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In vitro antioxidant activity of nicotinic acid hydrazides: Experimental and theoretical study•

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Abstract: The formation of reactive oxygen species (ROS) in the human body can lead to cell damage. Despite the body's natural defences, including the enzyme superoxide dismutase, novel antioxidant small molecules are needed. In this work, *in vitro* antioxidant activity of seven nicotinic acid amides (NcAs) derived from nicotinic acid and *mono*-thiocarbohydrazones was investigated using DPPH, ABTS, CUPRAC and TAC assays. The compounds exhibited IC_{50} values between 0.202 to 1.297 mM in the DPPH assay, which improved to 0.114–0.638 mM upon the addition of water in the system. In the ABTS assay, IC_{50} values ranged from 0.107 to 0.365 mM. CUPRAC and FRAP assays indicated reducing antioxidant power of 1.973–4.650 and 1.564–3.472 mM L^{-1}, respectively. Moderate antioxidant activity was also observed in the phosphomolybdenum assay for total antioxidant capacity. The density functional theory calculations revealed that the S–H bond of thioenol 1 tautomer, with a low bond dissociation enthalpy (*BDE*) of around 270 kJ mol^{-1}, is the favourable site for hydrogen atom transfer (HAT) to reactive free radicals. Additionally, all compounds exhibited high stability constants with Fe^{2+} and Fe^{3+} ($K_s \approx 10^8$), forming complexes with ML stoichiometry.

Keywords: biological activity; ROS; DFT; metal complexes; imino derivatives; pyridinecarboxylic acid derivatives.

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•This article is dedicated to the memory of our esteemed colleague Jasmina Nikolić whose scholarly contributions and dedication greatly enriched this work.
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INTRODUCTION

The human body is a complex system involving numerous processes, including metabolism, cellular repair and the transport of vital substances through the bloodstream.[1] Oxygen plays the main part in these processes, particularly in the production of adenosine triphosphate (ATP), which is essential for energy transfer within cells. ATP facilitates oxygen transport by haemoglobin and supports respiration.[2] While oxygen is crucial for cellular metabolism, its metabolic transformation into reactive oxygen species (ROS) presents potential downsides. ROS can lead to cell inflammation, cell death[3] and oxidative stress, which have both harmful and beneficial effects on the body. At regulated levels, ROS can inhibit tumour growth and neutralize pathogens. However, excessive ROS levels contribute to oxidative stress, which is linked to numerous diseases including cancer, cardiovascular disease and neurodegenerative disorders. Alongside ROS, reactive nitrogen species (RNS)[4] also play significant role in the oxidative stress. The most abundant ROS and RNS species include superoxide ($O_2^{\bullet-}$), hydroxyl ($HO^{\bullet}$), hydroperoxyl ($HO^{\bullet}_2$) and nitric oxide ($NO^{\bullet}$) radicals.

An example of free radical formation is the interaction of gamma radiation with water in tissue cells. The energy absorbed splits the O–H bonds homolytically, producing hydrogen atom (H) and OH·radical.[5] ROS and RNS can be generated both enzymatically and non-enzymatically. Enzymatic production involves enzymes such as peroxidase, NADPH oxidase, xanthine oxidase and others. Non-enzymatic formation occurs during oxidative phosphorylation in the mitochondria, where ATP is produced.[4] When mitochondria are dysfunctional, there is an increased production of $O_2^{\bullet-}$, leading to the elevated levels of ROS and an increased risk of DNA mutations, which accelerate aging.[6]

Superoxide dismutase (SOD) is a key enzyme that reduces the harmful effects of free oxygen radicals. It neutralizes superoxide radicals by converting them to into H_2O_2 and oxygen. However, H_2O_2 can be toxic to cells, as it can damage DNA through the reactions involving Fe^{2+}/Fe^{3+}.[1] SOD plays an important role in preventing diseases such as cancer, inflammatory conditions and rheumatoid arthritis, and current research focuses on its potential use in medical treatments.[7]

The human body system defends against ROS through three main mechanisms: 1) the use of antioxidants, 2) the action of SOD and 3) preventing the formation of free radicals with metal-ion binding proteins. Antioxidants can be either lipophilic or hydrophilic. Many fruits and vegetables contain hydrophilic antioxidants, such as vitamins and minerals, which are easily absorbed and eliminated from the body. In contrast, lipophilic antioxidants require fat for absorption and are not as rapidly eliminated.

