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Synthesis of alkoxy-isoflavones as potential α -glucosidase inhibitors

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Abstract

The aim of the present study was to synthesize isoflavone-enaminones **3a-c** and 7-alkoxyisoflavones **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 a greater inhibition of α -glucosidase than the positive control acarbose. The series of isoflavones **3a-c** and **4a-c** showed a higher inhibitory activity (IC_{50} = 6.3 - 87.6 μ M) than the parental 7-hydroxyisoflavones **2a-c** (IC_{50} = 109.4 - 173.2 μ M), 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 the inhibitory activity by hydrophobic effects according to docking calculations.

Graphical abstract

Key words: α -Glucosidase; diabetes mellitus; enaminone; isomaltase; alkoxy-isoflavones

Introduction

α -Glucosidase is an enzyme that catalyzes the hydrolysis of glycosidic bonds from the non-reducing portion of the oligosaccharide substrates, playing a key role in the digestion of dietary carbohydrates and the processing of glycoproteins. The inhibition of this enzyme decreases the digestion of starch and other dietary sugars, thus helping to avoid hyperglycemia by maintaining blood glucose at normal levels [1]. Hence, α -glucosidase is an attractive therapeutic target for the treatment of type 2 diabetes [2], cardiovascular diseases [3], and cancer [4, 5].

Diabetes is a metabolic disease characterized by high levels of blood glucose (hyperglycemia) derived from a deficiency in the secretion and/or action of insulin, a condition that affects around 422 million people worldwide [6]. Therefore, α -glucosidase inhibitors represent an effective therapeutic strategy for reducing post-prandial hyperglycemia.

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 have the potential to 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 factors 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].

Fig. 1 A) Structure of enaminone-chromones **1** and natural isoflavones **2**; B) Enaminone-isoflavones **3** and alkoxy-isoflavones **4** as α -glucosidase inhibitors

In accordance with these antecedents, the 7-hydroxy group and its 7-alkoxylated isoflavone derivatives as well as the 6-methoxy-free or the 6-methoxy-containing analogues 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. Hence, taking into consideration 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, and focusing particular attention on the role played by the enaminone moiety, 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 and antimicrobial effects. The analysis of the results provided insights into the structure-activity relationship of the isoflavone-based derivatives in the inhibition of α -glucosidase. Additionally, docking studies were carried out to better understand the mechanism of binding of the present 7-alkoxy-isoflavone derivatives to isomaltase, an α -glucosidase of *Saccharomyces cerevisiae*.

Results and discussion

Chemistry

Compounds **7a-c** were prepared in good yields by acylation of resorcinols **5a-c** with 2-(4-methoxyphenyl)acetic acid (**6**), in the presence of boron trifluoride diethyl etherate ($\text{BF}_3 \cdot \text{OEt}_2$). The *O*-alkylation reaction of 2,4-dihydroxyacetophenones **7a-c** employing 2-bromo-1-(4-chlorophenyl)ethanone (**8**) provided 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).

Scheme 1 Synthesis of enaminone-isoflavones **3a-c**

On the other hand, 2,4-dihydroxyacetophenones **7a-c** were treated with DMFDMA to give the series of isoflavones **2a-c** in high yields. Furthermore, 7-methoxyisoflavones **10a-b** were isolated as by-products in low yields [32]. Finally, target compounds **4a-c** were synthesized by *O*-alkylation of isoflavones **2a-c** with **8** (Scheme 2).

Scheme 2 Synthesis of 7-arylalkoxy-isoflavones **4a-c**

The structure of all the test compounds was elucidated by 1D and 2D NMR techniques and HRMS. The intramolecular cyclization and condensation of the α -arylacetophenones **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].

Evaluation of the antioxidant activity

Antioxidants are helpful agents responsible for protecting the body against oxidative stress and many other disorders such as diabetes. To determine the potential of presently synthesized compounds as antioxidants, their free radical scavenging was examined by the DPPH radical assay, with butylated hydroxytoluene (BHT) as the positive control. The reduction in color intensity represented the percentage of inhibition of DPPH (Table 1), findings 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, being 61% at 2.5 mM, suggesting that this effect is mainly related to hydroxyl groups substituted in the benzene ring.

In vitro α -glucosidase inhibition

All the test compounds were assessed for their inhibition of α -glucosidase. The IC_{50} 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** and **9c** was better. Indeed, **9a** ($IC_{50} = 20.0 \mu M$) proved to have a 15-fold greater activity than acarbose (**12**) ($IC_{50} = 309.2 \mu M$). 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, being 23-fold more active 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 whether 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**. It turns out that the best inhibition was found with **4a**, with an IC_{50} value (6.3 μ M) indicating a 49-fold greater inhibition of α -glucosidase than **12**.

