Short thesis for the degree of doctor of philosophy (PhD)

Expression and characterization of human *O*-(*N*-acetyl-β-D-glucosaminyl)-L-serine/threonine *N*-acetylglucosaminyl hydrolase, synthesis and evaluation of its new inhibitors

by Mariann Kiss

Supervisors: Dr. László Somsák, Dr. Teréz Barna

UNIVERSITY OF DEBRECEN

Doctoral School of Chemistry

Debrecen, 2022

1. Introduction and objective

O-GlcNAcylation is a unique post-translational modification of proteins in which a single *O*-linked β-*N*-acetyl-D-glucosamine (*O*-GlcNAc) unit is attached to Ser/Thr side chains. The *O*-GlcNAc modification is catalyzed by only two enzymes: uridine diphospho-*N*-acetylglucosamine:polypeptide β-*N*-acetylglucosaminyltransferase (OGT) catalyses the incorporation of β-*O*-GlcNAc while [protein]-3-*O*-(*N*-acetyl-D-glucosaminyl)-L-serine/threonine *N*-acetylglucosaminyl hydrolase (OGA) catalyses the removal of *O*-GlcNAc from the glycosylated substrates. Aberrant hypo-*O*-GlcNAcylation can lead to neurodegeneration such as Alzheimer's disease. PUGNAc (**2**)* is one of the first OGA inhibitors (K_i = 46 nM), however, it has no selectivity against β-*N*-acetylhexosaminidases (HexA and HexB) which also cleave *O*-GlcNAc but from oligosaccharide substrates. Applying selective OGA inhibitors may provide a therapeutic treatment for the above-mentioned disease, therefore, the study of OGA inhibitors and the development of new inhibitors have received considerable attention nowadays. In my doctoral work, PUGNAc (**2**) was chosen as the lead structure for the design of new inhibitors. Substitution in the aromatic group, but not an increase in its size, has been previously studied and, in addition, it has been found that the use of longer *N*-acyl chains at position 2 increases the selectivity against hexosaminidases. However, there are no examples in the literature for altering the linker between the sugar ring and the aromatic group. Considering these, synthesis of 2-acetamido-2-deoxy-D-gluco-1,5-lactone hydrazone derivatives (target compounds **39** in Figure 1) was planned. We intended to examine the effect of the linker (X = CONH: semicarbazones, X = CSNH: thiosemicarbazones, X = SO_2: sulfonyl-hydrazones) between the sugar ring and the aromatic group on binding. Changes in the electron density and size of the aromatic moiety (Ar = phenyl, substituted phenyl, naphthyl) can also affect binding.

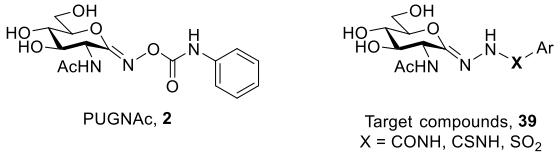


Figure 1. PUGNAc (**2**) and target compounds (**39**)

* In this short thesis, the same compound numbers are used as in the dissertation.

To investigate the inhibitory properties of the synthesized compounds, we planned the heterologous expression of the hOGA enzyme in an *E. coli* expression system and the biochemical characterization of the recombinant enzyme. In order to test the selectivity of the compounds, it was necessary to determine the inhibitory properties not only against OGA but also against hexosaminidases.

2. Methods

During the course of this work macro-, semimicro- and micro methods of modern preparative organic chemistry were applied. The reactions were monitored by using thin layer chromatography (TLC), the products were purified by column chromatography or by crystallization. Purity of the isolated products was checked by TLC and their ^{1}H and ^{13}C NMR spectra. For the characterization of the new compounds, physical constants such as melting point and optical rotation were measured, their structural elucidation was based on their NMR and MS spectra.

By molecular- and microbiological methods the *E. coli* strains were produced that carried the vector construct containing the gene encoding the full length hOGA isoenzyme. The conditions, which affect the intracellularly expressed recombinant enzyme production in small- and large-scale cell culture growth, were optimized. The lysis of the bacterial cell was carried out by ultrasound and enzymatic treatment. The recombinant enzyme was isolated in a single-step affinity chromatography. Bioanalitical methods were used to follow the efficiency of the bacterial expression and the isolation procedures: denaturing polyacrylamide electrophoresis and enzyme activity assay based on using chromophore substrate. The inhibition constants (K_i) of the compounds against the hOGA, HexA/HexB and hHexB enzymes were determined by Dixon method and nonlinear regression analysis, while the mode of inhibition was determined by Cornish-Bowden method and Lineweaver-Burk double reciprocal representation.

