# SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF PICOLINE DERIVATIVES

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*SUMMARY: Six different phenacyl halide derivative of β-picoline were synthesized and studied for their antibacterial activity against twenty four gram negative and twelve gram positive microorganisms. Compound I, III and IV showed almost broad spectrum activity, whereas rest of three compounds did not exhibit so promising results. Spectroscopic techniques, such as 1H-NMR, EIMS, UV and IR spectroscopy were utilized for their structure elucidation.*

*Key Words : ß-picoline, phenacyl halides, antibacterial.*

## INTRODUCTION

In order to assess the biological properties of some quaternary ammonium salts of heterocyclic bases, Hartwell *et al.* (4) prepared three different series of ammonium salts by adding the side chain of phenacyl, p-methoxyphenacyl and β-phenethyl halides to a number of heterocyclic bases of these compounds showed promising results in the course of studies in the chemotherapy of cancer. Quaternary ammonium salts (5) of dimethylpyridine were evaluated for antimicrobial activity against *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumonia, Bacillus subtilis, B. megaterium* and *Staphylococcus aureus.* Furan derivatives of pyridine and picoline have also demonstrated antibacterial properties (3). p-Fluorophenacyl bromide salts of γ-picoline was reported as anticancer agent by Bahner *et al.* (1) and antitubercular studies (6) in derivatives of 1-(4-nitrophenacyl)-4-alkylpyridinium bromides prompted us to undertake the synthesis and antibacterial activity of six derivatives of substituted 1-phenacyl-β-picolinium bromide.

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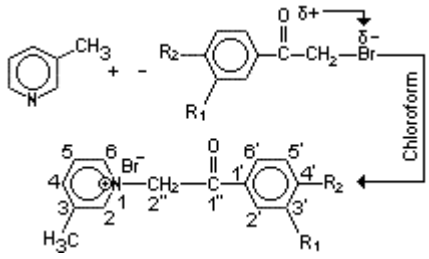
## EXPERIMENTAL

Unless otherwise stated all measurements were made as follows : Solvents and reagents were of analytical grade and used without further purification. Melting points were recorded on Gallenkamp melting-point apparatus and are uncorrected. $^1$H-NMR were recorded in D2O on a Bruker AM 300 spectrometer operating at 300 MHz. The chemical shifts are reported in s (ppm) and coupling constant in Hz. IR and UV spectra were recorded on JASCO IRA-1 and Pye-unicam SP-800 spectrometers respectively. Mass spectra were measured on Finnigan MAT 112.

**General Procedure**

Equimolar quantities of six derivatives of phenacyl bromides (2-bromo-3'4'-dihydroxyacetophenone, 2-bromo-3',4'-dihydroxyacetophenone, 2-bromo-3'-methoxyacetophenone, 2,4'-dibromoacetophenone, 2-bromo-4'chloroacetophenone, 2 bromo-4'-methoxy-acetophenone, 2-bromo-4'-methylacetophenone) and β-picoline were dissolved separately in CHCl3 (50 ml) in a round bottom flask and mixed together. The reaction mixture was stirred at room temperature for 30 min, then it was allowed to stand at room temperature for overnight at a dark place. Chloroform was removed under reduced pressure and residue was extracted with methanol to give the corresponding salts, I-VI.




The scheme of reaction, melting point and yield of each product is shown below :

| Comp No | R1 | R2 | Molecule Formula | Mol. Wt. | m.p. (°C) | Yield % |
|---|---|---|---|---|---|---|
| I | OH | OH | $(C_{14}H_{14}N^{+}O_3)Br^{-}$ | 324 | 202 | 23 |
| II | OMe | H | $(C_{15}H_{16}N^{+}O_2)Br^{-}$ | 322 | 123 | 19 |
| III | H | Br | $(C_{14}H_{13}N^{+}OBr)Br^{-}$ | 371 | 197 | 62 |
| IV | H | Cl | $(C_{14}H_{13}N^{+}OCl)Br^{-}$ | 326 | 242 | 76 |
| V | H | OMe | $(C_{15}H_{16}N^{+}O_2)Br^{-}$ | 322 | 213 | 86 |
| VI | H | Me | $(C_{15}H_{16}N^{+}O)Br^{-}$ | 306 | 186 | 72 |

