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***N*-2 Alkylated analogues of aza-galactofagomine as potential inhibitors of β -glucosidase**

FILIP ĐURKOVIĆ¹, MARIO ZLATOVIĆ^{1#}, DUŠAN SLADIĆ^{1#}, IRENA NOVAKOVIĆ^{2#},
FILIP BIHELOVIĆ^{1*} and ZORANA FERJANČIĆ^{1***}

¹University of Belgrade – Faculty of Chemistry, Studentski trg 12–16, Belgrade, Serbia and

²University of Belgrade – Institute of Chemistry, Technology and Metallurgy, Njegoševa 12, Belgrade, Serbia

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Abstract: The synthesis of four *N*-2-alkylated aza-galactofagomine (AGF) analogues was achieved by intermolecular reductive hydrazination or alkylation of suitably protected AGF. The synthesized compounds were evaluated as potential β -glucosidase inhibitors. The preliminary screening of inhibitor activity, conducted with sweet almond β -glucosidase immobilized in agar, as well as the standard inhibition assay with the same enzyme, showed the inhibitory potency of the synthesized analogues. In addition, these results are in a good agreement with the docking analysis of the human acid β -glucosidase, the enzyme implicated in Gaucher's disease.

Keywords: iminosugars; glycosidase inhibitor; Gaucher's disease.

INTRODUCTION

Glycosidases represent a large group of hydrolytic enzymes that catalyse the breaking of glycosidic bonds in various sugars. As enzymatic hydrolysis of carbohydrates is a widespread biological process, therefore glycosidase inhibitors have a wide range of applications, from agriculture to medicine. Glycosidases are involved in the biosynthesis of oligosaccharide chains of glycoproteins in the endoplasmic reticulum and the Golgi apparatus. Inhibition of these glycosidases has an impact on post-translational modifications, transport and secretion of glycoproteins, which play a role in communication between cells and cell–virus recognition, among other things. Inhibition of glycosidases results in an impact on these processes, and glycosidase inhibitors can potentially be used to treat viral diseases, cancer and genetic disorders.¹

* Corresponding authors. E-mail: (*)filip@chem.bg.ac.rs; (**)zferjan@chem.bg.ac.rs

Serbian Chemical Society member.

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Glucosidase inhibitors are currently of interest owing to their promising therapeutic potential in the treatment of disorders such as diabetes, human immunodeficiency virus (HIV) infection, metastatic cancer, and lysosomal storage diseases.²

Over the last two decades polyhydroxylated azaheterocycles, also known as iminosugars,^{3,4} have attracted notable interest among scientific community because of their ability to act as pharmacological chaperones (PC) for a variety of glycosidases.⁵ This concept is very attractive from the medical point of view and considered as a promising new tool for the treatment of a range of pathologies known as lysosomal storage disorders.⁶ The mutations associated with these diseases cause various glycosidases from lysosome to fold improperly, resulting in partially or totally inactive enzymes, that cannot pass quality control in endoplasmic reticulum. In cases where the disease is caused by misfolding of glycosidases, pharmacological chaperone therapy (PCT) is often effective.⁷

β -Glucosidase catalyses the hydrolysis of various β -glucosidic bonds within di- and oligosaccharides, as well as glucoconjugates. It is significant from a medical aspect, as the deficiency of lysosomal acid β -glucosidase leads to the accumulation of glucocerebroside in some cells, which is characteristic for Gaucher's disease.^{8–10} Furthermore, β -glucosidase has been confirmed to be overexpressed in breast, liver and gastric cancer cells.¹¹ In breast cancer, it has been shown that the inhibition of β -glucosidase might be an alternative therapeutic strategy to overcome chemoresistance.¹² On the other hand, malfunction or deficiency of lysosomal β -galactocerebrosidase causes progressive demyelination in the central nervous system and neurodegeneration.¹³ This rare and severe genetic neurological disorder is known as the Krabbe disease.¹⁴ Given that both of these glycosidases act in lysosomes, there is a possibility that some glucosidase inhibitors may act as multi-target molecules. The fact that aza-galactofagomine (**1**) (AGF, Fig. 1) acts as a competitive inhibitor of β -galactocerebrosidase,^{15,16} prompted us to investigate whether AGF derivatives can also be inhibitors of β -glucosidases. Recently, we have developed an enantioselective method for the preparation of AGF (**1**), that relies on a tactical combination of organocatalyzed aldolization/reductive hydrazination and this short synthesis offers an easy access to *N*-2 analogues of AGF.¹⁷ Therefore, our goal was to synthesize *N*-2 AGF analogues containing hydrophobic alkyl chains of different length, including those with aromatic ring, and to test them as the inhibitors of β -glucosidases. Our preliminary modelling studies suggested that interactions of

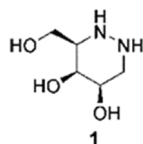


Fig. 1. Structure of aza-galactofagomine **1** (AGF).

these hydrophobic groups with enzyme should be relevant for the activity. A commercially available β -glucosidase from sweet almond (*Prunus dulcis*) was selected as a widely used model enzyme, as it shows a broad substrate specificity. The results of this study are presented herein.