Transition metals such as Fe^{2+}/Fe^{3+} and Cu^{+}/Cu^{2+} can react with O_2^{-} and H_2O_2 to produce $HO^{\bullet}$. When these metals interact with lipids, they degrade peroxides into peroxyl and alkoxyl radicals, further contributing to oxidative damage:[5]

$$\underset{(Cu^{+})}{\text{Lipid–OOH}} + Fe^{2+} \rightarrow \underset{\text{alkoxyl radical } (Cu^{2+})}{\text{Lipid–O}^{\bullet}\cdot} + Fe^{3+}{}^{\bullet}OH^{-} \quad \text{(I)}$$

$$\text{Lipid–OOH} + Fe^{3+} \rightarrow \underset{\text{peroxyl radical}}{\text{Lipid–OO}^{\bullet}\cdot} + Fe^{3+} + H^{+} \quad \text{(II)}$$

The redox activity of iron ions contributes to accumulation of ROS, and excessive iron can lead to cell death.[8] As a result, many studies have focused on iron chelation using various natural products. For instance, ginger contains curcumin, which chelates Fe^{2+} and prevents the formation of Fe^{2+}–ferrozine complex. This process help mitigate iron overload.[9] Moreover, the complexation of metal ions such as Al(III), Zn(II), Cu(II) and Fe(II) with natural antioxidants like flavonoids has proven to be an effective strategy for developing novel compounds with enhanced anti-inflammatory, anticancer and antioxidative properties compared to their uncomplexed forms. In particular, superior antioxidative activity of these metal complexes is often attributed to their ability to activate SOD.[1]

Thiosemicarbazones are well-known metal chelators due to the presence of sulphur and nitrogen atoms, which enable strong coordination with metal ions. These metal complexes exhibit potent anticancer activity[10] along with other valuable biological properties, such as antimicrobial effects.[11] Among various thiosemicarbazones, phenyl-thiosemicarbazones have been extensively studied.[12]

In this study, we report the antioxidant activity of novel amides synthesized *via* condensation between nicotinic acid and mono-thiocarbohydrazones. The antioxidant activity was assessed using several assays, including DPPH, ABTS, CUPRAC, FRAP and TAC methods. Moreover, we determined the stoichiometry and stability constants of complexes formed between Fe^{2+} and Fe^{3+} and the synthesized ligands. Finally, quantum chemical calculations were conducted to investigate the structure-antioxidant relationship of these compounds.

EXPERIMENTAL

Chemicals

All chemicals and solvents for synthesis were obtained from Sigma Aldrich and Merck and used without additional purification. Sodium phosphate, ammonium molybdate tetrahydrate, dimethyl sulfoxide (DMSO), 1,1-diphenyl-2-picrylhydrazil (DPPH$^{\bullet}$), 6-hydroxy-2,5,7,8--tetramethylchroman-2-carboxylic acid (Trolox) and 2,2'-azino-bis(3-ethylbenzothiazoline-6--sulfonic acid) (ABTS) were purchased from Sigma Aldrich. Neocuproine was obtained from Acros Organics. Copper(II) chloride dihydrate and ammonium acetate were purchased from Merck. All chemicals and reagents for antioxidant analysis were of commercial quality and used without further purification.

Structure of nicotinic acid amides with monothiocarbohydrazones (NcA)

Synthesis and structural characterization of compounds **1**–**7** (Fig. 1) were described in our previous work.[13] The analytical and spectral data for all synthesized compounds are given in the Supplementary material to this paper.