3. Results

3.1 We have developed a five-step, high-yielding, simple reaction sequence for the synthesis of 2-acetamido-2-deoxy-D-glucono-1,5-lactone hydrazone derivatives as new OGA inhibitors

The synthesis of one of the target compounds **39a** was published in the literature, so the syntheses were based on an adaptation of this procedure (Table 1). The known compound **33** was reacted with 4-arylsemicarbazides (**35a-h**) and arylsulfonylhydrazides (**35i-n**), available commercially or prepared by literature procedures, in the presence of 10 mol% *p*-toluenesulfonic acid in boiling chloroform.

In reactions of semicarbazides **35a-g** besides the expected **36a-g** the equilibrating open-chain tautomers **37a-g** were also observed in the ^{1}H NMR spectra, while the reactions with thiosemicarbazide **35h** and sulfonylhydrazides **35i-n** were devoid of this product. No attempt was made to separate compounds **36** and **37**. Oxidation of the mixtures containing **36** and **37** with CrO_3-pyridine complex gave good yields of the expected lactone semicarbazones **38a-g**. Regrettably, these oxidation conditions yielded complex reaction mixtures in case of the thio derivative **36h** and the sulfonylhydrazides **36i-n**. In these cases MnO_2 proved to be efficient to furnish the required compounds.

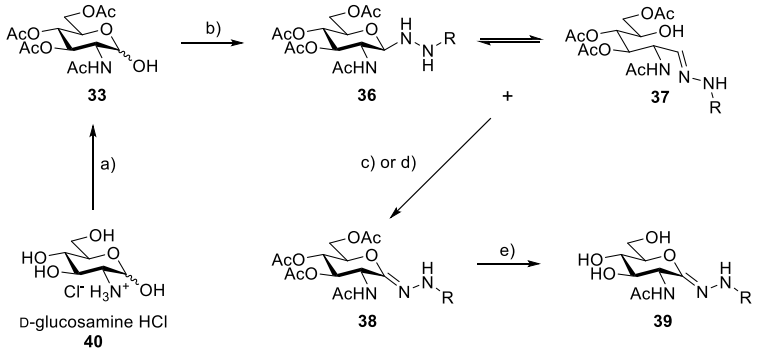
The lactone hydrazones **38h-n** were *O*-deprotected by NH_3/MeOH to result in the test compounds **39**. The overall yields are 15-55 % for five steps.

3.2 We have developed a heterolog expression method for producing the full-length recombinant hOGA enzyme in E. coli BL21(DE3) Rosetta strain. Stability factors were elucidated by which the half-life of hOGA has significantly increased. An isolation protocol was also worked out that has resulted in a homogenous hOGA enzyme sample with high catalytic efficiency.

3.2.1 Heterolog expression of hOGA in E. coli expression strains

The vector construct carrying the gene encoding full-length hOGA was kindly provided by prof. D. J. Vocadlo from Simon Fraser University (Burnaby, Canada). Five different *E. coli* strains were used for the expression of the long isomorph of hOGA: *E. coli* BL21(DE3), *E. coli* BL21(DE3) pLysS, *E. coli* BL21-CodonPlus (DE3)-RIPL, *E. coli* BL21 STAR(DE3) és *E. coli* BL21(DE3) Rosetta.

Table 1. Synthesis of target compounds



Reagents and conditions: a) 1. Ac_2O, abs. pyridine, r.t.; 2. 4Å MS, abs. MeOH, r.t. (83 % two steps); b) NH_2NHR (**35**), 0.1 eqv. $pTsOH \cdot H_2O$, $CHCl_3$, reflux; c) for **a-g**: CrO_3-pyridine, abs. CH_2Cl_2, 0°C → r.t.; d) for **h-n**: MnO_2, abs. CH_2Cl_2, reflux; e) NH_3/MeOH

