MR DENIS BABKOV (Orcid ID : 0000-0002-9645-3324)

Article type : Research Article

Synthesis and biological evaluation of 2-acylbenzofuranes as novel α-glucosidase inhibitors with hypoglycemic activity

Enzymatic study, docking, antidiabetic properties

Keywords: benzofurans, α-glucosidase, diabetes

Alexander A. Spasov[a]*, Denis A. Babkov[a], Tatyana Yu. Prokhorova[a], Ekaterina A. Sturova[a], Diana R. Muleeva[a], Maxim R. Demidov[b], Dmitry V. Osipov[b], Vitaly A. Osyanin[b], Yuri N. Klimochkin[b]

[a] *Volgograd State Medical University, Pavshikh Bortsov Sq. 1, 400131 Volgograd, Russian Federation;*

[b] *Department of Organic Chemistry, Samara State Technical University, Molodogvardeiskaya St. 244, 443100 Samara, Russian Federation.*

** Corresponding author: Alexander A. Spasov; +7(8442) 942-423; aspasov@mail.ru.*

Abstract

A series of benzofuran derivatives was synthesized as analogues of known natural α-glucosidase inhibitors. Their activity was evaluated in enzymatic assay and in rat model of diabetes mellitus. Newly identified inhibitors demonstrate significant potency with IC_{50} values ranging from 6.50 to 722.2 μM, as well as hypoglycemic activity exceeding the reference drug acarbose. Docking simulations provided insight to structure-activity relationships to direct further development of these novel hypoglycemic agents.

Introduction

α-Glucosidase is an exoenzyme that hydrolyzes α-1,4 bonds at the non-reducing end of α-1,4-glycans, cleaving glucose in its α-form. The principle of the action of oral α-glucosidase inhibitors is based on a competitive inhibition of the enzyme and slowing the release of glucose from complex carbohydrates, which leads to a decrease in postprandial hyperglycemia (1). Accordingly, several α-glucosidase inhibitors are clinically approved for the treatment of diabetes and obesity. The role

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/cbdd.13038

of cellular α-glucosidase in carbohydrate processing also enables a promising venue to fight other diseases including cancer (2, 3) and viral infections (4–6).

Clinically approved drugs for the treatment of type 2 diabetes via α-glucosidase inhibition include pseudosacharides acarbose, miglitol and voglibose (7). Their ability to improve glycemic control in diabetic patients is well documented (8) but accompanied with gastrointestinal side effects (9). Several cases of acarbose-induced hepatitis have been reported as well (10, 11). A wide range of structurally different inhibitors have been reported to date (12), including transition state analogues (13), small molecule synthetic compounds (14–17), and natural products (18–20).

Baicalein (Fig.1, **1**), a 5,6,7-trihydroxyflavone isolated from marjoram leaves of *O. majorana*, is a modest α-glucosidase inhibitor (IC_{50} 32 μM) that was further modified to yield 20 times more active compound **2** (21, 22). Anthocyanins **3** are another well-known source of α-glucosidase inhibitors (IC_{50} 27.6-36.2 μM) (23). Recently, hydroxyl-functionalized 2-arylbenzo[b]furans (24) and sulfur-containing benzofurans (25) were reported as α-glucosidase inhibitors. These observations prompted us to synthesize and explore structurally relevant benzofuran derivatives as analogues of naturally occurring compounds.

Methods and Materials

Chemistry

IR spectra were recorded on a Shimadzu IRAffinity-1 spectrometer in KBr pellets. ^{1}H and ^{13}C NMR spectra (including DEPT experiments) were acquired on a JEOL JNM-ECX 400 spectrometer (400 and 100 MHz, respectively) in DMSO-d_6 or in $CDCl_3$ with TMS as internal standard. Elemental analysis was performed on a EuroVector EA-3000 automated CHNS-analyzer. Melting points were determined by capillary method on an SRS OptiMelt MPA100 apparatus and are uncorrected. The reaction progress was monitored by TLC on aluminum foil-backed silica gel plates (Merck, Kiesgel 60 F254) with visualization under UV light and in iodine vapor.

The synthesis of compounds 6a-c, 8, 11

Dihydrobenzofurans were prepared according to previously described method (26).

trans-(4-Chlorophenyl)(3-phenyl-2,3-dihydrobenzofuran-2-yl)methanone (6a).

