

Chimeric design, synthesis, and biological assays of a new nonpeptide insulin-mimetic vanadium compound to inhibit protein tyrosine phosphatase 1B

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Abstract: Prior to its total synthesis, a new vanadium coordination compound, called TSAG0101, was computationally designed to inhibit the enzyme protein tyrosine phosphatase 1B (PTP1B). The PTP1B acts as a negative regulator of insulin signaling by blocking the active site where phosphate hydrolysis of the insulin receptor takes place. TSAG001, [V^VO₂(OH)(picolinamide)], was characterized by infrared (IR) and nuclear magnetic resonance (NMR) spectroscopy; IR: ν/cm^{-1} 3,570 (NH), 1,627 (C=O, coordinated), 1,417 (C–N), 970/842 (O=V=O), 727 δ (pyridine ring); ¹³C NMR: 5 bands between 122 and 151 ppm and carbonyl C shifted to 180 ppm; and ¹H NMR: 4 broad bands from 7.6 to 8.2 ppm and NH₂ shifted to 8.8 ppm. In aqueous solution, in presence or absence of sodium citrate as a biologically relevant and ubiquitous chelator, TSAG0101 undergoes neither ligand exchange nor reduction of its central vanadium atom during 24 hours. TSAG0101 shows blood glucose lowering effects in rats but it produced no alteration of basal- or glucose-induced insulin secretion on β cells during in vitro tests, all of which excludes a direct mechanism evidencing the extrapancreatic nature of its activity. The lethal dose (LD₅₀) of TSAG0101 was determined in Wistar mice yielding a value of 412 mg/kg. This value is one of the highest among vanadium compounds and classifies it as a mild toxicity agent when compared with literature data. Due to its nonsubstituted, small-sized scaffold design, its remarkable complex stability, and low toxicity; TSAG0101 should be considered as an innovative insulin-mimetic principle with promising properties and, therefore, could become a new lead compound for potential nonpeptide PTP1B inhibitors in antidiabetic drug research. In view of the present work, the inhibitory concentration (IC₅₀) and extended solution stability will be tested.

Keywords: inhibitors, PTP1B, computer assisted drug design (CADD), insulin, diabetes

Introduction

There are 2 clinical forms of diabetes mellitus.¹ Type 1 (or juvenile) diabetes is primarily due to autoimmune-mediated destruction of pancreatic β -cell islets, resulting in an absolute insulin deficiency. Patients with type 1 diabetes depend on a daily exogenous insulin administration (IM) to prevent the development of ketoacidosis. Its frequency is low when compared to that of type 2 diabetes, which accounts for over 90% of all reported cases worldwide. Type 2 diabetes is characterized by insulin resistance (peripheral or extrapancreatic) and/or abnormal insulin secretion (pancreatic), either of which may predominate. At the beginning, type 2 patients do not depend on exogenous insulin uptake, but may require it for controlling blood glucose levels if this cannot be

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achieved with diet alone or combined with oral hypoglycemic agents. Changes in living conditions, habits, and lifestyle over the last century have led not only to a dramatic increase in life span but also to the incidence of type 2 diabetes known as “diabesity”² or “metabolic syndrome.”³ In conjunction with genetic susceptibility, particularly in certain ethnic groups, type 2 diabetes is caused by environmental and behavioral factors such as sedentary lifestyle, overly rich nutrition, and obesity. The prevention of diabetes and control of its micro- and macrovascular complications will require an integrated approach for the significant reduction in the huge premature morbidity and mortality it causes.⁴

Current therapeutic approaches have largely been developed in the absence of defined molecular targets or even a solid understanding of disease pathogenesis. Within the recent past, our understanding of biochemical pathways related to the development of metabolic syndrome has expanded. Now, there is a steady growing range of molecular drug targets within these pathways. They are identified on the

basis of predicted roles in modulating one or more key aspects of the pathogenesis of diabetes or metabolic syndrome. Several mechanistic categories for new therapeutic approaches can be considered. In a key position are approaches aimed at reducing excessive glucose production by the liver, besides mechanisms to augment glucose-stimulated insulin secretion, specific molecular targets in the insulin signaling pathway, and new approaches to obesity and altered lipid metabolism, which offer the prospect of net improvements in insulin action (or secretion).⁵

The insulin signaling pathway starts with the binding of insulin to the extracellular α subunit of the transmembranal insulin receptor leading to a conformational change in the intracellular β subunit and promotion of the autophosphorylation at a number of tyrosine residues, $Y + P = PY$ (Figure 1). Although the receptor protein tyrosine kinase is a positive regulator of the signal transduction, the protein tyrosine phosphatase (PTP) represents a negative one. The insulin receptor itself acts as a tyrosine kinase by transferring

