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Original Article

Design, synthesis and biological evaluation of novel sulfonamide hydrazones as α-glucosidase and α-amylase inhibitors

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ABSTRACT

Background and Aims: Diabetes mellitus is among the major hazards to global public health due to increasing incidence worldwide, and new therapeutic agents are urgently needed for the control of the disease. In this study, a novel series of sulfonamide hydrazones (**3a-i**) were synthesized and evaluated, *in vitro*, for α-amylase and α-glucosidase inhibitor activities.
Methods: Target compounds were prepared according to a high-yielded synthetic route. The *in vitro* antidiabetic activity of the compounds was analyzed by evaluating the inhibitory abilities on α-glucosidase and α-amylase enzymes. Acarbose was chosen as a reference in this study.
Results: Compounds **3d**, **3e**, **3g** and **3h** exhibited better α-glucosidase inhibitory activity compared to reference antidiabetic drug acarbose. Compound **3g** was the most active analogue, possessing an IC_{50} value of 65.27 μg/mL. **3d**, **3e**, **3g** and **3h** showed similar α-amylase inhibitory activity compared to acarbose when tested at high concentrations. However, their IC_{50} values were much higher compared to that of reference acarbose.
Conclusion: The most active analogue **3g** was found to be two times more active than acarbose. The addition of a bulky group to the 4-position of the cyclohexane ring seemed to have a positive effect on antidiabetic activity. The new hydrazone derivatives reported in this study are potentially promising candidates for developing new antidiabetic agents.

Keywords: Antidiabetic activity, Sulfonamide, Hydrazone, α-amylase, α-glucosidase

INTRODUCTION

Diabetes mellitus is a metabolic disease identified by chronic hyperglycemia that causes defects in insulin secretion, insulin action or both. Diabetes is one of the major threats to global public health due to increasing incidence worldwide (Toniolo et al., 2019). Nearly 422 million people worldwide currently have diabetes, especially in low-and middle-income countries, and about 1.6 million deaths are linked to diabetes each year (WHO, 2021). Of the three major types of diabetes, type 2 diabetes is much more common (accounting for nearly 90% of all cases) than either type 1 or gestational diabetes. Type 2 diabetes, formerly called non-insulin-dependent, or adult-onset, is caused by insufficient use of insulin by the body (DeFronzo et al., 2015). One of the therapies for type 2 diabetes is the inhibition of the key enzymes that digest carbohydrates, such as α-amylase and α-glucosidase. α-Glucosidase inhibitors (AGIs) are a class of oral antidiabetic drugs that are widely used in the treatment of type 2 diabetes. Acarbose is the most commonly used AGI,

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and also the most widely studied one. Voglibose and miglitol are the other AGIs available commercially. AGIs are saccharides that competitively inhibit the α-glucosidase enzyme that converts complex non-absorbable carbohydrates into simple absorbable carbohydrates and result in a reducing effect on postprandial blood glucose and insulin levels (Hossain, Das, Ghosh, & Sil, 2020; Padhi, Nayak, & Behera, 2020). AGIs also inhibit the pancreatic α-amylase enzyme that hydrolyzes the starches into oligosaccharides, thus having a dual effect on complex carbohydrates (Proença, Ribeiro, Freitas, & Fernandes, 2020). However, their use has been limited due to severe gastrointestinal side effects and limited effect on fasting glucose levels. Thus, it is crucial to discover new AGIs as preclinical drug candidates that have fewer adverse reactions. A great number of new compounds with *in vitro* or *in vivo* α-glucosidase inhibitory activity has been reported in recent literature. Most of them are sugar-mimic compounds that have been designed on the basis of the structure of glucose like commercially available AGI drugs. There are also many compounds without a sugar-mimic framework that show favorable α-glucosidase inhibitory activity (Liu & Ma, 2017).

The hydrazone functional group is an important pharmacophore in medicinal chemistry, due to its chemical and biological properties, as well as its structural versatility. Many acyl/aryl/aroyl hydrazones with a diversity of heterocyclic spacers have been studied for their broad spectrum of biological activities, including anticancer (Nasr, Bondock, & Youns, 2014), antituberculosis (Koçyiğit-Kaymakçıoğlu et al., 2006), antimicrobial (Metwally, Abdel-Aziz, Lashine, Husseiny, & Badawy, 2006) and anti-inflammatory (Moldovan et al., 2011) properties. Two different series of aroyl hydrazone derivatives, 2-indolylcarbohydrazones (Taha et al., 2015) and benzimidazole hydrazones (Zawawi et al., 2016) have been reported to exert notable *in vitro* inhibitory activity against α-glucosidase enzyme. In a newly published study by Wang et al. (Wang et al., 2017), a chromone hydrazone derivative with 4-sulfonamide substitution at the phenyl part of the hydrazide was described as a promising inhibitory agent against α-glucosidase.

