

SYNTHESIS AND ANTIOXIDANT ACTIVITY OF A NEW 4-AMINOCOUMARIN DERIVATIVE

NOVICA RISTIĆ^{1*}, NIKO RADULOVIĆ², BILJANA DEKIĆ¹, MILENKO RISTIĆ¹, VIDOSLAV DEKIĆ¹

¹Faculty of Science and Mathematics, University of Priština, Kosovska Mitrovica, Serbia

²Department of Chemistry, Faculty of Sciences and Mathematics, University of Niš, Niš, Serbia

ABSTRACT

Synthesis, spectral characterization, and evaluation of *in vitro* antioxidant activity of a new coumarin derivative, 4-((1,3,4-thiadiazol-2-yl)amino)-3-nitro-2H-chromene-2-one, are described. The synthesis of the new product was performed in three reaction steps, with a good overall yield (78%). The structure was corroborated by detailed spectral analysis, including the 1D and 2D NMR experiments (¹H- and ¹³C NMR; ¹H-¹H COSY, NOESY, HSQC, and HMBC). The *in vitro* antioxidant activity was evaluated using the DPPH test. The synthesized compound possesses a good free-radical scavenging activity, IC₅₀=596.7±0.3 µg/ml, and can serve as a model for the synthesis of similar compounds with promising antioxidant effects.

Keywords: Coumarin derivative, Spectral characterization, NMR spectroscopy, Antioxidant activity, DPPH.

INTRODUCTION

Coumarin (2H-chromen-2-one) belongs to the benzopyrones, a group of heterocyclic compounds which have a condensed benzene and α -pyrone ring. These naturally occurring secondary metabolites enumerate about 1300 derivatives isolated from a number of plant species and bacteria (Venugopala et al., 2013). Coumarins are deriving the shikimic acid biosynthetic pathway. Some coumarin derivatives of natural origin already have a medical application, for example, novobiocin, warfarin, acenocoumarin and umbelliferone (Stefanachi et al., 2018). For decades, more efforts have been made to make similar coumarin derivatives by organic synthesis. These derivatives possess an exceptionally wide spectrum of pharmacological properties such as: anticancer (Nofal et al., 2000; Ayati et al., 2018), anti-inflammatory (Bansal et al., 2013; Hadjipavlou-Litina et al., 2007), antibacterial (Naik et al., 2017; Zaki et al., 2012; Radulović et al., 2015) antiviral/anti-influenza (Kostova et al., 2006; Su et al., 2006; Yu et al., 2003; Yeh et al., 2010), antifungal (Pasqualotto et al., 2010; Al-Amiery et al., 2012), anti-Alzheimer (Anand et al., 2012; Piazzini et al., 2008), anticoagulant (Danis et al., 2010), and antioxidant (Kotaiah et al., 2014; Manojkumar et al., 2009; Fylaktakidou et al., 2004).

The interactions between the substituent and the coumarin core determined the biological activity of the molecule itself, which results in its selectivity. Differently substituted derivatives of nitrocoumarin have a remarkable range of selectivity and possess pronounced pharmacological effects like antibacterial (Vaso et al., 2010; Aiyelabola et al., 2017), cytotoxic (Aiyelabola et al., 2017), and antioxidant (Parfěnov & Smirnov, 1991). Some of them have significance in the treatment of kidney cancer cells (Finn et al., 2002) and in the study of enzyme kinetics.

In the light of the mentioned studies of biologically active coumarins, as well as our previous work (Dekić et al., 2010; Dekić et al., 2010), in this research we put emphasis on the synthesis, assignment of ¹H and ¹³C NMR spectral data from an interpretation of results of 2D (¹H-¹H COSY, NOESY, HSQC and HMBC) experiments and evaluation of the antioxidant activity of a new 4-amino substituted-3-nitrocoumarin derivative.

EXPERIMENTAL

General chemistry

Reagents and solvents used in this research (A.R. grade) were of Sigma Aldrich (USA), Merck (Germany), Fluka (Germany) and Acros Organics (Belgium). Melting points were determined on MPM-HV2 (Germany) instrument. Recording of IR spectra was carried out on Thermo Nicolet model 6700 FTIR (USA) instrument and HR-MS(EI) spectra were recorded on a JEOL Mstation JMS 700 instrument (Germany). The reaction progress and purity of synthesized compounds checked by TLC, using silica gel plates (Kiesel 60 F₂₅₄, Merck). Visualization was performed by UV light or spraying the plates with 1:1 (v/v) aqueous sulfuric acid and then heating. The absorbance was measured using a UV-1800 Shimadzu spectrophotometer.