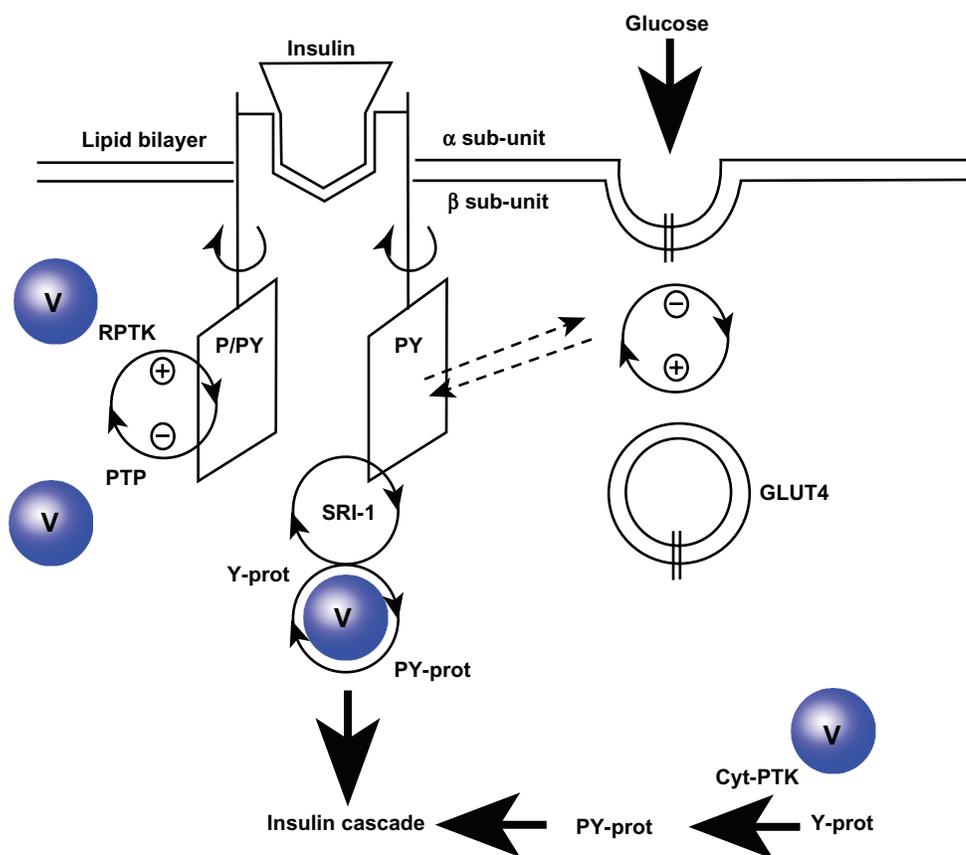


Figure 1 Schematic display of potential biomolecular targets of vanadium compounds within the human cell. The metabolic pathway of the insulin cascade is depicted. A double line symbolizes the cellular membrane, with the extracellular part above and the intracellular part below.

Abbreviations: Letter V, possible vanadium target; P/PY, tyrosine residue phosphorilated/nonphosphorylated; RPTK, receptor protein tyrosine kinase (positive regulator); PTP, protein tyrosine phosphatase (negative regulator); Y-prot, nonphosphorylated cytosolic protein; PY-prot, phosphorylated cytosolic protein; SRI-1, substrate 1 for the insulin receptor; Cyt-PTK, cytosolic protein tyrosine kinase.

phosphate to different receptor substrates, especially insulin receptor substrate 1, which in turn propagates the insulin signaling through phosphate transfer to cytoplasmic proteins (Y-prot), and all the metabolic effects further downstream. In the process, insulin increases glucose transport in fat and muscle cells by stimulating the translocation of the transporter GLUT4 from intracellular sites to the plasma membrane and thereby promoting its exocytosis.⁶

Among the hitherto known PTPs, protein tyrosine phosphatase 1B (PTP1B) has received much attention due to its role as a negative regulator of insulin signaling.^{7,8} Clearly, a selective control of the biological function of PTP1B is a challenging task, and an inhibitor must not only efficiently bind to the specific target enzyme but also do so without impeding the catalytic behavior of closely related enzymes. The design of small molecule PTP1B inhibitors to treat type 2 diabetes has received considerable attention.^{9–15}

Since the early discovery of the insulin-like activity of vanadate,^{16,17} VO_4^{3-} , vanadium salts have kept their rank among the highest inhibitory activities against PTP,^{8,18} although not in a selective manner. Other targets for monomeric vanadate or vanadyl are adenosine triphosphatases, adenylyl cyclases, and calcium channels,¹⁹ and F-actin in the case of decavanadate (V_{10}).²⁰ Nevertheless, the insulin-like activities of a number of vanadium coordination compounds have been well documented.^{17,21–23} Some of their effects were found as enhancement of glucose uptake, biosynthesis of glycogen, as well as inhibition of gluconeogenesis or lipolysis.^{24,25} The molecular action mechanism of vanadate and the vanadium complexes has not yet been sufficiently established. However, after different studies, it is accepted now that vanadium targets may be protein phosphatases,^{26,18,27–30} instead of insulin hormone itself or insulin receptors GLUT2 and GLUT4 messengers.³¹ Recently, literature evidence appears in support of the hypothesis that active vanadium compounds may have intrinsic affinity to the PTP1B catalytic site.^{32–35}

The insulin-like properties of vanadium have been demonstrated in a conclusive way based on in vitro experiments,³⁶ in vivo assays in rats^{37,38} or mice,³⁹ as well as in patients with diabetes.⁴⁰ It is noteworthy to state that as yet all clinical studies have been carried out successfully using inorganic salts of vanadium, albeit it is beyond doubt that the true potential lies in the use of organic vanadium complexes, judging from the higher activity and the lower toxicity rates.^{41–43} In addition, vanadium insulin-mimetic effects are superior to other heavy metals (Cr, Zn, and Cd),^{44,45} since vanadium research seeks to strengthen therapeutic benefits over toxicities.^{46,47} Especially vanadium seems not to present negative accumulation

effects.⁴⁸ Our present study also aims at the ongoing discussion on the prodrug role of vanadium coordination compounds (stability against ubiquitous citrate).⁴⁶ Different research teams have developed new organic compounds of vanadium to improve the drug properties. In the present work, due to the reported identification of a biomolecular target we could devise a so-called *de novo* design procedure. In a previous work,³⁵ we demonstrated that vanadium compounds stated in the literature as the most active antidiabetics are also potential PTP1B inhibitors.³⁵ Particularly, in the case of bis(maltolate) oxo-vanadium(IV) (BMOV) and ammonium bis(picolate) oxo-vanadium(V), we determined the active conformations during simulated docking into the target enzyme (PTP1B).^{35,49}

In the present work, we report the design, synthesis, bioassays, and toxicity tests for a new organic vanadium compound (TSAG0101).

