

SINTEZA I BIOLOŠKA SVOJSTVA NEKIH AZO BOJA NA BAZI 3-CIJANO-6-HIDROKSI-4-METIL-1-PROPIL-2-PIRIDONA

SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME AZO DYES BASED ON 3-CYANO-6-HYDROXY-4-METHYL-1-PROPYL-2-PYRIDONE

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Azo boje su najznačajnija grupa organskih sintetskih boja. Visoki molarni ekstencioni koeficijenti, koji karakterišu azo jedinjenja, doprinose njihovoj širokoj upotrebi kako u tradicionalnom bojenju materijala, tako i sve većoj primeni u novijim tehnologijama kao što su optički materijali i solarne ćelije. Mnoga istraživanja pokazuju da azo boje dobijene iz heterocikličnih molekula imaju biološku aktivnost. Arilazo piridonske boje pripadaju klasi disperznih boja koje ispoljavaju antibakterijsku, antifungalnu, antikancerogenu i antioksidativnu aktivnost. U ovom radu sintetisane su tri azo boje polazeći iz 2-, 3- i 4-aminofenola i 3-cijano-6-hidroksi-4-metil-1-propil-2-piridona. Struktura jedinjenja potvrđena je ATR-FTIR, NMR i UV-Vis podacima. Antioksidativna aktivnost jedinjenja ispitana je ABTS metodom. Fizičko-hemijski deskriptori i ADME parametri određeni su in silico pomoću SwissADME programa.

Ključne reči: hidrazon; ADME; antioksidativna svojstva

Azo dyes are the most important group of organic synthetic dyes. High molar extinction coefficients, which characterize azo compounds, contribute to their wide use in traditional dyeing of various materials, as well as the increasing application in new technologies such as optical devices and solar cells. Numerous studies show that azo dyes based on heterocyclic molecules possess biological activity. Arylazo pyridone dyes belong to the class of disperse dyes, which exhibit antibacterial, antifungal, anticancer and antioxidant properties. In this work, three azo dyes were synthesized starting from 2-, 3- and 4-aminophenol and 3-cyano-6-hydroxy-4-methyl-1-propyl-2-pyridone. The structure of dyes was confirmed by ATR-FTIR, NMR and UV-Vis spectroscopy. The antioxidant activity of compounds was examined by the ABTS method. Physico-chemical descriptors and ADME parameters were determined in silico using SwissADME program.

Key words: hydrazone; ADME; antioxidant properties

1 Introduction

The heterocyclic azo dyes are receiving constant attention over the years, due to its excellent coloration properties and broad spectrum of application in different fields, such as textile dyeing, photovoltaics and pharmaceuticals [1]. In comparison to simple arylazo dyes, heterocyclic azo compounds are more stable, brighter and ecologically acceptable [2]. Moreover, numerous studies have revealed that azo compounds derived from heterocyclic moieties exhibit prominent antibacterial, antifungal, antioxidant and anticancer activity [3].

Azo dyes based on 2-pyridone scaffolds are significant group of disperse dyes which display remarkable dyeing and biological properties. Recent studies have shown that 2-pyridone based azo compounds manifest cytotoxic action against several tumor cells and inhibit bacterial growth [4, 5]. Moreover, arylazo pyridone dyes may be strong antioxidants, especially if they have –OH or –NH groups in the structure. Antioxidants prevent biomolecules from undergoing oxidative damage

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through free radical mediated reactions, so they can be effective therapy for atherosclerosis, coronary heart disease, tumors, and aging itself [6].

In this work, three azo dyes have been synthesized starting from 2-, 3- and 4-aminophenol as diazo-component and 3-cyano-6-hydroxy-4-methyl-2-pyridone as coupling-component. The structure of dyes has been confirmed by melting points, ATR-FTIR, NMR and UV-Vis spectra. Antioxidant activity of the investigated dyes has been evaluated by ABTS method. The physico-chemical parameters and ADME (absorption, distribution, metabolism, and excretion) properties have been calculated using SwissADME, and druglikness was estimated.