EXPERIMENTAL

All chromatographic separations were performed on silica gel 60 (0.063–0.200 mm), Merck. Standard techniques were used for the purification of reagents and solvents. NMR spectra were recorded on Varian/Agilent 400 (^1H -NMR at 400 MHz; ^{13}C -NMR at 100 MHz) and on Bruker Avance III 500 (^1H -NMR at 500 MHz; ^{13}C -NMR at 125 MHz), in deuterated chloroform, if not otherwise stated. Chemical shifts are expressed in ppm (δ) using tetramethylsilane as internal standard, coupling constants (J) are in Hz. IR spectra were recorded on Thermo Scientific Nicolet Summit FT-IR instrument, and are expressed in cm^{-1} . Mass spectra were obtained on Orbitrap Exploris 240 spectrometer.

For all synthesized compounds detailed experimental procedures, spectral data and copies of NMR spectra are given in Supplementary material to this paper.

Chemistry

Four AGF analogues **12–14** and **22** were obtained from the key intermediate **6**. Compound **6** was obtained according to the slightly modified literature procedure.¹⁷ Compounds **12–14** were obtained by reductive hydrazination of **6**, followed by deprotection. Compound **22** was obtained by silylation of **6**, followed by alkylation/deprotection sequence.

Biochemical and computational methods

*Preliminary screening of β -glucosidase inhibitory activity.*¹⁸ Enzyme β -glucosidase from sweet almond was immobilized in agar by gelling. As a substrate, esculin was used, which is transformed into esculetin and glucose after an enzymatic reaction. The released esculetin forms complexes with the added FeCl_3 , developing a dark gel colour. If light spots appear on the agar after inoculation of the compounds and enzyme–substrate reaction, the compounds show enzyme inhibition. In 7 mL sodium acetate buffer (0.1 M, pH 5) 0.07 g of agar powder was dissolved at 80–100 °C. After cooling at 60 °C, 1.2 mL of 0.5 % FeCl_3 and 40 μL of enzyme β -glucosidase (0.02 IU mL^{-1}) were added. The final volume was adjusted to 10 mL with acetate buffer and the solution was poured into a Petri dish. After solidification of the agar, 5 μL of each compound (concentration of 1 μg μL^{-1}) were inoculated on the agar surface. As a positive control, conduritol β -epoxide was used. The plate was incubated for 15 min at room temperature to allow reaction between the enzyme and inhibitors, after which 7 mL of 0.2 % esculin solution was added to cover the agar surface. Afterwards, the plate was incubated for 30 min at room temperature for enzyme–substrate reaction, and clear zones were observed.

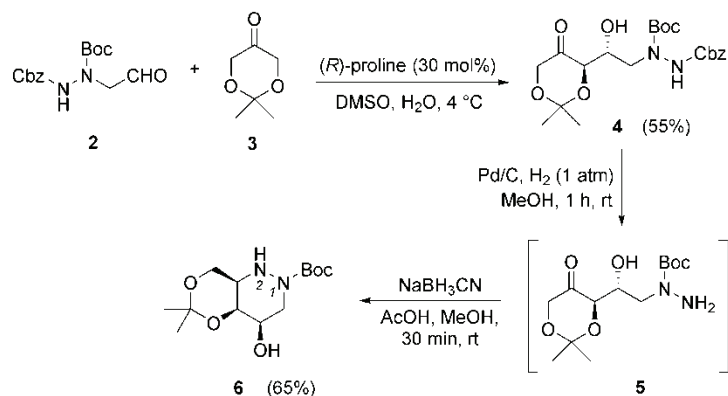
*β -Glucosidase inhibition assay.*¹⁹ The enzyme activity was determined by measuring (absorbance at 405 nm) the hydrolysis rate of *p*-nitrophenyl- β -D-glucopyranoside (pNGP). The amount of released *p*-nitrophenol (pNP) was determined from the previously constructed standard curve of absorbance vs. pNP concentration (the range of concentration was 1–7 mM). After incubation (15 min at 50 °C) of the enzyme (0.02 IU) with different concentrations of the tested compounds (the concentration range was 5–0.075 $\mu\text{g}/1.5$ mL reaction solution), the amount of released pNP was determined and the percentage of enzyme inhibition was calculated. From the curve of dependence of the percentage of inhibition vs. the compound con-

centrations, the IC_{50} value (concentration at which 50 % of the enzyme was inhibited) was calculated. 100 μL of β -glucosidase solution (0.03 IU mL^{-1}) was mixed with 5 μL of test compounds and incubated at 50 $^{\circ}\text{C}$ for 15 min. Then, 900 μL of pNPG solution (5 mM) in citrate buffer (50 mM, pH 7.0) was added to that solution and incubated for another 10 min at the same temperature, after which 495 μL of ice-cold sodium carbonate (0.5 M) was added and absorbance at 405 nm was measured. All experiments were performed in triplicate.