Methods

De novo design

Vanadium complexes were designed by a chimeric procedure of combinatory chemistry to obtain organic oxo-vanadium complexes of type VO_2L where V is the central vanadium atom and L stands for ligand. The former imitates the geometry of a phosphate anion, phosphatomimetic group,⁴⁶ whereas the latter is composed of unrelated organic rests (strong chelating groups). To this end, pharmacologically and chemically known molecular fragments (A, B, and Q in Figure 2) were combined to build an imaginary compound using Chem3D of the ChemOffice 5.0 tool box.⁵⁰ Each building block (fragment) follows a specific structural pattern and function:

1. Quelate fragment (Q): coordinates the dioxovanadate ion, VO_2^{2+} and interacts with the Cys215 from the PTP1B. Furthermore, these molecular fragments have aromatic interaction with residues Phe182 and Tyr46.
2. Basic fragment (B): interacts with the acid residue Asp181, at the center of the PTP1B cavity.
3. Acidic fragment (A): allows the molecular recognition of the substrate by the external PTP1B residues Arg45 and Arg47.

Geometry optimization of designed complexes

Density functional theory (DFT) with B3LYP hybrid exchange – correlation functional is a well-accepted standard procedure in computing of the equilibrium geometry. Especially, DFT/B3LYP is used for molecular geometry optimization of ligands. The basis set for all atoms is

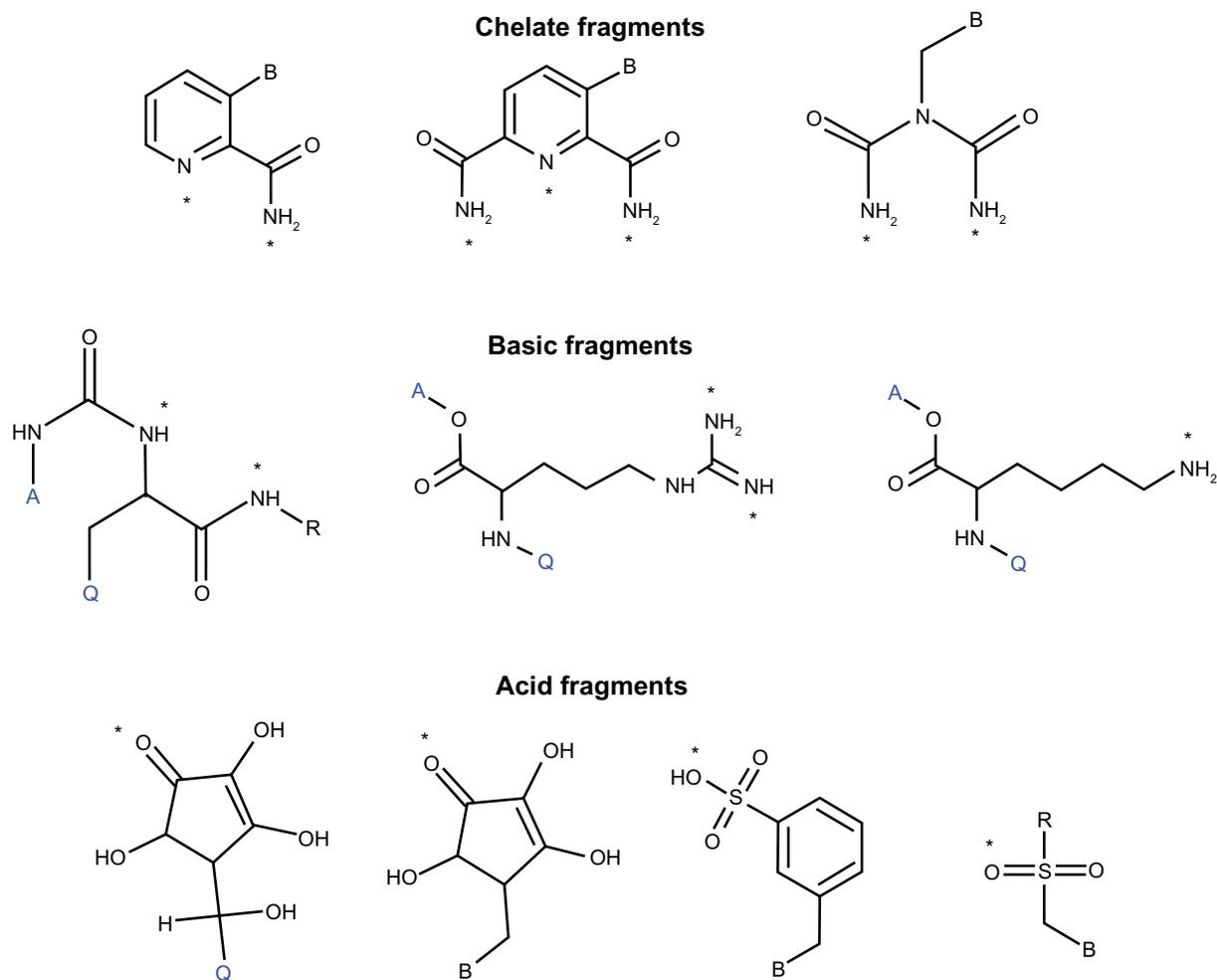


Figure 2 Molecular subunits used for the chimeric procedure. The design scheme allows the insertion of 2 or 3 fragments.

6–31 + G(d,p). A frequency computation is carried out using the optimized structures to provide a complete description of the molecular motions in normal mode. The absence of the imaginary frequencies after diagonalization of Hessian matrix confirmed that the optimized structure is the real minima on the ground state hyperdimensional surface. By means of visual inspection using the Gaussview program, the modes can be assigned to the irreducible representations of the point groups. All calculations have been done using Gaussian03 program suite,⁵¹ and Gaussview V3.09⁵² has been used for visualizing the conformers.

