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Synthesis, Characterization and Evaluation of Biological Activity of Pyridine Derivatives

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ABSTRACT

Here we report a new rapid method for the synthesis of novel pyridine derivatives. Synthesized compounds were characterized by FT-IR, ^{1}H NMR and ^{13}C NMR spectral analysis and evaluated for their antimicrobial activities and DNA binding ability. One of the derivatives has considerable DNA binding ability. Some of the compounds exhibit moderate to good activities against the tested the Gram-positive, Gram-negative bacterial strains as well as fungal strains.

Keywords: Pyridine, spectral characterization, DNA binding ability.

INTRODUCTION

Heterocyclic compounds constitute an important class of organic compounds. These compounds find applications in medicine for countless number of diseases. The medicinal drugs include anti-bacterial, antifungal, antioxidants, anticancer, anti-inflammatory etc[1].

Pyridine is a nitrogen heterocyclic organic compound that plays a key role catalyzing both biological and chemical systems. In the pharmaceutical industry, over 7000 existing drugs contain pyridine nucleus. Pyridine ring system is prevalent in nature, especially important alkaloids in some plants contain saturated pyridine nucleus[2]. Pyridine and its derivatives have large and wide biological activities, which include anticancer[3], antimicrobial[4], anti-inflammatory[5], anti-diabetic[6], anti-oxidant[7], enzyme inhibition[8], anti-malarial[9] and anti-amoebic[10].

Pyridine N-oxides are very useful synthetic reagent for the formation of various substituted pyridines and their derivatives[11]. The 3-hydroxypyridine ring system can be found in many alkaloids having biological activity of interest, for example the antibacterial agent pyridomycin[12].

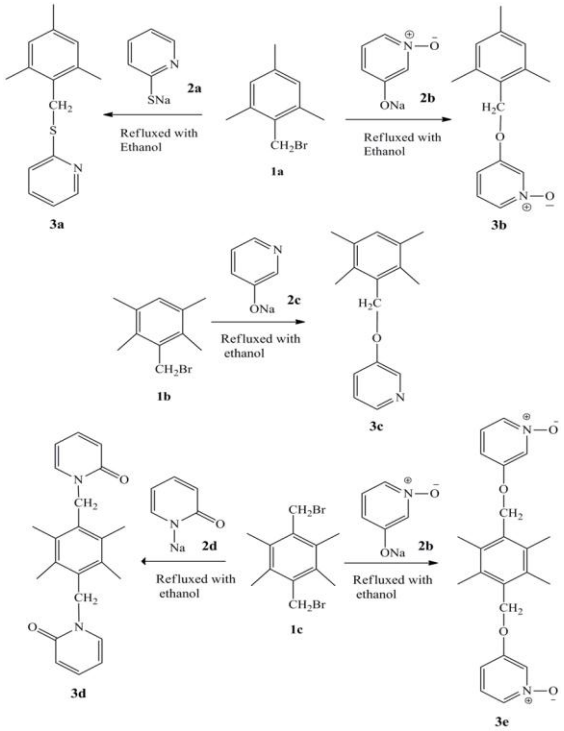
The purpose of the present study is to find new pyridine derivatives with biological activity. This work deals about the synthesis, characterization and evaluation of antimicrobial activity and DNA binding ability of pyridine derivatives.

2. EXPERIMENTAL

2.1 Chemicals and Reagents

Chemicals used for the synthesis were of AR grade of Merck, S.D. Fine and Aldrich. The melting points were measured in open capillary tube and are uncorrected. FT -IR spectra were recorded on a Jasco FT-IR-4600 Spectrophotometer (KBr, v_{max} in cm^{-1}).^{1}HNMR and ^{13}C NMR spectra were recorded on a Bruker AVV 300 instrument (300 MHz) using TMS as internal standard and $CDCl_3$-d3 as solvent. Chemical Shifts are given in parts per million (ppm). The reactions were monitored by TLC and visualized the spot in iodine chamber. The binding of CT DNA with the compound was studied using the UV absorption spectral method.