	R	Products and yields (%)				
		36 + 37	ratio of **36** : **37**[a]	**38**	**39**	Overall yield of **39** from D-glucosamine
a	CONHPh	90	5:1	83	79	49
b	CONH(*p*-MePh)	83	7:1	92	86	55
c	CONH(*p*-CF_3Ph)	81	5:1	26	87	15
d	CONH(*p*-OMePh)	87	7:3	86	83	52
e	CONH(*p*-ClPh)	77	4:1	78	74	37
f	CONH(1-naftil)	94	[b]	64	83	41
g	CONH(2-naftil)	80	[b]	93	75	46
h	CSNHPh	87[c]	-	78	83	47
i	SO_2(*p*-MePh)	77[c]	-	69	94	41
j	SO_2(*p*-CF_3Ph)	85[c]	-	60	83	35
k	SO_2(*p*-FPh)	86[c]	-	72	85	44
l	SO_2(*p*-ClPh)	89[c]	-	77	78	44
m	SO_2(1-naftil)	89[c]	-	66	80	39
n	SO_2(2-naftil)	76[c]	-	56	75	26

[a] Determined from the ^{1}H NMR spectra of the worked-up reaction mixtures. [b] Formation of the open-chain products **37f** and **37g** was detected by TLC but due to signal overlaps and small amounts of these compounds the **36**:**37** ratio was not established. [c] Compound **36** formed as a single product.

The small-scale expression trials of all transformed BL21(DE3) strains unravelled two main issues to be solved in this expression system. Namely, the high tendency of the recombinant hOGA to form inclusion body aggregates during the protein production phase and the significant proteolytic degradation of the recombinant enzyme observed both *in vivo* and *in vitro* (Figure 2). The worst performing strain was BL21 (DE3), where little amount of full-length recombinant protein was produced (Figure 2). Two strains, RIPL and Rosetta, showed increased recombinant enzyme production, however, adequate amounts of active, soluble protein were obtained with Rosetta only (Figure 2). The productivity of the Star and pLysS strains was very similar, with a relatively high yield of active hOGA compared to the aggregate form. Nevertheless, Star and pLysS showed the highest specific proteolytic activity.

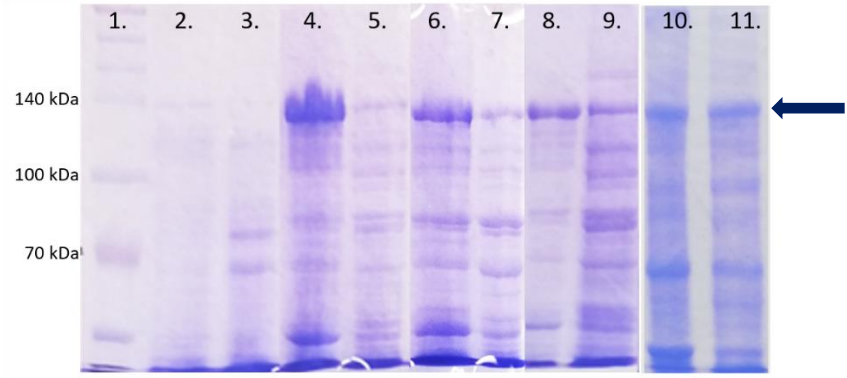


Figure 2. Gel electrophoretic (8% SDS-PAGE) analysis of the soluble and insoluble fractions of the cell lysates produced by different E. coli BL21(DE3) strains grown at 23 °C, induced at mid-exponential phase with 0.4mM IPTG and harvested at early stationary phase.
Lanes 1. protein marker; 2. E. coli BL21(DE3) insoluble fraction; 3. E. coli BL21(DE3) soluble fraction; 4. E. coli BL21(DE3) RIPL insoluble fraction; 5. E. coli BL21(DE3) RIPL soluble fraction; 6. E. coli BL21(DE3) Rosetta insoluble fraction; 7. E. coli BL21(DE3) Rosetta soluble fraction; 8. E. coli BL21 Star (DE3) insoluble fraction; 9. E. coli BL21 Star (DE3) soluble fraction; 10. E. coli BL21(DE3) pLysS insoluble fraction; 11. E. coli BL21(DE3) pLysS soluble fraction.