Modeling of the interaction PTP1B (receptor) – vanadium complexes (ligand)

Prior to manual ligand docking at the active site of the crystal structure⁵³ (PDB-code: 2HNP) of PTP1B,⁵⁴ the Tripos force field in Sybyl⁵⁵ was adapted for computing the steric and electrostatic energetics of ligand–receptor complex relaxations. In addition, modeling software packages MOE,⁵⁶

Hyperchem,⁵⁷ and ChemAxon⁵⁸ were used during consecutive stages of the work and at different places (see Acknowledgments) with the methods reported elsewhere.³⁵ In particular, chimeric candidates were fitted into the pharmacophore model based on intuitive grounds (guessing conformational entropy effects, hydrophobic burial, π -stacking, etc). Particularly, sensitive aspects of ligand docking like reliability and model limitations as well as target flexibility were considered and taken from the expert literature.^{59–61}

Synthesis of VO₂L complexes

The synthesis of VO₂L complexes was accomplished with the triethyl ester of vanadic acid, VO(OEt)₃. For its in situ preparation, a procedure was adapted from literature.⁶² Finely grounded V₂O₅(s) was refluxed in absolute ethanol overnight, the ethanol was previously treated with molecular sieve. The resultant olive green-colored slurry was cooled to room temperature and then filtered through a tared fritted glass filter to yield a clear, pale yellow solution of VO(OEt)₃. The filtered

solid was dried by air flux, and its mass was determined every hour until constant weight. Yield of $\text{VO}(\text{OEt})_3$ was calculated from the difference in the initial and recovered masses of V_2O_5 , assuming that the recovered solid was unreacted V_2O_5 . Previously prepared solution of $\text{VO}(\text{OEt})_3$ (filtered eventually) was stirred at room temperature. The ligand was added very slowly (stoichiometry 1:1) and dissolved in the minimum of ethanol. After ligand addition, there was a color change in the solution from pale green-yellow to a bright clear yellow. The resulting reaction mixture was stirred at room temperature for 1 hour after the addition of the ligand. Volume was reduced by half or until the appearance of solid, using a rotary evaporator with vacuum pump. At this point, the solution was put aside for slow crystallization by evaporation at room temperature, until the product had precipitated completely. Reaction mixture was decanted and ethanol was added to the slurry to double the volume. Ethanol was previously treated with a molecular sieve. The solid was washed vigorously with the ethanol and then put aside for precipitation, after the complete precipitation the mixture was decanted, these washes were done until the supernatant was not colored and a final wash was performed with a mixture 80:20 ethanol:acetone. Solution was decanted and the slurry was dried in a vial under vacuum. Spectroscopy characterization was done by infrared (IR [KBr]), ^1H and ^{13}C nuclear magnetic resonance (NMR) in deuterated water.

Solution stability of VO_2L complexes

For the solution stability tests, 2 solutions were prepared: one is vanadium compound in a concentration of 2.5×10^{-4} M, physiological pH 7.4 and the other one containing sodium citrate 1:1, which is a natural reducing and chelating agent for vanadium. The solutions were used for experiments of accumulation of spectra carried out for 27 hours.

Pancreatic activity of VO_2L complexes

Inbred, female Lewis rats (200 g) were purchased from Charles River (Sulzfeld, Germany). Islets were isolated by collagenase digestion⁶³ and purified by handpicking under the binocular. Batches of 5 freshly isolated islets were incubated in 1 mL of medium for 1 hour at 37°C. The incubation medium consisted of (mM) Na^+ , 122; K^+ , 4.7; Ca^{2+} , 2.5; Mg^{2+} , 1; HEPES, 10. The medium was supplemented with albumin (0.5%) and glucose. The pH was adjusted to 7.4 with NaOH. The test substance was dissolved in glucose-free incubation medium. Batches of 5 freshly isolated islets were incubated in 1 mL of incubation buffer for 1 hour at 37°C. After 1 hour of incubation, the samples were rapidly cooled on ice and an aliquot of medium was saved

for insulin determination. The insulin content of each sample was analyzed in duplicate by enzyme-linked immunosorbent assay (Merckodia AB, Sweden) and the duplicate mean was entered as one observation. Values are means + standard deviation observed in 3 independent islet preparations.

LD_{50} test of VO_2L complexes

The following lists the main facts of the lethal dose (LD_{50}) test in an order that follows the procedure:

Test substances

vanadium complex synthesized in this work; ammonium vanadate, NH_4VO_3 , as reference.

Method

Oral Acute Toxicity Up-And-Down-Procedure, published by the Organization for Economic Cooperation and Development (OECD), in the document: "Guideline for the Testing of Chemicals, SECTION 4: Health Effects Test No. 425, Oral Acute Toxicity: Up-and-Down Procedure."⁶⁴

