Accepted Manuscript

Polyoxometalates: Study of inhibitory kinetics and mechanism against α -glucosidase



Guoxiang Chi, Li Wang, Bingnian Chen, Jian Li, Jingjing Hu, Shuxia Liu, Meijuan Zhao, Xiaomei Ding, Yue Li

PII:	S0162-0134(19)30292-2
DOI:	https://doi.org/10.1016/j.jinorgbio.2019.110784
Article Number:	110784
Reference:	JIB 110784
To appear in:	Journal of Inorganic Biochemistry
Received date:	11 May 2019
Revised date:	13 July 2019
Accepted date:	15 July 2019

Please cite this article as: G. Chi, L. Wang, B. Chen, et al., Polyoxometalates: Study of inhibitory kinetics and mechanism against α -glucosidase, Journal of Inorganic Biochemistry, https://doi.org/10.1016/j.jinorgbio.2019.110784

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Polyoxometalates: Study of Inhibitory Kinetics and

Mechanism Against α-Glucosidase

Guoxiang Chi^a, Li Wang^{a,†}, Bingnian Chen^{b,†}, Jian Li^a, Jingjing Hu^a, Shuxia Liu^{c,†}, Meijuan Zhao^a, Xiaomei Ding^a, Yue Li^a

^a College of Food and Biological Engineering, Jimei University, Xiamen, 361021, P.R. China ^b Xiamen University Hospital, Xiamen 361005, P.R. China

^c Key Laboratory of Polyoxometalate Science of Ministry of Education, College of Chemistry, Northeast Normal University, Changchun, Jilin 130024, P.R. China

Abstract

Alpha-glucosidase is considered to be an important target for the treatment of noninsulin-dependent diabetes. In this work, the inhibitory effects of polyoxometalates (POMs) affected by three different factors (heteroatom, transition metal substitution element and vanadium substitution number) on α -glucosidase were studied. We found that POMs with Keggin-type and vanadium-substituted Dawson-type structures act as effective and mostly competitive inhibitors for α -glucosidase (IC₅₀ values around 40-160 μ M), and most compounds can compete with the substrate for the active site of α -glucosidase. By analyzing and comparing the inhibitory effects of each series of POMs on α -glucosidase, the results demonstrated that the structure and composition of the POMs themselves may indirect influence on their inhibitory capabilities. Moreover, we gained initial information about the structure-inhibition relationship of different POMs. More intriguingly, molecular docking simulation suggested that all compounds bind into the active site of α -glucosidase by multiple van-der-Waals and hydrogen bond interactions. Our kinetic data

[†] Corresponding author. Tel.: +86-592-6181487; Fax.: +86-592-6181487

E-mail address: wanglimerry@jmu.edu.cn (Li Wang); 3421836578@qq.com (Bingnian Chen); liusx@nenu.edu.cn (Shuxia Liu)

demonstrate the considerable potential of POMs for the development of clinically valuable α -glucosidase inhibitors.

Keywords: Polyoxometalates; a-Glucosidase inhibitors; Enzyme kinetics; Molecular docking

1. Introduction

Type 2 diabetes (non-insulin-dependent diabetes) is a type of a chronic hyperglycemia disorder caused by insulin deficiency or insulin resistance [1, 2]. α -Glucosidase is a carbohydrate hydrolase which is considered to be an important target for the treatment of non-insulin-dependent diabetes mellitus [3]. In recent years, the development of safe and effective α -glucosidase inhibitors has become a hotspot of research for the treatment of non-insulin-dependent diabetes mellitus. Peng et al. [4] reported that kaempferol showed a notable inhibition activity on α -glucosidase. Taha et al. [5] designed and synthesized seventeen Coumarin based derivatives that showed outstanding α -glucosidase inhibitory potential many folds better than the standard acarbose. However, the reported research on α -glucosidase inhibitors mainly focuses on natural extracts and organic compounds, and few inorganic agents have been reported that can inhibit α -glucosidase. Moreover, the current therapeutic drugs (acarbose [6], miglitol [7] and voglibose [8]) on the market are incompletely satisfactory due to the high cost and toxic side effects (Table S1). Therefore, the design of high efficiency, low cytotoxicity and low cost α -glucosidase inhibitors is an attractive goal in the field of medicinal chemistry.

Polyoxometalates (abbreviated as POMs), due to their incomparable structural diversity, novel functional properties and multifaceted bioactivities, have shown good application prospects

that encompasses anticancer [9-12], antiviral [10, 13], antibacterial [14,15], against Alzheimer's disease [16-18] and enzyme inhibition [19-22] in the field of medicine. Many studies have validated that POMs possess significant inhibitory effects on the activities of kinase, nucleotidase, phosphatase, sialyltransferase, nuclease, sulfotransferase, acetylase and protease [23]. Gumerova et al. [24] studied the inhibitory effects of nine different polyoxotungstates (POTs) on P-type ATPases in vitro (Ca^{2+} -ATPase) and ex vivo (Na^{+}/K^{+} -ATPase), and the results reveal some POTs are potent ATPase inhibitors, with K₉(C₂H₈N)₅[H₁₀Se₂W₂₉O₁₀₃] showing high selectivity towards Ca²⁺-ATPase. Moreover, the information about POMs as anti-diabetic agents is rapidly increasing in the last 30 years thereby creating many highlights (See Scheme S1). We found that the research of scientists is mainly focused on the anti-diabetic effect of vanadate [25,26] and tungstate [25,27]. Most recently, our group has reported that the $H_3PMo_{12}O_{40}$ and three transition metal-substituted Keggin-type phosphomolybdic acids can be excellent inhibitors to α -glucosidase activity, and the $H_3PMo_{12}O_{40}$ possess the strongest inhibition effect [28]. However, the structure-activity relationship of POMs and fundamental questions regarding the binding mode of POMs inhibitors on enzymes is largely unknown, especially for α -glucosidase. In addition, POMs are characterized by species diversity, structural modification, and extensive inhibition of enzymes. Therefore, the screening of a large number of POMs, especially of the Keggin and Dawson-type with superior inhibitory properties and lower cytotoxicities [29-32] may result in clinically valuable α -glucosidase inhibitor drugs.