Systematic cultivation parameters were optimized for Rosetta strain. The effect of temperature on the recombinant protein production was investigated under 30°C, at three temperatures (16, 23 and 28°C, Figure 3). Decreasing the cultivation temperature favoured full-length and active enzyme production at 23°C only. At lower temperature (16°C), the misfolding of the human enzyme

was accelerated even though a temperature of 16°C is commonly used in the literature to produce hOGA.

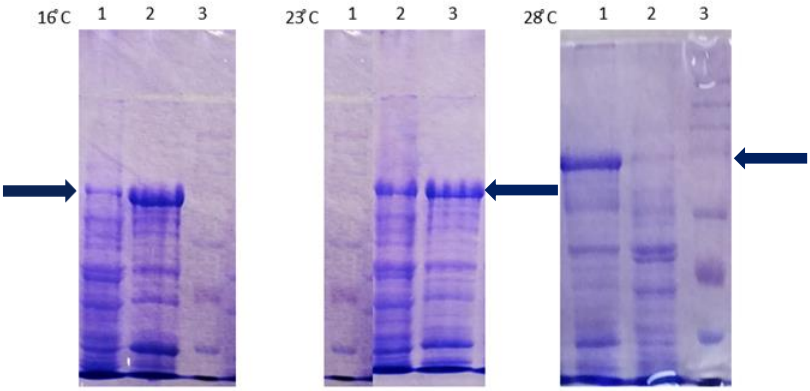


Figure 3. 8% SDS-PAGE analysis of the cell lysate obtained from *E. coli* Rosetta expression cultures grown at different temperature.
Sample lysates of 16°C: 1. soluble fraction, 2. insoluble fraction, 3. protein marker; Sample lysates of 23°C: 1. protein marker, 2. soluble fraction, 3. insoluble fraction; Sample lysates of 28°C: 1. insoluble fraction, 2. soluble fraction, 3. protein marker.

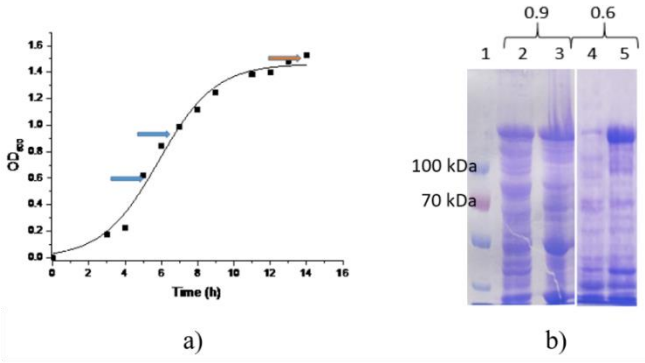


Figure 4. a) The growth curve of *E. coli* BL21(DE3) Rosetta culture at 23℃. The blue arrows indicate the induction phase (at OD_{600}= 0.6 and 0.9), the orange arrow shows the harvest phase. b) 8 % SDS-PAGE analysis of the cell lysates
Lanes: 1. protein marker; 2. the soluble, 3. the insoluble fraction of the later-phase induced culture. 4. the soluble, 5. the insoluble fraction of the earlier-phase induced culture.

The induction phase of the culture growth also influenced the yield of hOGA production. When the inducer was employed at later phase than the mid-exponential one (at OD_{600} 0.9 instead of 0.6), we could achieve 50% higher yield without being compromised by greater proteolytic degradation or formation of inclusion-body aggregates (Figure 4).

All the optimized conditions were built in the final heterologous expression protocol for hOGA, in which aggregate formation of the overproduced enzyme was minimized and an efficient full-length, active hOGA production was ensured.

3.2.2 Optimizing hOGA isolation conditions and finding stabilizing conditions

Cell lysis conditions were developed to extract intracellularly produced recombinant hOGA using ultrasound and lysozyme enzyme treatment. Experimentation of the appropriate lysis buffer and physical parameters of sonication were important for enzyme stability. Composition of a buffer of adequate stability: 50 mM potassium-phosphate buffer, pH 7.5, containing 1mM 2-hydroxy-1-methanethiol, 0.5 % Tween 20, Protease inhibitor cocktail (P8849, Sigma Aldrich, Hungary).

