Studies of antimicrobial activity of picryl amino pyridine N-oxides

D M Badgujar\textsuperscript{a}, M B Talawar\textsuperscript{b}, S N Asthana\textsuperscript{b} & P P Mahulikar*\textsuperscript{a}

\textsuperscript{a}School of Chemical Sciences, North Maharashtra University, Jalgaon 425 001, India
\textsuperscript{b}High Energy Materials Research Laboratory, Pune 411 021, India

E-mail: mahulikarpp@rediffmail.com

Received 16 July 2009; accepted (revised) 18 October 2010

Biologically active picryl amino pyridines and their N-oxide have been successfully synthesized and screened for their antimicrobial potency to set-up the structure activity relationships and found to possess better antibacterial activity than antifungal activity.

**Keywords**: Antimicrobial activity, nitro pyridines, 2- and 4-picrylamino pyridine-N-oxides, antimicrobial activity

The pyridine ring system occurs in the structures of many natural products, pharmaceutical and agrochemical compounds, and other commercial substances. Similarly, 2,5-di-substituted pyridines which appear to be important in many biologically active compounds have also been reported\textsuperscript{1}. The chemistry and applications of N-oxides have recently received much attention due to their usefulness as synthetic intermediates and their biological importance\textsuperscript{2}. Heterocyclic N-oxides are also useful as protecting groups, auxiliary groups, oxidants, ligands in metal complexes and catalysts\textsuperscript{3}.

![Scheme I — Synthesis of picryl amino pyridines and N-oxides](image)

The chemistry of heterocyclic N-oxides has aroused widespread interest owing to exceptionally high bioactivity of these compounds encompassing, among others, antibiotics, antibiotic antagonists and compounds exhibiting cancerostatic, mutagenic, sedative, anticonvulsive and fungistatic efficacy\textsuperscript{4}.

According to recent reports, the N-oxides\textsuperscript{5} of heterocyclic amines play an important role in reactions leading to the modification of enzymes and biological reductive systems, as well as factors substituting some functions of nucleic acids. The N-oxides\textsuperscript{6} possess an important feature which is, that the presence of the nitro group has been found crucial for developing antifungal efficacy of these compounds. In this respect, all studies related with the electronic structure of the N-oxides of heterocyclic amines substituted with the nitro group and with their protonated forms are of particular importance, as they may contribute to the understanding of the mechanism of antifungal activity of these compounds.

The pyridine N-oxide derivatives represent a peculiar class of antiviral compounds that qualify as promising novel drugs for exploration as potential anti-HIV agents\textsuperscript{7}. They have an entirely new mechanism of antiviral action and the capacity to retain antiviral activity\textsuperscript{7} against virus stains that have gained resistance to clinically used drugs such as NRTIs, NtRTIs, NNRTIs (depending on the particular structure of the pyridine N-oxide derivatives). Indeed it is clear that, whereas several members of this class of compounds...
functionally interact with HIV-1 RT as NNTRIs, other distinct members inhibit HIV-replication. They do so by interacting, additionally or alternatively, with a target in the HIV-replication cycle.

In view of these diverse applications, a systematic method has been developed for the synthesis of biologically active picryl amino pyridines and corresponding N-oxides (Scheme I). All synthesized compounds were found to possess excellent antimicrobial activity.

**Experimental Section**

**General procedure for the synthesis of picryl amino pyridine N-oxides**

To a stirred solution of amino substituted pyridines in dry ethanol (20 mL) was added the solution of picryl chloride (2.63 g, 0.0106 mole) with stirring at RT. Then the reaction mixture was refluxed for 6 hr. The reaction content was cooled, filtered and washed with cold ethanol and purified by recrystallization from ethanol to afford the desired compounds. Picryl amino pyridines in methanol (20 mL) was subjected for N-oxidation using H$_2$O$_2$ (30%, 10 mL) in glacial acetic acid (30%, 20 mL) to afford the different picryl amino pyridine N-oxides.

**2-picryl aminopyridine**: 2.10 g, (80%), m.p. 158-60°C; $^1$H NMR (CDCl$_3$+DMSO-d$_6$): δ 8.85 (s, 2H, picryl Ar-H), 8.08 (bs, 1H, NH), 7.74 (m, 2H, C$_5$ and pyridine C$_6$), 7.30 (d, 1H, pyridine C$_3$), 6.79 (d, 1H, pyridine C$_4$); IR (KBr): 3450 (NH), 3040 (Ar-CH), 1580, 1360 cm$^{-1}$ (NO$_2$).