Herein, the primary focus of this paper is to present the Keggin-type and vanadium-substituted Dawson-type POMs (Fig. S1) with potential pharmacological activity on α -glucosidase. Three structural crucial factors of POMs - heteroatom, transition metal substitution

element and vanadium substitution number - have been varied and the corresponding inhibition kinetics performed. Importantly, we initially analyzed the structure-inhibition relationship of POMs and fundamental questions regarding the mechanisms of the inhibitory capabilities of POMs on α -glucosidase. This work provides an enlightened strategy for the in-depth study of polyoxometalate medicinal chemistry and designing novel, safe and effective α -glucosidase inhibitors.

2. Materials and methods

2.1. Reagents

H₅GaMo₁₂O₄₀ [33], H₄SiMo₁₂O₄₀ [34], Na₇PMo₁₁MO₄₀ (M=Ni, Mn, Zn, Cr) [35, 36] and H_{6+n}P₂Mo_{18-n}V_nO₆₂ (n=1~5) [37, 38] (abbreviated as GaMo₁₂, SiMo₁₂, PMo₁₁M and P₂Mo_{18-n}V_n, respectively) were synthesized as previously described with slight modifications. Next, the structure of the synthetic compounds were characterized by IR (Jasco FT/IR-480) and UV/*Vis* (Cary-50) spectroscopy. Moreover, the structure of P₂Mo_{18-n}V_n (n=1~5) were further characterized by solid nuclear magnetic resonance spectrometry (UNITY+-300). α-Glucosidase of *S. cerevisiae* (EC 3.2.1.20), *p*-Nitrophenyl-α-D-glucopyranoside (*p*NPG), *p*-Nitrophenol (*p*NP) and Dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich Chemical Co. (USA). All other reagents were locally sourced and of analytical grade, and the ultra-pure water was used throughout.

2.2. Experiment procedure

2.2.1. Enzyme kinetics analysis

In this experiment, *p*NPG was used as a substrate to generate a yellow product (*p*NP) with catalysis by α -glucosidase, and the OD value after the reaction of the system was monitored by the

microplate reader (Synergy H1) at a wavelength of 405nm [28]. Briefly, the reaction system was sequentially supplemented with 133 µL of 0.1M phosphate buffer (Na₂HPO₄-NaH₂PO₄ buffer, pH 6.8), 7 µL of different concentrations of inhibitors completely dissolved in DMSO solution (the final concentration of DMSO in the test solution was 5%), and 10 µL of α -glucosidase solution that was added immediately. Next, the mixture was incubated at 37.0 °C for 10 min, and 20 µL of the substrate (*p*NPG) preheated at 37.0 °C for 10 min was added to the mixture. The reaction was carried out at 37.0 °C for 20 min, and then the reaction was terminated by the addition of an equal volume of Na₂CO₃ solution (1 M). The OD value of the 200 µL reaction solution was monitored in a 96-well plate by using the microplate reader at 405 nm [28]. As previously described [28], the inhibitory effect (IC₅₀), inhibition mechanism and inhibition type of three different factors (heteroatom, transition metal substitution element and vanadium substitution number) of POMs on α -glucosidase were studied.

2.2.2. Molecular docking study

To predict the binding mode of each series of synthesized compounds with α -glucosidase, a molecular docking study was carried out using MOE (Molecular Operating Environment) software package. According to our previous report [28, 39], the three-dimensional structures of the synthesized compounds were generated *via* using the builder tool in MOE. The generated compound was 3D protonated and energy minimized using the default parameters of the MOE (gradient: 0.05, Force Field: MMFF94X, pH 6.8). These compounds were then saved into an mdb file for further evaluation.

However, the 3D structure for α-glucosidase of *S. cerevisiae* has not been solved yet. The template structure; Herein, the crystal structure of isomaltase from *S. cerevisiae* (PDB 3AJ7) share

82% homology with the *S. cerevisiae* α -glucosidase, which is generally as considered excellent value for the selection of template [40]. Moreover, the homology modeling process of α -glucosidase and the details, and parameters of molecular docking in this paper, are the same as those of previously described [28, 41].

2.2.3. Statistical analysis

The experimental data were repeated five times under parallel determination by using SPSS version 17.0 and GraphPad Prism version 7.0 for analysis. Experimental drawings were completed using Origin 8.0 professional software. Error bars indicate \pm s.d.

3. Results and discussion

3.1. Analysis of Keggin-type POMs with different kinds of central atoms (heteroatom)

3.1.1. Spectroscopic characterization

Table S2 listed the IR spectra and the UV-*Vis* spectra data of the compounds $GaMo_{12}$ and $SiMo_{12}$. In the IR spectra, the two compounds showed most characteristic bands at 700-1100 cm⁻¹ due to molybdenum-oxygen stretching vibrations. In the UV-*Vis* spectra, the compounds showed two characteristic absorption bands at 195 nm and 310 nm, respectively. The data in the table strongly indicated that these two complexes have Keggin-type structures, which is consistent with the results of references [33, 34].

3.1.2. The inhibitory effect of compounds on α -glucosidase

To compare the inhibitory effects of POMs with different central atoms on α -glucosidase, we selected compounds GaMo₁₂ and SiMo₁₂ as inhibitors to study the effect of POMs on the activity of α -glucosidase-catalyzed *p*NPG. As shown in Fig. S2, the α -glucosidase activity revealed a