NMR measurement

¹H NMR (400 MHz) and ¹³C NMR (100.6 MHz) spectra were recorded on Bruker Avance III spectrometer (Switzerland) at 25°C in DMSO-*d*₆, using a TMS as an internal standard. Chemical shifts are reported in ppm (δ) values relative to TMS (δ _H 0 ppm) in ¹H or signals of residual solvents in ¹³C and heteronuclear 2D NMR spectra (residual DMSO-*d*₆ δ _H 2.54 ppm and ¹³CD₃SOCD₃ δ _C 40.45 ppm). The coupling constant (*J*) are reported in Hz. Multiplicities of proton resonance are designated as singlet (s), broad singlet (brs), a doublet of doublets (dd), a

* Corresponding author: risticnovica@gmail.com

doublet of doublets of doublets (ddd) and a triplet of doublets (td). 2D spectra (^1H - ^1H COSY, NOESY, HSQC, and HMBC) are performed on the same instrument with a standard pulse sequence.

Synthesis

Synthesis of 4-hydroxy-3-nitrocoumarin (2)

According to an already known procedure (Savel'ev et al., 1973), in suspension of 4-hydroxycoumarin (1) (5 g, 30.8 mmol) and glacial acetic acid (20 ml) the mixture of 72% NHO_3 (3 ml) and glacial acetic acid (2.6 ml) was added. The obtained suspension was heated on a water bath until all of 4-hydroxycoumarin are dissolved and does not show the brown vapors of nitrogen oxides. After cooled on ice bath yellow precipitate is obtained, then filtered and washed with saturated solution of sodium-bicarbonate and absolute ethanol. Recrystallization from absolute ethanol gives yellow crystals of 4-hydroxy-3-nitrocoumarin (2) (yield 84%, m.p. 171-172 °C).

Synthesis of 4-chloro-3-nitrocoumarin (3)

In *N,N*-dimethylformamide (2 ml, 26 mmol), cooled to 10°C, during the 15 min, with stirring, POCl_3 (4 g, 26 mmol) was added. The reaction then continued at room temperature for another 15 min. After the expiry of this period, 4-hydroxy-3-nitrocoumarin (2) (5.3 g, 26 mmol) dissolved in *N,N*-dimethylformamide (12.5 ml) was added dropwise. The addition of cold water (15 ml), after 15 min, stopped the reaction. The resulting yellow precipitate was filtered and washed with saturated sodium bicarbonate and water, to obtain the final product of 4-chloro-3-nitrocoumarin (3) (yield 87%, m.p. 162-163 °C) as a yellow crystals (Kaljaj et al., 1987), by recrystallization from the benzene:hexane mixture (1:1, v/v).

Synthesis of 4-((1,3,4-thiadiazol-2-yl)amino)-3-nitro-2H-chromen-2-one (5)

In the solution of 4-chloro-3-nitrocoumarin (3) (1 g, 4.4 mmol) in *N,N*-dimethylformamide (10 ml), 1,3,4-thiadiazol-2-amine (4) (0.45 g, 4.4 mmol) and sodium bicarbonate (0.75 g) was added. The mixture was stirred, and progress of the reaction was monitored by TLC. After 90 min, reaction was stopped by the addition of 20 ml of cold water. The formed light yellow solid precipitate was filtered and washed with water, to obtain 4-((1,3,4-thiadiazol-2-yl)amino)-3-nitro-2H-chromen-2-one (5) as yellow fine powder (yield 78%, m.p. 237-239 °C). IR (neat): 3262 (N-H), 3066 (Ar-H), 1745 (C=O), 1606 (C=C), 1543 and 1339 (NO_2), 1284, 1123, 867, 753 cm^{-1} . HR-MS(EI): M^+ ($\text{C}_{11}\text{H}_6\text{N}_4\text{O}_4\text{S}$) 290.0109, requires 290.0104 ($\Delta = + 0.4$ mmu).

DPPH assay

Antioxidant activity of synthesized compound (5) was determined by DPPH (2,2-diphenyl-1-picrylhydrazyl) using the previously described method with slight modification (Braca et

al., 2001). In a test tube with 1 ml of a methanolic solution of tested compound (1.96-1000 $\mu\text{g/ml}$), 2 ml of 0.004% DPPH methanolic solution was added. Test tubes left to stand for 35 min. in the dark at room temperature. After the expiry of this period, absorbance was read at 517 nm. The control was constituted by methanol instead of tested compounds. The same procedure was repeated for the solutions of ascorbic acid (1.96-1000 $\mu\text{g/ml}$) which used as a standard. The percentage of inhibition of the DPPH radical was calculated using the equation (1):

$$\% \text{ of inhibition} = [(Ac-As)/Ac] \cdot 100 \quad (1)$$

where *Ac* is the absorbance of control solution (2 ml of DPPH radical and 1 ml of methanol) and *As* is the absorbance of the methanolic solution of tested compound (2 ml DPPH and 1 ml of a methanolic solution of the tested compounds). Results were expressed as IC_{50} values in $\mu\text{g/ml}$ (concentration of the tested compound required to decrease the initial DPPH concentration by 50%) and estimated from % inhibition versus the concentration sigmoidal curve, using a non-linear regression algorithm.

