International Journal of Secondary Metabolite
2023, Vol. 10, No. 3, 361–369
https://doi.org/10.21448/ijsm.1196712
Published at https://dergipark.org.tr/en/pub/ijsm
Research Article

In-vitro anti-diabetic, anti-Alzheimer, anti-tyrosinase, antioxidant activities of selected coumarin and dihydroisocoumarin derivatives

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Abstract: Benzo-α-pyrone structured coumarin derivatives are secondary metabolites first obtained from *Coumarouna odorata* in 1822. Coumarin and its structural isomer dihydroisocoumarin derivatives are found in many different sources in nature. Several different bioactivities of these compounds have been reported. In this study, preliminary activity screening and comparison of four purchased coumarin derivatives (esculetin, esculin monohydrate, umbelliferon, scoparone) and four previously isolated 3-phenyl-3,4-dihydroisocoumarin derivatives (thunberginol C, scorzocreticoside I, scorzocreticoside II, scorzopygmaecoside) from a medicinal plant were carried out by *in-vitro* methods. α-Glucosidase, acetylcholinesterase, butyrylcholinesterase, tyrosinase inhibitor activities and antioxidant potentials of the compounds were evaluated. Consequently, thunberginol C (free – not glycosylated form of 3,4-dihydroisocoumarin structure) showed better potential in all enzyme inhibitory activities compared to coumarin structure. Particularly, α-glucosidase inhibitory activity of this compound with a very low IC_{50} value (94.76±2.98 µM) compared to standard acarbose (1036.2±2.70 µM) should be noted. Glycosylation and/or methoxy substitution of 3,4-dihydroisocoumarin structure resulted a significant decrease in all tested enzyme inhibitory activities. The structures of esculin MH, umbelliferone, scoparone, scorzocreticoside I, and scorzopygmaeceoside might be considered in further synthetic studies as selective acetylcholinesterase inhibitors. Thunberginol C has a promising potential in tyrosinase inhibitory activity. Esculetin and thunberginol C showed the best results with high potentials in antioxidant activity via 2,2-diphenyl-1-picryl-hydrazyl-hydrate free radical scavenging, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid cation radical decolorization, and cupric ion reducing antioxidant capacity assays compared to the standards.

ARTICLE HISTORY

Received: Oct. 31, 2022
Revised: Apr. 27, 2023
Accepted: May 24, 2023

KEYWORDS

Coumarins,
Dihydroisocoumarins,
Enzyme inhibitory,
Antioxidant,
Thunberginol C

1. INTRODUCTION

The use of medicinal plants is thought to be as old as first humans. The isolation of pure active compounds from these plants and turning them into drugs dates back to the 19th century. To date, many different secondary metabolites have been identified and presented to the usage and research in the pharmaceutical industry. The first of the coumarin derivatives, one of the important secondary metabolite groups, was isolated from tonka beans (*Dipteryx odorata* (Aubl.) Willd.) in 1822. Coumarin compounds, which are essentially in the benzo-α-pyrone

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e-ISSN: 2148-6905 / **© IJSM 2023**

structure, are found both in glycoside and free form in many different plants. Coumarin derivatives are compounds with different characteristic odors as in freshly mown grass. The reported pharmacological activities of coumarin derivatives have a broad spectrum. Examples of these are anti-microbial, anti-HIV, anticancer, MAO inhibitory, anti-diabetic, anti-inflammatory, anti-parasitic, antihypertensive, anti-Alzheimer, anti-convulsant, and antioxidant activities (Bruneton, 1995; Evans & Evans, 2009; Anand *et al*., 2012; Venkata Sairam *et al*., 2016; Srikrishna *et al*., 2018).

The new compounds that are formed as a result of the interchange of the oxygen atom and the keto group in the α-pyrone ring of the coumarin compounds are called isocoumarins. Isocoumarins and their 3,4-dihydro derivatives are natural compounds like coumarins and have been isolated from different sources such as plants, microbial strains, venoms, insects, and marine organisms. The biological activities of isocoumarins, which is a smaller group than coumarins, may show more diversity. The presence of both sweetening (phyllodulcin) and bitter (mellein) isocoumarins with the same main structure may be proof of this. Some of the reported pharmacological properties of isocoumarins are anti-microbial (anti-biotic, anti-malarial, anti-fungal), hepatoprotective, gastroprotective, neuroprotective, anti-inflammatory, anti-diabetic, sweetening, anti-allergic, and immunomodulatory activities. Among the 3,4-dihydroisocoumarins, 3-phenyl substituted derivatives have drawn attention with their prevalence in nature and similar pharmacological properties including above-mentioned activities (Braca *et al*., 2012; Saeed, 2016; Saddiqa *et al*., 2017; Çiçek *et al*., 2018).