This report is based on the synthesis and structural characterization of new cyclohexanone benzoylhydrazone derivatives carrrying a sulfonamide moiety on the benzene ring. The newly synthesized compounds were screened for inhibition of *in vitro* α-glucosidase and α-amylase activities.

MATERIAL AND METHODS

Chemistry

The chemicals were provided by Sigma-Aldrich. Melting points (m.p.) were uncorrected and determined with a Buchi B-540 melting point apparatus. Spectroscopic data were recorded as follows: Shimadzu IRAffinity-1 FTIR spectrophotometer for IR, Varian Mercury-400 MHz for ^{1}H NMR (DMSO-d_6) spectra, Varian^{UNITY} INOVA-125 MHz for ^{13}C-NMR (APT) (DMSO-d_6) spectra. Elemental analyses were run on a Thermo Finnigan Flash EA 1112 Series elemental analyzer (cy: cyclohexane, phenyl: ph).

2-Methoxy-4-sulfamoylbenzhydrazide (2)

$NH_2NH_2.H_2O$ (98%, 0.008 mol) was added to a solution of **1** (1.22 g, 0.005 mol) in ethanol (25 mL). The reaction mixtures were heated under reflux for 8 hours. The resulting mixture was cooled and allowed to stand overnight. The precipitate was filtered, washed with water and used without further purification.

General procedure for the synthesis of 4-(2-(3,4-(non) substituted cyclohexylidene)hydrazinecarbonyl)-3-methoxybenzene-1-sulfonamide (3a-i)

A mixture of compound **2** (1.22 g, 0.005 mol) with an appropriate cyclohexanone (0.006 mol) in absolute ethanol (10 mL) was refluxed for 5-7 hours. The reaction was followed up by TLC. After cooling, the reaction mixture was filtered. The purification was done with washing or recrystallization from ethanol.

4-(2-cyclohexylidenehydrazinecarbonyl)-3-methoxybenzene-1-sulfonamide (3a)

White powder (85%); m.p: 231-234 °C; IR (KBr): υ_{max} 3315, 3275, 3182 (N-H), 1643 (C=O), 1633, 1595, 1508, 1483 (C=N, C=C), 1325, 1161 (S=O). ^{1}H NMR (DMSO-d_6): δ 10.74, 10.61 (1H, 2s, NH), 8.11 (1H, d, *J*=2.5 Hz, H2), 7.87 (1H, dd, *J* = 8.7, 2.5 Hz, H6), 7.32 (2H, s, SO_2NH_2), 7.30 (1H, d, *J*= 8.7 Hz, H5), 3.94, 3.78 (3H, 2s, OCH_3), 2.23-2.40 (4H, m, CH_2-cy), 1.75-1.46 (6H, m, CH_2-cy). ^{13}C-NMR (DMSO-d_6): δ 163.53, 160.85 (C=N, C=O), 159.44 (C_3), 136.88 (C1), 130.19, 128.66 (C5,6), 123.62 (C4), 112.89 (C2), 57.25 (OCH_3), 35.38, 27.84, 27.19, 26.03, 25.50 (cy-C). Anal. calcd. for $C_{14}H_{19}N_3O_4S$ (325.38) C: 51.68, H: 5.89, N: 12.91. Found C: 51.82, H: 6.10, N: 12.89.

3-methoxy-4-[2-(3-methylcyclohexylidene)hydrazinecarbonyl]benzene-1-sulfonamide (3b)

