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Д.В.Сокольский атындағы «Жанармай,
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ИЗВЕСТИЯ

НАЦИОНАЛЬНОЙ АКАДЕМИИ НАУК
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АО «Институт топлива, катализа и
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Қазақстан Республикасы Ұлттық ғылым академиясы "ҚР ҰҒА Хабарлары. Химия және технология сериясы" ғылыми журналының Web of Science-тің жаңаланған нұсқасы Emerging Sources Citation Index-те индекстелуге қабылданғанын хабарлайды. Бұл индекстелу барысында Clarivate Analytics компаниясы журналды одан әрі the Science Citation Index Expanded, the Social Sciences Citation Index және the Arts & Humanities Citation Index-ке қабылдау мәселесін қарастыруда. Webof Science зерттеушілер, авторлар, баспашылар мен мекемелерге контент тереңдігі мен сапасын ұсынады. ҚР ҰҒА Хабарлары. Химия және технология сериясы Emerging Sources Citation Index-ке енуі біздің қоғамдастық үшін ең өзекті және беделді химиялық ғылымдар бойынша контентке адалдығымызды білдіреді.

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SYNTHESIS AND ANTI-MICROBIAL ACTIVITY OF N'-(2-HYDROXY-5-NITROBENZYLIDENE) ISONICOTINOHYDRAZIDE

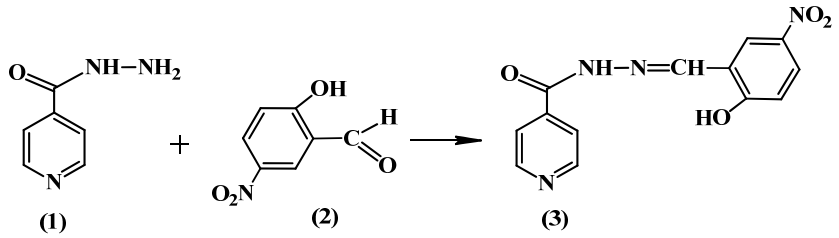
Abstract. The paper presents data on the synthesis, structure and bioactivity of N '- (2-hydroxy-5-nitrobenzylidene) isonicotinohydrazide based on isonicotinic acid hydrazide. The structure of the new hydrazone was studied by ^{1}H and ^{13}C NMR spectroscopy, as well as two-dimensional COSY (^{1}H-^{1}H) and HMQC (^{1}H-^{13}C) spectra. The values of chemical shifts, multiplicity and integral intensity of ^{1}H and ^{13}C signals in one-dimensional NMR spectra are determined. Using spectra in COSY (^{1}H-^{1}H) and HMQC (^{1}H-^{13}C) formats, homo- and heteronuclear interactions have been established, confirming the structure of new isonicotinic acid derivatives. Studies of the synthesized compound for antimicrobial activity against drug-sensitive museum strains of bacteria and fungi have been carried out. According to the results of bioassays, it was shown that a new hydrazone derivative of isonicotinic acid in a dose of 1 μg has a weak potential for antimicrobial activity, and the diameters of growth inhibition zones for *in vitro* test cultures averaged 12 ± 1.0 mm.

Key words: isonicotinic acid, hydrazones, chemical modification, antimicrobial activity.

Isonicotinic acid hydrazide (INH) is today one of the main widely used and fairly inexpensive tuberculostatics, however, it still does not meet the requirements for modern drugs in many clinical settings. On the basis of INH, a large number of various derivatives have been synthesized with a wide variation of the antituberculosis activity and toxicity of the compounds [1-3]. Thus, in 1951, in the Soviet Union, a method was developed for the synthesis of the drug ftivazid by the interaction of isonicotinic acid hydrazide with vanillin [4]. Ftivazide, due to its low toxicity and good individual tolerance, and currently occupies a leading place in the treatment of various forms of tuberculosis [4]. In clinical practice, other isonicotinic acid hydrazones, such as methazide, saluzid, and larusan, are widely used today. However, difficulties in the treatment of tuberculosis are associated with the development of drug resistance of Mycobacterium tuberculosis and, as a result, the drug loses its therapeutic effect. In this regard, the synthesis of new derivatives of isonicotinic acid hydrazones continues, and the search for new highly effective anti-TB drugs is still an urgent task [5].

