6th International Electronic Conference on Medicinal Chemistry
1-30 November 2020
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In vitro antioxidant, DNA-protective and cytotoxic effects of 2,3-substituted quinazolinone-derived Schiff bases

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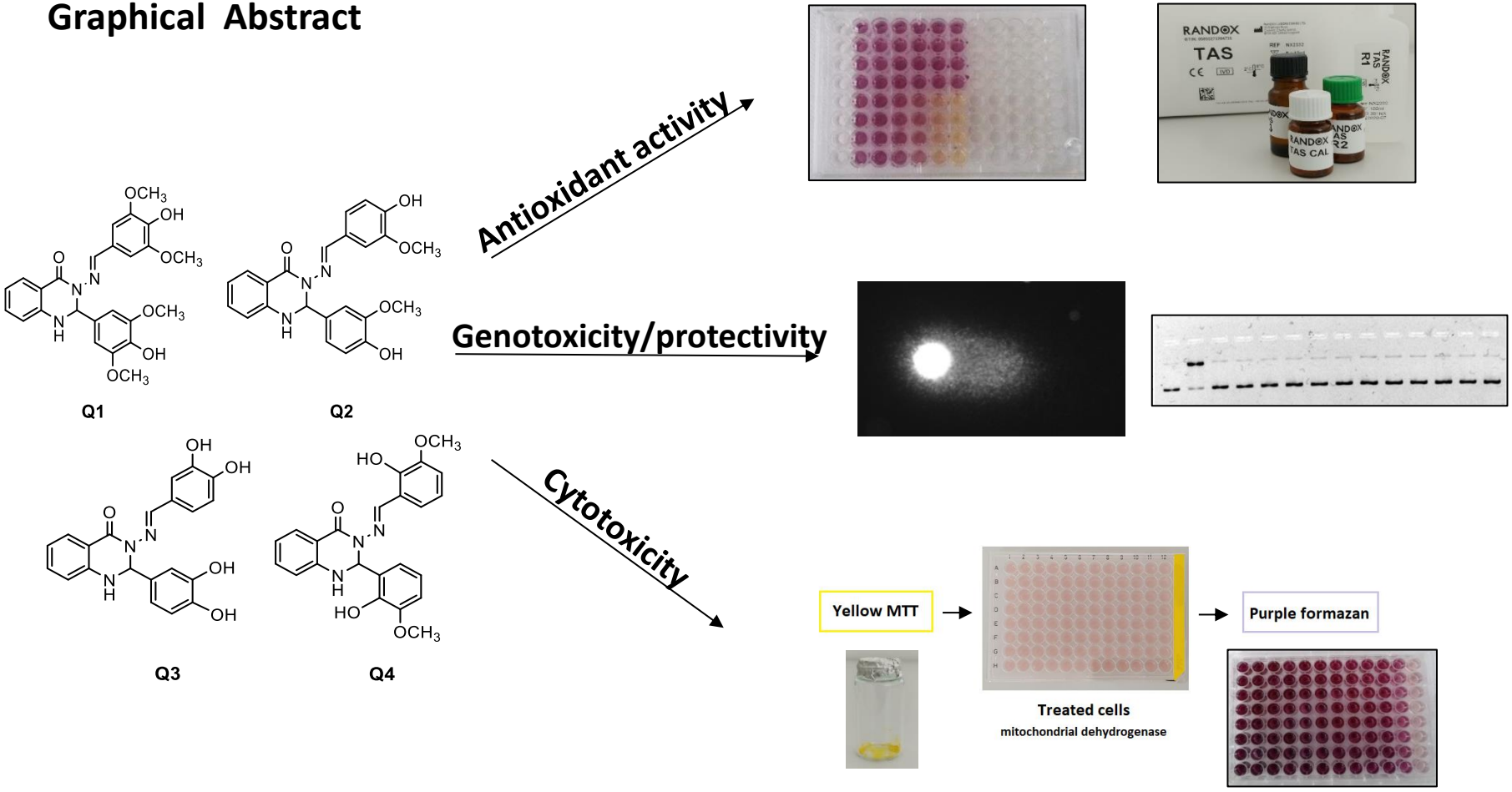
Graphical Abstract



