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Synthesis of nicotine derivatives and evaluation of their anti-bacterial activity

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ABSTRACT: Using a convergent synthetic method, a series of nicotine derivatives were synthesized from the basic materials nicotine-N-oxide in good yields. The structures of the synthesized compounds were confirmed by spectral methods of analysis (FT-IR, 1H-NMR, and 13C-NMR). Most of the target compounds were tested for antibacterial activity against five kinds of bacteria; the tested compounds exhibited varying levels of activity against both gram-negative and gram-positive bacteria. The results of bioactivities showed that some of the target compounds exhibited good antibacterial activities against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Klebsiella pneumoniae*. In addition, the broad spectrum anti-microbial action of nicotine derivatives developed in the present study may find immense applications in formulating new disinfection or decontamination strategies against widely spreading pathogens of clinical significance.

Keywords: Nicotine; Antibacterial properties; Effects of nicotine; Nicotine derivatives.

1. INTRODUCTION

Nicotine is an amine that is composed of pyridine and pyrrolidine rings [1]. With a pKa of 7.9, it is a weak base that is soluble in both water and lipids. The kidney excretes nicotine partially unaltered, but mostly in the form of twenty or more distinct metabolites with an intact pyridine ring. Nicotine has been proven to pass biological membranes, including the blood-brain barrier. Nicotine is extensively processed by the liver into a variety of major and minor metabolites once ingested [2-5]. Nicotine is found in a wide variety of plants and is transformed into many biologically relevant chemicals during harvesting and fermentation [6]. Furthermore, cigarettes and nicotine replacement therapies such as transdermal nicotine patches and nicotine-containing gum are the most common sources of nicotine exposure [7].

The most abundant alkaloid isolated from *Nicotiana* plants is (S)-nicotine. It was named after Jean Nicot, the French envoy to Portugal in the 16th century, who introduced tobacco to France [8,9]. Nicotine was originally isolated in 1828 by Posselt and Reimann, and Melsens proposed its chemical empirical formula in 1843. Pinner presented the structure of nicotine to the German Chemical Society in 1893. Pictet and Rotshy [11] were the first to synthesize nicotine in 1904, but it was not until 1978 that Pitner discovered the peculiar orientation of natural (S)-nicotine.

Tobacco-specific nitrosamines are the most prominent [12]. Nicotine's effects have been studied extensively in humans, animals, and a range of cell systems. Nicotine causes an increase in pulse rate, blood pressure, and plasma free fatty acids, as well as mobilization of blood sugar and an increase in the level of catecholamines in the blood in entire intact animals and humans [13-15]. Furthermore, nicotine has been shown to disrupt antioxidant defense mechanisms in rats on a high-fat diet. The stimulation of nicotinic receptors causes an increase in the production and exocytic release of many hormones, including epinephrine and epinephrine, at the cellular level [11,16]. Chronic nicotine administration has been demonstrated to activate tyrosine hydroxylase, the first and rate-limiting enzyme in catecholamine production, in addition to the release of these hormones [10,17]. Nicotinic receptor activation has also been demonstrated to stimulate the transcription factors c-fos and c-jun and to stabilize transforming growth factor intracellular levels [18,19]. Increased expression of heat shock proteins, stimulation of sister chromatids exchange and chromosome abnormality, reduction of cell growth, and suppression of apoptosis are among nicotine's additional biological effects [20,21].

In this research, we produced four nicotine derivatives. This type of combination and rebuilding of these heterocyclic compounds are expected to have high biological activity largely as antimicrobial agents and we compared the antibacterial activity results of these compounds with the analogous containing the same structural units.