In addition, it is known that hydrazones are generally widely used in synthetic chemistry due to the simple method of their production [6] and various biological activities besides anti-tuberculosis action (plant growth regulators, herbicides, fungicides, antioxidants) [7, 8]. Continuing research on the modification of isonicotinic hydrazide, it seemed interesting to obtain new hydrazones based on INH, containing additional pharmacophoric groups.

The purpose of this work was to study the reaction of the interaction of isonicotinic acid hydrazide **(1)** with 2-hydroxy-5-nitrobenzaldehyde **(2)**. The synthesis of the new N'-(2-hydroxy-5-nitrobenzylidene)isonicotinohydrazide **(3)** was carried out in ethyl alcohol at 50-60 °C for 4 hours.

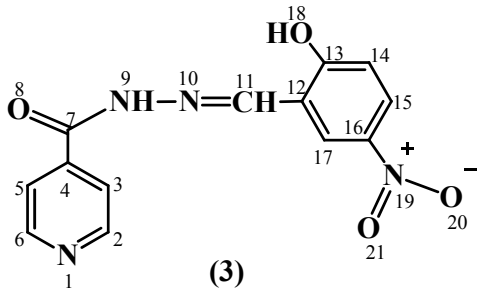


The reaction product **(3)** is a well-crystallized substance, soluble in many organic solvents, the yield of the compound is 92.0%.

The composition and structure of N'-(2-hydroxy-5-nitrobenzylidene)-isonicotinohydrazide **(3)** are confirmed by the data of IR-, ^{1}H- and ^{13}C- NMR spectroscopy, as well as two-dimensional spectra of COSY (^{1}H-^{1}H) and HMQC (^{1}H-^{13}C).

In the IR spectrum of compound **(3)** there is an intense absorption band in the region of 3028–3094 cm^{-1}, corresponding to valence N–H vibrations, in the region of 3016–3049 cm^{-1} - to valence C–H vibrations of the pyridine ring, 1870–1955 cm^{-1} - the overtones of the pyridine ring, 1642-1651 cm^{-1} - the vibrations of the C=O bond, the vibrations of the C=N bond are recorded in the region of 1543 cm^{-1}.

The ^{1}H NMR spectrum of the compound **(3)** is characterized by the presence in the aromatic region at 7.05 ppm doublet with an intensity of 1H with ^{3}J 9.2 Hz of the atom H^{14} of the benzene nucleus. The H^{15} proton, adjacent to H^{14}, as a result of spin-spins splitting by a neighbor, also manifests itself as a doublet signal at 8.11 ppm with an integrated intensity of 1H with ^{3}J 8.6, characteristic of aromatic compounds. H^{17} proton of the benzene nucleus, not having proton containing neighbors, is manifested by a singlet at 8.69 ppm with an intensity of 1H. Equivalent protons of the pyridine ring $H^{2.6}$ and $H^{3.5}$ resonated with doublet signals with an intensity of two protons at 8.75 (^{3}J 5.5 Hz) and 7.80 (^{3}J 5.5 Hz) ppm respectively. The proton H^{11}, which is unable to interact with other protons through three bonds, respectively, manifested itself in the form of a singlet signal at 8.54 ppm with an integrated intensity of 1H. The protons of the hydroxyl group and the amide bond manifested themselves in the form of broadened singlets with an integral 1H each in the weakest field of the spectrum at 12.55 and 12.37 ppm respectively.