significant decrease with increased the concentrations of the inhibitors ($GaMo_{12}$ and $SiMo_{12}$). The concentrations of $GaMo_{12}$ and $SiMo_{12}$ when the α -glucosidase activity was decreased by 50% (IC_{50}) were 615.10 ± 17.00 μ M and 33.71 ± 0.76 μ M, respectively. Compared with our previously reported PMo₁₂ [28], the inhibitory effects of these three Keggin-type POMs are $GaMo_{12} < SiMo_{12}$ < PMo12. From the above results, the IC50 revealed a significant decrease with increased the heteropolyanions charge (Ga: -3, Si: -4 and P: -5), which indicated a significant indirect influence of the central atom charge on the inhibition behaviour. In addition, the acid strength of POMs were determined by alkaline method as $GaMo_{12} < SiMo_{12} < PMo_{12}$, and studies have shown that the oxidizability of Keggin-type POMs with the same structure are mainly affected by the electrostatic effect of the anionic charge, which decreases with the oxidation state of the central atom, and its oxidizability decreases as the negative charge of the POMs increases [42,43]. Based on the above theory, we found that the different central atomic species of POMs change their own redox property and acidity, which may cause significant differences in the inhibition behaviour of the three POMs on α -glucosidase. We also found that the inhibitory effects of GaMo₁₂ and SiMo₁₂ on α -glucosidase were less effective than those of PMo₁₂, but the inhibitory effects of these two inhibitors were more significant than those of standard commercial acarbose (IC₅₀=750 \pm 1.5 μ M) [44]. Among them, the inhibitory strength of SiMo₁₂ is approximately 23 times higher than that of acarbose.

In addition, we also studied the inhibitory mechanism and inhibitory type of inhibitors on α -glucosidase activity. The results are shown in Table 1. From Fig. S3, in the reaction system, the plots of the remaining enzyme activity versus the concentrations of the enzyme produced several adjacent straight lines, which all passed through the origin. Next, a linear decrease with increasing

concentrations of inhibitors indicated that the inhibition mechanisms of these two inhibitors on α -glucosidase were reversible [28, 45]. Therefore, these two inhibitors are non-covalently attached to the active center of the enzyme by multiple polar and non-polar interactions. Moreover, the inhibition type of the GaMo₁₂ and SiMo₁₂ on the α -glucosidase activity was determined using the Lineweaver-Burk (double-reciprocal) plots of the reciprocal of the reaction rate versus the reciprocal of the substrate concentration. The inhibitory constant (K₁ or K₁₅) of the inhibitors was further calculated through secondary mapping, and the results are shown in Fig. S4. As shown in Fig. S4, the graph of 1/ ν versus 1/[s] represented a set of straight lines which all intersected at or extremely close to the Y-axis. Next, the linear slope increased gradually as the concentrations of the GaMo₁₂ and SiMo₁₂ inhibitors compete with the substrate for the active site of the α -glucosidase [45]. Furthermore, the inhibitory constants K₁ of the GaMo₁₂ and SiMo₁₂ were calculated by further plotting the linear slopes versus the inhibitor concentrations of 0.630 mM and 0.032 mM, respectively.

<Table 1.>

3.1.3. Analysis of molecular docking results

Different inhibitory effects on the target protein may occur due to differences in the chemical structure or the mechanism of action of the inhibitor itself. To vividly represent the binding forms and interactions of the ligands (GaMo₁₂ and SiMo₁₂) in the active site of α -glucosidase, the experimental results were analyzed by the MOE (Fig. 1AB) and the PyMOL (Fig. 1CD) software.

As shown in Fig. 1AB, the amino acid residues surrounding the compound are represented by pink and green circles. GaMo₁₂ and SiMo₁₂ accurately fit into the deep pocket of the active site and is largely surrounded by the polypeptide. After docking scoring, the scores for GaMo₁₂ and SiMo₁₂ docking with α -glucosidase were -11.68 and -13.62 kcal/mol, respectively. The score meant that the docking of these two compounds with α -glucosidase was successful. In addition, the smaller the numerical value is, the better is the ability of the compound to bind to the biomacromolecule. Therefore, in combination with the previous report [28], the binding ability of the compound to α -glucosidase is GaMo₁₂ < SiMo₁₂ < PMo₁₂ (Table S3). Further molecular docking study validated the experimental results of the enzyme kinetics.

<Fig. 1. (A, B, C and D)>

Furthermore, as shown in Fig. 1, SiMo₁₂ forms five hydrogen bonds with the amino acid residues (Ser240, His280, Pro312 and Arg315) in the active region, while GaMo₁₂ forms only three hydrogen bonds with the amino acid residues (Ser240 and Asp242) in the active region (Table S3), so the inhibition of SiMo₁₂ is greater than that of GaMo₁₂. Similarly, the inhibition of PMo₁₂ is greater than that of SiMo₁₂ and GaMo₁₂. In addition to the hydrogen bonding interactions with the enzyme, the compounds also form several van der Waals interactions which significantly contributes to the strong binding of the compounds. However, we found no significant difference in the number of amino acid residues involved in van der Waals interactions among the three compounds. According to the molecular simulation, the SiMo₁₂ and GaMo₁₂ does bind to the enzyme active groups in the form of hydrogen bonding and van der Waals interactions. Therefore,

the catalytic group of the enzyme is generally not destroyed, and the enzyme is not inactivated, which proves that both compounds are a reversible inhibitor [28].

3.2. Analysis of different transition metal-substituted Keggin-type POMs

3.2.1. Spectroscopic characterization

Table S4 listed the IR spectra (four characteristic bands at 700-1100 cm⁻¹) and the UV-*Vis* spectra (two characteristic absorption bands at 200 nm and 260 nm) data of the compounds PMo₁₁M (M=Ni, Mn, Zn, Cr). These data in the table strongly indicated that these complexes have Keggin-type structures, which is consistent with the results of references [35, 36]. Among them, the peak positions of the compounds in the table were shifted, which may be due to the substitution of transition metals.