Molecular docking studies. Crystal structure of human acid β -glucosidase from lysosome (PDB ID 2NSX)²⁰ was downloaded from RSCB database.²¹ Protein structure was prepared using Protein Preparation Wizard from Schrödinger Suite 2021-3 and chain B was used. Small molecules were prepared using Maestro and their pK_a values were determined using Schrödinger Suite 2021-3.²² Figures were prepared using the academic version of Maestro viewer from Schrödinger Suite 2024-1.²³

RESULTS AND DISCUSSION

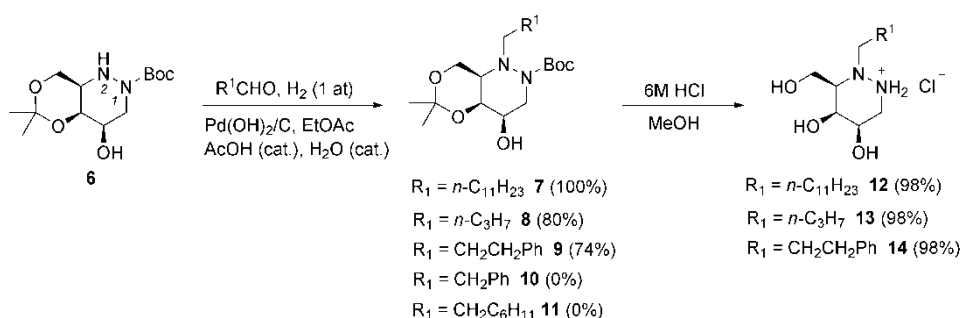
Our synthetic pathway toward AGF analogues started with organocatalyzed asymmetric aldol reaction between 2,2-dimethyl-1,3-dioxan-5-one (**3**, dioxanone) and protected hydrazinoaldehyde **2** (Scheme 1). The reactions proceed smoothly when (*R*)-proline was used as a catalyst, giving optically pure aldol adduct **4** in 55 % yield. Removal of the Cbz protecting group in aldol **4** was effected by catalytic hydrogenation using a 10 % Pd/C catalyst in methanol to give compound **5**, which is sufficiently pure for the use in the next step without further purification. Treatment of crude **5** with excess NaBH_3CN , in a mixture of AcOH/ methanol = 4:1 volume ratio, gave the cyclic product of reductive hydrazination **6** in good yield (65 %, over two steps). Compound **6**, with free *N*-2 and protected *N*-1 atoms on the piperazine ring, is a suitable precursor of *N*-2 analogues of AGF.



Scheme 1. Synthesis of compound **6** – a precursor of *N*-2 analogues of AGF.

We decided to derivatize *N*-2 nitrogen atom in **6** through reductive hydrazination or protection/alkylation sequence. First, we explored the possibility of reductive hydrazination by applying the protocol that Lopez developed for the alkylation of aza-fagomine (Scheme 2).²⁴ A mixture of the compound **6** and a proper

aldehyde was exposed to a hydrogen atmosphere, with palladium on charcoal as a catalyst. Under these conditions the intermediate **6** reacted smoothly with unhindered aldehydes and the products **7–9** were obtained in high yields. However, β -branched aldehydes, such as 2-phenylacetaldehyde and 2-cyclohexylacetaldehyde, failed to react, probably due to the presence of a bulky Boc protective group on the *N*-1 atom of hydrazine. Moreover, the reductive hydrazination of **6** with NaBH_3CN , as an alternative reagent, was also ineffective with branched aldehydes (similar results were obtained by Lopez on aza-fagomine).²⁴ Removal of both protecting groups was carried out under acidic conditions, giving analogs of AGF **12–14** in high yields, in the form of hydrazine hydrochloride salts.

