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Synthesis, spectroscopicand antimicrobial activityof some new 7-methyl-2-phenylimidazo[1,2-a]pyridin-3-amine derivatives

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Abstract
The new series of Schiff base were synthesized from 7-methyl-2-phenylimidazo[1,2-a]pyridin-3-amine condensed with different substituted aldehydes (4-Hydroxybenzaldehyde, Vanillin, 3-Nitrobenzaldyde, 4-Nitrobenzaldehyde)in presence of catalytic quantities of acetic acid. So the structure offers for this synthesis well coincide with the results obtained bydifferent spectroscopic techniques (IR, ^{1}H NMR, ^{13}C NMR and Mass spectroscopy). These new compounds were evaluated for their antibacterial, againstGram positive and Gram negative bacteria, and antifungal activityagainst yeasts and moulds, using micro-dilution tests. The biological activityof the synthesized compoundswere compared with standard drugs. The best results are observed in the amine starting (new 7-methyl-2-phenylimidazo [1,2-a]pyridin-3-amine), it showed interesting antibacterial activity of all tested strains, particularly against Proteus strain is a Gram -.

*Keywords:*7-methylimidazo[1,2-a]pyridine; Schiff bases; antifungal activity; antibacterial activity.

Introduction
Imidazo[1,2-a]pyridines derivatives are considered among the most importantmolecules activate in postponing discipline of the pharmaceutical industry [1]. We find them in certain medicinal formulations (Figure 1) that are available at present on the market such as Alpidem (anxiolytics) [2] andzolpidem (hypnotic) [3] .These molecules present several activities such as antiviral [4-5], anti-inflammatory [6], analgesic, antipyretic, antiulcer and antibacterial [7]. They are also β-amyloid formation inhibitors, benzodiazepine receptor agonists [4] and cardiotonic agents [8].
Given to the interests of the imidazo[1, 2-a]pyridine we were interested in the synthesis, in one step and good yield using acetic acid as a catalyst, of new series of compounds namedSchiff base, which they are known for their important therapeutic properties [9-16]. The latest antibacterial and antifungal studies of excesses of imidazo [1,2-a] pyridine showed low activity [17, 18] in this work we are interested in evaluating this activity to different bacterial strains, by these molecules have significant corrosion activity [19, 20].

2. Material and methods
2.1. Reagents and chemical products preparation
All reagents were commercially available and used without any purification. Melting points were recorded on Digital Melting Point Apparatus WRS-1B and are uncorrected. IR spectra were recorded on a Bruker-EQUINOX55 spectrometer. Mass spectra (EI, 70 ev) were measured with SHIMADZU GCMS-QP2010 Plus. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Brucker AC 300 instrument using $CDCl_3$or DMSO as the solvent at room temperature. The coupling constant J is given in Hz. All reactions were conducted using standard Schlenk techniques. Column chromatography was performed using the EM Silica gel 60 (300-400 mesh).