To a mixture of 2-(acetoxy(phenyl)methyl)phenyl acetate **4a** (1.180 g, 4.15 mmol) and 1-(2-(4-chlorophenyl)-2-oxoethyl)pyridin-1-ium bromide **5a** (1.297 g, 4.15 mmol) in acetonitrile (20 ml) DBU (1.26 g, 8.3 mmol) was added. The solution obtained was refluxed under argon atmosphere for 10 h and then was evaporated *in vacuo*. The residue was purified by column chromatography using $CHCl_3$ as eluent, further recrystallization from ethanol gave **6a** as colorless crystals (1.069 g, 76%). mp: 109–110 ºC (EtOH). FT-IR (KBr; cm^{-1}): 3024, 1690 (C=O), 1593, 1481, 1462, 1400, 1238, 1165, 1092, 1011, 968, 887, 841, 818, 756, 702. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 5.01 (d, J = 6.6 Hz, 1H), 5.73 (d, J = 6.6 Hz, 1H), 6.90 (td, J_1 = 0.9 Hz, J_2 = 7.6, 1H), 6.96 (d, J = 8.0 Hz, 1H), 7.01 (d, J = 7.4 Hz, 1H), 7.19–7.36 (m, 6H), 7.43 (d, J = 8.9 Hz, 2H), 7.90 (d, J = 8.7 Hz, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 50.7 (CH), 90.7 (CH), 110.1 (CH), 121.9 (CH), 125.5 (CH), 127.6 (CH), 128.2 (2CH), 129.0 (CH), 129.12 (2CH), 129.14 (2CH), 129.2 (C), 130.9 (2CH), 132.9 (C), 140.4 (C), 142.1 (C), 158.9 (C), 193.7 (C=O). Elemental analysis for $C_{21}H_{15}ClO_2$: Calculated: C, 75.34; H, 4.52; Found: C, 75.25; H, 4.48.

(1,2-Dihydronaphtho[2,1-*b*]furan-2-yl)(3-hydroxyadamantan-1-yl)methanone (6b). A mixture of 2-((dimethylamino)methyl)naphthalen-1-ol **4b** (604 mg, 3 mmol) and 1-(2-(3-hydroxyadamantan-1-yl)-2-oxoethyl)pyridin-1-ium bromide **5b** (1.057 g, 3 mmol) was refluxed in 10 ml of a mixture of acetonitrile-DMF (3:1) for 10 h and then was evaporated *in vacuo*. The residue was purified by column chromatography using $CHCl_3$, further recrystallization from CCl_4 gave **6b** as colorless crystals (721 mg, 69%); mp: 137–139 ºC (CCl_4). FT-IR (KBr cm^{-1}): 3600–3200 (OH), 3059 (CH Ar), 2920, 2855 (CH Ad), 1705 (C=O), 1632, 1601, 1578, 1520, 1466, 1373, 1335, 1312, 1261, 1246, 1184, 1161, 1138, 1115, 1076, 1015, 976, 957, 907, 810, 745. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.55–1.98 (m, 13H, Ad, OH), 2.33–2.35 (m, 2H, Ad-2), 3.58 (dd, J_1 = 7.8 Hz, J_2 = 15.6 Hz, 1H), 3.66 (dd, J_2 = 10.6 Hz, J_2 = 15.6 Hz, 1H), 5.64 (dd, J_1 = 7.8 Hz, J_2 = 10.6 Hz, 1H), 7.15 (d, J = 8.7 Hz, 1H), 7.32 (ddd, J_1 = 1.2 Hz, J_2 = 6.9 Hz, J_3 = 8.2 Hz, 1H), 7.47 (ddd, J_1 = 1.2 Hz, J_2 = 6.9 Hz, J_3 = 8.2 Hz, 1H), 7.55 (d, J = 8.2 Hz, 1H), 7.70 (d, J = 8.7 Hz, 1H), 7.80 (d, J = 8.2 Hz, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 30.3 (2CH), 32.2 (CH_2), 35.1 (CH_2), 36.8 ($2CH_2$), 44.4 ($2CH_2$), 45.4 (CH_2), 49.5 (C_{Ad}-1), 68.5 (C_{Ad}-3), 82.4 (CH), 111.9 (CH), 117.0 (C), 122.7 (CH), 123.3 (CH), 127.0 (CH), 128.8 (CH), 129.5 (CH), 129.6 (C), 130.5 (C), 156.7 (C), 187.3 (C=O). Elemental analysis for $C_{23}H_{24}O_3$: Calculated: C, 79.28; H, 6.94. Found: C, 79.34; H, 6.93.