Upon comparing compounds **3a-b** and **4a-b**, the analysis of the structure-activity relationship indicated that the polar enaminone group is not as relevant as the (4-chlorophenyl)-2-oxoethoxy group for increasing the inhibitory effect. In contrast, the enaminone group and the chlorine atoms in **3c** improves 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 play 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** leads to higher inhibitory activity than the presence of a methoxy group in **3b**, **4b** and **9b** or a chlorine atom in **4c** and **9c**.

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

Compound	% DPPH scavenging activity (2.5 mM)	α -Glucosidase inhibition	
		% (400 μ M)	(IC ₅₀ μ M)
2a	1.86 $\pm$ 0.79	99.51 $\pm$ 0.69	109.4 $\pm$ 0.19
2b	10.0 $\pm$ 0.15	99.15 $\pm$ 0.05	173.2 $\pm$ 0.37
2c	15.3 $\pm$ 2.19	99.80 $\pm$ 0.17	170.4 $\pm$ 0.28
3a	0.15 $\pm$ 0.05	97.44 $\pm$ 0.27	18.8 $\pm$ 0.13
3b	14.6 $\pm$ 1.32	98.40 $\pm$ 0.46	87.6 $\pm$ 0.99
3c	11.3 $\pm$ 0.82	99.98 $\pm$ 0.06	13.0 $\pm$ 0.011
4a	5.02 $\pm$ 0.04	99.97 $\pm$ 0.05	6.3 $\pm$ 0.11
4b	6.4 $\pm$ 0.32	99.59 $\pm$ 0.27	45.5 $\pm$ 0.08
4c	4.2 $\pm$ 0.98	98.70 $\pm$ 0.02	84.3 $\pm$ 0.01
7a	61.36 $\pm$ 0.63	92.18 $\pm$ 0.18	143.8 $\pm$ 0.14
7b	20.37 $\pm$ 0.52	14.24 $\pm$ 1.87	>400
7c	21.3 $\pm$ 0.21	20.42 $\pm$ 1.76	>400
9a	34.02 $\pm$ 0.03	99.53 $\pm$ 0.32	20.0 $\pm$ 0.29
9b	17.86 $\pm$ 1.12	82.61 $\pm$ 2.73	199.1 $\pm$ 0.12
9c	6.72 $\pm$ 1.03	95.20 $\pm$ 0.42	34.1 $\pm$ 0.013
10a	2.40 $\pm$ 0.10	31.28 $\pm$ 1.15	>400
10b	6.72 $\pm$ 1.03	6.72 $\pm$ 1.03	>400
BHT	85.02 $\pm$ 3.33 ^a	nt	nt
Acarbose	nt	63.18 $\pm$ 0.7	309.2 $\pm$ 0.9

^a IC₅₀ = 0.84 $\pm$ 0.08 mM; nt, not tested.

***In vitro* antimicrobial activity**

Invasive fungal infections represent a continuous and serious threat to human health. These infections are very common in patients immunocompromised by aggressive therapies for certain diseases, including diabetes, cancer, and HIV/AIDS [33]. *o*-Hydroxyaryl ketones are versatile building blocks for the preparation of benzopyrans such as chromones and isoflavones, for which antifungal effects have been described [8, 26, 34-36]. The test compounds were fungicidal, since the use of resazurin revealed no redox metabolism in fungal cells at the reported MIC. Redox metabolism is known to be a biochemical indicator of cell survival [37].

The antifungal activity of the present α -glucosidase inhibitors was poor against *C. albicans*, *S. cerevisiae* and *S. typhi*, considering that the compounds were applied in their pure form and that a high concentration was required to obtain good inhibition. Inhibition was expressed with MIC values, which oscillated between 500-3000 $\mu\text{g/mL}$ (Table S1). Nevertheless, **3a** showed an acceptable MIC for *S. cerevisiae*, and further modifications of its chemical scaffold may improve the antifungal effect. In a previous study, the antimicrobial activity of a series of 3-alkyl chromone derivatives was evaluated against *C. albicans* strains. According to the results, the heterocycle and a short aliphatic chain at the C-3 position of the chromone ring promotes the antifungal effect [15].

