Synthesis of alkoxy-isoflavones as potential α-glucosidase inhibitors

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Received: 11 February 2022 / Accepted: 15 May 2022 / Published online: 25 May 2022
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Abstract
The aim of the present study was to synthesize isoflavone-enaminones 3a-c and 7-alkoxy-isoflavones 4a-c, evaluate their inhibition of α-glucosidase, and analyze the bioisosteric effect of the presence versus absence of aromatic moieties in these benzopyran derivatives. All the test compounds exhibited greater inhibition of α-glucosidase than the positive control acarbose. These series of isoflavones 3a-c and 4a-c showed higher inhibitory activity (IC50 = 6.3–87.6 µM) than the parental 7-hydroxyisoflavones 2a-c (IC50 = 109.4–173.2 µM), suggesting that the attachment of a 4'-chloroacetophenone moiety to the 7-hydroxyl group of 2a-c is an efficient way to increase the inhibition of α-glucosidase. Furthermore, the aromatic moieties of the series of compounds 3 and 4 enhance inhibitory activity by hydrophobic effects, according to docking calculations.

Graphical abstract

Keywords α-Glucosidase · Diabetes mellitus · Enaminone · Isomaltase · Alkoxy-isoflavones

Supplementary information The online version contains supplementary material available at https://doi.org/10.1007/s00044-022-02910-1.

Introduction
α-Glucosidase is an enzyme that catalyzes the hydrolysis of glycosidic bonds from the non-reducing portion of the oligosaccharide substrates. It plays a key role in the digestion of dietary carbohydrates and the processing of glycoproteins. By inhibiting this enzyme, the digestion of starch and other dietary sugars decreases, which helps to avoid hyperglycemia by maintaining blood glucose at normal levels [1]. The reduction of sugar digestion makes α-glucosidase an attractive therapeutic target for the treatment of type 2 diabetes [2], cardiovascular diseases [3], and cancer [4, 5]. Regarding diabetes, about 422 million people worldwide are affected by this metabolic disease, characterized by high levels of blood glucose (hyperglycemia) derived from a deficiency in the secretion and/or action of insulin [6]. Therefore, α-glucosidase inhibitors may represent an effective therapeutic strategy for reducing postprandial hyperglycemia.

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In the search for α-glucosidase inhibitors, oxygenated heterocyclic compounds containing a 3-phenylbenzopyran-4-one scaffold (e.g., isoflavones or chromones) have attracted attention. These compounds, which occur in leguminous plants, have a broad range of biological properties, acting as antioxidant [7], anticancer [8, 9], anti-inflammatory [10], antimicrobial [11], antiviral [12], and antidiabetic agents [13, 14].

Recently, our group reported that enaminone-chromone derivatives can potentially act as α-glucosidase inhibitors [15]. Several interactions were found between the chromone derivatives and α-glucosidase, including π-stacking and hydrophobic effects. Such interactions are key for enhancing inhibitory activity. For instance, enaminone-chromone 1c bears a phenyl group at the C-3 position of the chromone ring system (isoflavone skeleton) and a 4-chlorophenyl group at the enaminone moiety. It displays considerably greater inhibition of α-glucosidase than compounds 1a-b (Fig. 1A).

According to the structural analysis, the benzopyran framework of isoflavone 1c is like that of the natural isoflavones formononetin (2a) and afromorsin (2b) (Fig. 1A). These isoflavones, isolated from leguminous plants, have shown α-glucosidase inhibition [13, 14, 16–18], antioxidant [19], antihypertensive [20], anticancer [21–25], antimicrobial [26], and antihyperglycemic activity [27, 28]. Consequently, α-glucosidase inhibitors from food sources (e.g., legumes) have become an attractive therapeutic approach for the treatment of metabolic disorders and cancer [29–31].

In accordance with the aforementioned, the 7-hydroxy group and its 7-alkoxylated isoflavone derivatives as well as 6-methoxy-containing or 6-methoxy-free analogs may generate a significant inhibition of α-glucosidase. This largely depends on the nature of the C-7 alkoxy groups and the C-6 substituents in such compounds. For the choice of compounds to be tested presently, it was taken into account that functionalized benzopyrans with a 2-(4-chlorophenyl)-2-oxoethoxy group at the C-7 position (i.e., compound 1c) increased the inhibition of the enzyme, focusing particular attention on the role played by the enaminone moiety. Hence, the aim of the current contribution was to synthesize a series of enaminone-isoflavones 3a-c and 7-(2-(4-chlorophenyl)-2-oxoethoxy)isoflavones 4a-c, evaluate them as potential inhibitors of α-glucosidase (Fig. 1B), and test them for their antioxidant effect. The analysis of the results on the inhibition of α-glucosidase provided insights into the structure-activity relationship of the isoflavone-based derivatives. Additionally, docking studies were carried out to better understand the mechanism of binding of the 7-alkoxy-isoflavone derivatives to the crystal structure of isomaltaose, an α-glucosidase of *Saccharomyces cerevisiae*.

**Results and discussion**

**Chemistry**

Compounds 7a-c were prepared in good yields by the acylation of resorcinols 5a-c with 2-(4-methoxyphenyl) acetic acid (6), in the presence of boron trifluoride.
diethyl etherate (BF₃·OEt₂). The O-alkylation reaction of 2,4-dihydroxyacetophenones 7a-c with 2-bromo-1-(4-chlorophenyl)ethanone (8) generated the series of α-arylacetophenones 9a-c, respectively, in good yields. Subsequently, these compounds were treated with DMFDMA to afford the corresponding enaminone-isoflavones 3a-c [15] (Scheme 1).

On the other hand, the treatment of 2,4-dihydroxyacetophenones 7a-c with DMFDMA gave the series of isoflavones 2a-c in high yields, along with 7-methoxyisoflavones 10a-b as by-products in low yields [32]. Then O-alkylation of isoflavones 2a-c with 8 provided the target compounds 4a-c (Scheme 2).

The structure of all test compounds was elucidated by 1D and 2D NMR techniques and HRMS. The intramolecular cyclization and condensation of the α-arylcetophenones 9a-c led to the enaminone-isoflavones 3a-c as single stereoisomers. The Z geometry of the latter compounds was established by NOE experiments. The irradiation of the signal assigned to the methyl protons of the dimethylamino group produced an enhancement of the signal corresponding to the H-6 (for 3a) and H-8 protons of the benzene ring of the chromen-4-one scaffold. This stereoselectivity has also been observed in similar systems, which is probably due to the higher stability achieved by the planar π-conjugated acrylate system when the bulky dimethylamino group is located at the opposite side of the carbonyl group [15].