RESULTS AND DISCUSSION

Chemistry

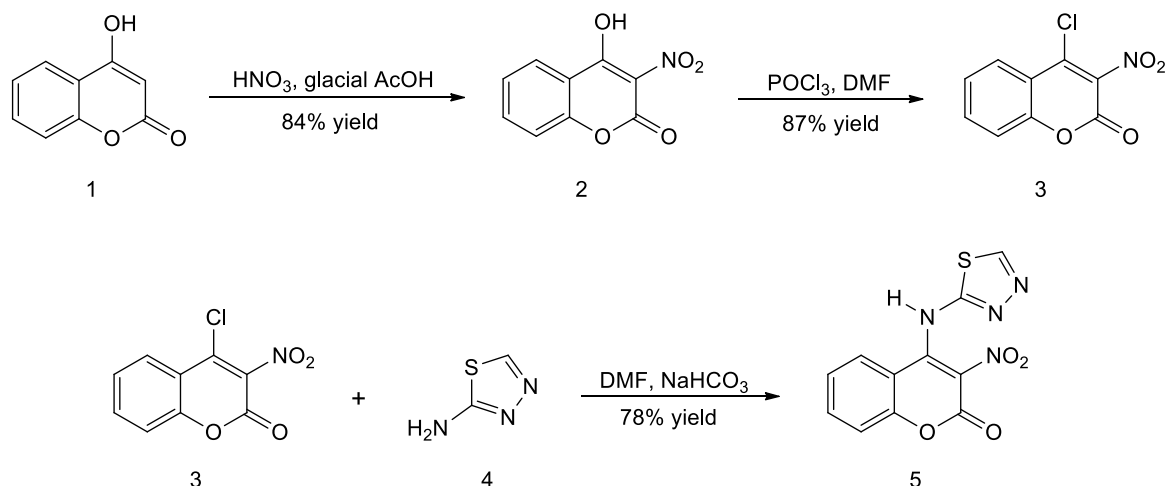
The synthesis of the new coumarin derivative was performed in three steps to give 4-((1,3,4-thiadiazol-2-yl)amino)-3-nitro-2H-chromen-2-one (5) as the main product (Scheme 1). The structure of the obtained compound was confirmed by IR spectroscopy and HR-MS(EI), and also complete assignation of the ^1H and ^{13}C NMR spectra was performed, by combining the data obtained with 1D and 2D techniques NMR (^1H and ^{13}C NMR, ^1H - ^1H COSY, NOESY, HSQC, and HMBC).

The IR spectrum of the synthesized compound showed the bands that confirm his structure. At 3262 and 3066 cm^{-1} appeared the bands of N-H and Ar-H bonds, respectively. The carbonyl group gives a strong vibration at 1745 cm^{-1} , while the band characteristic of the C=C bond occurs at 1606 cm^{-1} . The nitro group showed two strong bands at 1339 and 1543 cm^{-1} . The molecular weight based on HR-MS(EI) (290.0109) indicated the molecular formula of $\text{C}_{11}\text{H}_6\text{N}_4\text{O}_4\text{S}$, which additionally confirmed the structure of this compound.

In the ^1H NMR spectrum of the synthesized compound appeared six signals, two doublet of doublets with chemical shifts at 7.56 and 8.10 ppm, one triplet of doublets at 7.49 ppm, one doublet of doublets of doublets at 7.67 ppm, one wide singlet at 9.25 ppm and one singlet at 9.35 ppm (Fig. 1). The assignment of these signals can be determined based on the position of the hydrogen atoms in the assumed structure. The singlet corresponds to hydrogen atoms from the ring of the substituent and the secondary amino group. Based on the shape of the signal

(a wide singlet) can be concluded that the signal at 9.25 ppm corresponding to hydrogen bound to the nitrogen atom, so the

signal at 9.34 ppm corresponds to the proton at the position H-5'.



Scheme 1. Reaction steps in synthesis of 4-((1,3,4-thiadiazol-2-yl)amino)-3-nitro-2H-chromene-2-one.

This assumption is in accordance with the NOESY (Fig. 2) and the HSQC (Fig. 3) spectral data. The correlation in the NOESY spectrum showed only the singlet at 9.25 ppm. The cross peak of this signal with the doublet of doublets at 8.10 ppm, which corresponds to the proton bonded to the aromatic nucleus, confirms that it is near to the coumarin part of the molecule. On the other hand, the HSQC spectrum occurs interaction only for the signal at 9.35 ppm, which at the same time is assigned to C-5' at 151.1 ppm, while the interaction for proton at 9.25 ppm is absent, due to the fact that this proton is not bound to carbon, already for nitrogen.