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 served as the target protein because of the lack of an available crystal structure for α -glucosidase from *S. cerevisiae*. Two competitive inhibitors of isomaltase, maltose (**11**) and acarbose (**12**) were employed as positive controls. 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 isomaltase inhibitors [38-41]. In all the compounds, the two residues involved in catalysis (Glu277 and Asp352) are present, suggesting that the derivatives of 7-alkoxy- and 7-hydroxyisoflavones bind to the active site of the enzyme to exert their mechanism of the inhibition of α -glucosidase [38].

Maltose (11)

4a

9c

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**. In the 3D models, the hydrogen bonds are illustrated, as well as 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 - π 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)

The binding energy values (ΔG) and the type of interactions are shown in Table 2. The best values ΔG were found for **2a** (-7.51 Kcal/mol), **3a** (-9.82 Kcal/mol), **4a** (-9.58 Kcal/mol),

and **9c** (-9.83 Kcal/mol), being better than the corresponding value for the competitive maltose (**11**) inhibitor (-5.55 Kcal/mol). Derivative **9c** exhibited the best binding to the active site of isomaltase. 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 the maltose inhibitor are those formed between some substituent of the compounds and the Asp352 or Arg442 residues. The hydrophilic interaction with Phe178 is present in the four compounds evaluated, as observed in other studies [39-41].

Table 2 Docking results of isoflavones **2a**, **3a**, and **4a**, aryloxy carbonyl compound **9c**, maltose (**11**) and acarbose (**12**) at the active site of isomaltase

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 $\cdots$ O (Asp69) O-H $\cdots$ O (Gln182) O $\cdots$ H-N (Arg213) C-H $\cdots$ O (Asp215) O-H $\cdots$ O (Glu277) C-H $\cdots$ O (Glu277) C-H $\cdots$ O (Asp352) O $\cdots$ H-N (Arg442)	-
12	-7.78	Asp69, Tyr72, His112, Tyr158, Phe159, Phe178, Arg213, Asp215, Val216, Glu277, Gln279, His 280, Phe303, Asp307, Arg315, Tyr316, His351, Asp352, Gln353, Glu411, Arg442, Arg446	C-H $\cdots$ O (Asp69) O-H $\cdots$ O (Asp215) O $\cdots$ H-N (Gln279) O-H $\cdots$ O (Asp307) O $\cdots$ H-N (His351) O-H $\cdots$ O (Glu411) O $\cdots$ H-N (Arg446)	-
2a	-7.51	Asp69, Tyr72, His112, Phe159,		π - π stacked Phe178

3a	-9.82	Phe178, Gln182, Asp215, Val216, Glu277, Gln279, Phe303, Thr306, Asp307, Arg315, Asp352, Gln363, Arg442.	O-H····O (Asp69) O-H····O (Gln182) C-H····O (Thr306) C-H····O (Gln353) O····H-N (Arg442)	π-alkyl Val 216 π-π stacked Phe303
		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, Val 216, Arg315
4a	-9.58	Asp69, Tyr72, His112, Lys156, Ser157, Tyr158, Phe159, Gly160, Phe178, Gln182, Asp215, Val216, Glu277, Gln279, Phe303, Phe314, Asp352, Glu411, Asp415, Arg442		π-π stacked Tyr158
			O····H-N (Gln279) C-H····O (Asp352) C-H····O (Asn415)	π-alkyl Tyr72, His112, Lys156, Phe178
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-alkyl Phe159, Phe178, Val216, Arg315
				π-π Stacked Phe314

The results of the docking study coincide with those of the enzyme inhibition assays, evidenced by the greater inhibition of the α -glucosidase enzyme that was generally produced by compounds **3a**, **4a**, and **9c**. The corresponding IC₅₀ values were $18.8 \pm 0.13 \mu\text{M}$, $6.3 \pm 0.11 \mu\text{M}$, and $34.1 \pm 0.013 \mu\text{M}$, respectively, much lower than the IC₅₀ of **12** ($309.2 \pm 0.9 \mu\text{M}$). Considering these findings, modifications of the structure of the test compounds could

possibly give rise to a higher anti-glucosidase activity, and therefore these scaffolds provide promise for the development of new drugs to treat diabetes mellitus.

Conclusions

The series of isoflavones **2-4** were easily synthesized in good yields. They were evaluated *in vitro* as α -glucosidase inhibitors and antioxidant agents, showing a greater inhibition of α -glucosidase 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 plays a major role in the interaction with the active site of the enzyme. Compound **7a** showed slight antioxidant activity. Hence, the series of α -arylacetophenones and isoflavones herein prepared can be considered promising candidates 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 ^{13}C (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-GCMatell instrument. Acarbose, α -glucosidase from *Saccharomyces cerevisiae*, dimethyl sulfoxide (DMSO), *p*-nitrophenyl- α -D-glucopyranoside (PNP) and the reagents used for the 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 described [42].