**In vitro α-glucosidase inhibition**

All the test compounds were assessed for their inhibition of α-glucosidase. The IC₅₀ values obtained were compared to the corresponding value of acarbose (12) (Table 1). While the inhibitory effect of 2-acylphenol 7a was moderate, that of α-arylacetophenone compounds 9a-c was better. Indeed, 9a (IC₅₀ = 20.0 µM) produced a 15-fold greater effect than acarbose (12) (IC₅₀ = 309.2 µM). As can be appreciated, the incorporation of the (4-chlorophenyl)-2-oxoethoxy moiety significantly increased the inhibition of α-glucosidase. Interestingly, enaminone-isoflavones 3a-c also displayed good inhibition, generating a 23-fold greater effect than 12.

Compared to their precursors 7a-c, compounds 2a-c (with a benzopyran ring moiety) showed greater inhibition of α-glucosidase. In order to explore the possibility that the enaminone moiety in 3a-c favors inhibition, derivatives 4a-c (without this fragment) were evaluated. The latter compounds exhibited better inhibitory activity than compounds 3a and 3b. The best inhibition was found with 4a, the IC₅₀ value (6.3 µM) of which indicated a 49-fold greater inhibition of α-glucosidase than 12.

Regarding the inhibitory effect of compounds 3a-b and 4a-b, the analysis of the structure-activity relationship revealed that the polar enaminone group is not as relevant as the (4-chlorophenyl)-2-oxoethoxy group. In contrast, the enaminone group and the chlorine atoms in 3c favored improved inhibition with respect to 4c. Furthermore, the
π-stacking or hydrophobic effect of the additional aromatic rings at C-3 and C-7 of the isoflavone ring (series 3 and 4) may have played a more important role than the H-bond donor effect of 7-OH (series 2) in the interaction with the enzyme. At the C-6 position, the absence of a substituent in the isoflavone and benzene rings in 3a, 4a, and 9a led to better inhibitory activity than the presence of a methoxy group in 3b, 4b, and 9b or a chlorine atom in 4c and 9c.

### Evaluation of the antioxidant activity

Antioxidants are responsible for protecting the body against oxidative stress and many disorders (e.g., diabetes). To determine the potential of the presently synthesized compounds as antioxidants, their free radical scavenging was examined by the DPPH radical assay, with butylated hydroxytoluene as the positive control. The reduction in color intensity represented the percentage of inhibition of DPPH (Table 1), finding that the 2-acylphenols 7b-c, α-arylacetophenones 9a-c, and isoflavones 2a-c, 3a-c, 4a-c, and 10a-b did not have any significant antiradical activity up to the maximum concentration tested. Only compound 7a showed some inhibition (61% at 2.5 mM), suggesting that this effect is mainly related to the hydroxyl groups substituted in the benzene ring.

### Docking results

To identify the binding mode of the compounds with the greatest inhibition of α-glucosidase, molecular docking studies were carried out. The crystal structure of isomaltase from S. cerevisiae served as the target protein because it is a type of α-glucosidase. The positive controls were maltose (11) and acarbose (12), two competitive inhibitors of isomaltase. The results of the interactions are illustrated in 2D and 3D (Fig. 2). Compounds 2a, 3a, 4a, and 9c as well as the controls 11 and 12 recognized many of the amino acid residues at the active site. Among such residues were Asp64, His112, Arg213, Asp215, Glu277, His351, Asp352, and Arg442, as reported in other studies on isomaltose inhibitors [33–36]. For all the compounds, the two residues

### Table 1 DPPH-scavenging and α-glucosidase inhibition of the test compounds

| Compound | % DPPH scavenging activity (2.5 mM) | α-Glucosidase inhibition % (400 µM) (IC50 µM) |
|----------|-----------------|----------------------------------|
| 2a       | 1.86 ± 0.79     | 99.51 ± 0.69                    |
| 2b       | 10.0 ± 0.15     | 99.15 ± 0.05                    |
| 2c       | 15.3 ± 2.19     | 98.80 ± 0.17                    |
| 3a       | 0.15 ± 0.05     | 97.44 ± 0.27                    |
| 3b       | 14.6 ± 1.32     | 98.40 ± 0.46                    |
| 3c       | 11.3 ± 0.82     | 99.98 ± 0.06                    |
| 4a       | 5.02 ± 0.04     | 99.97 ± 0.05                    |
| 4b       | 6.4 ± 0.32      | 99.59 ± 0.27                    |
| 4c       | 4.2 ± 0.98      | 98.70 ± 0.02                    |
| 7a       | 61.36 ± 0.63    | 92.18 ± 0.18                    |
| 7b       | 20.37 ± 0.52    | 14.24 ± 1.87                    |
| 7c       | 21.3 ± 0.21     | 20.42 ± 1.76                    |
| 9a       | 34.02 ± 0.03    | 99.53 ± 0.32                    |
| 9b       | 17.86 ± 1.12    | 82.61 ± 2.73                    |
| 9c       | 6.72 ± 1.03     | 95.20 ± 0.42                    |
| 10a      | 2.40 ± 0.10     | 31.28 ± 1.15                    |
| 10b      | 1.27 ± 0.03     | 16.02 ± 1.13                    |
| BHT      | 85.02 ± 3.33ᵃ   | nt                               |
| Acarbose | nt              | nt                               |

ⁿt not tested
ᵃIC₅₀ = 0.84 ± 0.08 mM
Fig. 2 Portrayal of the interactions within the active site of isomaltase by isoflavone derivatives 2a, 3a, and 4a, aryloxy carbonyl compound 9c, and controls 11 and 12. The 3D models illustrate the hydrogen bonds and the amino acid residues at the active site of the enzyme. The non-hydrogen bonds were omitted for clarity. In the 2D model, the following bonds are displayed with dotted lines: conventional hydrogen (green), carbon-hydrogen (brown), π-sigma (purple), π-μ T-shaped (fuchsia), π-alkyl (pink), and halogens (cyan). The amino acids are depicted with circles: basic (pink), acid (orange), polar (dark blue), and non-polar (yellow).
involved in catalysis (Glu277 and Asp352) are active in the binding process, suggesting that the derivatives of 7-alkoxy-
and 7-hydroxyisoflavones bind to the active site of the enzyme to inhibit α-glucosidase [33].