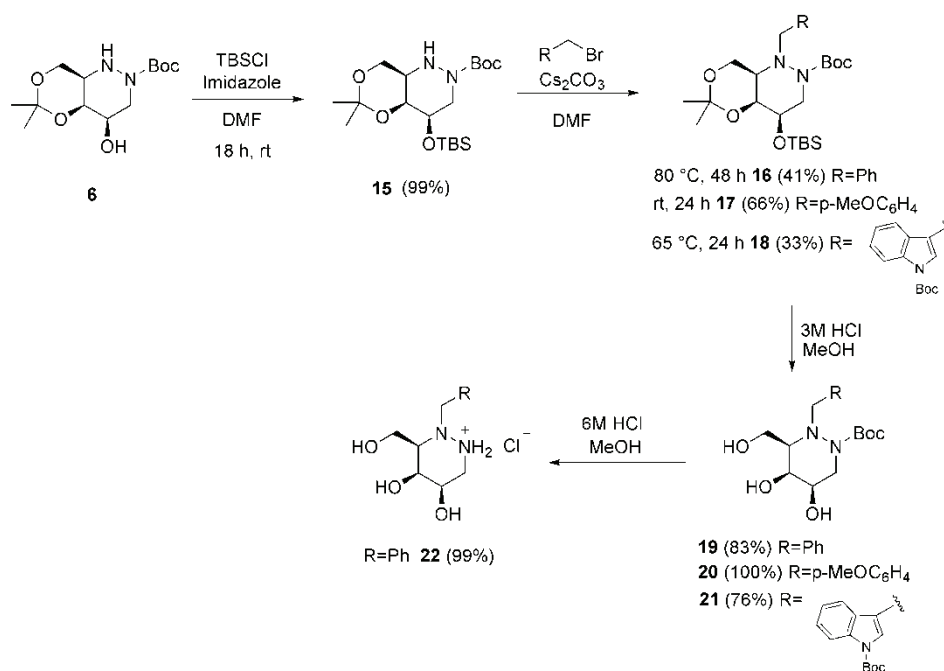


Scheme 2. Synthesis of *N*-2 alkylated analogues **12–14** of aza-galactofagomine by reductive hydrazination.

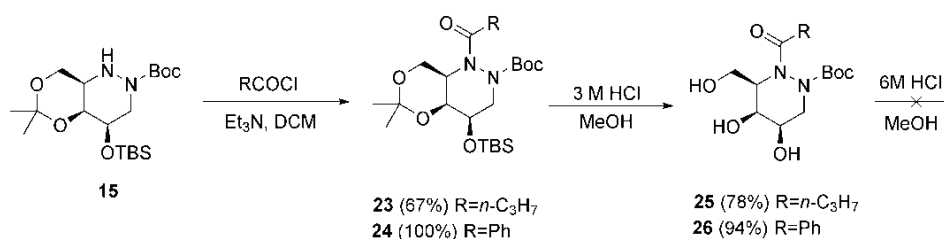
Alternatively, *N*-2 substitution in **6** can be accomplished by alkylation, albeit only with highly reactive alkylating agents (Scheme 3). The free OH group in compound **6** was protected as a silyl ether, and the resulting product **15** was treated with benzyl or alkyl halide in the presence of cesium carbonate at elevated temperature. Although an excess of both the base and the alkylating agent were used, the reaction does not go to completion even with prolonged reaction time. In addition to the reaction products **16–18**, obtained in moderate yields in all cases, smaller amounts of the starting substrate **15** were isolated. Removal of silyl and acetal protecting groups was effected with methanolic HCl, furnishing **19–21**. Boc deprotection of **19** with 6M HCl afforded AGF analogue **22-HCl** in quantitative yield. Unfortunately, under the same reaction conditions **20** and **21** gave a complex mixture of products.

In the end, derivatization of the *N*-2 position was also attempted through acylation (Scheme 4). Treatment of the intermediate **15** with benzoyl or butanoyl chloride gave *N*-2-acylated derivatives **23** and **24** in high yields. Acetal and TBS groups were cleanly removed with a diluted acid (3M HCl, MeOH), resulting in excellent yields of compounds **25** and **26**. However, the attempts to deprotect *N*-1

atom in **25** or **26** were unsuccessful: with either 6M HCl/MeOH or TFA/CH₂Cl₂ a complex mixture of products was obtained.



Scheme 3. Synthesis of *N*-2 alkylated analogue **22** of aza-galactofagomine by alkylation.



Scheme 4. *N*-2 Acylation of compound **15**.

Preliminary screening of potential β -glucosidase inhibitors

Despite the fact that the enzyme is immobilized in agar and the migration of compounds is aggravated, this test is good for rapid screening of potential enzyme inhibitors. The preliminary test results, presented in Fig. 2, confirmed that the synthesized compounds inhibit β -glucosidase, given that light spots appeared on the agar after the inoculation of the compound and the enzyme–substrate reaction.

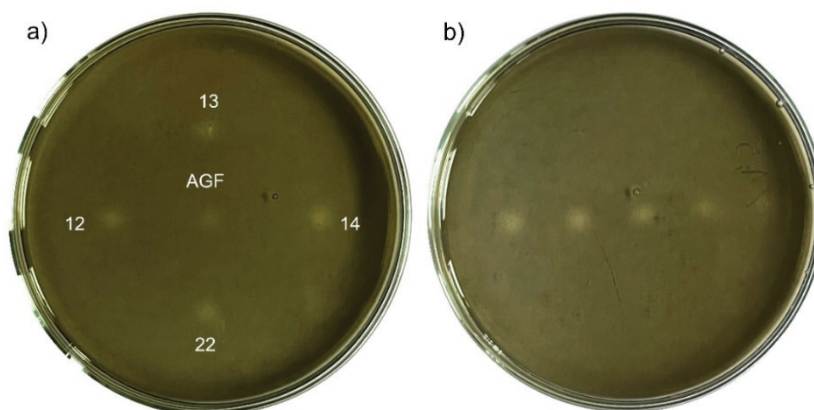


Fig. 2. β -Glucosidase inhibition using the agar plate method. a) Tested compounds were inoculated on agar surface in doses of 5 μ g. b) Conduritol β -epoxide in different doses: 2.5, 1, 0.5, 0.1 and 0.05 μ g, from left to right.