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 x 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 98:2) 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. *R_f* 0.85 (hexane/EtOAc, 8:2), mp 154-155 °C (Lit. 158-160 °C [43, 44]). ¹H NMR (300 MHz, DMSO-*d*₆): δ 3.72 (s, 3H, OCH₃), 4.20 (s, 2H, CH₂-2), 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-*d*₆): 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. *R_f* 0.31 (hexane/EtOAc, 7:3); mp 114-115 °C (Lit., 122-124 °C [45]). 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 (s, 1H, OH-C2'). ¹³C NMR (125 MHz, CDCl₃): δ 44.4 (CH₂), 55.2 (OCH₃), 56.4 (OCH₃), 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**) [46]. Following the general method, a mixture of **5c** (2.00 g, 13.38 mmol), **6** (2.76 g, 16.60 mmol), and BF₃•OEt₂ (3.93 g, 27.67 mmol) gave **7c** (3.46 g, 86%) as a white solid. *R_f* 0.28 (hexane/EtOAc, 7:3); mp 149-150 °C. ¹H NMR (500 MHz, CDCl₃): δ 3.80 (s, 3H, OCH₃), 4.14 (s, 2H, CH₂), 6.11 (brs, 1H, OH-C4'), 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-C2'). ¹³C NMR (125 MHz, CDCl₃): δ 44.0 (CH₂), 55.3 (OCH₃), 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 α-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 K₂CO₃ (1.5 mol equiv) in dried acetone (25 mL), which was stirred for 15 minutes. 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 K₂CO₃ (0.289 g, 2.09 mmol) delivered **9a** (0.382 g, 80%) as a white solid. *R*_f 0.82 (hexane/EtOAc, 7:3), mp 155-156 °C. ¹H NMR (600MHz, CDCl₃): δ 3.72 (s, 3H, CH₃O), 4.15 (s, 2H, CH₂-2''), 5.27 (s, 2H, CH₂-2), 6.37 (d, *J* = 2.4 Hz, 1H, H-2''), 6.52 (dd, *J* = 9.0, 2.4 Hz, 1H, H-6''), 6.85-6.89 (m, 2H, H-3^{IV}), 7.16-7.19 (m, 2H, H-2^{IV}), 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). ¹³C NMR (150 MHz, CDCl₃): δ 44.0 (CH₂-2''), 55.2 (CH₃O), 70.2 (CH₂-2), 101.9 (C-2''), 107.9 (C-6''), 113.8 (C-4''), 114.1 (C-3^{IV}), 126.0 (C-1^{IV}), 129.3 (C-3'), 129.4 (C-2'), 130.3 (C-2^{IV}), 132.2 (C-5''), 132.3 (C-1'), 140.0 (C-4'), 158.6 (C-4^{IV}), 164.0 (C-1''), 165.5 (C-

3''), 192.0 (CO-1), 202.4 (CO-1'''). HRMS (EI) [M⁺] Calculated for C₂₃H₁₉ClO₅: 410.0921; found: 410.0909.

1-(4-Chlorophenyl)-2-(5-hydroxy-2-methoxy-4-(2-(4-methoxyphenyl)acetyl)phenoxy)ethanone-1-one (**9b**). Following the general method, mixing **7b** (0.50 g, 1.73 mmol), **8** (0.485 g, 2.08 mmol), and K₂CO₃ (0.431 g, 3.12 mmol) resulted in **9b** (0.61 g, 80%) as a white solid. *R*_f 0.31 (hexane/EtOAc, 7:3); mp 152-154 °C. ¹H NMR (600 MHz, DMSO-*d*₆): δ 3.72 (s, 3H, CH₃O-4^{IV}), 3.80 (s, 3H, CH₃O-2''), 4.29 (s, 2H, CH₂-2''), 5.70 (s, 2H, CH₂, H-2), 6.53 (s, 1H, H-6''), 6.88-6.90 (m, 2H, H-3^{IV}), 7.20-7.23 (m, 2H, H-2^{IV}), 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). ¹³C NMR (150 MHz, DMSO-*d*₆): δ 43.9 (CH₂-2'''), 55.1 (CH₃O-4^{IV}), 56.6 (CH₃O-2''), 70.6 (CH₂-2), 101.8 (C-6''), 111.3 (C-3''), 113.1 (C-4''), 113.9 (C-3^{IV}), 127.0 (C-1^{IV}), 129.1 (C-2'), 130.0 (C-3'), 130.7 (C-2^{IV}), 132.9 (C-1'), 138.9 (C-4'), 141.7 (C-2''), 155.0 (C-1''), 158.1 (C-4^{IV}), 158.8 (C-5''), 192.7 (CO-1), 202.6 (CO-1'''). HRMS (EI) [M⁺] Calculated for C₂₄H₂₁ClO₆: 440.1027; found: 440.1029.