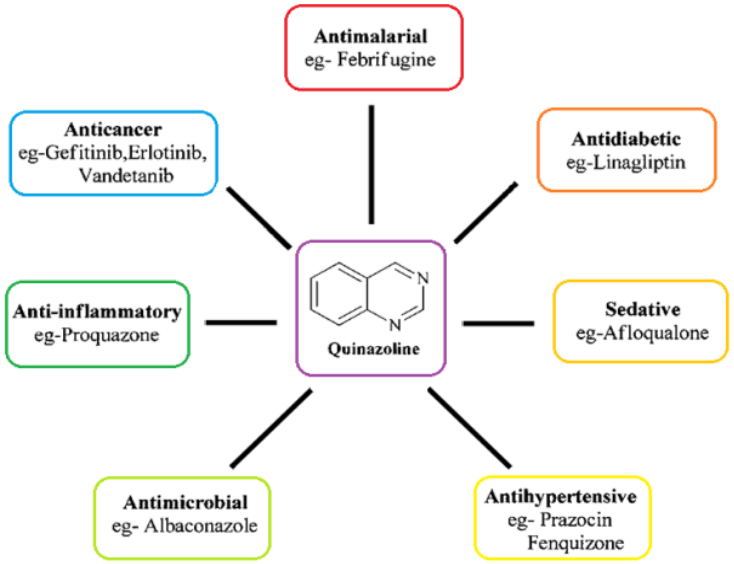
Abstract: Group of 2,3-ring-substituted quinazolinone-derived Schiff bases (Q1-Q4) were prepared and tested for cytotoxicity and DNA-protective potential against free radical-induced oxidative damage. The *in vitro* cytotoxic effect of compounds was assessed in human renal proximal tubular epithelial (TH-1) and hepatocellular carcinoma (HepG2) cells by MTT assay. The radical-scavenging and reducing potency was determined by DPPH and reducing power assay. The DNA-protective potential against oxidants (Fe^{2+}, H_2O_2) was screened by DNA topology and Comet assay. Antioxidant mode of action - total antioxidant cell status (TAS) and activity of individual antioxidant enzymes (SOD, CAT, GPx) were screened on TH-1 cells. The results suggest that Q1-Q4 affected TH-1 and HepG2 cell viability in a dose-dependent manner. Treatment of cells with Q1-Q4 increases TAS, GPx, SOD, and CAT levels. Compounds exhibit significant antioxidant properties, and high DNA-protective ability. The structure-biological activity relationships revealed the relation between structure and efficacy of studied compounds. The modification of the C-2/N-3 positions of the quinazolinone nucleus, by differently substituted phenyl moieties seems to be crucial for the DNA-protective potential of these compounds. Quinazolinones Q1-Q4 can be considered as an effective antioxidants, non-genotoxic and DNA-protective agents with potential medicinal applications.

Keywords: Quinazolinones; Antioxidant effect; DNA protectivity; Cytotoxicity

Introduction

Quinazolinones

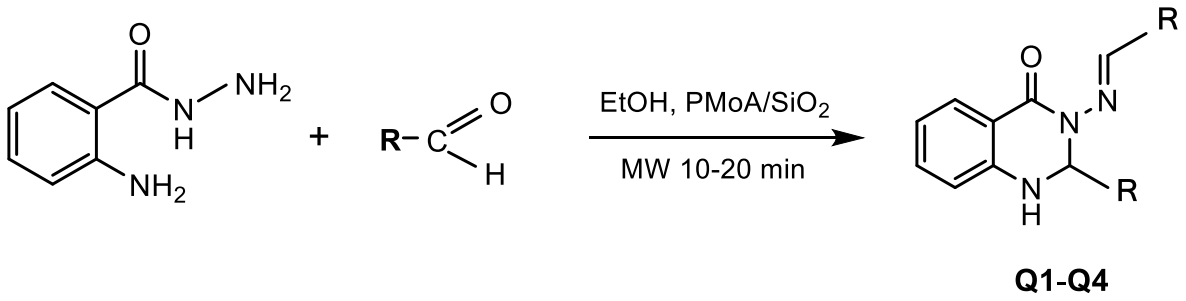
- applications in medicinal chemistry
- quinazoline-based alkaloids (vasicine, febrifugine) and synthetic analogues
- wide range of biological activities: antibacterial, antimalarial, antioxidant, anti-depressant, anti-inflammatory, vasodilating, anticancer agents [1-3].