3.2.3 Purification of the hOGA enzyme

The sequence containing 6 histidine (His_6-tag) on the *N*-terminus allows the protein to bind to the IMAC column. The transition metal ion used was shown to be significant in the IMAC chromatography of hOGA. The commonly used Ni^{2+} ion resulted in a significant decrease in enzyme activity in our case. Therefore, other metal ions were tested, such as Cu^{2+} and Co^{2+}, the latter did selectively bind the recombinant hOGA protein with His_6-tag. The efficiency of chromatography was greatly influenced by the sample loading conditions (sample concentration, temperature, flow rate) and other chromatographic parameters such as the salt concentration to weaken the binding of individual proteins and the use of a surfactant. One of the most important parameters was the initial imidazole concentration used during sample application, which largely determined the separation of proteolytically damaged hOGA fragments from the full-length hOGA protein. (Figure 5). The buffer composition developed for

lysis also proved to be suitable for long-term storage of the enzyme. In this buffer, hOGA was stable for at least two years at -20°C with the addition of 30% glycerol.

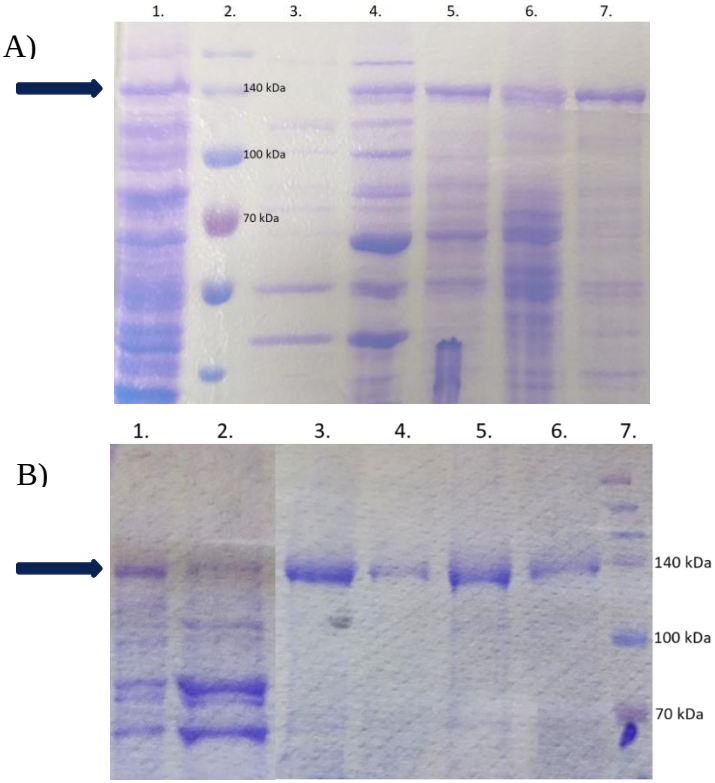


Figure 5. Purification of hOGA produced in *E. coli* BL21(DE3) Rosetta strain under optimized conditions by Co^{2+} ion IMAC affinity chromatography on a HiPrep IMAC Fast Flow column.
A) The binding buffer of the sample loaded to the column contained 10 mM imidazole. Elution with an imidazole gradient elution buffer containing 100-500 mM imidazole.
Lanes: 1. Sample loaded to the column; 2. protein marker, 3. 100 mM imidazole / l .; 4. 100 mM imidazole / 2 .; 5. 200 mM imidazole / l .; 6. 200 mM imidazole / 2 .; 7. 500 mM imidazole.
B) The binding buffer of the sample loaded to the column contained 50 mM imidazole. Elution with an imidazole gradient elution buffer containing 100-500 mM imidazole.
Lanes: 1. Sample loaded to the column; 2. fraction eluted with binding buffer, 3. 100 mM imidazole; 4. 150 mM imidazole; 5. 200 mM imidazole; 6. 500 mM imidazole; 7. protein marker.

3.3 We have determined the kinetic parameters of the enzymes used in the inhibition assays

The hOGA enzyme was expressed and purified as described above. The HexA/HexB heterodimeric enzyme from bovine kidney was obtained commercially, while the hHexB enzyme was provided by the staff of the Biotransformation Laboratory of the Institute of Microbiology of the Czech Academy of Sciences. I had the opportunity to perform the experiments with hHexB in the laboratory of Prof. Vladimír Křen in Prague.* Michaelis-Menten kinetic parameters for *p*NP-GlcNAc substrate in the presence of hOGA established in potassium-phosphate buffer at pH 7.5 resulted in 20 μM for K_M and 1.6 s^{-1} for k_{cat} values (Table 2). The determined k_{cat} value correlated well with the previously published data. However, the enzyme in this study displayed much higher binding affinity toward *p*NP-GlcNAc than the one reported previously for different conditions.

