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A NEW COMPOUND EMBELOSIDE A WITH HYPOGLYCEMIC POTENTIAL FROM THE FRUITS OF *EMBELIA OBLONGIFOLIA* HEMSL.

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Abstract – A total of eleven compounds were isolated from the ripe and dried fruits of *Embelia oblongifolia* Hemsl. Compound **1** was a new one named embeloside A, in addition, compounds **2**-**4**, **6** and **10** were isolated from this genus for the first time. Their molecular structures were elucidated by 1D/2D NMR spectroscopic analysis, HR-ESI-MS spectral data and charged aerosol detector (CAD). Further, hypoglycemic activity evaluation showed that compound **1** could reduce the fasting blood glucose levels in diabetic rats. Therefore, the results suggest that compound **1** might provide a theoretical basis for the development of potential hypoglycemic drugs. The discovery of compounds **1**-**11** might provide experimental guidance for the further development of *Embelia oblongifolia* Hemsl.

In recent years, increasing researches have focused on the evaluation of traditional ethnic medicinal materials. *Fructus Embeliae*, the dry and ripe fruit of *Embelia oblongifolia* Hemsl. of the Myrsinaceae family, is a commonly used Chinese ethnic medicine widely distributed in Yunnan, Guangxi, Jiangxi Province and other places in China. In addition, it is also distributed in some regions in Southeast Asia.[1,2] As a common medicine used in ethnic minorities, modern pharmacological studies have indicated that it has various pharmacological activities, including anti-tumor, anti-inflammatory and bacteriostatic as well as hypoglycemic.[3,4] Diabetes mellitus is a medical and social problem caused by the rapid spread of the disease and the development of serious complications, which significantly reduce the quality and life

expectancy of patients.[5] Considering various factors, today's research also discovered that medicines of natural source are potential hypoglycemic agents for their low cost, high efficacy and low toxicity.[6] In previous research, studies on *Embelia oblongifolia* Hemsl. at home and abroad mainly focused on the chemical composition of roots and leaves of it,[7,8] whereas relatively few studies on the fruit part were found and the detailed information regarding its chemical composition was unavailable.

Furthermore, the search for medicinal plants with a long history of use and small side effects is of great interest to our society. Thus, in this paper, *Embelia oblongifolia* Hemsl. was selected as the research object and 11 compounds were isolated by systematic chemical extraction from it. One of the specific objectives of our study was to conduct the hypoglycemic effect assays of the new compound, so we reported on the structural elucidation of all the isolated compounds based on ^{1}H-NMR and ^{13}C-NMR spectroscopy, and on the pharmacological activity results of the new compound **1** on treating the cognitive dysfunction and social behavioral disorder of experimental rats.

Eleven compounds (**1–11**) were isolated from the fruits part of *Embelia oblongifolia* Hemsl. by a combination of chromatographic techniques (**Figure 1**).