[1] Hameed A., Al-Rashida M., Uroos M., Ali S.A. (2018) Expert Opin Ther Pat. 28, 281
[2] Gupta T., Rohilla A., Pathak A., et al. (2018). Synth Commun. 48, 1099
[3] Rakesh K.P., Manukumar H., Gowda D.C. (2015) Bioorg Med Chem Lett. 25, 107

- new C–2, N–3 di-substituted quinazolinone derivatives were designed and synthesized as novel antioxidants and potential DNA-protective agents

- Quinazolinone-derived Schiff bases (Q1-Q4) were synthesized by microwave-assisted, phosphomolybdic acid catalysed condensation reaction of 2-amino benzhydrazide with differently substituted aromatic aldehydes (R). The molecular structures of Q1-Q4 were characterized by analytical, and spectroscopic methods (NMR, FT IR, UV-Vis, EPR, HRMS) [4].



[4] Hricovíniová Z., Hricovíni M., Kozics K. (2018) Chem Pap. 72, 104

Material and methods

- Compounds: C-2,N-3-ring-substituted quinazolinone derived Schiff bases (**Q1-Q4**)

- Cell lines: Human renal proximal tubular epithelial cells (**TH-1**)
  Human hepatocellular carcinoma (**HepG2**) cells

Antioxidant activity:

- 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay
- Reducing power (RPA) assay
- Antioxidant enzymes activity assays (SOD, CAT, GPx)
- Total antioxidant status (TAS)

DNA genotoxicity/protectivity:

- DNA topology assay
- Single cell gel electrophoresis (Comet assay)

Cytotoxic activity:

- MTT assay

Results and discussion

Studied compounds Q1-Q4 showed promising antioxidant effect (**DPPH** assay, **Fig.1.**) and reducing power (**RPA** test, **Fig.2.**) in comparison with ascorbic acid (AA). The relative DPPH radical-scavenging activity of tested compounds increased in the following order: Q4 < Q2 ~ Q1~ Q3 < AA.

Derivative Q3 bearing four OH groups showed the highest Fe^{2+} reducing activity: Q4 < Q2 < Q1 < AA < Q3.

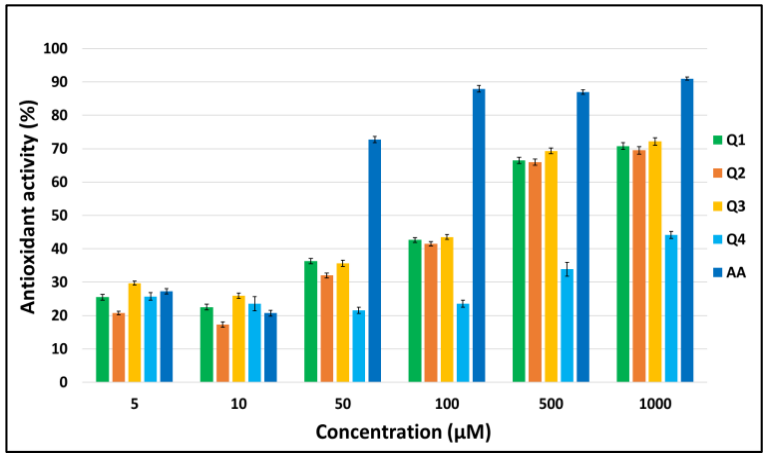


Fig.1. DPPH assay – Evaluation of radical scavenging activity of compounds Q1–Q4.

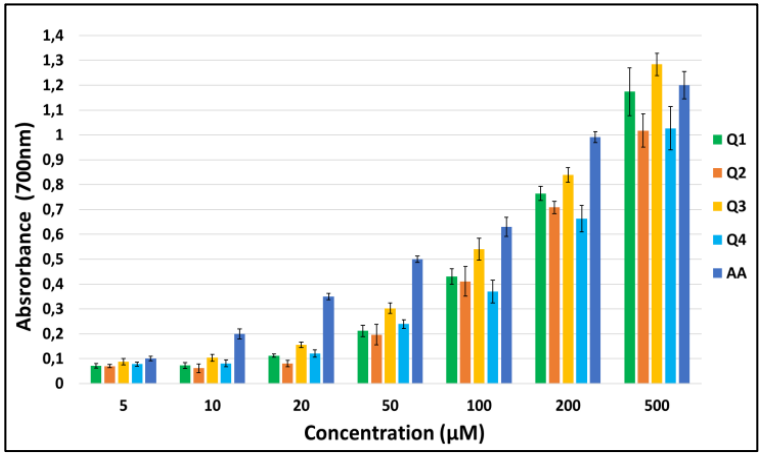


Fig. 2. Reducing power assay – Evaluation of antioxidant activity of compounds Q1–Q4.