3.2.2. The inhibitory effect of compounds on α -glucosidase

To compare the effects of different transition metal-substituted Keggin-type POMs on α -glucosidase, four transition metal-substituted Keggin-type POMs were synthesized and used as inhibitors to study their inhibitory effects on α -glucosidase. As shown in Fig. S5, the α -glucosidase activity gradually decreased as the concentration of the inhibitors PMo₁₁M (M=Ni, Mn, Zn, Cr) increased. Compared with the previously studied inhibitors (PMo₁₁V, PMo₁₁Co and PMo₁₁Fe) [28], the inhibitory effects of the seven Keggin-type POMs on α -glucosidase were PMo₁₁Fe < PMo₁₁Cr < PMo₁₁Co < PMo₁₁Zn < PMo₁₁V < PMo₁₁Mn < PMo₁₁Ni. Next, compared with standard acarbose (IC₅₀=750 ± 1.5 μ M) [44], the inhibition potency of PMo₁₁Ni (IC₅₀=37.29 ± 1.72 μ M) was approximately 20 times higher than that of acarbose, so the synthesized inhibitors in our laboratory possess significant inhibitory effects on α -glucosidase. It was revealed that, as compared to the IC₅₀ of each compound, we found that the inhibitory effects

of the three compounds (PMo₁₁Ni, PMo₁₁Mn and PMo₁₁V) on α -glucosidase were generally much stronger than the other four, which may be mainly due to the substitution of metal elements of POMs to α -glucosidase has different inhibition behaviour. The substitution of different transition metal elements may also change their own redox properties, which may indirect influence the inhibition behaviour of the POMs on α -glucosidase, and the need for further research is emphasized. Moreover, the reason why the inhibitory effect of PMo₁₁Fe on α -glucosidase is worse than other compounds may be that its inhibition type is different from others. Similarly, the inhibition mechanism of the four compounds we synthesized were all reversible (Fig. S6). Except that PMo₁₁Ni showed mixed-type inhibition, the inhibition types of the other three compounds were all competitive (Fig. S7). In the mixed-type inhibition, the inhibitor PMo₁₁Ni can affect the affinity of the enzyme to the substrate and affect the catalytic action of the enzyme. PMo₁₁Ni can bind to both the free enzyme and the enzyme-substrate complex, but this affects its inhibitory effect on α -glucosidase. Furthermore, Table 1 lists the inhibition constants (K₁ and K₁₅) of the four compounds for α -glucosidase.

3.2.3. Analysis of molecular docking results

In this part, we selected the best inhibitory compound $PMo_{11}Ni$ for α -glucosidase, and further researched the binding forms and interactions between $PMo_{11}Ni$ and α -glucosidase at the molecular level by molecular simulation, as shown in Fig. 2. The interactions between the ligand $PMo_{11}Ni$ and the most important amino acids generated by MOE are visualized in Fig. 2AC. The inhibitor perfectly matches to the active site pocket of α -glucosidase (Fig. 2AB), and the docking score was -13.35 kcal/mol after docking. The low score can be interpreted as measure for a strong enzyme-inhibitor binding and therefore as a reliable docking event.

<Fig. 2. (A, B and C)>

In addition, PMo₁₁Ni forms five hydrogen-bond interactions with the amino acid residues (Ser240, His280, Ser311, Pro312 and Arg315) in the active region (Fig. 2AC). PMo₁₁Ni forms van der Waals interactions with the amino acid residues (Tyr158, Asp242, Leu246, Gln279, Asp307, Thr310, Leu313 and Phe314) around the active region (Fig. 2AC). In the docking interaction residue analysis, the four polar amino acids around the active binding site of the enzyme are Tyr158, Gln279, Asp307 and Thr310, respectively. These polar amino acid residues significantly contributes to the strong binding of the compound.

3.3. Analysis of vanadium-substituted Dawson-type POMs

3.3.1. Spectroscopic characterization

Elemental analysis (%): Anal. Calc. (Found) for $P_2Mo_{17}V$: P 1.80 (1.77), Mo 47.34 (47.23), V 1.48 (1.47); Anal. Calc. (Found) for $P_2Mo_{16}V_2$: P 1.80 (1.77), Mo 44.66 (44.54), V 2.91 (2.95); Anal. Calc. (Found) for $P_2Mo_{15}V_3$: P 1.74 (1.73), Mo 40.28 (40.17), V 4.28 (4.33); Anal. Calc. (Found) for $P_2Mo_{14}V_4$: P 1.81 (1.76), Mo 39.26 (39.29), V 5.96 (5.94) and Anal. Calc. (Found) for $P_2Mo_{13}V_5$: P 1.88 (1.83), Mo 37.73 (37.56), V 7.72 (7.70). These data results were consistent with the results of reference [37].

In addition, Table S5 listed the IR spectra (four characteristic bands at 700-1100 cm⁻¹) and the UV-*Vis* spectra (two characteristic absorption bands at 210 nm and 310 nm, repectively) data of the compounds $P_2Mo_{18-n}V_n$ (n=1~5), and the ³¹P-NMR spectra was shown in Fig. S8. These data strongly indicated that these compounds were Dawson-type vanadium-substituted POMs, which

was consistent with the results of references [37, 38].

3.3.2. The inhibitory effect of compounds on α -glucosidase

Compared with Keggin-type structure, Dawson-type structure of POMs are relatively less studied and their redox ability is stronger. Therefore, a series of vanadium-substituted Dawson-type POMs were synthesized and used as inhibitors to study their inhibitory effects on α -glucosidase. As shown in Fig. S9, the α -glucosidase activity revealed a significant decrease with increased the concentrations of the inhibitors P2Mo18-nVn (n=1~5). Comparing the IC50 of each compound (Table 1), it was found that the inhibitory effects of the five Dawson-type POMs on α -glucosidase were $P_2Mo_{17}V < P_2Mo_{14}V_4 < P_2Mo_{16}V_2 < P_2Mo_{13}V_5 < P_2Mo_{15}V_3$. It was revealed that, as compared to the acarbose (IC₅₀=750 \pm 1.5 μ M) [44], the inhibition potency of P₂Mo₁₅V₃ $(IC_{50}=57.01 \pm 2.11 \ \mu M)$ was approximately 13 times higher than that of acarbose, so the synthesized inhibitors possess significant inhibitory effects on a-glucosidase. Moreover, the V is easier reduced than the Mo, so that the Mo in the Dawson-type POMs is substituted by the V, and its oxidizability is markedly enhanced [37]. Therefore, we analyzed that the inhibitory effect of vanadium-substituted POMs on α -glucosidase is $P_2Mo_{17}V < P_2Mo_{16}V_2 < P_2Mo_{15}V_3$, because the increase in the number of vanadium substitutions may enhance the oxidizability of the compounds, which may indirect influence their binding affinities to the active site of the α -glucosidase. From this, it is speculated further that the inhibition of α -glucosidase is related to the elemental composition of the POMs, and the more the V-containing component (not more than three), the stronger the inhibitory effect on α -glucosidase. Similarly, we also found that the inhibition mechanisms of these five vanadium-substituted Dawson-type POMs are reversible (Fig. S10), and their inhibition types are all competitive (Fig. S11). In addition, Table 1 lists the inhibition

constants (K_I) of these five compounds for α -glucosidase.