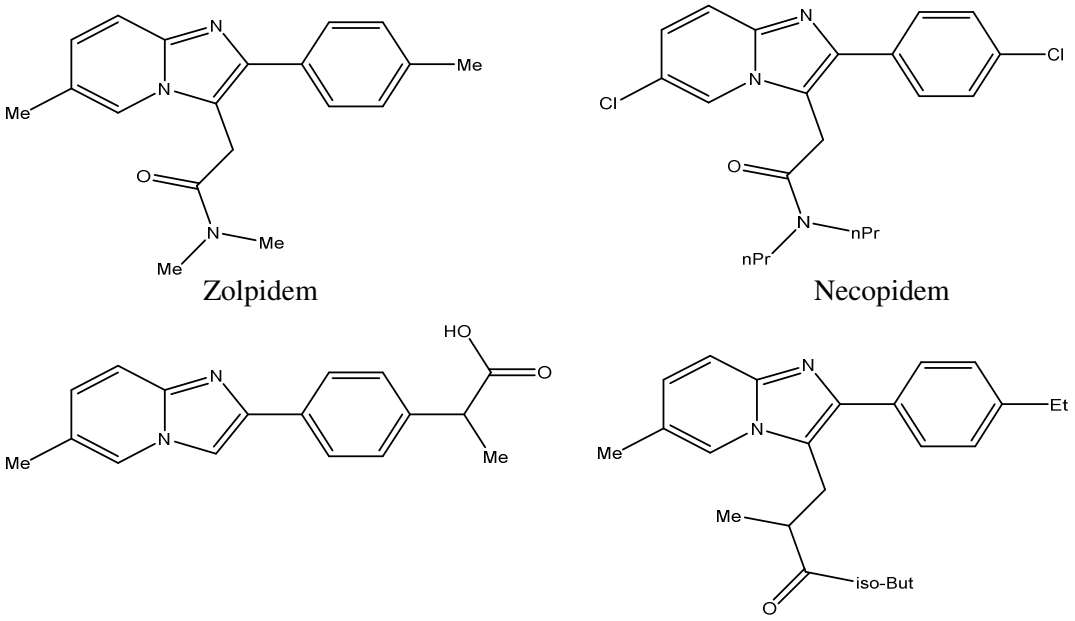


Figure1: Imidazo[1,2-a]pyridine-based drugs.

2.2. Antimicrobial activity measurement:

The strains used in antimicrobial activity measurement of the compounds studied were *Staphylococcus aureus*, *Listeria monocytogenes, Lactobacillus plantarum* for Gram positive bacteria and *Escherichia coli* and *Proteus sp* for Gram negative bacteria, and *Aspergillusniger* and *Candida pelliculosa*for yeasts and moulds.

The evaluation of the antimicrobial activity of different extracts were previously carried out by the disk diffusion method on Muller-Hinton agar (MHA) [21] to identify the activate extracts on which future studies will be conducted. The test solutions were prepared at concentration of 10 mg/ml with DMSO. Microbial suspensions in exponential growth phase (0.5 on the McFarland scale, about 1.5 10^6 cells/ml) were pouring plated on sterile MHA medium. Discs impregnated with 20 µl (10 mg/ml) of the product, 20 µL of DMSO for negative control and the reference antibiotic disc. In this case the gentamycin (30 µg / disc) have has been used has have positive control for the bacteria and Cycloheximide (25 µg / disc) was used for fungi. These controls were deposited on the MHA previously inoculated agar. After a pre-diffusion of 30 min at room temperature, the plates were incubated for 18-24 hours at 37 °C. All the essays were made in triplicate. The diameters of the microbial growth inhibition zone were measured, and the average and the standard deviations were calculated. The compounds showing inhibition zone higher than or equal to 12 mm (including disk 6 mm) [22], were selected for the determination of IC_{50}.

The method used is based on liquid culture medium in tubes. The compound stock solution, which was prepared using sterile distilled water with 20% DMSO as an emulsifier was sterilized by filtration through millipore membrane 0.45um. From this stock solution, serial dilutions of compound were prepared in liquid culture medium, sterilized in advance at 121°C/15 minutes. These serial dilutions were then inoculated with overnight cultures (aged 18 hours) previously prepared on the same culture medium used for measuring the IC50. The final volume of the tube was 4 ml culture. The culture media and the incubation conditions used in this section are the same as those used in the screening test.The biomass was evaluated by reading the OD at 625 nm of each solution (each tube) using white previously stored at 4 ° C. Plotting the OD curve as a function of the concentration of the test product permittedto determinethe concentration allowing 50% of inhibition.