(1,2-Dihydronaphtho[2,1-*b*]furan-2-yl)(4-hydroxyphenyl)methanone (6c). A mixture of 2-((dimethylamino)methyl)naphthalen-1-ol **4b** (604 mg, 3 mmol) and 1-(2-(4-hydroxyphenyl)-2-oxoethyl)pyridin-1-ium bromide **5c** (882 mg, 3 mmol) was heated in DMF (5 ml) at 90 °C for 15 h. Solution obtained was cooled, poured into water, the formed solid was filtered off, washed with water and dried. The product was purified by column chromatography using $CHCl_3$/EtOAc (3:1), further recrystallization from isopropyl alcohol gave **6c** as colorless crystals (435 mg, 50%); mp: 147–149 ºC (*i*-PrOH). FT-IR (KBr cm^{-1}): 3206, 1666 (C=O), 1632, 1601, 1578, 1516, 1466, 1369, 1285, 1242, 1173, 968, 810. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 3.54 (dd, J_1 = 6.9 Hz, J_2 = 15.9 Hz, 1H), 3.85 (dd, J_1 = 11.2 Hz, J_2 = 15.9 Hz, 1H), 6.38 (dd, J_1 = 6.9 Hz, J_2 = 11.2 Hz, 1H), 6.88–6.92 (m, 2H), 7.20 (d, J = 8.8 Hz, 1H), 7.28–7.32 (m, 1H), 7.43–7.47 (m, 1H), 7.61 (d, J = 8.2 Hz, 1H), 7.75 (d, J = 8.8 Hz, 1H), 7.85 (d, J = 8.2 Hz, 1H), 7.93–7.96 (m, 2H), 10.56 (s, 1H, OH). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 32.1 (CH_2), 82.7 (CH), 112.4 (CH), 116.1 (2CH), 117.9 (C), 123.4 (CH), 123.7 (CH), 125.9 (C), 127.4 (CH), 129.1 (CH), 129.4 (C), 129.6 (CH), 130.7 (C), 132.1 (2CH), 157.1 (C), 163.4 (C), 194.0 (C=O). Elemental analysis for $C_{19}H_{14}O_3$: Calculated: C, 78.61; H, 4.86. Found: C, 78.75; H, 4.84.

2-Nitrobenzofuran **8** was prepared from the diacetate of salicylic alcohol **4c** and potassium trinitromethanide **7** (27).

(3-Hydroxybenzofuran-2-yl)(phenyl)methanone (11). Phenacyl bromide **10** (1.09 g, 5.5 mmol) and K_2CO_3 (2.09 g, 15 mmol) were added to a solution of methyl salicylate **9** (0.76 g, 5.0 mmol) in dry acetone (6 ml). The mixture obtained was heated under reflux for 6 h, cooled, filtered and washed with acetone. Then the solid was suspended in water (30 ml) and acidified with 3M HCl. The precipitated yellow crude product was filtered and crystallized from EtOH. The mother liquor was evaporated *in vacuo* and purified by column chromatography using $CHCl_3$/EtOAc (4:1) as eluent for additional crop of the product **11**. Total yield 1.07 g (90%). Spectral characteristics and melting point are identical to literature data (28).

α-Glucosidase inhibition assay

In a 96-well plate the 0.01 mg/mL enzyme solution (EC 3.2.1.20, expressed in *S. cerevisiae*, Sigma; catalog #G5003) was incubated with test compounds in 0.1 M phosphate buffer (pH 6.8) at 37 °C for 5 min. Then, 25 µL of 5 mM substrate solution, i.e. *p*-nitrophenyl-α-D-glucopyranoside (Sigma; catalog #N1377), was added and the change in absorbance was recorded for 15 min at 400 nm with Infinite M200 PRO microplate reader (Tecan, Austria)(29). Test compounds were

replaced with 0.1 M phosphate buffer (pH 6.8) in negative control experiments. Acarbose was used as the positive control.

Oral maltose tolerance test

All animal studies were conducted with a previous approval from the VolgSMU Animal Care and Use Committee. Male Wistar rats aged 9–12 weeks were injected i.p. with 45 mg/kg streptozotocin (STZ) dissolved in 0.1 M citrate buffer (pH 4.5) 1 week before the experiment. Rats were fasted overnight and then administered orally with 2 g/kg maltose. Immediately after, 5 mg/kg acarbose, 2.2 mg/kg **6c**, or 1.8 mg/kg **11** suspended in 1% sodium carboxymethyl cellulose solution were orally administered to the respective groups consisting of five rats. Control group received the same volume of vehicle. Blood sampling from the tail vein was performed at 0, 60 and 120 min (30). The blood glucose levels were measured at each time interval using Biosen C_line glucose analyzer (EKF Diagnostics, Germany).

Docking simulations

Ligands were prepared with Marvin Scetch 16.2.22 (ChemAxon) (31). Protein (PDB ID: 1LWJ) and ligand structures followed standard preparation procedure using AutoDocTools 1.5.6. We used AutoDock Vina 1.1.2 (32) to perform all docking runs. Cubic grid box centered on cognate ligand was adjusted to include the entire concave region around the ligand and the solvent accessible entrance of the pocket. Only top-score binding pose were used in subsequent analysis. First, saxagliptin was docked in the native protein conformation to assess the ability of docking and scoring functions to reproduce crystallographic binding pose. Second, auxiliary ligand was docked in the same binding site. Protein-ligand interactions were analyzed with Discovery Studio 4.5 Visualizer (Accelrys) (33).