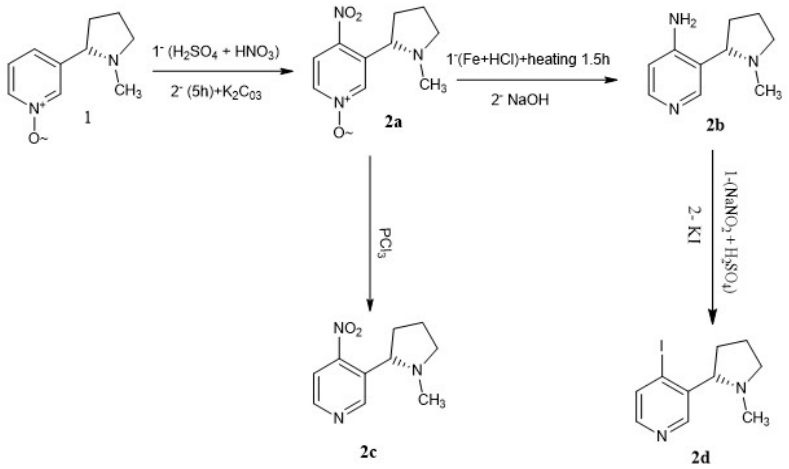
2. MATERIALS AND METHODS

2.1. Materials and instruments

Melting points were determined on a Gallen Kamp melting apparatus (Beijing Tech Instrument Co., China). 1H NMR and 13C NMR spectra were measured on a Bruker 80 TM 400 MHz Digital NMR Spectrometer (Bruker Company, Billerica, MA, US.) in DMSO as a solvent and recorded relative to internal standard tetramethylsilane. Infrared Spectrometer (FT-IR), Shimadzu FT-8201 PC. The course of the reactions was monitored by thin-layer chromatography (TLC) analysis on silica gel GF254. All reagents and solvents meet the standards of analytical reagent before use. H_2SO_4 (98%), HNO_3 (80%), Na_2SO_4 (97%), HCl (36%); Fe (98%), K_2CO_3 (99.5%), $Si(CH_3)_4$ (99%), KI (99%), $C_{10}H_{14}N_2$ (98%).

2.2. Chemistry

The nicotine derivatives were prepared according to Scheme 1.



Scheme 1. Synthetic route for preparation of compounds (2a-2d).

2.3. Synthesis of 4-nitronicotine-N-oxide (2a) [22]

An amount of nicotine-N-oxide (1.25 g, 7 mmol) was slowly added to an equivolume (14 mL) mixture of concentrated H_2SO_4 and fuming HNO_3. The mixture was then put under refluxing for 5 h. Subsequently, the mixture was carefully poured into 36 g of ice water and neutralized by K_2CO_3, and then extracted with chloroform. The extract was dried under vacuum with concentrated Na_2SO_4 a and then recrystallized with chloroform. The final product that was formed is 4-nitrocotinine-N-oxide weights 1.5 g, which represents a yield of 94% that manifests itself as yellow needles, m_P = 128-130°C.

^{1}H-NMR (DMSO-d$_6$, δ, ppm): 7.66 (d, 1H), 7.54 (s, 1H, J = 5.2 Hz), 7.10 (d, 1H, J = 5.2 Hz), 3.57 (t, 1H, J = 8.4 Hz), 2.36 (s, 3H), 3.12-2.22 (t, 2H), 2.00-1.84 (m, 2H), 2.00-2.14 (m, 2H).

^{13}C-NMR (DMSO -d$_6$, δ, ppm): 139.57, 119.1, 151.71, 124.19, 138.02, 70.01, 30.15, 20.61, 56.58, 40.09.

FTIR: 2750(C-H (sp^3)), 2700(C-H (sp^2)), 1720(N=O), 1650(N=C_{Ar}), 1450(C=C_{Ar}) cm^{-1}.

2.4. Synthesis of 4-aminonicotine (2b) [23]

The 4-nitronicotine-N-oxide (0.006 mol) was dissolved in 10 mL of ethanol followed by the addition of 0.4 mL hydrochloric acid (18%) with 2 g of iron. The mixture was heated under reflux for 1.5 h. Then, a dilute solution of sodium hydroxide was added to ensure a basic medium (pH ~12). The white precipitated formed was then filtrated and washed with water. The obtained product was finally dried and recrystallized from ethanol [21-24], m_P = 124-126°C.

