GLP-1 and HSA, which can be administrated once weekly and significantly mimics GLP-1 receptor-dependent effects on regulating glucose homeostasis,64 while there are still two proteins CJC-1131 and CJC-1134-PC in the clinical research,5,6) and at least one protein E2HSA in the preclinical stage.7) Of course, other macromolecules, such as the human immunoglobulin (Ig)G2 and IgG4, are also adopted in the development of GLP-1-based long-acting drugs. Dulaglutide, a GLP-1 analog with an amino acid substitution and linked to an Fc fragment of human IgG4, is the fifth approved GLP-1-based drug, which could also be dosed once weekly.5b)

GW002 is a novel GLP-1 derivative that expressed in pichia pastoris by fusing the C-terminal region of human glucagon-like peptide-1 (GLP-1) with a peptide linker. This study aims to evaluate its anti-diabetic effects both in vitro and in vivo. The GLP-1 receptor-dependent luciferase reporter plasmid was transiently transfected in NIT-1 cells to calculate the half-maximal concentration (EC50) for GLP-1 receptor activation, and normal ICR mice and diabetic KKAy mice were acutely injected with GW002 (1, 3, 9 mg/kg) subcutaneously to evaluate the hypoglycemic action, while the diabetic KKAy and db/db mice were treated with GW002 once daily for 7 weeks to evaluate the effects on glucose metabolism. The results showed that GW002 activated GLP-1 receptor in NIT-1 cells with higher EC50 versus exendin-4 (467 vs. 7.89 nm), and single subcutaneous injection of GW002 at doses of 1, 3 and 9 mg/kg efficiently restrained the glycemia variation after oral glucose loading in ICR mice for at least 4 d, as well as reducing the non-fasting blood glucose in KKAy mice for about 2 d, while repeated injections of GW002 significantly improved abnormal glycaemia, hemoglobin (Hb)A1c levels, oral glucose intolerance and β-cell function in diabetic db/db mice. These results suggested that GW002 showed prolonged hypoglycemic action by activating its cognate receptor and provided efficient control of glucose metabolism. Thus GW002 may be a potential treatment for the management of type 2 diabetes.

Key words glucagon-like peptide-1; long-acting analog; type 2 diabetes mellitus; β-cell function

Materials and Methods

Materials GW002 (Patent No. CN201310331182.6, Clinical trial approval No. 2016L10182), blank media and exendin-4 were provided by Jiangsu T-mab Biopharma (China). The reagents used included exendin (9–39) (Sigma, U.S.A.), lipofectamine™ 2000 (Invitrogen, U.S.A.), luciferase chemiluminescence detection kit (Vigorous Biotechnology, China), insulin (mouse) ultrasensitive enzyme-linked immunosorbent
assay (ELISA) kit (ALPCO, U.S.A.), glucagon (mouse) ELISA kit and rat anti-insulin primary antibody (R&D, U.S.A.), rab-
bit anti-glucagon primary antibody (CST, U.S.A.). Equipments
used included microplate reader (BioTek, U.S.A.), peristaltic
pump (ISATEC, Switzerland), fluorescence microscope
(Olympus, Japan), glucose meter (ACCU-CHERK Active,
Germany), 8-well E-plates and iCELLigence Real-Time Cell
Analyser system (ACEA Biosciences Inc., China).

Cells and Plasmid  The pancreatic β-cell line NIT-1 was
purchased from ATCC and cultured with Dulbecco's modi-
fied Eagle's medium (DMEM)/F12 containing 10% (v/v) fetal
bovine serum (FBS) and 1% (v/v) antibiotics (100 U/mL peni-
cillin and 1 mg/mL streptomycin) at 37°C, 5% CO₂. Plasmid
Peak 12 6×RIP-CRE luciferase was constructed by inserting
six copies of cAMP-response element (CRE)-like sequence of
rat insulin promoter (RIP-CRE, 5'-TGA CGT CC-3') upstream
of the luciferase reporter gene. 9)

Experimental Animals  ICR male mice weighing 20-22 g
(Vital River Laboratory Animal Technology Co., Ltd.) and female spontaneous type 2 diabetic KKAy mice aged 11-12
weeks (Institute of Laboratory Animal Science, CAMS &
PUMC) were used in the acute experiment. Female spontane-
ous type 2 diabetic db/db mice aged 9-10 weeks (Chang Zhou
Cavens Laboratory Animal Ltd.) and another batch of KKAy
mice (as above) were adopted in the chronic experiment. All
animals were housed five per cage with free access to
water and food, and handled in accordance with the standards
for laboratory animals (GB14925-2001) and the guideline on
the humane treatment of laboratory animals (MOST 2006a)
established by the People's Republic of China.