The kinetic parameters of the HexA/HexB enzyme from bovine kidney were determined for *p*NP-GlcNAc substrate in potassium phosphate buffer at pH 5.8, while the parameters of hHexB enzyme were examined in citrate-phosphate buffer at pH 5.0. For both hexosaminidases the K_M is 1.1 mM.

For the hOGA enzyme, Michaelis-Menten kinetic parameters were determined also in the presence for the fluorescent 4-MU-GlcNAc substrate. In this case, K_M = 12 μM in potassium phosphate buffer at pH 7.5.

Table 2. Michaelis-Menten kinetic parameters of hOGA for *p*NP-GlcNAc

Buffer	50 mM K_2HPO_4 - KH_2PO_4 buffer, pH 7.5	PBS (10 mM f phosphate buffer,137 mM NaCl, 2.7 mM KCl) pH 7.4	0.5 M citrate-phosphate buffer, pH 6.5
K_M	20 μM ± 2	206 μM	1.1 mM
k_{cat} (s^{-1})	1.6 ± 0,1	1.5	3.4

* Short term scientific mission was supported by COST Action 18132.

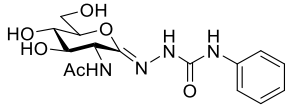
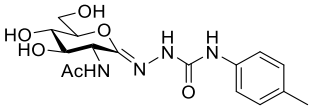
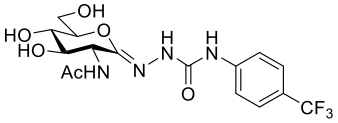
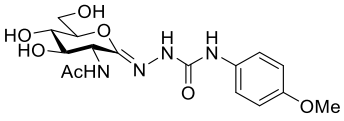
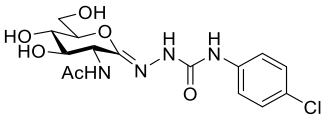
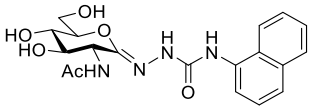
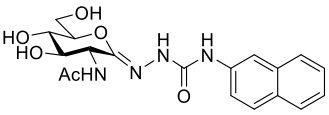
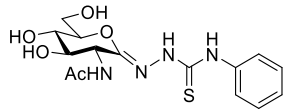
3.4 In kinetic studies we have established the inhibition manner of the synthesized compounds and their inhibitory potentials. All compounds competitively inhibited the enzymes - hOGA, HexA/HexB (from bovine kidney) and hHexB - in the nanomolar range.

The synthesized compounds are competitive inhibitors of both hOGA, HexA/HexB and hHexB enzymes. For the lactone semicarbazones **39a-h**, *p*NP-GlcNAc substrate was used to determine the inhibition constants against the hOGA enzyme, except for the tight-binding derivatives **39e** and **39g**, where the fluorescent 4-MU-GlcNAc substrate was used, thus we were able to reduce the enzyme concentration. The fluorescent substrate was also used for the lactone sulfonylhydrazones **39i-n**.

The inhibition constants of compounds **39** against the hOGA enzyme are in the low micromolar, in some cases in the nanomolar range. The inhibition constants of the lactone semicarbazones **39a-h** are summarized in Table 3. Of these compounds, **39e** and **39g** showed the best inhibitory effect, while the thio derivative **39h** was the weakest inhibitor. Slightly higher K_i values were obtained for the HexA/HexB enzyme, no significant selectivity was observed between the two enzymes. It is worth noting the considerable difference in the inhibition by naphthyl derivatives **39f** and **39g**, of which the 2-naphthyl **39g** is more than one order of magnitude more potent than the 1-naphthyl **39f** counterpart. On the other hand, **39f** is more selective towards HexA/HexB.