3.3.3. Analysis of molecular docking results

The above enzyme kinetics showed that the compound $P_2Mo_{15}V_3$ had the best inhibitory effect on α -glucosidase. Therefore, we further researched the binding forms and interactions of $P_2Mo_{15}V_3$ and α -glucosidase at the molecular level by the MOE (Fig. 3AB) and the PyMOL (Fig. 3C) software. As shown in Fig. 3AB, the $P_2Mo_{15}V_3$ accurately fit into the deep pocket of the active site and is largely surrounded by the polypeptide, and the docking score was -19.55 kcal/mol after docking. The low score can be interpreted as measure for a strong enzyme-inhibitor binding and therefore as a reliable docking event. Furthermore, $P_2Mo_{15}V_3$ forms six hydrogen bonds with the amino acid residues (Tyr158, Asp242, His280 and Ser311) in the active region (Fig. 3AC). In addition to the hydrogen-bond interactions with the enzyme, the compound also forms several van der Waals interactions which significantly contributes to the strong binding of the compound. $P_2Mo_{15}V_3$ forms van der Waals interactions with the amino acid residues (Val232, Ser240, Pro243, Leu246, Pro312, Leu313, Phe314 and Arg315) around the active region (Fig. 3AC). Similarly, in the docking interaction residue analysis, these polar amino acid residues (Ser240 and Arg315) significantly contributes to the strong binding of the compound.

<Fig. 3. (A, B and C)>

4. Conclusions

In summary, to gain initial information about the structure-inhibition relationship of different POMs, three structural crucial factors of POMs - heteroatom, transition metal substitution element

and vanadium substitution number - have been varied and the corresponding inhibition kinetics and molecular simulation performed. Enzyme kinetics demonstrated that our synthetic compounds PMo_{12} (6.41 ± 0.38 μ M) [28], $PMo_{11}Ni$ (IC_{50} =37.29 ± 1.72 μ M) and $P_2Mo_{15}V_3$ (IC_{50} =57.01 ± 2.11 μ M) has the strongest inhibitory effect on α -glucosidase in their respective series, and their inhibitory effect was approximately 117, 20 and 13 times higher than that of standard acarbose, respectively. In addition, we found that all synthetic compounds except PMo₁₁Ni (reversible mixed-type) and PMo₁₁Fe (reversible noncompetitive) exhibited reversible competitive inhibition. Table 1 listed the inhibition constants (K_I and K_{IS}) of all compounds for α -glucosidase. More intriguingly, we found that the IC_{50} revealed a significant decrease with increased the heteropolyanions charge (Ga: -3, Si: -4 and P: -5), which indicated a significant indirect influence of the central atom charge on the inhibition behaviour. The structure and composition of POMs may change their own oxidation-reduction, acidity, and electrostatic effects of anion charge, which may also indirect influence the inhibition behaviour of POMs on α -glucosidase. Moreover, molecular docking simulation demonstrated that the compounds we synthesized competitively bind to the active site of α -glucosidase mainly through multiple van-der-Waals and hydrogen bond interactions. The involvement of more polar amino acid residues significantly contributes to the strong binding of POMs on a-glucosidase. To some extent, molecular simulations validated the experimental results of enzyme kinetics, and further expound upon the mechanisms of action at the molecular level. Through future experiments, we will further study the effects of factors such as counter cations and lattice water on the structure-activity relationship of POMs, and further expand our investigation with the human α -glucosidase and the model enzyme isomaltase. Furthermore, we will determine the X-ray structure of α -glucosidase-POMs complex for

comparison and for evaluating the accuracy of the modelling data. In this sense, this work further advances our understanding of the design and synthesis of POMs as therapeutic agents for type 2 diabetes. Also, this work opens up a window toward the biomedical applications of polyoxometalate chemistry.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Grant No. 21871110) and the National Key Research and Development Program of China (Grant No. 2018YFD0901004).

References

 [1] Ley, S. H., Ardisson Korat, A. V., Sun, Q., Tobias, D. K., Zhang, C., Qi, L., Willett, W. C., Manson, J. E., Hu, F. B. Contribution of the Nurses' Health Studies to uncovering risk factors for type 2 diabetes: diet, lifestyle, biomarkers, and genetics[J]. *American Journal of Public Health*, 2016, 106(9): 1624-1630.

https://doi.org/10.2105/AJPH.2016.303314

[2] Migdal, A., Abrahamson, M., Peters, A., Vint, N. Approaches to rapid acting insulin intensification in patients with type 2 diabetes mellitus not achieving glycemic targets[J]. *Annals of Medicine*, 2018: 1-27.

https://doi.org/10.1080/07853890.2018.1493216

[3] Olennikov, D. N., Chirikova, N. K., Kashchenko, N. I., Nikolaev, V. M., Kim, S. W., Vennos, C. Bioactive phenolics of the genus artemisia (asteraceae): HPLC-DAD-ESI-TQ-MS/MS profile

of the siberian species and their inhibitory potential against α -amylase and α -glucosidase[J]. *Frontiers in Pharmacology*, 2018, 9: 756-783.

https://doi.org/10.3389/fphar.2018.00756

[4] Peng, X., Zhang, G., Liao, Y., & Gong, D. Inhibitory kinetics and mechanism of kaempferol on α-glucosidase[J]. *Food Chemistry*, 2016, 190: 207-215.