Observing the structure of the compounds, it is easy to conclude that doublet of doublets at 7.56 and 8.10 ppm, respectively, and doublet of doublets of doublets at 7.67 ppm corresponding to protons bonded to the coumarin core.

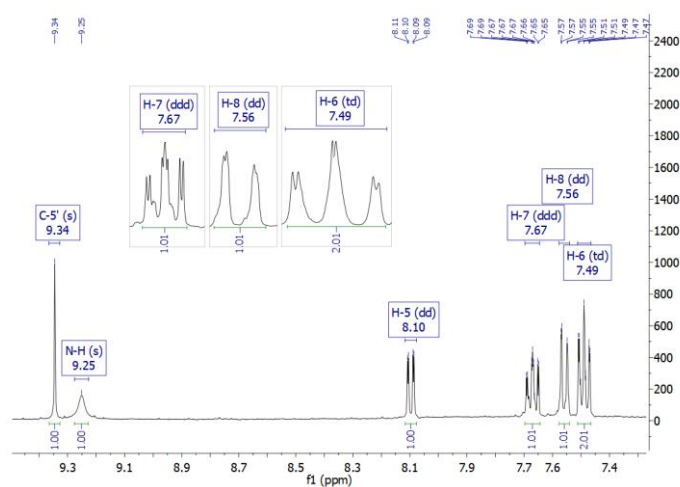


Figure 1. ^1H NMR (CDCl_3 , 400 MHz) spectrum of compound (5).

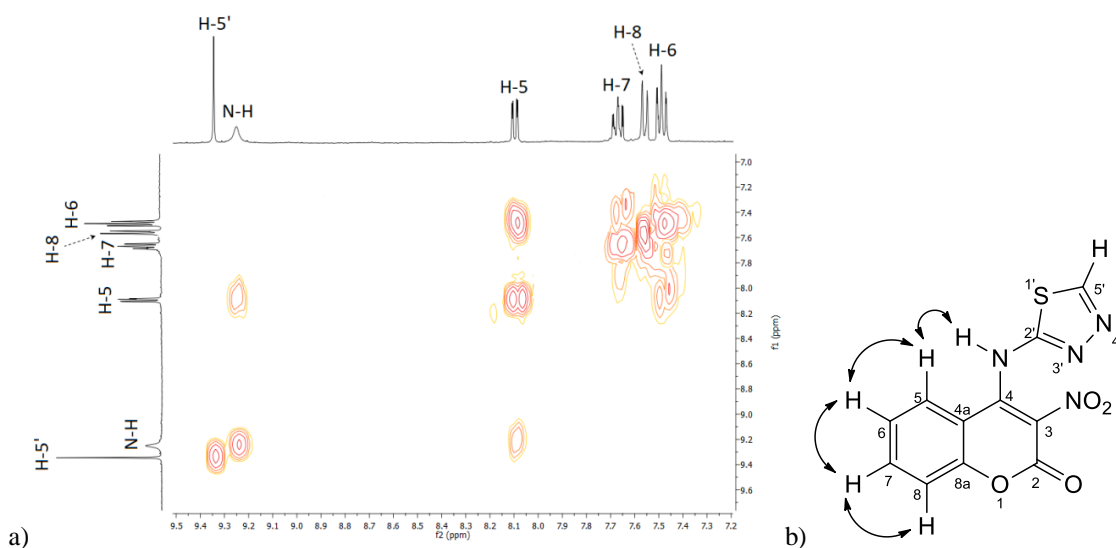


Figure 2. NOESY a) spectrum and b) correlations of compound (5).

Assignments can be performed starting from the signal which showed correlation with the hydrogen from an amino group (9.25 ppm) and doublet of doublets at 8.10 ppm. Based on the spatial disposition in the molecule, it is clear that this signal belongs to the proton H-5. The signal shape corresponding to the position of the proton in the molecule, since it is coupling with one vicinal (H-6, $J = 8.0$ Hz) and one distant (N-7, $J = 1.6$ Hz) proton. Signals of other protons, bound to the coumarin core are assigned based on their correlations in the NOESY spectrum, in a similar manner. Chemical shifts of the carbon atoms to which these protons are attached (C-5, δ 123.3; C-6, δ 125.7; C-7, δ 131.8; C-8, δ 118.0) were determined based on the HSQC spectrum. This assignment is in agreement with data obtained from the HMBC spectrum (correlation of hydrogen and carbon through three chemical bonds) (Fig. 4).