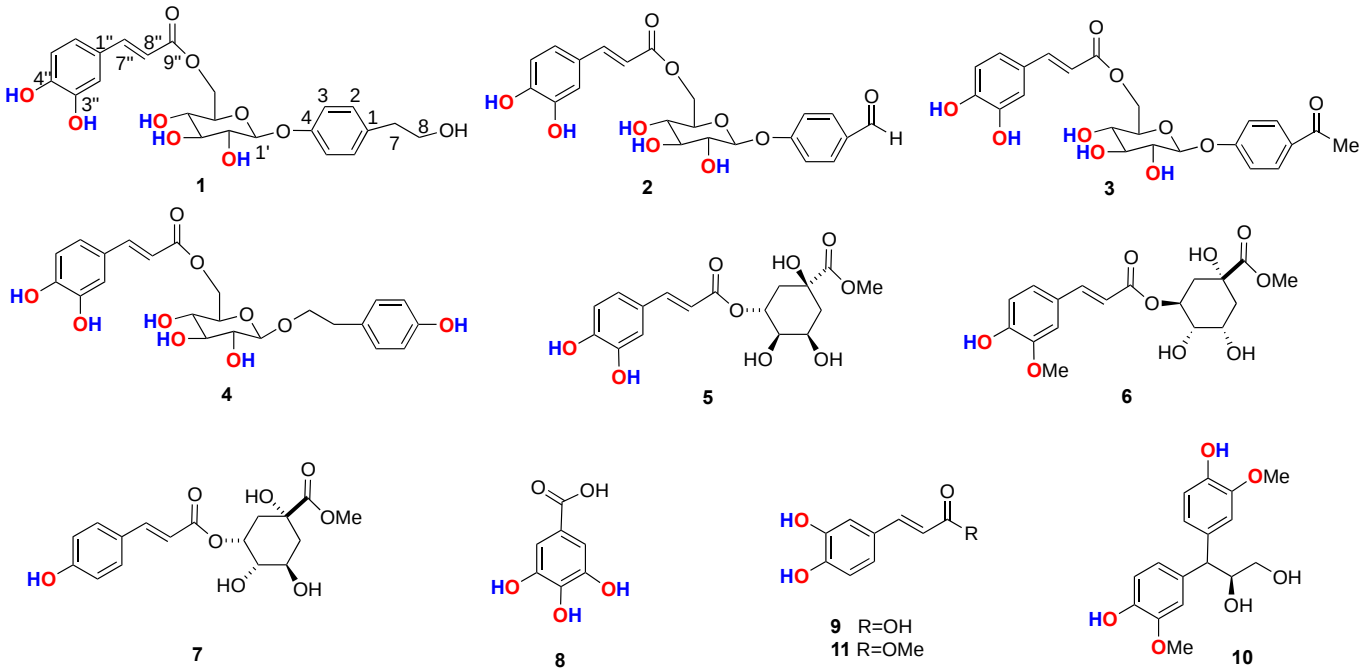

Figure 1. Structures of compounds **1–11** from *Embelia oblongifolia* Hemsl.

Compound **1** was isolated as an oily solid (MeOH) with dark spots at UV 254 nm and a gray dark spot upon exposure to a 10% solution of EtOH-H_2SO_4 (v/v). The ^{1}H-NMR (400 MHz, DMSO-d_6) spectrum of compound **1** (**Table 1**) displayed characteristic signals of trans double bonds at δ_H 7.47 (1H, d, J = 15.9 Hz) and 6.25 (1H, d, J = 15.9 Hz). Combined the hydrogen signals at δ_H 7.06 (1H, d), δ_H 6.99 (1H, dd, J = 8.1, 1.5 Hz) and 6.77 (1H, d, J = 8.1 Hz) presumably there was a 1,2,4-substituted benzene ring structure.

Meanwhile, on the basis of δ_H 7.06 (2H, d) and δ_H 6.92 (2H, d, J = 8.6 Hz), we speculated that there was a 1,4-substituted benzene ring structure also, and the ^{13}C-NMR data further validated our conjectures. A typical terminal hydrogen signal appeared at δ_H 4.84 (1H, d, J = 7.3 Hz) was also observed, it suggested that there might be a glucose structure in the structure. Along with ^{13}C-NMR spectrum exhibited the presence of two connected methylene groups in the structure were at δ_H 3.50 (2H, t, J = 7.1 Hz) and 2.59 (2H, t, J = 7.1 Hz), and according to the chemical shift values of the two were relatively large, thus we speculated that they should be connected with the benzene ring or hydroxyl group.

Table 1. ^{1}H-NMR and ^{13}C-NMR spectral data of compound **1** in DMSO-d_6 (δ in ppm, J in Hz)

No.	**1**	
	δ_H (J in Hz)	δ_C
1		132.85
2	7.06 (2H, d)	129.58
3	6.92 (2H, d, 8.6)	116.00
4		155.42
5	6.92 (2H, d, 8.6)	116.00
6	7.06 (2H, d)	129.58
7	2.59 (2H, t, 7.1)	38.11
8	3.50 (2H, t, 7.1)	62.23
1’	4.84 (1H, d, 7.3)	100.44
2’	3.25 (1H, m)	73.15
3’	3.28 (1H, m)	76.39
4’	3.22 (1H, m)	70.00
5’	3.64 (1H, m)	73.72
6’	4.43 (1H, m)/4.16 (1H, dd, 11.9, 7.0)	63.33
1’’		125.29
2’’	7.06 (1H, d)	115.74
3’’		145.66
4’’		148.63
5’’	6.77 (1H, d, 8.1)	114.69
6’’	6.99 (1H, dd, 8.1, 1.5)	121.33
7’’	7.47 (1H, d, 15.9)	145.21
8’’	6.25 (1H, d, 15.9)	113.67
9’’		166.30

