 1H), 7.55 (d, 5H), 7.44 (d, 2H), 7.34 (s, 1H), 7.28 (s, 1H), 7.22 (m, 1H), 6.49 (d, 1H), 3.76 (s, 3H).
F-4: $^1$H NMR (400 MHz, CDCl$_3$) δ 8.72 (d, $J$ = 4.4 Hz, 1H), 8.50 (d, $J$ = 4.6 Hz, 1H), 8.42 (d, $J$ = 8.4 Hz, 1H), 8.35 (d, $J$ = 8.1 Hz, 1H), 8.02 (d, $J$ = 15.8 Hz, 1H), 7.55-7.40 (m, 7H), 7.34 (dd, $J$ = 7.8, 4.9 Hz, 1H), 6.69 (d, $J$ = 15.8 Hz, 1H), 3.81 (s, 3H).
F-5: $^1$H NMR (400 MHz, CDCl$_3$) δ 8.43 (d, $J$ = 4.6 Hz, 1H), 8.24 (d, $J$ = 7.4 Hz, 1H), 7.79 (d, $J$ = 15.5 Hz, 1H), 7.56 (dd, $J$ = 23.2, 6.9 Hz, 4H), 7.43 (d, $J$ = 6.7 Hz, 2H), 7.38-7.29 (m, 2H), 7.25-7.17 (m, 1H), 7.09 (t, $J$ = 7.3 Hz, 1H), 6.52 (d, $J$ = 15.4 Hz, 1H), 3.76 (s, 3H).

Scheme 1: Synthetic route of F series. a: LDA, THF, 0 °C, 24 h. b: CH$_3$I, NaH, DMF, rt, 24 h. c: POCl$_3$, DMF, 0 °C, 24 h. d: hexahydropyridine, malonic acid, Py, refluxed, 4 h. e: EDCI, HOBT, triethylamine, rt, 0.5 h.
Figure 1: Continued.
Figure 1: Preliminary efficacy test. (a, b) Bodyweight and Blood glucose changes in mice. (c–f) Intraperitoneal glucose tolerance test (IGTT) evaluation.
Figure 2: Bodyweight, the ratio of liver weight to bodyweight and the ratio of fat weight to bodyweight were tested. (a) Bodyweight changes in mice at different times. (b) Bodyweight changes in mice in different groups. (c, d) The ratio of liver weight to bodyweight and the ratio of fat weight to bodyweight were detected (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).
Figure 3: Fasting blood glucose, intraperitoneal injection glucose tolerance (IGTT), and insulin tolerance (ITT) were measured (* p < 0.05, ** p < 0.01, *** p < 0.001).
F-6: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.85 (d, $J = 8.7$ Hz, 1H), 8.79 (s, 1H), 8.48 (m, 2H), 8.11 (d, $J = 15.5$ Hz, 1H), 7.73 (s, 1H), 7.60–7.38 (m, 6H), 6.71 (d, $J = 15.5$ Hz, 1H), 3.82 (s, 3H).

F-7: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.85 (d, $J = 8.4$ Hz, 1H), 8.79 (s, 1H), 8.48 (m, 2H), 8.11 (d, $J = 15.2$ Hz, 1H), 7.73 (s, 1H), 7.61 (m, 3H), 7.50 (s, 2H), 7.38 (s, 1H), 6.71 (d, $J = 15.5$ Hz, 1H), 3.82 (s, 3H).

Figure 4: Contents of AST, ALT, TC (total cholesterol), TG (triglyceride), HDL, and LDL in serum. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).
F-8: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.85 (d, $J$ = 8.6 Hz, 1H), 8.79 (d, $J$ = 3.6 Hz, 1H), 8.49 (dd, $J$ = 8.3, 4.2 Hz, 2H), 8.11 (d, $J$ = 15.5 Hz, 1H), 7.64–7.45 (m, 7H), 6.89 (d, $J$ = 15.5 Hz, 1H), 3.82 (s, 3H), 2.53 (s, 3H).

F-9: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.49 (dd, $J$ = 8.3, 4.2 Hz, 3H), 8.04 (dd, $J$ = 16.4, 8.0 Hz, 2H), 7.76 (t, $J$ = 7.8 Hz, 1H), 7.65–7.59 (m, 3H), 7.53–7.45 (m, 2H), 7.37 (dd, $J$ = 7.8, 4.9 Hz, 1H), 3.81 (s, 3H), 2.92 (d, $J$ = 29.7 Hz, 3H).