Statistical analysis

The GraphPad Prism 6 (GraphPad Software Inc.) was employed for data analysis and graph preparation. The experimental data of biological activity was expressed as mean values ± standard error of mean (mean±SEM). The IC_{50} value is defined as the concentration of an inhibitor which caused 50% reduction of the enzyme activity under specific assay conditions and was calculated with a nonlinear regression analysis. Each point in the constructed graphs represents the mean of three experiments. Mann-Whitney U-test or two-way ANOVA were used for comparisons of sample values versus control. $P < 0.05$ was considered statistically significant.

Results

Chemistry

The synthesis of condensed furans and dihydrofurans is presented in Scheme 1. Compounds **6a-c** and **8** were synthesized via formation of *ortho*-quinone methide intermediates from corresponding 2-(acetyloxy)benzyl acetates **4a** and **4c** (for **6a** and **8**) or naphtholic Mannich base **4b** (for **6b** and **6c**) and further cascade heterocyclization (34). Dihydroarenofurans **6a-c** were formed through Michael-type addition of *in situ* generated pyridinium ylides from salts **5a-c** to *ortho*-quinone methides followed by intramolecular nucleophilic substitution. Compound **6a** was obtained exclusively as the *trans*-diastereomer; such diastereoselectivity was explained in previous publication (26). 2-Nitrobenzofuran **8** was synthesized according to earlier developed method with the use of potassium nitroformate **7** in the presence of triethylamine in aqueous methanol (27). Benzofuran **11** was prepared from methyl salicylate **9** and phenacyl bromide **10** in dry acetone in the presence of K_2CO_3 in 90% yield. The structures of all synthesized compounds were confirmed

with elemental analysis and spectroscopic methods including ^{1}H, ^{13}C and DEPT NMR spectroscopy and IR spectroscopy.

Inhibitory effect of 2-acylbenzofuran derivatives against α-glucosidase activity

Synthesized compounds were first evaluated *in vitro* for α-glucosidase inhibition as previously described (35). Results are represented in Table 1. A bulky substituent might be required for activity. Indeed, as the structure-activity relationships show, a 2-benzoyl substituent provides optimal steric properties in comparison to adamantane counterpart **6b** and 2,3-disubstituted **6a**. Introduction of a 2-(4-hydroxybenzoyl) group resulted in sharp potency increase rendering compound **6c** as the most potent with IC_{50} value of 6.50 μM.

Docking studies

In order to rationalize the activity data and gain structural insight into the binding of the novel inhibitors to α-glucosidase, they were docked into the active site of the enzyme and compared to the acarbose binding conformation. Due to the unavailability of three-dimensional X-ray structures of α-glucosidase enzymes commonly used in biological assays, such as the native or complexed protein of *S. cerevisiae*, we employed a recently constructed model of an α-glucosidase homologue based on a 4-α-glucanotransferase of *T. maritima* (36), the latter showing a high sequential identity with the α-glucosidase of *S. cerevisiae* (37).

Fig. 2 shows the experimental binding mode of acarbose and the predicted top-scored conformation of **6c** in the active site of α-glucosidase. As revealed by interaction analysis, acarbose forms an extensive hydrogen bond network, while inhibitor **6c** seem to be stabilized through the interactions with aromatic side chains of Trp131, Phe150 and Trp218. It is of interest that, the simple yet well-tuned stacking interactions provide affinity that is sufficient for inhibiting the enzyme. Along with that simple scaffold of identified 2-acylbenzofuranes provide multiple opportunities for modification to further enhance the potency. Considering the hydrophilic nature of the binding site introduction of hydrogen bond donors is the most apparent way to improve the affinity. However, there may be an enthalpic penalty for displacement of buried water molecules (38).

Hypoglycemic activity of 2-acylbenzofurane derivatives

In order to confirm the potency of the newly identified α-glucosidase inhibitors in animals, the oral maltose tolerance test was carried out using the male Wistar rat streptozotocin diabetes model (39). All groups demonstrated marked hyperglycemia prior to the experiment. In the control group, the blood glucose level increased from 15.44±2.07 to 21.66±4.30 mM 60 minutes after the maltose challenge (Fig. 3). Administration of the compounds **6c** and **11** was found to effectively prevent postprandial hyperglycemia. Moreover, they proved to be significantly superior to acarbose at the specified time points ($p < 0.05$).

Discussion

We have developed an efficient and versatile synthetic route to the novel 2-acylbenzofuran derivatives as analogues of naturally occurring flavonoids. An alternative method was reported earlier (40), however our attempts to reproduce the described results were unsuccessful. Among the target derivatives four compounds were identified as inhibitors of α-glucosidase. Compound **6c** inhibits α-glucosidase *in vitro* with IC_{50} of 6.50 μM and exceeds hypoglycemic activity of acarbose in streptozotocin-induced diabetic rats. Recently, structurally related 2-arylbenzo[*b*]furans (41) and oxadiazole-substituted benzofurans (42) have been reported as potent inhibitors of α-glucosidase.