2.2 Synthesis of pyridine derivatives



Scheme 1. Synthesis of novel pyridine derivatives

Compounds such as mesitylene and durene as starting material were used for the synthesis of Mono(bromomethyl)mesitylene, Mono(bromomethyl)durene and 1, 4-Bis (bromomethyl)durene [**1a-1c**] following available literature procedure[13]. Pyridine compounds were converted to the corresponding sodium salt **(2a-2d)** using sodium ethanol. These on subsequent reaction with **1a-1c** gave the products **3a-3e** (Scheme 1). The structural identities were confirmed on the basis of spectral data.

3. SPECTRAL ANALYSIS OF SYNTHESIZED COMPOUNDS

The synthesis of the novel pyridine derivatives **(3a-3e)** are described in the reaction schemes 1. The identities of the newly synthesized compounds have been established on the basis of their FT-IR, ^{1}H NMR and ^{13}C NMR spectral data.

3.1 FT-IR

In compounds **3a-e** aromatic stretch appears in the region 2915-3059 cm^{-1} Aromatic bent is seen around 1572-1668 cm^{-1}, C=C stretch appear at 1474-1479 cm^{-1}. Alkyl stretch and alkyl bend appear around 2779-2964 cm^{-1} and 1380-1398 cm^{-1} respectively. The N-O stretching appears in the compound (**3b and 3e**) around 1566-1567 cm^{-1}. The carbonyl stretching appears in **3d** at 1653 cm^{-1}.

3.2 ^{1}H NMR and ^{13}C NMR

2-(2,4,6-Trimethylbenzylsulfanyl)pyridine (3a)
Colour: White. ^{1}H NMR ($CDCl_3$): **δ ppm**: Ar-H and Py-H 8.75-6.86 (m,6H), S-CH_2-Ar 4.85-4.78 (m,2H) and -CH_3 2.44-2.04 (m,9H). ^{13}C NMR (300 MHz $CDCl_3$): **δ ppm:** $\underline{C}H_3$-19.55 and 20.78, S-$\underline{C}H_2$-32.89, Ar carbon- 125.73, 129.14, 138.11 and 141.93, Py carbon-120.72, 124.48, 137.6, 143.42 and 158.55.

3-((2,4,6Ttrimethylbenzyl)oxy)pyridine N-oxide (3b)
Colour: White. ^{1}H NMR ($CDCl_3$): **δ ppm**: Ar-H and Py-H 8.25-6.74 (m,6H),O-CH_2-5.02 (s,2H) and CH_3- 2.25-1.88 (m,4H).

3-(2,3,5,6-Tetramethylbenzyloxy)pyridine (3c)
Colour: White. ^{1}H NMR ($CDCl_3$): **δ ppm**: Ar-H and Py-H 8.52-6.89 (m,5H), O-CH_2- 5.40-5.12 (m,2H) and CH_3-2.33-2.14 (m,12H). ^{13}C NMR (300 MHz $CDCl_3$): **δ ppm**: -$\underline{C}H_3$-15.34 and 20.20, O-$\underline{C}H_2$-65.59, Ar carbon-126.70, 131.31, 134.35 and 134.95, Py carbon-121.10, 123.38, 137.65, 141.93 and 155.19.

1,1'-((2,3,5,6-Tetramethyl-1,4-phenylene)bis(methylene))bis(pyridin-2(1H)-one) (3d)
Colour: White. M.Pt^{o}C: 220.5. ^{1}H NMR ($CDCl_3$): **δ ppm**: Py-H 7.41-6.01 (m,8H), N-CH_2-5.27-5.15 (m,4H) and -CH_3 2.32-2.12 (m,12H). ^{13}C NMR (300 MHz $CDCl_3$): **δ ppm**: $\underline{C}H_3$-16.30, N-$\underline{C}H_2$-46.42, Ar carbon-131.58 and 138.96, Py carbon-105.98, 120.4, 134.19, 135.46 and 162.8.