(3)

In the ^{13}C NMR spectrum of N'-(2-hydroxy-5-nitrobenzylidene)-isonicotinohydrazide **(3)**, signals of the aromatic ring are observed at 117.60 (C^{14}), 120.51 (C^{12}), 127.35 (C^{15}), 140.34 (C^{16}), 145.48 (C^{17}) и 162.12 (C^{13}) ppm. The carbon atoms of the pyridine fragment resonated at 122.05 ($C^{3,5}$), 150.89 ($C^{2,6}$) и 140.45 (C^{4}) ppm. Chemical shift signal at 123.91 ppm corresponds to the carbon atom bound by a double bond with the nitrogen atom. In the field of a weak field at 163.09 ppm marked signal carbonyl atom C^{7}. The structure of compound **(3)** was also confirmed by the methods of two-dimensional NMR spectroscopy COSY (^{1}H-^{1}H) and HMQC (^{1}H-^{13}C), which allows one to establish spin-spin interactions of a homo- and heteronuclear nature. The observed correlations in the molecule **(3)** are shown in Figures 1 and 2. In the COSY (^{1}H-^{1}H) spectra of the compound **(3)** spin-spin correlations are observed through three proton bonds of the neighboring methylene groups H^{2}-H^{3} and H^{5}-H^{6} of the pyridine ring cross-peaks with coordinates at 8.73, 7.80 and 7.78, 8.75. Coordinates 8.10, 7.05 and 7.04, 8.11 correspond to the homolytic interaction through three bonds of the neighboring aromatic protons $H^{14,15}$. Heteronuclear interactions of protons with carbon atoms through one bond were established using HMQC (^{1}H-^{13}C) spectroscopy for all pairs present in the compounds: H^{14}-C^{14} (7.03, 117.61), $H^{3,5}$-$C^{3,5}$ (7.78, 122.07), H^{15}-C^{15} (8.09, 127.25), H^{11}-C^{11} (8.52, 123.89), H^{17}-C^{17} (8.68, 145.45), $H^{2,6}$-$C^{2,6}$ (8.73, 150.85).

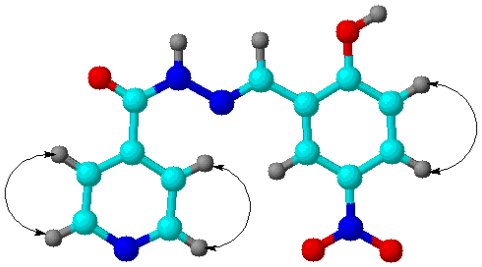


Figure 1 - Scheme of COSY (^{1}H-^{1}H) correlations compounds **(3)**

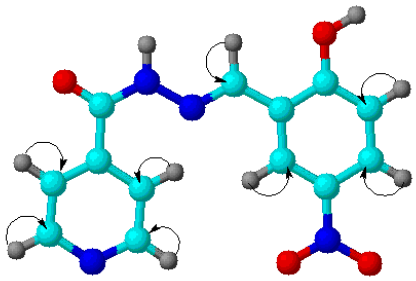


Figure 2 - Scheme of HMQC (^{1}H-^{13}C) correlations of compound **(3)**

In order to study the biological activity of the synthesized new derivative of isonicotinic acid hydrazide, a bioscreening was conducted for the presence of antimicrobial activity against drug-sensitive museum strains of bacteria and fungi. Antimicrobial activity was assessed by the minimum inhibitory concentration (1 μg), and the concentration of reference drugs was 1 mg.

The sensitivity of bacteria *S. aureus, Bacillus subtilis 6633, E. Coli, Ps was determined. Aerugiosa ATCC 9027* and the yeast fungus *Candida albicans* to the test sample **(3)** by the diffusion method using discs, solvent 96% ethyl alcohol. The antibiotic benzylpenicillin sodium, gentamicin and the third-generation cephalosporin antibiotic, ceftriaxone, and nystatin, antifungal activity, have been selected as standards for evaluating antibacterial activity. Test cultures were sown using the lawn method on nutrient media: JSA, Endo medium, nutrient agar and Saburo medium. Then the Petri dishes were incubated for 24 hours at 28-37 °C.