GLP-1 Receptor Activation in Vitro  NIT-1 cells were
transiently transfected with the reporter gene plasmid Peak12
6×RIP-CRE luciferase using Lipofectamine™ 2000 for 20h,
then treated with GW002 ranging from 0.1 nm to 3.5 µM and
exendin-4 from 3.0 pm to 2.4 µM for 24h. To clarify the speci-
cicity of GW002 for GLP-1 receptor, NIT-1 cells were also co-
incubated with GLP-1 receptor antagonist exendin (9-39) and
GW002 or exendin-4 for 24h. The expression of luciferase
was determined by adding chemiluminescence substrate,
the activation ratio and EC₅₀ were calculated. Phosphate buffered
saline (PBS) was included as negative reference. The activa-
tion ratio was defined as the chemiluminescence value of treat-
ment divided by that of the negative reference.

Acute Injection of GW002 in Normal ICR Mice and Di-
betic KK/Ay Mice  All the ICR mice were grouped randomly
and fasted overnight with water ad libitum before the experi-
ment. GW002 at doses of 1, 3 and 9 mg/kg was subcutaneous-
lly injected, followed by two oral glucose loadings (2.0 g/kg)
15 min and 5 h later. Blood was collected from tail tip prior to
injection (0 min), 30, 60 and 120 min after the first glucose
loading, and at 30 min after the second glucose loading. In
order to evaluate how long the hypoglycemic effect could last,
the blood glucose was dynamically monitored in the following
6d by determining the blood glucose with feed removed for
5 h but water ad libitum (0 min), and that 30 min later of the
oral glucose loading.

Another batch of ICR mice were grouped randomly and
treated with GW002 as above, then the non-fasting blood glu-
cose was dynamically measured at 1, 5h and in the following
4d after injection with food and water ad libitum. The diabetic
KK/Ay mice were grouped based on fasting blood glucose,
non-fasting blood glucose and body weight, and treated with
GW002 as above. The non-fasting blood glucose was dynami-
cally determined at 5h and on day 2, 3, 4 and 5 after injec-
tion. The normal group (Nor) and diabetic control group (Con)
were injected with blank media.

Chronic Treatment of GW002 in the Diabetic db/db and
KK/Ay Mice  The diabetic db/db and KKAy mice were both
grouped based on blood glucose (BG), triglyceride, total
cholesterol, body weight and the decreasing percent of BG at
40 min in the insulin tolerance test (0.4 U/kg), and then were
injected with GW002 at doses of 1, 3 and 9 mg/kg once daily
at 9:00 am for 7 weeks. Exendin-4 (2 µg/kg) was adopted as
positive control and injected twice daily at 9:00 am and 5:00
pm. The blank media was used as negative control. All the
peptide dosing solution was freshly made before injection.

The non-fasting blood glucose levels were dynamically
monitored every week and expressed as blood glucose varia-
tion ratio that calculated as [100−(BG_con average−BG_treatment)/
BG_con average]×100, while BG_treatment means the blood glucose
of every mouse in treatment group and BG_con average means
the average blood glucose of mice in the control group (Con).
The hemoglobin (Hb)A1c levels were measured after 7 weeks and
6 weeks of treatment in db/db and KKAy mice, respectively.
The oral glucose tolerance test was carried out 3 weeks after
injection in db/db mice as mentioned in the acute experiment
in ICR mice, and the fasting blood insulin and glucagon levels
were separately measured 2 and 7 weeks after injection. The
insulin tolerance test was performed 4 weeks after treatment
in db/db mice as follows: mice were fasted for 4 h with water
ad libitum before blood was collected (0 min), followed by
subcutaneous injection of insulin (0.4 U/kg). Then the blood
was taken 40 and 90 min later. The body weight, food and
water intake of db/db mice were monitored every week.