The binding energy values (ΔG) and the type of interactions are shown in Table 2. The best ΔG values were observed for 2a (−7.51 Kcal/mol), 3a (−9.82 Kcal/mol), 4a (−9.58 Kcal/mol), and 9c (−9.83 Kcal/mol), each being
better than the corresponding value for maltose (11) (−5.55 Kcal/mol), a competitive inhibitor. The best binding to the active site of isomaltase was found with derivative 9c. There were hydrophilic and hydrophobic interactions in isoflavones 2a, 3a, 4a, and 9c, but not in the two controls. The predominant hydrophilic interactions in all the derivatives and maltose (11) are those between some substituent of the compounds and the Asp352 or Arg442 residues. There is a hydrophobic interaction with Phe178 for these four derivatives, as reported previously [34–36].

The results of the docking study coincide with those of the enzyme inhibition assays. That is, among the four aforementioned compounds with the binding energy, 3a, 4a, and 9c produced the greatest inhibition of the α-glucosidase enzyme. The corresponding IC_{50} values were 18.8 ± 0.13, 6.3 ± 0.11, and 34.1 ± 0.013 µM, respectively, much lower than the IC_{50} of 12 (309.2 ± 0.9 µM). The analysis of the docking results for compounds 3a, 4a, and 9c showed that the 4-methoxyphenyl group at C-3 of the benzopyran ring (C-1 for derivative 9c) exhibits a hydrophilic interaction with a hydrogen atom of group methoxy between carboxamide group of Asn415. The hydrophobic interactions π-π stacked and π-alkyl were observed with Arg315, Phe314, and Lys156. The presence

| Compound | Binding energy ΔG (kcal/mol) | Residues interacting with the ligand | Polar interactions | Hydrophobic interactions |
|----------|-----------------------------|------------------------------------|--------------------|-------------------------|
| 11       | −5.55                       | Asp69, Tyr72, His112, Phe159, Phe178, Gln182, Arg213, Asp215, Val216, Glu277, His351, Asp352, Arg442 | O-H—O (Asp69) O-H—O (Gln182) O−H-N (Arg213) C-H—O (Asp215) O-H—O (Glu277) C-H—O (Asp352) O−H-N (Arg442) | —                        |
| 12       | −7.78                       | Asp69, Tyr72, His112, Tyr158, Phe159, Phe178, Arg213, Asp215, Val216, Glu277, Gln279, His280, Phe303, Asp307, Arg315, Tyr316, His351, Asp352, Gln353, Glu411, Arg442 | C-H—O (Asp69) O-H—O (Asp215) O−H-N (Gln279) O-H—O (Asp307) O−H-N (His351) O-H—O (Glu411) O−H-N (Arg442) | —                        |
| 2a       | −7.51                       | Asp69, Tyr72, His112, Phe159, Phe178, Gln182, Asp215, Val216, Glu277, Gln279, Phe303, Thr306, Asp307, Arg315, Asp352, Glu363, Arg442 | O-H—O (Asp69) O-H—O (Gln182) O−H-N (Asp307) O−H-N (Glu353) O−H-N (Arg442) | π-π stacked Phe178 π-alkyl Val216 π-π stacked Phe303 |
| 3a       | −9.82                       | Lys156, Tyr158, Gly160, Phe178, Val216, Gln253, Glu277, Phe303, Thr306, Asp307, Leu313, Phe314, Arg315, Asp352, Asn415, Arg442 | C-H—O (Thr306) C-H—O (Gln353) C-H—O (Asn415) C-H—O (Arg442) | π-alkyl Lys156, Phe178, Val216, Arg315 |
| 4a       | −9.58                       | Asp69, Tyr72, His112, Lys156, Ser157, Tyr158, Phe159, Gly160, Phe178, Glu182, Asp215, Val216, Glu277, Gln279, Phe303, Phe314, Asp352, Glu411, Asp415, Arg442 | O−H-N (Gln279) C-H—O (Asp352) C-H—O (Asn415) | π-π stacked Tyr158 π-alkyl Tyr72, His112, Lys156, Phe178 π-π stacked Asp69 |
| 9c       | −9.83                       | Asp69, Lys156, Tyr158, Phe159, Gly160, Phe178, Asp215, Val216, Glu277, Gln279, Phe303, Phe314, Arg315, Asp352, Glu411, Asn415, Arg442 | O−H-N (Gln279) C-H—O (Asp352) C-H—O (Arg442) | π-π Stacked Phe314 Halogen bonding Asp69 π-alkyl Phe159, Phe178, Val216, Arg315 Halogen bonding Phe178 π-alkyl Val216 Halogen bonding Asp69

The results of the docking study coincide with those of the enzyme inhibition assays. That is, among the four aforementioned compounds with the binding energy, 3a, 4a, and 9c produced the greatest inhibition of the α-glucosidase enzyme. The corresponding IC_{50} values were 18.8 ± 0.13, 6.3 ± 0.11, and 34.1 ± 0.013 µM, respectively, much lower than the IC_{50} of 12 (309.2 ± 0.9 µM). The analysis of the docking results for compounds 3a, 4a, and 9c showed that the 4-methoxyphenyl group at C-3 of the benzopyran ring (C-1 for derivative 9c) exhibits a hydrophilic interaction with a hydrogen atom of group methoxy between carboxamide group of Asn415. The hydrophobic interactions π-π stacked and π-alkyl were observed with Arg315, Phe314, and Lys156. The presence
Table 3 Calculated physicochemical properties of compounds 2a-c, 3a-c, 4a-c, 10a-b, maltose (11), and acarbose (12)