F-10: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.85 (d, $J$ = 8.7 Hz, 1H), 8.79 (d, $J$ = 3.6 Hz, 1H), 8.49 (dd, $J$ = 8.3, 4.2 Hz, 2H), 8.11 (d, 1H), 7.63–7.45 (m, 7H), 6.89 (d, $J$ = 15.5 Hz, 1H), 3.82 (s, 3H), 2.31 (s, 3H).

F-11: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.44 (d, $J$ = 4.7 Hz, 1H), 8.23–8.13 (m, 4H), 7.81 (d, $J$ = 15.5 Hz, 1H), 7.56 (d, $J$ = 6.2 Hz, 3H), 7.45 (d, $J$ = 7.5 Hz, 2H), 6.85 (d, $J$ = 4.9 Hz, 1H), 6.51 (d, $J$ = 15.6 Hz, 1H), 3.77 (s, 3H), 2.36 (s, 3H).

F-12: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.85 (d, $J$ = 8.2 Hz, 1H), 8.79 (d, $J$ = 3.6 Hz, 1H), 8.49 (dd, $J$ = 8.3, 4.2 Hz, 2H), 8.11 (d, $J$ = 15.5 Hz, 1H), 8.04 (d, $J$ = 14.9 Hz, 1H), 7.62–7.45 (m, 7H), 3.82 (s, 3H), 2.80 (s, 3H).

F-13: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.85 (d, $J$ = 8.7 Hz, 1H), 8.79 (s, 1H), 8.49 (dd, $J$ = 13.1, 6.3 Hz, 2H), 8.12 (d, $J$ = 12.3 Hz, 2H), 7.61 (d, $J$ = 6.4 Hz, 3H), 7.50 (d, $J$ = 8.1 Hz, 3H), 6.42 (d, $J$ = 8.5 Hz, 1H), 3.82 (s, 3H).

F-14: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.85 (d, $J$ = 8.2 Hz, 1H), 8.79 (d, $J$ = 3.6 Hz, 1H), 8.49 (dd, $J$ = 8.3, 4.2 Hz, 2H), 8.11 (d, $J$ = 15.5 Hz, 1H), 8.04 (d, $J$ = 14.9 Hz, 1H), 7.74–7.38 (m, 5H), 6.69 (d, $J$ = 8.5 Hz, 1H), 3.82 (s, 3H).

F-15: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.85 (d, $J$ = 8.6 Hz, 1H), 8.79 (s, 1H), 8.59–8.36 (m, 2H), 8.11 (d, $J$ = 15.6 Hz, 1H), 7.73 (s, 1H), 7.61 (t, $J$ = 6.8 Hz, 4H), 7.50 (s, 2H), 7.38 (s, 1H), 3.82 (s, 3H).

F-16: $^1$H NMR (400 MHz, DMSO) $\delta$ 8.82 (d, $J$ = 3.4 Hz, 1H), 8.74 (dd, $J$ = 20.9, 8.1 Hz, 2H), 8.53 (s, 1H), 7.85 (d, $J$ = 15.8 Hz, 1H), 7.66–7.56 (m, 6H), 7.45 (d, $J$ = 6.3 Hz, 1H), 6.95 (d, $J$ = 15.5 Hz, 1H), 3.76 (s, 3H).

F-17: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.85 (d, $J$ = 8.4 Hz, 1H), 8.79 (d, $J$ = 4.3 Hz, 1H), 8.49 (dd, $J$ = 12.5, 6.3 Hz, 2H), 8.11 (d, $J$ = 15.6 Hz, 1H), 7.73 (dd, $J$ = 8.4, 4.4 Hz, 1H), 7.62 (t, $J$ = 7.6 Hz, 4H), 7.53–7.45 (m, 2H), 7.38 (dd, $J$ = 7.9, 4.8 Hz, 1H), 3.82 (s, 3H).

F-18: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.85 (d, $J$ = 8.4 Hz, 1H), 8.79 (s, 1H), 8.48 (m, 2H), 8.11 (d, $J$ = 15.6 Hz, 1H), 7.73 (s, 1H), 7.61 (m, 3H), 7.50 (s, 2H), 7.38 (s, 1H), 6.71 (d, $J$ = 8.5 Hz, 1H), 3.82 (s, 3H).

F-19: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.85 (d, $J$ = 8.6 Hz, 1H), 8.77 (s, 1H), 8.48 (m, 2H), 8.11 (d, $J$ = 15.6 Hz, 1H), 7.75 (s, 1H), 7.61 (m, 3H), 7.55 (s, 2H), 7.38 (s, 1H), 6.71 (d, $J$ = 8.5 Hz, 1H), 3.82 (s, 3H).

F-20: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.82 (d, $J$ = 8.7 Hz, 1H), 8.75 (s, 1H), 8.48 (m, 2H), 8.11 (d, $J$ = 15.5 Hz, 1H), 7.73 (d, 1H), 7.66–7.56 (m, 6H), 6.71 (d, $J$ = 8.5 Hz, 1H), 3.82 (s, 3H).