White needles (81%); m.p: 237-240 °C; IR (KBr): υ_{max} 3346, 3323, 3176 (N-H), 1668 (C=O), 1633, 1593, 1537, 1479 (C=N, C=C), 1332, 1170 (S=O). ^{1}H NMR (DMSO-d_6): δ 10.74, 10.62 (1H, 2s, NH), 8.11 (1H, 2d, *J* = 2.5 Hz, H2), 7.87 (1H, 2dd, *J* = 8.7, 2.5 Hz, H6), 7.31-7.30 (3H, m, H5 and SO_2NH_2), 3.94, 3.77 (3H, 2s, OCH_3), 2.69 (1H, t, *J*=13 Hz, CH/CH_2-cy), 2.43-2.28, 2.21-2.09 (1H, m, CH/CH_2-cy), 2.00-1.78 (2H, m, CH/CH_2-cy), 1.76-1.53 (3H, m, CH/CH_2-cy), 1.52-1.30 (1H, m, CH/CH_2-cy), 1.26-1.06 (1H, m, CH/CH_2-cy), 0.83, 0.95 (3H, 2d, *J* = 6.4 Hz, CH_3). ^{13}C-NMR (DMSO-d_6): 163.27, 160.87 (C=N, C=O), 159.41 (C_3), 136.89 (C1), 130.20, 128.68 (C5,6), 123.70 (C4), 112.89 (C2), 57.25 (OCH_3), 35.73, 34.89, 24.81 (cy-C), 33.70, 32.78 (cy-C3), 22.37 (CH_3). Anal. calcd. for $C_{15}H_{21}N_3O_4S$ (339.40) C: 53.08, H: 6.24, N: 12.38. Found C: 53.14, H: 6.55, N: 12.37.

3-methoxy-4-[2-(4-ethylcyclohexylidene)hydrazinecarbonyl]benzene-1-sulfonamide (3c)

White powder (84%); m.p: 238-240 °C; IR (KBr): υ_{max} 3340, 3184 (N-H), 1660 (C=O), 1598, 1483 (C=N, C=C), 1334, 1168 (S=O). ^{1}H NMR (DMSO-d_6): δ 10.73, 10.61 (1H, 2s, NH), 8.12 (1H, d, *J*=2.5 Hz, H2), 7.88 (1H, dd, *J* = 8.7, 2.5 Hz, H6), 7.32 (2H, s, SO_2NH_2), 7.31 (1H, d, *J*=8.7 Hz, H5), 3.94, 3.78 (3H, 2s, OCH_3), 2.95, 2.75 (1H, 2d, *J*= 14.0 Hz, CH/CH_2-cy), 2.45-2.34 (1H, m, CH/CH_2-cy), 2.23 (1H, td, *J*=13.7, 4.9 Hz, CH/CH_2-cy), 2.06-1.70 (4H, m, CH/CH_2-cy), 1.52-0.98 (4H, m, CH/CH_2-cy and 4-CH_2CH_3-cy), 0.87 (3H, t, *J*= 7.4 Hz, CH_3). ^{13}C-NMR (DMSO-d_6): 163.63, 160.82 (C=N, C=O), 158.62 (C3), 136.89 (C1), 130.21, 128.68 (C5,6), 123.58 (C4), 112.89 (C2), 57.25 (OCH_3), 37.96 (cy-C4), 34.53, 32.72, 26.93, 24.81 (cy-C), 31.61 (CH_2), 11.98 (CH_3). Anal. calcd. for $C_{16}H_{23}N_3O_4S$ (353.43) C: 54.37, H: 6.56, N: 11.89. Found C: 54.05, H: 6.83, N: 11.99.

3-methoxy-4-[2-(4-propylcyclohexylidene)hydrazinecarbonyl]benzene-1-sulfonamide (3d)

White powder (90%); m.p: 248-250 °C; IR (KBr): υ_{max} 3338, 3190 (N-H), 1658 (C=O), 1598, 1525, 1483 (C=N, C=C), 1334, 1166 (S=O). ^{1}H NMR (DMSO-d_6): δ 10.74, 10.61 (1H, 2s, NH), 8.12 (1H, d, *J*=2.5 Hz, H2), 7.88 (1H, dd, *J* = 8.8, 2.5 Hz, H6), 7.32 (2H, s, SO_2NH_2), 7.30 (1H, d, *J*= 8.8 Hz, H5), 3.94, 3.77 (3H, 2s, OCH_3), 2.94, 2.74 (1H, 2d, *J* = 14.0 Hz, CH/CH_2-cy), 2.45-2.35 (1H, m, CH/CH_2-cy), 2.23 (1H, td, *J*=13.3, 4.9 Hz, CH/CH_2-cy), 2.03-1.72 (3H, m, CH/CH_2-cy), 1.60-1.46 (1H, m, CH/CH_2-cy), 1.38-1.00 (6H, m, CH/CH_2-cy and 4-$CH_2CH_2CH_3$-cy), 0.86 (3H, t, *J*= 7.2 Hz, CH_3). ^{13}C-NMR (DMSO-d_6): 163.58, 160.82 (C=N, C=O), 159.44 (C3), 136.89 (C1), 130.20, 128.68 (C5,6), 123.56 (C4), 112.97 (C2), 57.24 (OCH_3), 38.26, 34.55, 33.09, 32.22 (cy-C), 36.03 (cy-C4), 26.97, 20.13 (CH_2), 11.98 (CH_3). Anal. calcd. for $C_{17}H_{25}N_3O_4S$ (367.46) C: 55.57, H: 6.86, N: 11.44. Found C: 55.78, H: 7.23, N: 11.48.