1-(5-Chloro-4-(2-(4-chlorophenyl)-2-oxoethoxy)-2-hydroxyphenyl)-2-(4-methoxyphenyl)ethan-1-one (**9c**). Following the general method, mixing **7c** (0.20 g, 0.68 mmol), **8** (0.19 g, 0.82 mmol), and K₂CO₃ (0.14 g, 1.02 mmol) led to **9c** (0.123 g, 81%) as a beige solid. *R*_f 0.52 (hexane/EtOAc, 7:3); mp 192-193 °C. ¹H NMR (500 MHz, CDCl₃): δ 3.80 (s, 3H, CH₃O), 4.14 (s, 2H, CH₂-2), 5.53 (s, 2H, CH₂-1''), 6.33 (s, 1H, H-3'), 6.86-6.92 (m, 2H, H-3^{IV}), 7.14-7.20 (m, 2H, H-2^{IV}), 7.46-7.52 (m, 2H, H-3'''), 7.86 (s, 1H, H-6'), 7.91-7.97 (m, 2H, H-2'''), 12.55 (s, 1H, OH). ¹³C NMR (125 MHz, CDCl₃): δ 44.0 (CH₂-2), 55.2 (CH₃O), 71.1 (CH₂-1''), 101.9 (C-3'), 113.4 (C-5'), 113.8 (C-1'), 114.3 (C-3^{IV}), 125.4 (C-1^{IV}), 129.3

(C-3'''), 129.6 (C-2'''), 130.3 (C-2^{IV}), 131.6 (C-6'), 132.2 (C-1'''), 140.8 (C-4'''), 158.8 (C-4^{IV}), 159.2 (C-4'), 163.9 (C-2'), 191.3 (CO-2''), 201.9 (CO-1). HRMS (EI) [M⁺] Calculated for C₂₃H₁₈Cl₂O₅: 444.0531; found: 444.0531.

General method for the synthesis of enaminone-isoflavones (**3a-c**)

A mixture of the corresponding substituted α -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)-4*H*-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. *R*_f 0.32 (hexane/EtOAc, 1:1), mp 200-201 °C. ¹H NMR (600 MHz, CDCl₃): δ 3.07 (s, 6H, N(CH₃)₂), 3.84 (s, 3H, CH₃O), 6.92 (brd, *J* = 1.8 Hz, 1H, H-8), 6.94-6.98 (m, 2H, H-3'), 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)₂), 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 $C_{27}H_{22}ClNO_5$: 475.1186; found: 475.1194.

(*Z*)-7-((3-(4-Chlorophenyl)-1-(dimethylamino)-3-oxoprop-1-en-2-yl)oxy)-6-methoxy-3-(4-methoxyphenyl)-4*H*-chromen-4-one (**3b**). Following the general method, a mixture of **9b** (0.20 g, 0.45 mmol) and DMFDMA (0.16 g, 1.36 mmol) furnished **3b** (0.21 g, 92%), as a yellow solid. *R*_f 0.34 (hexane/EtOAc, 1:2); mp 205-206 °C. ¹H NMR (600 MHz, CDCl₃): δ 3.07 (s, 6H, N(CH₃)₂), 3.84 (s, 3H, CH₃O-4'), 3.99 (s, 3H, CH₃O-6), 6.89 (s, 1H, H-8), 6.95-6.97 (m, 2H, H-3'), 7.11-7.23 (brs, 1H, H-1''), 7.31-7.33 (m, 2H, H-3'''), 7.46-7.49 (m, 2H, H-2'), 7.63-7.67 (m, 3H, H-2'''), H-5), 7.88 (s, 1H, H-2). ¹³C NMR (150 MHz, CDCl₃): δ 41.7 (N(CH₃)₂), 55.3 (CH₃O-4'), 56.4 (CH₃O-6), 102.5 (C-8), 106.0 (C-5), 113.9 (C-3'), 118.9 (C-4a), 124.2 (C-3), 124.3 (C-1'), 126.2 (C-2''), 128.2 (C-3'''), 129.8 (C-2'), 130.1 (C-2'''), 136.6 (C-1'''), 137.5 (C-4'''), 141.0 (C-1''), 147.4 (C-6), 151.85 (C-8a), 151.87 (C-2), 153.3 (C-7), 159.5 (C-4'), 175.4 (CO-4), 186.4 (CO-3''). HRMS (EI) [M^+] Calculated for $C_{28}H_{24}ClNO_6$: 505.1292; found: 505.1300.