Total antioxidant status (TAS) of Q1–Q4 and the **activity of individual enzymes** – superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) in TH-1 cells after 24 h treatment (**Table 1**.).

Tab.1. Control (−) untreated TH-1 cells; control (+) ascorbic acid. The levels of CAT and GPx and TAS, in TH-1 cells treated with Q1–Q4, were significantly higher in comparison with the negative control. Data represent the mean ± SD of three independent experiments. *P < 0.05; **P < 0.01; ***P < 0.001 indicate significant differences compared to the untreated control cells.

	TAS	SOD (U/mg prot)	GPx (U/mg prot)	CAT (U/mg prot)
Control (−)	0.795±0.120	0.957±0.070	0.0104±0.0008	142.109±2.290
Control (+)	3.320±0.080	2.520±0.520	0.039±0.002	585±54
Q1				
10µM	0.828±0.040	1.471±0.130	0.0187±0.0028*	237.274±1.948***
20 µM	0.992±0.025	0.804±0.210	0.0161±0.0008**	217.756±16.280*
50 µM	1.016±0.060	1.537±0.290	0.0229±0.0023**	285.640±0.956***
Q2				
10 µM	0.755±0.090	1.041±0.180	0.0158±0.0015**	232.940±7.437**
20 µM	1.091±0.240	1.415±0.440	0.0312±0.0029**	489.357±27.782**
50 µM	2.678±0.110**	2.084±0.001*	0.0349±0.0006**	544.981±29.630**
Q3				
10 µM	1.033±0.410	0.980±0.070	0.0140±0.0007*	243.603±16.312**
20 µM	1.247±0.420	1.023±0.180	0.0121±0.0007	240.114±3.354***
50 µM	1.615±0.110*	1.031±0.150	0.0197±0.0018**	261.402±2.166***
Q4				
10 µM	0.841±0.350	0.792±0.140	0.0162±0.0012**	232.392±6.750***
20 µM	1.040±0.040	0.869±0.010	0.0149±0.0007*	244.104±1.994***
50 µM	1.636±0.110*	1.077±0.030	0.0202±0.0021**	323.904±8.911***

DNA-protective/damaging effect of Q1-Q4, was assessed by **DNA topology assay** (**Fig.3**) and **Comet assay** (**Fig.4**). All studied compounds were able to protect **pBR322 plasmid DNA** as well as the **DNA of TH-1 cells** against oxidative DNA damage induced by oxidants (Fe^{2+}ions, H_2O_2).

DNA topology assay. Treatment of pBR322 plasmid DNA with Q1–Q4 in selected concentration range (5-500 µM) did not change the mobility of the supercoiled pDNA, indicating the non-genotoxic effect of tested compounds.