3,3'-((2,3,5,6-Tetramethyl-1,4-phenylene)bis(methyleneoxy))bis(pyridine N-oxide) (3e)

Colour; White. M.Pt^{o}C: 105.6. ^{1}H NMR ($CDCl_3$): **δ ppm:** Py-H 8.09-7.21(m,8H), O-CH_2-5.19-5.09 (m,4H) and -CH_3 2.38-1.99 (m,12H). ^{13}C NMR (300 MHz $CDCl_3$): **δ ppm**: -$\underline{C}H_3$-15.55, O-$\underline{C}H_2$-65.66,Ar carbon- 132.09 and 134.50, Py carbon-105.30, 124.71, 139.07, 141.01 and 156.58.

4. RESULT AND DISCUSSION

The newly synthesized compounds (**3a-3e**) were characterized on the basis of FT-IR, ^{1}H NMR and ^{13}C NMR spectral data.

The absence of S-H stretching (2500-2640 cm^{-1}) in 3a and the absence of O-H stretching (3600-3200 cm^{-1}) in **3b,3c** and **3e** indicate the formation of new compounds. In **3d** the presence of C=O stretching suggest the keto group.

In ^{1}H NMR spectra of the compounds (**3a-3e**) there are four signals corresponding to the protons of Aromatic ring, pyridine ring, methylene and methyl group. Aromatic and pyridine ring protons are merged and overlapped and appear as multiplet in the region of 8.75-6.89 ppm[14]. The methyl protons are observed as multiplet in the range of 2.38-1.99 ppm.

3a is formed by the reaction between 2-mercapto pyridine and monobromo methyl mesitylene (**2a**). Hence S-CH_2 bond is formed in **3a** and has its proton signal appear in the region of 4.85-4.78 ppm. In **3b** O-CH_2 signal appears as a singlet in 5.02 ppm[15]. In **3c** and **3e** O-CH_2 protons observed as a multiplet in the range of 5.40-5.09 ppm. **3d** exist in keto form as it is prepared from 2-hydroxy pyridine which is predominately present in the keto form rather than the enolic form of the tautomer. Hence bonding occurs at pyridine N and N-CH_2 bond is formed. The N-CH_2 protons (**3d**) are appearing in the range of 5.27-5.15 ppm[16].

^{13}C NMR of the compound **(3a-3e)** show chemical shift between 15-162 ppm. The signals of pyridine and aromatic carbon appear from 105-162 ppm. The carbon of methyl has signals from 15-20 ppm. The signals of S-CH_2, N-CH_2 and O-CH_2 carbons appear in 32, 46 and 66 ppm respectively.

4.1 Evaluation of antimicrobial screening

All the synthesized compounds of series **3a-3e** were screened against some microorganisms to evaluate their antimicrobial activities. The agar well diffusion method was used for antibacterial assay against the gram-positive bacteria *S.aureus* and the gram-negative becteria, *E.coli* and *P.aeruginosa*. For antifungal screening disc diffusion method was used against fungi *Candida albicans* and *Aspergillus niger*. Amikacin was used as standard drug for antibacterial screening and nystatin and flucanazole was used as the standard drug for antifungal screening. The compound **3d** and **3e** shows mild activity towards all the bacteria. In **3c** and **3e** shows good activity towards the fungus species *Candida albicans.*