^{1}H-NMR (DMSO -d$_6$, δ, ppm): 8.06 (d, 1H), 8.00 (s, 1H, J = 5.2 Hz), 6.40 (d, 1H, J = 5.2 Hz), 3.15 (t, 1H, J = 8.4 Hz), 2.17 (s, 3H), 3.11-2.21 (t, 2H), 2.21-1.84 (m, 2H), 2.02-1.83 (m, 2H).

^{13}C-NMR (DMSO -d$_6$, δ, ppm): 149.82, 119.28, 152.45, 110.1, 148.85, 70, 30.14, 22.62, 56.58, 40.09.

FTIR: 2750(C-H (sp^3)), 2700(C-H (sp^2)), 1720(N=O), 1600(N=C_{Ar}), 1450(C=C_{Ar}) cm^{-1}.

2.5. Synthesis of 4-nitronicotine (2c)

A 100 mL Schlenk flask equipped with a reflux condenser was charged with a stir bar, 4-nitronicotine-N-oxide (4.5 mmol), phosphorus trichloride (2 mL), and chloroform (15 mL). The resulting solution was heated at reflux for 1 h. After cooling, clean a dilute solution of sodium hydroxide to pH >12. 4-nitronicutine is extracted using chloroform. Chloroform is evaporated and dried with sodium persulfate. Recrystallization with chloroform yielded the desired product as yellow crystals (0.9 g, 98%), m_P= 134-136°C.

^{1}H-NMR (DMSO -d$_6$, δ, ppm): 8.65 (s, 1H), 8.12 (d, 1H, J = 5.2 Hz), 7.90 (d , 1H, J = 5.2 Hz), 3.63 (s, 1H, J = 8.4 Hz), 2.26 (s, 3H), 2.42-2.30 (t, 2H), 2.00-1.75 (m,2H), 1.64-1.54 (m, 2H)

^{13}C-NMR (DMSO -d$_6$, δ, ppm): 149.7, 133.2, 156.8, 120.5, 149.5, 71.2, 33.4, 23, 60.30, 43.3.

FTIR: 2820(C-H (sp^3)), 2750(C-H (sp^2)), 1620(N=O), 1550(N=C_{Ar}), 1450(C=C_{Ar}) cm^{-1}.

2.6. Synthesis of 4-iodonicotine (2d)

1.25 g of 4-amino nicotine was converted into a conical flask that contains 10 mL, 1% sulfuric acid under gentle stirring for 10 min. After that, 0.63 g sodium nitrite, and 2.5 g potassium iodide solutions were also added to the previous solution, respectively, with keeping at 10°C in an ice bath. Then, the obtained precipitate was rinsed many times with ultrapure water before being dried at room temperature. Lastly, the 4-iodonicotine has been synthesized and recrystallized with ethanol (a yield of 64%), m_P = 96-98°C [25].

^{1}H-NMR (DMSO -d$_6$, δ, ppm): δ 8.60 (s, 1H), 8.04(d, 1H, J = 5.2 Hz), 7.07 (d, 1H, J = 5.2 Hz), 3.83(t, 1H, J = 8.4 Hz), 2.46-2.32 (m, 2H), 2.24 (s, 3H), 1.98-1.78 (m, 2H), 1.58-1.46 (m, 2H).

^{13}C-NMR (DMSO -d$_6$, δ, ppm): 148.5, 111.51, 149.9, 134.4, 142.1, 72.49, 32.98, 23.01, 57.12, 40.81.

FTIR: 2967-2831(C-H (sp^3)), 1676(N=C), 1560(C=C), 1058(C-N) cm^{-1}.