2 Experimental

2.1 Materials and methods

All chemicals were obtained from Merck, Fluka and Acros and were used without further purification. Fourier transform infrared spectroscopy (FT-IR) spectra of the dyes were recorded using a Nicolet™ iSTM 10 FT-IR Spectrometer (Thermo Fisher SCIENTIFIC) spectrometer, with Smart iTR™ Attenuated Total Reflectance (ATR) sampling accessories. The ATR-FTIR spectra were recorded in the 500–4000 cm^{-1} range with 20 scans per spectrum. The melting points were determined on the melting point apparatus Electrothermal. The ^1H NMR spectra were taken on a Bruker Ascend 400 apparatus (400 Hz) in deuterated dimethylsulfoxide ($\text{DMSO-}d_6$) with tetramethylsilane (TMS) as an internal standard. The ultraviolet–visible (UV–Vis) absorption spectra were recorded on a Shimadzu 1700 spectrophotometer, at concentration of the solutions $4 \cdot 10^{-5}$ mol/L. All spectroscopic measurements were carried out at room temperature (25 °C).

2.2 Synthesis of azo dyes 1-3

The azo dyes **1-3** (Figure 1) were synthesized *via* classical diazo-coupling reaction, starting from 2-, 3- and 4-aminophenol as diazo-component, and 3-cyano-6-hydroxy-4-methyl-1-propyl-2-pyridone as coupling-component. Target 2-pyridone was prepared according to previously reported method [7]. The corresponding aminophenol (10 mmol) was dissolved in concentrated hydrochloric acid (2.5 mL) and cooled to 0 °C. Sodium nitrate (11 mmol) was dissolved in cold water (4 mL) and added dropwise to the aminophenol solution. The mixture was stirred for an hour to give related diazonium salt. The obtained 2-pyridone (10 mmol) was dissolved in an aqueous potassium hydroxide solution and cooled to 0 °C. Then corresponding diazonium salt was added dropwise to the solution of 2-pyridone. The mixture was stirred for 3 hours at temperature 0–5 °C, and then left in the refrigerator overnight. The crude dyes were filtrated, washed with water and air dried. The azo dyes **1-3** were recrystallized from ethanol. The overall synthesis is presented in Figure 1.

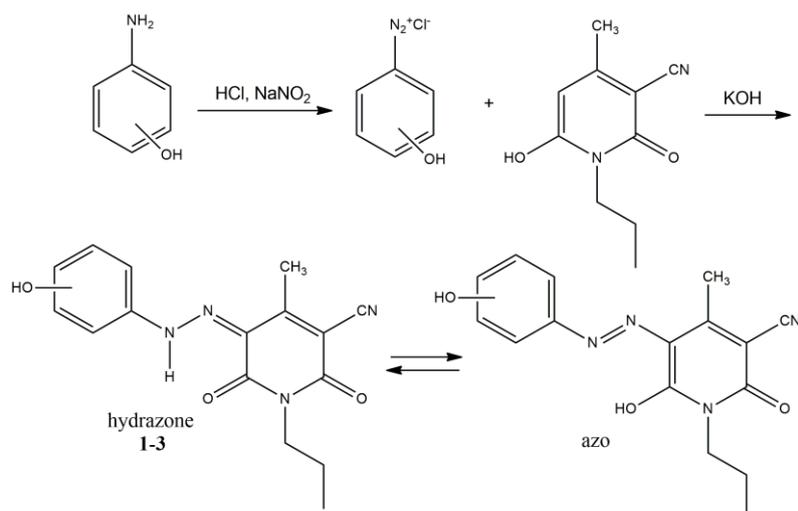


Figure 1. The synthesis of arylazo pyridone dyes **1-3** (**1**: 2-OH, **2**: 3-OH, **3**: 4-OH).

5-(2-Hydroxyphenylazo)-3-cyano-6-hydroxy-4-methyl-1-propyl-2-pyridone (**1**): Dark red powder; yield 70%; m.p. 252-253 °C; ATR-FTIR (ν/cm^{-1}): 3213 (NH), 2224 (CN), 1659 (CO), 1625

(CO), 1509 (NH); $^1\text{H NMR}$ (400 MHz, $\text{DMSO-}d_6$, δ/ppm): 0.87 (3H, t, $J = 7.4$ Hz, CH_3), 1.51-1.60 (2H, m, CH_2), 2.52 (3H, s, CH_3), 3.80 (2H, t, $J = 7.4$ Hz, CH_2), 6.94–7.01 (2H, m, Ar–H), 7.16 (1H, t, $J = 7.8$ Hz, Ar–H), 7.70 (1H, d, $J = 8.0$ Hz, Ar–H), 10.86 (1H, s, OH), 14.98 (1H, s, NH hydrazone). UV-Vis (EtOH) ($\lambda_{\text{max}}/\text{nm}$ ($\log \epsilon/\text{mol}^{-1}\text{dm}^3\text{cm}^{-1}$): 459.5 (4.40).