| Compound   | MW (g/mol) | Log P  | Log S | PSA  | H-A | H-D |
|------------|------------|--------|-------|------|-----|-----|
| maltose (11) | 180.15 | −6.26 | 0.25 | 110.38 | 6 | 5 |
| acarbose (12) | 645.60 | −7.17 | 0.58 | 321.17 | 19 | 14 |
| 2a | 268.26 | 2.24 | −3.33 | 55.76 | 4 | 1 |
| 2b | 298.29 | 2.17 | −3.35 | 64.99 | 5 | 1 |
| 2c | 302.72 | 2.85 | −4.07 | 55.76 | 4 | 1 |
| 3a | 475.92 | 4.35 | −6.05 | 65.07 | 6 | 0 |
| 3b | 505.95 | 4.28 | −6.07 | 74.3 | 7 | 0 |
| 4a | 510.37 | 4.95 | −6.79 | 65.07 | 6 | 0 |
| 4b | 420.84 | 4.09 | −6.00 | 61.83 | 5 | 0 |
| 4c | 450.87 | 4.02 | −6.02 | 71.06 | 6 | 0 |
| 7a | 455.29 | 4.69 | −6.73 | 61.83 | 5 | 0 |
| 7b | 258.27 | 2.21 | −2.86 | 66.76 | 4 | 2 |
| 7c | 288.29 | 2.14 | −2.87 | 75.99 | 5 | 2 |
| 9a | 292.71 | 2.81 | −3.59 | 66.76 | 4 | 2 |
| 9b | 410.85 | 4.05 | −5.52 | 72.83 | 5 | 1 |
| 9c | 440.87 | 3.98 | −5.54 | 82.06 | 6 | 1 |
| 10a | 410.82 | 4.05 | −5.52 | 72.83 | 5 | 1 |
| 10b | 312.30 | 2.45 | −3.66 | 53.99 | 5 | 0 |
|     | 316.73 | 3.13 | −4.38 | 44.74 | 6 | 0 |

MW molecular weight, HD hydrogen bond donor, HA hydrogen bond acceptor, Log P octanol/water partition coefficient, Log S aqueous solubility, PSA topological polar surface area

of the (4-chlorophenyl)-2-oxyethoxy fragment at C-7 of the isoflavone ring presents hydrophobic interactions with the active site of α-glucosidase such as π-sigma interactions with Val216, π-alkyl with Tyr72, His112 and Phe178, and halogen bonding with Asp69. Hydrophilic interactions of conventional hydrogen bond type and carbon-hydrogen bond were observed with Gln279 with Asp352, respectively.

Considering these findings, the presence of polar methoxy or hydroxy groups on the aromatic ring at C-3 of the benzopyran system could increase the affinity with α-glucosidase. Furthermore, analogs with a chlorine substituent at C-6 of the benzopyran ring increase their affinity for the enzyme. On the other hand, bioisosteric replacement of the (4-chlorophenyl)-2-oxyethoxy group by a (4-chlorophenyl)-2-oxyacetamide group at C-7 of the isoflavone ring could be explored, since that oxoacetamide moiety may show hydrophilic interactions that stabilize binding energies at the active site of the enzyme.

These results suggest that isoflavones 2a and 4a (without a substituent at C-6), as well as compounds 7a and 9a (without a substituent on the catechol ring), exhibited a greater inhibitory effect than their chlorine-substituted analogs (2c, 4c, 7c, and 9c) and methoxy-substituted analogs (2b, 4b, 7b, and 9b). In addition, compounds 2c and 3c with the chlorine substituent at C-6 of the benzopyran ring (C-5 for compound 9c) exhibited better enzyme inhibition than compounds 2b and 3b with the methoxy substituent (C-5 for compound 9b), which indicates that the negative inductive effect of the chlorine atom increases its affinity with the enzyme compared to the mesomeric effect of the methoxy group. Therefore, these scaffolds provide promise for the development of new drugs to treat diabetes mellitus.

Physicochemical properties

The physicochemical properties used to evaluate the drug-likeness of the synthesized compounds are shown in Table 3. The octanol-water partition coefficient Log P (expressed as Log Po/w) is defined as the partition coefficient of an un-ionized compound in two immiscible phases (octanol and water/buffer) at equilibrium.

Log S is a descriptor of the octanol-water distribution co-efficient for the partitioning of ionizable species in biphasic media. It reflects the true behavior of ionizable compounds in solution at a given pH value or range [39]. Compounds 2a-c, 3a-c, 4a-c, 7a-c, 9a-c, and 10a-b showed a slight to moderate solubility in water with their Log S ranging from −2.86 to −6.79. The controls, 11 and 12, exhibited values of 0.25 and 0.58, respectively (their polar groups form hydrogen bonds with the water).

The polar surface area (PSA) defines the sum of the surfaces of the polar atoms (N and O) and slightly polar atoms (S and P), and the hydrogen atoms attached to them. This parameter predicts intestinal absorption and the capacity to cross the blood-brain barrier [40]. A PSA below 90 Å² is indicative of the capacity of a drug to cross the blood-brain barrier. All of the present derivatives meet this requirement. Interestingly, the compounds with a methoxy group at the C-6 position of the benzopyran ring showed higher PSA values than those with a chlorine or hydrogen atom. For acarbose (12), the PSA value obtained indicates poor penetration of the central nervous system.

Finally, the bioavailability of these compounds was evaluated using Lipinski’s rule of 5, which states that a
molecule must have: (1) molecular weight < 500 g/mol; (2) Log P < 5; (3) < 5 hydrogen bond donors (NH, OH or SH); and (4) < 10 hydrogen bond acceptors [38]. As can be appreciated in Table 3, the synthesized compounds are in agreement with these guidelines (except 3b and 3c with a molecular weight > 500 g/mol).

Conclusions

The series of isoflavones 2-4 were easily synthesized in good yields. In vitro assays showed a greater inhibition of α-glucosidase by these derivatives than the positive control acarbose (12). Furthermore, alkoxy-isoflavone derivatives 4a-b displayed better inhibition than enaminone-isoflavones 3a-b. Docking studies revealed that the π-stacking and hydrophobic effects of the aromatic moieties at C-3 and C-7 of the chromone ring play a major role in the interaction with the active site of the enzyme. Compound 7a showed a low degree of antioxidant activity. Hence, the series of α-arylacetophenones and isoflavones herein prepared can be considered promising scaffolds for the development of new compounds with enhanced pharmacological activity.

Materials and methods

General experimental procedures

Raw data measurements of melting points were taken on an Electrothermal apparatus and are uncorrected. NMR spectra of 1H (300, 500, 600 or 750 MHz) and 13C (75, 125, 150 or 187.5 MHz) were recorded on Varian Mercury (300 MHz), Varian VNMR (500 MHz), Bruker 600AVANCE III (600 MHz), and Bruker Ascend (750 MHz) spectrometers. The chemical shifts (δ) are expressed in ppm relative to TMS as internal standard. Multiplicities were denoted as follows: s (singlet), d (doublet), t (triplet), dd (double of doublets), brs (broad singlet), brd (broad doublet), q (quartet), and m (multiplet). Coupling constants (J) are expressed in Hz. High-resolution mass spectra (HRMS, in electron ionization mode) were acquired on a Jeol JSM-I-Class® mass spectrometer (Waters Corporation, Milford MA, USA) coupled to a quadrupole time-of-flight mass spectrometer (Waters Xevo G2-XT QTOF; electrospray ionization mode ESI-tandem quadrupole) was used for UPLC and mass spectrometer analyses (LC-MS/MS). The UPLC/MS purity of all evaluated compounds was confirmed to be higher than 95%, and retention time values (tR) are given in minutes. Acarbose, α-glucosidase from Saccharomyces cerevisiae, dimethyl sulfoxide (DMSO), p-nitrophenyl-α-D-glucopyranoside (PNP), and the reagents used for all syntheses were purchased from Sigma-Aldrich. Anhydrous solvents were obtained by a distillation process. Thin-layer chromatography was carried out with precoated silica gel plates (Merck 60F254), and flash chromatography with silica gel (230–400 mesh). The synthesis of compounds 2a-c and 7a-c has been reported [41].