In KKAy mice, the hyperglycemic clamp test was per-
formed to evaluate β-cell function. Firstly, the mice were in-
traperitoneal injected with pentobarbital sodium (60 mg/kg)
after a 10 h fast. Then the right jugular vein was catheterized
and a bolus of d-glucose (100 mg/kg) was infused 30 min
later, followed by continuous infusion of glucose (10%) at
10–20 µL/min. Blood samples were collected every 5 min from
the tail tip to determine glucose levels using glucose meter.
The infusion rate of glucose was adjusted according to the
blood glucose levels to achieve a steady state with hypergly-
cemia nearly 14±0.5 mmol/L. 10) The first phase insulin secre-
tion was measured at 10 min after glucose infusion and the glucose
infusion rate (GIR) was calculated. From three to five mice
were used in each group.

Immunofluorescence  Db/db mice were sacrificed 7 weeks
after treatment. The pancreas were dissected and fixed in
Bouin solution, then embedded in the paraffin. Serial 5µm
paraffin sections were prepared and stained for insulin and
glucagon as follows. The paraffin sections were dewaxed by
xylene, and rehydrated through different concentrations of
ethyl alcohol, then boiled in the Tris–ethylene diaminetetra-
acetic acid (EDTA) (pH 9.0) to make antigen retrieval, fol-
lowed by sealing off at room temperature for 1 h and incuba-
tion overnight with a cocktail of rabbit anti-glucagon antibody
(1:25) and rat anti-insulin antibody (1:45) at 4°C. The pri-
mary antibodies were incubated with a cocktail of fluorescein
conjugated secondary antibody and rhodamine conjugated
secondary antibody at 37°C for 1 h, and then observed under
the fluorescence microscope equipped with a charge-coupled device camera. The ratio of insulin and glucagon positive cells to total islet area were respectively calculated from digitized images captured under 20× objective using Image J software. From three to five mice were analyzed in the groups of Nor, Con, GW002 (9 mg/kg) and exendin-4.

**Cell Growth Assay** NIT-1 cells were planted in 8-well E-plates and cultured in DMEM/F12 medium (containing 10% fetal bovine serum, 100 U/mL penicillin and 100 µg/mL streptomycin) for 24 h. Then the cells were incubated with GW002 at the concentrations of 10^{-6} and 10^{-8} M, as well as exendin-4 at the concentration of 10^{-6} M, in the culture media containing 25 mM glucose and 0.5 mM palmitic acid, followed by dynamic monitoring of cell growth through the iCELLigence Real-Time Cell Analyser system. The cells incubated with 25 mM glucose, 0.5 mM palmitic acid and phosphate-buffered saline were adopted as negative control (Con), and that incubated with 25 mM glucose and phosphate-buffered saline were adopted as normal control (Nor).

**Statistical Analysis** The EC_{50} values were determined from the curves plotting activation ratio versus sample concentrations using the GraphPad Prism 7.01 (GraphPad Software, San Diego, CA, U.S.A.) and expressed as mean±standard error (S.E.) with four identical experiments. The other data was expressed as the mean±S.E. and analyzed by one way ANOVA with Bonferroni’s correction in the Student’s t-test, except the data of food and water intake in db/db mice was expressed as mean. The value of p<0.05 was accepted as being statistically significant.

**RESULTS**

**GW002 Activates GLP-1 Receptor in NIT-1 Cells** To assess whether recombinant fusion with HSA could influence the binding and activation of GLP-1 to its cognate receptor, GLP-1 receptor activation and specificity were measured. GW002 produced a dose-dependent induction of luciferase expression in NIT-1 cells, and the maximal activation multiple was almost equivalent to exendin-4 (3.9 for GW002 at 3.5×10^{-6} M vs. 3.0 for exendin-4 at 2.4×10^{-6} M), but the EC_{50} was increased compared to exendin-4 (46.7 nM for GW002 vs. 7.89 nM for exendin-4). GLP-1 receptor antagonist exendin (9-39) ranging from 0.2 to 1.0 µM evidently inhibited the activation effect of GW002 at 0.1 µM in a dose-dependent manner (Fig. 1). This indicated that GW002 could specifically activate GLP-1 receptor with similar efficacy but lower potency than exendin-4.

