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Synthesis of 2-Amino-3-cyanopyridine Derivatives and Investigation of Their Antibacterial and Antifungal Properties

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Abstract

The aim of this study was to examine of antibacterial and antifungal activity of 2-amino-3-cyanopyridine derivatives against some microorganisms. The investigated compounds **2**, **4**, **6** exhibit promising antibacterial and antifungal activities. The high biological activities of the indicated compounds can be probably caused by the simultaneous presence of bromine atoms, nitro and amino groups in the molecules.

Keywords: Multicomponent reactions, aminopyridine, microorganism, medicinal, pharmaceutical

INTRODUCTION

Preparing of effective and new synthetic methods for constructing polyfunctional heterocyclic compounds have importance in organic and medicinal chemistry (Gouda et al., 2014; Purwaningsih et al., 2023; Zakaria et al., 2023; Fadei et al., 2023). The pyridine ring is one of the most prevalent heterocyclic fragments, which we can find in nature, pharmaceutical preparations and functional materials (Michael, 2005). So different approaches for their synthesis have been developed (Vaghei et al., 2013; Karimi, 2017; Khalifeh, & Ghamari, 2016; Behrouz, 2016; Yahyazadeh et al., 2018; Akbarpoor et al., 2020; Mirjalili et al., 2020; Momeni, & Vaghei, 2023). Pyridine derivatives have considerable attention due to their synthetic and pharmaceutical importance (Murata et al. 2003).

2-Amino-3-cyanopyridines also have different biological and pharmacological activities, particularly antimicrobial, antidepressant, cardiotonic and anticancer activity (Shi et al., 2005; Liao et al., 2015; Alazawi et al., 2021; Achagar et al., 2022; Mamedov et. all. 2020).

According to the concise literature survey above, cyanopyridines **1**–**6** were tested (at a single concentration of 1 mg/ml) against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans.*

METHODOLOGY

Materials and Instrumentals

All the chemicals were obtained from commercial sources (Aldrich) and used as received.

NMR experiments have been performed on a BRUKER FT NMR spectrometer (UltraShieldTM Magnet) AVANCE 300 (300.130 MHz for ^{1}H and 75.468 MHz for ^{13}C) with a BVT 3200 variable temperature unit in 5 mm sample tubes using Bruker Standard software (TopSpin 3.1). The ^{1}H and ^{13}C chemical shifts were referenced to internal tetramethylsilane (TMS); the experimental parameters for ^{1}H: digital resolution = 0.23 Hz, SWH = 7530 Hz, TD = 32 K, SI = 16 K, 90^{0} pulse-length = 10 μs, PL1= 3dB, ns-=1, ds=0, d1=1 s; for ^{13}C: digital resolution = 0.27 Hz, SWH=17985 Hz, TD=64K, SI = 32K, 90^{0} pulse-length=9μs, PL1=1.5dB, ns=100, ds=2, d1=3s. NMR-grade DMSO-d_6 was used for solutions **1-6** (Figure 1-6). FT-IR spectra were obtained as KBr pellets on a Perkin-Elmer 781 spectrophotometer.

The purity of the synthesized compounds was confirmed by thin-layer chromatography (TLC) on commercial aluminum-backed plates of silica gel (60 F254), and iodine vapor was used as visualizing agent, eluent- 5:2 hexane/ethyl acetate.

Methods

General procedure for the synthesis of 2-amino-3-cyanopyridines (1-6).

Acetophenone (or 4-bromoacetophenone, 2 mmol) together with the respective aromatic aldehyde (2 mmol), malononitrile (2 mmol) and ammonium acetate (15 mmol) were refluxed for 10-14 h in ethanol (20 mL). The precipitate formed was filtered, washed with ethyl alcohol and recrystallized from DMF-methyl alcohol (1: 10).

Antibacterial and antifungal testing.

Compounds **1-6** were evaluated for their in vitro antibacterial, antifungal activities by the disc-diffusion method (used Tryptone Soy Agar (*TSA*), Mueller-Hinton (*MH*), Baird-Parker (*BP*), Endo (*En*) and Sabouraud agars (*SA*) growth mediums). Stock solutions of test compounds were diluted in dimethyl sulfoxide (DMSO) to give a final concentration of 1 mg/ml. The DMSO alone was used as a control and it was revealed, that solvent doesn't influence antibacterial-antifungal properties (the zone of inhibition was 1-1.5 mm). The plates with bacterial suspensions and disk of investigated compounds were incubated at 37°C, for 24 hours for the bacteria and fungi. After incubation, growth was surveyed by measuring the diameter of the growth inhibition zones.