Antimicrobial activity of the samples was assessed by the diameter of the zones of growth inhibition of test strains (mm). The diameters of the zones are less than 10 mm and continuous growth in the cup was evaluated as the absence of antimicrobial activity, 10-15 mm – weak activity, 15-20 mm – moderately pronounced activity, more than 20 mm – pronounced. The main sample was tested in three parallel experiments. Statistical processing was performed by parametric statistics with the calculation of the arithmetic mean and standard error. The results of the identified growth delays on the media are shown in the table.

Table – Antimicrobial activity of N'-(2-hydroxy-5-nitrobenzylidene)-isonicotinohydrazide. Diameters of growth inhibition of test strains, mm

Test substances	S.aureus	B.subtilis 6633	E.coli	Ps.aerugiosa ATCC 9027	C.albicans
N'-(2-hydroxy-5-nitrobenzylidene)-isonicotinohydrazide (3)	12±1.0	13±1.0	13±1.0	11±1.0	12±1.0
Ethyl alcohol 96%	9±1.0	9±1.0	9±1.0	9±1.0	9±1.0
Benzylpenicillin sodium salt	15±1.0	-	16±1.0	12±1.0	-
Gentamicin	22±1.0	30±1.0	31±1.0	30±1.0	-
Ceftriaxone	30±1.0	30±1.0	29±1.0	22±1.0	-
Nystatin	-	-	-	-	25±1.0

These pharmacological studies have shown that N'-(2-hydroxy-5-nitrobenzylidene) isonicotinhydrazide **(3)** at a dose of 1 μg has a weak antimicrobial activity potential, since the diameters of the in vitro growth inhibition zones for test cultures averaged 12 ± 1.0 mm. Chemical modification of derivatives of isonicotinic acid hydrazide can ensure the production of new biologically active substances with antimicrobial activity.

Experimental part

The ^{1}H and ^{13}C NMR spectra of the compounds **(3)** were recorded on a JNN-ECA 400 spectrometer from Jeol (Japan) in a DMSO-d_6 solution relative to the internal TMS standard with a frequency of 400 and 100 MHz on the ^{1}H and ^{13}C cores, respectively. IR spectra were taken on a Cary 600 Series FTIR spectrometer manufactured by Agilent Technologies (USA) using a single reflection attachment on a

Gladiatr diamond manufactured by PIKE (USA). All measurements were carried out at a resolution of 4.0 cm^{-1}, the number of scans was 40. Melting points were determined on an OptiMelt instrument. TLC analysis was performed on Silufol UV-254 plates, the manifestation of iodine vapor. Biological tests of samples of the synthesized compounds for antibacterial and antifungal activity were carried out at the base of the Department of Microbiology of Karaganda State Medical University.

N'-(2-hydroxy-5-nitrobenzylidene) isonicotinohydrazide (3). To a mix-ture of 0.7 g (0.004 mol) of isonicotinic acid hydrazide in 10 ml of ethanol, 0.8 g (0.004 mol) of 2-hydroxy-5-nitrobenzaldehyde dissolved in 10 ml of ethanol was added with stirring at room temperature. Then the reaction mixture was stirred for 4 hours at a temperature of 50-60 °C under reflux. The completion of the reaction was monitored by TLC. The solution was cooled, the precipitated precipitate was filtered. Received 1.3 g (92,0%) of the product (**3**) yellow with a melting point of 285-287 °C. Found, %: C 54.65; H 3.31; N 19.63. Calculated, %: 54.54; H 3.49; N 19.58. $C_{13}H_{10}N_4O_4$. NMR ^{1}H (DMSO-d_6), δ, ppm: 7.05 d (1H, H^{14}, $^3J_{HH}$ 9.2 Hz), 8.11 d (1H, H^{15}, $^3J_{HH}$ 8.6 Hz), 8.69 c (1H, H^{17}), 8.75 d (2H, $H^{2.6}$, $^3J_{HH}$ 5.5 Hz), 7.80 d (2H, $H^{3,5}$, $^3J_{HH}$ 5.5 Hz), 8.54 c (1H, H^{11}), 12.15 br. with (1H, OH), 12.37 br. with (1H). NMR ^{13}C (DMSO-d_6), δ, ppm: 117.60 (C^{14}), 120.51 (C^{12}), 127.35 (C^{15}), 140.34 (C^{16}), 145.48 (C^{17}), 162.12 (C^{13}), 122.05 ($C^{3,5}$), 150.89 ($C^{2,6}$), 140.45 (C^4), 123.91 (N=CH), 163.09 (C=O).