Computational methods

Bond dissociation enthalpy (BDE) is calculated by optimizing the geometries of a molecule, corresponding radical and H atom at the B3LYP/6-311++g(d,p) level of theory. The unrestricted DFT calculations are performed for radicals, with multiplicity set to 2. The frequency calculations confirmed that there are no negative vibrational frequencies, so each geometry obtained corresponds to the minimum. The zero point energy correction is applied to the molecule and corresponding radical. All calculations were done in Gaussian 09, version B.01.[14]

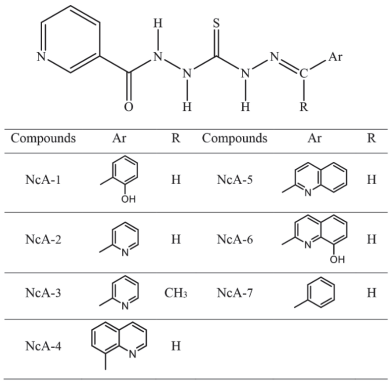

Fig. 1. Structures of synthesized NcA derivatives.

BDE was calculated by the following equation:

$$BDE = H_{\text{radical}} + H_{\text{H\_atom}} - H_{\text{molecule}} \quad (1)$$

Herein, H_{radical}, $H_{\text{H\_atom}}$, H_{molecule} represent enthalpies of radical species, H atom and ground state, respectively.

Antioxidant methods

DPPH$^{\bullet}$ and ABTS free radical scavenging assays were conducted by measuring the changes in the absorbance of DPPH$^{\bullet}$ at 517 nm and ABTS$^{\bullet+}$ at 734 nm, as described in the literature.[15] Ascorbic acid was used as a control and the scavenging activity was calculated as:

$$\text{Scavenging activity} = 100\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \quad (2)$$

where A_{sample} and A_{control} refer to the absorbances at 517 and 734 nm of DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ in the samples and control solutions, respectively.

The cupric ion reducing antioxidant capacity (CUPRAC) assay method was prepared according to the method[16] by measuring the changes in the absorbance at 450 nm. Trolox was used as a control. The results were reported as Trolox equivalent antioxidant capacities (*TEAC*) and calculated as ratio of molar absorptivity of each tested compounds and molar absorptivity of Trolox using the following equation:

$$TEAC = \frac{\varepsilon_{\text{sample}}}{\varepsilon_{\text{Trolox}}} \quad (3)$$

where ε_{sample} and ε_{Trolox} are molar absorptivities of sample and Trolox solutions, respectively.

Ferric reducing antioxidant power assay (FRAP) method was prepared according to the methods given in reference[17] by measuring the changes in the absorbance at 593 nm. $FeSO_4 \cdot 7H_2O$ was used as a control to construct a calibration curve (0.1, 0.4, 0.8, 1.0, 1.12, 1.5 mM) and the results are expressed as mM Fe^{2+}/L of the compounds.

Phosphomolybdenum assay, which defines the total antioxidant capacity (*TAC*), was performed according to the method described elsewhere.[17] The absorbance at 695 nm was recorded on Shimadzu 1800 UV/Vis spectrophotometer. The antioxidant capacity was expressed as the mass equivalents of ascorbic acid.

Mole ratio method – determination of stability constant

The stoichiometry and stability constants of the metal complexes formed between Fe^{2+} and Fe^{3+} and nicotinic acid amides (NcAs) were determined according to the procedure reported in the literature.[18] Stock solutions of metal ions (1.0×10^{-2} M) were prepared using Fe^{2+} ($FeSO_4 \cdot 7H_2O$) and Fe^{3+} (($Fe(NO_3)_3 \cdot 9H_2O$). Stock solutions of compounds NcA1–NcA7 were prepared in a mixture of deionized water (10 ml) and DMSO (2 ml). Working solutions of compounds were prepared within a concentration range that ensured the absorbance of 0.5–0.6 at λ_{max}. Following the procedure, 3 ml of the compound solution was mixed with 2 µl of the stock metal solution, and the reaction mixture was mixed using the magnetic stirrer for 10 min at 200 rpm. After the equilibration, the UV spectra were recorded. The compounds were titrated with the stock metal solution until the UV spectra stabilised, indicating the endpoint of the reaction.

RESULTS AND DISCUSSION

Antioxidant capacity of nicotinic acid amides (NcAs)

The antioxidant activity of nicotinic acid amide derivatives was investigated *in vitro* using various assays, including DPPH, ABTS, CUPRAC, FRAP and *TAC*. Ascorbic acid and Trolox were used as standard antioxidants. The tested derivatives exhibited varying degrees of antioxidant activity, ranging from weak to strong, with the results summarized in Table I.