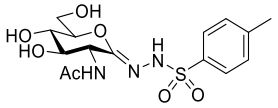
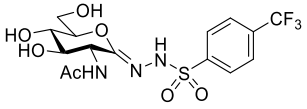
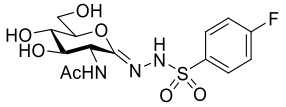
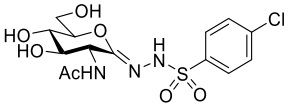
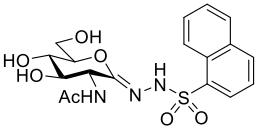
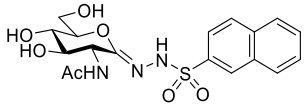
The lactone sulfonylhydrazones **39i-n** are better inhibitors of hOGA than lactone semicarbazones **39a-h** (Table 4). The most potent inhibitors are the naphthyl derivatives **39m** and **39n**, while **39j** proved to be the weakest one. On the other hand, lower K_i values against hHexB were obtained for all but one of the compounds, indicating that these compounds are better inhibitors of hHexB than of hOGA. Sulfonylhydrazone **39m** is the strongest inhibitor of both enzymes and the most selective towards hHexB after **39j** (**39j** is also the least potent inhibitor for both enzymes).

Table 3. The binding affinities of 2-acetamido-2-deoxy-D-glucono-1,5-lactone semicarbazone derivatives (**39a-h**) toward hOGA and HexA/HexB enzymes (K_i [μM])

	Compound	hOGA	HexA/HexB
39a		0.190 ± 0.008	0.205 ± 0.014 (lit.: 0.130 ± 0.020)
39b		0.155 ± 0.003	0.332 ± 0.017
39c		0.167 ± 0.006	0.125 ± 0.004
39d		0.270 ± 0.010	0.413 ± 0.014
39e		0.083±0.004‡	0.170 ± 0.004
39f		0.506 ± 0.020	0.154 ± 0.006
39g		0.036±0.001‡	0.047± 0.002
39h		27 ± 3	30 ± 2

‡ 4-MU-GlcNAc was used as substrate

Table 4. The binding affinities of 2-acetamido-2-deoxy-D-glucono-1,5-lactone sulfonylhydrazone derivatives (**39i-n**) toward hOGA and hHexB enzymes (K_i [nM]).

	Compound	hOGA	hHexB
39i		78 ± 1	21 ± 2
39j		230 ± 17	48 ± 4
39k		95 ± 11	45 ± 3
39l		70 ± 3	39 ± 2
39m		27 ± 7	6.8 ± 1.8
39n		30 ± 2	30 ± 3

4. Possible future applications of the results

The five-step synthetic route we have developed for the new inhibitors uses simple, easy-to-perform reaction conditions, in most cases yielding the target compounds in ~40% or greater overall yield. This synthetic route allows the preparation of other variously substituted compounds of similar structure.

The synthesized target compounds **39** are similar good inhibitors of the OGA enzyme as PUGNAc (**2**), although they do not have significant selectivity for hexosaminidases. The simple synthetic route provides an opportunity to develop modifications that are expected to increase selectivity, which are currently under investigation in our research team. If these are successful and selective inhibitors can be prepared, they can later serve as synthetic and biochemical guides.

Our developed heterologous expression system provided functional, full-length recombinant hOGA enzyme. The subsequent isolation protocol elaborated in this work resulted in a high catalytic efficiency enzyme suitable for kinetic studies. We also elucidated new stabilizing conditions in which the affinity of the artificial *p*NP-GlcNAc substrate for the active site of the enzyme was significantly increased compared to previously reported results. These procedures are expected to be used by other researchers, which may facilitate the investigation and biomedical application of the enzyme and other new inhibitors.

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

Registry number: DEENK/331/2022.PL
Subject: PhD Publication List

Candidate: Mariann Kiss
Doctoral School: Doctoral School of Chemistry
MTMT ID: 10083958

List of publications related to the dissertation

Foreign language scientific articles in international journals (2)

1. **Kiss, M.**, Timári, I., Barna, T., Mészáros, Z., Slámová, K., Bojarová, P., Křen, V., Hayes, J. M., Somsák, L.: 2-Acetamido-2-deoxy-d-glucono-1,5-lactone Sulfonylhydrazones: Synthesis and Evaluation as Inhibitors of Human OGA and HexB Enzymes.
   Int. J. Mol. Sci. 23 (3), 1-18, 2022. ISSN: 1661-6596.
   DOI: http://dx.doi.org/10.3390/ijms23031037
   IF: 5.923 (2020)