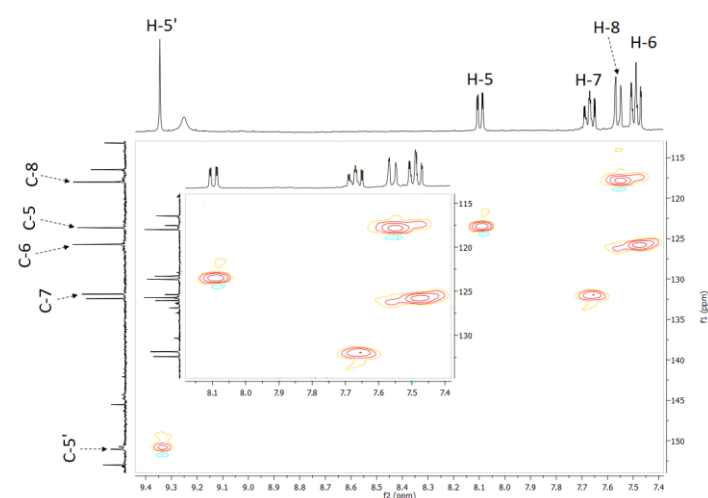


Figure 3. HSQC spectrum of compound (5).

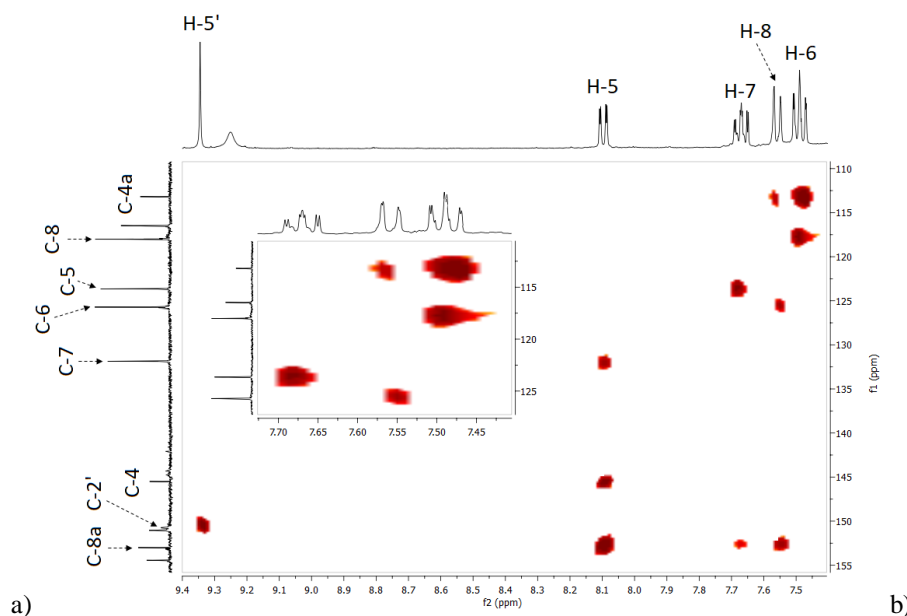


Figure 4. HMBC a) spectrum and b) correlations of compound (5).

Based on interactions with the H-5 in the same spectrum, the chemical shifts of quaternary carbons, C-4a and C-8a, 113.2 and 153.0 ppm, respectively, were assigned. Proton H-5 in this spectrum showed a once more correlation, with the signal at 145.5 ppm, which assigned carbon at position C-4. The chemical shift of the only remaining quaternary carbon atom from the ring of the substituent, C-2' at 150.7 ppm (Table 1), was determined based on his HMBC correlation with the proton on position H-5'.

Table 1. NMR data of compound (5) recorded at 101 (^{13}C NMR) and 400 MHz (^1H NMR).

Position	δ_{H} , m (J, Hz)	δ_{C}	NOESY*	HMBC**
2		154.4		
3		116.5		
4		145.5		
4a		113.2		
5	8.10 dd (1.6, 8.0)	123.7	H-6	C-4, C-7, C-8a
6	7.49 td (0.8, 8.0)	125.7	H-5, H-7	C-4a, C-8a
7	7.67 ddd (1.6, 8.0, 8.4)	131.9	H-6, H-8	C-5, C-8a
8	7.56 dd (0.8, 8.4)	118.0	H-7	C-4a, C-6, C-8a
8a		153.0		
2'		150.7		
N-H	9.25 brs			
5'	9.34 s	151.1		C-2'

*NOESY interactions of the hydrogen from the column "Position" with the hydrogen from the column "NOESY"

**HMBC interactions of the hydrogen from the column "Position" with the carbons from the column "HMBC"

In this way, the chemical shifts of all hydrogen and carbon atoms were determined, except for carbons at position C-2 and C-3, which showed no correlation in all of these two-dimensional NMR spectra. The two last unassigned signals in the ^{13}C NMR spectrum at 154.4 and 116.5 ppm (Fig. 5) were attributed to the C-2 and C-3, respectively, based on the expected values and compared with similar compounds of previous studies (Dekić et al, 2010; Dekić et al., 2016).