This work used differential microorganisms and culture media from the company "*Liofil-chem*" (Italy): *Staphylococcus aureus (ATCC 99213)*, *Escherichia coli (ATCC 25922)* and *Candida albicans (ATCC 90028).*

RESULTS AND DISCUSSION

The ^{1}H NMR spectra of compound **1 (**Figure 1**)** showed the presence of the singlets at 7.15 and 8.15 ppm, also multiplets at 7.35-7.75 ppm appeared for amine and aromatic protons respectively.

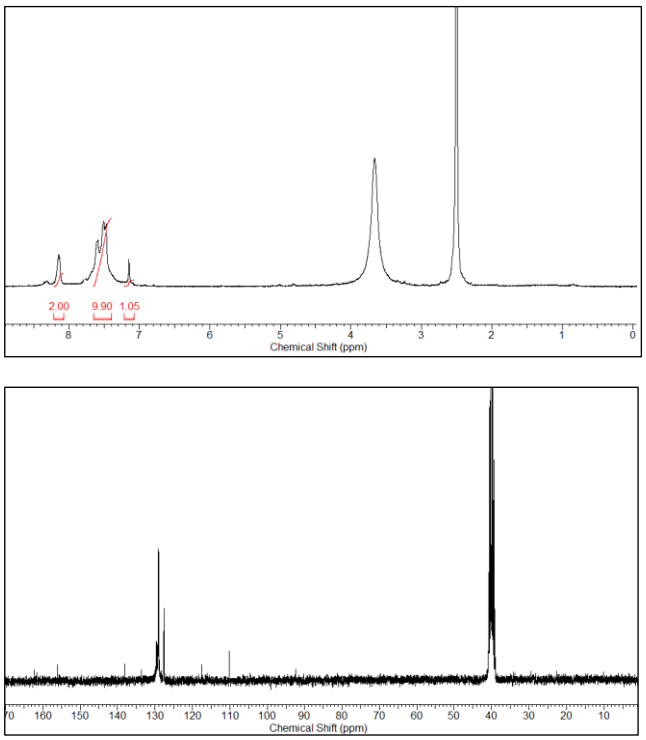


Figure 1**.** ^{1}H and ^{13}C NMR spectra of compounds 1 in DMSO-*d*6

2-Amino-4,6-diphenylnicotinonitrile (1). White powder, yield 73%, mp: 123°C; IR (KBr, cm^{-1}): 3362, 3085, 1655, 1621; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 7.15 (s, 1H), 7.35-7.75 (m, 10H), 8.15 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 92.5, 110.5, 118.5, 126.7, 127.3, 127.8, 128.3, 128.7, 129.1, 134.5, 138.5, 156.1, 161.5, 162.1.

The ^{1}H NMR spectra of compound **2** (Figure 2) showed the presence of the singlets at 7.15 and 8.15 ppm, also multiplets at 7.45-7.63 ppm appeared for amine and aromatic protons respectively.

