European Journal of Medicinal Chemistry 45 (2010) 2327–2335

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

A new insulin-enhancing agent: [*N*,*N*′-bis(4-hydroxysalicylidene)-o-phenylene-diamine]oxovanadium(IV) and its permeability and cytotoxicity

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ARTICLE INFO

Article history:
Received 29 September 2009
Received in revised form
29 January 2010
Accepted 2 February 2010
Available online 10 February 2010

Keywords:
Insulin-enhancing
Salen-type ligands
Caco-2 cells

ABSTRACT

A new insulin-enhancing agent: [*N*,*N*′-bis(4-hydroxysalicylidene)-o-phenylene-diamine] oxovanadium (IV) (BPOV) was synthesized and characterized by X-ray crystallography. BPOV was administered intragastrically to STZ-diabetic rats for 4 weeks. The results showed that BPOV could significantly decrease the blood glucose level and ameliorated impaired glucose tolerance in STZ-diabetic rats. BPOV has been further tested on insulin, glycogen and serum lipid studies. The results suggested BPOV has glucose-lowering activity in diabetic rats, as well as improved the disorder of lipid metabolism in diabetes. BPOV had permeability above 10^{-5} cm s^{-1}. It was suggested good lipophilic properties. The cytotoxicity of BPOV on Caco-2 cells was measured by MTT assay which suggested BPOV have higher effect on impairment of cellular associated with lower level capacity of cellular accumulation.

1. Introduction

Vanadium is a trace element that plays an essential role of its physiological effects and medical applications. Vanadium compounds, as a nonspecific phosphotyrosine phosphatase inhibitor, have been known to possess insulin-mimetic and/or enhancing effects both in vitro and in vivo [1–3]. Studies testing compounds in animal model systems [4–8] and in human beings [9–11] show that simple vanadium salts and V-complexes alleviate the symptoms of diabetes. Several studies have shown that vanadium compounds improve not only hyperglycemia in human subjects and animal models of type 1 diabetes but also glucose homeostasis in type 2 diabetes [12,13].

Vanadium salts, at doses ranging from 0.1 to 0.7 mM kg^{-1} d^{-1} [14–16], in a variety of animal models of diabetes [17], normalized blood glucose and lipid levels, corrected thyroid hormone deficiency, improved insulin sensitivity, and prevented or reversed secondary complications, such as cardiomyopathy, cataract development, impaired antioxidant status and excessive food and fluid intake [18,19]. In order to reduce the toxicity and improve the absorption, tissue uptake and efficiency of vanadium, the organic ligand with less or no toxicity is to be great interest. A lot of organic vanadium complexes have been synthesized and demonstrated to be effective. A large class of compounds based on V(IV) chelate complexes [20] have been extensively studied mainly by the Sakurai and Orvig/McNeill groups, respectively [21–23]. In 1992, bis(maltolato)oxovanadium(IV) BMOV was reported two to three times more effective acutely than vanadyl sulfate as a glucose-lowering agent, was better tolerated than inorganic vanadium salts, and resulted in reliable glucose-lowering in all animal models of diabetes [24–27]. Furthermore, the first Phase II clinical trial of a designed vanadium-based pharmaceutical agent (bis(ethylmaltolato)oxovanadium(IV), BEOV), was completed by Medeval Ltd., Manchester, UK [28]. Several vanadium complexes of the Schiff base sal_2en {*N*,*N*′-ethylenediaminebis(salicylideneiminato)} and related ligands have been proposed as insulin-enhancing agents, and for the treatment of obesity and hypertension [29]. At present, only [$V^{IV}O(sal_2en)$] has been tested in vivo for insulin-mimetic activity.

In our work, salen-type ligands were study with it present versatile steric, electronic and lipophilic properties. A new insulin-enhancing vanadyl complex [*N*,*N*′-bis(4-hydroxysalicylidene)-o-phenylene-diamine]oxovanadium(IV) (BPOV) has been synthesized, ensuring a balanced lipophilicity and hydrophilicity for orally available for vanadium-based pharmaceuticals and its insulin-mimetic activity investigated. Our studies have shown BPOV could

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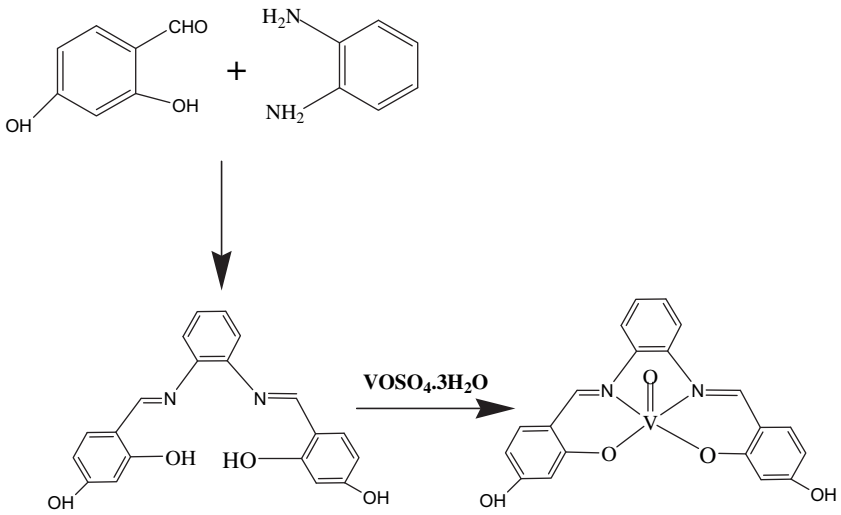
doi:10.1016/j.ejmech.2010.02.010



Scheme 1. Synthesis procedure of the complex BPOV.

significantly decrease the blood glucose level and ameliorated impaired glucose tolerance in STZ-diabetic rats. Furthermore, we have taken on insulin, glycogen and serum lipid studies of the complex, attempted to know the in vivo anti-diabetes activity relationships of BPOV with $VO(O_2N_2)$ coordination mode.

To investigate the factors leading to the different bioavailability of vanadium compounds and their relation to the cytotoxicity, the mechanism of permeation and effects on Caco-2 and the cell monolayer were studied with vanadium compounds [30,31]. In this paper, the permeability and toxicity of BPOV were assessed using the Caco-2 cell monolayer to explore its absorption, distribution, metabolism, elimination, and toxicity (ADME/T) properties.

2. Chemistry

Chemicals and solvents were reagent grade and were used without further purification unless otherwise noted. O-phenylene-diamine, 2,4-dihydroxybenzaldehyde and vanadyl sulfate hyrate ($VOSO_4 \cdot 3H_2O$,) were purchased from Aldrich (purity $\geq$ 98%). The complex [*N*,*N*′-bis(4-hydroxysalicylidene)-o-phenylene-diamine] oxovanadium(IV) (BPOV) was synthesized by 2,4-dihydroxybenzaldehyde and o-phenylenediamine with vanadyl sulfate, produced a monomeric V^{IV} complex. The reaction sequences are outlined in Scheme 1. The block shaped well defined green colour crystals of BPOV suitable for X-ray studies were obtained from the mother liquor.

Table 1
Crystal data and structural refinements for the complex.