β -Glucosidase inhibition assay

Based on the results obtained by β -glucosidase inhibition assay (Table I) it can be concluded that the strongest inhibition effect was observed for compound **14**. This compound expressed only two times weaker inhibition than the control compound conduritol β -epoxide. A somewhat lower degree of inhibition was shown by compound **12**, while compounds **13** and **22** showed some inhibitory potential, but at slightly higher micromolar concentrations. If the structures of the compounds and their inhibitory capacity are compared, it can be observed that longer substituents on the iminosugar moiety increase the inhibition of the enzyme. By comparing the activity of compounds **14** and **22**, it can be seen that a longer linker between the bulky phenyl group and the iminosugar core, also increases the inhibitory potential of the compound. Previously, it was reported that compound **1** showed strong inhibitory effect on β -glucosidase from almond.²⁵ In the enzyme assay that we applied all synthesized compounds showed better enzyme inhibition than the parent compound AGF (**1**). Such encouraging preliminary results can provide guidelines for the rational design and the synthesis of new compounds that would show better grade of inhibition.

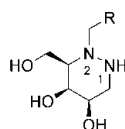
TABLE I. Inhibition of β -glucosidase results

Compound	12	13	14	22	AGF (1)	Conduritol β -epoxide
IC_{50} / μ M	3.03 \pm 0.04	10.72 \pm 0.13	2.08 \pm 0.05	5.90 \pm 0.09	14.29 \pm 0.07	1.06 \pm 0.06

Molecular docking studies

In order to investigate the possibility of bioactivity of synthesized compounds in humans, we simulated binding of five derivatives to human acid- β -

-glucosidase using molecular docking. As a target protein we used previously prepared structure of human acid β -glucosidase from lysosome (PDB ID 2NSX).²⁰ Having in mind that lysosomes maintain rather acidic environment of pH 4.5–5.0,²⁶ pK_a values for nitrogen atoms in small molecules were determined using Epik module from Schrödinger Suite 2021-3 at pH 5.00 \pm 2.00. Results showed that compounds **12–14** would have a protonated substituted nitrogen atom at pH 5.00 \pm 2.00 in given environment. For the structure **22** a small energy difference between neutral and protonated form was predicted, in favour of the protonated molecule, and structure of compound AGF (**1**) is predominantly neutral (Table II). The protonation state does influence binding, as we also considered binding of less probable, neutral forms. Binding energies in protonated state are twice as large as those where docking simulations were done with the neutral form. As for cellular uptake, all compounds are neutral at pH 7.4, so the compounds retain membrane permeability. Only when the compounds pass the lysosome membrane, they enter acidic environment, and the protonation becomes likely.

TABLE II. pK_a values predicted by Epik

Compound	pK_a N-2	pK_a N-1
12	5.83 \pm 2.22	5.23 \pm 2.22
13	5.82 \pm 2.22	5.21 \pm 2.22
14	5.57 \pm 2.22	5.10 \pm 2.22
22	4.93 \pm 2.22	4.47 \pm 2.22
AGF (1)	4.98 \pm 0.94	4.41 \pm 0.94

Docking simulations were performed using Glide docking from Schrödinger Suite 2021-3,²² using XP precision. The best docking poses for all tested molecules were selected and investigated. The results of docking simulations, the both values of Glide score and E_{model} values were in good agreement with the biological assay (Table III), with small discordance for molecules **12** and **14**, where docking scores showed better bonding for molecule **12**, although the results of biological assay showed that molecule **14** binds stronger. This kind of disagreement can emerge because of different structures of human lysosomal acid β -glucosidase and enzyme used in tests. Unfortunately, there is no crystal structure of sweet almond β -glucosidase, that was used in bioassay, and we could not compare bonding to those two proteins. Nevertheless, from the results of bioassay and docking structures, it is clear that molecules with similar structures can have significant potential as inhibitors of lysosomal acid β -glucosidase. Better binding

can be expected from molecules with bulkier, nonpolar substituent at *N*-2, preferably with a moiety capable of various π -interactions with amino acids nearby.