3-methoxy-4-[2-(4-phenylcyclohexylidene)hydrazinecarbonyl]benzene-1-sulfonamide (3e)

White needles (94%); m.p: 275-279 °C; IR (KBr): υ_{max} 3350, 3190 (N-H), 1666 (C=O), 1598, 1521, 1487 (C=N, C=C), 1334, 1168 (S=O). ^{1}H NMR (DMSO-d_6): δ 10.83, 10.69 (1H, 2s, NH), 8.12 (1H, d, *J*=2.5 Hz, H2), 7.88 (1H, dd, *J* = 8.8, 2.5 Hz, H6), 7.40-7.09 (8H, m, H5, ph-H and SO_2NH_2), 3.94, 3.81 (3H, 2s, OCH_3), 2.97-2.73 (2H, m, CH/CH_2-cy), 2.59-2.36 (2H, m, CH/CH_2-cy and DMSO-d_6), 2.23-1.86 (3H, m, CH/CH_2-cy), 1.77-1.47 (2H, m, CH/CH_2-cy). ^{13}C-NMR (DMSO-d_6): 162.57, 160.96 (C=N, C=O), 159.45 (C3), 146.22, 126.62 (ph-C), 136.88 (C1), 130.21, 128.86 (C5,6), 123.66 (C4), 112.89 (C2), 57.24 (OCH_3), 42.94 (cy-C4), 35.15, 34.27, 33.22, 27.53 (cy-C). Anal. calcd. for $C_{20}H_{23}N_3O_4S$. 1/4H_2O (406.07) C: 59.18, H: 6.16, N: 10.35. Found C: 59.07, H: 5.85, N: 10.46.

3-methoxy-4-{2-[4-(trifluoromethyl)cyclohexylidene] hydrazinecarbonyl}benzene-1-sulfonamide (3f)

White powder (78%); m.p: 210-214 °C; IR (KBr): υ_{max} 3340, 3205 (N-H), 1662 (C=O), 1600, 1529, 1485 (C=N, C=C), 1338, 1166 (S=O). ^{1}H NMR (DMSO-d_6): δ 10.85, 10.68 (1H, 2s, NH), 8.09 (1H, d, *J*=2.5 Hz, H2), 7.88 (1H, dd, *J* = 8.8, 2.5 Hz, H6), 7.33 (2H, s, SO_2NH_2), 7.31 (1H, d, *J*= 8.7 Hz, H5), 3.94, 3.78 (3H, 2s, OCH_3), 2.86 (1H, d, *J* = 14.3 Hz, CH/CH_2-cy), 2.72-2.58 (1H, m, CH/CH2-cy), 2.36 (1H, td, *J* = 14.2, 4.9 Hz, CH/CH_2-cy), 2.16-1.88 (4H, m, CH/CH_2-cy), 1.55-1.31 (2H, m, CH/CH_2-cy). ^{13}C-NMR (DMSO-d_6): 161.24, 161.02 (C=N, C=O), 159.41 (C3), 136.86 (C1), 130.23, 128.64 (C5,6), 128.28 (q, *J*=277 Hz, CF_3), 123.67 (C4), 112.88 (C2), 57.22 (OCH_3), 40.16 (d, *J*= 20 Hz, C4), 32.76, 25.52, 25.14, 23.99 (cy-C). Anal. calcd. for $C_{15}H_{18}F_3N_3O_4S$ (393.38) C: 45.80, H: 4.61, N: 10.68. Found C: 45.50, H: 4.30, N: 11.05.