5-(3-Hydroxyphenylazo)-3-cyano-6-hydroxy-4-methyl-1-propyl-2-pyridone (**2**): Red powder; yield 75%; m.p. > 300 °C; ATR-FTIR (ν/cm^{-1}): 3367 (NH), 2220 (CN), 1667 (CO), 1621 (CO), 1501 (NH); $^1\text{H NMR}$ (400 MHz, $\text{DMSO-}d_6$, δ/ppm): 0.87 (3H, t, $J = 7.4$ Hz, CH_3), 1.53–1.59 (2H, m, CH_2), 2.52 (3H, s, CH_3), 3.80 (2H, t, $J = 7.4$ Hz, CH_2), 6.70 (1H, d, $J = 8.4$ Hz, Ar–H), 7.12 (2H, d, $J = 8.0$ Hz, Ar–H), 7.24-7.28 (1H, m, Ar–H), 9.88 (1H, s, OH), 14.52 (1H, s, NH hydrazone). UV-Vis (EtOH) ($\lambda_{\text{max}}/\text{nm}$ ($\log \epsilon/\text{mol}^{-1}\text{dm}^3\text{cm}^{-1}$): 440.0 (4.41).

5-(4-Hydroxyphenylazo)-3-cyano-6-hydroxy-4-methyl-1-propyl-2-pyridone (**3**): Dark orange powder; yield 70%; m.p. > 300 °C; ATR-FTIR (ν/cm^{-1}): 3367 (NH), 2222 (CN), 1662 (CO), 1624 (CO), 1505 (NH); $^1\text{H NMR}$ (400 MHz, $\text{DMSO-}d_6$, δ/ppm): 0.87 (3H, t, $J = 7.4$ Hz, CH_3), 1.52-1.58 (2H, m, CH_2), 2.51 (3H, s, CH_3), 3.80 (2H, t, $J = 7.2$ Hz, CH_2), 6.87 (2H, d, $J = 8.8$ Hz, Ar–H), 7.59 (2H, d, $J = 8.4$ Hz, Ar–H), 9.94 (1H, s, OH), 14.88 (1H, s, NH hydrazone). UV-Vis (EtOH) ($\lambda_{\text{max}}/\text{nm}$ ($\log \epsilon/\text{mol}^{-1}\text{dm}^3\text{cm}^{-1}$): 468.5 (4.44).

2.3 Antioxidant activity

The antioxidant activity of dyes **1–3** was determined by the ABTS radical-scavenging assay [8]. A stock solution of the $\text{ABTS}^{+\cdot}$ radical cation was prepared in the reaction of ABTS (4.912 mL, 7 mM in phosphate-buffered saline (PBS)) and potassium persulfate (0.088 mL, 140 mM in distilled water). After 16 h of incubation in the dark, the stock solution was diluted with methanol until absorbance recorded at 734 nm was 0.700 ± 0.02 . Subsequently, 20 μL of the methanolic dye solutions (5 mM) were mixed with 2 mL of the ABTS radical solution, shaken and stored in the dark for 10 min. Afterwards the absorbance was measured at 734 nm. Each test was done in triplicate. The inhibition percentage of $\text{ABTS}^{+\cdot}$ was calculated using the formula:

$$\text{Inhibition (\%)} = (A_c - A_s) / A_c \times 100,$$

where A_c is the absorbance of the control solution (20 μL of methanol in 2 mL of ABTS solution) and A_s is the absorbance of the sample solution. Ascorbic acid was used as a standard antioxidant. The antioxidant activity was further evaluated by determination of the IC_{50} values. The methanolic solutions of dyes and ascorbic acid were prepared at concentrations 5, 2.5, 1.25 and 0.5 mM, and obtained IC_{50} were compared.