Empirical formula	$C_{24}H_{30}N_2O_9S_2V$
F_w	605.56
Temperature/K	293(2)
Wavelength/nm	0.71073
Crystal system	Triclinic
Space group	P-1
Unit cell dimensions (nm, °)	$a = 9.325(3)$ $\alpha = 78.464(5)$
	$b = 12.312(4)$ $\beta = 72.067(6)$
	$c = 12.829(4)$ $\gamma = 85.072(6)$
Volume/nm^3	1187.3(11)
Z	2
Density (calc.)/g cm^{-3}	1.465
μ/mm^{-1}	0.567
F (000)	630
Crystal size/mm^3	0.20 × 0.16 × 0.12
θ range/°	2.16–26.46
Limiting indices	$-8 \leq h \leq 11$, $-15 \leq k \leq 15$, $-16 \leq l \leq 13$
Reflection collected	8035
Independent reflection	5595 [R(int) = 0.0345]
Max. and min. transmission	0.790 and 0.745
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	5595/0/349
Goodness-of-fit on F^2	1.001
Final R indices [$I > 2\sigma(I)$]	R1 = 0.0608, wR2 = 0.1138
R indices (all data)	R1 = 0.1314, wR2 = 0.1364
Largest diff. peak and hole/e·Å^{-3}	0.562 and −0.348

2.1. Crystallographic data collection and structure analysis

X-ray structure determinations Intensity data for single crystals of complex was collected on a BRUKER SMART 1000 CCD detector with graphite-monochromatized Mo Ka radiation ($\lambda = 0.071073$ nm). For BPOV, a total of 8035 independent reflections were collected, of which 5595 [R(int) = 0.0345] were considered as observed [$I > 2\sigma(I)$]. The structure was solved by direct method using the program SHELXS-97 [32] and subsequent Fourier difference techniques, and refined anisotropically by full-matrix least-squares on F^2 using SHELXL-97 [33]. Crystal data and structural refinements of complex and selected bond lengths and angles are shown in Tables 1 and 2. Crystallographic data of the complex has been deposited at the Cambridge Crystallogrphic Data Center, CCDC Nos. 252952. Any queries relating to the data can be e-mailed to deposit@ccdc.cam.ac.uk.

Table 2
Bond lengths [A] and angles [°] around the vanadium atom in [*N*,*N*′-1,3-propyl-bis (salicyladimine)]oxovanadium (IV), BPOV.

V(1)–O(5)	1.592 (3)	O(5)–V(1)–O(3)	110.44 (13)
V(1)–O(3)	1.915(3)	O(5)–V(1)–O(1)	108.63 (12)
V(1)–O(1)	1.926 (2)	O(3)–V(1)–O(1)	84.26 (11)
V(1)–N(1)	2.049(3)	O(5)–V(1)–N(2)	104.61 (12)
V(1)–N(2)	2.045(3)	O(3)–V(1)–N(2)	88.37 (12)
		O(1)–V(1)–N(2)	146.46 (11)
		O(5)–V(1)–N(1)	106.62 (13)
		O(3)–V(1)–N(1)	142.71 (12)
		O(1)–V(1)–N(1)	87.97 (11)
		N(1)–V(1)–N(2)	78.35 (12)
		O(5)–V(1)–N(1)–C7	93.4 (3)

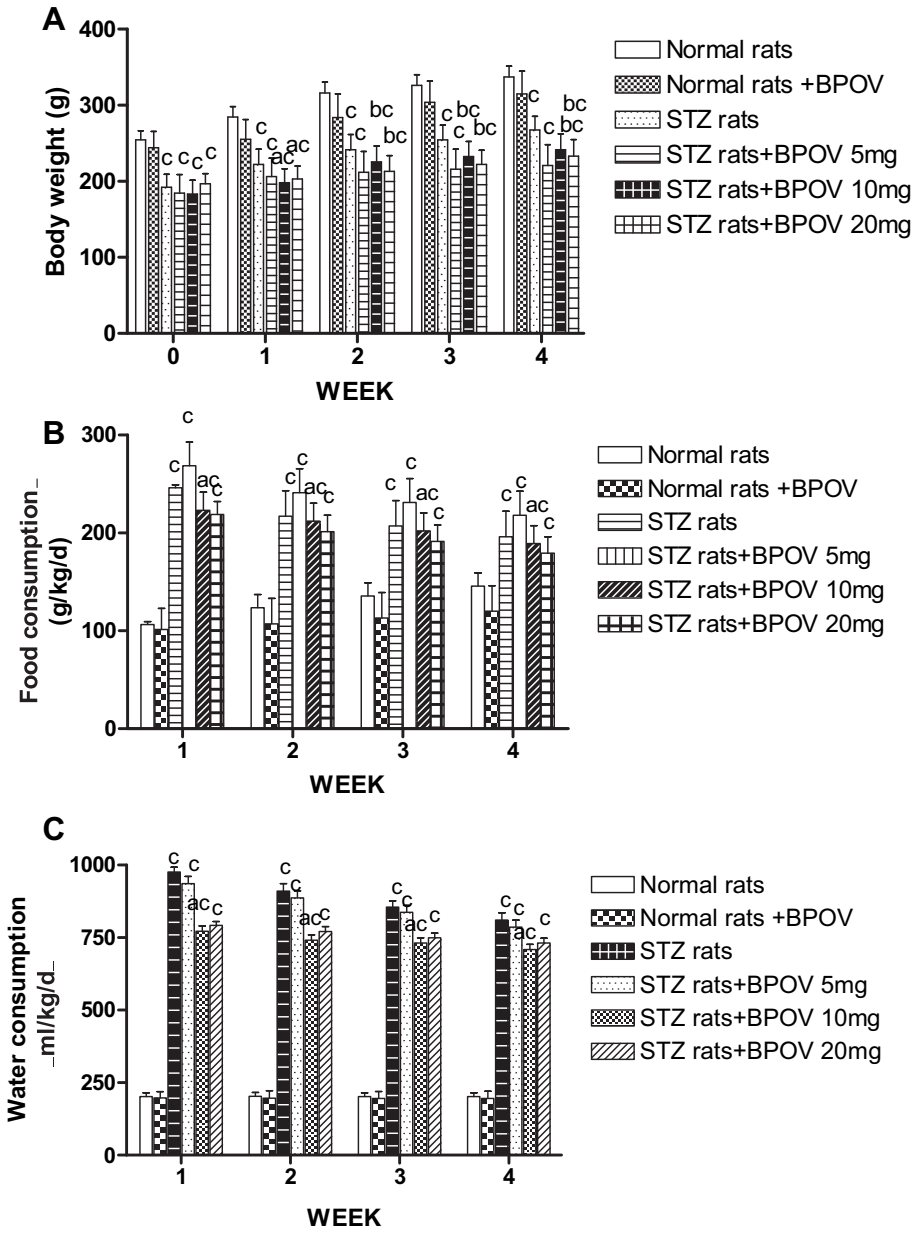


Fig. 1. Effects of complex BPOV on body weight (A), food (B) and water (C) intake in normal and STZ-induced diabetic rats ($n = 9$–10). The diabetic rats were induced by a single intraperitoneal injection of STZ 50 mg kg^{-1}. CON: normal control group, 0.9% NaCl solution ig; CON + BPOV: treated normal group, BPOV 3 10 mg V kg^{-1} ig; DM: diabetic group, 0.9% NaCl solution ig; DM + BPOV L, M, H: treated diabetic group, BPOV 5, 10, 20 mg V kg^{-1} ig, respectively. Values are mean ± SD. $^{a}P < 0.05$, $^{b}P < 0.01$, vs. normal control; $^{c}P < 0.01$, vs. DM control (Dunnett's test).