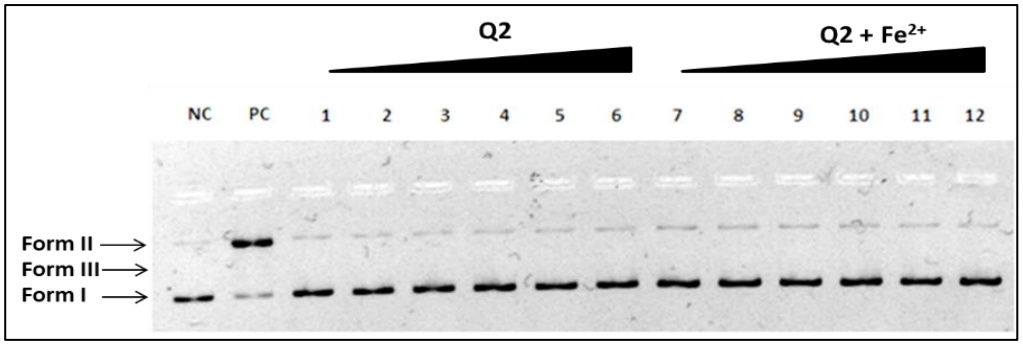


Fig. 3. Electrophoretic monitoring of topological changes induced in the structure of plasmid DNA with compound **Q2**. Plasmid DNA treated only with **Q2** in selected concentrations (5, 10, 20, 50, 100, 500 µM) (lanes 1–6); treatment with increasing concentrations (5–500 µM) of **Q2** in the presence of Fe^{2+} ions (lanes 7–12); negative control (NC): intact pBR322; positive control (PC): pBR322 treated with Fe^{2+} ions. Treatment of pBR322 with Fe^{2+} ions induce DNA breaks resulting in the conversion of plasmid topology from supercoiled (form I) to single-strand breaks (form II) or double-strand breaks (form III).

Comet assay. For the induction of DNA single strand breaks in TH-1 cells, H_2O_2 (500 µM) was used (**Fig.4**). Three non-genotoxic concentrations (10, 20 and 50 µM) were selected from standard comet assay (Fig.4, inserted panel). The H_2O_2-induced DNA damage (% of DNA in the tail) was about 45%. Pre-treatment of TH-1 cells with derivatives Q1-Q4 showed promising DNA-protective activity against H_2O_2, as they significantly decreased the levels of DNA lesions in comparison with the positive control.

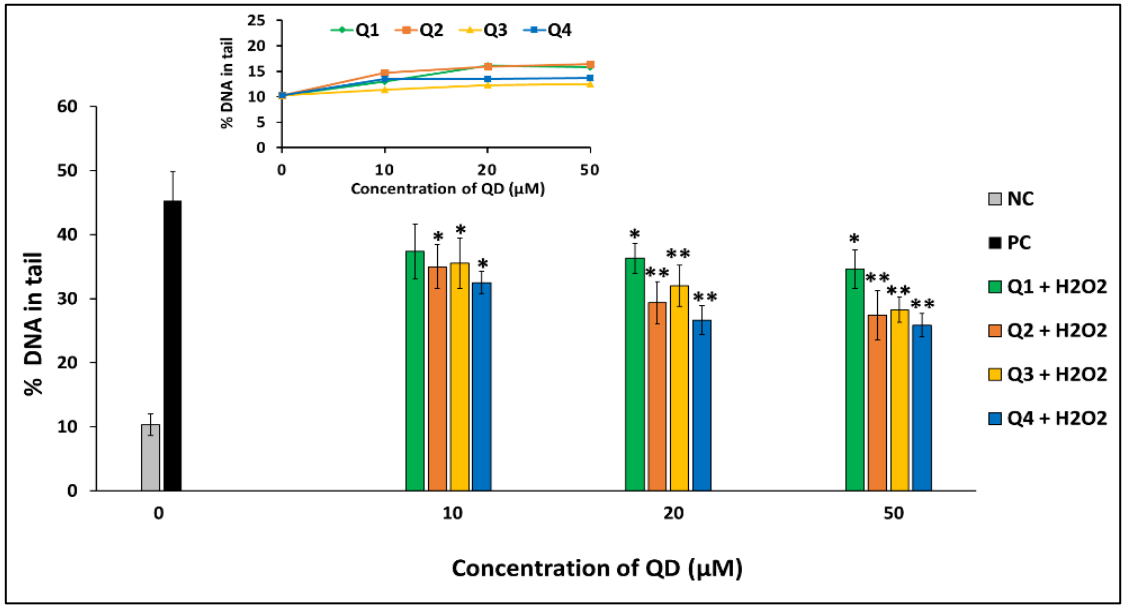


Fig. 4. Comet assay. DNA-protective effect of derivatives Q1–Q4 against H_2O_2. TH-1 cells were pre-treated with Q1–Q4 for 24 h and then treated with 500 µM H_2O_2. TH-1 cells only with PBS were used as negative control (NC); cells incubated only with 500 µM H_2O_2 correspond to a positive control (PC). *P < 0.05; **P < 0.01; ***P < 0.001 indicate significant differences compared to the positive control.