Software

Oral Acute Toxicity Statistical Program (Guideline 425; AOT425StatPgm).⁶⁵

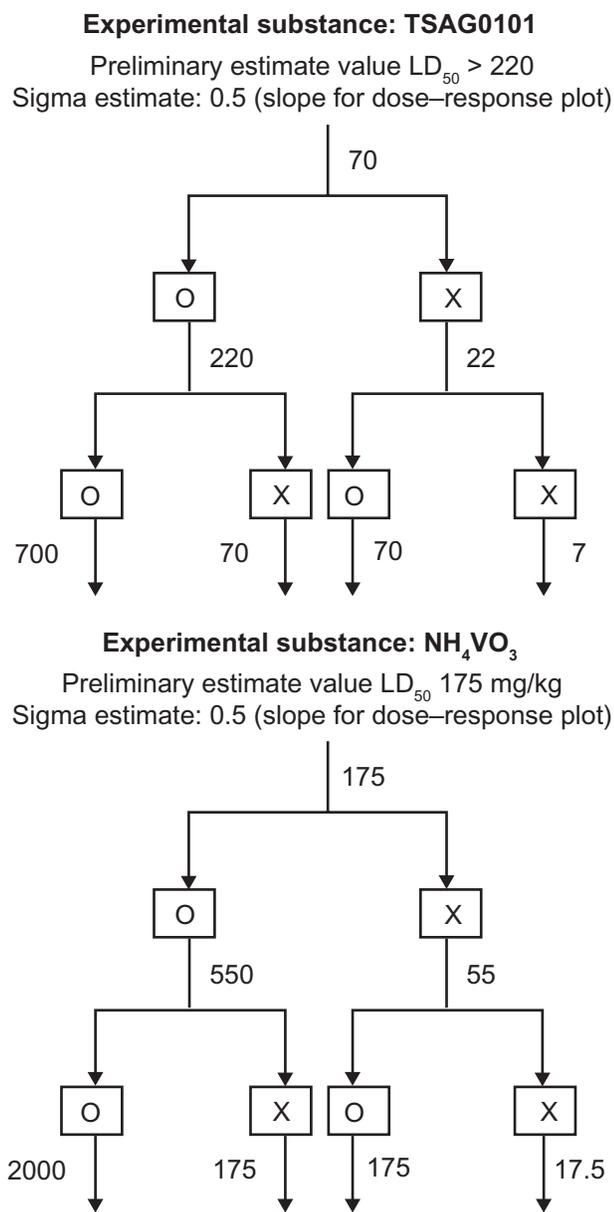
Animal model: 15 mice for substance, provided by the Bioterio "Claude Bernard," BUAP.

Administration

intra-gastric, dose according to program, diluted in 2 cm³ of solution of NaCl 0.3%.

Procedure

According to the OECD "Guideline for the Testing of Chemicals,"⁶⁵ with a preliminary value of 175 mg/kg and a factor of progression of dose of 3.2 for NH_4VO_3 . In the case of the tested vanadium compound, the reference value of LD_{50} was that of the compound BMOV, 220 mg/kg.⁶⁶ Test consists of the dosage of the animals, one at a time, in intervals of 48 hours. The first animal receives a dose a step behind from the reference value of LD_{50} . If the animal survives, the dose for the following animal is increased by an adaptor factor of dose progression (default, 3.2 times). Dosification tree is shown in Scheme 1. Each animal should be observed carefully for 48 hours, before making a decision about its short-term survival. All animals are kept under observation for 14 days in total, to establish their long-term survival. The survival data in the short run for each animal are registered in the software AOT425StatPgm, and the program assesses the next dose until the program recognizes



Scheme 1 Dosification tree for the LD_{50} experiment (O, survivors; X, deaths).

1 of the 3 preestablished situations to stop the calculation loop. Once the dose assessment has been stalled, the results of long-term test data (14 days) are registered in the program. The animals were killed to end their suffering by humanitarian means before the end of the observation period, and were counted as dead. The LD_{50} values are calculated according to the statistical method of maximum probability.^{67,68}

Results and discussion

Computer assisted drug design

In the first stage of the drug design study, the modeler of the group (TS) previously devised a pharmacophore model based on PTP1B inhibitors^{9–15} in collaboration and documented in

a graduate thesis.⁴⁹ Then, few suitable vanadium complexes (ligands) were created for the chimeric procedure described earlier. The geometries were optimized by Gaussian 03. A docking procedure inside the cavity was performed to accommodate the ligand in the best position. A criterion was established to look for the best binders and docked positions inside the active site: during manual docking, all those chimeric combinations with large repulsion due to steric hindrance were discarded. From the literature, certain experimental findings were taken into account to improve the final pharmacophore models: planar sandwich or face-to-face parallel-stacked geometry under an attractive charge-transfer interaction between aromatic systems showing electron-deficiency and electron-rich areas, with an energy gain of 1.5 kcal/mol at a distance between ring centers 5 nm.⁶⁹ Similar observations were made by Gung et al⁷⁰: ΔH of -2 kcal/mol and a ΔS of -3 kcal/mol. Especially, π -stacking with edge-to-face (T-shaped), also OH- π arrangements, cation- π interactions, and Coulombic enzyme–substrate interactions are reported by Meyer et al.⁷¹ An empirical study observed that the aromatic side chains of amino acids prefer an off-centered parallel alignment in proteins (parallel displaced π -stacking).⁷² Moreover, calculations show that organocationic compounds interact with π bonds very strongly (10 – 22 kcal/mol⁻¹), and are even more stable than ion-pair interactions, like salt bridges between tetra-alkylammonium ions and anionic residues in aqueous solution. This is an important finding for molecular recognition by cation- π interactions.⁷³

As a final result, a small-sized nonsubstituted vanadium compound, TSAG0101, constitutes a potential lead compound to build a novel series of insulin-mimetic drug candidates. Figure 3 displays the receptor–ligand complexes for the chimeric

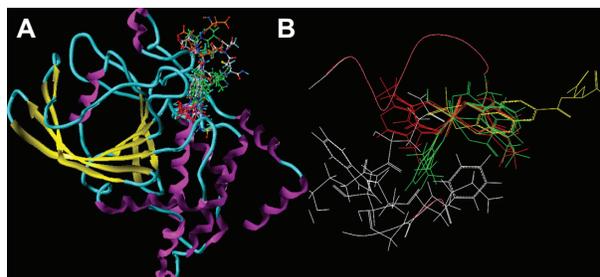


Figure 3 A) Superposition of peptidic inhibitors found in crystal complexes (PDB codes: IBZH, IBZC, IPTU, IC83, 2HNP, and 2HNQ) with their common target PTP1B. This model helps (1) to visualize structural diversity and similarities to define a consensus pharmacophore pattern regarding volume, shape, polar/nonpolar, and basic/acidic fields, (2) to identify interacting amino acids at the binding site, and (3) to identify unoccupied pockets, and finally estimate conformational entropic effects upon binding. **B)** Final active site poses of the chimeric series (TSAG0101, TSAG0203, TSAG0302, TSAG0701, TSAG0702, TSAG0703, and TSAG0704) after manual docking.

