Chromeno-pyrimidine-type compounds (part II): *in vitro* evaluation of antioxidant potential

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DOI: 10.46793/ICCBI23.690A

Abstract: The chromeno and pyrimidine classes compounds include a variety of hybrid molecules displaying diverse biological actions. Although they have been examined for many years, these compounds are still of interest due to their facile chemical transformations. The presence of chromeno and pyrimidine structural motifs in many drugs, prompted us to investigate the antioxidant features of compounds 5-(7-bromo-2,4-dioxo-1,3,4,5-tetrahydro-2*H*-chromeno[2,3-d]pyrimidin-5-yl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-tri-one (**CP-1**) and 8,9-dihydroxy-2*H*-chromeno[2,3-d]pyrimidine-2,4(3*H*)-dione (**CP-2**). In this paper, we investigated *in vitro* antioxidant properties of selected chromeno-pyrimidine derivatives. The percentage activity of the tested compounds **CP-1** and **CP-2**, as well as the quercetin standard, NDGA, against the DPPH radical in concentrations of 25 μM, 50 μM and 100 μM was tested. Compound **CP-2** was found to have an exceptional efficacy of 92% at a concentration of 25 μM. In addition, the IC_{50} value confirms a high antiradical power against DPPH radicals for the compound **CP-2** (IC_{50} = 3.5 μM) and a moderate activity for the compound **CP-1** (IC_{50} = 55.4 μM).

Keywords: Chromeno-pyrimidine derivatives, Antioxidant activity assay, DPPH radical

1. Introduction

Nitrogen-containing heterocyclic pyrimidines and their fused derivatives serve an essential function in medicinal chemistry and have been employed as drug development scaffolds [1–6]. The benzopyrano[2,3-d]pyrimidines is an important pharmacore which contains two fused benzopyran and pyrimidine rings. For this class of compounds, a large spectrum of biological and pharmacological properties is known [7–15]. The benzopyrans (4H-chromene) have shown a wide range of biological activities such as cytotoxic [7], antibacterial [8], antioxidant [9], and antigenotoxic [10]. On the other hand, pyrimidine scaffold is the base of many bioactive molecules [11-13]. Consequently,

synthetic methodologies for the synthesis of novel chromeno-pyrimidines are of particular interest to organic and medicinal chemists. Barbituric acid is a pyrimidine heterocyclic molecule with an active methylene group that can be involved in condensation reactions with aldehydes, ketones and α, β-unsaturated carbonyl compounds forming other heterocycles compounds with an outstanding biological activity.

In this study, the antioxidant features of chromeno-pyrimidine derivatives (CP), 5-(7-bromo-2,4-dioxo-1,3,4,5-tetrahydro-2H-chromeno[2,3-d]pyrimidin-5-yl)pyrimidine-2,4,6(1H,3H,5H)-tri-one (CP-1) and 8,9-dihydroxy-2H-chromeno[2,3-d]pyrimidine-2,4(3H)-dione (CP-2), obtained in ionic liquid catalysed reaction of different substituted salicylaldehydes with barbituric acid are investigated. The synthesis and structural characterization of these compounds is described in the chromene-pyrimidine study part 1.

2. Results and discussion

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging test is one of the most frequently used assays and the first step in evaluating antioxidant activity. Thus, antioxidant molecules can neutralize DPPH free radicals by donating hydrogen atoms or electrons, resulting in a decrease in 517 nm absorbance.

In this work, compounds **CP-1** and **CP-2** (Figure 1) were subjected to *in vitro* antioxidant screening using DPPH assay (Table 1). The obtained DPPH assay results are displayed as an IC_{50} value, which is defined as the effective concentration of antioxidants required to reduce the initial concentration of free radicals by 50 %.

First, the percentage activity of the investigated compounds **CP-1** and **CP-2**, as well as the standards of quercetin, NDGA, on the DPPH radical was tested at concentrations of 25 μM, 50 μM and 100 μM, and for the duration of incubation of 20 and 60 minutes, as shown in Table 1. The Table shows that compound **CP-2** has a remarkable degree of efficacy, with a notable activity level of 92 %, even when administered at low concentrations of 25 μM. Conversely, compound **CP-1** shows much less activity at the same concentration. In the second step, inhibitory concentrations were determined and expressed as IC_{50} values. The IC_{50} value for compound **CP-2** is 3.5 μM, indicating excellent antioxidant potential. The activity of this compound is slightly lower than that of the positive control quercetin and NDGA. Chromeno-pyrimidine derivative **CP-1** showed a moderate activity with IC_{50} of 55.4 μM. The better activity of the compound **CP-2** can be attributed to the presence of the catechol fragment in the structure of this compound.

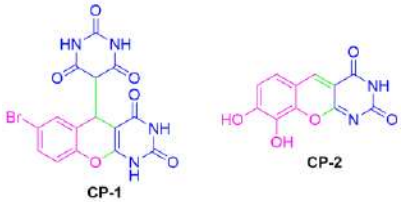

Figure 1. Structure of investigated chromeno-pyrimidine derivatives.

Table 1. *In vitro* interactions of selected compounds **CP-1** and **CP-2** with DPPH radical.

Compound	DPPH scavenging ability (%)						IC_{50} (μM)
	25 μM		50 μM		100 μM		
	20 min	60 min	20 min	60 min	20 min	60 min	
CP-1	40.9 ± 1.4	54.7 ± 3.1	52.2 ± 1.2	69.7 ± 0.5	65.2 ± 1.3	83.1 ± 1.2	55.4 ± 2.5
CP-2	91.3 ± 0.5	91.5 ± 3.0	93.5 ± 0.2	93.9 ± 1.8	96.9 ± 0.8	97.1 ± 1.9	3.5 ± 0.1
NDGA	94.6 ± 0.7	94.6 ± 0.6	94.2 ± 0.7	94.2 ± 0.7	94.5 ± 0.2	94.1 ± 0.7	1.7±0.1
Quercetin	95.3 ± 0.8	95.1 ± 0.9	96.8 ± 1.0	96.5 ± 0.9	95.1 ± 0.9	95.4 ± 0.8	1.9±0.1

3. Experimental

The 2,2-diphenyl-1-picrylhydrazyl (DPPH), nordihydroguaiaretic acid (NDGA), quercetin and methanol were purchased from from Merck. The UV-Vis determinations were performed on PerkinElmer, Lambda 365 UV/Vis Spectrophotometer.

*3.1 Antioxidant activity of compounds **CP-1** and **CP-2***

The antioxidant potency of chromeno-pyrimidine derivatives was determined using DPPH method [16]. The samples were prepared by mixing the methanolic solution of DPPH radical (0.05 mM, 1 mL) with the tested compound (20 μL of different concentrations in dimethyl sulfoxide (DMSO) and 980 μL of methanol). After the incubation period (twenty and sixty minutes in a dark room at room temperature), the absorbance was determined spectrophotometrically at 517 nm. Quercetin and nordihydroguaiaretic acid were used as reference compounds, whereas methanol was a control solution. All measurements were made in triplicate. The obtained results are presented as mean values ± standard deviation (SD) of three independent measurements.

4. Conclusions

The results of the *in vitro* DPPH examination showed that both investigated compounds, **CP-1** and **CP-2**, expressed antioxidant activity. It should be mentioned that especially compound **CP-2**, with an activity slightly lower than the reference compounds NDGA and quercetin, acts as an excellent radical scavenger.

Acknowledgment

This research is funded by the Ministry of Education and Ministry of Science, Technological Development and Innovation, Republic of Serbia, Grants: No. 451-03-47/2023-01/200378.

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