3.2. Bioassays. The preliminary efficacy test was studied by investigating the change of weight and blood glucose of high-fat diet (HFD, 60% of calories derived from fat)-induced NAFLD model rats after treatment, we found F-9 and
F-15 exhibited higher potency of regulating bodyweight and blood glucose than others (Figures 1(a) and 1(b)) (* *p < 0.05, **p < 0.01). The results of the intraperitoneal glucose tolerance test (IGTT) indicated that F-9 and F-15 could significantly antagonize the increase and fluctuation of blood glucose in mice caused by exogenous glucose (Figures 1(c)–1(f)). Therefore, the two compounds were selected for further in vivo pharmacological evaluation.

After 35 days of continuous administration, we found the bodyweight of treatment groups decreased to varying degrees (Figures 2(a) and 2(b)). The mean bodyweight of F-9(10), F-9(50), F-15(10), and F-15(50) was reduced by the loss values of 10.6%, 32.0%, 3.8%, and 14.9%, respectively. Importantly, the ratio of liver weight to bodyweight in the high-dose treatment group was higher, proving that the therapeutic drugs were less toxic to the liver (Figure 2(c)). Meanwhile, the ratio of fat weight to bodyweight (epididymal) of F-9(10), F-9(50), F-15(10), and F-15(50) decreased by 79.1%, 92.5%, 81.0%, and 84.5%, respectively, indicating the adipose tissue was degraded. Especially, F-9(50) was better than other treatment groups (Figure 2(d)).

Because of the close relationship between blood glucose and fat metabolism, we further evaluated the serum level of blood glucose in HFD-induced NAFLD rats. As shown in Figure 3, the serum level of blood glucose increased during the 5 weeks high-fat feeding. After the treatment of F-9 and F-15, the concentration of fasting blood glucose was reduced to the normal level. Among them, F-9 (50) had the lowest blood glucose value, which means that it has the best hypoglycemic effect (Figure 3(a)). To further investigate the integrity of β cell function in pancreatic islets and the ability of the body to regulate blood glucose in high-fat diet-induced obese mice. After 35 days of treatment, IGTT and ITT were tested in mice. Compared with the model group, the treatment group significantly reduced blood glucose. Especially F-9 (50) is even lower than the normal group of mice (Figure 3(b)). In the ITT test, the mice of the treatment groups increased their sensitivity to exogenous insulin. At 120 min, the blood glucose in the F-9 (50) group was the lowest (Figure 3(c)).

We also measured another six serum levels of triglycerides, AST, ALT, total cholesterol, HDL, and LDL, which played a vital role in the fat liver. The levels of AST and ALT in the model group and vehicle group were higher than the normal group, indicating the liver function of model rats was impaired. After 35 days of treatment, compared to the data of the model group, F-9 and F-15 could significantly reduce the serum concentrations of AST, ALT, LDC, HDL, TC, and TG (Figure 4). Importantly, F-9 showed a more potent ability to regulate HFD-induced abnormal serum biomarkers to normal levels than F-15.

According to the result of H&E staining of the liver (Figure 5), the liver of the normal group had no obvious histological changes. However, numerous lipid droplets in liver tissues from HFD-induced NAFLD rats severely ruined liver histology, presented as ballooning degeneration and further led to the formation of steatosis. Fortunately, the liver tissue structure and liver cell size and shape of rats in all treatment groups basically returned to normal, and fatty liver symptoms were alleviated. The most remarkable reduction was observed in the F-9 treatment group.

4. Conclusions

In this study, 18 novel small-molecule SIS3 derivatives have been designed, synthesized, and evaluated for pharmacological activity. F-9 and F-15 were found to possess a potent weight-lowering effect on NAFLD rats. Importantly, intraperitoneal administration of F-9 and F-15 improved effectively the progression of NAFLD and reduced the weight of the body and fat as well as modulating serum parameters of LDL, HDL, ALT, AST, TC, and TG. The results of H&E staining also revealed that F-9 and F-15 reduced fat deposition in liver tissues and suppressed the increase of adipocyte size in adipose tissues from NAFLD rats. Especially, F-9 alleviated the clinical symptoms of obesity and regulated serum biomarkers to appropriate ranges. These surprisingly pharmacological activities demonstrate that F-9 as a candidate constitutes an attractive approach to the treatment of obesity-related NAFLD and is valued for further development.

Data Availability

All the data included in this study are available upon request by contact with the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors’ Contributions

Jialin Ma and Jing Li contributed equally to the article.

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