However, animal studies were not reported to support these findings. Considering the *in vivo* hypoglycemic activity the most active compound **6c** could be further pursued for the improved potency and ADMET properties. Docking experiments have elucidated the binding of **6c** to the enzyme and could facilitate future optimization efforts.

Acknowledgments

The biological part of this work received financial support from the Russian Science Foundation (project 14-25-00139); the synthetic part was supported by the Russian Foundation for Basic Research (project 15-43-02304 r_povolzhye_a).

Conflict of Interest

The authors declare no conflicts of interest.

References

1. Truscheit E., Frommer W., Junge B., Müller L., Schmidt D.D., Wingender W. (1981) Chemistry and Biochemistry of Microbialα-Glucosidase Inhibitors. Angew Chemie Int Ed English;20:744–761. doi: 10.1002/anie.198107441
2. Humphries M.J., Matsumoto K., White S.L., Olden K. (1986) Inhibition of experimental metastasis by castanospermine in mice: blockage of two distinct stages of tumor colonization by oligosaccharide processing inhibitors. Cancer Res;46:5215–22. doi: 3093061
3. Chakroun M., Khemakhem B., Mabrouk H. Ben, El Abed H., Makni M., Bouaziz M., Drira N., Marrakchi N., Mejdoub H. (2016) Evaluation of anti-diabetic and anti-tumoral activities of bioactive compounds from Phoenix dactylifera L's leaf: In vitro and in vivo approach. Biomed Pharmacother;84:415–422. doi: 10.1016/j.biopha.2016.09.062
4. Sayce A.C., Alonzi D.S., Killingbeck S.S., Tyrrell B.E., Hill M.L., Caputo A.T., Iwaki R., Kinami K., Ide D., Kiappes J.L., Beatty P.R., Kato A., Harris E., Dwek R.A., Miller J.L., Zitzmann N. (2016) Iminosugars Inhibit Dengue Virus Production via Inhibition of ER Alpha-Glucosidases—Not Glycolipid Processing Enzymes. PLoS Negl Trop Dis;10:e0004524. doi: 10.1371/journal.pntd.0004524
5. Howe J.D., Smith N., Lee M.J.-R., Ardes-Guisot N., Vauzeilles B., Désiré J., Baron A., Blériot Y., Sollogoub M., Alonzi D.S., Butters T.D. (2013) Novel imino sugar α-glucosidase inhibitors as antiviral compounds. Bioorg Med Chem;21:4831–4838. doi: 10.1016/j.bmc.2013.03.014
6. Durantel D., Alotte C., Zoulim F. (2007) Glucosidase inhibitors as antiviral agents for hepatitis B and C. Curr Opin Investig Drugs;8:125–9. doi: 17328228
7. Yee H.S., Fong N.T. A review of the safety and efficacy of acarbose in diabetes mellitus. Pharmacotherapy;16:792–805.
8. Floris Alexander van de Laar (2008) Alfa-glucosidase inhibitors in the early treatment of type 2 diabetes. Vasc Heal Risk Manag;1189–1195.
9. Derosa G. M.P. (2012) α-Glucosidase inhibitors and their use in clinical practice. Arch Med Sci;899–906. doi: 10.5114/aoms.2012.31621
10. Diaz-Gutierrez F.L., Ladero J.M., Diaz-Rubio M. (1998) Acarbose-Induced Acute Hepatitis. Am J Gastroenterol;93:481–481. doi: 10.1111/j.1572-0241.1998.481_1.x

11. Hsiao S.-H. (2005) Hepatotoxicity Associated with Acarbose Therapy. Ann Pharmacother;40:151–154. doi: 10.1345/aph.1G336

12. Borges de Melo E., da Silveira Gomes A., Carvalho I. (2006) α- and β-Glucosidase inhibitors: chemical structure and biological activity. Tetrahedron;62:10277–10302. doi: 10.1016/j.tet.2006.08.055

13. Lillelund V.H., Jensen H.H., Liang X., Bols M. (2002) Recent Developments of Transition-State Analogue Glycosidase Inhibitors of Non-Natural Product Origin. Chem Rev;102:515–554. doi: 10.1021/cr000433k

14. Taha M., Ismail N.H., Imran S., Mohamad M.H., Wadood A., Rahim F., Saad S.M., Rehman A. ur, Khan K.M. (2016) Synthesis, α-glucosidase inhibitory, cytotoxicity and docking studies of 2-aryl-7-methylbenzimidazoles. Bioorg Chem;65:100–109. doi: 10.1016/j.bioorg.2016.02.004

