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Exploring the inhibitory potential of 2-styrylchromones on pancreatic α-amylase

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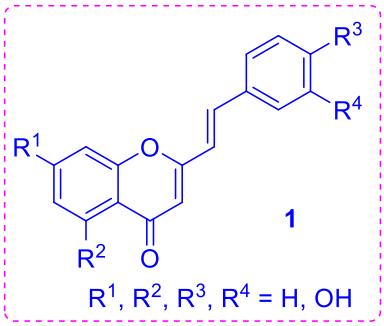
Diabetes *mellitus* is a metabolic disorder that afflicts about 537 million people worldwide and this number is predicted to rise to 643 million by 2030, according to the International Diabetes Federation.[1] It is characterized by hyperglycemia, caused by the deficiency in the secretion of insulin and/or in the action of this pancreatic hormone. To date, the best therapeutic strategy known consists of inhibiting carbohydrate-hydrolyzing enzymes, namely the α-amylase enzyme. The currently marketed inhibitors (e.g., acarbose, miglitol, and voglibose) are based on carbohydrate-related structures, with moderate affinity for the enzyme and with disturbing side effects.[2] Thus, an active pursuit for novel and more effective anti-diabetic drugs has been carried out and a wide variety of structurally diverse heterocyclic compounds has been studied. Chromones are among the oxygenated 6-membered heterocycles evaluated and the results exhibited by some 2-arylchromones point out the relevance of this class of compounds in the inhibition of α-amylase enzymatic activity.[3] Nonetheless, a detailed investigation of the effects of the restricted group of chromones known as 2-styrylchromones (2-SC) has not been conducted to date. With this rationale in mind and as part of our on-going project, the aim of the present study is to investigate the effect of a panel of twelve 2-SC **1** on pancreatic α-amylase activity and their mechanism of inhibition, to infer about the importance of this class of compounds in the management of type 2 diabetes and its complications.



α-Amylase was exposed to different concentrations of 2-SC **1** and the hydrolysis of the substrate 2-chloro-*p*-nitrophenyl-α-D-maltotriose was monitored spectrophotometrically at 405 nm. Acarbose was used as the standard inhibitor. In addition, the study of the inhibition type was carried out through nonlinear regression Michaelis-Menten enzymatic kinetics and the corresponding Lineweaver-Burk plot.[4]

The results showed that the IC_{50} values obtained ranged from 26 to 174 μM, considerably higher than the positive control acarbose (IC_{50} = 0.62 ± 0.07 μM). All active compounds revealed a competitive type of inhibition while for the positive control a mixed type of inhibition was obtained. More details concerning the structure-activity relationship will be presented and discussed in this communication.

Acknowledgements
This work received support from national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through the projects of CIMO (UIDB/00690/2020 and UIDP/00690/2020), SusTEC (LA/P/0007/2021), REQUIMTE (UIDB/50006/2020 and UIDP/50006/2020) and through the project with the reference EXPL/MED-QUI/0815/2021. Carina Proença acknowledges funding from FCT through the project, EXPL/MED-QUI/0815/2021. Marisa Freitas acknowledges her contract under the Scientific Employment Stimulus - Individual Call (CEEC Individual) 2020.04126.CEECIND/CP1596/CT0006 and LAQV-REQUIMTE for her contract under the reference LA/P/0008/2020.

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EXPLORING THE INHIBITORY POTENTIAL OF 2-STYRYLCHROMONES ON PANCREATIC α-AMYLASE

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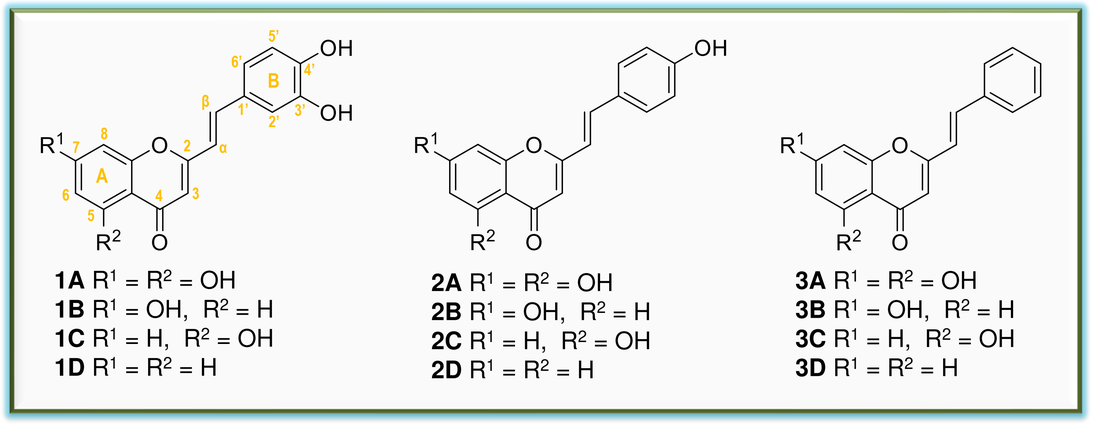
INTRODUCTION