General method for the synthesis of 2-4-dihydroxyacetophenones (7a-c)

Under a nitrogen atmosphere and at 0 °C, BF₃·OEt₂ (2.0 mol equiv.) was added to a solution of phenols 5a-c (1.0 mol equiv.) and 2-(4-methoxyphenyl)acetic acid (6) (1.2 mol equiv.), and the resulting mixture was stirred at 80 °C for 3 h. The reaction crude was poured into cold water (10 ml) and adjusted to neutral pH with an aqueous saturated solution of NaHCO₃ (10%), followed by extraction with EtOAc (3 × 30 ml). The organic layer was dried (Na₂SO₄) and concentrated under vacuum. The residue was purified by flash chromatography over silica gel (hexane/EtOAc: 8:2 or 9:8) to provide the corresponding products 7a-c.

1-(2,4-Dihydroxyphenyl)-2-(4-methoxyphenyl)ethan-1-one (7a)

Following the general method, a mixture of 5a (2.50 g, 22.70 mmol), 6 (4.52 g, 27.24 mmol), and BF₃·OEt₂ (6.44 g, 4.54 mmol) afforded 7a (4.39 g, 75%) as a crystalline reddish solid. Rf 0.85 (hexane/EtOAc, 8:2), mp 154–155 °C (Lit. 158–160 °C [42, 43]). 1H NMR (300 MHz, DMSO-d6): δ 3.72 (s, 3H, OCH₃), 4.20 (s, 2H, CH₂-O), 6.26 (d, J = 2.4 Hz, 1H, H-3'), 6.39 (dd, J = 8.7, 2.4 Hz, 1H, H-5'), 6.86–6.90 (m, 2H, H-3''), 7.19–7.23 (m, 2H, H-2''), 7.95 (d, J = 8.7 Hz, 1H, H-6'), 10.46–11.26 (br, 1H, OH-4'), 12.57 (s, 1H, OH-2'). 13C NMR (75 MHz, DMSO-d6): δ 43.2 (CH₂-2'), 55.0 (OCH₃), 102.5 (C-3''), 108.3 (C-5'), 112.0 (C-1''), 113.8 (C-3''), 127.0 (C-1'), 130.5 (C-2''), 133.6 (C-6''), 158.0 (C-4''), 164.7 (C-4''), 165.1 (C-2''), 202.5 (C=O).

1-(2,4-Dihydroxy-5-methoxyphenyl)-2-(4-methoxyphenyl)ethan-1-one (7b)

Following the general method, a mixture of 5b (3.00 g, 21.40 mmol), 6 (4.27 g, 25.69 mmol), and BF₃·OEt₂ (6.07 g, 42.81 mmol) furnished 7b (4.94 g, 80%) as a beige solid. Rf 0.31 (hexane/EtOAc, 7:3); mp 114–115 °C (Lit., 122–124 °C [44]). 1H NMR (500 MHz, CDCl₃): δ 3.79 (s, 3H, OCH₃), 3.85 (s, 3H, OCH₃), 4.13 (s, 2H, CH₂), 6.30 (brs, 1H, OH-C4'), 6.50 (s, 1H, H-3'), 6.86–6.90 (m, 2H, H-3''), 7.15 (s, 1H, H-6''), 7.16–7.20 (m, 2H, H-2''), 12.55
arylacetophenones (9a-c). $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 44.4 (CH$_2$), 55.2 (OCH$_3$), 56.4 (OCH$_3$), 103.6 (C-3'), 110.4 (C-6'), 112.0 (C-1'), 114.2 (C-3''), 126.4 (C-1''), 130.2 (C-2''), 139.6 (C-5'), 153.6 (C-4''), 158.6 (C-4''), 160.8 (C-2''). 201.6 (C = O).

1-(5-Chloro-2,4-dihydroxyphenyl)-2-(4-methoxyphenyl) ethan-1-one (7c) [45]

Following the general method, a mixture of 5c (2.00 g, 13.38 mmol), 6 (2.76 g, 16.60 mmol), and BF$_3$• OEt$_2$ (3.93 g, 27.67 mmol) gave 7c (3.46 g, 86%) as a white solid. $Rf$ 0.28 (hexane/EtOAc, 7:3); mp 149–150 °C. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 3.80 (s, 3H, OCH$_3$), 4.14 (s, 2H, CH$_2$), 6.11 (t, 1H, OH-C$_5'$), 6.59 (s, 1H, H-3'), 6.87–6.92 (m, 2H, H-3''), 7.15–7.21 (m, 2H, H-2''), 7.82 (s, 1H, H-6'), 12.46 (s, 1H, OH-C-2'). $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 44.0 (CH$_3$), 55.3 (OCH$_3$), 104.8 (C-3'), 111.1 (C-5'), 114.1 (C-1'), 114.3 (C-3''), 125.6 (C-1'''), 130.4 (C-2''), 130.7 (C-6'), 157.7 (C-4''), 158.8 (C-4''), 164.2 (C-2''), 201.8 (C = O).

General method for the preparation of $\alpha$-arylacetophenones (9a-c)

At room temperature (rt), 2-bromo-4'-chloroacetophenone (8) (1.2 mol equiv) was added dropwise to a solution of 2,4-dihydroxyarylacetophenones 7a-c (1.0 mol equiv), and K$_2$CO$_3$ (1.5 mol equiv) in dried acetonitrile (25 ml), which was stirred for 15 min. After refluxing at 60 °C for 3 h, the reaction mixture was filtered, and the solvent removed under vacuum. The crude residue was purified by flash chromatography over silica gel (hexane/EtOAc, 7:3) to generate the corresponding products 9a-c.