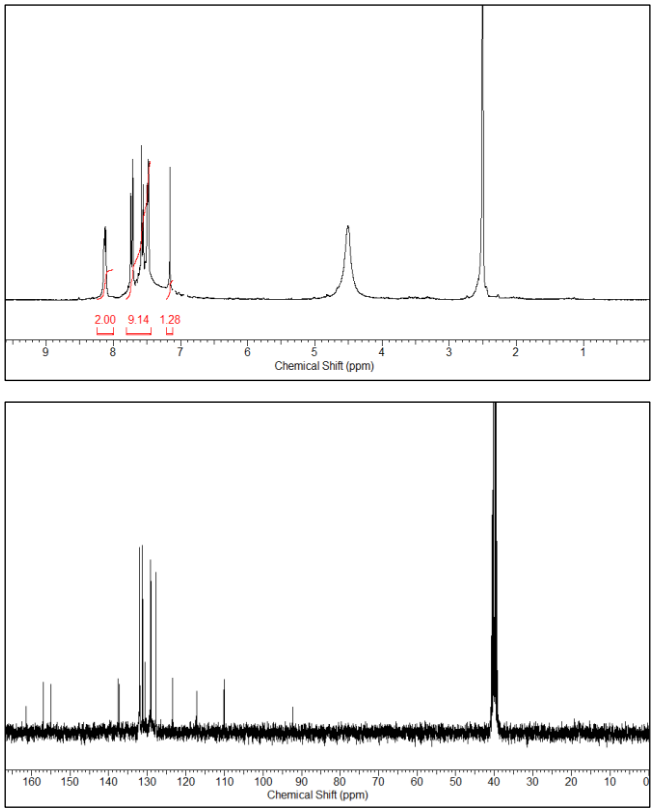


Figure 2**.** ^{1}H and ^{13}C NMR spectra of compounds **2** in DMSO-d_6

2-Amino-6-(4-bromophenyl)-4-phenylnicotinonitrile (2). Yellow powder, yield 78%, mp: 150ºC; IR (KBr, cm^{-1}): 3398, 3076, 1647, 1609; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 7.15 (s, 1H), 7.45-7.63 (m, 5H), 7.64 (d, J = 8.3 Hz, 2H), 7.75 (d, J = 8.3 Hz, 2H),), 8.15 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 92.8, 110.1, 117.5, 123.7, 127.3, 128.8, 128.9, 132.7, 137.1, 137.9, 138.5, 154.1, 157.5, 160.8.

The ^{1}H NMR spectra of compound **3** (Figure 3) showed the presence of the singlets at 6.85 and 6.98 ppm, also multiplets at 7.50-7.75 ppm appeared for amine and aromatic protons respectively.

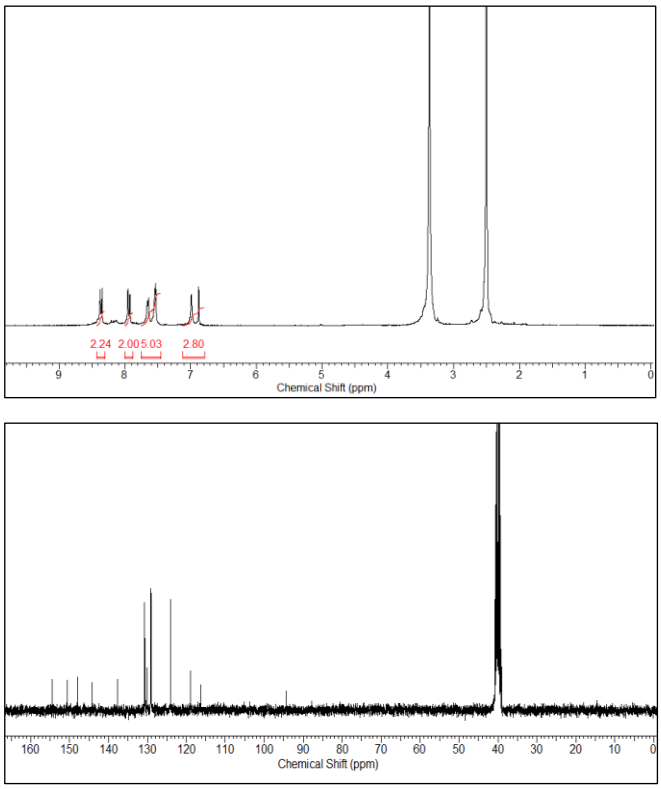
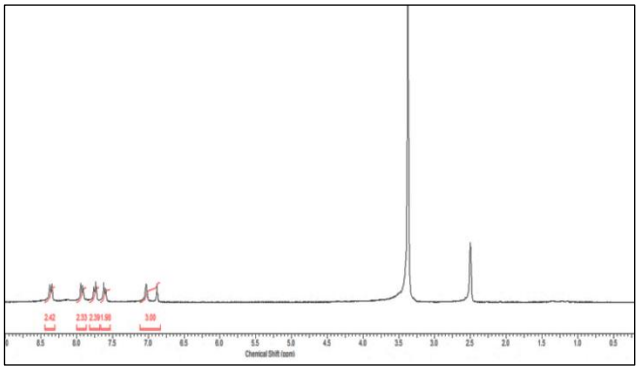


Figure 3. ^{1}H and ^{13}C NMR spectra of **3** in DMSO-d_6

2-Amino-4-(4-nitrophenyl)-6-phenylnicotinonitrile (3). Yellow powder, yield 75%, mp: 141ºC; IR (KBr, cm^{-1}): 3325, 3078, 1649, 1619; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 6.85 (s, 1H),), 6.98 (s, 2H), 7.50-7.75 (m, 5H), 7.95 (d, J = 8.6 Hz, 2H), 8.35 (d, J = 8.3 Hz, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 94.5, 116.5, 119.3, 129.1, 129.9, 138.1, 144.6, 148.3, 150.6, 154.8, 138.5, 154.1, 157.5, 160.8.