**Acute Injection of GW002 Shows Long-Lasting Hypoglycemic Effects in ICR Mice and KKAY Mice** To investigate the hypoglycemic effects and duration, GW002 was acutely

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Fig. 1. GW002 Displays Similar Efficacy but Lower Potency than Exendin-4 in Activating GLP-1 Receptor

The luciferase values of treated groups are expressed as activation ratio divided by that of negative control. (A) GLP-1 receptor activation curve of GW002 and exendin-4. (B) Effect of exendin (9–39) on GLP-1 receptor activation by GW002 and exendin-4. Values are expressed as mean±S.E., n=4. *p<0.05, **p<0.01, ***p<0.001 vs. vehicle without exendin (9–39) GW002 or exendin-4. ###p<0.001 vs. GW002 at the corresponding concentration, $$$p<0.001 vs. exendin-4 at the corresponding concentration.
administered by subcutaneous injection in normal ICR mice and diabetic KKAy mice. In ICR mice, GW002 at doses of 3 and 9 mg/kg could significantly reduce the glycemic excursion following the first oral glucose challenge given 15 min after the acute injection, but 1 mg/kg did not (Fig. 2A). However, all the doses of GW002 (1, 3, and 9 mg/kg) could significantly decrease the 30 min blood glucose following the second oral glucose loading performed 5 h after acute injection (Fig. 2B). Furthermore, all doses of GW002 could continuously restrain the glucose excursion after oral glucose challenge performed...
in the following 4 d (from days 2 to 5), while 9 mg/kg still significantly decrease the blood glucose even on the 7th day after injection (Fig. 2C).

In addition, acute administration of GW002 at doses of 3 and 9 mg/kg for 1 h also evidently decreased the non-fasting blood glucose in ICR mice and lasted for at least 4 d, while 1 mg/kg did not work at 1 h but significantly acted 5 h later (Fig. 3A). Nevertheless, single injection of GW002 at doses of 1, 3 and 9 mg/kg for 5 h had no effect on the non-fasting blood glucose, but lowered the blood glucose level in a dose-dependent manner on the 2nd and 3rd day after injection in diabetic KKAy mice (Fig. 3B). This suggested that GW002 displays prolonged hypoglycemic action though with different onset time and duration in ICR mice and KKAy mice.

**Chronic Administration of GW002 Remarkably Regulates the Blood Glucose Variation in db/db and KKAy Mice**

To assess whether repeated treatment with GW002 could control blood glucose fluctuation, the diabetic db/db and KKAy mice were injected with GW002 once daily for 7 weeks. In db/db mice, GW002 dose-dependently reduced the non-fasting blood glucose 5 h after the first injection and consistently reduced the non-fasting blood glucose during the following 7 weeks (Fig. 4A). Moreover, GW002 at the dose of 9 mg/kg evidently decreased the HbA1c levels by 1.84% (6.34±1.50 vs. 4.50±1.50%, Fig. 4B). In KKAy mice, GW002 also repressed the blood glucose variation to some extent, especially at the dose of 9 mg/kg with a reduction of HbA1c by 1.78% though without statistical significance (6.25±2.81 vs. 4.47±2.33%, Figs. 4C, D).

**Chronic Administration of GW002 Improves β-Cell Function and Increases Insulin Secretion in db/db and KKAy Mice**

The benefit of GW002 on β-cell function was also ascertained during the chronic treatment in both db/db and KKAy mice. In db/db mice, chronic treatment with GW002 at doses of 1, 3 and 9 mg/kg for 3 weeks significantly reduced the fasting blood glucose, ameliorated the glycemic excursion following oral glucose loading, and lowered the area of blood glucose curve (AUC) in a dose-dependent manner (Figs. 5A, B), suggesting an improvement of impaired oral glucose tolerance in diabetic status. GW002 also markedly increased the fasting blood insulin level and decreased the glucagon level in db/db mice, so as to correct the imbalance of insulin and glucagon in regulation of glucose metabolism (Figs. 5C, D). Nevertheless, GW002 hardly decreased the blood glucose variation ratio at 40 min compared to fasting blood glucose (0 min) in the insulin tolerance test, though significantly decreased the fasting blood glucose and that at 40 min after insulin injection at the dose of 9 mg/kg, as well as AUC (Figs. 5E, F), suggesting a failure of improving insulin sensitivity.