https://doi.org/10.1016/j.foodchem.2015.05.088

[5] Taha, M., Shah, S. A. A., Afifi, M., Imran, S., Sultan, S., Rahim, F., & Khan, K. M. Synthesis, α-glucosidase inhibition and molecular docking study of coumarin based derivatives[J]. *Bioorganic Chemistry*, 2018, 77: 586-592.

https://doi.org/10.1016/j.bioorg.2018.01.033

- [6] Clissold, S. P., & Edwards, C. Acarbose: a preliminary review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential[J]. *Drugs*, 1988, 35(3): 214-243. https://doi.org/10.2165/00003495-198835030-00003
- [7] Scott, L. J., & Spencer, C. M. Miglitol: a review of its therapeutic potential in type 2 diabetes[J]. *Drugs*, 2000, 59 (3): 521-549.
 https://doi.org/10.2165/00003495-200059030-00012
- [8] Kaku, K. Efficacy of voglibose in type 2 diabetes[J]. Expert Opinion on Pharmacotherapy, 2014, 15(8): 1181-1190.

https://doi.org/10.1517/14656566.2014.918956

[9] Bijelic, A., Aureliano, M., Rompel, A. Polyoxometalates as potential next-generation metallodrugs in the combat against cancer[J]. *Angewandte Chemie International Edition*, 2018, 57: 2-22.

https://doi.org/10.1002/anie.201803868

- [10] Hasenknopf, B. Polyoxometalates: introduction to a class of inorganic compounds and their biomedical applications[J]. *Frontiers in Bioscience*, 2005, 10(1-3): 275-287.
 https://doi.org/10.2741/1527
- [11] Pérez-Álvarez, L., Ruiz-Rubio, L., Artetxe, B., dM Vivanco, M., Gutiérrez-Zorrilla, J. M., & Vilas-Vilela, J. L. Chitosan nanogels as nanocarriers of polyoxometalates for breast cancer therapies[J]. *Carbohydrate Polymers*, 2019, 213: 159-167.

https://doi.org/10.1016/j.carbpol.2019.02.091

[12] Li, X. H., Chen, W. L., Wei, M., Liu, J., Di, Y., Liu, L., ... & Wang, E. B. Polyoxometalates nanoparticles improve anti-tumor activity by maximal cellular uptake[J]. *Inorganica Chimica Acta*, 2019, 486: 104-112.

https://doi.org/10.1016/j.ica.2018.10.046

- [13] Liu, J., Mei, W. J., Xu, A. W., Tan, C. P., Shi, S., Ji, L. N. Synthesis, characterization and antiviral activity against influenza virus of a series of novel manganese-substituted rare earth borotungstates heteropolyoxometalates[J]. *Antiviral Research*, 2004, 62(1): 65-71. https://doi.org/10.1016/j.antiviral.2003.12.004
- [14] Bijelic, A., Aureliano, M., Rompel, A. The antibacterial activity of polyoxometalates: structures, antibiotic effects and future perspectives[J]. *Chemical Communications*, 2018, 54(10): 1153-1169.

https://doi.org/10.1039/c7cc07549a

[15] Marques-da-Silva, D., Fraqueza, G., Lagoa, R., Vannathan, A. A., Mal, S. S., & Aureliano, M. Polyoxovanadates inhibition of *Escherichia coli* growth shows a reverse correlation with

Ca²⁺-ATPase inhibition[J]. New Journal of Chemistry, 2019.

https://doi.org/10.1039/C9NJ01208G

[16] Ma, M., Gao, N., Sun, Y., Du, X., Ren, J., Qu, X. Redox-activated near-infrared-responsive polyoxometalates used for photothermal treatment of Alzheimer's disease[J]. Advanced Healthcare Materials, 2018: e1800320.

https://doi.org/10.1002/adhm.201800320

[17] Gao, N., Dong, K., Zhao, A., Sun, H., Wang, Y., Ren, J., Qu, X. Polyoxometalate-based nanozyme: Design of a multifunctional enzyme for multi-faceted treatment of Alzheimer's disease[J]. *Nano Research*, 2016, 9(4): 1079-1090.

https://doi.org/10.1007/s12274-016-1000-6

[18] Gao, N., Sun, H., Dong, K., Ren, J., Duan, T., Xu, C., Qu, X. Transition-metal-substituted polyoxometalate derivatives as functional anti-amyloid agents for Alzheimer's disease[J]. *Nature Communications*, 2014, 5: 3422-3422.

https://doi.org/10.1038/ncomms4422

[19] Lee, S. Y., Fiene, A., Li, W., Hanck, T., Brylev, K. A., Fedorov, V. E., Lecka, J., Haider, A., Pietzsch, H., Zimmermann, H., Sévigny, J., Kortz, U., Stephan, H., Müller, C. E. Polyoxometalates-potent and selective ecto-nucleotidase inhibitors[J]. *Biochemical Pharmacology*, 2015, 93(2): 171-181.

https://doi.org/10.1016/j.bcp.2014.11.002

[20] Liu, W., Al-Oweini, R., Meadows, K., Bassil, B. S., Lin, Z., Christian, J. H., Dalal, N. S., Bossoh, A. M., Mbomekallé, I. M., de Oliveira. P., Iqbal, J. Cr^{III}-substituted heteropoly-16tungstates [Cr^{III}₂ (B-β-X^{IV}W₈O₃₁)₂]¹⁴⁻(X=Si, Ge): Magnetic, biological, and electrochemical

studies[J]. Inorganic Chemistry, 2016, 55(21): 10936-10946.

https://doi.org/10.1021/acs.inorgchem.6b01458

[21] Arefian, M., Mirzaei, M., Eshtiagh-Hosseini, H., Frontera, A. A survey of the different roles of polyoxometalates in their interaction with amino acids, peptides and proteins[J]. *Dalton Transactions*, 2017, 46(21): 6812-6829.

https://doi.org/10.1039/C7DT00894E

[22] Fraqueza, G., Fuentes, J., Krivosudský, L., Dutta, S., Mal, S. S., Roller, A., ... & Aureliano, M. Inhibition of Na⁺/K⁺-and Ca²⁺-ATPase activities by phosphotetradecavanadate[J]. *Journal of Inorganic Biochemistry*, 2019, 197: 110700.