TABLE I. IC_{50} values (mM) determined by DPPH and ABTS methods, and the results of CUPRAC, *TAC* and FRAP assays

Compound	DPPH	DPPH DMSO+H_2O	ABTS	CUPRAC *TEAC*	*TAC* Phosphomolybdenum test	*FRAP* mM/L
NcA-1	0.232	0.138	0.365	3.791	0.490	3.321
NcA-2	0.202	0.114	0.107	4.650	0.484	3.472
NcA-3	0.186	0.241	0.279	1.973	0.754	1.564
NcA-4	0.215	0.283	0.147	4.373	0.670	3.460
NcA-5	0.952	0.638	0.152	3.894	0.697	2.278
NcA-6	1.297	0.281	0.112	4.516	0.897	3.470
NcA-7	1.584	0.135	0.142	4.138	0.727	3.454
Ascorbic acid	0.094	0.211	0.150	–	–	–
Trolox	–	–	–	1.000	–	–

In the DPPH assay, compound NcA-3 demonstrated the highest activity among the derivatives, with an IC_{50} value of 0.183 mM (Table I), although this activity was still lower than that of ascorbic acid (IC_{50} = 0.0939). While all derivatives showed lower antioxidant activity compared to the standards, the derivatives displayed notable antioxidant potential in the DMSO/water, ranging from 0.114 to 0.638 mM at the same concentration. Specifically, compounds NcA-1, NcA-2 and NcA-7 were more potent toward $DPPH^{\bullet}$ than ascorbic acid. As reported by Assaleh *et al.*[19] the addition of water to DMSO led to increased antioxidative activity of thiocarbohydrazones in the DPPH assay. This effect was ascribed to the *E*/*Z* isomerism around the imine bond, thione/thiol tautomerism, and hydrogen bonding interactions between the studied compounds and solvent.

The antioxidant activity of NcAs varied across the different assays used (Table I). In the ABTS assay, all compounds effectively scavenged $ABTS^{\bullet+}$, with NcA-1 and NcA-3 showing comparatively lower activities. On the other hand, higher activities – *i.e.*, lower IC_{50} values – were observed for the ABTS assay compared to the DPPH and DPPH (DMSO + H_2O) assays. Similar activities of NcA-2 and NcA-4–NcA-7 derivatives were identified by ABTS method (Table I).

The antioxidant activity is influenced by the presence and position of substituents, as well as the nature of electron-accepting and electron-donating groups and the inclusion of heteroatoms in the aromatic core. In particular, the presence of a hydroxyl group in the ortho position on the quinoline ring significantly increased activity, as evidenced by the literature.[15,20] The results suggest that extended π-delocalization in the most potent derivatives, along with the electron-donating effect of the hydroxyl group in NcA-6, play a crucial role in the resonance stabilization of the radical, contributing to the higher activity than NcA-5 in the ABTS assay. Additionally, intramolecular hydrogen bonding (IHB) between the phenolic –OH group and imino nitrogen in NcA-1 may hinder efficient hydrogen atom transfer, which likely contributes to its reduced antioxidant activity across all free radical assays.

The CUPRAC assay, which evaluates antioxidative potential based on reduction reactions, is particularly suitable for compounds with chelating groups. Trolox was used as the standard, and the results were expressed as *TEAC*, based on the molar absorptivity ratios of the tested compounds compared to Trolox (Table I and Fig. S-36 of the Supplementary material). Among the derivatives, NcA-2, NcA-6 and NcA-4 exhibited the higher activities, while NcA-3 showed the lower.

The FRAP assay, which measures the ability of compounds to reduce Fe(III) to Fe(II), indicated that all tested derivatives displayed good reducing power, with values ranging from 1.564 and 3.472 mM/L. NcA-2 was identified as the most potent derivative in this assay (Table I).

In the TAC, which evaluates the total antioxidant capacity, the tested nicotinamides showed promising antioxidative activity relative to ascorbic acid. The results, expressed as ascorbic acid equivalents, are presented in Table I. NcA-6 demonstrated the higher antioxidant potential, with 1 mg of the compound equivalent to 0.897 mg of ascorbic acid.