(*Z*)-6-Chloro-7-((3-(4-chlorophenyl)-1-(dimethylamino)-3-oxoprop-1-en-2-yl)oxy)-3-(4-methoxyphenyl)-4*H*-chromen-4-one (**3c**). Following the general method, a mixture of **9c** (0.05 g, 0.11 mmol) and DMFDMA (0.040 g, 0.33 mmol) gave **3c** (0.045 g, 79%), as a brown solid. *R*_f 0.30 (hexane/EtOAc, 1:2); mp 211-213 °C. ¹H NMR (750 MHz, CDCl₃): δ 3.09 (s, 6H, N(CH₃)₂), 3.83 (s, 3H, CH₃O), 6.91 (s, 1H, H-8), 6.92-6.97 (m, 2H, H-3'), 7.23-7.37 (m, 3H, H-1'', H-3'''), 7.42-7.47 (m, 2H, H-2'), 7.63-7.72 (m, 2H, H-2'''), 7.87 (s, 1H, H-2), 8.26 (s, 1H, H-5). ¹³C NMR (187.5 MHz, CDCl₃): δ 41.3 (N(CH₃)₂), 55.3 (CH₃O), 102.9 (C-8), 113.9 (C-3'), 119.6 (C-4a), 120.7 (C-6), 123.6 (C-1'), 124.9 (C-3), 127.8 (C-5), 128.3 (C-

3'''), 129.8 (C-2'''), 130.0 (C-2'), 136.9 (C-1'''), 137.5 (C-4'''), 141.0 (C-1''), 152.1 (C-2), 155.8 (C-8a), 158.0 (C-7), 159.6 (C-4'), 174.7 (CO-4), 186.2 (CO-3''). HRMS (EI) [M⁺] Calculated for C₂₇H₂₁Cl₂NO₅: 509.0797; found: 509.0799.

7-Hydroxy-3-(4-methoxyphenyl)-4*H*-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. *R*_f 0.57 (hexane/EtOAc, 7:3); mp 256-258 °C (Lit. 257-258 °C [43, 44]). ¹H NMR (300 MHz, DMSO-*d*₆): 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-2). ¹³C NMR (75 MHz, DMSO-*d*₆): 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 (C=O).

7-Hydroxy-6-methoxy-3-(4-methoxyphenyl)-4*H*-chromen-4-one (**2b**). 6,7-Dimethoxy-3-(4-methoxyphenyl)-4*H*-chromen-4-one (**10a**). Following the method of preparation for **3a**, a mixture of **7b** (3.00 g, 10.41 mmol) and DMFDMA (3.72 g, 31.21 mmol) was heated at 120 °C for 3 h to provide **2b** (2.5 g, 80%) as a beige solid and **10a** (0.256 g, 8%) as a yellow solid. Data for **2b**: *R*_f 0.15 (hexane/EtOAc, 7:3); mp 222-224 °C (Lit. 225-226 °C [45]). ¹H NMR (600 MHz, DMSO-*d*₆): δ 3.78 (s, 3H, CH₃O-4'), 3.88 (s, 3H, CH₃O-6), 6.95 (s, 1H, H-8), 6.96-6.99 (m, 2H, H-3'), 7.44 (s, 1H, H-5), 7.49-7.53 (m, 2H, H-2'), 8.32 (s, 1H, H-2), 10.60 (s, 1H, OH). ¹³C NMR (150 MHz, DMSO-*d*₆): δ 55.1 (CH₃O-4'), 55.1 (CH₃O-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). Data for **10b**: *R_f* 0.22 (hexane/EtOAc, 7:3); mp 173-175 °C (Lit. 174-176 °C [47]). ¹H NMR (600 MHz, DMSO-*d*₆): δ 3.79 (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-*d*₆): δ 55.1 (CH₃O-4'), 55.7 (CH₃O-6), 56.3 (CH₃O-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)-4*H*-chromen-4-one (**2c**). 6-Chloro-7-methoxy-3-(4-methoxyphenyl)-4*H*-chromen-4-one (**10b**). Following the method of preparation for **3a**, a mixture of **7c** (2.37 g, 8.10 mmol) and DMFDMA (2.89 g, 24.3 mmol) was heated at 120 °C for 3 h to deliver **2c** (2.01 g, 82%) as a beige solid and **10b** (0.23 g, 9%) as a white solid. Data for **2c**: *R_f* 0.27 (hexane/EtOAc, 7:3); mp 232-233 °C [46]. ¹H NMR (600 MHz, DMSO-*d*₆): 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-*d*₆): 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). Data for **10b**: *R_f* 0.29 (hexane/EtOAc, 7:3); mp 217-219 °C (Lit. 216-218 °C [9]). ¹H NMR (600 MHz, DMSO-*d*₆): 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-*d*₆): 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)-4*H*-chromen-4-one (**4a**).