15. Kumar J.A., Tiwari A.K., Ali A.Z., Madhusudhana K., Reddy B.S., Ramakrishna S., China Raju B. (2010) New antihyperglycemic, alpha-glucosidase inhibitory, and cytotoxic derivatives of benzimidazoles. J Enzyme Inhib Med Chem;25:80–86. doi: 10.3109/14756360903017122

16. Khairunissa N., Abdullah N., Taha M., Ahmat N., Wadood A., Hadiani N., Rahim F., Sikander S., Abdullah N. (2016) Benzimidazole derivatives as new a -glucosidase inhibitors and in silico studies. Bioorg Chem;64:29–36. doi: 10.1016/j.bioorg.2015.11.006

17. Khan M., Yousaf M., Wadood A., Junaid M., Ashraf M., Alam U., Ali M., Arshad M., Hussain Z., Khan K.M. (2014) Discovery of novel oxindole derivatives as potent ??-glucosidase inhibitors. Bioorganic Med Chem;22:3441–3448. doi: 10.1016/j.bmc.2014.04.033

18. Du Z., Liu R., Shao W., Mao X., Ma L., Gu L., Huang Z., Chan A.S.C. (2006) α-Glucosidase inhibition of natural curcuminoids and curcumin analogs. Eur J Med Chem;41:213–218. doi: 10.1016/j.ejmech.2005.10.012

19. Benalla W., Bellahcen S., Bnouham M. (2010) Antidiabetic Medicinal Plants as a Source of Alpha Glucosidase Inhibitors. Curr Diabetes Rev;6:247–254. doi: 10.2174/157339910791658826

20. Tarling C.A., Woods K., Zhang R., Brastianos H.C., Brayer G.D., Andersen R.J., Withers S.G. (2008) The Search for Novel Human Pancreatic α-Amylase Inhibitors: High-Throughput Screening of Terrestrial and Marine Natural Product Extracts. ChemBioChem;9:433–438. doi: 10.1002/cbic.200700470

21. Kawabata J., Mizuhata K., Sato E., Nishioka T., Aoyama Y., Kasai T. (2003) 6-Hydroxyflavonoids as α-Glucosidase Inhibitors from Marjoram (Origanum majorana) Leaves. Biosci Biotechnol Biochem;67:445–447. doi: 10.1271/bbb.67.445

22. Gao H., Kawabata J. (2005) α-Glucosidase inhibition of 6-hydroxyflavones. Part 3: Synthesis and evaluation of 2,3,4-trihydroxybenzoyl-containing flavonoid analogs and 6-aminoflavones as α-glucosidase inhibitors. Bioorg Med Chem;13:1661–1671. doi: 10.1016/j.bmc.2004.12.010

23. Johnson M.H., De Mejia E.G., Fan J., Lila M.A., Yousef G.G. (2013) Anthocyanins and proanthocyanidins from blueberry-blackberry fermented beverages inhibit markers of inflammation in macrophages and carbohydrate-utilizing enzymes in vitro. Mol Nutr Food Res;57:1182–1197. doi: 10.1002/mnfr.201200678

24. Hsieh J.F., Lin W.J., Huang K.F., Liao J.H., Don M.J., Shen C.C., Shiao Y.J., Li W.T. (2015) Antioxidant activity and inhibition of α-glucosidase by hydroxyl-functionalized 2-arylbenzo[b]furans. Eur J Med Chem;93:443–451. doi: 10.1016/j.ejmech.2015.02.024

25. Liu Z., Xia G., Chen S., Liu Y., Li H., She Z. (2014) Eurothiocin A and B, Sulfur-Containing Benzofurans from a Soft Coral-Derived Fungus Eurotium rubrum SH-823. Mar

Drugs;12:3669–3680. doi: 10.3390/md12063669

26. Osyanin V.A., Osipov D. V., Klimochkin Y.N. (2013) Reactions of o -Quinone Methides with Pyridinium Methylides: A Diastereoselective Synthesis of 1,2-Dihydronaphtho[2,1- b]furans and 2,3-Dihydrobenzofurans. J Org Chem;78:5505–5520. doi: 10.1021/jo400621r

27. Osyanin V.A., Osipov D. V., Demidov M.R., Klimochkin Y.N. (2014) Potassium Trinitromethanide as a 1,1-Ambiphilic Synthon Equivalent: Access to 2-Nitroarenofurans. J Org Chem;79:1192–1198. doi: 10.1021/jo402543s

28. Zhou Z.-Z., Deng Y.-H., Jiang Z.-H., Chen W.-H. (2010) Microwave-Assisted Dieckmann Reaction: Efficient One-Step Synthesis of 2-Aroylbenzofuran-3-ols. Adv Synth Catal;352:1909–1913. doi: 10.1002/adsc.201000256