2.4 ADME evaluation

The molecular structures of hydrazones **1–3** were converted into SMILES database using ChemDraw Ultra 12.0. Then, these SMILES were inserted in SwissADME [9] website to calculate the physicochemical descriptors, ADME (absorption, distribution, metabolism, and excretion) and drug-like properties.

3 Results and discussion

3.1 ATR-FTIR and NMR results

The synthesized arylazo pyridone dyes **1–3**, contain hydroxy group in the pyridone moiety, in the *ortho*- position to the azo bond, which enables intramolecular proton transfer, and thus the existence of azo and hydrazone tautomeric forms (Figure 1) [10]. The ATR-FTIR and NMR spectra of dyes **1–3** suggest the existence of the hydrazone tautomeric form (Figure 1) in the solid state, as well as, in the $\text{DMSO-}d_6$ solution. The stretching vibrations of two carbonyl groups in pyridone ring, appear in the ATR-FTIR spectra in the region of 1667–1659 and 1625-1621 cm^{-1} . The N-H stretching vibrations of the hydrazone group appear in the region of 3367–3213 cm^{-1} . Additional confirmation of the presence of the hydrazone tautomer is intensive band appearing in the region of 1509–1501 cm^{-1} which is ascribed to mutual stretching of $\text{C}=\text{N}$ and bending of N–H vibrations. The $^1\text{H NMR}$ spectra of all dyes, obtained in $\text{DMSO-}d_6$ solution, contain the signal of the hydrazone N-H group in the range of 14.98–14.52 ppm.

3.2 UV-Vis analysis

The UV-Vis absorption spectra of investigated dyes, in ethanol, are shown in Figure 2. The obtained spectra, also, suggest the existence of hydrazone tautomeric form in case of all dyes [11]. The absorption bands at 459.5 (**1**), 440.0 (**2**) and 465.8 (**3**) nm are ascribed to π - π^* transitions between pyridone and phenyl moiety in hydrazone tautomer. Hydroxy group in phenyl moiety manifests positive resonance effect in *para*- and *ortho*- positions and causes a bathochromic shift in comparison to *meta*- substituted dye where only negative inductive effect is involved. *Ortho*- substituted dye (**1**) have displayed a hypsochromic shift, comparing to *para*- substituted dye (**3**), due to the steric effect which prevents effective delocalization [10].

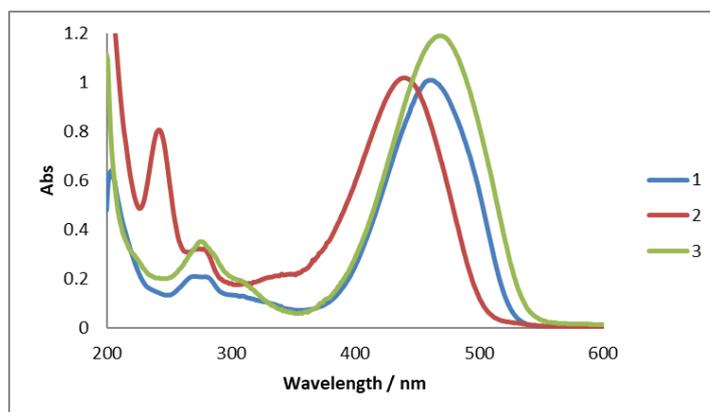


Figure 2. UV-Vis spectra of dyes 1-3 in ethanol

3.3 Antioxidant activity

Antioxidant properties of dyes **1-3** have been assayed using the ABTS method. The scavenging activity of azo dyes was compared to the activity of ascorbic acid (Figure 3). The results have shown remarkable activity of the compounds, indicating that -OH group in the phenyl ring affect the oxidant ability of investigated molecules. Namely, **1** (89.7%), **2** (94.3%) and **3** (95.0%) expressed excellent ability to scavenge the ABTS^{•+} radical cation, comparing to the inhibition of ascorbic acid (95.3%).