3-methoxy-4-[2-(4-(2-methylbutan-2-yl)cyclohexylidene)hydrazinecarbonyl]benzene-1-sulfonamide (3g)

White powder (80%); m.p: 228-232 °C; IR (KBr): υ_{max} 3340, 3246, 3186 (N-H), 1651 (C=O), 1598, 1514, 1483 (C=N, C=C), 1336, 1165 (S=O). ^{1}H NMR (DMSO-d_6): δ 10.74, 10.61 (1H, 2s, NH), 8.13 (1H, d, *J*=2.5 Hz, H2), 7.88 (1H, dd, *J* = 8.8, 2.5 Hz, H6), 7.31 (2H, s, SO_2NH_2), 7.30 (H, d, *J*= 8.8 Hz, H5), 3.95, 3.77 (3H, 2s, OCH_3), 2.87-2.77 (1H, m, CH/CH_2-cy), 2.45-2.38 (1H, m, CH/CH_2-cy), 2.22 (1H, td, *J*= 13.4, 4.8 Hz, CH/CH_2-cy), 2.04-1.69 (3H, m, CH/CH_2-cy), 1.46-1.01 (5H, m, CH/CH_2-cy and 4-$C(CH_3)_2CH_2CH_3$-cy), 0.80-0.74 (9H, m, CH_3). ^{13}C-NMR (DMSO-d_6): 163.58, 160.75 (C=N, C=O), 159.45 (C3), 136.91 (C1), 130.24, 128.74 (C5,6), 123.58 (C4), 112.91 (C2), 57.28 (OCH_3), 44.09 (cy-C4), 35.05, 32.74, 27.35 (cy-C), 24.58 (CH), 8.52 (CH_3). Anal. calcd. for $C_{19}H_{29}N_3O_4S$ (395.51) C: 57.70, H: 7.39, N: 10.62. Found C: 57.54, H: 7.58, N: 10.67.

3-methoxy-4-[2-(4-cyano-4-phenylcyclohexylidene)hydrazinecarbonyl]benzene-1-sulfonamide (3h)

White powder (82%); m.p: 257-263 °C; IR (KBr): υ_{max} 3394, 3352, 3209 (N-H), 1643 (C=O), 1597, 1566, 1485 (C=N, C=C), 1338, 1166 (S=O). ^{1}H NMR (DMSO-d_6): δ 10.98, 10.78 (1H, 2s, NH), 8.11 (1H, d, *J*=2.5 Hz, H2), 7.89 (1H, dd, *J* = 8.8, 2.5 Hz, H6), 7.57 (2H, d, *J*= 7.9 Hz, ph-H2,6), 7.44 (2H, t, *J*= 7.6 Hz, ph-H3,5), 7.37 (1H, d, *J*= 7.9 Hz, ph-H4), 7.34 (2H, s, SO_2NH_2), 7.32 (1H, d, *J*=8.9 Hz, H5), 3.95, 3.81 (3H, 2s, OCH_3), 3.05-2.95 (1H, m, CH_2-cy), 2.69-2.57 (2H, m, CH_2-cy), 2.37-2.07 (5H, m, CH_2-cy). ^{13}C-NMR (DMSO-d_6): 161.19, 159.50 (C=N, C=O), 159.06 (C3), 140.07, 129.54, 126.16 (ph-C), 122.23 (CN), 136.88 (C1), 130.31, 127.08 (C5,6), 123.58 (C4), 112.91 (C2), 57.23 (OCH_3), 43.67 (cy-C4), 36.57, 36.32, 35.37, 32.42, 25.29 (cy-C). Anal. calcd. for $C_{21}H_{22}N_4O_4S$ (426.48) C: 59.14, H: 5.20, N: 13.14. Found C: 59.19, H: 5.39, N: 12.99.

3-methoxy-4-[2-(4-acetylaminocyclohexylidene)hydrazinecarbonyl]benzene-1-sulfonamide (3i)