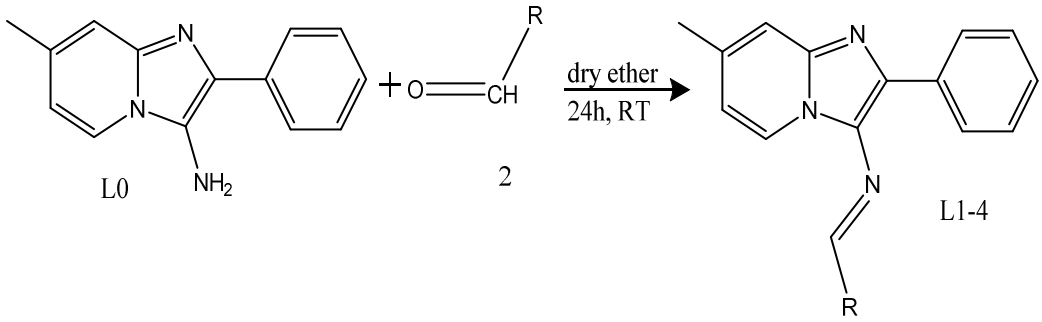
3. Results and discussion

3.1. General procedure for the preparation of 7-Methyl-2-phenylimidazo [1, 2-a] pyridin-3-amine:

A mixture of 4-methylpyridin-2-amine (1 mmol) and 2-Bromoacetophenone (1.2mmol) at 60°C for 30min, the cooled to room temperature and the residue was treated by $NaHCO_3$, extraction with CH_2Cl_2, The final purification was performed by re-crystallization from hot acetone to give white powder [18].

To the solution of 7-methyl-2-phenylimidazo[1, 2-a] pyridine (1.0 mmol) in acetic acid (25 ml), sodium nitrite (1.5 mmol) was added at room temperature under stirring for 5h, the residue solid was treated with water, filtered, and crystallized from acetonitrile.
The last step is a reduction Nitroso group. So a solution containing HBr cold -10 ° C is added in small fractions of tin (2 eq) allowing stirred for 10 min, after we added the product nitroso (7-methyl-3-nitroso-2-phenylimidazo[1, 2-a]pyridine (1 eq)) by small fraction, allowed the reaction was stirred for 2 h at -10°C, after 24 hours at room temperature. The resulting solution was filtered and treated with Na_2CO_3, extraction with CH_2Cl_2 crystallized from acetonitrile to give pure product (Yield 50%).

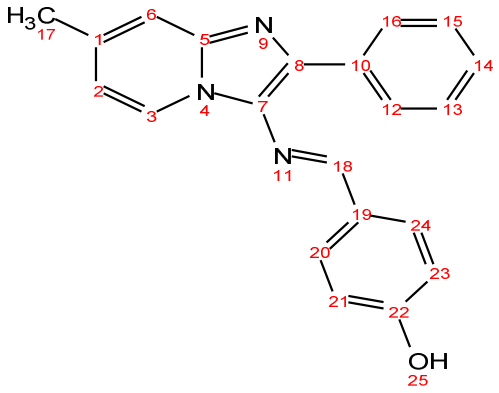
3.2. General procedure for the preparation of Schiff base heterocyles of Imidazo [1,2-a] Pyridine nucleus:
7-methyl-2-phenylimidazo[1,2-a]pyridin-3-amine (2.39 mmol) dissolved in 20 ml of dry diethyl ether and to this two drops of acetic acid as catalyst (0.3 ml) solution were added and stirred for 15-20 minutes at room temperature. To this substituted of aldehyde (2.39 mmol) was added. Stirred the above reaction mixture for 24 hours at room temperature. The reaction was monitored by TLC. The formed product was filtrated and washed with dry ether. The final purification was performed by recrystallization from hot methanol to give powder.



R= 4-hydroxybenzaldehyd **(L1)**
R= 4-hydroxy-3-methoxybenzaldehyde **(L2)**
R= 4-nitrobenzaldehyde **(L3)**
R= 3-nitrobenzaldehyde **(L4)**

Scheme 1: Synthesis of Schiff bases from 7-methyl-2-phenylimidazo [1,2-a] pyridin-3-amine.