3. Pharmacology

3.1. Effects of BPOV on blood glucose in both normal and STZ-induced diabetic rats

In this experiment, the STZ-diabetic rats exhibited a significant increase in the fluid and food consumption, compared with the normal rats. After administration, BPOV markedly reduced the intake of fluid in STZ-diabetic rats but not in normal rats. The food intake was transitory decreased in the BPOV-treated STZ-diabetic rats, compared with the STZ-diabetic rats. The mean body weight in STZ-diabetic rats was lower than that in normal rats. After administration of BPOV, the tendency of decrease in the body weight was observed both in the diabetic and normal rats. 4 Weeks administration of BPOV (20.0 mg V kg^{-1} ig) significantly reduced the body weight in the diabetic group, compared with the diabetic control group ($P < 0.05$) (Fig. 1).

In present study, the blood glucose level in normal control group was maintained in the normal range. When normal rats received a daily oral administration of the complex at a dose of 10.0 mg V kg^{-1} for 4 weeks, the blood glucose level did not decrease as compared with the untreated normal rats ($P > 0.05$), suggesting that BPOV did not affect the blood glucose level of normal rats.

The STZ-diabetic rats exhibited significant hyperglycemia. After four-weeks administration with the complex, the blood glucose level was significantly decreased in a dose-dependent manner, compared with the diabetic control group ($P < 0.05$, 0.01). It was suggested that the complex has hypoglycemic effect on STZ-

Table 3
Effects of intragastric BPOV on blood glucose levels in both normal and STZ-diabetic rats.

Group	Dose (mg V kg^{-1})	Blood glucose (mmol L^{-1})				
		0	1	2	3	4 (Week)
Normal rats	Saline	3.97 ± 0.26	3.52 ± 0.23	3.44 ± 0.33	3.98 ± 0.28	4.14 ± 0.27
Normal rats + BPOV	10	4.06 ± 0.46	3.56 ± 0.29	3.22 ± 0.42	3.93 ± 0.33	3.79 ± 0.52
STZ-rats	Saline	11.25 ± 0.42^{c}	11.52 ± 1.04^{c}	12.48 ± 0.53^{c}	12.41 ± 0.88^{c}	16.09 ± 1.58^{c}
STZ-rats + BPOV	5	11.67 ± 0.70^{c}	11.30 ± 0.86^{c}	13.41 ± 1.01^{c}	12.33 ± 0.91^{c}	13.77 ± 1.92^{c}
	10	11.85 ± 1.52^{c}	10.51 ± 1.44^{c}	10.66 ± 1.02^{bc}	11.34 ± 0.64^{ac}	12.67 ± 1.57^{bc}
	20	11.24 ± 1.29^{c}	11.05 ± 0.75^{c}	10.88 ± 1.06^{bc}	10.25 ± 1.56^{bc}	11.46 ± 2.43^{bc}

The diabetic rats were induced by a single intraperitoneal injection of STZ 50 mg kg^{-1}. Data were expressed as mean ± standard deviations for 10 rats in each group. $^{a}P < 0.05$ or less vs. normal rats; $^{b}P < 0.05$ or less vs. SZT-diabetic rats (Dunnett's test).

diabetic rats (Table 3). Moreover, it was also suggested that the hypoglycemic effect was achieved maybe by increasing the sensitivity of insulin and not by enhancing the excretion of insulin.

3.2. Oral glucose tolerance test (OGTT) [34]

OGTT was performed to investigate the effect of BPOV on glucose tolerance. One hour after treatment with the loaded glucose, the blood glucose increased to the peak value (4.86 mmol L^{-1}) and decreased to the fasting blood glucose level after 2 h in normal control group. When normal rats received BPOV at a dose of 10.0 mg V kg^{-1}, the oral glucose tolerance curve was not significantly changed as compared with normal control group (Fig. 2). It was suggested that BPOV did not influence glucose tolerance in normal rats.

In diabetic control group, the blood glucose levels at 0, 15, 30, 60 and 120 min were much higher than that in normal control group, and reached the peak value with 21.63 mmol L^{-1} at 30 min after glucose loading. Even at 120 min, the blood glucose still was high to 17.97 mmol L^{-1}. After BPOV administration for 4 weeks, the oral glucose tolerance curve became flattened in a dose-dependent manner. BPOV at 10 and 20 mg V kg^{-1} marked lowered the blood glucose levels, compared with diabetic group ($P < 0.05$ or $P < 0.01$) (Fig. 3). It showed that the complex could accelerate the glucose clearance and improve glucose tolerance in STZ-diabetic rats.

3.3. Insulin, glycogen and serum lipid studies

As shown in Fig. 3, normal rats received a daily oral administration of BPOV at dose of 10.0 mg V kg^{-1} for 4 weeks, whereas the serum insulin was significantly decreased in the non-diabetic rats treated with BPOV at the end of experiment. The STZ-induced diabetic rats exhibited significant hyperglycemia and lower serum insulin concentration. After administration, BPOV markedly decreased the blood glucose concentrations in a dose-dependent manner, compared with diabetic rats ($P < 0.05$ and 0.01). However, the serum insulin concentration did not increase along with the decrease of blood glucose and even decreased in the diabetic rats treated with BPOV 20.0 mg V kg^{-1} body weight. Moreover, this suggested BPOV has insulin-mimetic activities or increased sensitivity of insulin-accepter. This may suggest that BPOV has glucose-lowing effect because it itself has insulin-mimetic activities or increased sensitivity of insulin-accepter, which it did not excrete insulin via enhancing injured insulin β cells (Fig. 3.)

Glycosylated hemoglobin was found to increase in patients with diabetes mellitus and the amount of increase is directly proportional to blood glucose concentration [35]. Therefore, glycosylated hemoglobin is used to indicate blood glucose concentration of diabetes in a long time (4–10 weeks) and not affected by the temporary fluctuation of blood glucose concentration. Administration of BPOV complex could decrease the increased glycosylated hemoglobin in STZ-diabetic rats, suggesting that the complex controlled the glycation of hemoglobin. While there was no changes in BFOV-treated non-diabetic rats. The results further manifested that BPOV has glucose-lowering activity in diabetic rats.

Fig. 4 demonstrated the content of glycogen in liver and diaphragmatic muscle of experimental rats after 4 weeks administration of BPOV. A significant increase was observed in the hepatic and muscle glycogen of BPOV-treated diabetic rats, but not in treated non-diabetic rats, compared with diabetic rats. Moreover, the hepatic glycogen contents in diabetic rats were higher than that in non-diabetic rats. Thus it suggests that BPOV can regulate serum lipid and ameliorate metabolism turbulence on the lipid of diabetic animals. Administration of BPOV increased hepatic and muscle glycogen contents, which was beneficial to reduce blood glucose concentration. But it was not clear that this was a result of enhanced glycogenesis or inhibited glycogenolysis.