- Diabetes mellitus is a metabolic disorder that afflicts about 537 million people worldwide and this number is predicted to rise to 643 million by 2030, according to the International Diabetes Federation [1]. It is characterized by hyperglycemia, caused by the deficiency in the secretion of insulin and/or in the action of this pancreatic hormone. To date, the best therapeutic strategy known consists of inhibiting carbohydrate-hydrolyzing enzymes, namely the α-amylase enzyme. The currently marketed inhibitors (e.g., acarbose, miglitol, and voglibose) are based on carbohydrate-related structures, with moderate affinity for the enzyme and with disturbing side effects [2].

- An active pursuit for novel and more effective anti-diabetic drugs has been carried out and a wide variety of structurally diverse heterocyclic compounds has been studied. Chromones are among the oxygenated 6-membered heterocycles evaluated and the results exhibited by some 2-arylchromones point out the relevance of this class of compounds in the inhibition of α-amylase enzymatic activity [3]. Nonetheless, a detailed investigation of the effects of the restricted group of chromones known as 2-styrylchromones (2-SC) has not been conducted to date.

OBJECTIVES

The main goal of the present study is to investigate the effect of a panel of twelve 2-SC (Scheme 1) on pancreatic α-amylase activity and their mechanism of inhibition, using a spectrophotometric screening methodology, to infer about the importance of this class of compounds in the management of type 2 diabetes and its complications [4].



Scheme 1. Chemical structures of the studied 2-SC.

INHIBITORY ASSAY

The α-amylase inhibitory assay was carried out in a 96-well plate, by incubating porcine pancreatic α-amylase, 2SC **1-3** dissolved in DMSO and the substrate 2-chloro-4-nitrophenyl-α-D-maltotrioside (CNPG3) at 37 °C, and monitoring the α-amylase-mediated transformation of the substrate CNPG3 into 2-chloro-4-nitrophenol (CNP), spectrophotometrically at 405 nm for 30 min. Acarbose was used as positive control. The obtained results were expressed as the mean % inhibition ± SEM and, when possible, calculated the respective IC_{50} values (Table 1 and Figure 1).

Table 1. Inhibitory effects of 2-SC **1-3** on the pancreatic α-amylase activity.

	IC_{50} (μM) or Inhibition (%)
Compounds	**α-amylase**
1A	29 ± 2
1B	68 ± 3
1C	25.9 ± 0.9
1D	88 ± 2
2A	62 ± 3
2B	174 ± 6
2C	< 20% [200 μM*]
2D	33 ± 3% [200 μM*]
3A	28 ± 4% [200 μM*]
3B	48 ± 3% [200 μM*]
3C	25 ± 3% [200 μM*]
3D	24 ± 3% [200 μM*]
Positive control	
Acarbose	0.62 ± 0.07

*The value represents the percentage of inhibition for the highest tested concentration (in superscript).

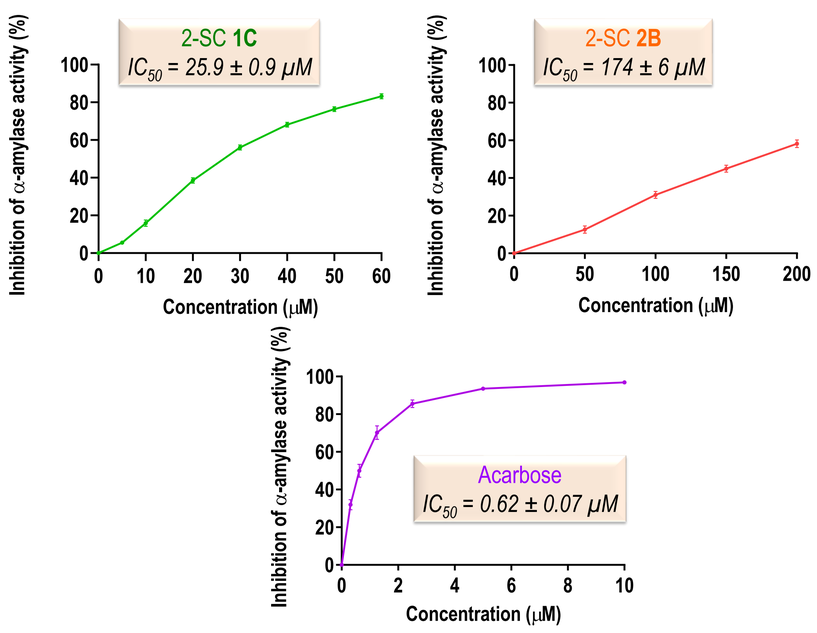


Figure 1. α-Amylase inhibition by 2-SC **1C**, 2-SC **2B** and the positive control, acarbose.