1-(4-Chlorophenyl)-2-(3-hydroxy-4-(2-(4-methoxyphenyl)acetyl) phenoxy) ethan-1-one (9a)

Following the general method, a mixture of 7a (0.30 g, 1.16 mmol), 8 (0.325 g, 1.39 mmol), and K$_2$CO$_3$ (0.289 g, 2.09 mmol) delivered 9a (0.382 g, 80%) as a white solid. $Rf$ 0.82 (hexane/EtOAc, 7:3); mp 155–156 °C. $^1$H NMR (600 MHz, CDCl$_3$): $\delta$ 3.72 (s, 3H, OCH$_3$), 4.15 (s, 2H, CH$_2$-2''), 5.27 (s, 2H, CH$_2$-2''), 6.37 (dd, $J = 2.4$ Hz, 1H, H-1''), 6.52 (dd, $J = 9.0$, 2.4 Hz, 1H, H-6''), 6.85–6.89 (m, 2H, H-3''), 7.16–7.19 (m, 2H, H-2''), 7.47–7.50 (m, 2H, H-3''), 7.78 (d, $J = 9.0$ Hz, 1H, H-5''), 7.90–7.93 (m, 2H, H-2''). 12.68 (s, 1H, OH). $^{13}$C NMR (150 MHz, CDCl$_3$): $\delta$ 44.0 (CH$_2$-2''), 55.2 (CH$_3$O), 70.2 (CH$_2$-2), 101.9 (C-2''), 107.9 (C-6''), 113.8 (C-4''), 114.1 (C-3'''), 126.0 (C-1'''), 129.3 (C-3''), 129.4 (C-2''), 130.3 (C-2'''), 132.2 (C-5''), 132.3 (C-1''), 140.0 (C-4''), 158.6 (C-4'''), 164.0 (C-1''), 165.5 (C-3''), 192.0 (CO-1), 202.4 (CO-1 ''). HRMS (EI) [M$^+$] Calculated for C$_{23}$H$_{19}$ClO$_5$: 410.0921; found: 410.0909. LC/MS (ESI) [M + H]$^+$ Calculated for C$_{23}$H$_{19}$ClO$_5$: 411.09938; found: 411.09984; purity: 100%; $\tau_R = 0.49$.

1-(5-Chloro-2,4-dihydroxyphenyl)-2-(4-methoxy-2-methoxyphenyl)acetyl)phenoxy) ethan-1-one (9b)

Following the general method, mixing 7b (0.50 g, 1.73 mmol), 8 (0.485 g, 2.08 mmol), and K$_2$CO$_3$ (0.431 g, 3.12 mmol) resulted in 9b (0.61 g, 80%) as a white solid. $Rf$ 0.31 (hexane/EtOAc, 7:3); mp 152–154 °C. $^1$H NMR (600 MHz, DMSO-d$_6$): $\delta$ 3.72 (s, 3H, CH$_3$O-4''), 3.80 (s, 3H, CH$_2$O-2''), 4.29 (s, 2H, CH$_2$-2''), 5.70 (s, 2H, CH$_2$-H-2), 6.53 (s, 1H, H-6''), 6.88–6.90 (m, 2H, H-3'''), 7.20–7.23 (m, 2H, H-2'''), 7.47 (s, 1H, H-3''), 7.63–7.65 (m, 2H, H-3''), 8.00–8.03 (m, 2H, H-2''), 12.32 (s, 1H, OH). $^{13}$C NMR (150 MHz, DMSO-d$_6$): $\delta$ 43.9 (CH$_2$-2''), 55.1 (CH$_3$O-4'''), 56.6 (CH$_2$O-2''), 70.6 (CH$_2$-2), 101.8 (C-6''), 111.3 (C-3''), 113.1 (C-4''), 113.9 (C-3'''), 127.0 (C-1'''), 129.1 (C-2''), 130.0 (C-3''), 130.7 (C-2'''), 132.9 (C-1''), 138.9 (C-4'''), 141.7 (C-2'''), 155.0 (C-1''), 158.1 (C-4'''), 158.8 (C-5''), 192.7 (CO-1), 202.6 (CO-1''). HRMS (EI) [M$^+$] Calculated for C$_{25}$H$_{24}$ClO$_6$: 440.1027; found: 440.1029. LC/MS (ESI) [M + H]$^+$ Calculated for C$_{25}$H$_{24}$ClO$_6$: 441.10994; found: 441.11079; purity: 100%; $\tau_R = 0.48$.

General method for the synthesis of enaminoisoflavones (3a-c)

A mixture of the corresponding substituted $\alpha$-arylacetophenones 9a-c (1.0 mol equiv) and DMFDMA (3.0 mol equiv) at room temperature (rt) was poured into a threaded
ACE glass pressure tube with a sealed Teflon screw cap. It was heated at 120 °C for 12 h and diluted with CH₂Cl₂ (30 ml) before removing the solvent under vacuum. The residue was purified by flash chromatography over silica gel (hexane/EtOAc, 4:6) to generate the corresponding products 3a-c.

(Z)-7-(3-(4-Chlorophenyl)-1-(dimethylamino)-3-oxoprop-1-en-2-yl)oxy)-3-(4-methoxyphenyl)-4H-chromen-4-one (3a)

Following the general method, a mixture of 9a (0.10 g, 0.24 mmol) and DMFDMA (0.087 g, 0.73 mmol) afforded 3a (0.086 g, 75%) as a white solid. Rf 0.32 (hexane/EtOAc, 1:1), mp 200–201 °C. ¹H NMR (600 MHz, CDCl₃): δ 3.07 (s, 6H, N(CH₂)₃), 3.84 (s, 3H, CH₃O), 6.92 (brd, J = 1.8 Hz, 1H, H-8), 7.06 (brd, J = 8.7 Hz, 1H, H-6), 7.13–7.28 (brs, 1H, H-1'''), 7.34 (brd, J = 7.8 Hz, 2H, H-3'''), 7.45–7.50 (m, 2H, H-2'''), 7.62 (brd, J = 7.8 Hz, 2H, H-2'''), 7.89 (s, 1H, H-2), 8.20 (d, J = 8.7 Hz, 1H, H-5). ¹³C NMR (150 MHz, CDCl₃): δ 41.7 (N(CH₃)₃), 55.3 (CH₃O), 102.4 (C-8), 113.9 (C-3''), 114.2 (C-6), 119.3 (C-4a), 124.0 (C-1''), 124.8 (C-3), 126.1 (C-2''), 128.3 (C-5, C-3''), 129.8 (C-2''), 130.0 (C-2''), 136.6 (C-4'''), 137.5 (C-1'''), 144.7 (C-1''), 152.0 (C-2), 157.7 (C-8a), 159.5 (C-4''), 163.0 (C-7), 175.7 (CO-4), 187.0 (CO-3'). HRMS (IE) [M⁺] Calculated for C₂₈H₂₄ClNO₆: 506.13649; found: 506.13794; purity: 100%, Rf = 0.47.