TABLE III. Docking scores and bioassay activities of investigated molecules

Compound	Activity	Glide score, kJ mol ⁻¹	E_{model} / kJ mol ⁻¹
12	3.03±0.04	-44.42	-266.45
14	2.08±0.05	-41.13	-243.25
22	5.90±0.09	-39.99	-228.12
13	10.72±0.13	-31.37	-194.34
AGF (1)	14.29±0.07	-23.89	-168.32

The results in Table III indicate that we can expect ligands with the same basic structure, with a longer *N*-substituent and a structure capable of aromatic interactions, to be more active at the binding site. From ligand interactions schemes (Fig. 3, other figures are in the Supplementary material) it can be seen that there are several key amino acids involved in bonding of the main scaffold. Compounds **12**, **14** and **22** bind almost identically, as seen in Fig. 4. The only possible difference was observed for the behaviour of hydroxymethyl group on C3. Hydroxyl moiety from that group can be involved in the intramolecular H-bonding with OH group from C4, or in the case of ligand **22**, in the intramole-

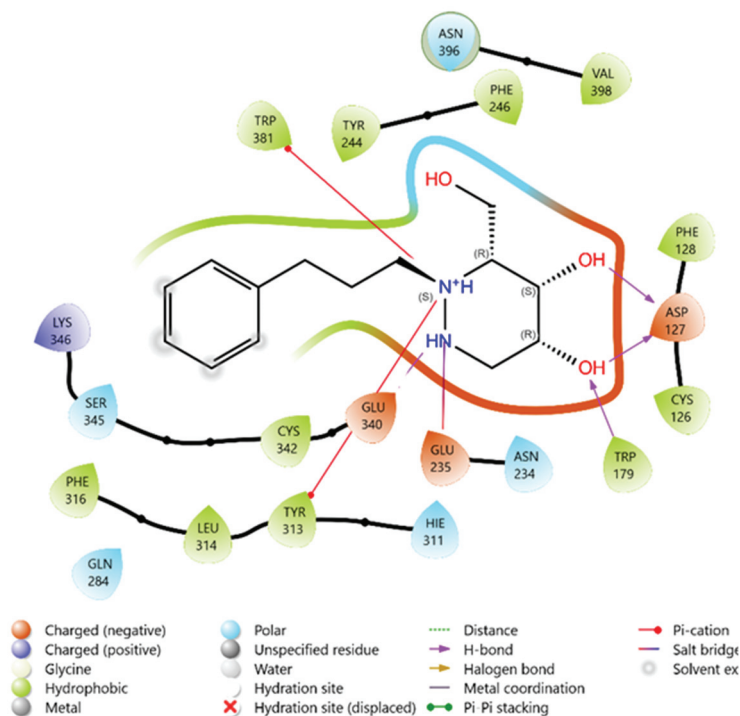


Fig. 3. Ligand interactions for molecule **14** bound to β -glucosidase.

cular π -interaction with the aromatic ring. Structures **13** and AGF (**1**) bind in the slightly different position, especially structure AGF (**1**), Fig. 5. Both have less interactions, mainly charged ones with lower binding scores, resulting in weaker binding and thus in lower activities.

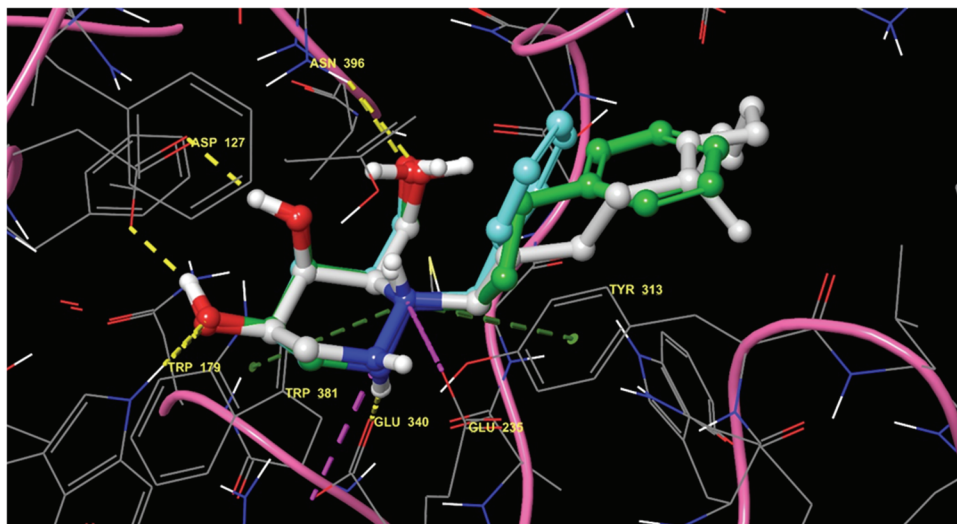


Fig. 4. Compounds **12** (grey), **14** (green) and **22** (cyan) in the binding site of human lysosomal acid β -glucosidase.