The study of the enzymatic inhibition mechanism was carried out through nonlinear regression Michaelis-Menton enzymatic kinetics and the corresponding Lineweaver-Burk plots (Table 2 and Figure 2).

Table 2. Type of inhibition (using Solver™ supplement) of the active 2-SC **1-2** and acarbose against α-amylase activity and respective kinetic parameters values: V_{max}, K_m, K_{ic} and K_{iu} (mean ± SEM).

Compounds	Type of inhibition	V_{max} (ΔAbs/min)	K_m (μM)	K_{ic} (μM^{-1})	K_{iu} (μM^{-1})
1A	competitive	43.9 ± 0.6	909 ± 22	20.1 ± 0.5	–
1B	competitive	50 ± 1	1130 ± 41	53.7 ± 0.9	–
1C	competitive	49 ± 2	1053 ± 48	18.6 ± 0.6	–
1D	competitive	53 ± 3	1188 ± 86	70 ± 2	–
2A	competitive	51 ± 1	1082 ± 37	62 ± 1	–
2B	competitive	51.8 ± 0.8	1167 ± 26	128 ± 2	–
Acarbose	mixed	41 ± 1	942 ± 53	2.6 ± 0.2	0.37 ± 0.01

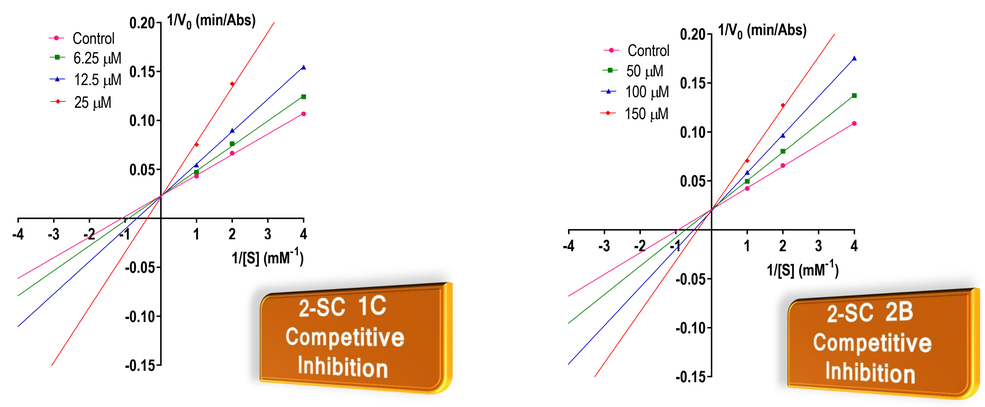


Figure 2. Lineweaver–Burk plots of α-amylase inhibition by 2-SC **1C** and **2B**.

CONCLUSIONS

- The most active compounds tested were those from group 1 (with 3',4'-$(OH)_2$ in B-ring), being **1C and 1A** the most effective compounds of the group, with almost similar effects.
- Only 2-SC **2A** and **2B** from group 2 (with 4'-OH in B-ring), have shown a concentration-dependent activity, although 2-SC **2A** (IC_{50} = 62 ± 3 μM) was noticeably more potent than 2-SC **2B** (IC_{50} = 174 ± 6 μM).
- Compounds from group 3 (no substitution in B-ring) were the less efficient group of compounds tested, recording low inhibitory activities from 24% to 48%, up to the maximum tested concentrations (200 μM).
- . For the type of inhibition mechanism, compounds **1A-D**, **2A** and **2B** behaved as competitive inhibitors of α-amylase.
- **The present work can open a promising area of research based on the design of novel chromone derivatives for targeting key enzymes enrolled in glucose metabolism and therefore in the management of type 2 DM.**

ACKNOWLEDGEMENTS

This work received support from national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through the projects of CIMO (UIDB/00690/2020 and UIDP/00690/2020), SusTEC (LA/P/0007/2021), REQUIMTE (UIDB/50006/2020 and UIDP/50006/2020) and through the project with the reference EXPL/MED-QUI/0815/2021.
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