7-Hydroxy-3-(4-methoxyphenyl)-4H-chromen-4-one (2a)

Following the method of preparation for 3a, a mixture of 7a (2.61 g, 10.11 mmol) and DMFDMA (3.61 g, 30.32 mmol) was heated at 120 °C for 3 h. The residue was purified by flash chromatography over silica gel (hexane/EtOAc, 8:2) to produce 2a (2.5 g, 92%) as a crystalline beige solid. Rf 0.57 (hexane/EtOAc, 7:3); mp 256–258 °C (Lit. 257–258 °C [42, 43]). ¹H NMR (300 MHz, DMSO-d₆): δ 3.78 (s, 3H, CH₃O), 6.86 (d, J = 2.1 Hz, 1H, H-8), 6.94 (dd, J = 8.9, 2.4 Hz, 1H, H-6), 6.96–7.01 (m, 2H, H-3''), 7.47–7.54 (m, 2H, H-2''), 7.97 (d, J = 8.9 Hz, 1H, H-5), 8.33 (s, 1H, H-1). ¹³C NMR (75 MHz, DMSO-d₆): δ 55.1 (CH₃O), 102.1 (C-8), 113.6 (C-3''), 115.2 (C-6), 116.5 (C-4a), 123.1 (C-3), 124.2 (C-1''), 127.3 (C-5), 130.1 (C-2''), 153.1 (C-2), 157.4 (C-8a), 158.9 (C-4''), 162.7 (C-7), 174.6 (CO-5). LC/MS (ESI) [M + H⁺] Calculated for C₁₉H₁₉ClNO₄: 269.08084; found: 269.08122; purity: 100%, Rf = 0.46.
2H, H-2'), 8.32 (s, 1H, H-2), 10.60 (s, 1H, OH). 13C NMR (150 MHz, DMSO-d6): δ 55.1 (CH2O-4'), 55.1 (CH2O-6), 102.8 (C-8), 104.7 (C-5), 113.6 (C-3'), 116.2 (C-4a), 122.6 (C-1'), 124.4 (C-3), 130.0 (C-2'), 146.9 (C-6), 151.7 (C-8a), 152.8 (C-2'), 152.9 (C-7), 158.9 (C-4'), 174.2 (C=O).

LC/MS (ESI) [M + H]+ Calculated for C24H24O6: 299.09140; found: 299.09259; purity: 100%; \( t_R = 0.46 \) .

Data for 10b: \( Rf \) 0.22 (hexane/EtOAc, 7:3); mp 173–175 °C (Lit. 174–176 °C [46]). 1H NMR (600 MHz, DMSO-d6): δ 3.70 (s, 3H, CH3O-4'), 3.87 (s, 3H, CH3O-6), 3.92 (s, 3H, CH3O-7) 6.98–7.01 (m, 2H, H-3'), 7.21 (s, 1H, H-8), 7.44 (s, 1H, H-5), 7.52–7.55 (m, 2H, H-2'), 8.41 (s, 1H, H-2). 13C NMR (150 MHz, DMSO-d6): δ 55.1 (CH2O-4'), 55.7 (CH2O-6), 56.3 (CH2O-7), 100.3 (C-8), 104.0 (C-5), 113.6 (C-3'), 116.9 (C-4a), 122.8 (C-1'), 124.3 (C-3), 130.0 (C-2'), 147.4 (C-6), 151.7 (C-8a), 153.1 (C-2), 154.2 (C-7), 158.9 (C-4'), 174.2 (C=O).

6-Chloro-7-hydroxy-3-(4-methoxyphenyl)-4H-chromen-4-one (2c). 6-Chloro-7-methoxy-3-(4-methoxyphenyl)-4H-chromen-4-one (10b)

Following the method of preparation for 3a, a mixture of 7c (2.37 g, 8.10 mmol) and DMFDMAL (2.89 g, 24.3 mmol) was heated at 120 °C for 3 h to deliver 2c (2.01 g, 82%) as a white solid and 7c (0.23 g, 9%) as a white solid. Data for 2c: \( Rf \) 0.27 (hexane/EtOAc, 7:3); mp 232–233 °C [45]. 1H NMR (600 MHz, DMSO-d6): δ 3.78 (s, 3H, CH3O), 6.97–6.99 (m, 2H, H-3'), 7.05 (s, 1H, H-8), 7.48–7.49 (m, 2H, H-2'), 7.99 (s, 1H, H-5), 8.35 (s, 1H, H-2), 11.76 (brs, 1H, OH). 13C NMR (150 MHz, DMSO-d6): δ 55.1 (CH3O), 103.6 (C-8), 113.6 (C-3'), 117.1 (C-4a), 119.6 (C-6), 123.1 (C-3), 123.8 (C-1'), 126.1 (C-5), 130.1 (C-2'), 153.4 (C-2'), 155.6 (C-8a), 157.9 (C-7), 159.1 (C-4'), 173.8 (C=O). LC/MS (ESI) [M + H]+ Calculated for C16H16ClO4: 293.04186; found: 293.04311; purity: 100%; \( t_R = 0.47 \) .

Data for 10b: \( Rf \) 0.29 (hexane/EtOAc, 7:3); mp 217–219 °C (Lit. 216–218 °C [9]). 1H NMR (600 MHz, DMSO-d6): δ 3.79 (s, 3H, CH3O-4'), 4.01 (s, 3H, CH3O-7), 6.91 (s, 1H, H-8), 6.95–7.02 (m, 2H, H-3'), 7.41 (s, 1H, H-5), 7.49–7.56 (m, 2H, H-2'), 8.06 (s, 1H, H-5), 8.49 (s, 1H, H-2). 13C NMR (150 MHz, DMSO-d6): δ 55.1 (CH3O-4'), 57.3 (CH3O-7), 101.6 (C-8), 113.6 (C-3'), 117.8 (C-4a), 120.0 (C-6), 123.3 (C-3), 123.7 (C-1'), 125.7 (C-5), 130.0 (C-2'), 153.8 (C-2), 155.9 (C-8a), 158.6 (C-7), 159.1 (C-4'), 173.7 (C=O).