MTT assay. The **cytotoxic effect** of Q1–Q4 was investigated *in vitro* in the human TH-1 and HepG2 cell lines. The results of the MTT assay showed that the 24 h treatment of cells with Q1–Q4 affected cell viability in a dose-dependent manner. Derivatives exhibited variable potencies (IC_{50}).

Studied compounds exhibited different cytotoxic activity against tested cell lines (TH-1 **Fig.5**) and (HepG2 **Fig.6**).

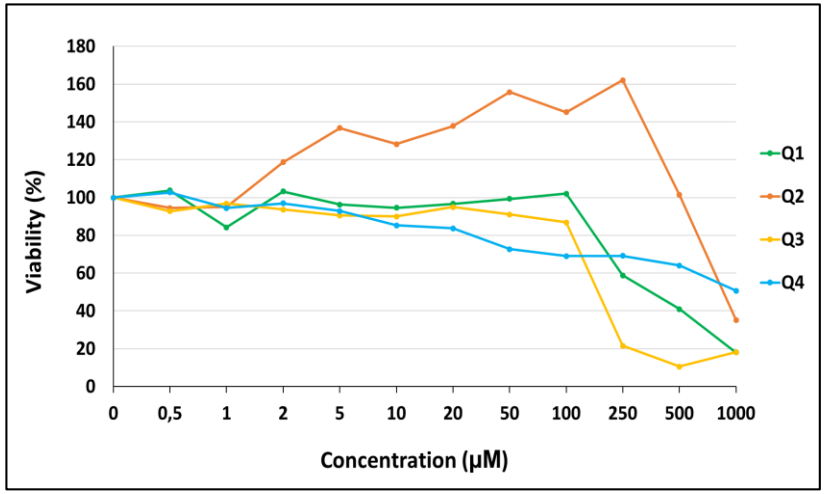


Fig.5 MTT assay – Evaluation of cytotoxic effects of compounds Q1–Q4 on TH-1 cells.

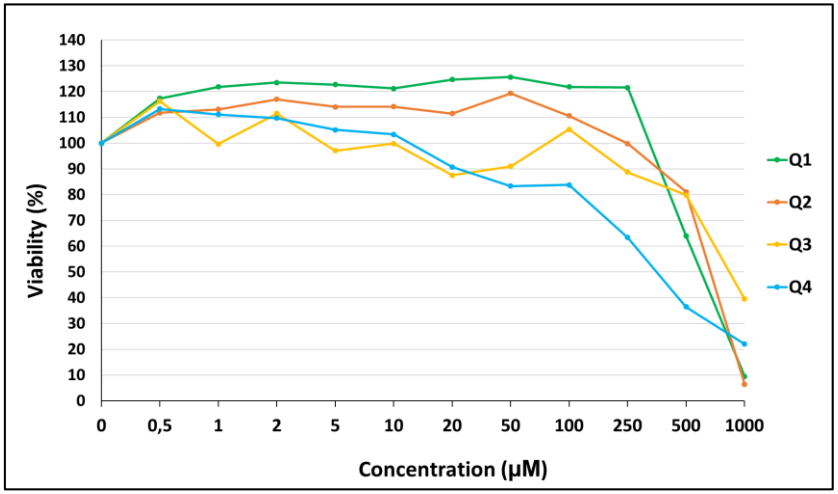


Fig.6 MTT assay – Evaluation of cytotoxic effects of compounds Q1–Q4 on HepG2 cells.

Conclusions

- C-2,N-3 substituted quinazolinone-derived Schiff bases (Q1-Q4) were characterized by chemical and biological assays
- High radical-scavenging, antioxidant and DNA-protective effects were observed
- Efficient DNA-protection correlates with enzymatic (SOD, CAT, GPx, TAS) and non-enzymatic (DPPH, RPA assay) antioxidant defence
- Studied compounds were able to protect the DNA of TH-1 cells as well as pBR322 plasmid DNA against oxidative DNA damage induced by oxidants
- SAR analysis revealed the relation between molecular structure and efficiency of studied derivatives
- Their potential applications in pharmacology will require additional validation using further *in vitro* and *in vivo* studies

Acknowledgments

The authors would like to acknowledge financial support from the Slovak Grant Agency, VEGA grants No. 2/0022/18 and 2/0055/20.