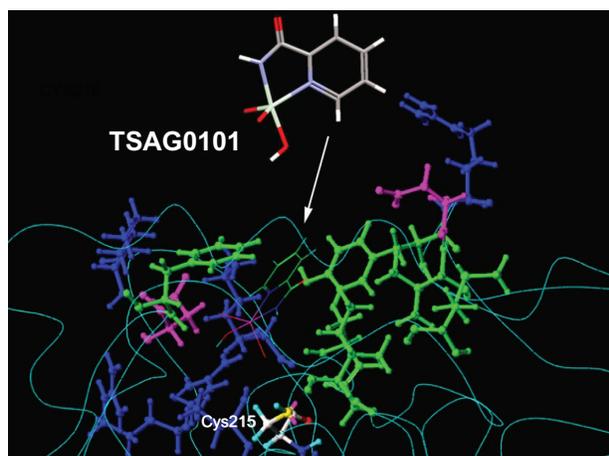


Figure 4 3D-model structure of ligand TSAG0101 and its complex with the target structure (PDB code: IPTU). In particular, Cys215 residue interacts with the vanadate oxo-oxygen.

series and Figure 4 displays the structure of TSAG0101 docked into the phosphate-binding cleft of the PTP1B target enzyme.

Synthesis and characterization of TSAG0101

In the next step, the lead candidate TSAG0101 was synthesized and a product with a greenish yellow color

was obtained. The product was a powder stable in air. The yield was of 65% with regard to vanadium. IR spectrum (KBr; Figure 4) showed the following bands: ν/cm^{-1} 3,570 (NH), 1,627 (C=O, co-ordinated), 1,417 (C–N), 970/842 (O=V=O), 727 δ (pyridine ring).

The successful complex formation of TSAG0101 was proven in the IR spectrum for the displacement of the carbonyl band from its original position in $1,664\text{ cm}^{-1}$, up to $1,627\text{ cm}^{-1}$ (Figure 5). Formation of the complex is evident from 2 bands corresponding to the group of dioxovanadate, O=V=O, in 970 and 842 cm^{-1} . The $^1\text{H NMR}$ spectrum (Figure 5) reflects a group of 4 broad bands from 7.6 to 8.2 ppm due to the effect of the vanadium that has a quadrupolar nuclear magnetic moment and NH_2 shifted to 8.8 ppm. The solvent signal did not show the same broadening, given that no free vanadium ions are present in solution. $^{13}\text{C NMR}$: 5 bands between 122 and 151 ppm and carbonyl C shifted to 180 ppm (not shown). The data discussed above agree with the formula $[\text{V}^{\text{V}}\text{O}_2(\text{OH})$ (picolinamide)] and the structure is shown in Figure 5.

Solution stability of TSAG0101

The solution stability of the complex was studied by the accumulation of ultraviolet (UV)-spectra in aqueous solution.