7-(2-(4-Chlorophenyl)-2-oxoethoxy)-3-(4-methoxyphenyl)-4H-chromen-4-one (4a)

Following the method of preparation for 9a, mixing 2a (0.20 g, 0.74 mmol), and K2CO3 (0.20 g, 0.74 mmol) in 2H, H-2'), 8.32 (s, 1H, H-2), 10.60 (s, 1H, OH). 13C NMR (150 MHz, DMSO-d6): δ 55.1 (CH2O-4'), 55.1 (CH2O-6), 102.8 (C-8), 104.7 (C-5), 113.6 (C-3'), 116.2 (C-4a), 122.6 (C-1'), 124.4 (C-3), 130.0 (C-2'), 146.9 (C-6), 151.7 (C-8a), 152.8 (C-2'), 152.9 (C-7), 158.9 (C-4'), 174.2 (C=O).
159.1 (C-4'), 173.7 (CO-4), 192.1 (CO-2'). HRMS (EI) [M+] Calculated for C_{35}H_{15}Cl_{13}O_{17}: 454.0375; found: 454.0382. LC/MS (ESI) [M + H]^+ Calculated for C_{35}H_{16}Cl_{13}O_{17}: 455.04476; found: 455.04582; purity: 100%, r_{f} = 0.49.

**Biological evaluation**

**Inhibition of α-glucosidase**

α-Glucosidase inhibition was determined according to a method described by Salehi et al. with some modifications [47]. For this assay, α-glucosidase from *S. cerevisiae* was employed. A reaction was prepared by mixing 20 µl of α-glucosidase solution (0.5 unit/ml), 120 µl of 0.1 M phosphate buffer (pH 6.9), and 10 µl of the sample at several concentrations. The solution was incubated in a 96-well microplate at 37 °C for 15 min. Subsequently, the enzymatic reaction was initiated by adding 20 µl of 5 mM PNP solution in 0.1 M phosphate buffer (pH 6.9), followed by incubation at 37 °C for 15 min. The reaction was stopped by adding 80 µl of 0.2 M sodium carbonate solution and absorbance was read at 405 nm in a microplate reader (Epoch, BioTek®). The reaction system without any test compounds was used as the control, and the system without α-glucosidase served as the blank for correcting the background absorbance. The rate of inhibition on α-glucosidase by the sample was calculated with Eq. (1):

$$\% \text{ of inhibition} = \left( \frac{\text{Control absorbance} - \text{sample absorbance}}{\text{Control absorbance}} \right) \times 100$$

(DPPH radical scavenging assay)

The scavenging of free radicals by the synthesized compounds was assessed with the previously reported 2,2-diphenyl-1-picyrylhydrazyl radical (DPPH) assay, with some modifications [48]. After a solution of each compound was elaborated at a concentration of 2.5 mM in DMSO, a solution of DPPH (133.33 µM in absolute ethanol) was added at a ratio of 1:3 (v/v). The mixture was incubated at 37 °C for 30 min before reading the absorbance in a microplate reader (Epoch, BioTek®) at 517 nm. Butylhydroxytoluene served as the positive control. Scavenging capacity was expressed as the percent decrease in DPPH at 2.5 mM:

$$\text{SC} \% = \left[ \left( A_{\text{control}} - A_{\text{test}} \right) / A_{\text{control}} \right] \times 100$$

where $A_{\text{control}}$ is the absorbance of the DPPH solution (control) and $A_{\text{test}}$ is the absorbance of the DPPH solution plus a compound.

**Molecular docking studies**

Molecular docking simulations were carried out on the AutoDock 4 program [49]. The α-glucosidase homology model was constructed by using the crystal structure of isomaltase from *Saccharomyces cerevisiae* in complex with its competitive inhibitor maltose (Protein Data Bank (PDB): 3A4A), which was retrieved from the PDB (http://www.rcsb.org/). Docking was validated with maltose to identify the main side chains present at the active site of the enzyme. Water molecules were removed, hydrogen atoms were added to the polar atoms (considering pH at 7.4), and Kollman charges were assigned with AutoDock Tools 1.5.6. The 3D structures of maltose and acarbose were downloaded from the ZINC 15 database [50]. The alkoxy-isoflavone derivatives were sketched in two dimensions with ChemSketch (https://www.acdlabs.com/resources/freeeware/chemsketch/) and converted into 3D mol2 format in the Open Babel GUI program [51]. The maltose and acarbose ligands were optimized with PM6 on Gaussian 98/75 software to obtain the minimum energy conformation for the docking studies. All the possible rotatable bonds, torsion angles, atomic partial charges and non-polar hydrogens were determined for each ligand. The grid dimensions in AutoDockTools were 60 × 72 × 66 Å with points separated by 0.375 Å, centered at: $X = 24.0, Y = -8.0$ and $Z = 23.028$. The hybrid Lamarckian genetic algorithm was applied for minimization, utilizing default parameters. A total of 100 docking runs were conducted, adopting the conformation with the lowest binding energy (kcal/mol) for all further simulations. AutoDockTools was used to prepare the script and files as well as to visualize the docking results, which were edited in Discovery 4.0 Client.

**Physicochemical properties**

The physicochemical properties of compounds 2a–c, 3a–c, 4a–c, 7a–c, 9a–c, 10a–b, maltose (11), and acarbose (12) were generated in silico. These parameters were determined with the OSIRIS DataWarrior V4.7.2 program (http://www.organic-chemistry.org/prog/peo/) [52]. The drug-likeness was evaluated by using Lipinski’s rule of five.

**Acknowledgements** We thank Dr. Elvia Becerra (Laboratorio Multi-disciplinario de Caracterización del Centro de Nanociencias y Micro y Nanotecnologías del IPN) for her assistance in recording the NMR (750 MHz) spectra and Bruce A. Larsen for proofreading. MCC-L, FEI-M, JT, and AM-M gratefully acknowledge the financial support provided by the SIP-IPN (grants 20195786, 20201314, 20195228, 20200227, and 20210700), CONACYT (grants 300520 and A1-S-17131), and TECNM (grant 9ff8r1 (9991.21-P)). CHE and RUG are grateful to CONACYT for awarding them graduate and research stay scholarships and thank the SIP/IPN (BEIFI) and the Ludwig K. Hellweg Foundation for scholarship complements. MCC-L, FEI-M, OG-G, JT, and AM-M are fellows of the EDI-IPN and/or COFAA-IPN programs.
Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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