Following the method of preparation for **9a**, mixing **2a** (0.20 g, 0.74 mmol), **8** (0.21 g, 0.89 mmol), and K₂CO₃ (0.15 g, 1.11 mmol) resulted in **4a** (0.264 g, 84%) as a colorless crystalline solid. *R*_f 0.57 (hexane/EtOAc, 7:3); mp 224-225 °C. ¹H NMR (500 MHz, DMSO-*d*₆): δ 3.79 (s, 3H, CH₃O), 5.80 (s, 2H, CH₂-1''), 6.97-7.01 (m, 2H, H-3'), 7.17 (dd, *J* = 9.0, 2.5 Hz, 1H, H-6), 7.28 (d, *J* = 2.5 Hz, 1H, H-8), 7.51-7.55 (m, 2H, H-2'), 7.66-7.70 (m, 2H, H-3'''), 8.03-8.09 (m, 3H, H-5, H-2'''), 8.41 (s, 1H, H-2). ¹³C NMR (125 MHz, DMSO-*d*₆): δ 55.1 (CH₃O), 70.7 (CH₂-1''), 101.7 (C-8), 113.6 (C-3'), 114.9 (C-6), 117.9 (C-4a), 123.3 (C-3), 124.0 (C-1'), 126.9 (C-5), 128.9 (C-3'''), 129.8 (C-2'''), 130.0 (C-2'), 132.8 (C-1'''), 138.7 (C-4'''), 153.5 (C-2), 157.2 (C-8a), 158.9 (C-4'), 162.3 (C-7), 174.5 (CO-4), 192.6 (CO-2''). HRMS (EI) [M⁺] Calculated for C₂₄H₁₇ClO₅: 420.0764; found: 420.0763.

7-(2-(4-Chlorophenyl)-2-oxoethoxy)-6-methoxy-3-(4-methoxyphenyl)-4*H*-chromen-4-one (**4b**). Following the method of preparation for **9a**, mixing **2b** (0.10 g, 0.335 mmol), **8** (0.094 g, 0.400 mmol), and K₂CO₃ (0.069 g, 0.50 mmol) led to **4b** (0.133 g, 88%) as a white solid. *R*_f 0.57 (hexane/EtOAc, 7:3); mp 207-209 °C. ¹H NMR (600 MHz, DMSO-*d*₆): δ 3.79 (s, 3H, CH₃O-4'), 3.91 (s, 3H, CH₃O-6), 5.80 (s, 2H, CH₂-1''), 6.96-7.01 (m, 2H, H-3'), 7.30 (s, 1H, H-8), 7.49 (s, 1H, H-5), 7.51-7.55 (m, 2H, H-2'), 7.66-7.69 (m, 2H, H-3'''), 8.03-8.08 (m, 2H, H-2'''), 8.39 (s, 1H, H-2). ¹³C NMR (150 MHz, DMSO-*d*₆): δ 55.1 (CH₃O-4'), 55.9 (CH₃O-6), 70.9 (CH₂-1''), 101.7 (C-8), 104.5 (C-5), 113.6 (C-3'), 117.3 (C-4a), 122.8 (C-3), 124.3 (C-1'), 128.9 (C-3'''), 129.9 (C-2'''), 130.0 (C-2'), 132.9 (C-1'''), 138.8 (C-4'''), 147.4 (C-6), 151.4 (C-8a), 152.7 (C-7), 153.2 (C-2), 158.9 (C-4'), 174.2 (CO-4), 192.5 (CO-2''). HRMS (EI) [M⁺] Calculated for C₂₅H₁₉ClO₆: 450.0870; found: 450.0862.