Mechanism of antioxidative activity – bond dissociation enthalpy and spin density distribution of NcA compounds

The molecular structure plays a crucial role in determining the radical scavenging properties of antioxidants. To correctly ascribe the solution structure of thiocarbohydrazones, it is essential to consider both their *E*/*Z* isomerism and tautomerism. In the solid-state, bis-thiocarbohydrazones predominantly exist as the thione (thioketo) form.[21,22] However, the presence of the thioenol tautomers cannot be completely ruled out, as the experimentally determined bond C–S length (1.66–1.67 Å) falls between the typical values for a C–S single bond (1.82 Å) and a C=S double bond (1.56 Å).[23]

In a solution, solvent-solute interactions may alter the isomeric or tautomeric preferences of a compound. While the thioenol tautomers are less prevalent than the thioketo form, it may play a more significant role in the interaction between the studied compounds and free radicals. Our previous experimental and theoretical studies confirmed the presence of thioenol tautomer of bis-thiocarbohydrazones in aqueous solution.[19]

Accordingly, we hypothesize that the S–H bond in the thioenol tautomer is a favourable site for attack by reactive free radicals such as $DPPH^{\bullet}$ and $ABTS^{\bullet+}$. This hypothesis was tested by calculating the *BDE* of several bonds in thioketo and two thioenol tautomeric forms of NcA-2 (Fig. 2), where the bond with the lowest *BDE* would indicate the most reactive site for the interaction with free radicals. As shown in Fig. 2, the S–H bond in the thioenol 1 form is more susceptible to free radical attack compared to the N–H bonds. Due to significant differences in *BDE*s, we focused exclusively on the thioenol 1 form for all other compounds. Table II lists the *BDE* values of the S–H bonds in the thioenol 1 form for compounds NcA1–NcA7, along with the *BDE*s for phenolic O–H bonds in compounds that contain this functional group.

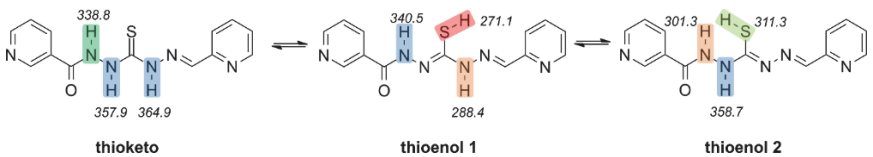

Fig. 2. Tautomeric equilibrium of NcA-2 and bond dissociation enthalpies (*BDE* in kJ mol^{-1}) of the three most reactive heteroatom-hydrogen (X–H) bonds in each tautomer.

TABLE II. *BDE* values (kJ mol^{-1}) for S–H bond of thioenol 1 tautomer of all compounds, and O–H group for NcA-1 and NcA-6

Compound	S–H	O–H
NcA-1	269.43	326.02
NcA-2	271.11	
NcA-3	272.24	
NcA-4	289.95	
NcA-5	291.03	
NcA-6	290.25	318.18
NcA-7	269.08	

According to Eq. (1), the resonance stabilization of radical formed by H atom abstraction decreases the enthalpy of the radical, which results in lower *BDE* values. The extent of this resonance stabilization can be elucidated by visualizing the electron spin density (*ESD*) distribution of the radical. The 2D representation of the *ESD* distribution of S radical of thioenol 1 tautomer (Fig. 3a) shows that the spin is delocalized over the entire thiocarbazone moiety. Only 17.6 % of the spin remains at the S atom, while most of the *ESD* is delocalized over the neighbouring N atom. Accordingly, the extent of O radical delocalization for NcA-1 is lower (Fig. 3b), where 34 % of the initial spin density is retained on the O atom, and the largest part of α and β spin densities is delocalized over the corresponding aromatic ring. The 3D representation of *ESD* distribution provides better visualization of this effect (Fig. 3c and d).