According to ^{13}C-NMR spectrum (100 MHz, DMSO-d_6) which displayed 23 carbon signals, incorporating the ^{1}H-NMR data we analyzed, the structure of the *para* substituted benzene ring was determined by the carbon signals which were at δ_C 129.58 (C-2, C-6) and 116.00 (C-3, C-5). Whereas the existence of an ABX coupling system was validated by the carbon signals at δ_C 121.33 (C-6''), 115.74 (C-2'') and 114.72 (C-5''), combining the signals of a *trans* double bond and δ_C 166.30 (C-9'') also demonstrated the presence of a caffeic acid structure, at the same time we conjectured it to be attached to the glucopyranose ring. In addition, a methylene group (C-7) was attached at the para substituted benzene ring and followed by a -CH_2OH, which was illustrated by at δ_C 63.33 (C-8) and 38.11 (C-7).

To further ascertain the linkage sequence of compound **1**, and to confirm whether the two methylene groups were linked to the glucopyranose ring or were in the para position, the deduction was confirmed through an HSQC experiment, in which the correlations of δ_C 38.11 (C-7) with δ_H 2.59 (H-7) and δ_C 62.23 (C-8) with δ_H 3.50 (H-8) demonstrated two directly linked methylene groups. The HSQC correlations of δ_C 70.00 (C-4') with δ_H 3.22, δ_C 73.15 (C-2') with δ_H 3.25 (H-2') and δ_C 76.39 (C-3') with δ_H 3.28 (H-3') indicated signals at the three locations of 2 ', 3', 4 'on glucose, respectively, while its specific connectivity sequence needed to be judged in combination with HMBC spectrum. Besides the above-mentioned signals on glucose, the correlations of δ_C 73.72 (C-5') with δ_H 3.64 (H-5') and δ_C 63.33 (C-6') with δ_H 4.16 and 4.43 also suggested signals on glucose (C-5' and C-6'). In the low-field region, the carbon signals on the end groups were confirmed by the correlations from δ_C 100.44 (C-1') to δ_H 4.84 (H-1'), apart from these characteristic signals, resonances for a *trans* double bond was also observed and its position was assigned by the correlations between δ_C 113.67 (C-8'') with δ_H 6.25 and δ_C 145.21 with δ_H 7.47. The linkage positions of the two methylene groups were confirmed by HMBC spectrum, in which the oxophenyl ring carbon signal (δ_C 155.52) showed correlation with the terminal hydrogen signal (δ_H 4.84), indicating that a C-glycoside structure was attached at the 4-position of the benzene ring, whereas the methylene groups (C-7, C-8) should be linked to the 1-position.

Taken together, these spectral data revealed that the structure of compound **1** was a *p*-hydroxyphenylethyl alcohol. Its molecular formula of $C_{23}H_{26}O_{10}$ was determined from HR-ESI-MS showing the molecular ion at *m/z* 461.1506 $[M-H]^-$ (calcd 462.1526).

The configuration of glucose was determined by sugar hydrolysis experiments. 5 mg of compound **1** was taken and configured into 2 mol/L solution of HCl. Samples were prepared by taking 5 mL of the configured solution, heating reflux in the oil bath at 100 °C for 2 h (magnetic stirring), extracting three times with CH_2Cl_2 after complete hydrolysis, discarding the lower solution, and adding water constantly to the aqueous layer to distill under reduced pressure to pH neutrality. The determination of sugar configuration was performed on a BEH HILIC C_{18} column coupled with charged aerosol detector (CAD). The column was eluted by using a gradient elution of 5%−40% 0.1% formic acid and 95%−60% acetonitrile at a flow rate

of 0.6 mL/min. The column temperature was maintained at 40 °C, and the injection volume was 4.0 μL. Peak time alignments were performed on the optical rotatory liquid phase with sugar standards. After comparing the retention time of standard samples and compound **1** in chromatograms（**Figure S6**), as well as peak orientations, the sugar unit of compound **1** was identified as *D*-glucopyranoside.