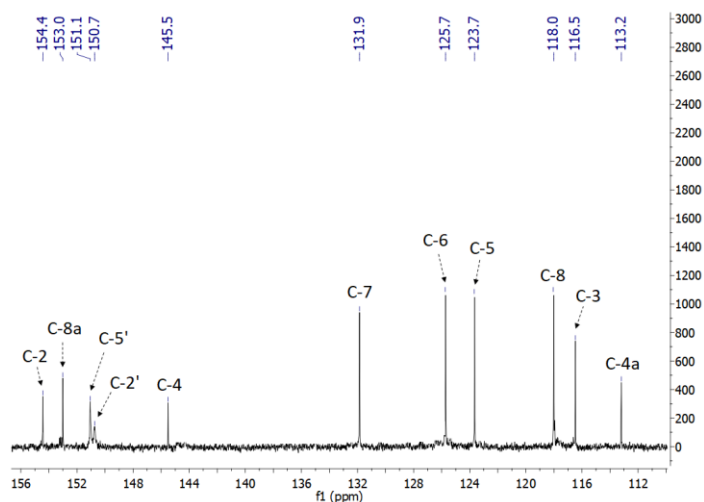


Figure 5. ^{13}C NMR (CDCl_3 , 100.6 MHz) spectrum of compound (5).

DPPH assay

The DPPH assay is a simple, fast and reliable method for determining the antioxidant activity of the compounds. The percentage inhibition of DPPH radicals, depending on the concentration of the tested compound (5), is showed at Fig. 6. Also, the IC_{50} value for the tested compound was determined, with the amount of $596.7 \pm 0.3 \mu\text{g/ml}$.

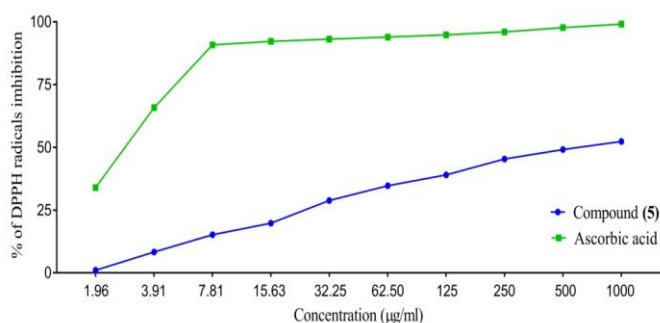


Figure 6. Percentage of inhibition (%) of DPPH radical depending on the concentration of the tested compound. Ascorbic acid was used as a positive control.

Ascorbic acid, known as an exceptional antioxidant, inhibits 50% of the DPPH radicals at a concentration of $3.0 \pm 0.1 \mu\text{g/ml}$. By comparing the result obtained for the tested compound

with the result for ascorbic acid, it can be concluded that the tested compound showed a very good antioxidant potential.

CONCLUSION

Briefly, synthesis, spectral characterization and antioxidant activity of the new coumarin derivative 4-((1,3,4-thiadiazol-2-yl)amino)-3-nitro-2*H*-chromene-2-one, is described. The structure of the synthesized compound was confirmed by the complete assignment of ^1H and ^{13}C NMR spectra, based on data obtained by 1D and 2D NMR techniques. Determination of antioxidant activity was performed by DPPH assay. The tested compound showed a good antioxidant potential, which provides a strong basis for further research.

ACKNOWLEDGMENTS

This work was financially supported by the Ministry of Education, Science and Technological Development of Serbia [Project No. 172061 and 45022].