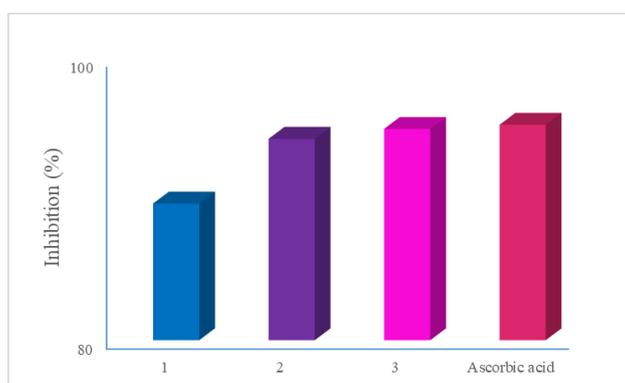


Figure 3. Antioxidant properties of investigated dyes

Thus, all investigated molecules have been further evaluated by determination of IC₅₀ values, which corresponds to the concentration of sample able to scavenge 50% of ABTS radicals in the solution (Figure 4). It is known that low IC₅₀ values suggest high antioxidant activity. The antioxidant activity based on IC₅₀ values of studied dyes was found to be as follows: **3** (0.3 mM) > **2** (0.45 mM) > **1** (2.80 mM). Furthermore, the antioxidant capacity of dyes **2** and **3** was better than ascorbic acid (IC₅₀ value 1.25 mM), indicating that related molecules are promising antioxidants.

The computational study of the synthesized dyes **1-3** was performed to evaluate physicochemically and ADME properties, using SwissADME [9]. With respects to the physicochemical properties (Table 1), all synthesized compounds have zero violations for Lipinski's rule, for orally active drugs [12]. All topological polar surface areas (TPSA) are less than 112 Å² indicating good cell membrane permeability. In addition, absorption (%ABS) was calculated by the equation % ABS = 109 - (0.345

x TPSA) [13], and obtained value of 72.5% demonstrates that these dyes have good cell permeability and bioavailability. All compounds have 4 rotatable bonds, which indicate molecular flexibility to their bio-target. Regards to the pharmacokinetic and medicinal chemistry results evaluated by SwissADME, it was found that all azo molecules have high gastro-intestinal absorption and have no permeation to the blood brain barrier, which ensures no side effects to CNS.

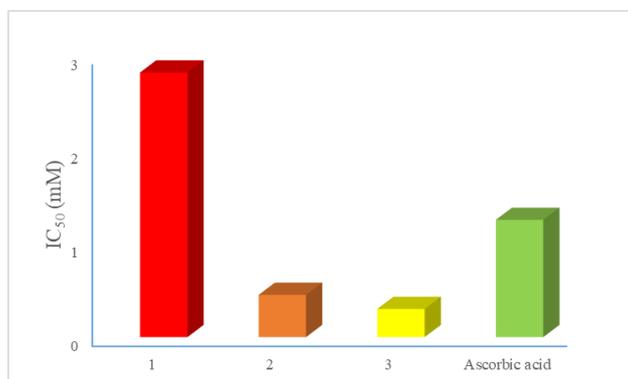


Figure 4. IC₅₀ values of studied compounds

3.4 *In silico* evaluation of physicochemical and ADME properties

Table 1. The physicochemical properties of investigated dyes calculated using SwissADME

Dye	HBD ^a	HBA ^b	M log P ^c	MW ^d	Lipinski's violations ^e	TPSA [Å ²] ^f	% ABS ^g	Rot. Bond ^h
1-3	2	5	0.76	312.32	0	105.79	72.50	4

^aThe number of hydrogen bond donors; ^bThe number of hydrogen bond acceptors;

^cThe octanol/water partition coefficient; ^dThe molecular weight;

^eThe number of Lipinski's rule violation; ^fThe topological polar surface area;

^gThe absorption; ^hThe number of rotatable bonds.

4 Conclusion

In this work three hydroxy substituted 5-phenylazo-3-cyano-6-hydroxy-4-methyl-1-propyl-2-pyridones were synthesized and their structures have been confirmed by ATR-FTIR, NMR and UV-Vis data. The ATR-FTIR and NMR spectra confirmed that synthesized dyes **1–3** exist in hydrazone tautomeric form, in solid state, as well as in DMSO-*d*₆ solution. The UV-Vis analysis in ethanol showed that all investigated dyes have one absorption maximum ascribed to the hydrazone tautomer. The antioxidant assay evinced that synthesized azo dyes **1–3** have excellent antioxidant properties comparing to antioxidant ability of ascorbic acid. Furthermore, IC₅₀ values of dyes **2** and **3** were lower than IC₅₀ value of ascorbic acid indicating remarkable antioxidant properties. The last, but not the least, ADME evaluation *in silico* has shown that all investigated compounds may be orally bioavailable with no permeation to the blood brain barrier.