The hyperglycemic clamp technique was a gold standard test for evaluating β-cell function and was carried out in KKAy mice. Clearly, repeated treatment with GW002 at doses of 1 and 9 mg/kg markedly increased the glucose infusion rate (GIR), suggesting an amelioration of β-cell sensitivity to glucose stimulating, even though the middle dose 3 mg/kg without statistical significance. GW002 also dose-dependently enhanced the first phase insulin level, indicating increased in-
Repeated Administration of GW002 Significantly Improves β-Cell Function in Diabetic db/db Mice and KKAy Mice

(A) Blood glucose curve of the oral glucose tolerance test (OGTT) performed 3 weeks after treatment in db/db mice. (B) The area under the blood glucose curve (AUC) of OGTT in db/db mice. (C) Fasting blood insulin level determined after treatment for 2 weeks in db/db mice. (D) Fasting blood glucagon level measured after treatment for 7 weeks in db/db mice. (E) Blood glucose curve of the insulin tolerance test (ITT) performed 4 weeks after treatment in db/db mice. (F) AUC of ITT in db/db mice. (G) GIR, glucose infusion rate at the glucose steady state in KKAy mice. (H) First phase insulin level in KKAy mice. Values are expressed as mean±S.E., n=10–13 for db/db mice and 3–5 for KKAy mice. *p<0.05, **p<0.01, ***p<0.001 vs. Con.
sulin storage in β cells though without statistical significance for less sample (n=3–5) (Figs. 5G, H).

GW002 Promoted β-Cell Survival and Corrected the Distribution of β- and α-Cell  To evaluate whether repeated injections of GW002 could influence the proliferation and distribution of β- and α-cell, and explore the reasons for variations of fasting blood insulin and glucagon levels, the histological analysis of pancreatic islets was carried out and the insulin- and glucagon-positive stained areas were defined as β- and α-cell, respectively. In db/db mice, the ratio of insulin positive β-cell area (green) to total islet decreased accompanied by abnormal distribution of α- and β-cell. Repeated injection of GW002 significantly increased β-cell area ratio and decreased α-cell area ratio to total islet, and corrected the distribution of α-cell that should set outside the islet (Figs. 6A–C), suggesting an improvement of β-cell proliferation and islet morphology.

To confirm the protection of β-cell mass by GW002, the growth of NIT-1 cells were dynamically monitored in vitro. The results showed that the growth of NIT-1 cells were significantly inhibited by 25mM glucose and 0.5mM palmitate, and GW002 ameliorated the inhibition and increased cell growth in a dose-dependent manner to some extent but without statistically significant differences, which indicated that GW002 may promote cell survival in face of damage but needs further confirmation (Fig. 6D).

Chronic Treatment with GW002 Probably Ameliorated the Polydipsia, Polyphagia and Obesity in db/db Mice  Repeated injections with GW002 at doses of 1, 3 and 9mg/kg for 1 week decreased the body weight by 5.0, 9.3 and 9.1%, respectively, while separately lowered food intake by 31.4, 40.9 and 56.3%, as well as reducing water consumption by...
42.5, 55.1 and 72.4%, respectively, but the actions gradually attenuated, especially those on the body weight and food intake (Fig. 7).

**DISCUSSION**

As a member of the incretin family, the major advantages of GLP-1 over conventional anti-diabetic drugs, such as thiazolidinedione, metformin, sulfonylurea, mainly lie in mitigating hypoglycemia risks and promoting β-cell proliferation and survival. Thus GLP-1 has sparked widespread interest in its potential as an anti-diabetic treatment. Furthermore, there are still large ongoing prospective clinical trials to ascertain the effects of GLP-1-based drugs on diabetes-related complications in patients with T2DM, which further establishes its pivotal role in anti-diabetic therapeutic research.

GW002 is a GLP-1 analog fused by connecting the C-terminal region of human GLP-1 to the N-terminal region of HSA with a peptide linker aimed to be injected once weekly in patients with T2DM. The reporter gene assay demonstrated that GW002 could significantly activate GLP-1 receptor with nearly equivalent efficacy though reduced potency compared to exendin-4, the first approved GLP-1-based drug that injected twice daily, and the effect was markedly inhibited by GLP-1 receptor antagonist exendin (9–39). This suggests that recombinant expression with HSA did not affect the activation of GLP-1 receptor but demanded higher EC₅₀ for stereospecific blockade, and the action of GW002 possessed GLP-1 receptor specificity.