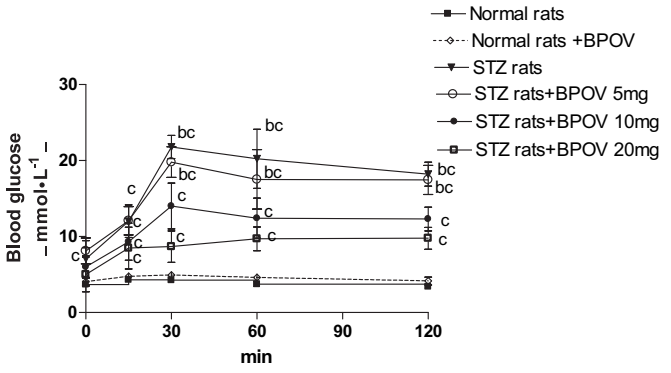


Fig. 2. Effect of BPOV on glucose tolerance in normal rats and STZ-induced diabetic rats. The diabetic rats were induced by a single intraperitoneal injection of STZ 50 mg kg^{-1}. CON: normal control group, 0.9% NaCl solution ig; CON + BPOV: treated normal group, BPOV 10 mg V kg^{-1} ig; DM: diabetic group, 0.9% NaCl solution ig; DM + BPOV L, M, H: treated diabetic group, BPOV 5, 10, 20 mg V kg^{-1} ig, respectively. $n = 9$ or 10 rats in each group. Mean ± SD. $^{a}P < 0.05$, $^{b}P < 0.01$, vs. DM; $^{c}P < 0.01$, vs. CON (Dunnett's test).

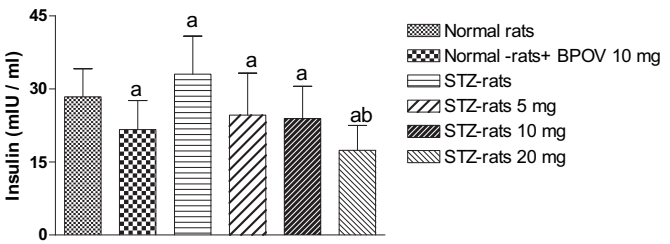


Fig. 3. Effect of intragastric BPOV for 4 weeks on serum insulin levels in normal rats and STZ-rats. Normal rats, STZ-rats, BPOV. Values are meanTS.E. for seven rats in each group. $^{a}P < 0.05$ vs. Normal rats; $^{b}P < 0.05$ vs. STZ-rats (ANOVA).

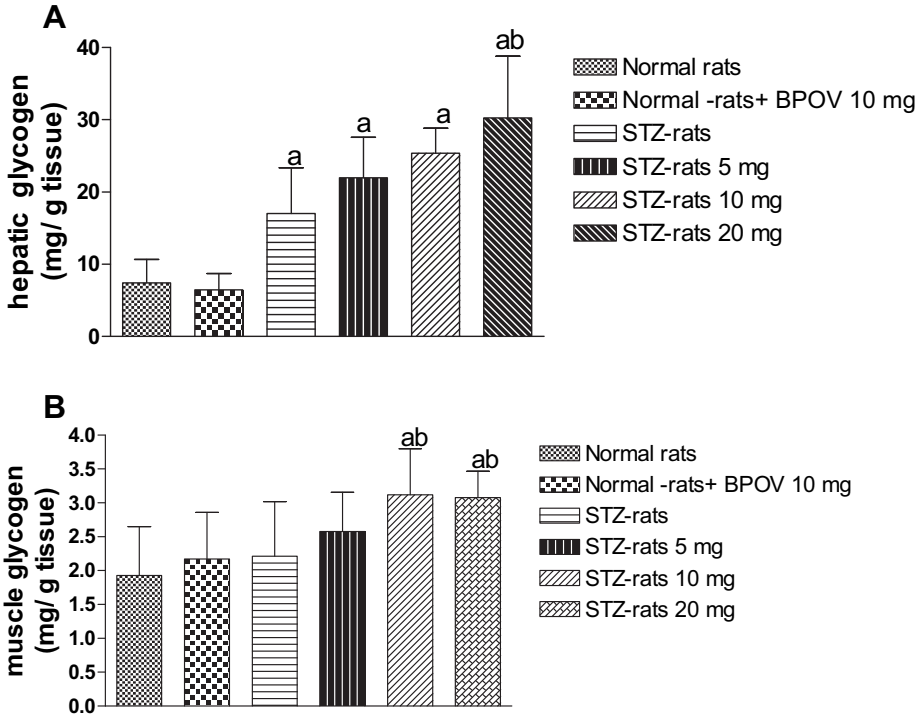


Fig. 4. Changes of the hepatic (A) and muscle glycogen (B) content in normal rats and STZ-rats. Normal rats, STZ-rats, BPOV. Values are meanTS.E. for seven rats in each group. [a] $P < 0.05$ vs. Normal rats; [b] $P < 0.05$ vs. STZ-rats (ANOVA).

Table 4 shows the changes in the concentration of serum lipid of control and experimental rats. There was a significant increase of the serum total cholesterol, triglycerides and low-density lipoprotein–cholesterol in STZ-diabetic rats. Administration of BPOV to diabetic rats markedly reversed the changes and increased serum high-density lipoprotein–cholesterol concentration. No significant alterations were observed in BPOV-treated non-diabetic rats.

Changes in plasma lipid concentration are a frequent complication in patients with diabetes mellitus [36,37], which contributes to the development of vascular disease. In the present study, STZ-diabetic rats exhibited hypertriglyceridemia and hypercholesterolemia, while BPOV treatment significantly decreased serum total cholesterol and triglycerides concentration as well as increased serum high-density lipoprotein–cholesterol concentrations in diabetic rats, suggesting that BPOV could improve the disorder of lipid metabolism in diabetes.

3.4. Permeation of vanadium complexes across Caco-2 monolayer

Within 1 h, the vanadium complex BPOV permeated through the Caco-2 cell monolayer. The apparent permeability coefficients (P_{app}), the bidirectional transport between apical and basolateral side, and the intracellular accumulation of BPOV are shown in Table 5. BPOV showed transmembrane permeability of 3.7×10^{-5} cm s^{-1}. The P_{app} for BPOV was independent of the corresponding compound concentrations (Fig. 5). The ratio of P_{app} in the apical to basolateral direction (A → B) to the reversed direction (B → A) was 0.5–0.8. BPOV had permeability above 10^{-6} cm s^{-1}, suggesting BPOV has better gastrointestinal stress for oral administration.

The P_{app} constants for BPOV were found to be concentration independent (Fig. 5). The permeability of BPOV in the apical to basolateral (P_{app}, A to B) was about twice that of the basolateral to apical directions (P_{app}, B to A). The experimental results illuminate that BPOV passes through Caco-2 cell monolayer via the simple passive diffusion. The proof includes: (i). The rates of BPOV increased linearly with the concentration and did not show saturation up to 0.5 mM (Fig. 5), indicating that this permeation process is driven by the concentration gradient. (ii) No efflux was observed for BPOV. The AP to BL permeability is larger than the reversed direction. A ratio of ~2 for A to B permeability over that of B to A is unexpected for the simple diffusion process. The reasons might be the differentiated Caco-2 cells are polarized, the microvilli membrane side has a relatively larger surface and thus possesses a much higher binding capacity than the basolateral membrane side. When B to A transport is carried out, more binding of

Table 4
Effects of 4 weeks of intragastric of BPOV on the concentration of serum lipid in normal rats and STZ-rats.