White needles (76%); m.p: 239-244 °C; IR (KBr): υ_{max} 3317, 3211, 3190 (N-H), 1658 (C=O), 1595, 1529, 1479 (C=N, C=C), 1332, 1165 (S=O). ^{1}H NMR (DMSO-d_6): δ 10.79, 10.64 (1H, 2s, NH), 8.09 (1H, d, *J*=2.5 Hz, H2), 7.90-7.75 (2H, m, H6 and N**H**$COCH_3$), 7.33 (2H, s, SO_2NH_2), 7.30 (1H, d, *J*= 8.6 Hz, H5), 3.93, 3.79 (3H, 2s, OCH_3), 2.74-2.63 (1H, m, CH/CH_2-cy), 2.46-2.25 (2H, m, CH/CH_2-cy), 2.19-1.84 (4H, m, CH/CH_2-cy), 1.78 (3H, s, NHCOC**H**$_3$), 1.50-1.18 (2H, m, CH/CH_2-cy). ^{13}C-NMR (DMSO-d_6): 169.22 ($NHCOCH_3$), 162.29, 161.01 (C=N, C=O), 159.45 (C3), 136.84 (C1), 130.21, 128.59 (C5,6), 123.74 (C4), 112.87 (C2), 57.22 (OCH_3), 46.40 (cy-C4), 32.94, 32.30, 31.15, 25.40 (cy-C), 23.21 ($COCH_3$). Anal. calcd. for $C_{16}H_{22}N_4O_5S$. 1/2H_2O (391.43) C: 49.10, H: 6.13, N: 14.32. Found C: 49.62, H: 5.89, N: 14.50.

Biological activity

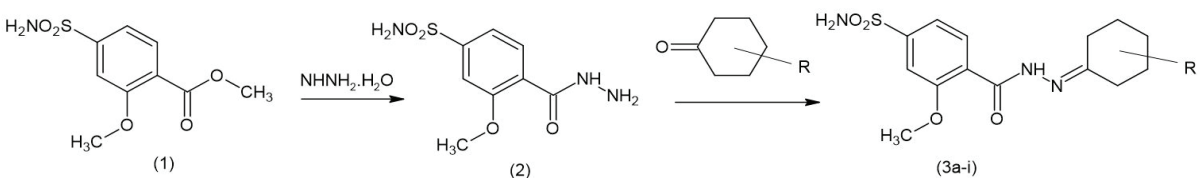
The antidiabetic activity

The antidiabetic activity of compounds was analyzed by measuring the inhibitory effects on α-glucosidase and α-amylase enzymes. α-Amylase, α-glucosidase, 3,5-dinitrosalicylic acid (DNS), acarbose, dimethyl sulfoxide (DMSO), *p*-nitrophenyl α-D-glucopyranoside (*p*NPG) and starch were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals used were of analytical grade. For the assays, the synthesized compounds and acarbose were dissolved in DMSO to form a 5 mg/mL stock solution. Different concentrations of the compounds and acarbose were prepared for use in the analyses by dilution of the stock solution with DMSO.

α-Glucosidase inhibitory activity

The α-glucosidase inhibitory activities of the compounds were determined by Bothon et al.'s method, with some modifications (Bothon et al., 2013). 10 µL of the compounds' solution, 90 µL of Na-phosphate buffer (pH 6.8) and 50 µL of α-glucosidase solution (1 U/mL) were mixed and incubated at 37°C for 10 min. After incubation, 50 µL of *p*NPG solution was added and the absorbance increase was measured at 405 nm. Acarbose

Scheme 1. Synthesis of compounds 3a-i.



Compound	R	Compound	R	Compound	R
3a	H	**3d**	4-C_3H_7	**3g**	4-$C(CH_3)_2CH_2CH_3$
3b	3-CH_3	**3e**	4-C_6H_5	**3h**	4-CN, 4-C_6H_5
3c	4-C_2H_5	**3f**	4-CF_3	**3i**	4-$NHCOCH_3$

was used as the standard. The α-glucosidase inhibitory activity (%) was calculated according to the following formula:

$$Inhibition\ level\ (\%) = \left(1 - \frac{Reaction\ rate\ of\ sample\ at\ 405\ nm}{Reaction\ rate\ of\ control\ at\ 405\ nm}\right) \times 100$$

α-Amylase inhibitory activity

The α-amylase inhibitory activities of the compounds were determined by Ali et al.'s method, with some modifications (Ali, Houghton, & Soumyanath, 2006). 10 µL of the compounds' solution, 40 µL of Na-phosphate buffer (pH 6.8) and 50 µL of α-amylase solution (3 U/mL) were mixed and incubated for 10 min at 25°C. Starch solution (50 µL, 0.75%) was added to the mixture and kept at 25°C for 5 min. Then, 75 µL of DNS reagent was added to stop the reaction. The mixture was kept at 85°C for 15 min and diluted with distilled water after cooling. The absorbances were measured at 540 nm against the blank. Acarbose was used as the standard. The α-amylase inhibitory activity (%) was calculated according to the following formula:

$$Inhibition\ level\ (\%) = \left(1 - \frac{Absorbance\ of\ sample\ at\ 540\ nm}{Absorbance\ of\ control\ at\ 540\ nm}\right) \times 100$$