Compound **1** was identified as a new compound not reported in the literature by SciFinder search, so it was elucidated as a new one with the name embeloside A.

Furthermore, ten known compounds were also isolated and identified from their spectroscopic data following comparison with those reported in the literature. They were elucidated as: 6'-*O*-caffeoyl-*p*-hydroxybenzaldehyde-4-*O*-*β*-*D*-glucopyranoside (**2**),[9] 6'-*O*-caffeoyl-*p*-hydroxyacetophenone-4-*O*-*β*-*D*-glucopyranoside (**3**),[10] 6'-*O*-caffeoyl-*p*-hydroxyphenylethyl-4-*O*-*β*-*D*-glucopyranoside (**4**),[11,12] methyl chlorogenate (**5**),[13] 3-*O*-feruloylquinate (**6**),[14] 5-*O*-*p*-*trans*-coumaroylquinic acid methyl ester (**7**),[15] gallic acid (**8**),[16] caffeic acid (**9**),[17,18] (2'*R*)-3',3'-di-(4-hydroxy-3-methoxyphenyl)propane-1',2'-diol (**10**),[19] and caffeic acid methyl ester (**11**).[20]

HYPOGLYCEMIC EFFECT ASSAY

The experimental data of the research group show that the blood glucose levels of the drug administered rats were significantly lower compared with those of the model rats after intragastric injection of high (800 $mg \cdot kg^{-1} \cdot d^{-1}$, 80 mg/mL), medium (400 $mg \cdot kg^{-1} \cdot d^{-1}$, 40 mg/mL) and low (200 $mg \cdot kg^{-1} \cdot d^{-1}$, 20 mg/mL) doses of compound **1** into diabetic model rats, and the blood glucose level of the medium dose group was close to that of the positive control drug (Metformin) group (**Table 2**).

Table 2. Effects of compound **1** on blood glucose values in diabetic rats (mean±SD)

Group	Blood glucose values（mmol/L）				
	0 W	1 W	2 W	3 W	4 W
1	7.41±2.43	6.90±0.68	8.62±1.56	7.54±0.34	8.04±0.43
2	23.44±4.76**	25.55±3.55**	28.57±3.32**	26.93±3.46**	28.97±3.66**
3	23.43±6.54	21.34±4.49	21.06±3.51$^{\#}$	19.52±3.45$^{\#}$	18.75±3.37$^{\#}$
4	22.73±5.31	21.03±4.49	22.09±3.34$^{\#}$	20.98±4.57$^{\#}$	21.92±3.43$^{\#}$
5	23.5±4.45	25.83±3.51	23.97±4.39	24.56±3.47	24.55±3.60
6	23.7±3.46	20.61±3.52	18.88±3.38$^{\#}$	18.06±3.39$^{\#}$	16.86±3.49$^{\#}$

1: the control group; 2: the model group; 3: high-dose group; 4: medium-dose group; 5: low-dose group; 6: the positive drug control group.

$^{*}P < 0.05$, $^{**}P < 0.01$ when compared to normal; $^{\#}P < 0.05$ when compared to diabetic.

These above findings showed that compound **1** could improve the symptoms of diabetes in experimental rats with a hypoglycemic effect. No statistically significant difference (P>0.05) between the results in the average speed of swimming of the model rats and that of the control group was observed by the Morris water maze test (MWM), and interference of the swimming speed with the escape latency could be eliminated (**Table 3**). And the escape latency parameters in MWM test were presented in the **Table 4**, indicating that rats of the model group searched for the platform more than approximately 2 times longer than that of the control group (P<0.01). At the same time, further observation of rats in the administered and positive drug groups revealed that the escape latencies of them were significantly shorter compared with the model group rats (P<0.05). In the spatial exploration test, compared with the control group, there was no statistical difference in the mean swimming speed of rats in the model group (P>0.05), while the number of platform crossings and the activity time around the platform of rats in the model group were significantly decreased (P<0.05). Meanwhile, there was no statistical difference in the mean swimming speed of rats in the administered and positive drug groups (P>0.05), and we also observed the significant increase both in their numbers of platform crossings and activity time around the platform compared with the model group rats (P<0.05) (**Table 5**).