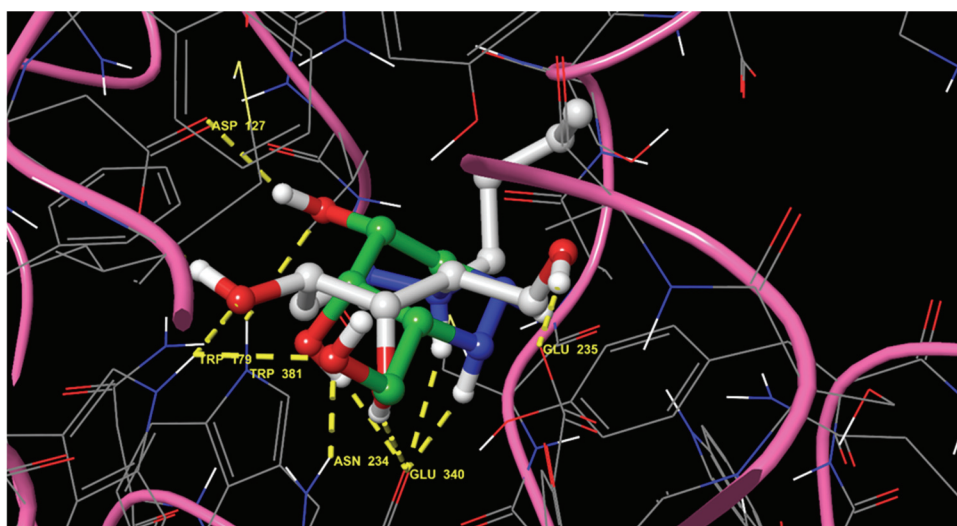


Fig. 5. Compounds **13** (grey) and AGF (**1**) (green) in the binding site of human lysosomal acid β -glucosidase.

CONCLUSION

To summarize, we have reported a synthesis of four *N*-2 alkylated aza-galactofagomines, relying on the intermolecular reductive hydrazination of the suitably protected heterocyclic core, obtained in a tactical sequence comprising of enantioselective organocatalytic aldolization and intramolecular reductive hydrazination. It was shown that these analogues, containing nonpolar alkyl- or phenylalkyl-side chains are potent inhibitors of β -glucosidase. Compound **14**, possessing 3-phenylpropyl side-chain showed the highest inhibitory activity (2.08 μ M), which is comparable to conduritol β -epoxide, which was a control compound. The enzyme assay results correspond well with the results of docking analysis of the human acid β -glucosidase. It is worth noting that β -glucosidase from sweet almond is a good and cheap model enzyme for preliminary testing of potential inhibitors of human acid β -glucosidase from lysosomes. The obtained results are encouraging for the rational design and synthesis of new iminosugar derivatives as the potential inhibitors of a human acid β -glucosidase, which is one of the key enzymes for Gaucher's disease.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: <https://www.shd-pub.org.rs/index.php/JSCS/article/view/12846>, or from the corresponding author on request.

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ИЗВОД

АЛКИЛОВАНИ АНАЛОЗИ АЗА-ГАЛАКТОФАГОМИНА КАО ПОТЕНЦИЈАЛНИ ИНХИБИТОРИ БЕТА-ГЛУКОЗИДАЗА

ФИЛИП ЂУРКОВИЋ¹, МАРИО ЗЛАТОВИЋ¹, ДУШАН СЛАДИЋ¹, ИРЕНА НОВАКОВИЋ², ФИЛИП БИХЕЛОВИЋ¹
и ЗОРАНА ФЕРЈАНЧИЋ¹

¹Универзитет у Београду – Хемички факултет, Студентски тир 12–16, Београд и ²Универзитет у Београду – Институт за хемију, технологију и металургију, Њевошева 12, Београд

Четири *N*-2 алкилована аналога аза-галактофагомина су синтетисана применом интермолекуларног редуктивног хидразиновања или алкиловања адекватно заштићеног облика аза-галактофагомина. Испитивано је инхибиторно дејство синтетисаних аналога према β -глюкозидази. Прелиминарни тестови инхибиторне активности су урађени на β -глюкозидази имобилисану у агару. Овај тест, као и стандардни инхибиторни есеј са истим ензимом, указују на снажно инхибиторно дејство синтетисаних аналога. Добијени резултати се добро корелишу са резултатима докинг анализе хумане киселе β -глюкозидазе, ензима битног за Гошеову болест.

