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Der Pharma Chemica, 2012, 4(6):2283-2287

(http://derpharmachemica.com/archive.html)

ISSN 0975-413X

CODEN (USA): PCHHAX

Synthesis of 3,5-diaryl-isoxazole-4-carbonitriles and their efficacy as antimicrobial agents

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ABSTRACT

*Nitrile oxides generated in situ by the oxidative dehydrogenation of aromatic aldoximes (**1a-g**) undergo 1,3-dipolar cycloaddition reaction with 3-(4-methoxyphenyl)propiolonitrile (**2**) to afford the new cycloadducts 3-aryl-5-(4-methoxyphenyl)-isoxazole-4-carbonitriles (**3a-g**) in moderate yield. The new synthesized cycloadducts were tested for their antifungal and antibacterial activity.*

Key words: Isoxazoles, chloramine-T, cycloaddition, antifungal, antibacterial.

INTRODUCTION

Isoxazoles and isoxazolines are very useful heterocycles in organic and medicinal chemistry. Isoxazolines also serves as important building blocks for the synthesis of various biologically active molecules. The isoxazoles are known to exhibit significant number of biological applications such as hypoglycemic, analgesic, anti-inflammatory and HIV-inhibitory activity [1], also found to exhibit antibacterial [2], antifungal [3], antioxidant [4], potent selective agonists at human cloned dopamine D4 receptors [5], $GABA_A$ antagonist [6], COX-2 inhibitory [7], antinociceptive [8], anticancer [9] activities. They serve as prodrug for the anti-arithretic agent [10]. The most convenient synthesis of isoxazole ring system has been executed in the via 1,3-dipolar cycloaddition reactions of alkenes and alkynes with nitrile oxides [11-14]. It was reported that nitrile oxides preferentially adds to olefinic C-C double or C-C triple bond than C-N triple bond of acrylonitrile to form isoxazolines [15-16].

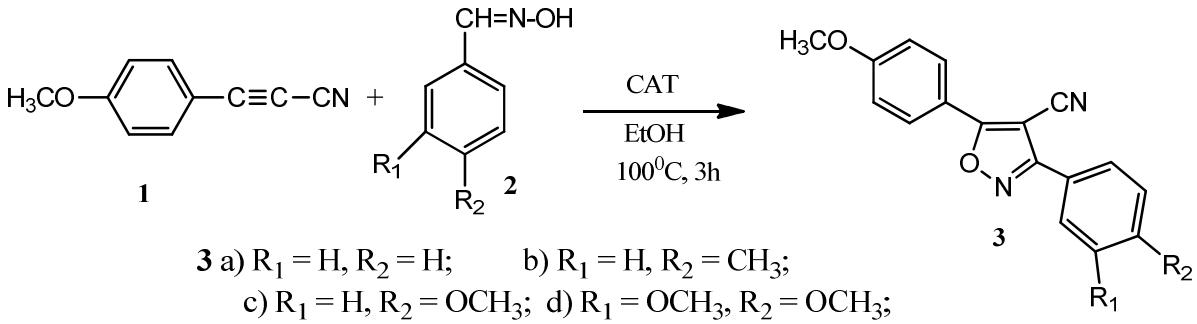
This paper describes the synthesis of 3-aryl-5-(4-methoxyphenyl)-isoxazole-4-carbonitriles via 1.3-dipolar cycloaddition reactions and *in vitro* screening results of their antifungal, antibacterial activity and their minimum inhibitory concentrations.

MATERIALS AND METHODS

The chemicals used were purchased from sigma-aldrich chemicals (India) and Merck Chemicals (India). IR spectra were recorded on a Nujol mull on Shimadzu 8300 spectrometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker supercon 400 MHz spectrophotometer using $CDCl_3$ as solvent and TMS as an internal standard. The Chemical shifts are expressed in δ ppm. Mass spectra were obtained on Shimadzu LCMS-2010A spectrophotometer (chemical ionization) and the important fragments are given with the relative intensities in the bracket. Elemental analysis was obtained on a Thermo Finnigan Flash EA 1112 CHN analyser. Thin layer chromatography (TLC) were performed on a pre-coated Silica Gel sheets (HF 254, sd-fine) using benzene:ethyl acetate (7:2) eluent and visualization of the spots was done in iodine vapour and UV light. Chromatographic separations were carried out on silica gel (70-230 mesh, Merck) column using hexane:ethyl acetate (6:1) as eluent.