Together, these data indicated that diabetes made the cognitive function of rats impaired, and the results experimentally substantiated the positive effect of compound **1** on the cognitive dysfunction exhibited by diabetic rats to some extent.

Table 3. Effects of compound **1** on swimming speed in diabetic rats (mean±SD)

Group	n	Swimming speed（$mm•s^{-1}$）
the control group	20	207.34±34.50
the drug administration group	20	223.69±52.66
the positive drug control group	20	224.13±42.88
the model group	20	222.28±54.02

$^{*}P < 0.05$, $^{**}P< 0.01$ when compared to normal; $^{\#}P< 0.05$ when compared to diabetic.

Table 4. Effect of compound **1** on escape latency of diabetic rats in the MWM test (mean±SD)

Group	n	Escape latency（s）
the control group	20	28.34±30.13
the drug administration group	20	$45.22±25.84^{\#}$
the positive drug control group	20	$32.48±29.34^{\#}$
the model group	20	$68.34±24.50^{*}$

$^{*}P < 0.05$, $^{**}P< 0.01$ when compared to normal; $^{\#}P< 0.05$ when compared to diabetic.

Table 5. Effect of compound **1** on diabetic rats in the spatial exploration test (mean±SD)

Group (n=20)	Mean swimming speed（mm•s^{-1}）	Number of platform crossings	Activity time around the platform（s）
1	213.19±34.61	4.3±1.3	20.39±5.87
2	222.83±41.10	2.3±1.7$^{\#}$	14.34±1.95$^{\#}$
3	217.85±38.52	3.1±1.7$^{\#}$	15.83±2.89$^{\#}$
4	218.32±36.14	1.7±1.6*	10.94±4.07*

1: the control group; 2: the drug administration group; 3: the positive drug control group; 4: the model group.
*P < 0.05, **P< 0.01 when compared to normal; $^{\#}$P< 0.05 when compared to diabetic.

The parameters in the social interaction behavior test were presented in the **Table 6**, which showed that there was a significant difference between the rats in the control group and the model group. Specifically, the control group increased the time and frequency of social interaction compared with the model group, which indicated that diabetes impaired social behavior in rats. By comparing the model group with the positive drug and administration group, we found that both of the positive drug and administration group increased significantly the number of social interaction, as well as extended the duration of social behavior after treatment. All of above results demonstrated that rats in the model group with diabetes had social behavior disorders, which could be treated to some extent by compound **1**.

Table 6. Effect of compound **1** on social interaction of diabetic rats (mean±SD)

Group (n=20)	Frequency of contacts with metal cage	Time of contacts with metal cage (s)
the control group	11.34±3.03	90.53±30.28
the drug administration group	7.08±1.55$^{\#}$	50.67±13.48$^{\#}$
the positive drug control group	8.72±1.45$^{\#}$	65.29±17.44$^{\#}$
the model group	4.58±1.21**	31.32±12.95**

*P < 0.05, **P< 0.01 when compared to normal; $^{\#}$P< 0.05 when compared to diabetic.

Towards the goal of targeted search for new bioactive natural products, a solvent extract of *Embelia oblongifolia* Hemsl. has afforded eleven compounds, among them embeloside A (**1**) was a new compound not previously reported and compounds **2**-**4**, **6** and **10** were isolated from this genus for the first time. The results of pharmacological activity assays showed that compound **1** had significant hypoglycemic effects in diabetes rats. Taken together, the above findings enriched the content of chemical compounds in the

genus of *Embelia* plants, as well as laid a theoretical foundation for exploitation of *Embelia oblongifolia* Hemsl. extracts as a promising candidate for further research on the treatment of diabetes mellitus.