29. Elya B., Basah K., Mun'im A., Yuliastuti W., Bangun A., Septiana E.K. (2012) Screening of α-Glucosidase Inhibitory Activity from Some Plants of Apocynaceae, Clusiaceae, Euphorbiaceae, and Rubiaceae. J Biomed Biotechnol;2012:1–6. doi: 10.1155/2012/281078

30. Suzuki Y.A., Murata Y., Inui H., Sugiura M., Nakano Y. (2005) Triterpene Glycosides of Siraitia grosvenori Inhibit Rat Intestinal Maltase and Suppress the Rise in Blood Glucose Level after a Single Oral Administration of Maltose in Rats. J Agric Food Chem;53:2941–2946. doi: 10.1021/jf0478105

31. MarvinSketch 16.2.22.0, ChemAxon Ltd. (2016).

32. Trott O., Olson A.J. (2009) AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem;31:455–461. doi: 10.1002/jcc.21334

33. Discovery Studio Visualiser, Release 4.5, San Diego: Accelrys Software Inc. (2016).

34. Osipov D. V, Osyanin V.A., Klimochkin Y.N. (2017) ortho-Quinone methides as key intermediates in cascade heterocyclizations. Russ Chem Rev. doi: 10.1070/RCR4679

35. Morkovnik A.S., Spasov A.A., Kuz'menko T.A., Kucheryavenko A.F., Divaeva L.N., Koshchienko Y. V., Anisimova V.A., Kuzmina L.G., Rogova N. V., Kuznetsova V.A., Chepljaeva N.I., Solovyova O.A., Taran A.S., Vorobiev E.S., Aleshin D.A., Sirotenko V.S., Gajdukova K.A., Bogoslavtseva M. V. (2015) Prototropic equilibrium in 1(11)H-2, 3, 4, 5-tetrahydro[1, 3]diazepino[1, 2-a]benzimidazole, synthesis and pharmacological properties of its N-substituted derivatives. Russ Chem Bull;64:2622–2631. doi: 10.1007/s11172-015-1200-3

36. Roujeinikova A., Raasch C., Sedelnikova S., Liebl W., Rice D.W. (2002) Crystal structure of Thermotoga maritima 4-alpha-glucanotransferase and its acarbose complex: implications for substrate specificity and catalysis. J Mol Biol;321:149–62. doi: 10.2210/pdb1lwj/pdb

37. Tomich C.H., da Silva P., Carvalho I., Taft C.A. (2005) Homology modeling and molecular interaction field studies of alpha-glucosidases as a guide to structure-based design of novel proposed anti-HIV inhibitors. J Comput Aided Mol Des;19:83–92. doi: 10.1007/s10822-005-1486-6

38. Michel J., Tirado-Rives J., Jorgensen W.L. (2009) Prediction of the Water Content in Protein Binding Sites. J Phys Chem B;113:13337–13346. doi: 10.1021/jp9047456

39. Pisarev V.B., Snigur G.L., Spasov A.A., Samokhina M.P., Bulanov A.E. (2009) Mechanisms of Toxic Effect of Streptozotocin on β-Cells in the Islets of Langerhans. Bull Exp Biol Med;148:937–939. doi: 10.1007/s10517-010-0856-9

40. Cadona L., Dalla Croce P. (1976) A Convenient Synthesis of 2-Acyl- or 2-Aroyl-substituted 2,3-Dihydrobenzofurans and 1,2-Dihydronaphtho[2,1-b]furans. Synthesis (Stuttg);1976:800–801. doi: 10.1055/s-1976-24204

41. Hsieh J.-F.F., Lin W.-J.J., Huang K.-F.F., Liao J.-H.H., Don M.-J.J., Shen C.-C.C., Shiao Y.-

J.J., Li W.-T.T. (2015) Antioxidant activity and inhibition of α-glucosidase by hydroxyl-functionalized 2-arylbenzo[b]furans. Eur J Med Chem;93:443–451. doi: 10.1016/j.ejmech.2015.02.024

42. Taha M., Ismail N.H., Imran S., Wadood A., Rahim F., Saad S.M., Khan K.M., Nasir A. (2016) Synthesis, molecular docking and α-glucosidase inhibition of 5-aryl-2-(6′-nitrobenzofuran-2′-yl)-1,3,4-oxadiazoles. Bioorg Chem;66:117–123. doi: 10.1016/j.bioorg.2016.04.006

Figure legends and Tables

Figure 1: Flavonoid α-glucosidase inhibitors.

Scheme 1: Synthesis of the target compounds.