2. **Kiss, M.**, Szabó, E., Bocska, B., Sinh, L. T., Fernandes, C. P., Timári, I., Hayes, J. M., Somsák, L., Barna, T.: Nanomolar inhibition of human OGA by 2-acetamido-2-deoxy-d-glucono-1,5-lactone semicarbazone derivatives.
   Eur. J. Med. Chem. 223, 1-14, 2021. ISSN: 0223-5234.
   DOI: http://dx.doi.org/10.1016/j.ejmech.2021.113649
   IF: 6.514 (2020)

DEBRECENI EGYETEM * Egyetemi és Nemzeti Könyvtár *

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

List of other publications

Foreign language scientific articles in international journals (1)

3. Csire, G., Timári, S., Asztalos, J., Király, J. M., **Kiss, M.**, Várnagy, K.: Coordination, redox properties and SOD activity of Cu(II) complexes of multihistidine peptides.
J. Inorg. Biochem. 177, 198-210, 2017. ISSN: 0162-0134.
DOI: http://dx.doi.org/10.1016/j.jinorgbio.2017.08.033
IF: 3.063

Total IF of journals (all publications): 15,5
Total IF of journals (publications related to the dissertation): 12,437

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

09 June, 2022

DEBRECENI EGYETEM * Egyetemi és Nemzeti Könyvtár *

Lectures in the subject of the PhD thesis

1. **Kiss M.** Potenciális OGA inhibitorok szintézise. XXXIX. Kémiai Előadói Napok, Szeged, 2016. október 17-19.
2. **Kiss, M.**; Barna, T.; Somsák, L. Inhibition of wild type human OGA enzyme by 2-acetamido-2-deoxy-D-glucono-1,5-lactone semicarbazones. MTA Szénhidrát, Nukleinsav és Antibiotikumkémiai Munkabizottsági ülés 2017. Mátraháza, 2017. május 31 - június 2.
3. **Kiss M.**; Barna T.; Somsák L. A humán OGA enzim inhibíciós vizsgálata a szerkezet-funkció kapcsolatának feltárására. II. Debreceni Röntgendiffrakciós Kerekasztal, Debrecen, 2018. január 18-19.
4. **Kiss, M.**; Barna, T.; Somsák, L. Potent inibition of wild-type human OGA by aldonolactone hydrazone derivatives. Annual meeting of the Working Committee for Carbohydrates, Nucleic Acids and Antibiotics, Mátrafüred, 2018. május 23-25.
5. **Kiss, M.**; Somsák, L.; Barna, T. Nanomolar inhibition of human OGA by 2-acetamido-2-deoxy-D-glucono-1,5-lactone hydrazone derivatives. 29th International Carbohydrate Symposium, Lisszabon (Portugália), 2018. július 14-19.

Lecture in other subject

1. **Kiss M.** A szuperoxid-dizmutáz (SOD) enzimet modellező peptidek réz(II)- és nikkel(II)-komplexei. XXXI. Országos Tudományos Diákköri Konferencia, Kémiai és Vegyipari Szekció, Eger 2013. április 4-6.

Posters in the subject of the PhD thesis

1. **Kiss M.**; Barna T.; Somsák L. A humán OGA enzim inhibíciós vizsgálata 2-acetamido-2-dezoxi-D-glükono-1,5-lakton hidrazon származékokkal. I. Fiatal Kémikusok Fóruma Szimpózium, Debrecen, 2019. április 3-5.
2. **Kiss M.**; Barna T.; Somsák L. *N*-Acil-glükózamin származékok szintézise és inhibíciós vizsgálata humán OGA enzimmel szemben. MKE Vegyészkonferencia 2019, Eger, 2019. június 24-26.
3. **Kiss, M.**; Barna, T.; Somsák, L. Synthesis of *N*-acyl-glucosaminyl derivatives for inhibition of human OGA. Eurocarb XX. Leiden (Hollandia), 2019. június 30 - július 4.

Poster in other subject

1. Várnagy, K.; Timári, S.; Serfőző, D.; Asztalos, J.; **Kiss, M.** Coordination ability of small multihistidine peptides. International Symposium on Metal Complexes, Lisszabon (Portugália), 2012. június 18-22.