

Evaluation of DNase I inhibitory activity on synthetic halogenated chalcone derivatives

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Introduction

Chalcones represent a class of bioactive, naturally occurring compounds, consisting of two aromatic rings (ring A and ring B) linked by α,β -unsaturated three-carbon chain (Table 1). In nature, chalcones are abundant in fruits, vegetables and various plants, many of which have found their place in traditional herbal medicine for the therapy of various medical conditions (Orlikova et al., 2011). Chalcone derivatives, either from natural sources or synthetically obtained, have demonstrated numerous bioactivities, and are considered as privileged scaffolds in medicinal chemistry. It has been shown that this group of compounds exerts antioxidant, antineoplastic, antiangiogenic, hypolipidemic, antimicrobial, antiinflammatory, tyrosine inhibitory, vasorelaxant and other activities. Chalcones have also been reported to possess enzyme inhibitory activities towards numerous pharmacologically significant enzymes including cholinesterases, xanthine oxidase, cyclooxygenase, lipoxygenase, etc. (Zhuang et al., 2017).

Deoxyribonuclease I (DNase I) is one of the most important enzymes in the human body which catalyzes DNA hydrolysis forming 5'-oligonucleotides. This enzyme is one of the main nucleases responsible for DNA fragmentation during programmed cell death (apoptosis) (Oliveri et al., 2001; Samejima and Earnshaw, 2005). Elevated levels of DNase I can lead to increased DNA fragmentation and excessive cell death, thus contributing to the development of numerous pathological conditions (cardiovascular, autoimmune, neurodegenerative) (Oliveri et al., 2001).

Having in mind the pathophysiological importance of DNase I, the relatively small number of known organic inhibitors of this enzyme, as well as the fact that there is no inhibitor defined as a "gold standard", there is a need for finding new efficient DNase I inhibitors.

To the best of our knowledge, there is no information of chalcones as DNase I inhibitors, therefore this experiment represents an entry into an unexplored field which will hopefully result in increased interest for further research on this topic and discovery of new active chalcone structures.

Materials and methods

Synthetic procedure

Chalcones have been synthesized by Claisen-Schmidt condensation reaction according to the procedure previously described (Wani et al., 2018), with minor modifications. A mixture of chalcone bearing acetophenones (0.003 mol) and corresponding benzaldehydes (0.003 mol) was stirred in ethanol (12 mL) and then 60% aqueous solution of sodium hydroxide (3 mL) was added to it dropwise with continuous stirring at 0°C. The mixture was stirred for 2-3 hours in the ice-bath, and was afterwards diluted with ice-cold water, filtered under reduced pressure and washed with cold water until neutral pH. Precipitate was dried in air and recrystallised from 96% ethanol. Obtained chalcone derivatives were characterized using ¹H- and ¹³C-NMR experiments and their purity was determined by high-performance liquid chromatography (HPLC).

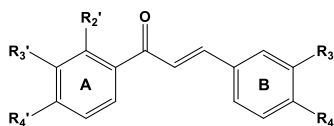
Evaluation of DNase I inhibitory activity

Chalcone derivatives were investigated for the inhibitory effect towards bovine pancreatic DNase I. The *in vitro* evaluation of DNase I inhibition is based on spectrophotometric measurement of acid-soluble nucleotides formation at 260 nm according to the method previously described (Ilić et al., 2018) using crystal violet as a positive control.

Results and discussion

A small series consisting of seven chalcone bearing chalcones (**1-7**) was synthesized (Table 1).

Table 1. Synthesized chalcone derivatives **1-7**



	R2'	R3'	R4'	R3	R4
1	H	Br	H	H	OCH ₃
2	H	H	Br	H	OCH ₃
3	Br	H	H	OCH ₃	OCH ₃
4	H	Br	H	OCH ₃	OCH ₃
5	H	H	Br	OCH ₃	OCH ₃
6	Cl	H	H	H	OCH ₃
7	Cl	H	H	OCH ₃	OCH ₃

In the enzyme inhibition test, among all tested compounds, only 2'-chloro-3,4-dimethoxy-chalcone (compound **7**, Table 1) inhibited DNase I in the tested concentration range ($IC_{50} < 250 \mu M$). With an IC_{50} value of $214.53 \pm 21.64 \mu M$, this compound showed better DNase I inhibitory properties compared to the positive control crystal violet ($IC_{50} = 351.82 \pm 29.41 \mu M$). In this particular series of seven compounds, no structure-activity relationship (SAR) studies could be conducted. However, this study represents a good starting point for the evaluation of chalcone derivatives as DNase I inhibitors.

Conclusion

Chalcones represent a class of bioactive compounds with various bioactivities, including the inhibition of numerous clinically important enzymes. This is the first experiment evaluating the inhibitory activity of chalcones

towards DNase I and it resulted in finding one derivative (2'-chloro-3,4-dimethoxy-chalcone) with stronger inhibitory effect than positive control. Taking into account the involvement of DNase I in the pathophysiological processes of many diseases, these results indicate the therapeutic potential of chalcones as DNase inhibitors.

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Author Contributions

VG, experimental work on synthesis and first-draft preparation; **AM**, DNase I experiments and data curation; **JL**, recording and analyzing spectral data, data curation, resources and final writing; **AS**, resources. All authors read and approved the final manuscript.

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