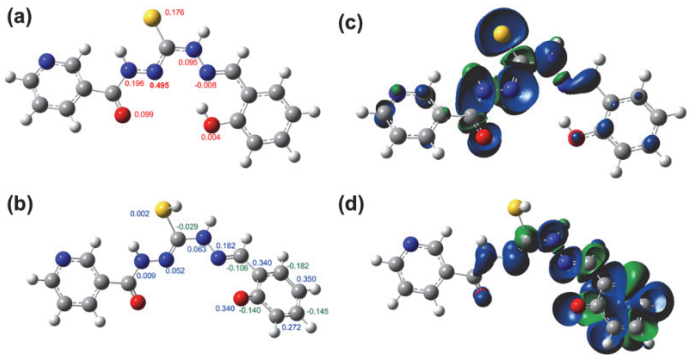

Fig. 3. Mulliken atomic spin densities for compound NcA-1 calculated on B3LYP/6-311++g(d,p) level of theory. a) 2D representation of electron spin density (*ESD*) for S radical of thioenol 1 form; b) 2D representation of *ESD* for O radical of thioenol 1 form; c) 3D *ESD* distribution of S radical; d) 3D *ESD* distribution of O radical.

In the case of thienol 2 form, the most favourable site for H-atom abstraction is the amidic N_1–H bond (*BDE* = 301.3 kJ mol^{-1}), while the S–H and N_2–H are less reactive, with *BDE*s of 311.3 and 358.7 kJ mol^{-1}, respectively. The exceptional reactivity of the amidic N–H bond can be attributed to pronounced spin

delocalization in the resulting radical, where only 45.9 % of spin is retained on N_1 atom and the rest is distributed, mostly over thiocarbohydrazide moiety. In contrast, the reactivity of the amidic N–H bond toward H-atom transfer is significantly reduced in the thioketo and thioenol 1 tautomers, with corresponding BDEs of 338.8 and 340.5 kJ mol^{-1}, respectively.

Given the low *BDE* value of NcA-1 in its thioenol 1 form, it would be expected to exhibit stronger antioxidative activity. Indeed, the IC_{50} value decreases from 0.232 to 0.138 mM with the addition of water to the solvent system. However, despite the fact that the *BDE* of the NcA-2 derivative is 1.5 kJ mol^{-1} higher than that of NcA-1, NcA-2 demonstrates greater antioxidant activity. This can be attributed to the formation of an intramolecular, six-membered hydrogen-bonded ring between the phenolic –OH group and the imino-N atom, which stabilizes the ground state and consequently reduces its reactivity toward free radicals.

Iron chelating ability

We next investigated the iron-chelating capabilities of the synthesized nicotinamides toward biologically relevant Fe^{2+} and Fe^{3+}. Compounds containing functional groups such as C=O, C=S, C–OH and C–SH demonstrate high affinity for metal ion binding, allowing them to uptake iron ions from tissues and promote their excretion through urine and faeces. These compounds act as antioxidants by reducing the availability of iron ions available for $^{\bullet}$OH generation *via* the Fenton reaction.[24]

Nicotinamides show strong affinity for Fe^{2+} and Fe^{3+}, with stability constants (K_s) ranging from 10^7 to 10^9 M^{-1} (Table III and Fig. S-37 of the Supplementary material). The stoichiometry of the resulting complexes is 1:1, indicating that all complexes are of the ML-type. As discussed, the compounds with C=O and C=S groups form stable complexes with iron ions, and the nicotinamides studied here contain both moieties, resulting in high chelating abilities toward Fe^{2+} and Fe^{3+}.

TABLE III. Stability constants of nicotinamide complexes with Fe^{2+} and Fe^{3+}

Compound	$K_s(Fe^{2+})\times10^{-8}/M^{-1}$	$K_s(Fe^{3+})\times10^{-8}/M^{-1}$
NcA-1	3.89	0.211
NcA-2	5.32	77.3
NcA-3	12.1	9.5
NcA-4	13.2	2.97
NcA-5	7.76	9.69
NcA-6	37.9	47.8
NcA-7	8.76	33.2

Among the compounds, the NcA-6 formed the most stable complexes with iron ions, likely due to the presence of 8-hydroxy group on the 2-quinoline moiety, which may serve as an additional ligand for metal ions. NcA-1 exhibited the lowest iron-chelating ability, probably due to a strong intramolecular hydrogen bond and

the reduced coordination potential of its imino nitrogen atom and phenolic OH group.[19]