5 References

- [1] **Zollinger H.** Color chemistry: synthesis, properties and application of organic dyes and pigments. Weinheim: Wiley-VCH; 2003.
- [2] **Porobić SJ, Krstić AD, Jovanović DJ, Lađarević JM, Katnić ĐB, Mijin DŽ, Marinović-Cincović M.** Synthesis and thermal properties of arylazo pyridone dyes. *Dyes Pigments* 2019;170:107602. <https://doi.org/https://doi.org/10.1016/j.dyepig.2019.107602>.
- [3] **Wainwright M.** Dyes in the development of drugs and pharmaceuticals. *Dyes Pigments* 2008;76:582–9. <https://doi.org/https://doi.org/10.1016/j.dyepig.2007.01.015>.

- [4] **Tadić JD, Ladarević JM, Vitnik ŽJ, Vitnik VD, Stanojković TP, Matić IZ, Mijin DŽ.** Novel azo pyridone dyes based on dihydropyrimidinone skeleton: Synthesis, DFT study and anticancer activity. *Dyes Pigments* 2021;187:109123. <https://doi.org/https://doi.org/10.1016/j.dyepig.2020.109123>.
- [5] **Ashkar SM, El-Asasery MA, Touma MM, Elnagdi MH.** Synthesis of some novel biologically active disperse dyes derived from 4-methyl-2,6-dioxo-1-propyl-1,2,5,6-tetrahydropyridine-3-carbonitrile as coupling component and their colour assessment on polyester fabrics. *Molecules* 2012;17:8822–31. <https://doi.org/10.3390/molecules17088822>.
- [6] **Bendary E, Francis RR, Ali HMG, Sarwat MI, El Hady S.** Antioxidant and structure–activity relationships (SARs) of some phenolic and anilines compounds. *Ann Agric Sci* 2013;58:173–81. <https://doi.org/https://doi.org/10.1016/j.aoas.2013.07.002>.
- [7] **Tadić J, Mihajlović M, Jovanović M, Mijin D.** Continuous flow synthesis of some 6- and 1,6-substituted 3-cyano-4-methyl-2-pyridones. *J Serb Chem Soc* 2019;84:531–8. <https://doi.org/10.2298/JSC180703092T>.
- [8] **Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C.** Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol Med* 1999;26:1231–7. [https://doi.org/10.1016/S0891-5849\(98\)00315-3](https://doi.org/10.1016/S0891-5849(98)00315-3).
- [9] *** A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness, www.swissadme.ch; Swiss Institute of Bioinformatics 2019 [accessed April 2021].
- [10] **Mirković J, Rogan J, Poleti D, Vitnik V, Vitnik Ž, Ušćumlić G, Mijin D.** On the structures of 5-(4-, 3- and 2-methoxyphenylazo)-3-cyano-1-ethyl-6-hydroxy-4-methyl-2-pyridone: An experimental and theoretical study. *Dyes Pigments* 2014;104:160–8. <https://doi.org/https://doi.org/10.1016/j.dyepig.2014.01.007>.
- [11] **Mirković J, Božić B, Vitnik V, Vitnik Ž, Rogan J, Poleti D, Ušćumlić G, Mijin D.** Structural, spectroscopic and computational study of 5-(substituted phenylazo)-3-cyano-1-ethyl-6-hydroxy-4-methyl-2-pyridones. *Color Technol* 2017;134:33–43. <https://doi.org/10.1111/cote.12321>.
- [12] **Lipinski CA, Lombardo F, Dominy BW, Feeney PJ.** Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliver Rev* 1997;23:3–25. [https://doi.org/https://doi.org/10.1016/S0169-409X\(96\)00423-1](https://doi.org/https://doi.org/10.1016/S0169-409X(96)00423-1).
- [13] **Ismail MMF, Farrag AM, Harras MF, Ibrahim MH, Mehany ABM.** Apoptosis: A target for anticancer therapy with novel cyanopyridines. *Bioorg Chem* 2020;94:103481. <https://doi.org/10.1016/j.bioorg.2019.103481>.