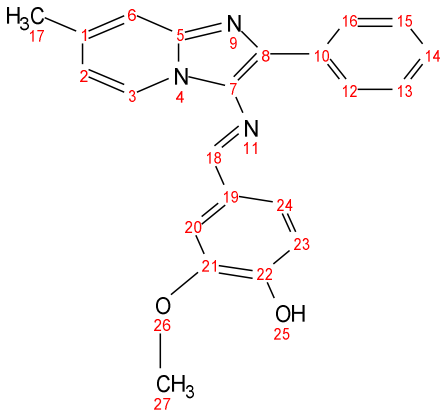
3.3. Spectral data of representative compound: (E)-4-(((7-methyl-2-phenylimidazo[1,2-a]pyridin-3-yl)imino)methyl)phenol (L1):
Green powder. Yield 92.15%. Mp> 270°C. Rf = 0.50 (silica, CH_2Cl_2/CH_3OH: 9/1). ^{1}HNMR (300 MHz, DMSO, δ ppm): 8.659 (s, 1H, HC_{18}=N); 8.322 (d, 1H, C_3H, J= 6.99Hz); 7.842 (d, 2H, $C_{12}H$ $C_{16}H$, J=7.29Hz); 7.654 (d, 2H, $C_{15}H$ + $C_{13}H$, J=22,2Hz); 7.35 (q, 4H, C_6H + $C_{14}H$, $C_{24}H$ + $C_{20}H$); 6.75 (d, 3H, C_2H + $C_{23}H$ + $C_{21}H$, J = 6.09Hz); 2.41 (s, 3H, $C_{17}H$). ^{13}C NMR (75 MHz, DMSO, δ ppm): 171.94; 160.87; 158.06; 142.28; 135.41; 134.78; 132.47; 130.38; 128.55; 128.48; 127.54; 127.26; 123.04; 115.80; 115.22; 115.02; 20.76. m/z (M+): 328.00. IR: ν (CH=N, imine) = 1655cm^{-1}; ν(OH)=3450cm^{-1}.



(E)-4-(((7-methyl-2-phenylimidazo[1,2-a]pyridin-3-yl)imino)methyl)phenol (L1)

3.4. Spectral data of representative compound:(E)-2-methoxy-4-(((7-methyl-2-phenylimidazo[1,2-a]pyridin-3-yl)imino)methyl)phenol (L2):

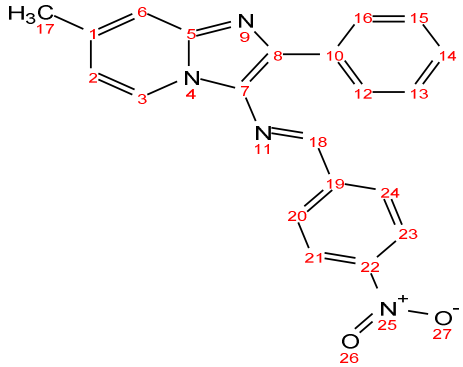
Green powder. Yield 90.25%. Mp: 213-215°C. Rf = 0.51 (silica, CH_2Cl_2/CH_3OH: 9/1). ^{1}HNMR (300 MHz, $CDCl_3$, δ ppm): 9.742 (s, 1H, $O_{25}H$); 8.653 (s, 1H, $C_{18}H$=N); 8.363 (d, 1H, C_3H, J= 6.99Hz); 7.846 (d, 2H, $C_{12}H$ + $C_{16}H$, J = 7.44 Hz); 7.541 (s, 1H, C_6H); 7.25 (m, 5H, $C_{13}H$ + $C_{14}H$ + $C_{15}H$ + $C_{23}H$ +$C_{24}H$); 6.735(d, 2H, C_2H + $C_{20}H$, J = 6.96 Hz); 3.813 (s, 3H, $C_{27}H$); 2.433 (s, 3H, $C_{17}H$). ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 157.78; 150.48; 148.13; 142.36; 135.46; 134.76; 132.75; 130.38; 128.46; 128.40; 128.06; 127.57;127.28; 123.81; 123.23;115.46; 115.25; 115.00; 110.11; 55.57; 20.77 m/z (M+): 358.1. IR: ν (CH=N, imine) = 1605cm^{-1}.