Treatment	Dose (mg V kg^{-1} d^{-1})	Serum lipid (mmol L^{-1})			
		TC	TG	LDL	HDL
Normal rats	Saline	2.34 ± 0.14	0.71 ± 0.12	1.09 ± 0.24	0.76 ± 0.15
Normal rats + BPOV	10.0	2.50 ± 0.46	0.50 ± 0.06[a]	1.20 ± 0.22	0.79 ± 0.17
STZ-rats	Saline	2.91 ± 0.36[a]	1.03 ± 0.13[a]	1.67 ± 0.33[a]	0.66 ± 0.11[a]
STZ-rats + BPOV	5.0	3.05 ± 0.48[a]	0.86 ± 0.11[b]	1.55 ± 0.41[b]	0.66 ± 0.07[a]
	10.0	2.19 ± 0.25[b]	0.71 ± 0.12[b]	1.10 ± 0.14[b]	0.80 ± 0.09[b]
	20.0	2.35 ± 0.33[b]	0.71 ± 0.16[b]	0.83 ± 0.12[b]	0.85 ± 0.10[b]

Normal control, STZ-rats, BPOV—[*N*,*N*′-bis(4-hydroxysalicylidene)-o-phenylene-diamine] oxovanadium(IV). Values are meanTS.E. for seven rats in each group. TC—total cholesterol, TG—triglycerides, LDH—low-density lipoprotein–cholesterol. HDL—high-density lipoprotein–cholesterol. [a] < 0.05 vs. Normal control. [b] < 0.05 vs. STZ-rats.

Table 5
The apparent permeability coefficients of BPOV.

Complex	P_{app} (10^{-5} cm s^{-1})[a]		
	A → B ($P_{app\ a-b}$)	B → A ($P_{app\ b-a}$)	P_{a-b}/P_{b-a}
BPOV	1.96 ± 0.1	0.53 ± 0.1	3.7

[a] Each data point was the average of three assays.

vanadium complex to the microvilli membrane could result in less vanadium permeation across monolayer than that in the reverse transport direction. (iii) The fact that the P_{app} was independent of vanadium concentration (Fig. 5) did not support involvement of an active transport mechanism.

The experimental results above suggested that simple diffusion could be the major pathway for organic vanadium compounds in oral administration. It is widely accepted that lipophilic drugs have better cellular permeability and absorption profiles than hydrophilic drugs. Therefore, lipophilicity could be one of the major determining factors for the oral bioavailability of vanadium compounds. The experimental results indicate that BPOV with salen-type ligands has good lipophilic properties.

3.5. The cytotoxicity and of vanadium complex BPOV

The cytotoxicity of the vanadium complex BPOV on Caco-2 cells was measured by decrease of cell viability using the MTT assay as described in the Experimental protocols Section. The data of cell viability decrease was fitted to the Hill model and the calculated IC_{50} value was 1.52×10^{-5} mmol L^{-1}.

Our data showed that the IC_{50} value of BPOV was higher to the neutral charge organic complexes, e.g., bis(maltolato) oxovanadium ([VO(ma)$_2$]) [38]. It suggested BPOV has effect on impairment of cellular associated with lower level capacity of cellular accumulation.

4. Results and discussion

Structure of BPOV is based on elemental analyses, spectroscopic (IR, UV/vis, EPR) data and X-ray diffraction analyses.

4.1. Single crystal X-ray diffraction studies

The asymmetric unit of BPOV consists of a monomeric vanadium (IV) complex, two dimethyl sulfoxide (DMSO) molecules and two water molecules(Fig. 6). The V^{IV} atom is five-coordinate in a distorted square-pyramidal environment. The basal square plane is constituted by the *N*,*N*′-bis(salicylidene)-o-phenylene-diamine molecule, which acts as a tetradentate ligand through its o-phenylene-diamine N atoms and its deprotonated phenol O atoms. The V atom is located 0.5997 (2) Å above the mean plane defined by atoms N1/N2/O1/O3. This distance is very similar to those observed for VO(acac)$_2$ (0.55 Å) [39] and [VO(acen)] [acen is *N*,*N*-ethylenebis (acetylacetoneiminate); 0.58 Å] [40]. The apical position is occupied by the oxo ligand; the V–O_{oxo} bond distance of 1.592 (3) Å is typical for five-coordinate vanadyl species. Similar to other VO (salen) [*N*,*N*′-ethylenebis(salicylaldiminato)]oxovanadium(IV)-type compounds [41,42], the title complex is best described as having purely square-pyramidal geometry, because of its ζ value of 0.06. The ζ parameter was introduced by Cornman [43] to measure the distortion of a square-pyramidal structure toward trigonal-bipyramidal.

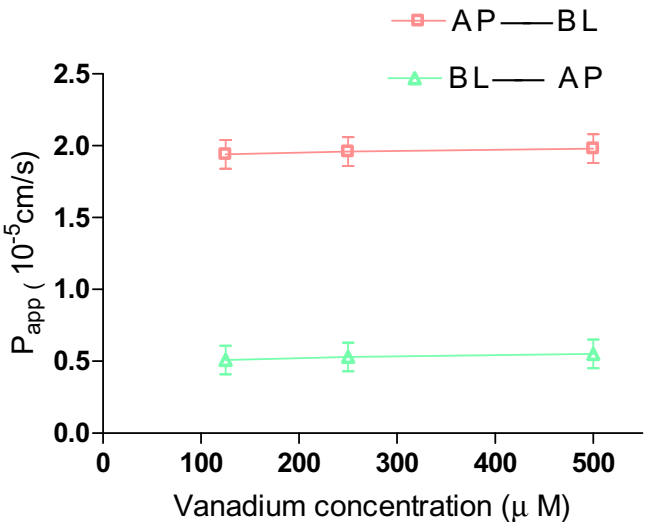


Fig. 5. Plot of apparent permeability coefficient, P_{app} of BPOV vs. the concentration in the absorptive (AP–BL) direction or the exsorptive (BL–AP) direction.

4.2. Spectra

BPOV exhibits a magnetic moment at room temperature and 110 K in the solid state. With an electron configuration of [Ar]3d^1, V (IV) has one unpaired electron for which the spin-only formula predicts a magnetic moment of 1.73 BM. BPOV has an effective magnetic moment (μ_{eff}) of 1.75 BM at 300 K, and this is a typical value for a V(IV) complex with $S = 1/2$. This confirmed that BPOV is in the mononuclear vanadyl state. The ESR spectrum of BPOV at room temperature exhibited the characteristic widely spaced eight-lined pattern due to the coupling of the unpaired electron with large moment of the ~100% abundant ^{51}V nucleus ($I = 7/2$), indicated only one mononuclear vanadium (IV) species predominates in the complex solution examined.

In the visible absorption spectrum of the complex, four absorption bands at 265, 332, 415 and 695 nm were observed. Absorption bands at 265 and 332 nm can be assigned to $\pi \rightarrow \pi^*$ transition based on the ligand. Generally, vanadyl complexes with lower symmetry than C_{4v} usually give rise to three d–d bands, due to the splitting of the degenerate d_{xz} and d_{yz} [44]. Two or three absorption bands assigned to d–d transitions [$d_{xy} \rightarrow (d_{xz},d_{yz})$, $d_{xy} \rightarrow d_{x2-y2}$, and $d_{xy} \rightarrow d_{z2}$ in the order of decreasing wavelength] were observed in the visible region [45]. Absorption bands at 695 nm can be assigned to a $d_{xy} \rightarrow (d_{xz},d_{yz})$ transition and the absorption bands at 415 nm can be assigned to a $d_{xy} \rightarrow d_{x2-y2}$ transition. The increase of the ligand field strength is responsible for a blue shift of the absorption bands. Therefore, the excitation to the orbital occurs at 350–400 nm and can be masked by intraligand or charge transfer bands. The IR absorption band due to V=O stretching frequency was found at 905 cm^{-1}.