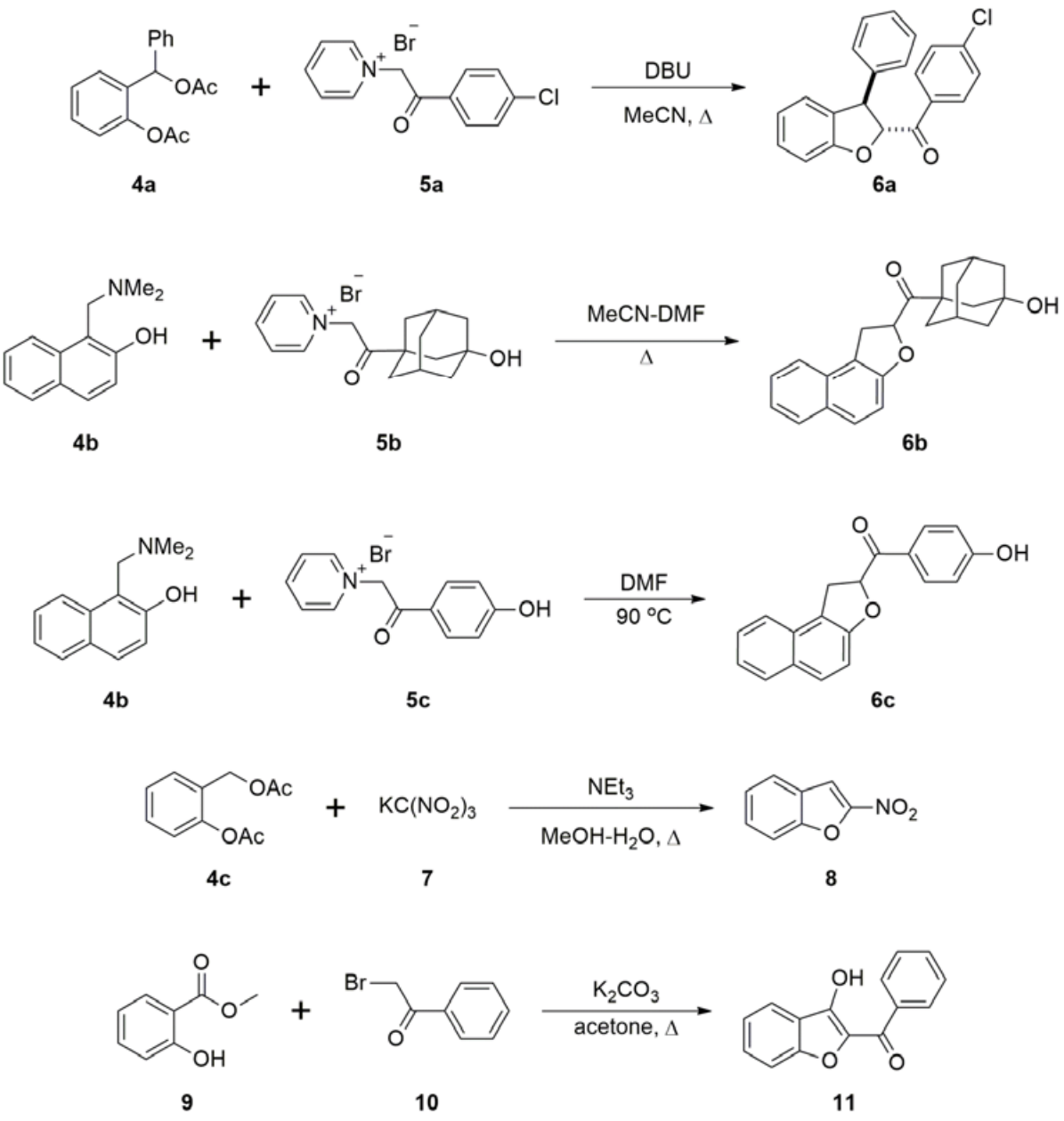
Figure 2: Proposed binding mode of compound **6c** (B) in comparison with acarbose (A).

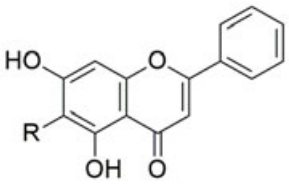
Figure 3: Effect of compounds **6b** and **11** on plasma glucose levels during oral maltose tolerance test in diabetic rats (n = 5). Statistical significance versus diabetic control ($^{*}p < 0.05$) and diabetic animals treated with acarbose ($^{\#}p < 0.05$).

Table 1: Activity of the target compounds against α-glucosidase *in vitro*

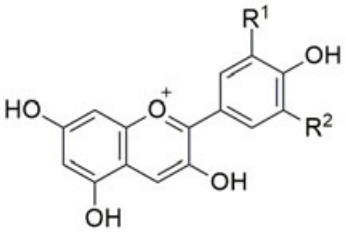
Comp.	IC_{50} (μM)
6a	451.4
6b	722.2
6c	6.50
8	n.a.
11	167.0
Acarbose	543.6

* Statistical significance ($p < 0.001$) versus negative control (Mann-Whitney U-test); n.a. – not analyzed.





1, R = OH
2, R= NH_2



3, R^1 = R^2 = H, OH, OMe