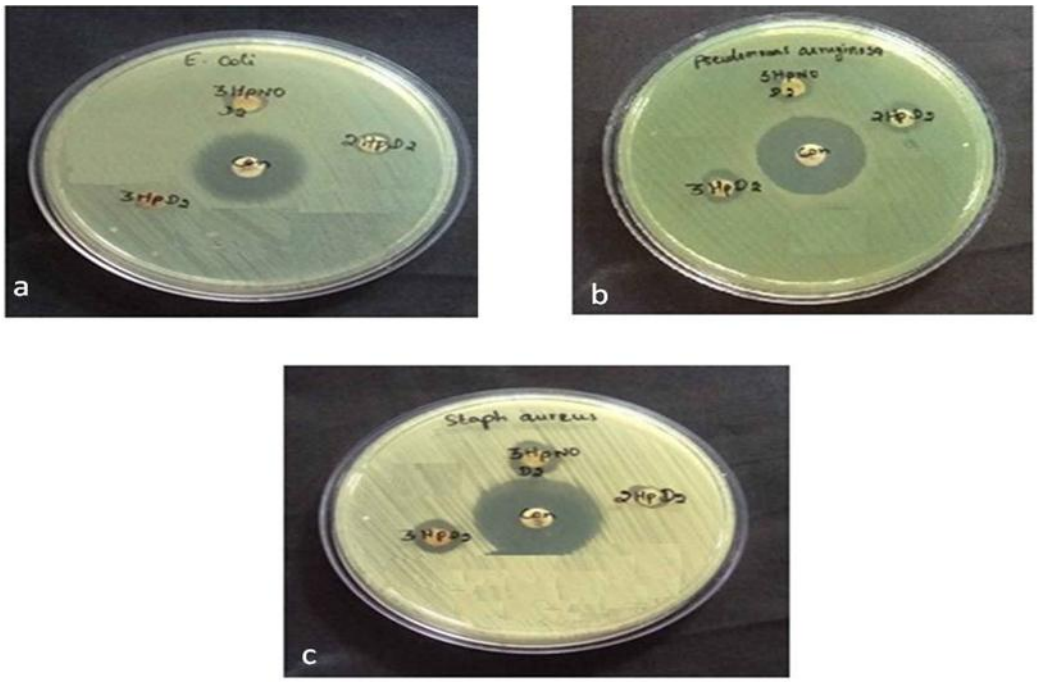


Figure 1. Antibacterial assay: Zone of inhibition against a) *E.coil*, b) *P.aeruginosa* and c) *S.aureus*

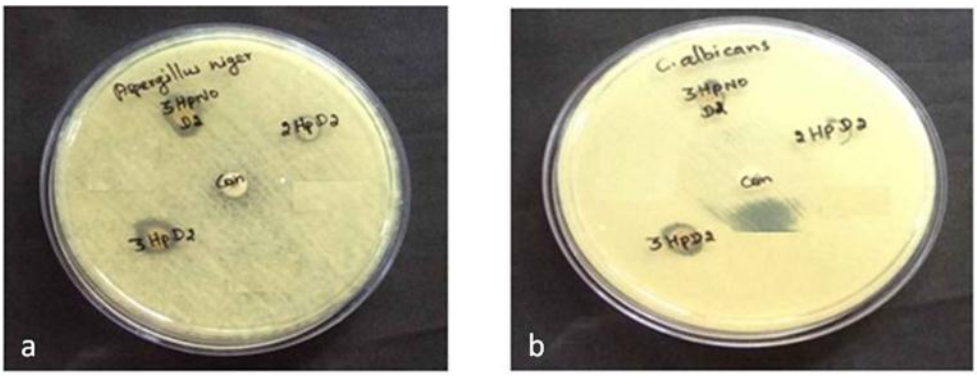


Figure 2. Anti-fungi assay: Zone of inhibition against a) *A.niger* and b) *C.albicans*

4.2 DNA binding study

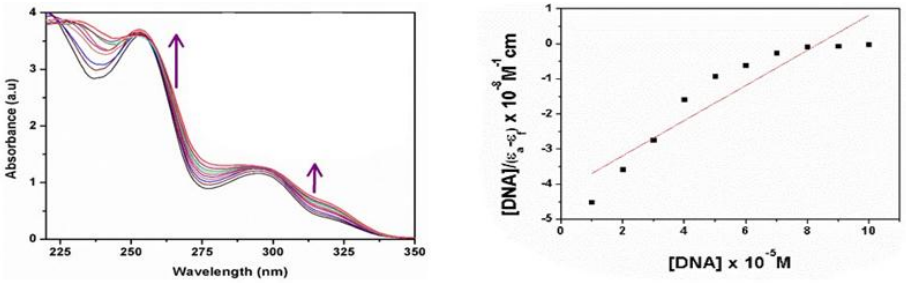
Binding strength of the compounds with DNA is ascertained by examining the changes in the absorbance of Compound – DNA solutions. Quantification of DNA binding strength is made from intrinsic association constant *K*b values. The intrinsic association constant, Kb was determined by the following equation.