RESULTS AND DISCUSSION

Chemistry

A convenient method for the synthesis of hydrazones is illustrated in Scheme 1. The structures of the newly synthesized compounds were elucidated by spectrometric methods (microanalysis, IR, ^{1}H-NMR and APT). The IR spectra of the new hydrazone compounds (**3a–i**) exhibited the N-H and C=O stretching vibrations in the 3394-3176 cm^{-1} and 1668-1643 cm^{-1}, respectively. The presence of the characteristic signals for aliphatic protons (Cihan-Üstündağ & Çapan, 2012; Kocabalkanlı et al., 2017; Cihan-Üstündağ, Mataracı-Kara, & Çapan, 2019) in the cyclohexylidene residue at about δ 0.98-2.97 ppm in the ^{1}H NMR spectrum of **3a–i** confirmed the formation of the hydrazone compounds. The splitting patterns of the H2, H5 and H6 hydrogens on the aromatic ring were in accordance with the 1,2,4-trisubstituted benzene system. The NH protons resonated at about δ 10.98-10.61 ppm and OCH_3 protons resonated at about δ 3.95-3.77 ppm as two separate singlets. The multiplicity in these signals pointed to the presence of two isomeric forms due to the restricted rotation about the C=N double bond and the partial double bond character of CONH (C-N) bond, in accordance with earlier reports (Ulusoy-Güzeldemirci, Şatana, & Küçükbasmacı 2015; Ulusoy-Güzeldemirci, Pehlivan, Halamoğlu, & Kocabalkanlı, 2016; Apaydın, 2018; Cihan-Üstündağ et al., 2019). Previous X-ray crystallographic studies on 4-methyl/4-ethylcyclohexanone derived indole-hydrazones revealed that these compounds existed as two crystallographically independent molecules due to the restricted rotation and these pair of molecules were found to be connected to each other, forming N—H ··· O dimers (Türktekin-Çelikesir, Akkurt, Cihan-Üstündağ, Çapan, & Büyükgüngör, 2013; Akkurt, Türktekin-Çelikesir, Cihan-Üstündağ, Çapan, & Büyükgüngör, 2013). The ^{1}H-NMR spectrum of compound **3b** displayed two sets of signals for most of the protons, including aromatic and 3-CH_3 protons, as well as NH and OCH_3 protons. It is assumed that the methyl substituent at 3-position interrupts the symmetry of the molecule and gives rise to the formation of *E* and *Z* isomers for compound **3b** (Montalvo-Gonzalez, Montalvo-Gonzalez, & Ariza-Castolo, 2008; Cihan-Üstündağ et al., 2019). Carbon assignments were evaluated by performing APT experiments. The new C=N carbon signals resonated with the C=O signals at δ 163.63-159.50 ppm region and further supported the formation of hydrazone derivatives. The detailed spectral data of compounds **3a–i** are given in the Materials and Methods section.

Biological activity

α-Glucosidase and α-amylase inhibitory activity

The novel benzoylhydrazones (**3a–i**) were tested for *in vitro* antidiabetic activity against α-glucosidase and α-amylase enzymes. The inhibitory activity test results were expressed as percentage inhibition and IC_{50} values. IC_{50} values indicate the concentration that inhibits enzyme activity by 50%. IC_{50} values were calculated from dose-response curves (Figure 1), using Microsoft Excel software. The antidiabetic drug acarbose was used as the standard α-glucosidase and α-amylase inhibitor in the tests.

Compounds **3d**, **3e**, **3g** and **3h** showed high α-glucosidase inhibitory activity compared to acarbose, especially at concentrations of 125 and 250 µg/mL. **3e** and **3g** showed similar α-glucosidase inhibitory activity compared to acarbose at a concentration of 62.5 µg/mL. The most active analogue **3g**, with a *tert*-pentyl group at the cyclohexane ring, exhibited the highest α-glucosidase inhibitory activity with an IC_{50} of 65.27 µg/mL, while the IC_{50} value of the reference acarbose was found to be 122.25 µg/mL (Table 1). **3d**, **3e**, **3g** and **3h** showed similar

