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Short communication

Antioxidant properties of a novel triazole ligand

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Introduction

Two of the most chemically active, hence harmful, physiologically significant reactive species (RS) are the hydroxyl radical (•OH) and the hydroperoxyl radical (•OOH) (Kell et al., 2009). They tend to attack a variety of molecular sites, especially such that contain conjugated double bonds. They are associated with lipid peroxidation, breaking down lipids to malondialdehyde (MDA) and other MDA-like products (Moore and Roberts, 1998). Lipid peroxidation and the associated chain reactions can be prevented with the help of antioxidants. Antioxidants are molecules that are able to interact with RS and, via hydrogen atom transfer (HAT) or electron transfer reactions, "neutralize" the unpaired electrons of RS, thus making them less harmful to living systems. The present work deals with an *in-vitro* investigation of the impact of two triazole derivatives - sodium 2-(4-chlorophenyl)-5-(pyrrolidin-1-yl)-2H-1,2,3-triazole-4-carboxylate and 2-(4-chlorophenyl)-5-(pyrrolidin-1-yl)-2H-1,2,3-triazole-4-carboxylic acid (named BNP01 and BNP03, respectively) - on the degradation of 2-deoxyribose, caused by UV-induced water radiolysis. Additionally, the ability of BNP01 and BNP03 to participate in HAT and electron-transfer reactions would be tested in order to attempt to identify the molecular sites, responsible for the possible antioxidant activity.

Materials and methods

Materials

All materials were of highest grade (pro analysis) SIGMA-ALDRICH (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany). Bi-distilled water and 95% ethanol were used as solvents. For the Deoxyribose Degradation Assay the following solutions were prepared: 6.0 mM water solution of deoxyribose; 1% water solution of thiobarbituric acid (TBA); 3% water solution of trichloroacetic acid (TCA); 50mM K-Na phosphate saline solution (PBS) with pH=7.4. The antioxidant capacity by way of HAT was measured using a stock ethanol solution of 2,2-diphenyl-1-picrylhydrazyl radical (•DPPH), prepared according to well-established protocols (Kedare and Singh, 2011; Molyneux, 2004). The electron-exchange potential of the substances was established using the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic free radical ($ABTS^{\bullet+}$) assay. For that purpose, as per Erel (2004a, b) were prepared the following solutions: 0,4 mmol acetate buffer, pH-5.8; 0.03 mmol acetate buffer solution, pH=3.6 with ABTS and H_2O_2 in order to produce the stable $ABTS^{\bullet+}$.

A Shimadzu UV1601 spectrophotometer was utilized for all UV-VIS measurements. Kinetic setting was used with 10 second lag time and 300 second measuring time. Results were calculated using the data obtained after 300 seconds.

For each tested concentration, three parallel measurements were taken, each one representing an individual datapoint. Averages and standard deviations were calculated. Relative changes within the limits of experimental error were not discussed. The impact of the varying concentrations on the results obtained was statistically verified using one-way ANOVA, followed by Bonferroni post-test.

Deoxyribose Degradation Assay

The investigation utilized a modified protocol by Halliwell et al. (1987). •OH and •OOH are generated by UV-induced water radiolysis (Burns and Sims, 1981). Degradation of 2-deoxyribose to MDA-like products is estimated with the TBA assay. The following molarities of each substance were tested: $1*10^{-4}$ M, $3*10^{-5}$ M, $1*10^{-5}$ M, $3*10^{-6}$ M and $1*10^{-6}$ M. The degree of 2–deoxyribose

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degradation was calculated as a Spectrophotometric Scavenging Index (SPh-SI):

$$SPh - SI, \% = \frac{A_{sample}}{A_{control}} * 100$$

DPPH Assay

For each concentration, the absorbance of three types of samples was measured at λ=517 nm – "blank", "control" and "sample". The HAT activity of the substances was calculated as Radical Scavenging Activity (RSA):

$$RSA, \% = \frac{A_{control} - (A_{sample} - A_{blank})}{A_{control}} * 100$$

The following molarities of each substance were tested: $1*10^{-4}$ M, $3*10^{-5}$ M and $1*10^{-5}$ M.

ABTS Assay

For each concentration, the absorbance of three types of samples was measured at λ=660 nm – "blank", "control" and "sample". The participation in electron-exchange reactions was presented as RSA, calculated with the same formula as the DPPH test. The following molarities of each substance were tested: $1*10^{-4}$ M, $3*10^{-5}$ M and $1*10^{-5}$ M.

Results and discussion

Both substances significantly suppress the degradation of 2-deoxyribose at the highest concentration ($1*10^{-4}$ M). At $3*10^{-5}$ M the sodium salt (BNP01) behaves as a more potent antioxidant than its conjugate acid (BNP03). At concentrations of $1*10^{-5}$ M and below, both substances seem to exhibit a very slight antioxidant effect.

BNP01 displayed very close to zero tendency to participate in HAT reactions with DPPH within the entire range of investigated concentrations. BNP03 interacted with the stable radical to a small extent at the highest concentration. Its activity dropped in a concentration-dependent manner to practically zero at $1*10^{-5}$ M. Since both substances did not manifest any significant activity at $1*10^{-5}$ M, the authors deemed it unnecessary to test lower concentrations.

At the highest concentration both substances interacted with ABTS•$^{+}$ to a limited extent, the activity of BNP01 being slightly higher. The activities of both seem to decrease in a concentration-dependent manner to near zero within the limits of the experimental error at $1*10^{-5}$ M, therefore, the authors decided not to test lower molarities.

Conclusions

Based on the aforementioned experimental data, the following conclusions can be drawn:

- both BNP01 and BNP03 are *in-vitro* scavengers of free radicals, derived by UV radiolysis of water in presence of 2-deoxyribose. Their activity decreases in a concentration-dependent manner;

- BNP01 does not participate significantly in HAT reactions with DPPH, while BNP03 manifests an observable activity at concentrations above $1*10^{-5}$ M. The authors propose that the higher observable HAT activity of BNP03 is due to the active hydrogen from the carboxyl group;

- both compounds interact with ABTS•$^{+}$ - BNP01 seems to be a better scavenger of the stable radical at $1*10^{-4}$ M compared to its conjugate acid. The activity decreases in a concentration-dependent manner to practically zero at $1*10^{-5}$ M.

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