5. Conclusions

A newly prepared complex [*N*,*N*′-bis(4-hydroxysalicylidene)-o-phenylene-diamine] oxovanadium(IV) (BPOV) is found to be a potent insulin-enhancing and antidiabetic vanadyl compound. It was characterized by elemental analysis, IR, UV–vis, EPR and X-ray crystallographic analysis. The V^{IV} atom is five-coordinate in a distorted square-pyramidal environment. Based on in vivo evaluations, a 4 weeks administration with BPOV resulted in a decrease of the blood glucose levels in STZ-diabetic rats.

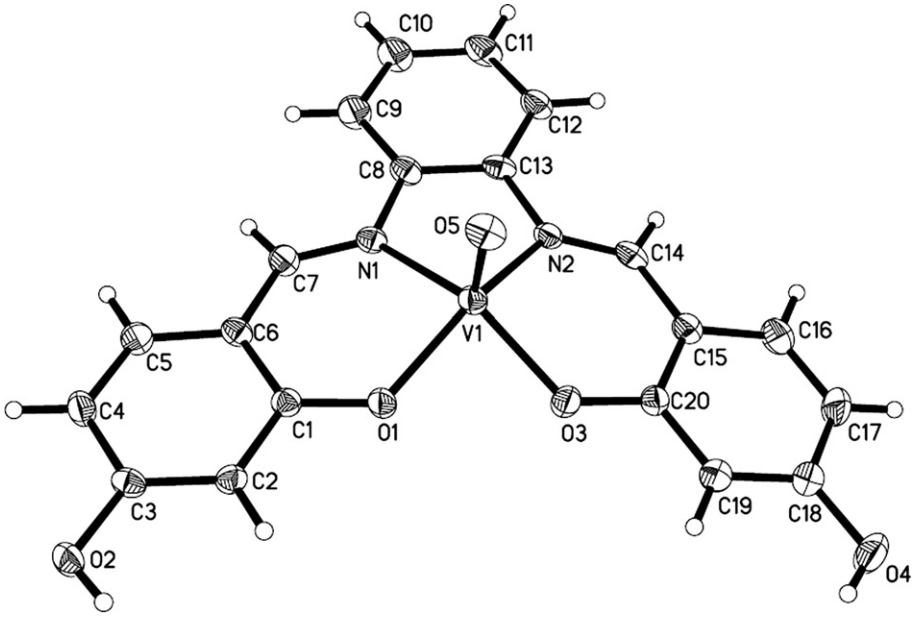


Fig. 6. A view of BPOV molecule with the atomic labelling scheme. Displacement ellipsoids are shown at the 30% probability level. The water and DMSO molecules have been omitted.

Furthermore, OGTT demonstrated that the impaired glucose tolerance in STZ-diabetic rats was ameliorated by BPOV treatment. As tested on insulin, glycogen and serum lipid studies with BPOV, BPOV has proved its antidiabetic potency due to its blood glucose-lowering and glycogenesis actions, its abilities to improve lipid metabolism. BPOV had permeability above 10^{-5} cm s^{-1}. It was suggested BPOV with salen-type ligands has better gastrointestinal stress for oral administration and good lipophilic properties. The cytotoxicity of BPOV on Caco-2 cells was measured by decrease of cell viability using the MTT assay which implyed salen-type ligands for vanadium complexes have higher effect on impairment of cellular associated with lower level capacity of cellular accumulation.

6. Experimental protocols

6.1. General

Streptozotocin (STZ) was purchased from Sigma (purity $\geq$ 98%). Glucose reagent kit was a product of Shanghai institute of Biological Products (purity $\geq$ 98%).

Caco-2 cells were obtained from Cell Culture Center of Peking Union Medical Science. Fetal bovine serum and non-essential amino acid mixture were purchased from Hyclone. Dulbecco_s modified eagle medium (DMEM, high glucose) was purchased from Gibco. Hank's balance salt solution (HBSS), Earlier's balance salt solution (EBSS), phosphate buffer saline (PBS), MTT, *N*-2-hydroxyethylpiperazine-ethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), 2′,7′-dichlorofluorescein diacetate (DCFH-DA), ethylenediaminetetra-acetate (EDTA), and trypsin were purchased from Sigma. Transwells were purchased from Corning Costar (Cambridge, MA, USA). Solutions of BPOV were freshly prepared by dissolving the compounds in HBSS (pH 7.2) or EBSS (pH 6.5) buffer to 1.0 mM and immediately diluting to the desired concentrations. DCFH-DA solution was prepared by dissolving in dimethyl sulfoxide (DMSO) and diluting with PBS buffer to the final concentration of 10 μM. MTT was also prepared in DMSO at the working concentration of 0.5 mg ml^{-1}.

6.2. Chemistry

Elemental analyses were carried out using a Carlo-Erba 1106 Elemental analyzer. IR spectra were recorded on a Nicolet 170SXFT-IR using a KBr disk technique. UV–vis spectra were recorded on a Shimadzu UV-2000 at room temperature. HPLC spectra were recorded on a Shimadzu LC-10A at room temperature. Biological activity experiments were measured on CL-770 Shimadzu Clinic Spectrometer, BS Semi-Automation Biochemistry analyzer which was purchased from Beijing Biochem Analyze Ltd., MP4R and OM8464 US-IEC freezing centrifugation instrument.

6.2.1. Perparation of [N,N′-bis(4-hydroxysalicylidene)-o-phenylene-diamine] oxovanadium(IV) (BPOV)

A methanol solution (10 mL) of o-phenylene-diamine (0.309 g, 3.0 mmol) was added to a methanol solution (10 mL) of 2,4-dihydroxybenzaldehyde (0.828 g, 6.0 mmol) and the resulting mixture was refluxed for 1 h. The ligands were not isolated from the methanol solution and the solution mixture was added first to an aqueous solution of $VOSO_4 \cdot 3H_2O$ (0.783 g, 3.0 mmol), then to an Et_3N (0.602 g, 6.0 mmol) solution, and refluxed for 2 h. The green precipitate was collected by filtration. Crystals of suitable quality for X-ray analysis were obtained by slow evaporation of a dimethyl sulfoxide solution. $C_{24}H_{30}N_2O_9S_2V$, $M = 605.56$. Anal. Found: C, 47.42; H, 4.57; N, 4.16. Calc.: C, 47.55; H, 4.95; N, 4.65%. UV–vis (DMSO) $\lambda_{max} = 265$ ($\epsilon = 2136$ M^{-1} cm^{-1}), 332 ($\epsilon = 1683$ M^{-1} cm^{-1}), 415 ($\epsilon = 43$ M^{-1} cm^{-1}) nm, 695 ($\epsilon = 31$ M^{-1} cm^{-1}) nm. IR (KBr disk): ν (cm^{-1}) = 905($\nu_{v=o}$), 523ν_{v-o}). ESR (RT, DMSO): 8-Line pattern, g $0 = 2.01 \pm 0.001$, A $0 = 101.6 \pm 0.1 \times 10^{-4}$ $cm^{-}1$. Magnetic moment (solid): 1.75 BM (one unpaired electron). Purity: ≥96% (HPLC: used a C_{18} column, methanol–water (5/95,V/V) as the mobile phase at 25 °C with detection wavelength at 210 nm).