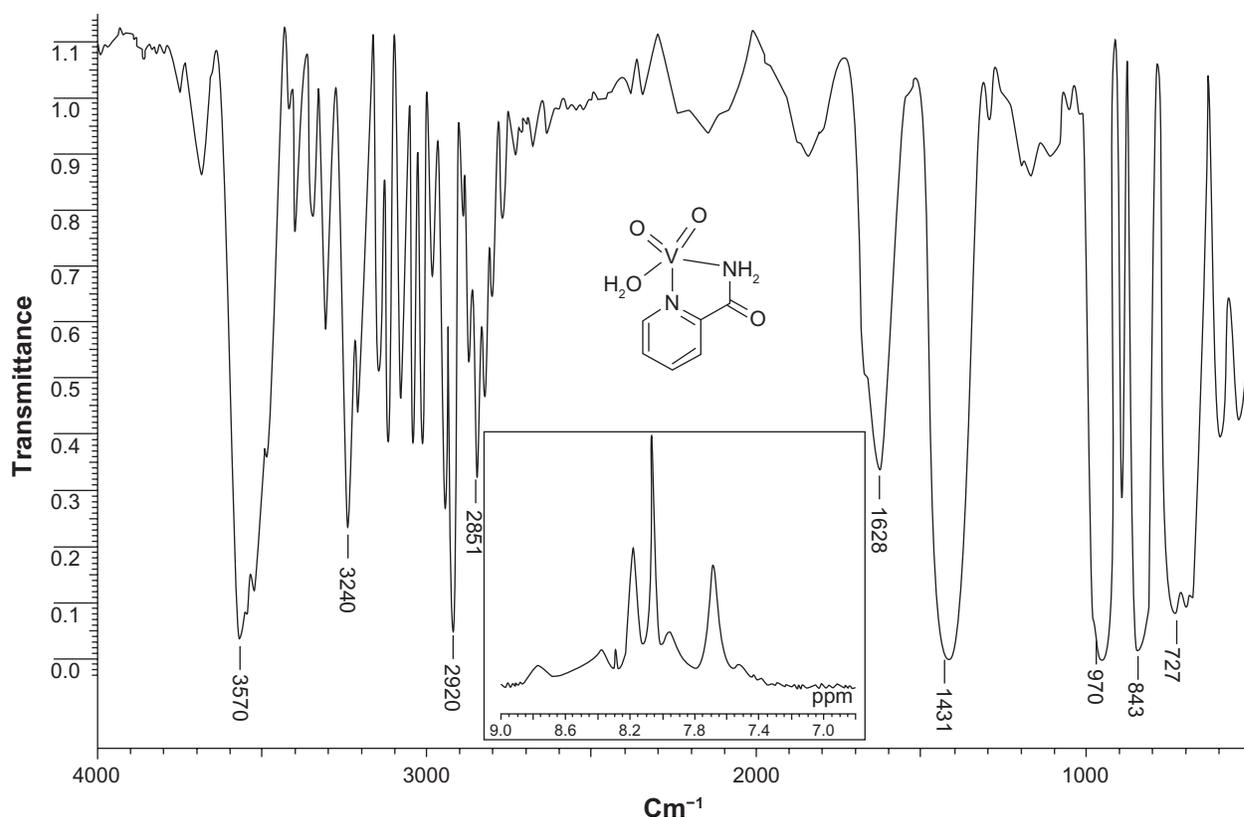


Figure 5 IR spectra of lead candidate TSAG0101 after synthesis. In the insert pane: $^1\text{H NMR}$ -spectra of the same compound.

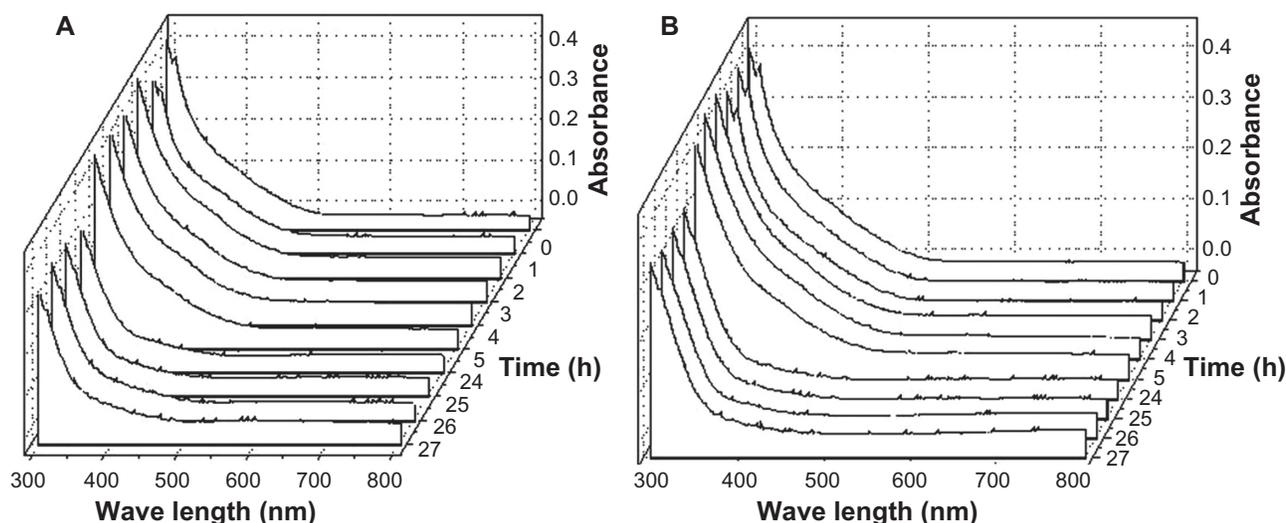


Figure 6 **A)** Cumulative UV-visible spectra of TSAG0101 in 2.5×10^{-4} M aqueous solution. **B)** Cumulative UV-visible spectra of 2.5×10^{-4} M TSAG0101 and 2.5×10^{-4} M citrate in aqueous solution.

Complexes with vanadium in oxidation state +5 display a band in the UV region from a charge transfer from ligand to metal, whereas those complexes with vanadium in oxidation state +4 possess 3 bands in the visible region.^{74,75} Cumulative spectra for TSAG0101 in aqueous solution with and without sodium citrate are shown in Figure 6. In the first case (Figure 6a), the spectrum changed very slowly with time, the charge transfer band near 400 nm fade away, and a new band appears in the visible region, possibly due to a change in oxidation state of the vanadium ion from +5 to +4. The spectrum for the solution with citrate behaved in the same way (Figure 6b), indicating that citrate did not form a new compound. Citrate is a very competitive reducer for metal ions; therefore, the experimental compound TSAG0101 is stable with respect to the exchange of ligands but it slowly reduces the oxidation state of vanadium from +5 to +4. The band observed at 400 nm also could be related with the presence of decavanadate. Nevertheless, this last species is not the most stable form of free vanadium at neutral pH and it has to be introduced from a stock solution to be present at low concentration.²²

Pancreatic activity

Rat islets responded with a 20-fold increase in insulin secretion to a change from substimulatory to stimulatory glucose concentrations. No effects of the test substance on basal- or glucose-induced insulin release were observed. There was a tendency to inhibit glucose-induced insulin secretion at the highest concentrations of test substance, this effect was, however, not significant. (Note: further samples with 16.7 mM glucose are available but could not be analyzed in this work due to inappropriate dilution preparations.) The data also indicate

an inhibition at the highest concentration because the secretion data with a 100 μ M test substance are in the range of detection, whereas all other values are still above that range (Table 1).

Glucose-induced insulin secretion in static incubation is a standard test to determine the metabolic activity of isolated islets. The 20-fold increase of glucose-induced insulin secretion demonstrates that the examined preparations consisted of very active islets. The high secretory activity in the presence of 11 mM glucose is a sensitive measure to detect a possible influence of the test substance on β -cell metabolism. No alteration of basal- or glucose-induced insulin secretion was observed excluding a direct effect of the test substance on β cells and, therefore, the observed blood glucose lowering effect in rats should be an extrapancreatic activity.