Chemistry

In a general 1,3-dipolar cycloaddition reaction, a mixture of aromatic aldoximes **2,** 3-(4-methoxyphenyl) propiolonitrile **1**, and chloramine-T in ethyl alcohol was refluxed on water bath for 3 hours. The progress of the reaction was monitored by TLC. After the completion of the reaction and usual work up, the reaction mixture gave one major spot corresponding to the product 3-aryl-5-(4-methoxyphenyl)-isoxazole-4-carbonitriles **3** in TLC, and two minor spots corresponding to the un-reacted precursors. The products were separated by column chromatography using hexane:ethyl acetate (8:1 v/v) (scheme-1).



3 a) $R_1 = H, R_2 = H$; b) $R_1 = H, R_2 = CH_3$;
c) $R_1 = H, R_2 = OCH_3$; d) $R_1 = OCH_3, R_2 = OCH_3$;
e) $R_1 = H, R_2 = F$; f) $R_1 = H, R_2 = Cl$.

Scheme 1

General procedure for the cycloaddition: A mixture of oxime **2** (5 mmol), 3-(4-methoxyphenyl)propiolonitrile **1** (5 mmol) and chloramine-T trihydrate (5.5 mmol) was refluxed on a water bath for 3-4 hours. The progress of the reaction was monitored by TLC. After completion of the reaction, the salts formed were filtered off; the solvent was evaporated in vacuum. The residual mass was extracted into ether (1 x 25 mL), washed successively with water (3 x 20 mL), 5% sodium hydroxide (2 x 10 mL), brine solution (1 x 15 mL) and dried over anhydrous sodium sulphate. Evaporation of the solvent afforded crude yellow oily substance **3**, which was purified by column chromatography using benzene: ethyl acetate (6:1) as eluent.

Antimicrobial activity: Antimicrobial activity of the synthesized compounds (**3a-f**) was done by paper disc diffusion method [17-19]. The synthesized compounds were tested against *Escherichia coli*, *Bacillus substilis*, *Staphylococcus aureus* bacterial strains; the antibiotic streptomycin was used as standard drug against bacteria. The compounds were tested against *Aspergillus niger*, *Candida albicans*, *Fusarium oxysporium* fungi species; the antibiotic Nystatin was used as standard drug against fungi. The screening tests were performed in triplicate and the results were taken as a mean of three determinations. Minimum inhibitory concentrations (MICs) were determined by broth dilution technique.

Experimental

5-(4-Methoxyphenyl)-3-phenyl-isoxazole-4-carbonitrile **3a:** Obtained as a light yellow oil in 56% yield. IR (Nujol): 1670 cm^{-1} C=N (str), 2235 cm^{-1} CN (str). ^{1}H NMR ($CDCl_3$): δ 3.82 (s, 3H, OCH_3), 6.91-6.93 (dd, 2H, Ar-H), 7.22-7.22 (dd, 2H, Ar-H), 7.56-7.78 (m, 5H, Ar-H). ^{13}C NMR ($CDCl_3$): δ 55.9 (1C, O<u>C</u>H$_3$), 100.1 (1C, 4-<u>C</u>), 114.0 (2C, Ar-<u>C</u>), 116.5 (1C, <u>C</u>N), 127.2 (2C, Ar-<u>C</u>), 128.4 (2C, Ar-<u>C</u>), 128.8 (2C, Ar-<u>C</u>), 131.0 (1C, Ar-<u>C</u>), 133.1 (1C, Ar-<u>C</u>),

133.7 (1C, Ar-C), 156.8 (1C, 5-C), 159.3 (1C, Ar-C), 164.5 (1C, 3-C). MS (relative abundance) m/z: 277 (MH^+, 100), 250 (18), 218 (26), 172 (28), 155 (32). Anal. Cacld. for $C_{17}H_{12}N_2O_2$, C, 73.90, H, 4.38, N, 10.14%; Found: C, 73.80, H, 4.21, N, 10.02%.

5-(4-Methoxyphenyl)-3-(4-Methylphenyl)-isoxazole-4-carbonitrile **3b:** Obtained as a light yellow oil in 56% yield. IR (Nujol): 1670 cm^{-1} C=N (str), 2235 cm^{-1} CN (str). ^1H NMR ($CDCl_3$): δ 2.45 (s, 3H, CH_3), 3.80 (s, 3H, OCH_3), 6.91-6.94 (dd, 2H, Ar-H), 7.10-7.12 (dd, 2H, Ar-H), 7.22-7.24 (dd, 2H, Ar-H), 7.64-7.67 (dd, 2H, Ar-H). ^{13}C NMR ($CDCl_3$): δ 21.2 (1C, CH_3), 55.4 (1C, OCH_3), 99.9 (1C, 4-C), 114.2-114.4 (2C, Ar-C), 116.7 (1C, CN), 124.1 (1C, Ar-C), 127.2-127.4 (2C, Ar-C), 130.0 (1C, Ar-C), 131.4 (1C, Ar-C), 132.9 (1C, Ar-C), 133.3 (1C, Ar-C), 136.6 (1C, Ar-C), 140.2 (1C, Ar-C), 156.3 (1C, 5-C), 159.5 (1C, Ar-C), 164.4 (1C, 3-C). MS (relative abundance) m/z: 291 (MH^+, 100), 260 (20), 236 (24), 172 (28), 155 (38). Anal. Cacld. for $C_{18}H_{14}N_2O_2$, C, 74.47, H, 4.86, N, 9.65%; Found: C, 74.34, H, 4.81, N, 9.56%.