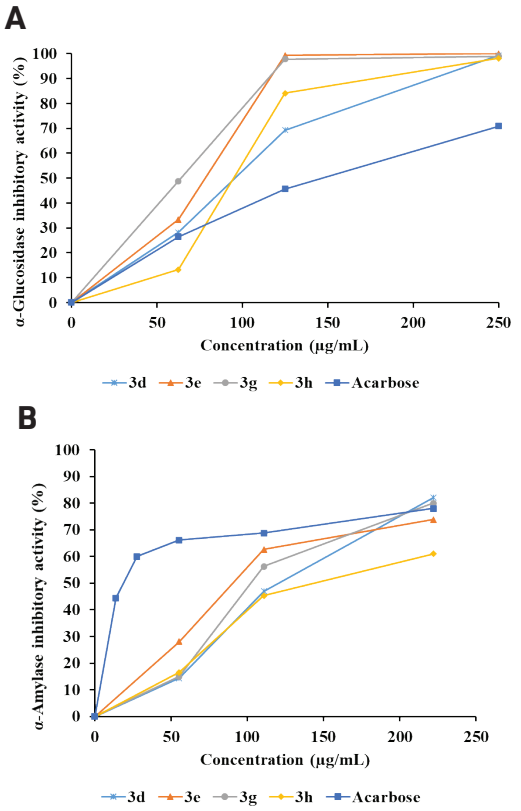


Figure 1. α-Glucosidase (**A**) and α-amylase (**B**) inhibitory activities (%) of compounds at different concentrations.

α-amylase inhibitory activity compared to acarbose at high concentrations, 222 and 111 μg/mL. However, they showed weak inhibitory activity at a concentration of 55.5 μg/mL and their IC_{50} values were much higher compared to that of reference acarbose (Table 2). None of the other tested hydrazone compounds had a meaningful efficacy on α-glucosidase and α-amylase enzyme activity at the highest concentrations tested. Introduction of a bulky substituent at position 4- of the cyclohexane system seemed to have a positive effect on antidiabetic activity.

CONCLUSION

This report is based on the synthesis and characterization of a new series of cyclohexanone benzoylhydrazones. The new hydrazones were evaluated for their *in vitro* α-glucosidase and α-amylase inhibitory activity. Of the nine compounds tested, four derivatives, **3d**, **3e**, **3g** and **3h,** were found to have an inhibitory effect on the enzymes tested. Compounds **3d**, **3e**, **3g** and **3h** exhibited better α-glucosidase inhibitory activity compared to reference antidiabetic drug acarbose. Compound **3g** was the most active agent tested (IC_{50}:65.27 μg/mL), being two-fold more active than acarbose. **3d**, **3e**, **3g** and **3h** showed similar α-amylase inhibitory activity compared to acarbose when tested at high concentrations. However, they were found to be weakly active at a concentration of 55.5 μg/mL and their IC_{50} values were much higher compared to that of reference acarbose. The existence of a bulky group at the 4-position of the cyclohexane system seemed to cause an increase in antidiabetic activity.

Table 1. α-Glucosidase inhibitory activity of compounds 3d, 3e, 3g and 3h.

	Inhibition (%)			IC_{50} (μg/mL)[a]
Compounds	**250 μg/mL**	**125 μg/mL**	**62.5 μg/mL**	
3d	99.37±0.77	69.26±0.99	28.27±2.43	102.28±2.44
3e	100.00±0.00	99.31±0.41	33.43±2.23	78.18±1.61
3g	98.94±0.84	97.82±0.16	48.87±3.04	65.27±1.46
3h	98.16±0.81	84.08±1.63	13.28±0.92	94.92±1.13
Acarbose	70.96±2.56	54.49±2.99	36.77±1.20	122.25±5.05

Table 2. α-Amylase inhibitory activity of compounds 3d, 3e, 3g and 3h.

	Inhibition (%)			IC_{50} (μg/mL)[a]
Compounds	**222 μg/mL**	**111 μg/mL**	**55.5 μg/mL**	
3d	82.04±1.30	46.96±1.10	14.31±1.42	135.30±1.76
3e	73.80±1.08	62.65±0.39	28.11±1.14	110.13±1.87
3g	80.02±2.68	56.24±2.22	14.98±1.89	128.55±3.17
3h	60.94±0.56	45.33±0.77	16.59±1.41	166.10±0.24
Acarbose	77.98±1.66	68.74±0.75	66.20±0.13	18.06±1.81

[a] IC_{50} values indicate the concentration that inhibits enzyme activity by 50%. IC_{50} values were calculated from dose-response curves (by plotting the percentage of inhibition against concentration) using Microsoft Excel software.
*Values represent the means of three replicates ± standard deviation.

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