6-Chloro-7-(2-(4-chlorophenyl)-2-oxoethoxy)-3-(4-methoxyphenyl)-4*H*-chromen-4-one (**4c**). Following the method of preparation for **9a**, mixing **2c** (0.13 g, 0.43 mmol), **8** (0.12 g, 0.51 mmol), and K₂CO₃ (0.089 g, 0.64 mmol) gave **4c** (0.18 g, 90%) as a white solid. *R*_f 0.45 (hexane/EtOAc, 7:3); mp. 244-245 °C. ¹H NMR (750MHz, DMSO-*d*₆): δ 3.78 (s, 3H, CH₃O), 5.94 (s, 2H, H-1''), 6.97-7.01 (m, 2H, H-3'), 7.50-7.56 (m, 3H, H-2', H-8), 7.66-7.70 (m, 2H, H-3'''), 8.04-8.07 (m, 2H, H-2'''), 8.09 (s, 1H, H-5), 8.46 (s, 1H, H-2). ¹³C NMR (187.5 MHz, DMSO-*d*₆): δ 55.1 (CH₃O), 71.7 (C-1''), 102.9 (C-8), 113.6 (C-3'), 118.1 (C-4a), 120.2 (C-6), 123.3 (C-3), 123.7 (C-1'), 125.9 (C-5), 128.9 (C-3'''), 129.9 (C-2'''), 130.0 (C-2'), 132.8 (C-1'''), 138.8 (C-4'''), 153.9 (C-2), 155.7 (C-8a), 157.4 (C-7), 159.1 (C-4'), 173.7 (CO-4), 192.1 (CO-2''). HRMS (EI) [M⁺] Calculated for C₂₄H₁₆Cl₂O₆: 454.0375; found: 454.0382.

Biological evaluation

DPPH radical scavenging assay

The scavenging of free radicals by the synthesized compounds was assessed with the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) assay as previously reported, 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 (SC) was expressed as the percent decrease in DPPH at 2.5 mM:

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

Where A_{control} is the absorbance of the DPPH solution (control) and A_{test} is the absorbance of the DPPH solution plus a compound.

Inhibition of α -glucosidase

α -Glucosidase inhibition was determined according to a method described by Salehi et al. (2013) with some modifications [49]. 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 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 Equation 1.

$$\% \text{ of inhibition} = \left(\frac{\text{Control absorbance} - \text{sample absorbance}}{\text{Control absorbance}} \right) \times 100 \quad (1)$$

Antimicrobial activity

Compounds **2a-c**, **3a-b**, **4a-b**, **7a**, **7c** and **9a-b** were evaluated for their inhibition of the growth of fungal strains *Candida albicans* and *Saccharomyces cerevisiae*, and Gram-negative bacterium *Salmonella typhi*. The broth microdilution method was utilized with resazurin as an indicator of cell viability [37]. Different concentrations of the test compounds (500-3000 µg/mL, always using 5×10^5 CFU) were placed in each well of a 96-wells microplate (quintuplicate, n = 15) containing PDB medium and resazurin at a final volume of 0.3 mL. The plate was incubated at 30 °C for 12 h. Absorbance was measured at 545 nm, fluconazole as the reference (0.1-200 µg/mL). The minimum inhibitory concentration (MIC) was considered as the initial concentration capable of avoiding any change in absorbance in the dose response curves. The inhibitory efficiency of each chromone was validated by ANOVA and the Tukey test ($p < 0.05$) on GraphPad Prism 7.0 software.

Statistical analysis

Significant differences between the treatment with each compound and the DMSO control were determined with the ANOVA test. MIC calculations were performed on GraphPad Prism 7.0 software.

Molecular docking studies

Molecular docking simulations were carried out on the AutoDock 4 program [50]. The α -glucosidase homology model was constructed by using the crystal structure of isomaltase from *Saccharomyces cerevisiae* in complex with its competitive inhibitor maltose (PDB: 3A4A), which was retrieved from the Protein Data Bank (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 [51]. The alkoxy-isoflavone derivatives were sketched in two dimensions (2D) with ChemSketch (<https://www.acdlabs.com/resources/freeware/chemsketch/>) and converted into 3D, mol2 format in the Open Babel GUI program [52]. The maltose and acarbose ligands were optimized with PM6 on Gaussian 9875 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 $60 \times 72 \times 66 \text{ \AA}^3$ with points separated by $0.375 \text{ \AA}$, 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 one hundred 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.

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Conflict of Interest

The authors declare no conflict of interest.

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