**2-picrylaminopyridine N-oxide**: 2.78 g, (76%), m.p. 180-82°C; IR (KBr): 3450 (NH), 3040 (Ar-CH), 1626(C = C), 1350(NO$_2$), 2990, 1620 (Ar-CH), 1360 cm$^{-1}$ (NO$_2$).
1250,1190 cm\(^{-1}\) (N-O). **4-picryl aminopyridine**: 2.30 g. (80%), m.p. 225°C; \(^1\)H NMR (CDCl\(_3\)+DMSO-d\(_6\)): \(\delta\) 9.55 (s, 2H, Ar-H), 9.06 (bs, 1H, NH), 8.66 (dd, 2H, pyridine C\(_2\) and C\(_6\) pyridine), 7.77 (dd, 2H, pyridine C\(_3\) and C\(_5\)); IR (KBr): 3480 (NH), 3030 (Ar-CH), 1565, 1360 cm\(^{-1}\) (C-NO\(_2\)). **4-picryl aminopyridine N-oxide**: 2.78 g. 78%, m.p. 208°C; \(^1\)H NMR (CDCl\(_3\)+DMSO-d\(_6\)): \(\delta\) 9.20 (bs, 1H, -NH), 7.92 (d, 2H, pyridine C\(_2\) and C\(_6\)), 7.40 (s, 2H, Ar-H), 6.84 (d, 2H, pyridine C\(_3\) and C\(_5\)); FT-IR (KBr): 3450 (NH), 3030, 1610 (Ar-CH), 1589, 1360 cm\(^{-1}\) (C-NO\(_2\)). **2,6-bis-picrylaminopyridine**: 3.70 g. (76.17%), m.p. 305°C (dec.); \(^1\)H NMR (CDCl\(_3\)+DMSO-d\(_6\)): \(\delta\) 9.11 (bs, 1H, Ar-H), 8.79 (d, 1H, pyridine C\(_4\)), 7.39 (d, 1H, pyridine C\(_4\)), 6.75 (1H, d, pyridine C\(_4\)); FT-IR (KBr): 3340 (NH), 2990 (Ar-CH), 1626 (C=C), 1589, 1360 cm\(^{-1}\) (N-O). **2,6-bis-picrylaminopyridine N-oxide**: 1.10 g. (80%), m.p. 260°C; FT-IR (KBr): 3350 (NH), 3040 (Ar-CH), 1490, 1350 (NO\(_2\)), 1250, 1180 cm\(^{-1}\) (N-O). **5-nitro-2-picrylaminopyridine**: 1.05 g. (76%), m.p. 208°C; \(^1\)H NMR (DMSO-d\(_6\)): \(\delta\) 9.62 (s, 1H, Ar-H), 8.79 (dd, 2H, pyridine C\(_3\) and C\(_5\)), 8.70 (d, 1H, pyridine C\(_4\)), 7.89 (d, 1H, pyridine C\(_3\)); IR (KBr): 3340 (NH), 3030, 1610 (Ar-CH), 1589, 1555 cm\(^{-1}\) (NO\(_2\)). **5-nitro-2-picrylaminopyridine N-oxide**: 3.35 g. (80%), m.p. 197°C; \(^1\)H NMR (DMSO-d\(_6\)): \(\delta\) 9.00 (bs, 1H, NH), 8.90 (s, 2H, Ar-H), 8.54 (d, 1H, pyridine C\(_3\)), 7.92 (d, 1H, pyridine C\(_3\)), 7.26 (m, 1H, pyridine C\(_3\)); IR (KBr): 3480 (NH), 3010 (Ar-CH), 1580, 1450 (NO\(_2\)), 1230 cm\(^{-1}\) (N-O).

**Antimicrobial Assay**

All synthesized compounds were evaluated for their antimicrobial activity, namely, antifungal activity against *Pacillomycus lilacinus*, *Aspergillus awamorii* and *Colletricum falcatum* and *Agrobacterius tumefaciam*, *Pseudomonas putida* and *Escherichia coli* using paper disc agar method. For antimicrobial study, fungi were grown on Czepadox broth growth and bacteria on nutrient Agar. In antifungal bioassay, to each petriplate 20 mL of sterilized medium was added. After the agar had set, 10% of inoculum (suspension culture) was added to each petriplate and spread thoroughly by rotary motion of the plate. Sterilized Whatman No.1 filter paper discs (diameter 6 mm) were thoroughly moistened with a 5 mg/mL solution of the compound(s) in methanol and placed on the seeded agar plates. Paper discs moistened with ethanol were considered as a control. The plates were incubated at 27°C for 2-3 hr. A clear zone of inhibition around the paper disc demonstrated the relative susceptibility of the fungi to the synthesized derivatives. The fungicidal activity is proportional to the diameter (mm) of the zone of inhibition. The antibacterial activity was determined as described for antifungal activity; only the plates were incubated at 37°C for 24 hr. The antibacterial and antifungal activity results are represented in Figures 1 and 2, respectively.

**Results and Discussion**

Various amino pyridines in dry ethanol (20 mL) and picryl chloride with stirring at RT and at reflux conditions afforded picryl amino pyridines, which on N-oxidation using H\(_2\)O\(_2\) (30%, 10 mL) in glacial acetic acid (30%, 20 mL) gave the corresponding picryl amino pyridine N-oxides.

The picryl amino pyridine N-oxides were found to be more effective against microbes. On comparing the antibacterial and antifungal activities, the overall antimicrobial testing results revealed that the synthesized different picryl amino pyridine N-oxides showed better antibacterial activity than antifungal activity.

**Acknowledgement**

The authors are thankful to Ministry of Defence, DRDO, New Delhi for financial assistance and HEMRL, Pune for characterizations of the products.

**References**