REFERENCES

- Aiyelabola, T., Akinkunmi, E., Obuotor, E., Olawuni, I., Isabirye, D., & Jordaan, J. 2017. Synthesis Characterization and Biological Activities of Coordination Compounds of 4-Hydroxy-3-nitro-2 H -chromen-2-one and Its Aminoethanoic Acid and Pyrrolidine-2-carboxylic Acid Mixed Ligand Complexes. *Bioinorganic Chemistry and Applications*, pp. 1-9. doi:10.1155/2017/6426747
- Al-Amiery, A.A., Kadhum, A.A.H., & Mohamad, A.B. 2012. Antifungal Activities of New Coumarins. *Molecules*, 17(5), pp. 5713-5723. doi:10.3390/molecules17055713
- Anand, P., Singh, B., & Singh, N. 2012. A review on coumarins as acetylcholinesterase inhibitors for Alzheimer's disease. *Bioorganic and Medicinal Chemistry*, 20(3), pp. 1175-1180. doi:10.1016/j.bmc.2011.12.042
- Ayati, A., Oghabi, B. T., Moghimi, S., Esmaeili, R., Majidzadeh-A, K., Safavi, M., Firoozpour, L., Emami, S., & Foroumadi, A. 2018. Synthesis and biological evaluation of new coumarins bearing 2,4-diaminothiazole-5-carbonyl moiety. *European Journal of Medicinal Chemistry*, 155, pp. 483-491. doi:10.1016/j.ejmech.2018.06.015
- Bansal, Y., Sethi, P., & Bansal, G. 2013. Coumarin: a potential nucleus for anti-inflammatory molecules. *Medicinal Chemistry Research*, 22(7), pp. 3049-3060. doi:10.1007/s00044-012-0321-6
- Braca, A., De Tommasi, N., Bari, L. D., Pizza, C., Politi, M., & Morelli, I. 2001. Antioxidant Principles from *Bauhinia t arapotensis*. *Journal of Natural Products*, 64(7), pp. 892-895. doi:10.1021/np0100845
- Danis, O., Yuce-Dursun, B., Gunduz, C., Ogan, A., Sener, G., Bulut, M., & Yarat, A. 2011. Synthesis of 3-amino-4-hydroxy coumarin and dihydroxy-phenyl coumarins as novel anticoagulants. *Arzneimittelforschung*, 60(10), pp. 617-620. doi:10.1055/s-0031-1296335
- Dekić, B., Dekić, V., Radulović, N., Vukićević, R., & Palić, R. 2010. Synthesis of new antimicrobial 4-aminosubstituted 3-