There are many molecules from these groups that have become natural, semi-synthetic, synthetic drugs or drug candidates (Kontogiorgis *et al*., 2012). However, more studies are needed for these derivatives to be used more in the pharmaceutical industry. It is particularly important to screen new natural derivatives for related activities and to compare these pharmacologically important scaffolds. This study aims to investigate the inhibitory potential against several enzymes (α-glucosidase, AChE, BChE, tyrosinase) and antioxidant activity of selected four coumarin derivatives (esculetin, esculin monohydrate, umbelliferon, and scoparone) and four previously isolated 3-phenyl-3,4-dihydroisocoumarin derivatives (thunberginol C, scorzocreticoside I, scorzocreticoside II, and scorzopygmaecoside) from a medicinal plant considering the reported pharmacological properties of the involved structures.

2. MATERIAL and METHODS

2.1. Chemicals and Compounds

PNPG (p-nitrophenol, α-D-glycopyranoside), enzyme α-glucosidase type I (E.C. 3.2.20), disodium hydrogen phosphate, sodium azide, sodium dihydrogen phosphate, acarbose, DMSO, AChE (acetylcholinesterase), BChE (butyrylcholinesterase), and tyrosinase from mushroom (E.C. 1.14.18.1) were obtained from Sigma–Aldrich/Merck. Acetylthiocholine iodide was purchased from Applichem and butyrylthiocholine iodide was Fluka branded. All other chemicals were of analytical grade.

Esculetin, esculin monohydrate, umbelliferone, and scoparone were purchased from Sigma-Aldrich. Thunberginol C, scorzocreticoside I, scorzocreticoside II, and scorzopygmaecoside were isolated and identified previously (Şahin *et al*. 2020a; Şahin *et al*. 2020b).

2.2. Anti-Diabetic Activity

α-Glucosidase inhibitory activity was employed for determination of anti-diabetic potential of the compounds (Trinh *et al*., 2016). Na_2HPO_4, NaH_2PO_4 and ultra-pure water were used to prepare a buffer with pH 7.5 containing NaN_3 (0.02 %). In brief, 100 µL of the compounds dissolved in buffer with 10 % DMSO (8 concentrations between 800 – 6.25 µM), and 80 µL of enzyme solution were added to all wells. After incubation (28 °C, 10 min) 20 µL of PNPG

(substrate) was added. The blank wells contained enzyme, substrate, and buffer with 10 % DMSO. No background well was used since using slopes instead of absorbance eliminates the potential absorbances due to the color of the compounds. Absorbance measurement at 405 nm every 40 s for 35 min and incubations were performed with a microplate photometer BioTek Power Wave XS branded. An oral inhibitor, acarbose, was employed as control. α-Glucosidase inhibition % was calculated using the following formula:

$$\text{Inhibition \%} = (\text{Slope}_{\text{blank}} - \text{Slope}_{\text{sample}}) / \text{Slope}_{\text{blank}} \times 100$$

2.3. Anti-Alzheimer Activity

A colorimetric method developed by Ellman *et al*. was used with minor changes to evaluate AChE and BChE inhibitory activities of the compounds (Ellman *et al*., 1961; Yıldız *et al*., 2022). Each well finally contained 150 µL of buffer, 10 µL of compound solutions, 20 µL of enzyme solution (BChE or AChE), 10 µL of DTNB [5,5-dithiobis (2-nitro benzoic acid)], and 10 µL of the either acetylthiocholine iodide or butyrylthiocholine iodide. The incubation times at 25 °C were 15 minutes before DTNB was added and 10 minutes after iodides were added. Above-mentioned plate reader was used for incubations and measuring the absorbances at 412 nm. Galantamine was used as positive control and sample solvent was used as blank. % inhibitions were calculated according to following equation.

$$\text{Inhibition \%} = (A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}} \times 100 \text{ (A: Absorbance)}$$

2.4. Anti-Tyrosinase Activity

Another colorimetric method was used to determine the anti-tyrosinase potentials of the compounds (Hearing & Jiménez, 1987). L-DOPA (0.5 mM) was the substrate for tyrosinase enzyme. A phosphate buffer with pH 6.8 was used. Compounds were prepared in a series of concentration and a pre-incubated with enzyme solution for 10 minutes at room temperature. The substrate was added to start the enzymatic reaction. 20 minutes incubation was carried out and the absorbance was measured at 475 nm at 37 °C. Kojic acid was employed as control. Same equation given in anti-Alzheimer activity section was used to calculate the percentage of the inhibitory activity of the samples.