EXPERIMENTAL

General experimental procedures. The NMR spectral data were recorded on Bruker AV-600 (400 MHz for ^{1}H and 100 MHz for ^{13}C) and Bruker-ARX-400 (400 MHz for ^{1}H and 100 MHz for ^{13}C) with tetramethylsilane (TMS) as the internal standard (Bruker Co.). High-performance liquid chromatography (HPLC) was performed on Shimadzu LC-10ATvp with analytical columns (YMC-Pack ODS-A, 5 μm, 250 × 4.6 mm; Diamons C_{18}, 5 μm, 250 × 4.6 mm), Shimadzu LC-8A with a semi-preparative column (YMC-Pack ODS-A, 5 μm, 250 × 10 mm), and Middle-pressure liquid chromatography (Agela Technologies Inc.). Samples were concentrated in a SB-35 rotary evaporator (Physicochemical Co., Tokyo, Japan). The HR-ESI-MS data were obtained on the Micromass AutoSpec-UltimaE TOF mass spectrophotometer (Bruker Co. Ltd.). The determination of sugar configuration was performed on an ACQUITY UPLC BEH HILIC C_{18} column (2.1 mm × 100 mm, 1.7 μm, Waters). A blood glucose meter was obtained from Johnson Co. (New Brunswick, NJ, USA).

Plant material. *Embelia oblongifolia* Hemsl. was purchased from Qinghai Chinese medicinal market, in northwest of China, and identified by Research Associate Ying Xu (Pharmaceutical Engineering Technology Research Center, Harbin University of Commerce). The voucher specimen (No. Hsd20200906) was deposited at the Pharmaceutical Engineering Technology Research Center, Harbin University of Commerce, China.

Extraction and isolation. The ripe and dried fruits of *Embelia oblongifolia* Hemsl. (30 kg) were extracted under heating reflux three times with 95% industrial EtOH (2 h each time) at room temperature. The extracts were combined and concentrated under reduced pressure, and the resulting extractums were dispersed with hot water and sequentially extracted with equal volumes of petroleum ether, CH_2Cl_2 and EtOAc. Then these extractums were recovered and concentrated under reduced pressure to give 90 g of CH_2Cl_2 extractum and 80 g of EtOAc extractum, respectively. The specific experimental procedures could be seen in **Figure S7** and **S8.**

The EtOAc extract (80 g) was chromatographed on a silica gel column (eluted with CH_2Cl_2/MeOH, from 100:0 to 100:100, v/v) to yield four subfractions (Fr.C-E). Fr.C was eluted by ODS column (MeOH: H_2O, 40:60, v/v) to yield compound **8** (6.3 mg) as well as purified by repeated Sephadex LH-20 to give compound **9** (18 mg). Fr.D separated out white amorphous powders, which was repeatedly washed by MeOH to yield compound **7** (5.2 mg). Fr.E1 was chromatographed on a silica gel column (eluted with CH_2Cl_2/MeOH, 100:10, v/v) and recrystallized from solvent to yield compound **2** (28 mg). And Fr.E1 was further purified by silica gel column (eluted with CH_2Cl_2/MeOH, 100:7, v/v) and HPLC (MeOH:H_2O, 39:61, v/v) to afford

compounds **1** (7 mg), **3** (4.6 mg), **4** (7 mg) and **6** (13.4 mg), while Fr.E2 was further purified by Sephadex LH-20 to afford compound **5** (25 mg).

The CH_2Cl_2 extract (90 g) was chromatographed on a silica gel column eluting with petroleum ether–acetone (100:0-100:100, v/v), and we found that a large number of crystals and oils appeared in the 100:0-100:5 flow fractions, after thin-layer chromatography (TLC) and polarity judging this part of the compounds as the fatty acids and a large amount of nandinine, so that a detailed separation was performed starting at 100:10. Two fractions (Fr.A-B) were eluted by silica gel column chromatography, then compounds **10** (4.7 mg) and **11** (3 mg) were obtained by recrystallization and liquid chromatography.

Compound 1： Oily solid (MeOH); dark spots at UV 254 nm and a gray dark spot upon exposure to a 10% solution of EtOH-H_2SO_4 (v/v); HR-ESI-MS: 461.1506 ([M-H]$^-$, calcd for $C_{23}H_{26}O_{10}$, 461.1506); ^{1}H-NMR (400 MHz, DMSO-d_6) data and ^{13}C-NMR (100 MHz, DMSO-d_6) data were shown in **Table 1**.

Animals. All experiments involving animals in this study were carried out in accordance with the China National Institutes of Health Guidelines for the care and use of laboratory animals, and male Wistar rats (240±20 g, 10-12 weeks, SPF) were obtained from YiSi Experimental Animal Technology Co., Ltd (Changchun, China).