- nitrocoumarins. *Chemical Papers*, 64(3). doi:10.2478/s11696-010-0004-z
- Dekić, B., Radulović, N., Ristić, M., & Dekić, V. 2016. The synthesis and NMR spectral assignments of 3-nitro-4-((6-nitrobenzothiazol-2-yl)amino)-2H-chromen-2-one. *The University Thought - Publication in Natural Sciences*, 6(1), pp. 32-38. doi:10.5937/univtho6-10634
- Dekić, V., Radulović, N., Vukićević, R., Dekić, B., Skropeta, D., & Palić, R. 2010. Complete assignment of the ¹H and ¹³C NMR spectra of antimicrobial 4-arylamino-3-nitrocoumarin derivatives. *Magnetic Resonance in Chemistry*, 48(11), pp. 896-902. doi:10.1002/mrc.2681
- Fylaktakidou, K., Hadjipavlou-Litina, D., Litinas, K., & Nicolaidis, D. 2004. Natural and Synthetic Coumarin Derivatives with Anti-Inflammatory / Antioxidant Activities. *Current Pharmaceutical Design*, 10(30), pp. 3813-3833. doi:10.2174/1381612043382710
- Finn, G. J., Kenealy, E., Creaven, B. S., & Egan, D. A. 2002. In vitro cytotoxic potential and mechanism of action of selected coumarins, using human renal cell lines. *Cancer Letters*, 183(1), pp. 61-68. doi:10.1016/s0304-3835(02)00102-7
- Hadjipavlou-Litina, D., Kontogiorgis, C., Pontiki, E., Dakanali, M., Akoumianaki, A., & Katerinopoulos, H. E. 2007. Anti-inflammatory and antioxidant activity of coumarins designed as potential fluorescent zinc sensors. *Journal of Enzyme Inhibition and Medicinal Chemistry*, 22(3), pp. 287-292. doi:10.1080/14756360601073914
- Kaljaj, V., Trković, M., & Stefanović-Kaljaj, L. 1987. Synthesis of new heterocyclocoumarins starting with 3-cyano-4-chlorocoumarin. *Journal of the Serbian Chemical Society*, 52(4), pp. 183-185.
- Kostova, I., Raleva, S., Genova, P., & Argirova, R. 2006. Structure-Activity Relationships of Synthetic Coumarins as HIV-1 Inhibitors. *Bioinorganic Chemistry and Applications*, pp. 1-9. doi:10.1155/bca/2006/68274
- Kotaiah, Y., Nagaraju, K., Harikrishna, N., Venkata, R. C., Yamini, L., & Vijjulatha, M. 2014. Synthesis, docking and evaluation of antioxidant and antimicrobial activities of novel 1,2,4-triazolo[3,4-b][1,3,4]thiadiazol-6-yl)selenopheno[2,3-d]pyrimidines. *European Journal of Medicinal Chemistry*, 75, pp. 195-202. doi:10.1016/j.ejmech.2014.01.006
- Manojkumar, P., Ravi, T., & Subbuettiar, G. 2009. Synthesis of coumarin heterocyclic derivatives with antioxidant activity and in vitro cytotoxic activity against tumour cells. *Acta Pharmaceutica*, 59(2), pp. 159-170. doi:10.2478/v10007-009-0018-7
- Naik, N. S., Shastri, L. A., Joshi, S. D., Dixit, S. R., Chougala, B. M., Samundeeswari, S., Holiyachi, M., Shaikh, F., Madar, J., Kulkarni, R., & Sunagar, V. 2017. 3,4-Dihydropyrimidinone-coumarin analogues as a new class of selective agent against *S. aureus*: Synthesis, biological evaluation and molecular modelling study. *Bioinorganic and Medicinal Chemistry*, 25(4), pp. 1413-1422. doi:10.1016/j.bmc.2017.01.001
- Nofal, Z., El-Zahar, M., & Abd, E. S. 2000. Novel Coumarin Derivatives with Expected Biological Activity. *Molecules*, 5(12), pp. 99-113. doi:10.3390/50200099
- Parfěnov, Ě. A., & Smirnov, L. S. 1991. Heterocyclic bioantioxidants. 2. Interaction of 3-nitro-4-substituted coumarins with mercaptans. *Chemistry of Heterocyclic Compounds*, 27(11), pp. 1190-1192. doi:10.1007/bf00471742
- Pasqualotto, A. C., Thiele, K. O., & Goldani, L. Z. 2010. Novel triazole antifungal drugs: focus on isavuconazole, ravuconazole and albaconazole. *Current Opinion in Investigational Drugs*, 11, pp. 165-174.
- Piazzini, L., Cavalli, A., Colizzi, F., Belluti, F., Bartolini, M., Mancini, F., Recanatini, M., Andrisana, V., & Rampa, A. 2008. Multi-target-directed coumarin derivatives: hAChE and BACE1 inhibitors as potential anti-Alzheimer compounds. *Bioorganic and Medicinal Chemistry Letters*, 18(1), pp. 423-426. doi:10.1016/j.bmcl.2007.09.100
- Radulović, N., Stojanović-Radić, Z., Stojanović, P., Stojanović, N., Dekić, V., & Dekić, B. 2015. A small library of 4-(alkylamino)-3-nitrocoumarin derivatives with potent antimicrobial activity against gastrointestinal pathogens. *Journal of the Serbian Chemical Society*, 80(3), pp. 315-327. doi:10.2298/jsc140619085r
- Savel'ev, V. L., Artamonova, O. S., Troitskaya, V. S., Vinokurov, V. G., & Zagorevskii, V. A. 1973. Investigations of pyrans and related compounds. *Khimiya Geterotsiklicheskikh Soedinenii*, 9(7), pp. 816-820. doi:10.1007/bf00471556
- Stefanachi, A., Leonetti, F., Pisani, L., Catto, M., & Carotti, A. 2018. Coumarin: A Natural, Privileged and Versatile Scaffold for Bioactive Compounds. *Molecules*, 23(2), p. 250. doi:10.3390/molecules23020250
- Su, C., Mouscadet, J., Chiang, C., Tsai, H., & Hsu, L. 2006. HIV-1 Integrase Inhibition of Biscoumarin Analogues. *Chemical and pharmaceutical bulletin*, 54(5), pp. 682-686. doi:10.1248/cpb.54.682
- Vaso, K., Behrami, A., & Krasniqi, I. 2010. Antibacterial Activity of Compounds Synthesized From 4-Chloro-3-nitro-2H-[1]-benzopyran-2-one. *Asian Journal of Chemistry*, 22(9), pp. 7313-7317.
- Venugopala, K. N., Rashmi, V., & Odhav, B. 2013. Review on Natural Coumarin Lead Compounds for Their Pharmacological Activity. *BioMed Research International*, pp. 1-14. doi:10.1155/2013/963248
- Yeh, J. Y., Coumar, M. S., Horng, J. T., Shiao, H. Y., Kuo, F. M., Lee, H. L., Chen, I. C., Chang, C. W., Tang, W. F., Tseng, S. N., Chen, C. J., Shih, S. R., Hsu, J. T., Liao, C. C., Chao, Y. S., & Hsieh, H. P. 2010. Anti-Influenza Drug Discovery: Structure-Activity Relationship and Mechanistic Insight into Novel Angelicin Derivatives. *Journal of Medicinal Chemistry*, 53(4), pp. 1519-1533. doi:10.1021/jm901570x
- Yu, D., Suzuki, M., Xie, L., Morris-Natschke, S. L., & Lee, K. 2003. Recent progress in the development of coumarin derivatives as potent anti-HIV agents. *Medicinal Research Reviews*, 23(3), pp. 322-345. doi:10.1002/med.10034
- Zaki, R. M., Elossaily, Y. A., & Kamal, E. A. M. 2012. Synthesis and antimicrobial activity of novel benzo[f]coumarin compounds. *Russian Journal of Bioorganic Chemistry*, 38(6), pp. 639-646. doi:10.1134/s1068162012040152