(E)-2-methoxy-4-(((7-methyl-2-phenylimidazo [1, 2-a] pyridin-3-yl)imino) methyl)phenol (L2)

3.5. Spectral data of representative compound: (E)-N-(7-methyl-2-phenylimidazo[1,2-a]pyridin-3-yl)-1-(4-nitrophenyl)methanimine (L3):

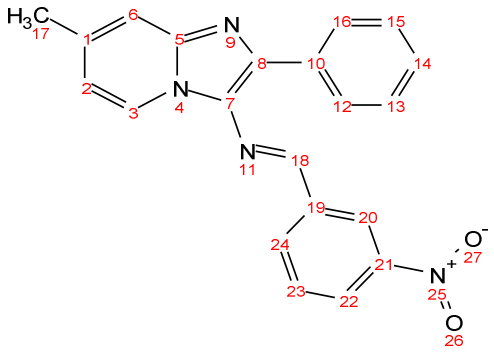
Red powder. Yield 96.28%. Mp: 208-210°C. Rf = 0.55 (silica, CH_2Cl_2/CH_3OH: 9/1). ^{1}HNMR (300 MHz, $CDCl_3$, δ ppm): 8.954 (s, 1H, $C_{18}H$=N); 8.670 (d, 1H, C_3H, J = 7.02Hz); 8.262 (d, 2H, $C_{21}H$ + $C_{23}H$, J = 8.67Hz); 8.03 (d, 4H, $C_{20}H$+ $C_{24}H$ + $C_{12}H$ + $C_{16}H$, J = 8.73Hz); 7.41 (t, 4H, $C_{13}H$+$C_{14}H$+ $C_{15}H$+C_6H, J = 7.68Hz); 6.88 (d, 1H, C_2H, J = 7.05Hz); 2.3 (s, 3H, $C_{17}H$). ^{13}C NMR(75 MHz, $CDCl_3$, δ ppm): 150.12; 148.10; 144.00. 142.24; 138.02; 137.52; 134.24; 128.71; 128.58; 128.33; 128.10; 126.57; 124.47; 124.03; 116.03; 115.62; 20.77. m/z (M+): 357. IR: ν(CH=N, imine) = 1655cm^{-1}.



(E)-N-(7-methyl-2-phenylimidazo[1, 2-a]pyridin-3-yl)-1-(4-nitrophenyl)methanimine (L3)

3.6. Spectral data of representative compound: (E)-N-(7-methyl-2-phenylimidazo[1,2-a]pyridin-3-yl)-1-(3-nitrophenyl)methanimine (L4):

Red powder. Yield 95.15%. Mp= 178-180°C,Rf = 0.45 (silica, CH_2Cl_2/CH_3OH, 9/1). ^{1}HNMR (300 MHz, DMSO, δ ppm): 9.024 (s, 1H, $C_{18}H$); 8.670 (d, 2H, C_3H + $C_{20}H$, J = 6Hz); 8.291 (t, 2H, $C_{22}H$ + $C_{24}H$, J = 15Hz); 8.00 (d, 2H, $C_{12}H$ + $C_{16}H$, J = 8.4Hz); 7.76 (t, 1H, $C_{23}H$, J= 15.9Hz); 7.43 (q, 4H, C_6H + $C_{13}H$ + $C_{14}H$ + $C_{15}H$, J = 29.7Hz); 6.9 (d, 1H, C_2H, J = 8.7Hz); 2.393 (s, 3H, $C_{17}H$). ^{13}C NMR (75 MHz, DMSO, δ ppm): 151.71; 148.76; 144.16; 138.99; 137.74; 137.58; 134.70; 134.02; 130.93; 129.03; 128.71; 128.45; 126.88; 125.37;124.86; 122.76; 116.37; 116.02; 21.25. m/z (M+): 357.15. IR ν(CH=N, imine) = 1655cm^{-1}