$[DNA]/(\varepsilon_a-\varepsilon_f) = [DNA]/(\varepsilon_b-\varepsilon_f)+1/K_b(\varepsilon_b-\varepsilon_f)$

where [DNA] is the concentration of DNA in base pairs, ε_a,ε_f and ε_b are the extinction coefficient of the apparent, free and bound compound respectively, K_b is given by the ratio of the slope to the intercept of the plot of [DNA]/[ε_a-ε_f] vs. [DNA][17,18].

In the UV absorption band there is a significant ''hyperchromic'' effect in the intraligand transition bands near 250-275 nm with moderate red shift of 2–3 nm for **3b, 3d,** and **3e**. The K_b values for the compounds **3b, 3d** and **3e** are found to be 1.20 x 10^4 M^{-1}, 4.17 x 10^3M^{-1} and 5.40 x 10^4M^{-1}, respectively.

Among the synthesized compound **3e** serves as a better DNA binding agent with high binding constant which suggests that the interaction of the complex with DNA is strong but lower than the classical intercalators[19].



a b

Figure 3. a) Absorption spectra of 3b b) Plot of [DNA]/(ε_a-ε_f) vs [DNA]

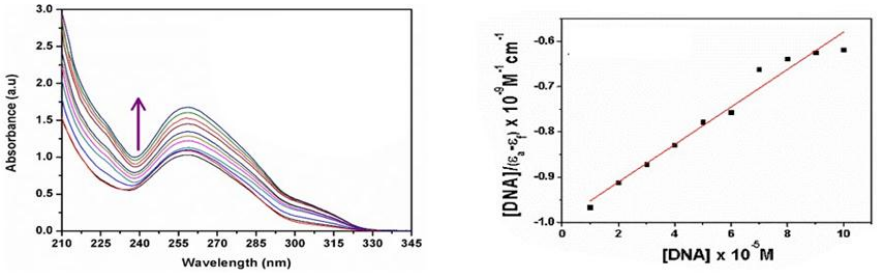


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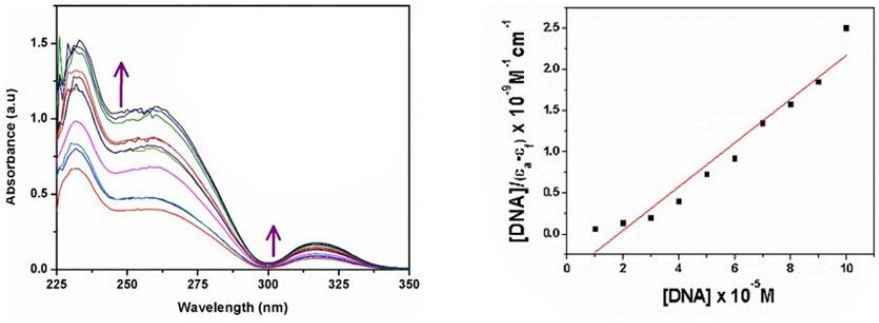
Figure 4. a) Absorption spectra of 3d b) Plot of [DNA]/(ε_a-ε_f) vs [DNA]



a b

Figure 5. a) Absorption spectra of 3e b) Plot of [DNA]/(ε_a-ε_f) vs [DNA]

5. CONCLUSION

In conclusion, this research has developed a convenient strategy for the synthesis of novel biologically active pyridine derivatives. Synthesized compounds were characterized by FT-IR, ^{1}H NMR and ^{13}C NMR spectral technique. The antimicrobial study revealed that compounds **3d** and **3e** shows mild activity towards all the bacteria and **3c** and **3e** shows good activity against pathogenic fungal strain *Candida albicans*. DNA binding ability for **3e** is moderately higher which is revealed from K_b value. Syntheses of new pharmaceutically/ biologically important pyridine derivatives are under progress in our research group.

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