2.7. Antibacterial activity *in vitro*

2.7.1. Agar disc diffusion assay

In this work, an antimicrobial test was performed according to the method of spreading the disc. Antimicrobial activity of compounds (2a-2d) was assessed *in vitro* against Gram-negative and Gram-positive bacteria. DMSO was used as solvent. The agar and Petri dishes were sterilized for 15 min at 121°C. These plates were incubated at 37°C for 24 hrs. The damping zones caused by the two components were examined by measuring the inhibition diameter of the inserted ruler (mm). Holes were filled with 100 µL of pre-prepared compounds (50 mg of melted compound in 1 mL of DMSO) and the remaining concentrations of 1, 10, 25 and 50 mg/mL were prepared [25,26].

2.7.2. Minimal inhibitory concentration (MIC)

To quantify the antimicrobial activity of 2a-2d compounds, Minimal Inhibitory Concentration (MIC) values of each compound was determined against all tested bacterial by the microdilution technique [27] with some modifications using 96-well plates. A volume of 100 µL of Muller-Hinton broth and 100 µL of each extract dissolved in 10% (v/v) of dimethyl sulfoxide or water, obtained from a stock solution of 30 mg/mL, was pipetted into the first row of the plate. Serial dilutions were consequently performed such that each well the test materiel in serially descending concentrations ranging from 15 to 0.007 mg/mL. To each well 30 µL of resazurin (0.015%) was added as an indicator of microbial growth [28]. Finally, 10 µL of bacterial (10^7 CFU/mL) was added to achieve a concentration of 10^6 CFU/mL in each well. The MIC values of the positive standards chloramphenicol and cycloheximide were determined in the same conditions using concentrations ranging from 5 to 0.001 mg/mL. A column with all solutions with the exception of the test compound and a column with all solutions with the exception of the bacterial solution adding nutrient broth instead were realized. The plates were prepared in triplicate and placed in an incubator set at 37°C/18-24 h for bacteria. After incubation, the MIC corresponding to the lowest concentration at which a change in the color occurred was visually determined.

3. RESULTS AND DISCUSSION

In general, the prepared nicotine derivatives showed inhibition zones from one type of bacteria to another and from one compound to another. The compounds have indeed inhibitory effects for most of the concentrations. Through the biological efficacy study, it was noted that the concentration 1 mg/mL was inhibitory for all investigated bacterial strains, whether they were Gram-negative or Gram-positive.

The compound **2a** gave the highest inhibition against *Pseudomonas*, the highest concentration was 21 mm and 14 mm for *L. monocytogenes*, 10 mm for *K. pneumoniae*, 8 mm for *E. coli* and *S. aureus* (Table 1).

Similarly, through the results depicted in Table 2, the compound 2b had a good inhibitory effect against the isolated bacteria, especially the Gram-negative ones, as it gave the highest inhibition against *P. aeruginosa*. At the highest concentration, the diameter of inhibition was 22 mm, 19 mm at the concentration 25 mg/mL and 10 mL. It also gave the highest inhibition against *L. monocytogenes* and *S. aureus*. The highest concentration was 12 mm, 13 mm for *K. pneumoniae* and 8 mm for *L. monocytogenes*. Thus, the importance of the compound is that it inhibits the growth of positive and negative bacteria on Gram stain. Its effectiveness is due to the fact that it is a compound of alkaloids, and alkaloids are known for their high toxicity.

Table 1. Inhibition zone diameters (mm) and MIC (mg/mL) of compound 2a.

Organisms	Method	Compound 2a				MIC (mg/mL)
		1 mg/mL	10 mg/mL	25 mg/mL	50 mg/mL	
Pseudomonas aeruginosa	Inhibition zone (mm)	0	6	13	21	4.21
Staphylococcus aureus		0	1	3	8	9.34
Listeria monocytogenes		0	4	9	14	6.82
Klebsiella pneumoniae		0	2	7	10	7.41
Escherichia coli		0	2	5	8	7.63

Table 2. Inhibition zone diameters (mm) and MIC (mg/mL) of compound 2b.