2.5. Antioxidant Activity

2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free radical scavenging, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{+}$) cation radical decolorization, and cupric ion reducing antioxidant capacity (CUPRAC) assays were preferred to evaluate the antioxidant potentials of the compounds. All assays were carried out according to previously published literature (Blois, 1958; Re *et al*., 1999; Apak *et al*., 2004; Yıldız *et al*., 2022).

All assays were performed in triplicates (Student's t-test $p<0.05$) and the results were reported as mean ± SD. IC_{50} calculations were performed with GraphPad Prism 8.0.1.

3. RESULTS and DISCUSSION

Enzyme inhibitory and antioxidant activities of selected coumarins and 3-phenyl-3,4-dihydroisocoumarins are presented in Table 1 and Table 2 respectively.

Enzyme inhibition is one of the most studied modes of action in the discovery of new drug molecules. It is therefore not surprising that many inhibitors of different enzymes are found in clinical use. Glucosidase inhibitors prevent α-glucosidase from hydrolyzing oligosaccharides to monosaccharides in human intestine. Thus, they contribute to the treatment/care of Diabetes mellitus (DM) patients by preventing postprandial hyperglycemia. Keeping the blood glucose level under control in these patients is very crucial, particularly for preventing/delaying of chronic complications of DM such as retinopathy and neuropathy (Maurya *et al*., 2020).

Selected coumarins; esculetin, esculin MH, umbelliferone, scoparone and dihydroisocoumarins; thunberginol C, scorzocreticoside I, scorzocreticoside II, scorzopygmaeacoside (Figure 1) were tested against this enzyme *in-vitro*. IC_{50} values of all tested compounds were above 800 µM except esculetin and thunberginol C (Table 1). These compounds showed higher potency than the standard acarbose which is a clinically used oral inhibitor of the enzyme. It should be noted that IC_{50} value of thunberginol C is approximately ten times lower than that of acarbose. Furthermore, the results suggest that glycosylation and/or methoxy substitution instead of free hydroxyl in both coumarins and dihydroisocoumarins decrease the activity significantly. Considering the potencies of esculetin, umbelliferon, and scoparone together, it can be deduced that at least two free hydroxyl substitution is crucial for higher activity. In addition to that, the same pattern in dihydroisocoumarins suggests that meta positioning of phenolic hydroxyl groups may result higher activity than that of ortho positioning. This is the first report on α-glucosidase inhibitory effect of the tested compounds except esculetin, umbelliferone and scoparone. Other data about these three compounds are in accordance with the previous literature (Nurul Islam *et al*., 2013; Karakaya *et al*., 2018).

Table 1. Enzyme inhibitory activity results of the compounds.