In vivo, single injection of GW002 significantly decreased the blood glucose following oral glucose loading in ICR mice and the effect could last for at least 7 d at the dose of 9 mg/kg. In addition, single injection of GW002 also evidently reduced the non-fasting blood glucose both in ICR mice and KKAy mice with prolonged duration but with different onset time. In ICR mice, single injection of GW002 at the dose of 1 mg/kg for 1 h had no effect on the non-fasting blood glucose but showed significantly hypoglycemic action 5 h after injection, while in KKAy mice, single injection of GW002 from 1 mg/kg to 9 mg/kg for 5 h did not decrease the non-fasting blood glucose, but displayed evident glucose-lowering effect in db/db mice, suggesting the efficacy of GW002 is largely determined by the basic glucose metabolism status of the animal model itself. In short, single injection of GW002 displayed long-lasting hypoglycemic action and may be given once weekly in clinic.

The most notable use for GLP-1 lies in its remarkable improvement of β-cell function and excellent glucose-dependent insulinotropic effects. Clinical studies have previously shown that exendin-4 could dose-dependently decrease the fasting and postprandial blood glucose, lower the HbA1c level, restore the blunted first phase insulin secretion, enhance the second phase insulin secretion and inhibit glucagon secretion. Chronic treatment with GW002 was assessed in diabetic db/db and KKAy mice by subcutaneous injection once daily. The results showed that chronic treatment with GW002 could significantly control the non-fasting blood glucose and HbA1c levels, increase insulin secretion and suppress glucagon secretion therefore improving the imbalance of insulin and glucagon in controlling glucose metabolism. Moreover, the oral glucose tolerance test and hyperglycemic clamp test, a gold standard in evaluating β-cell function, indicated that GW002 could improve β-cell sensitivity to glucose stimulation and appropriately increase the storage and secretion of insulin in diabetic mice.

But KKAy and db/db mice are model mice of T2DM that represent obese and insulin resistance with hyperinsulinemia, which seems contradictory with the increase of fasting blood insulin by GW002. The insulin tolerance test showed that
chronic treatment with GW002 did not increase the use of exogenous insulin to decrease blood glucose. Therefore, the hypoglycemic effect of GW002 mainly relies on promoting insulin secretion rather than improving insulin sensitivity. Furthermore, chronic treatment with GW002 could also decrease the body weight, food and water intake in db/db mice, which may contribute to decreasing blood glucose.

In addition, many studies in rodent models of diabetes show that exogenous administration of GLP-1 analogs have the ability to increase islet mass and preserve β-cell function by enhancing its proliferation and neogenesis, while at the same time inhibiting its apoptosis. The related mechanisms involve some key proteins, such as COUP-TFI (Chicken Ovalbumin Upstream Promoter Transcription Factor II), Skp2 (a substrate recognition component of SCF (Skp, Cullin, F-box) ubiquitin ligase), sirtuin deacetylase 1 and stromal cell-derived factor-1 (SDF-1)/chemokine (C-X-C motif) receptor 4 (CXCR4) axis.

Chronic treatment with GW002 significantly increased the fasting blood insulin level and appropriately the first phase insulin secretion, indirectly suggesting an increase of β-cell numbers. The histological analysis of islets showed significant decrease of β-cell-to-ilet area ratio in db/db mice compared to db/m mice, suggesting a decompensation of islet β-cell number though with hyperinsulinemia. Chronic treatment with GW002 at the dose of 9 mg/kg could significantly increase β-cell-to-islet area ratio and decrease α-cell-to-islet area, which was consistent with the increase of fasting and first phase blood insulin and reduction of fasting blood glucagon. Furthermore, GW002 remarkably ameliorated the inhibition of NIT-1 cells growth induced by high glucose and high fatty acid in a dose-dependent manner, directly suggesting the improvement of cell proliferation and protection of cell mass. But the related mechanisms are currently underway.

Although repeated injection with GW002 exhibited favorable anti-diabetic effects in db/db and KK-Ay mice, most of the benefits displayed gradual weakening with time, which may result from the production of antibodies due to species difference and large molecular weight. In theory, these long-term GLP-1 analogs, including GW002, could be administrated about once weekly in the chronic treatment according to pharmacokinetic and pharmacodynamics parameters. However some of them, such as CNOT736 and CJC-1134-PC, were both dosed subcutaneously once daily in animals for immunization, potentially long-acting GLP-1-based drug for the management of T2DM.

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