https://doi.org/10.1016/j.jinorgbio.2019.110700

- [23] Stephan, H., Kubeil, M., Emmerling, F., Müller, C. E. Polyoxometalates as versatile enzyme inhibitors[J]. *European Journal of Inorganic Chemistry*, 2013, 2013(10-11): 1585-1594. https://doi.org/10.1002/ejic.201201224
- [24] Gumerova, N., Krivosudský, L., Fraqueza, G., Breibeck, J., Al-Sayed, E., Tanuhadi, E., Bijelic, A., Fuentes, J., Aureliano, M., Rompel, A. The P-type ATPase inhibiting potential of polyoxotungstates[J]. *Metallomics*, 2018, 10: 287-295.
 https://doi.org/10.1039/c7mt00279c

[25] Hasenknopf, B. Polyoxometalates: introduction to a class of inorganic compounds and their biomedical applications[J]. *Frontiers in Bioscience*, 2005, 10: 275-287. https://doi.org/10.2741/1527

[26] Sánchez-Lara, E., Treviño, S., Sánchez Gaytán, B. L., Sánchez-Mora, E., Castro, M. E., Mel éndez-Bustamante, F. J., ... & González-Vergara, E. Decavanadate salts of cytosine and

metformin: a combined experimental-theoretical study of potential metallodrugs against diabetes and cancer[J]. *Frontiers in Chemistry*, 2018, 6: 402.

https://doi.org/10.3389/fchem.2018.00402

- [27] Ilyas, Z., Shah, H. S., Al-Oweini, R., Kortz, U., & Iqbal, J. Antidiabetic potential of polyoxotungstates: *in vitro* and *in vivo* studies[J]. *Metallomics*, 2014, 6(8): 1521-1526. https://doi.org/10.1039/C4MT00106K
- [28] Chi, G., Qi, Y., Li, J., Wang, L., & Hu, J. Polyoxomolybdates as α-glucosidase inhibitors: Kinetic and molecular modeling studies[J]. *Journal of Inorganic Biochemistry*, 2019, 193: 173-179.

https://doi.org/10.1016/j.jinorgbio.2019.02.001

- [29] Qi, Y., Xiang, Y., Wang, J., Qi, Y., Li, J., Niu, J., Zhong, J. Inhibition of hepatitis C virus infection by polyoxometalates[J]. *Antiviral Research*, 2013, 100(2): 392-398. https://doi.org/10.1016/j.antiviral.2013.08.025
- [30] Geng, J., Li, M., Ren, J., Wang, E., Qu, X. Polyoxometalates as inhibitors of the aggregation of amyloid β peptides associated with Alzheimer's disease[J]. *Angewandte Chemie International Edition*, 2011, 50(18): 4184-4188.

https://doi.org/10.1002/ange.201007067

- [31] Han, Z., Wang, E., Luan, G., Li, Y., Zhang, H., Duan, Y., Hua, C., Hu, N. Synthesis, properties and structural characterization of an intermolecular photosensitive complex: (HGly-Gly)₃PMo₁₂O₄₀·4H₂O[J]. *Journal of Materials Chemistry*, 2002, 12(4): 1169-1173. https://doi.org/10.1039/B107225K
- [32] Wu, Q., Wang, J., Zhang, L., Hong, A., Ren, J. Molecular recognition of basic fibroblast

growth factor by polyoxometalates[J]. Angewandte Chemie (International ed. in English), 2005, 44(26): 4048-4052.

https://doi.org/10.1002/anie.200500108

[33] Tsiganok, L. P., Vishnikin, A. B., & Maksimovskaya, R. I. UV, IR, ⁷¹Ga and ¹⁷O NMR Spectroscopic studies of the 12-Molybdogallate Heteropolyanion[J]. *Polyhedron*, 1989, 8(23): 2739-2742.

https://doi.org/10.1016/S0277-5387(00)80529-X

[34] Rocchiccioli-Deltcheff, C., Fournier, M., Franck, R., & Thouvenot, R. Vibrational investigations of polyoxometalates. 2. Evidence for anion-anion interactions in molybdenum (VI) and tungsten (VI) compounds related to the Keggin structure[J]. *Inorganic Chemistry*, 1983, 22(2): 207-216.

https://doi.org/10.1021/ic00144a006

[35] Lapkowski, M., Turek, W., Barth, M., & Lefrant, S. Changes in catalytic properties of substituted and unsubstituted heteropolyacids in conductive polymer matrix[J]. Synthetic Metals, 1995, 69(1-3): 127-128.

https://doi.org/10.1016/0379-6779(94)02386-D

[36] Mazari, T., Marchal, C. R., Hocine, S., Salhi, N., & Rabia, C. Oxidation of propane over substituted Keggin phosphomolybdate salts[J]. *Journal of Natural Gas Chemistry*, 2009, 3(18): 319-324.

https://doi.org/10.1016/S1003-9953(08)60111-5

[37] Wang, E. B., Gao, L. H., Liu, J. F., Liu, Z. X., & Yan, D. H. Preparation and properties of the molybdovanadophosphoric acids with Dawson structure[J]. *Acta Chimica Sinica*, 1988, 46(8):

757-762.