**Characterization of Compounds**

**1-(3', 4'-Dihydroxyphenacyl)-β-picolinium bromide (I)**

$^{1}$H-NMR ($D_2O$) σ 8.02 (1H, t, J=1.78 Hz, H-2), 7.99 (1H, dt, J=6.42, 1.54 Hz, H-6), 7.64 (1H, dd, J=8.04, 7.94 Hz, H-5), 7.21 (1H, dt, J=7.32, 2.02 Hz, H-4), 7.12 (1H, dd, J=7.43, 2.1 Hz, H-6'), 6.92 (1H, d, J=7.43 Hz, H-5', 6.83 (1H, d, J=2.16, H-2'), 2.70 (3H, S, Arm. $CH_3$).

EIMS m/z $M^{+1}$ 245, other important peaks at 230, 211 and 107.

IR $\nu$max (KBr) cm$^{-1}$ 3105 (Arm. CH), 1760 (C=0), 1560, 1450 (Arm. C=C), 1385 ($CH_3$), 850 (C=C). UV $\lambda$max (MeOH) 412, 252, and 201 nm.

**1-(3'-Methoxypenacyl)-β-picolinium bromide (II)**

$^{1}$H-NMR ($D_2O$) σ 8.63 (1H, t, J=1.57 Hz, H-2), 8.55 (1H, dt, J=6.33, 1.57 Hz, H-6), 8.50 (1H, dd, J=8.01, 6.80 Hz, H-5), 8.03 (1H, dt, J=7.74, 1.57 Hz, H-4), 7.95 (1H, dt, J=8.59, 1.42 Hz, H-2'), 7.83 (1H, t, J=8.43 Hz, H-5'), 7.52 (1H, dd, J=2.55, 1.43 Hz, H-6'), 7.37 (1H, ddd, J=7.69, 2.25, 1.43 Hz, H-4'), 3.66 (3H, S, Arm-$OCH_3$), 2.06 (3H, S, Arm-$CH_3$).

EIMS m/z $M^{+1}$ 243, important peaks at 228 and 215.

IR $\nu$max (KBr) cm$^{-1}$ 3020 (Arm. CH), 2900 (Ali. CH), 1690 (C=O), 1590, 1480 (Arm. C=C), 1360 ($CH_3$), 800, 840 (C=C). UV $\lambda$max (MeOH) 315, 255, 219 and 201 nm.

**1-(4'-Bromophenacyl)-β-picolinium bromide (III)**

$^{1}$H-NMR ($D_2O$) σ 8.52 (1H, t, J=1.61 Hz, H-2), 8.32 (1H, dt, J=6.42, 1.52 Hz, H-6), 8.21 (1H, dd, J=8.62, 7.04 Hz, H-5), 7.98 (1H, dt, J=7.62, 1.04 Hz, H-4) 7.68 (2H, d, J=8.24 Hz, H-3', H-5'), 7.42 (2H, d, J=7.62 Hz, H-2', H-6'), 2.25 (3H, S, Arm-$CH_3$).

EIMS m/z $M^{+1}$ 293, important peaks at 211, 107, and 135.

IR $\nu$max (KBr) cm$^{-1}$ 3100 (Arm. CH), 1600 (C=O), 1580, 1480 (Arm. C=C), 1380 ($CH_3$), 850 (C=C). UV $\lambda$max (MeOH) 418, 263, and 202 nm.

**1-(4'-Chlorophenacyl)-β-picolinium bromide (IV)**

$^{1}$H-NMR ($D_2O$) σ 8.31 (1H, t, J=1.52 Hz, H-6), 8.04 (1H, dt, J=6.32, 1.52 Hz, H-2), 7.98 (1H, dd, J=8.62, 7.62 Hz, H-5), 7.62 (1H, dt, J=7.96, 1.24 Hz, H-4) 7.42 (2H, d, J=8.62 Hz, H-3', H-5'), 7.21 (2H, d, J= 8.24 Hz,

H-2', H-6'), 2.72 (3H, S, Arm-$CH_3$).