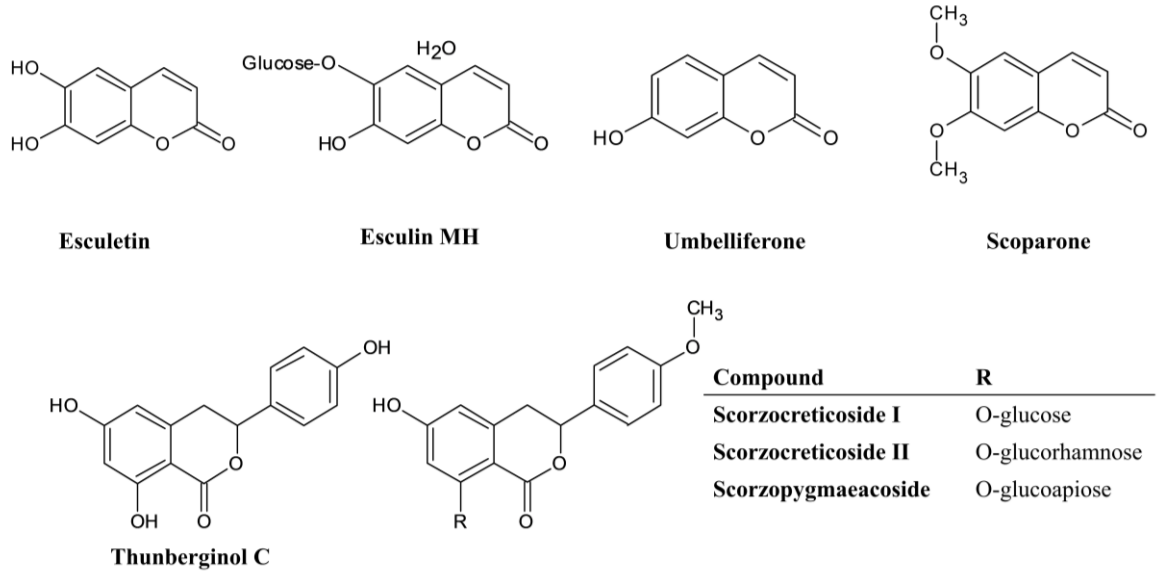
	IC_{50} (µM)			
	α-glucosidase	AChE	BChE	Tyrosinase
Esculetin	374.00 ± 0.70	135.53 ± 1.59	196.99 ± 0.36	NT
Esculin MH	>800	118.82 ± 2.20	>1000	>1000
Umbelliferone	>800	209.61 ± 0.74	>1000	NT
Scoparone	>800	236.96 ± 1.41	>1000	>1000
Thunberginol C	94.76 ± 2.98	82.41 ± 1.30	137.25 ± 1.01	90.25 ± 1.67
Scorzocreticoside I	>800	133.90 ± 0.43	>1000	531.16 ± 3.27
Scorzocreticoside II	>800	265.78 ± 0.42	340.20 ± 0.36	>1000
Scorzopygmaeacoside	>800	261.03 ± 1.38	>1000	240.91 ± 1.65
Acarbose[a]	1036.2 ± 2.70	NT	NT	NT
Galanthamine[b]	NT	5.78 ± 0.02	16.58 ± 0.18	NT
Kojic acid[c]	NT	NT	NT	15.72 ± 0.14

Values are means of three parallel measurements ± Standard deviation.
a Standard compound for α-glucosidase
b Standard compound for AChE and BChE
c Standard compound for Tyrosinase
NT: Not tested.

One of the approaches in the treatment of neurodegenerative Alzheimer's disease characterized by cholinergic abnormalities is the inhibition of AChE and BChE enzymes (Francis *et al*., 2010). All tested compounds showed low-moderate inhibitory activities on these enzymes (Table 1). Esculin MH, umbelliferone, scoparone, scorzocreticoside I and scorzopygmaeacoside were more selective towards AChE, while esculetin, thunberginol C and scorzocreticoside II acted as dual inhibitors. The most potent AChE inhibitor was thunberginol C. The glycosylation and/or methoxy substitution instead of free hydroxyl of this compound led to lower potentials just as in case of α-glucosidase inhibition. However, glycosylation resulted in higher selectivity towards AChE enzyme considering potentials of scorzocreticoside I and scorzopygmaeacoside. Similarly, glycosylation, methoxy substitution instead of free hydroxyl, and loss of a free hydroxyl group led to the same selectivity in coumarin derivatives. Current literature provides studies conducted on AChE/BChE inhibitory activities of umbelliferone, esculetin, scoparone and thunberginol C with different results. However, most of the studies report moderate-high potencies (Adhami *et al*., 2014; Ali *et al*., 2016; Hwang *et*

al., 2021). Although, none of the compounds showed comparable potential with positive standard galantamine, results may contribute to the field by being new models for selective synthetic inhibitors. Despite the decrease in AChE activity in advanced stages of Alzheimer's disease, the increase in BChE activity shows that selective inhibitors may be more useful in the treatment (Lane *et al*., 2006).

Figure 1. Structures of selected coumarin and dihydroisocoumarin derivatives.