Organisms	Method	Compound 2b				MIC (mg/mL)
		1 mg/mL	10 mg/mL	25 mg/mL	50 mg/mL	
Pseudomonas aeruginosa	Inhibition zone (mm)	4	10	19	22	0.26
Staphylococcus aureus		3	5	9	12	0.53
Listeria monocytogenes		1	3	7	8	0.92
Klebsiella pneumoniae		1	10	7	13	0.96
Escherichia coli		0	2	5	6	9.52

As well as through the results presented in Table 3, the compound 2c gave the highest inhibition against *Pseudomonas* at the highest concentration. The inhibition diameter was 20 mm, 11 mm for *S. aureus*, 7 mm for *E.coli*, 10 mm for *K. pneumoniae* and 8 mm for *L. monocytogenes*.

Table 3. Inhibition zone diameters (mm) and MIC (mg/mL) of compound 2c.

Organisms	Method	Compound 2c				MIC (mg/mL)
		1 mg/mL	10 mg/mL	25 mg/mL	50 mg/mL	
Pseudomonas aeruginosa	Inhibition zone (mm)	3	8	12	20	0.24
Staphylococcus aureus		2	4	7	11	0.37
Listeria monocytogenes		0	2	5	8	7.44
Klebsiella pneumoniae		1	3	7	10	0.63
Escherichia coli		0	1	5	7	9.41

Also, through the recorded results in Table 4, the compound 2d produced the highest inhibition against *Pseudomonas*, in the highest concentration, as it had diameters of inhibition, 12 mm for *S. aureus*, 11 mm for *L. monocytogenes*, 10 mm and 8 mm for *E. coli* and *K. pneumoniae.*

Table 4. Inhibition zone diameters (mm) and MIC (mg/mL) of compound 2d.

Organisms	Method	Compound 2d				MIC (mg/mL)
		1 mg/mL	10 mg/mL	25 mg/mL	50 mg/mL	
Pseudomonas aeruginosa	Inhibition zone (mm)	1	7	10	12	0.29
Staphylococcus aureus		2	4	8	11	0.19
Listeria monocytogenes		1	3	6	10	0.37
Klebsiella pneumoniae		0	3	5	8	7.12
Escherichia coli		0	2	5	8	8.36

In general, the anti-bacterial activity of nicotine derivatives depend on the type of microorganism, the type of compound, and the amount of concentration used. The areas of inhibition for *E. coli* bacteria reached 8 mm for the first and fourth compound, 7 mm for the third compound, and 6 mm for the second compound. Consequently, it can be affirmed that *E. coli* bacteria are weakly sensitive towards the four compounds.

The areas of inhibition for *P. aeruginosa* reached 22 mm compound (2b), 21 mm compound (2a), 20 mm compound (2c), and 12 mm compound (2d). It has also been proven that the compounds are effective and their ability to biologically influence is due to the presence of alkaloids, which have a high activity against microbes. From it, we can say that *P. aeruginosa* is highly sensitive, as the inhibition zones on S. aureus reached 12 mm for the compound (2b) and to 11 mm for compound (2c) and compound (2d) and to 8 mm for compound (2a). It can be said that the bacteria *S. aureus* is moderately sensitive to compounds 2b, 2c and 2d, and weakly sensitive to compound 2a.

The areas of inhibition on *L. monocytogenes* reached 14 mm for compound (2a), 8 mm for compound (2b) and compound (2c), and 10 mm for compound (2d). Therefore, it can be said that the bacteria *L. monocytogenes* are moderately sensitive to compound (2a) and compound (2d), while compound (2b) and compound (2c) are weakly sensitive.

The areas of inhibition on *K. pneumoniae* reached 10 mm for compound (2a) and for compound (2c), for compound (2b) to 13 mm, and for compound (2d) to 8 mm. As a result, it can be said that the bacteria *K. pneumoniae* are moderately sensitive to compound (2a), compound (2b), and compound (2c), and weakly sensitive to compound (2d).

It has been shown that nicotine derivatives participate in a wide number of biological processes. It was determined to be desirable to attempt the synthesis of the compounds in question because of the significance of the aforementioned heteroyl nuclei, as well as the potential for incorporating a nicotine moiety into heterocyclic compounds. The compounds in question are as follows: the structure-activity connection of these compounds and an examination of their antibacterial activity are now being researched further. A number of different compounds were manufactured with the intention of determining whether or not they have anti-microbial activity.