6.3. Glucose-lowering studies

Male Sprague-Dawley rats weighing 200–250 g were obtained from Experimental Animal Center, Kunming Medical Collage (Grade II, Certificate number 2001034). Rats were allowed free

access to standard solid food for laboratory animals and tap water. Diabetes was induced by a single intraperitoneal injection of STZ 50 mg kg^{-1} in 0.1 mol L^{-1} citrate buffer (pH 4.4). Seven days after STZ injection, blood samples for analysis of blood glucose were obtained from the tail vein of the rats and the blood glucose was measured by the glucose oxidase method. The STZ-rats with the blood glucose level $\geqq$11.1 mmol L^{-1} were considered as diabetic rats. Normal rats were injected with 0.1 M citrate buffer alone.

Rats, 1 week after STZ treatment, were used for the experiments. STZ-rats were allowed free access to standard solid food for laboratory animals and tap water. Body weights of STZ-rats were measured weekly during the experiments. Intakes of solid food and drinking water in each rat were checked every day throughout the experiments. At concentration 5, 10, 20 mg V kg^{-1}, BPOV was dissolved in water (solution were prepared fresh daily) and administered to STZ-rats for 4 weeks by ig. During the experiments, serum insulin of rats treated by BPOV was measured at 9:00 a.m. daily.

The experimental animals were randomly divided into six groups with ten rats each according to the blood glucose. Normal control group: normal rats treated with 0.9% saline; treated normal group: normal rats treated with 10 mg V kg^{-1} BPOV; diabetic control group: STZ-diabetic rats treated with 0.9% saline; treated diabetic group: STZ-diabetic rats treated with BPOV 5, 10, 20 mg V kg^{-1} ig. The substances above were administered intragastrically once a day at the volume of 10 mL kg^{-1} for 2 weeks. Once per week the blood samples were collected from the tail vein of the rats for the blood glucose assay.

6.4. Oral glucose tolerance test [34]

Normal rats and STZ-diabetic rats were prepared and randomly divided into six groups as described in Glucose-Lowering Studies. After the drugs were administered intragastrically in both normal and STZ-induced diabetic rats for 4 weeks, Oral Glucose Tolerance Test was undertaken. The experimental rats were fasted for 14 h and given an oral glucose challenge at a dose of 2.0 g kg^{-1} body weight [34]. Blood glucose levels were measured at 0, 15, 30, 60, 120 min after glucose loading.

6.5. Insulin, glycogen and serum lipid studies

STZ-rats were prepared and randomly divided into as described as above. After drug administered to STZ-rats for 4 weeks, blood was collected from the tail vein of the rats following fasting 12 h. Then centrifuged (3000 rpm) for 10 min, the blood serum were collected into refrigeratory at −20 °C. The insulin was measured by ^{125}I radioimmunoassay. Cholesterol (CHO) and Triglyceride (TG) were measured by enzyme colorimetry .The high-density lipid (HDL) and the low-density lipid (LDL) were measured by polyethylene sulfuric acid precipitation method and phospho-tungstic acid magnesium precipitation method, respectively. The result was done by student τ test.

6.6. Cell culture and monolayer preparation

Caco-2 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 4.5 g $^{-1}$ D-glucose and 3.7 g $^{-1}$ $NaHCO_3$, supplemented with 10% Fetal bovine serum, 1% non-essential amino acids, 100 U ml^{-1} penicillin and 100 μg mL^{-1} streptomycin [46]. The cells were incubated at 37 °C in a humidified atmosphere of 5% CO_2. Cells between passage 45 and 60 were used in the studies. The cell monolayer was prepared as the method described previously [46]. Briefly, Caco-2 cells were seeded on Transwell insert filter (surface area = 1.0 cm^2, pore size = 3.0 μm) at a density of about 1×10^5 cells cm^{-2}. Cells were left to grow for 21 days to reach confluence and differentiation. The trans-epithelial electric resistance (TEER) of the monolayer was measured with a Millicell electrode resistance system (Millipore) and TEER values of $>$700 Ω cm^2 were routinely observed.

6.7. Transport of complex BPOV across caco-2 monolayer

The filter inserts upon which caco-2 monolayer grew were rinsed twice with HBSS (pH 7.4). Transport experiment was initiated by adding 0–500 μM of the vanadium complexes to either the apical side (vanadium compound dissolved in EBSS, pH 6.0) or the basolateral side (vanadium compound dissolved in HBSS, pH 7.4). Then the samples were shaken (37 °C 50 rpm) and 100 μL aliquots were taken from the receiver side at desired time intervals of 0–1 h. Samples were determined by inductively coupled plasma mass spectrum (ICP-MS). The apparent permeability coefficient (P_{app}) across the caco-2 cell monolayers was calculated with the following equation: $P_{app} = (dQ/dt)/(A \cdot C_0)$;

where dQ/dt is the flux of drug across the monolayer (mol transported/s), A (cm^2) is the surface area of the inserts, and C_0 (M) is the initial drug concentration in the donor compartment. To measure the cellular uptake of vanadium complex in the Caco-2 monolayer, the Caco-2 cells were washed three times with 1 mL of ice-cold 5 mM of EDTA/PBS (pH 7.2) solution after the transport studies described above to remove residual medium and surface-bound metal ions. Then the cells were collected and completely digested with 0.5 mL ultrapure nitric acid. The amount of vanadium in the collected samples was determined with ICP-MS (Leeman Labs Inc., USA). To investigate the effects of ion pair reagents, triethanolamine hydrochloride or tetrabutylammonium chloride, was added to a final concentration of 1.0 mM at the donor side of the transwell. Then the transport experiments were carried out as described above.

6.7.1. Measurement of TEER (trans-epithelial electrical resistance) of Caco-2 cell monolayer

The Caco-2 monolayer was incubated with 0–0.5 mM of vanadium complex in EBSS on the apical side for 60 min at 37 °C. Then the sample was shaken at 50 rpm. The integrity of the monolayer was monitored by measuring the trans-epithelial electrical resistance (TEER) using an epithelial voltohmmeter (World Precision Instrument, Sarasota, FL, USA).

6.8. Cytotoxicity assay

The Caco-2 cells were seeded on 96-well microplate at 5×10^4 cells/well and cultured for 48 h. The cells were washed with DMEM once, then incubated with various concentrations of vanadium complexes from 0 to 2.7 mM in 100 μL DMEM for 24 h. Afterwards, the medium was removed and 100 μL MTT (0.5 mg ml^{-1} in PBS) was added to each well and incubated for 4 h. The MTT medium was subsequently removed and 200 μL DMSO was added to dissolve the formazan formed. The absorption at 570 and 630 nm was measured 15 min later on plate reader (TECAN SUNRISE, Switzerland). The 50% inhibition concentration (IC_{50}) was calculated by plotting the cell viability vs. vanadium concentrations and fitted to the Hill model using a MicroCal*e* Origin program (Lab Corporation, USA).

6.9. Statistical analysis

Statistics values were presented as means ± standard deviations. Statistical analysis was performed by one-way analysis of variance followed by Dunnett's multiple comparison tests using Prism version 4.0 software.

Acknowledgements

This work was supported by National Natural Science Foundation of China (30260118) and Yunnan Natural Science Foundation (2002C0019R) and (2009CD006) and Yunnan University Science Foundation (2005Q002A).

Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.ejmech.2010.02.010.