Tyrosinase is a multifunctional oxidase which mediates producing melanin from tyrosine. Thus, its inhibition has different potentials in skin-whitening, skin cancer, neurodegeneration, and undesired browning of foods (Bonesi *et al*., 2019). Selected coumarin derivatives revealed no activity against tyrosinase enzyme at tested concentrations (Table 1). Esculetin and umbelliferone were not tested against this enzyme since several studies reported these compounds as substrates for the enzyme (Munoz-Munoz *et al*., 2007; Garcia-Molina *et al*., 2013). No previous study has been found on the anti-tyrosinase activity of scoparone. However, low inhibitory activity result of esculin MH is in accordance with the previous literature (Masamoto *et al*., 2003). The most potent inhibitor against tyrosinase enzyme among the tested compounds was thunberginol C with a potential approximately 6 times weaker than the positive control kojic acid. The negative impact of the glycosylation and/or methoxy substitution of tested dihydroisocoumarins on the inhibitory activity was valid for this enzyme too.

Oxidative stress in humans caused by several reasons such as stress, unhealthy diet, chemicals etc., is associated with many diseases. Thus, antioxidants which can keep the oxidative stress in desired limits are suggested to decrease the risk of them. Main antioxidant sources of humans are natural phytochemicals provided by traditional medicinal and edible plants (Sen & Chakraborty, 2011). In this context, antioxidant activities of the selected compounds were evaluated and esculetin appeared to be the most potent antioxidant in every tested method with better potencies than used standards (Table 2). Furthermore, a significant decrease was determined in case of glycosylation, methoxy substitution instead of free hydroxyl, and loss of a free hydroxyl group in coumarin derivatives. This structure-activity relationship is valid for dihydroisocoumarin derivatives except scorzocreticoside II in DPPH method. Thunberginol C was determined as a promising antioxidant among the tested dihydroisocoumarins with high potencies comparable to that of the standards. Results are in accordance with previous studies reporting moderate to high antioxidant potentials of coumarin

and dihydroisocoumarin derivatives (Zidorn *et al*., 2005; Wu *et al*., 2007; Witaicenis *et al*., 2014; Mazimba, 2017).

Table 2. Antioxidant activity results of the compounds.

	EC_{50} (µM)		$A_{0.5}$ (µM)
	DPPH Free Radical	ABTS Cation Radical	CUPRAC
Esculetin	8.37 ± 0.22	6.29 ± 0.04	10.26 ± 0.19
Esculin MH	255.39 ± 1.21	201.62 ± 1.56	90.06 ± 1.84
Umbelliferone	539.16 ± 1.08	293.58 ± 2.71	556.33 ± 4.42
Scoparone	182.39 ± 2.72	>1000	>1000
Thunberginol C	126.38 ± 0.07	17.69 ± 1.16	62.22 ± 2.66
Scorzocreticoside I	641.03 ± 5.54	520.34 ± 6.18	>1000
Scorzocreticoside II	65.83 ± 1.22	455.31 ± 2.40	>1000
Scorzopygmaeacoside	536.27 ± 4.16	239.25 ± 2.83	298.69 ± 2.43
BHA[a]	45.31 ± 1.32	8.53 ± 0.24	25.91 ± 0.50
α-TOC[a]	49.88 ± 0.79	14.31 ± 0.32	38.89 ± 0.87
BHT[a]	270.42 ± 1.52	6.42 ± 0.38	32.74 ± 1.52

Values are means of three parallel measurements ± Standard deviation.
a Standard compounds

4. CONCLUSION

Consequently, thunberginol C (free form of 3,4-dihydroisocoumarin structure) showed better potential in all enzymes inhibitory activities compared to coumarin structure. Particularly, α-glucosidase inhibitory activity of this compound with a very low IC_{50} value compared to standard acarbose should be noted. Further toxicological and *in-vivo* activity studies might be considered on this compound to develop a more potent hypoglycemic agent. Glycosylation and/or methoxy substitution instead of free hydroxyl of 3,4-dihydroisocoumarin structure resulted in a significant decrease in all tested enzyme inhibitory activities. Esculin MH, umbelliferone, scoparone, scorzocreticoside I, and scorzopygmaeceoside might be considered in further studies as selective AChE inhibitors. Thunberginol C has a promising potential in tyrosinase inhibitory activity. Esculetin and thunberginol C showed the best results with high potentials in antioxidant activity via DPPH free radical, ABTS cation radical scavenging and CUPRAC assays compared to the standards.

Acknowledgments

The author kindly acknowledges Assoc. Prof. Dr. Mehmet Boğa for providing accession to needed laboratory facilities and materials.

Declaration of Conflicting Interests and Ethics

The author declares no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the author.

Authorship Contribution Statement

Hasan Şahin: Conceptualizing the study, Conduction of the assays, Interpreting of the results, and Writing the manuscript.

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