The ^{1}H NMR spectra of compound **4** (Figure 4) showed the presence of the singlets at 6.85 and 7.05 ppm, also duplets at 7.60, 7.75, 7.9, 8.35 ppm appeared for amine and aromatic protons respectively.



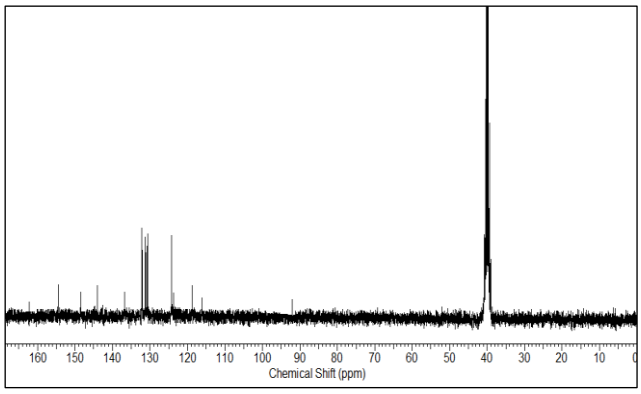
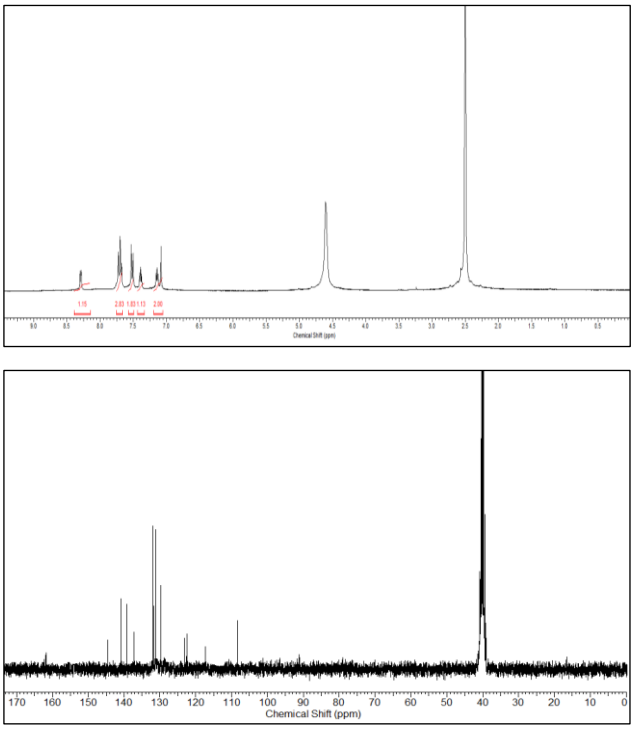


Figure 4**.** ^{1}H and ^{13}C NMR spectra of **4** in DMSO-d_6

2-Amino-6-(4-bromophenyl)-4-(4-nitrophenyl)nicotinonitrile (4). Yellow powder, yield 83%, mp: 166ºC; IR (KBr, cm^{-1}): 3333, 3087, 1651, 1614; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 6.85 (s, 1H),), 7.05 (s, 2H), 7.60 (d, J = 8.3 Hz, 2H), 7.75 (d, J = 8.3 Hz, 2H), 7.90 (d, J = 8.6 Hz, 2H), 8.35 (d, J = 8.6 Hz, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 92.3, 115.5, 118.8, 124.1, 125.3, 130.5, 131.6, 132.3, 136.5, 144.8, 148.5, 154.9, 157.5, 162.3.

The IR spectrum of compound **5** (Figure 5) exhibited absorption at 3323 cm^{-1} for NH_2, 3077, 3085, 1655, 1648 and 1614 for ArH. The ^{1}H NMR spectra of compound **5** showed the presence of the singlets at 7.10 and 8.29 ppm, also duplets at 7.2, 7.51, 7.55, 7.49 ppm, triplet at 7.75 ppm appeared for amine, thiophene and aromatic protons respectively.