The antimicrobial activity of the compounds ranges from fair to excellent; nevertheless, more research is required to draw any definitive conclusions on the compounds' medicinal potential (2a-2d). According to the findings, the majority of the compounds that were nicotine's of compounds were created and demonstrated antibacterial properties against gram-positive and Gram-negative bacteria. By determining the testing's zone of inhibition, the researchers were able to assess the effectiveness of the antibacterial investigation. In this case, the zone of inhibition, which was investigated using MIC values as well as many strains of bacteria, including *Escherichia coli, Pseudomonas aerogenes, Staphylococcus aureus, Listeria monocytogenes,* and *Klebsiella pneumoniae*. The effectiveness of the antibacterial treatment was determined by determining the zone of inhibition of the organism that was being tested. According to the results of the antibacterial investigations that were carried out, it was shown that the zone of inhibition expanded as the concentration of synthesized nicotine rose. A significant portion of pharmaceuticals and other compounds with important biological roles are heterocyclic in nature. In many cases, preference specificities in their biological reactions are impacted by the existence of hetero atoms or groups. Because of its potential use in medicine and agriculture, the chemistry and biology of studying heterocyclic compounds have been recognized as an attractive area for a significant amount of time. A diverse range of biological activity may be attributed to the presence of a variety of heterocyclic derivatives containing nitrogen and sulfur atoms. One of the most

important heterocycles in medicinal chemistry is pyridine. Pyridine has a wide range of applications, including activities that are antimicrobial, anti-inflammatory, anti-HIV, antiplasmodial, anti-tubercular, antibacterial, and anticonvulsant [29]. Additionally, pyridine has many other important biological significances. In the study of synthetic organic chemistry, the significance of heterocyclic molecules has been acknowledged for a significant amount of time. It is well knowledge that heterocyclic molecules containing nitrogen and sulfur display a broad spectrum of different types of biological activity. Researchers tested many different pyridine derivatives for their ability to inhibit tumor growth [30]. Nicotinamide has been proven to be useful in the treatment of papular and pustular acne, as well as an improvement in skin cancer [31]. In addition, nicotinamide has been demonstrated to help improve the condition of skin cancer. Nicotinamide, often known as nicotinic, has been put to use as a treatment for a variety of conditions, including schizophrenia and hypercholesterolemia [32]. Nicotinamide and its derivatives are also utilized to prevent type-1 diabetes in animal models and people since studies on both groups indicated that they have cytotoxic characteristics [32]. The vast number of possibilities and the widespread practical use of nicotine's substituted derivatives as a means of acquiring physiologically active drugs are responsible for the growing interest in the chemistry of nicotine and its related compounds. The investigation of whether or not derivatives of nicotine (2a-2d) exhibit antibacterial action is of interest. An investigation into the antibacterial activity of these compounds in vitro has been attempted.

4. CONCLUSION

In this work, our attention focused on the preparation and biological activity of some nicotine derivatives in the context of its evaluation. Initially, four new nicotine derivatives were prepared from nicotine-N-oxide. The new compounds were diagnosed using physics and spectroscopic tools, FT-IR, ^{1}H-NMR and ^{13}-C NMR, and some of their physical properties were measured. The prepared compounds showed biological activity against *P. aeruginosa*. The first and second compounds are less sensitive towards *S. aureus* bacteria, and the third compound is weak towards *S. aureus*, and also the three compounds are not effective against *E. coli* bacteria. Accordingly, it can be said that all compounds can play a role in the inhibitory ability as anti-bacterial *P. aeruginosa*. Therefore, this class of compounds could be a good starting point to develop new compounds for handling this pathogenic bacterial. In addition, the broad spectrum anti-microbial action of nicotine derivatives developed in the present study may find immense applications in formulating new disinfection or decontamination strategies against widely spreading pathogens of clinical significance.

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