[38] Jing, S., Wang, Z., Zhu, W., Guan, J., & Wang, G. Oxidation of cyclohexane with hydrogen peroxide catalyzed by Dawson-type vanadium-substituted heteropolyacids[J]. *Reaction Kinetics and Catalysis Letters*, 2006, 89(1): 55-61.

https://doi.org/10.1007/s11144-006-0086-3

- [39] Scholz, C., Knorr, S., Hamacher, K., Schmidt, B. DOCKTITE-a highly versatile step-by-step workflow for covalent docking and virtual screening in the molecular operating environment[J]. *Journal of Chemical Information and Modeling*, 2015, 55(2): 398-406. https://doi.org/10.1021/ci500681r
- [40] Islam, M. S., Barakat, A., Al-Majid, A. M., Ali, M., Yousuf, S., Choudhary, M. I., Khalil, R., UI-Haq, Z. Catalytic asymmetric synthesis of indole derivatives as novel α-glucosidase inhibitors *in vitro*[J]. *Bioorganic Chemistry*, 2018, 79: 350-354. https://doi.org/10.1016/j.bioorg.2018.05.004
- [41] Taha, M., Ismail, N. H., Imran, S., Wadood, A., Ali, M., Rahim, F., Khan, A. A., Riaz, M. Novel thiosemicarbazide-oxadiazole hybrids as unprecedented inhibitors of *yeast* α-glucosidase and in silico binding analysis[J]. *RSC Advances*, 2016, 6(40): 33733-33742. https://doi.org/10.1039/C5RA28012E
- [42] Pope M T. Heteropoly and isopoly oxometalates[J]. Springer-Verlag, 1983.
- [43] Timofeeva, M. N. Acid catalysis by heteropoly acids[J]. *Applied Catalysis A: General*, 2003, 256(1-2): 19-35.

https://doi.org/10.1016/S0926-860X(03)00386-7

[44] Mohammadi-Khanaposhtani, M., Rezaei, S., Khalifeh, R., Imanparast, S., Faramarzi, M. A.,

Bahadorikhalili, S., Safavi, M., Bandarian, F., Esfahani, E. N., Mahdavi, M., Larijani, B. Design, synthesis, docking study, α-glucosidase inhibition, and cytotoxic activities of acridine linked to thioacetamides as novel agents in treatment of type 2 diabetes[J]. *Bioorganic Chemistry*, 2018, 80: 288-295.

https://doi.org/10.1016/j.bioorg.2018.06.035

[45] Xing, R., Zheng, A., Wang, F., Wang, L., Yu, Y., Jiang, A. Functionality study of Na₆PMo₁₁FeO₄₀ as a mushroom tyrosinase inhibitor[J]. *Food Chemistry*, 2015, 175: 292-299. https://doi.org/10.1016/j.foodchem.2014.11.157

Figure captions

Figure 1. Docking conformations of ligand $GaMo_{12}$ and $SiMo_{12}$ in α -glucosidase. Two-dimensional ligand interaction map of compounds $GaMo_{12}(A)$ and $SiMo_{12}(B)$ docked into the binding site of α -glucosidase; Three-dimensional complex interaction of α -glucosidase and the ligands $GaMo_{12}(C)$ and $SiMo_{12}(D)$.

Figure 2. Binding mode of the ligand $PMo_{11}Ni$ with α -glucosidase residues. (A) Two-dimensional ligand interaction map of compound $PMo_{11}Ni$ docked into the binding site of α -glucosidase. (B) The ligand $PMo_{11}Ni$ in the active site of α -glucosidase. (C) Three-dimensional complex interaction of ligand $PMo_{11}Ni$ with α -glucosidase residues.

Figure 3. Binding mode of the ligand $P_2Mo_{15}V_3$ with α -glucosidase residues. (A) Two-dimensional ligand interaction map of compound $P_2Mo_{15}V_3$ docked into the binding site of α -glucosidase. (B) The ligand $P_2Mo_{15}V_3$ in the active site of α -glucosidase. (C) Three-dimensional complex interaction of ligand $P_2Mo_{15}V_3$ with α -glucosidase residues.

Table 1 Enzyme kinetic results of all synthetic compounds on α -glucosidase. Error bars indicate \pm

		1
C		a
- 03	٠	u.

Compound (POMs)		Inhibitory mechanism	Inhibitory type	Inhibitory constant		
	$IC_{50}/(\mu mol \cdot L^{-1})$			$K_{\rm I}/({\rm mmol}\cdot{\rm L}^{-1})$	$K_{\rm IS}/({\rm mmol}\cdot{\rm L}^{-1})$	Ref.
H ₅ GaMo ₁₂ O ₄₀	615.10 ± 17.00	reversible	competitive	0.630		
$H_4SiMo_{12}O_{40}\\$	33.71 ± 0.76	reversible	competitive	0.032		
$H_3PMo_{12}O_{40}$	6.41 ± 0.38	reversible	competitive	0.018		[19]
Na ₇ PMo ₁₁ NiO ₄₀	37.29 ± 1.72	reversible	mixed-type	0.049	0.109	
Na ₇ PMo ₁₁ MnO ₄₀	47.66 ± 1.15	reversible	competitive	0.079		
Na ₄ PMo ₁₁ VO ₄₀	52.33 ± 1.41	reversible	competitive	0.146		[19]
Na7PMo11ZnO40	99.23 ± 2.24	reversible	competitive	0.059		
Na ₇ PMo ₁₁ CoO ₄₀	103.10 ± 2.88	reversible	competitive	0.121		[19]
Na ₇ PMo ₁₁ CrO ₄₀	126.20 ± 3.70	reversible	competitive	0.278		
Na ₆ PMo ₁₁ FeO ₄₀	161.90 ± 7.68	reversible	noncompetitive	0.312	0.412	[19]
$H_7[P_2Mo_{17}VO_{62}]$	164.20 ± 14.20	reversible	competitive	0.328		
$H_8[P_2Mo_{16}V_2O_{62}]$	117.40 ± 3.64	reversible	competitive	0.278		
$H_9[P_2Mo_{15}V_3O_{62}]$	57.01 ± 2.11	reversible	competitive	0.024		
$H_8[P_2Mo_{14}V_4O_{62}H_2]$	127.13 ± 4.01	reversible	competitive	0.110		
$H_9[P_2Mo_{13}V_5O_{62}H_2]$	99.65 ± 2.45	reversible	competitive	0.053		

Highlights

- Keggin-type and vanadium-substituted Dawson-type polyoxometalates were synthesized.
- The structure-inhibition relationship of polyoxometalates were initially analyzed.
- Molecular modeling revealed the polyoxometalates binding mode.



TYR-158

SER-240

LYS-156

ASP-242

1415-280

GLN-279

LEU-246

ASP-242

PRO-312

LEU-313

TYR-158

SER-240

ARG-315

PHE-314

SER-311