EIMS m/z $M^{+1}$ 247, important peaks at 196, 135, and 231.

IR $\nu$max (KBr) $cm^{-1}$ 3110 (Arm. CH), 1600 (C=O), 1560, 1500 (Arm. C=C), 1380 ($CH_3$), 850 (C=C). UV $\lambda$max (MeOH) 410, 263, and 202 nm.

**1-(4'-Methoxyphenacyl)-β-picolinium bromide (V)**

$^1$H-NMR ($D_2O$) $\sigma$ 8.52 (1H, t, J=1.61 Hz, H-2), 8.22 (1H, dt, J=6.42, 1.54 Hz, H-6), 8.04 (1H, dd, J=7.94, 7.04 Hz, H-5), 7.41 (1H, dt, J=7.42, 2.02 Hz, H-4), 6.94 (2H, d, J=8.62 Hz, H-3', H-5'), 6.88 (2H, d, J=7.82 Hz, H-2', H-6'), 3.96 (3H, S, Arm-$OCH_3$), 2.42 (3H, S, Arm-$CH_3$).

EIMS m/z $M^{+1}$ 243, important peaks at 212 and 228.

IR $\nu$max (KBr) $cm^{-1}$ 3150 (Arm. CH), 1720 (C=O), 1580, 1460 (Arm. C=C), 1385 (CH3), 850 (C=C). UV $\lambda$max (MeOH), 432, 260, and 202 nm.

**1-(4'-Methylphenacyl)-β-picolinium bromide (VI)**

$^1$H-NMR ($D_2O$) $\sigma$ 8.82 (1H, t, J=1.74 Hz, H-2), 8.62 (1H, dt, J=7.24, 1.62 Hz, H-2), 8.38 (1H, dd, J=7.84, 6.09 Hz, H-5), 7.98 (1H, dt, J=7.62, 1.52 Hz, H-4), 7.62 (2H, d, J=7.86 Hz, H-3', H-5'), 7.42 (2H, d, J=7.46 Hz, H-2', H-6'), 3.42 (3H, S, Arm-$CH_3$), 2.76 (3H, S, Arm-$CH_3$).

EIMS m/z $M^{+1}$ 227, important peaks at 212, 197, and 98.

Table 1: Primary screening of β-picoline derivatives against gram positive micro-organisms.

| S.N. Micro-organisms | Zone of inhibition for Compound | | | | | |
|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI |
| 1. *C. diphtheriae* | 0 | 08 | 8 | 0 | 12 | 0 |
| 2. *C. hoffmanii* | 26 | 8 | 28 | 22 | 10 | 0 |
| 3. *C. Xerosis* | 20 | 12 | 24 | 10 | 12 | 0 |
| 4. *St. Pyogenes* | 18 | 10 | 26 | 12 | 14 | 14 |
| 5. *St. Fecalis* | 20 | 6 | 28 | 14 | 0 | 12 |
| 6. *S. aureus* | 14 | 6 | 30 | 24 | 6 | 10 |
| 7. *S. spidermidis* | 10 | 8 | 8 | 20 | 14 | 8 |
| 8. *B. subtilis* | 22 | 8 | 10 | 16 | 10 | 0 |
| 9. *B. anthracis* | 18 | 18 | 20 | 8 | 10 | 8 |
| 10. *B. bronchoseptica* | 20 | 10 | 28 | 10 | 0 | 12 |
| 11. *List. monocytogenes* | 8 | 16 | 24 | 16 | 8 | 13 |
| 12. *List. invanovii* | 16 | 10 | 20 | 18 | 8 | 15 |

**Determination of Antibacterial Activity**

All compounds were tested for their antibacterial activity by agar diffusion technique (2). The overnight broth culture of bacteria in Trypticase Soya broth containing 10 c.f.u./ml was uniformly inoculated on the sensitest agar plates to obtain a confluent lawn. Stock solution of each compound was prepared in DMSO and