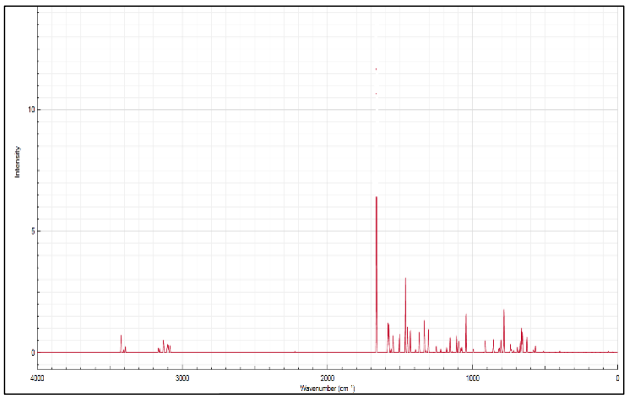
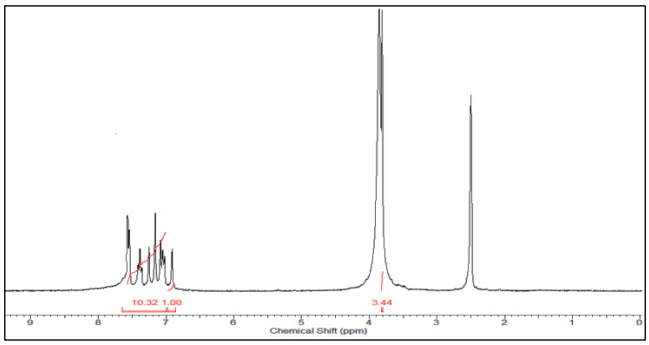


Figure 5. ^{1}H and ^{13}C NMR spectra of compounds 5 in DMSO-d_6; IR spectra of compounds 5

2-Amino-6-(4-bromophenyl)-4-(thiophen-2-yl)nicotinonitrile (5). Yellow powder, yield 63%, mp: 155ºC; IR (KBr, cm^{-1}): 3323, 3077, 3085, 1655, 1648, 1614; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 7.10 (s, 1H),), 7.2 (d, J = 4.5 Hz, 1H), 7.75 (t, J = 4.5 Hz, 1H), 7.51 (d, J = 8.3 Hz, 2H), 7.55 (d, J = 4.5 Hz, 1H), 7.59 (d, J = 8.3 Hz, 2H), 8.29 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 90.9, 108.5, 112.8, 122.3, 123.3, 129.9, 131.3, 131.6, 136.3, 139.8, 140.5, 149.9, 161.5, 161.7.

The IR spectrum of compound **6** (Figure 6) exhibited absorption at 3348 cm^{-1} for NH_2, 3087, 1653 and 1614 for ArH. The ^{1}H NMR spectra of compound **6** showed the presence of the singlets at 3.85, 6.95 and 7.81 ppm, also multiplets at 7.1-7.7 ppm appeared for methoxy, amine and aromatic protons respectively.

2-Amino-6-(4-bromophenyl)-4-(3-methoxy-phenyl) nicotinonitrile (6). Yellow powder, yield 63%, mp: 148ºC; IR (KBr, cm^{-1}): 3348, 3087, 1653, 1614; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.85 (s, 3H), 6.95 (s, 1H),), 7.1-7.7 (m, 8H), 7.81 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 55.5, 92.3, 109.8, 112.5, 119.7, 119.9, 123.3, 129.9, 131.3, 131.6, 131.8, 137.3, 139.5, 147.8, 151.7, 159.7, 161.9.



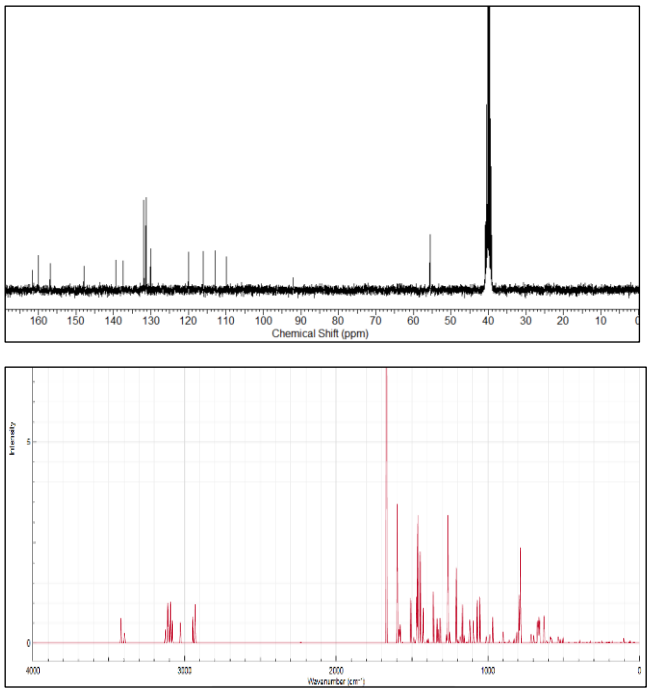


Figure 6. ^{1}H and ^{13}C NMR spectra of compounds 6 in DMSO-d_6; IR spectra of compounds 6

Our goal in this work were to examine of anti-microbial and antifungal activity of **1-6**. The synthesis route and mechanism of the 2-amino-3-cyanopyridne derivatives is given in Scheme 1 and 2 (Figure 7 and 8).