References

[1] D.C. Crans, J.J. Smee, E. Gaidamauskas, L. Yang, Chem. Rev. 104 (2004) 849–902.
[2] D. Rehder, Inorg. Chem. Commun. 6 (2003) 694–697.
[3] A.K. Srivastava, M.Z. Mehdi, Diabet. Med 22 (2004) 2–13.
[4] H. Sakurai, K. Tsuchiya, M. Nukatsuka, M. Sofue, J. Kawada, J. Endocrinol. 126 (1990) 451–459.
[5] J.H. McNeil, V.G. Yuen, H.R. Hoveyda, C. Orvig, J. Med. Chem. 35 (1992) 1489–1491.
[6] V.G. Yuen, P. Caravan, L. Gelmini, N. Glover, J.H. McNeill, I.A. Setyawati, Y. Zhou, C. Orvig, J. Inorg. Biochem. 68 (1997) 109–116.
[7] B.A. Reul, S.S. Amin, J.-P. Buchet, L.N. Ongemba, D.C. Crans, S.M. Brichard, Br. J. Pharmacol. 126 (1999) 467–477.
[8] D.C. Crans, L. Yang, J.A. Alfano, L.-H. Chi, W. Jin, M. Mahroof-Tahir, K. Robbins, M.M. Toloue, L.K. Chan, A.J. Plante, R.Z. Grayson, G.R. Willsky, Coord. Chem. Rev. 237 (2003) 13–22.
[9] G. Boden, X. Chen, J. Ruiz, G.D. van Rossum, S. Turco, Metabolism 4 (5) (1996) 1130–1135.
[10] M. Halberstam, N. Cohen, P. Shlimovich, L. Rossetti, H. Shamoon, Diabetes 45 (1996) 659–666.
[11] A.K. Goldfine, M.-E. Patti, L. Zuberi, B.J. Goldstein, R. LeBlanc, E.J. Landaker, Z. Y. Jiang, G.R. Willsky, C.R. Kahn, Metabolism 49 (2000) 400–410.
[12] V.G. Yuen, E. Vera, M.L. Battell, W.M. Li, J.H. McNeill, Diabetes Res. Clin. Pract. 43 (1999) 9–19.
[13] J. Meyerovitch, P. Rothenberg, Y. Shechter, S. Bonner-Weir, C.R. Kahn, J. Clin. Invest. 87 (1991) 1286–1294.
[14] M. Bendayan, D. Gingras, Diabetologia 32 (1989) 561–567.
[15] R.A. Pederson, S. Ramanadham, A.M. Buchan, J.H. McNeill, Diabetes 38 (1989) 1390–1395.
[16] S.M. Brichard, C.J. Bailey, J.-C. Henquin, Diabetes 39 (1990) 1326–1332.
[17] S.M. Brichard, A.M. Pottier, J.C. Henquin, Endocrinology 125 (1989) 2510–2516.
[18] K.H. Thompson, J.H. McNeill, Res. Commun. Chem. Pathol. Pharmacol. 80 (1993) 187–200.
[19] S. Dai, K.H. Thompson, J.H. McNeill, Pharmacol. Toxicol. 74 (1994) 101–109.
[20] H. Sakurai, K. Fujii, H. Watanabe, H. Tamura, Biochem. Biophys. Res. Commun. 214 (1995) 1095–1101.
[21] K.H. Thompson, J.H. McNeill, C. Orvig, Chem. Rev. 99 (1999) 2561–2572.
[22] H. Yasui, A. Tamura, T. Takino, H. Sakurai, J. Inorg. Biochem. 91 (2002) 327–328.
[23] K. Fukui, Y. Fujisawa, H. Ohya-Nishiguchi, H. Kamada, H. Sakurai, J. Inorg. Biochem. 77 (1999) 215–224.
[24] C. Orvig, K.H. Thompson, M. Battell, J.H. McNeill, Met. Ions Biol. Syst. 31 (1995) 575–594.
[25] P. Caravan, L. Gelmini, N. Glover, F.G. Herring, H. Li, J.H. McNeill, S.J. Rettig, I. A. Setyawati, E. Shuter, Y. Sun, A.S. Tracey, V.G. Yuen, C. Orvig, J. Am. Chem. Soc. 117 (1995) 12759–12770.
[26] V.G. Yuen, C. Orvig, J.H. McNeill, Can. J. Physiol. Pharmacol. 71 (1993) 263–269.
[27] K.H. Thompson, V.G. Yuen, J.H. McNeill, C. Orvig, Chemical and pharmacological studies of a new class of antidiabetic vanadium complexes. in: A. S. Tracey, D.C. Crans (Eds.), Vanadium Compounds. Chemistry, Biochemistry, and Therapeutic Applications, ACS Symposium Series, vol. 711. American Chemical Society, Washington, DC, 1998, pp. 329–343.
[28] K.H. Thompson, C. Orvig, Dalton Trans. (2006) 761–764.
[29] N. Durai, G. Saminathan, J. Clin. Biochem. Nutr. 22 (1997) 31–39.
[30] I.J. Hidalgo, T.J. Raub, R.T. Borchardt, Gastroenterology 96 (1989) 736–749.
[31] S. Yamashita, T. Furubayashi, M. Kataoka, T. Sakane, H. Sezaki, H. Tokuda, Eur. J. Pharm. Sci. 10 (2000) 195–204.
[32] G.M. Sheldrick, SHELXS97 and SHELXL97. University of Gottingen, Germany, 1997.
[33] G.M. Sheldrick, SHELXTL. Version 6.10. Bruker AXS Inc., Madison, Wisconsin, USA, 2000.
[34] P. Basnet, S. Kadota, M. Shimizu, Y. Takata, M. Kobayashi, T. Namba, Planta Med. 61 (1995) 402–405.
[35] R.J. Koenig, C.M. Peterson, R.L. Jones, C. Saudek, M. Lehrman, A. Cerami, N. Engl. J. Med. 295 (1976) 417–420.
[36] B.V. Howard, P.J. Savage, L.J. Bennion, P.H. Bennett, Atherosclerosis 30 (1978) 153–162.
[37] E.A. Nikkila, M. Kekki, Metabolism 22 (1973) 1–22.
[38] X.G. Yang, X.D. Yang, L. Yuan, K. Wang, D.C. Crans, Pharm. Res. 21 (2004) 1026–1033.
[39] R.P. Dodge, D.H. Templeton, A. Zalkin, J. Chem. Phys. 35 (1961) 55–67.
[40] D. Bruins, D.L. Weaver, Inorg. Chem. 9 (1970) 130–135.
[41] .M. Pasquali, Marchetti, C. Floriani, M. Cesari, Inorg. Chem. 19 (1980) 1198.
[42] P.E. Riley, V.L. Pecoraro, C.J. Carrano, J.A. Bonadies, K.N. Raymond, Inorg. Chem. 25 (1986) 154–160.
[43] C.R. Cornman, K.M. Geisre-Bush, S.R. Rowley, P.D. Boyle, Inorg. Chem. 36 (1997) 6401–6408.
[44] N.D. Chasteen, R.L. Belford, I.C. Paul, Inorg. Chem. 8 (1969) 408–418.
[45] C.J. Ballhausen, H.B. Gray, Inorg. Chem. 1 (1962) 111–122.
[46] J. Gao, E.D. Hugger, M.S. Bech-Westermeyer, R.T. Borchardt, in: S.J. Enna, M. Williams, J.W. Ferkany, T. Kenakin, R.D. Porsolt, J.P. Sullivan (Eds.), Current Protocols in Pharmacology, Wiley, New York, 2000, pp. 7.2.1–7.2.23.