LD₅₀ test

Table 2 shows the experimental LD₅₀ values determined for both, NH_4VO_3 and TSAG0101 compounds, using the AOT425StatPgm software.

The reliability of the methodology used to obtain LD₅₀ values was confirmed with the value obtained for NH_4VO_3 ,

Table 1 Effect of the test substance TSAG0101 on glucose-induced insulin secretion. Batches of 5 freshly isolated rat islets were incubated for 1 hour with glucose and test substance as indicated. Insulin secretion was measured in ng/mL units. Values are means \pm SD observed in 3 independent islet preparations

Test substance TSAG0101, μ M	Glucose concentration (mM)			
	0	1	10	100
Glucose 2.8 mM	1.1 \pm 0.5	1.1 \pm 0.7	1.3 \pm 0.8	1.4 \pm 0.7
Glucose 11.1 mM	19.2 \pm 0.6	18.4 \pm 2.3	19.0 \pm 0.7	16.1 \pm 2.1

Table 2 Results of LD₅₀ tests for NH₄VO₃ and TSAG0101

Test/substance	NH ₄ VO ₃ (ammonium vanadate)	TSAG0101
Preliminary estimate	175	220
LD ₅₀ , mg/kg		
Sigma estimate, mg/kg	0.5	0.5
Recommended dose progression	2000, 550, 175, 55, 17.5, 5.5, 1.75	2000, 700, 220, 70, 22, 7, 2.2, 0.7
Essay animal number	8	7
LD ₅₀ calculated, mg/kg	55	412
Confident interval to 95% probability, mg/kg	35.02–155	220–700

since it is only slightly above the value reported by Ida et al⁷⁶ (55 mg/kg vs 10 mg/kg), which is probably due to experimental conditions and/or mathematical treatments; however, the value lies within the narrow range for all known vanadium inorganic salts (10–75 mg/kg) with the exception of sodium vanadate (300 mg/kg).

When directly compared, TSAG0101 (LD₅₀ 412 mg/kg) is by far less toxic than caffeine (LD₅₀ 192 mg/kg) and BMOV (220 mg/kg). Besides, being one of the most soluble vanadium complexes, this determines that its value of nominal LD₅₀ becomes comparable with those of soluble substances (Table 3). In analogy, our observed LD₅₀ value for TSAG0101 lies reasonably inside the range of LD₅₀ values of other organic vanadium compounds that are classified as moderately toxic, according to the scales established in the literature.

Conclusions

Several vanadium compounds with hypoglycemic activity have already been patented.^{77–81} In particular, an ethyl-derivative of BMOV entered clinical trials.^{17,82} In addition, vanadium appears as bio-conjugates and in nutritional

supplements, as well as in cases of illegal doping. In drug research, its therapeutic performance is subjected to further scrutiny.^{83,84}

As a direct result of our chimerical *de novo* design, TSAG0101 is proposed as a new lead candidate for its structural simplicity and chemical derivation potential. Our *in silico* studies led theoretical insight into its pharmacophore pattern and molecular blocking action. The ligand was successfully docked into the phosphate binding site of its target, the intracellular PTP1B enzyme. In our *in vivo* and *in vitro* activity tests, our synthesized compound showed less toxicity. The complex was found to be sufficiently stable in presence of ubiquitous sodium citrate at the test concentration because no changes occurred within 24 hours. However, the prodrug function of the experimental compound TSAG0101 cannot be ruled out given the complex chemistry of vanadium compounds in aqueous solution. This finding could be interpreted against the hypothesis of prodrug function which cannot be ruled out for the more unstable organic vanadium compounds.⁴⁶ With regard to the patented compounds,^{77–81} an enhanced pharmacokinetic and dynamic drug profile of TSAG0101 can be expected; due to its experimental complex stability and theoretical binding specificity. In biological assays in rats, TSAG0101 showed insulin-like activity (insulin mimesis), also no pancreatic component could be observed, ie, its blood glucose lowering effect is of extrapancreatic nature. It is also less toxic than caffeine (LD₅₀ 192 mg/kg) and BMOV (220 mg/kg). Because of the present work which extends the known literature on nonpeptidic insulin mimetics, more studies can be conducted in the future to assess the inhibitory concentration (IC₅₀) activities, as well as extended stability test in aqueous solutions under varying concentrations and temperatures by NMR and electron paramagnetic resonance spectroscopy.

Table 3 Solubility data and LD₅₀ values for some vanadium salts and compounds

Vanadium compounds	Formula	Solubility in water g/L 20°C	LD ₅₀ mouse, mg/kg	References
Vanadium pentoxide; vanadium(V) oxide; divanadium pentoxide	V ₂ O ₅	8	23.4	76
Ammonium vanadate; ammonium metavanadate; vanadic acid ammoniacal salt	NH ₄ VO ₃	58	10 55	76 This work
Vanadyl sulphate; oxovanadium(IV) sulphate	VO ₂ SO ₄	Soluble	10	76
Sodium vanadate; sodium metavanadate	NaVO ₃	211 (25°C) 388 (75°C)	74.6	85
Sodium orthovanadate; sodium tetraoxovanadate	Na ₃ VO ₄	Slightly soluble	300	86
BMOV; bis-maltolateoxovanadate	VO(maltol) ₂	Soluble	220	87
TSAG0101	–	Soluble	412	This work
Caffeine		Soluble	192	88
Sodium cyanide	NaCN	Soluble	6.4	7

We also expect the new compound will contribute to shed some light into the ongoing prodrug discussion either identifying or discarding the existence of active specie(s). In addition, side chain derivation is thought to improve the target selectivity of our nonsubstituted lead candidate which we make accessible for future research work.

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Disclosure

The authors report no conflicts of interest in this work.

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