Table 2 : Primary screening of β-picoline derivatives against gram negative micro-organisms..

| S.N. Micro-organisms | Zone of inhibition for Compound | | | | | |
|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI |
| 1. *S. typhi* | 16 | 12 | 20 | 26 | 6 | 0 |
| 2. *S. typhi para A* | 18 | 12 | 4 | 28 | 6 | 0 |
| 3. *S. typhi para B* | 22 | 10 | 18 | 14 | 8 | 8 |
| 4. *S. typhimurium* | 0 | 8 | 20 | 12 | 4 | 10 |
| 5. *S. gallinarium* | 22 | 10 | 26 | 8 | 10 | 12 |
| 6. *S. pullorum* | 8 | 10 | 0 | 20 | 16 | 0 |
| 7. *Sh. dysenteriae* | 8 | 10 | 0 | 22 | 20 | 0 |
| 8. *Sh. flexneri* | 10 | 12 | 12 | 12 | 0 | 0 |
| 9. *Sh sonnei* | 10 | 16 | 10 | 10 | 0 | 6 |
| 10. *Sh. boydii* | 12 | 12 | 28 | 18 | 0 | 8 |
| 11. *E. coli* | 22 | 20 | 18 | 20 | 0 | 9 |
| 12. *Ent. aerogenes* | 26 | 10 | 20 | 10 | 8 | 14 |
| 13. *Ent. cloacae* | 28 | 10 | 14 | 12 | 7 | 18 |
| 14. *Kl. pneumoniae* | 14 | 10 | 16 | 20 | 18 | 8 |
| 15. *Kl. ozaenae* | 10 | 10 | 14 | 28 | 10 | 0 |
| 16. *Ps aeruginosa* | 18 | 12 | 14 | 30 | 8 | 0 |
| 17. *Vib. cholerae* | 20 | 10 | 22 | 14 | 0 | 0 |
| 18. *Vib. parahaemolyticus* | 20 | 0 | 24 | 10 | 0 | 10 |
| 19. *Prot. vulgaris* | 14 | 0 | 24 | 10 | 0 | 10 |
| 20. *Prot. mirabilis* | 16 | 0 | 24 | 10 | 0 | 10 |
| 21. *Ser marcescens* | 6 | 13 | 22 | 18 | 13 | 12 |
| 22. *Aero-hydrophila* | 24 | 14 | 20 | 22 | 14 | 18 |
| 23. *Acineto. calcoaceticus* | 12 | 0 | 16 | 18 | 0 | 20 |
| 24. *Citro. freundii* | 10 | 4 | 8 | 19 | 4 | 4 |

20 μl of each was applied to the sterile 6 mm filter paper discs. These were placed on the medium aseptically. Plates were incubated at 37°C for 24 hours and zones of inhibition were measured in mm (Results are enlisted in Tables 1 and 2).

## RESULTS AND DISCUSSION

All the synthesized compounds were screened for their antibacterial activity against twenty four strains of gram negative and twelve of gram positive micro-organisms. Results of these studies are presented in Tables 1 and 2.

Among all the tested compounds only three derivatives (I, III and IV) showed promising antibacterial activity against both gram positive and gram negative micro-organisms. Rest of three compounds were also active against most of test micro-organisms, but their zones of inhibition were small as compared to compound I, III and IV at the tested dilutions (20 mg/ml).

1-(3', 4'-Dihydroxyphenacyl)-β-picolinium bromide was proved to be the most active antibacterial agent amongst all the compounds Halogenated derivatives (III and IV) having bromo and chloro functions at the para position of phenacyl part of molecule exhibited almost same level of antibacterial activity.

Similarly compound II, V and VI having meta and para methoxy and para methyl functions respectively at the phenacyl moiety were unable to inhibit the growth of test organisms at the concentration of 20 mg/ml.

It is noteworthy that all the six derivatives did not significantly inhibit the growth of *C. diphtheria* and particularly I and IV remained absolutely inactive towards the said micro-organism.

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