(E)-N-(7-methyl-2-phenylimidazo[1,2-a]pyridin-3-yl)-1-(3-nitrophenyl)methanimine (L4)

3.7. Antimicrobial activity measurement

3.7.1. Screening test

The results of the inhibition zones of compounds against microbial strains obtained are reported on table 1. From these results we see that the L0 molecule has important activity against all the strains tested (*Listeriamonocytogenes*, *Proteussp*, *Staphylococcusaureus*, *Escherichiacoli*, *Lactobacillusplantarum*, *Aspergillusniger* and *Candidapelliculosa*. It should be emphasized that the result obtained with L0 was similar the inhibition zone obtained with Gentamycin (control) against Proteus. This finding indicates the possible use of this molecule (L0) in the medicinal field against the disease caused by this bacterium. Excepting the compound L2, showing an inhibition zone against *Candidapelliculosa*, all the compoundsstudied (L1, L2, L3 and L4) didn't show any inhibitory effect against the strains tested.

Table 1: Screening Test of antimicrobial activity of the compounds (Legend: the values correspond to diameter (mm) of the halo inhibition, 6: no inhibition halo).

	Averages of the diameter of the zones of inhibition (diameter of the record(disk) included 6 mm) in mm						
	Gentamicine	Cycloheximide	L0	L1	L2	L3	L4
Listeria monocytogenes	26 ± 0.01	-	16 ± 0.01	6 ± 0.01	6 ± 0.01	6 ± 0.01	6 ± 0.01
Proteus	22 ± 0.01	-	22 ± 0.01	6 ± 0.01	6 ± 0.01	6 ± 0.01	6 ± 0.01
Staphylococcus aureus	25 ± 0.01	-	20 ± 0.01	6 ± 0.01	6 ± 0.01	6 ± 0.01	6 ±0,01
Escherichia coli	25 ± 0.01	-	18 ± 0.01	6 ± 0.01	6 ± 0.01	6 ± 0.01	6 ± 0.01
Lactobacillus plantarum	21 ± 0.01	-	15 ± 0.01	6 ± 0.01	6 ± 0.01	6 ± 0.01	6 ± 0.01
Aspergillusniger	-	22 ± 0.01	15 ± 0.01	6 ± 0.01	6 ± 0.01	6 ± 0.01	6 ± 0.01
Candida pelliculosa	-	20 ± 0.01	11 ± 0.01	6 ± 0.01	13 ±0.01	6 ± 0.01	6 ± 0.01

3.8. Measurement of IC_{50}:

The measurement of $IC_{50}$was made for the L0 and L2 compounds showing significant antibacterial and antifungalactivities. The results obtained are reported in Table 2. The L0 compound showed lower IC_{50}against *Proteussp*more than those found with the other strains (bacteria, yeasts and moulds). The second compound L2 showed lower IC_{50} than L0 against *Candida pelliculosa.*

Table 2: IC_{50} measurement in µg/ml of tested chemical compounds

	IC $_{50}$ ug/ml	
	L0	L2
Listeria monocytogenes	2.50	-
Proteus	1.75	-
Staphylococcus aureus	2.48	-
Escherichia coli	2.80	-
Lactobacillus plantarum	2.20	-
Aspergillusniger	2.37	-
Candida pelliculosa	2.38	1.00

Conclusion

The structures of synthesized compounds were confirmed by IR, ^{1}HNMR, ^{13}C NMRand Masse spectroscopy. The study of antibacterial and antifungal activities of different products showed the high inhibitory effect of the compound L0, particularly against *Proteus sp*. This finding was confirmed by the IC_{50}values obtained. Hence the lowest values were obtained with L0 against *Proteus sp* and L2 against *Candidapelliculosa*, indicating the possible use of these compounds in medical and/or food technology domains.

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