Study of the hypoglycemic effect of compound 1. Seventy male Wistar rats were weighed and randomly divided into 2 groups. The normal control group (10 rats) was fed a normal chow diet during the whole experiment, whereas the diabetic model group (60 rats) was fed with high sugar and fat diet. After four weeks of feeding, rats in the diabetic model group were intraperitoneally injected freshly configured sodium citrate suspension (40 mg·kg^{-1}) containing 1% streptozotocin (STZ) to establish diabetic rats' model. The control group rats were treated with an equal volume of physiological saline by intraperitoneal injection after they were given normal chow diet for 4 consecutive weeks, during which time both groups of rats were kept in room having temperature 22±2 °C with normal drinking water. Furthermore, fasting blood glucose (FBG) was detected from blood collection by cutting rats' tail, and the rats whose blood glucose concentration was more than 11.1 mmol/L for three consecutive days were considered as modeling successful diabetic rats.

After the establishment of diabetic rats' model, we divided randomly the model group into 5 groups by intragastric administration, which were the positive control drug (Metformin) group (15 μmol·kg^{-1}·d^{-1}), high (800 mg·kg^{-1}·d^{-1}, 80 mg/mL), medium (400 mg·kg^{-1}·d^{-1}, 40 mg/mL) and low (200 mg·kg^{-1}·d^{-1}, 20 mg/mL) dose of drug administration group and the model group. Meanwhile, both the control and model group were administrated with an equal volume of saline, and all rats were continuously administrated for 4 weeks, while the value of FBG was measured by cutting the tails using scissors then allowing the blood to touch the test strip which was inserted into a calibrated glucose meter every other week.

Intervention effects on social behaviors in diabetic rats. The intervention effect of compound **1** on cognitive impairment in diabetic rats was further determined by the Morris water maze test (MWM). Eighty male Wistar rats were weighed and randomized into control group (20 rats) and diabetic model group (60 rats). According to the diabetes modeling method mentioned above, the rats of diabetic model group were randomly divided into three groups (the drug administration group, the positive drug group and the model group) of 20 rats each after modeling successfully. Animals in diabetic model and control group were intragastrically administered the same dose of normal saline solution twice daily, while those in drug administration group were intragastrically administered compound **1** (800 $mg \cdot kg^{-1} \cdot d^{-1}$, 80 mg/mL) and Donepezil (2 $mg \cdot kg^{-1} \cdot d^{-1}$), respectively.

MWM test was performed on all experimental rats beginning on Day 60 after successful diabetes model modeling. The day before formal experiments, all rats were allowed to swim freely for 2 min in the water maze without the platform and markers for acclimatization to avoid the stress reaction. The Morris water maze was consisted of a circular pool (diameter of 150 cm, height of 50 cm) filled with water (23.0±2.0 °C) where a platform was placed in the center of one of the quadrants. The platform was positioned 2 cm underneath the water surface. The maze was divided into four quadrants by four equally spaced points, and different markers were affixed to the center of the four quadrant pool walls as colored cards with triangles, squares, circles, and five pointed stars, respectively. In MWM location navigation experiment, experimental rats were randomly placed in 3 quadrants facing the pool wall other than the target quadrant (the quadrant where the platform was located) to find the platform from the pool wall. Their escape latencies were recorded, along with the whole experiment was carried out in a dimly lit and soundproof test room. During the training trials, rats were allowed to stay on the platform for 10 s after they had searched for the platform within 90 s, the time that rats took to reach the platform was recorded as escape latency. If rats reached the platform longer than 90 s, the escape latency was scored as 90 s, while they were carefully brought to it and allowed to rest for 10 s then perform spatial learning memory according to the markers of 4 quadrants, Furthermore, each rat was tested twice daily for 5 consecutive days. The platform was withdrawn after the location navigation experiment, and each rat was randomly placed into the water while the number of platform crossings within 90 s was recorded.

To further determine the pharmacological activity of compound **1**, we performed social interaction behavior test to investigate social situation of another 80 diabetes rats on the 60th day following the procedure mentioned above for the MWM test. Rats were placed in the social behavior box before the start of the experiment, and after being familiarized with the environment, the sociability of the rats was judged by detecting the times and frequencies of their proximity to another metal cage of the same rat in the box within 5 min.

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DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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