In a previous study by Assaleh *et al*.[18] cinnamic acid hydrazides in complexes with iron ions demonstrated high antimicrobial activity, especially against *Acinetobacter baumannii.* Trends in the observed biological activity correlated with the stability constants of the complexes, influenced by electron density distribution across the thiocarbohydrazone bridge and significant atomic charges on carbonyl oxygen, sulphur and nitrogen atoms. Given the structural similarity between our nicotinamides and cinnamic acid amides, iron complexation could play an important role in the antimicrobial mechanism of NcAs.[13] This aspect will be further explored in future research.

CONCLUSIONS

In this study the structure-antioxidant activity relationship of novel nicotinamides (NcAs) using DPPH, ABTS, CUPRAC, *TAC* and iron chelating assays was investigated. Quantum-chemical calculations of bond dissociation enthalpies (*BDE*s) provided further insights into their antioxidant mechanisms. Our findings revealed that the addition of water promotes thione/thiol isomerism and favours the formation of the S–H bond, a key site for hydrogen atom transfer due to its exceptionally low *BDE* value. Additionally, NcAs demonstrated strong iron-chelating properties, with the 8-hydroxy-2-quinoline derivative NcA-6 forming the most stable 1:1 complexes. In contrast, the intramolecular hydrogen bonding (IHB) in NcA-1 was found to reduce both its antioxidant capacity and iron-chelating ability. The future studies will focus on optimizing the antioxidant properties of NcAs, particularly by investigating their interaction with metal ions and how this influences their radical-scavenging activity.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/13117, or from the corresponding author on request.

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ИЗВОД

IN VITRO АНТИОКСИДАТИВНА АКТИВНОСТ ХИДРАЗИДА НИКОТИНСКИХ КИСЕЛИНА: ЕКСПЕРИМЕНТАЛНА И ТЕОРЕТСКА СТУДИЈА

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Формирање реактивних кисеоничних врста у организму може да оштети ћелије. Поред природних одбрамбених механизама као што је ензим супероксид-дизмутаза, потребни су и нови мали молекули као антиоксиданси. У овом раду испитивана је антиоксидативна активност више амида никотинске киселине, синтетисаних у реакцији са *mono*-тиокарбо-хидразонима, помоћу DPPH, ABTS, CUPRAC и TAC метода. IC_{50} вредности су биле између 0,202 и 1,297 mM за DPPH (0,114–0,638 mM уз додатак воде) и 0,107––0,365 mM за ABTS. Дистрибуција електронског спина је показала да је S–H веза тиоенолног облика испитиваних никотинамида погодно место за транфер атома водоника на реактивне слободне радикале. Сва једињења су показала високе константе стабилности комплекса типа ML са Fe^{2+} и Fe^{3+}.