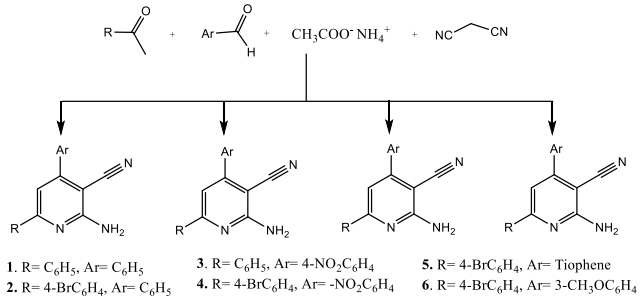


Figure 7. *Scheme 1*: Synthesis route of 2-amino-3-cyanopyridne derivatives (**1-6**)

The *in vitro* antibacterial activities of compounds **1-6** were evaluated against gram-positive and gram-negative bacteria and fungi using the cultures of different standard microorganisms: *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. Our investigations demonstrated that compounds **2**, **4** and **6** in minimal concentration exhibited better antibacterial, antifungal activities than **1**, **3** and **5** against investigated microorganisms in all growth mediums.

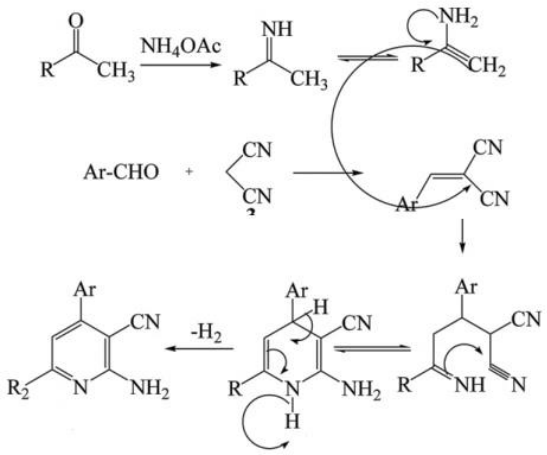


Figure 7. *Scheme 2:* The possible synthesis mechanism of the 2-amino-3-cyanopyridne derivatives

The high biological activities of the **2**, **4** and **6**, can be probably caused by the simultaneous presence of bromine atoms, nitro and amino groups in the molecules. Bromine-, methoxy-, nitro- and the amino groups in compounds exhibit antibacterial activity suggesting that the bromine participates in a hydrophobic (as a halogen bond donor), NH_2, OCH_3 and NO_2 in a hydrophilic interaction (as a hydrogen bond acceptor). The fact that indicates that hydrogen bonding impacts the antibacterial activity of **2**, **4** and **6**. Thus, halogen bonding, which has lately been demonstrated to be often beneficial for bioactivity, is most likely involved in the antibacterial activity of **2**, **4** and **6** (Wilcken et al., 2013; Mendez et al., 2017). The results are shown in Table 1.

Table 1. Antibacterial, antifungal activity of **5** and **6** in DMSO solution (10 mg/ml) (disc-diffusion method)

№ comp.	Microor-ms	Antimicrobial activity (zone of inhibition in mm)				
		MH	*TSA*	*BP*	*En*	*SA*
1	*S. aureus*	11	10	12		
	E. coli	9	10		12	
	C. albicans	6	4			9
2	*S. aureus*	14	12	15		
	E. coli	15	14		16	
	C. albicans	9	10			11
3	*S. aureus*	10	13	12		
	E. coli	11	9		13	
	C. albicans	6	7			8
4	*S. aureus*	12	11	14		
	E. coli	13	12		15	
	C. albicans	11	10			14
5	*S. aureus*	8	6	11		
	E. coli	12	9		13	
	C. albicans	7	6			8
6	*S. aureus*	14	12	15		
	E. coli	13	11		14	
	C. albicans	8	7			11

CONCLUSION

In the presented work, we demonstrated the studying of six practical compounds against microorganisms. This allows making to the fast testing of pharmacological activities of these 2-amino-3-cyanopyridine derivatives. Obtained results demonstrated the best antibacterial, antifungal activities of **2**, **4** and **6**.

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