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REFERENCES

1. N. Asano, *Glycobiology* **13** (2003) 93R (<https://doi.org/10.1093/glycob/cwg090>)
2. E. Borges de Melo, A. da Silveira Gomes, I. Carvalho, *Tetrahedron* **62** (2006) 10277 (<https://doi.org/10.1016/j.tet.2006.08.055>)
3. *Iminosugars: From Synthesis to Therapeutic Applications*, P. Compain, O. R. Martin, Eds., Wiley, Chichester, 2007 (ISBN: 978-0-470-03391-3)
4. R. J. Nash, A. Kato, C.-Y. Yu, G. W. J. Fleet, *Future Med. Chem.* **3** (2011) 1513 (<https://doi.org/10.4155/fmc.11.117>)
5. N. J. Leidenheimer, *Targeting Trafficking in Drug Development*. Springer, Cham, 2017, p. 135 (<https://doi.org/10.1007/978-3-319-74164-2>)
6. R. E. Boyd, G. Lee, P. Rybczynski, E. R. Benjamin, R. Khanna, B. A. Wustman, K. J. Valenzano, *J. Med. Chem.* **56** (2013) 2705 (<https://doi.org/10.1021/jm301557k>)
7. E. M. Sanchez-Fernandez, J. M. Garcia Fernandez, C. O. Mellet, *Chem. Commun.* **52** (2016) 5497 (<https://doi.org/10.1039/C6CC01564F>)
8. L. Smith, S. Mullin, A. H.V. Schapira, *Exp. Neurol.* **298** (2017) 180 (<https://doi.org/10.1016/j.expneurol.2017.09.010>)
9. J. M. Benito, J. M. Garcia Fernández, C. O. Mellet, *Expert Opin. Ther. Pat.* **21** (2011) 885 (<https://doi.org/10.1517/13543776.2011.569162>)
10. A. Trapero, A. Llebaria, *Future Med. Chem.* **5** (2013) 573 (<https://doi.org/10.4155/fmc.13.14>)
11. H. Martin, L. Ramírez Lázaro, T. Gunnlaugsson, E. M. Scanlan, *Chem. Soc. Rev.* **51** (2022) 9694 (<https://doi.org/10.1039/D2CS00379A>)
12. X. Zhou, Z. Huang, H. Yang, Y. Jiang, W. Wei, Q. Li, Q. Mo, J. Liu, *Biomed. Pharmacother.* **91** (2017) 504 (<https://doi.org/10.1016/j.biopha.2017.04.113>)
13. M. L. Feltri, N. I. Weinstock, J. Favret, N. Dhimal, L. Wrabetz, D. Shin, *Glia* **69** (2021) 2309 (<https://doi.org/10.1002/glia.24008>)
14. A. N. D'Agostino, G. P. Sayre, A. B. Hayles, *Arch. Neurol.* **8** (1963) 82 (<https://doi.org/10.1001/archneur.1963.00460010098012>)
15. C. H. Hill, A. H. Viuff, S. J. Spratley, S. Salamone, S. H. Christensen, R. J. Read, N. W. Moriarty, H. H. Jensen, J. E. Deane, *Chem. Sci.* **6** (2015) 3075 (<https://doi.org/10.1039/C5SC00754B>)
16. A. H. Viuff, H. H. Jensen, *Org. Biomol. Chem.* **14** (2016) 8545 (<https://doi.org/10.1039/C6OB01309K>)
17. J. Marjanovic Trajkovic, Z. Ferjancic, R. N. Saicic, *Tetrahedron* **73** (2017) 2629 (<https://doi.org/10.1016/j.tet.2017.03.052>)
18. S. Pandey, A. Sree, S.S. Dash, D.P. Sethi, *BMC Microbiol.* **13** (2003) 55 (<https://doi.org/10.1186/1471-2180-13-55>)
19. O. G. Korotkova, M. V. Semenova, V. V. Morozova, I. N. Zorov, L. M. Sokolova, T. M. Bubnova, O. N. Okunev, A. P. Simitsyn, *Biochemistry (Moscow)* **74** (2009) 569 (<https://doi.org/10.1134/S0006297909050137>)
20. R. Lieberman, B. Wustman, P. Huertas, A. C. Powe Jr, C. W. Pine, R. Khanna, M. G. Schlossmacher, D. Ringe, G. A. Petsko, *Nat. Chem. Biol.* **3** (2007) 101 (<https://doi.org/10.1038/nchembio850>)
21. RCSB PDB: Protein Data Bank, <https://www.rcsb.org>
22. *Schrödinger Release 2021-3: Protein Preparation Wizard; Maestro; Epik; Glide*, Schrödinger, LLC, New York, 2021
23. *Schrödinger Release 2024-1: Maestro*, Schrödinger, LLC, New York, 2024

24. O. L. Lopez, M. Bols, *ChemBioChem* **8** (2007) 657
(<https://doi.org/10.1002/cbic.200700012>)
25. H. H. Jensen, M. Bols, *J. Chem. Soc., Perkin Trans. 1* (2001) 905
(<https://doi.org/10.1039/B007973L>)
26. R. Chen, M. Jäättelä, B. Liu, *Cancers* **12** (2020) 2437
(<https://doi.org/10.3390/cancers12092437>).