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REFERENCES

1. P. Mucha, A. Skoczyńska, M. Malecka, P. Hikisz, E. Budzisz, *Molecules* **26** (2021) 4886 (https://doi.org/10.3390/molecules26164886)
2. E. Ortiz-Prado, J. F. Dunn, J. Vasconez, D. Castillo, G. Viscor, *Am. J. Blood Res*. **9** (2019) 1 (https://pubmed.ncbi.nlm.nih.gov/30899601/)
3. A. A. Dayem, M. K. Hossain, S. B. Lee, K. Kim, S. K. Saha, G. M. Yang, H. Y. Choi, S. G. Cho, *Int. J. Mol. Sci.* **18** (2017) 120 (https://doi.org/10.3390/ijms18010120)
4. L. A. Pham-Huy, H. He, C. Pham-Huy, *Int. J. Biomed. Sci.* **4** (2008) 89 (https://www.ijbs.org/User/ContentFullText.aspx?VolumeNO=4&StartPage=89)
5. B. Halliwell, *Br. J. Exp. Path*. **70** (I989) 737 (https://pubmed.ncbi.nlm.nih.gov/2557883/)
6. A. J. Pontes, O. de Almeida, J. C. Pinheiro, L. de Oliveira, L. V. da Silva Pontes, J. F. de Souza Júnior, T. Amanda, F. Gonçalves, S. H. Dantas, M. S. de Almeida Feitosa, A. O. Silva, I. A. Medeiros, *Oxid. Med. Cell. Longev.* **2022** (2022) 1225578 (https://doi.org/10.1155/2022/1225578)
7. H. Younus, *Int. J. Health Sci.* **12** (2018) 88 (https://pmc.ncbi.nlm.nih.gov/articles/PMC5969776/)
8. G. Özbolat, A. A. Yegani, *Cukurova Med. J.* **44** (2019) 947 (https://doi.org/10.17826/cumj.484672)
9. I. Gulcin, S. H. Alwasel, *Processes* **10** (2022) 132 (https://doi.org/10.3390/pr10010132)
10. X. G. Bai, Y. Zheng, J. Qi, *Front. Pharmacol.* **13** (2022) 1 (https://doi.org/10.3389/fphar.2022.1018951)
11. S. M. E. Khalil, M. Shebl, F. S. Al-Gohani, *Acta Chim. Slov.* **57** (2010) 716 (https://acta-arhiv.chem-soc.si/57/57-3-716.pdf)
12. J. H. Ajudiya, M. C. Shah, *Ymer* **21** (2022) 152 (https://ymerdigital.com/uploads/YMER211221.pdf)

13. B. Anić Marković, A. Marinković, J. Antić Stanković, S. Mijatović, I. Cvijetić, M. Simić, I. Arandjelović, *Pharmaceuticals* **17** (2024) 1084 (https://doi.org/10.3390/pharmaceutics16081084)
14. *Gaussian 09, revision B.01*, Gaussian, Inc., Wallingford, CT, 2009 (https://gaussian.com)
15. M. D. Milošević, A. D. Marinković, P. Petrović, A. Klaus, M. G. Nikolić, N. Ž. Prlainović, I. N. Cvijetić. *Bioorg. Chem.* **102** (2020) 104073 (https://doi.org/10.1016/j.bioorg.2020.104073)
16. M. Özyürek, K. Güçlü, E. Tütem, K. S. Başkan, E. Erçağ, S. Esin Çelik, S. Baki, L. Yıldız, Ş. Karaman, R. Apak, *Analit. Met.* **3** (2011) 2439 (https://doi.org/10.1039/c1ay05320e)
17. I. F. F.Benzie, J. J. Strain, *Meth. Enzym.* **299** (1999) 15 (https://doi.org/10.1016/S0076-6879(99)99005-5)
18. M. H. Assaleh, S. Jeremić, I. Cvijetić, A. Marinković, N. Prlainović, *J. Mol. Struct.* **1262** (2022) 1262 (https://doi.org/10.1016/j.molstruc.2022.133016)
19. M. H. Assaleh, A. R. Božić, S. Bjelogrlić, M. Milošević, M. Simić, A. D. Marinković. I. N. Cvijetić, *Struct. Chem.* **30** (2019) 2447 (https://doi.org/10.1007/s11224-019-01371-4)
20. M. O. Puskullu, B. Tekinel, S. Suzen, *Mini Rew. Med. Chem.* **13** (2013) 365 (https://doi.org/10.2174/138955713804999793)
21. S. L. A. Kumar, M. S. Kumar, S. J. Jenniefer, P. T. Muthiah, A. Sreekanth, *Phosphorus, Sulfur Silicon Rel. Elem.* **188** (2013) 1110 (https://doi.org/10.1080/10426507.2012.736100)
22. E. Manoj, M. R. P. Kurup, E. Suresh, *J. Chem. Crystallogr.* **38** (2008) 157 (https://doi.org/10.1007/s10870-007-9267-9)
23. A. Bacchi, A. Bonini, M. Carcelli, F. Ferraro, E. Leporati, C. Pelizzi, G. Pelizzi, *J. Chem. Soc. Dalton Trans.* **13** (1996) 2699 (https://doi.org/10.1039/DT9960002699)
24. J. P. Adjimani, P. Asare, *Toxicol. Rep.* **2** (2015) 721 (https://doi.org/10.1016/j.toxrep.2015.04.005).