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N'-(2-ГИДРОКСИ-5-НИТРОБЕНЗИЛИДЕН)ИЗОНИКОТИНО-ГИДРАЗИДІНІҢ СИНТЕЗІ МЕН МИКРОБҚА ҚАРСЫ БЕЛСЕНДІЛІГІ

Аннотация. Жұмыста изоникотин қышқылы гидразидінің негізінде алынған N'-(2-гидрокси-5-нитробензилден) изоникотиногидразидінің синтезі, құрылысы және био-белсенділігі бойынша деректер келтірілген. Жаңа гидразонның құрылысы ЯМР ^{1}Н- және ^{13}С-спектроскопия әдістерімен, сондай-ақ COSY (^{1}Н-^{1}Н) және HMQC (^{1}Н-^{13}С) екіөлшемді спектрлерінің деректерімен зерттелген. Бірөлшемді ЯМР спектрлерінде ^{1}Н және ^{13}С сигналдардың интегралдық қарқындылығы, мультиплеттілігі және химиялық ығысу мәндері анықталды. COSY (^{1}Н-^{1}Н) және HMQC (^{1}Н-^{13}С) форматтарындағы спектрлер көмегімен изоникотин қышқылының жаңа туындысының құрылымын растайтын гомо- және гетероядролық өзара әрекеттесулері орнатылды. Бактериялар мен зеңдердің дәрілік-сезімтал музей штаммдарына қатысты микробқа қарсы белсенділікке синтезделген қосылыстарға зерттеулер жүргізілді. Биосынау нәтижелері бойынша 1 мкг дозадағы изоникотин қышқылының жаңа гидразондық туындысы микробқа қарсы белсенділіктің әлсіз шамасына ие, *in vitro* тестілік дақылдардың өсуін кідірту аймағының диаметрлерінің орташа есеппен 12±1,0 мм құрайтыны көрсетілген.

Түйін сөздер: изоникотин қышқылының гидразиді, гидразондар, химиялық түрлендіру, микробқа қарсы белсенділік.

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СИНТЕЗ И ПРОТИВОМИКРОБНАЯ АКТИВНОСТЬ N'-(2-ГИДРОКСИ-5-НИТРОБЕНЗИЛИДЕН) ИЗОНИКОТИНОГИДРАЗИДА

Аннотация. В работе приведены данные по синтезу, строению и биоактивности N'-(2-гидрокси-5-нитробензилиден)изоникотиногидразида, полученного на основе гидразида изоникотиновой кислоты. Строение нового гидразона исследовано методами ЯМР ^{1}Н- и ^{13}С-спектроскопии, а также данными

двумерных спектров COSY (^{1}H-^{1}H) и HMQC (^{1}H-^{13}C). Определены значения химических сдвигов, мультиплетность и интегральная интенсивность сигналов ^{1}H и ^{13}C в одномерных спектрах ЯМР. С помощью спектров в форматах COSY (^{1}H-^{1}H) и HMQC (^{1}H-^{13}C) установлены гомо- и гетероядерные взаимодействия, подтверждающие структуру нового производного изоникотиновой кислоты. Проведены исследования синтезированного соединения на антимикробную активность в отношении лекарственно-чувствительных музейных штаммов бактерий и грибов. По результатам биоиспытаний показано, что новое гидразоновое производное изоникотиновой кислоты в дозе 1 мкг обладает слабым потенциалом противомикробной активности, диаметры зон задержки роста тестовых культур *in vitro* в среднем составляют 12±1,0 мм.

Ключевые слова: гидразиды изоникотиновой кислоты, гидразоны, химическая модификация, антимикробная активность.

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