3,5-Bis(4-methoxyphenyl)-isoxazole-4-carbonitrile **3c:** Obtained as a pale oil in 60% yield. IR (Nujol): 1655 cm^{-1} C=N (str), 2225 cm^{-1} CN (str). ^1H NMR ($CDCl_3$): δ 3.80 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 6.92-6.93 (dd, 2H, Ar-H), 6.99-6.70 (dd, 2H, Ar-H), 7.22-7.23 (dd, 2H, Ar-H), 7.74-7.75 (dd, 2H, Ar-H). ^{13}C NMR ($CDCl_3$): δ 55.1 (1C, OCH_3), 55.5 (1C, OCH_3), 100.5 (1C, 4-C), 114.0-114.2 (2C, Ar-C), 116.4 (1C, CN), 124.0 (1C, Ar-C), 127.0-127.2 (2C, Ar-C), 130.1 (1C, Ar-C), 131.3 (1C, Ar-C), 132.8 (1C, Ar-C), 133.4 (1C, Ar-C), 136.1 (1C, Ar-C), 140.7 (1C, Ar-C), 157.1 (1C, 5-C), 159.8 (1C, Ar-C), 164.3 (1C, 3-C). MS (relative abundance) m/z: 307 (MH^+, 100), 280 (26), 248 (30), 172 (25), 155 (40). Anal. Cacld. for $C_{18}H_{14}N_2O_3$, C, 70.58, H, 4.61, N, 9.15%; Found: C, 70.48, H, 4.56, N, 9.10%.

3-(3,4-Dimethoxyphenyl)-5-(4-methoxyphenyl)-isoxazole-4-carbonitrile **3d:** Obtained as a pale yellow oil in 61% yield. IR (Nujol): 1672 cm^{-1} C=N (str), 2238 cm^{-1} CN (str). ^1H NMR ($CDCl_3$): δ 3.81 (s, 6H, OCH_3), 3.81 (s, 3H, OCH_3), 6.92-6.93 (dd, 2H, Ar-H), 6.99 (dd, 1H, Ar-H), 7.23-7.24 (dd, 2H, Ar-H), 7.30 (s, 1H, Ar-H), 7.40 (dd, 1H, Ar-H). ^{13}C NMR ($CDCl_3$): δ 55.5 (1C, OCH_3), 55.8 (2C, OCH_3), 100.4 (1C, 4-C), 112.3 (1C, Ar-C), 114.5-114.7 (3C, Ar-C), 116.8 (1C, CN), 121.5 (1C, Ar-C), 127.3-127.5 (3C, Ar-C), 148.8 (1C, Ar-C), 149.5 (1C, Ar-C), 150.3 (1C, Ar-C), 156.6 (1C, 5-C), 159.0 (1C, Ar-C), 164.4 (1C, 3-C). MS (relative abundance) m/z: 337 (MH^+, 100), 310 (14), 278 (23), 172 (20), 155 (40). Anal. Cacld. for $C_{19}H_{16}N_2O_4$, C, 67.85, H, 4.79, N, 8.33%; Found: C, 67.78, H, 4.67, N, 8.24%.

3-(4-Fluorophenyl)-5-(4-methoxyphenyl)-isoxazole-4-carbonitrile **3e**: Obtained as light yellow oil in 62% yield. IR (Nujol): 1654 cm^{-1} C=N (str), 2228 cm^{-1} CN (str). ^1H NMR ($CDCl_3$): δ 3.84 (s, 3H, OCH_3), 6.90-6.93 (dd, 2H, Ar-H), 6.93-6.95 (dd, 2H, Ar-H), 7.38-7.40 (dd, 2H, Ar-H), 7.78-7.80 (dd, 2H, Ar-H). ^{13}C NMR ($CDCl_3$): δ 55.7 (1C, OCH_3), 100.0 (1C, 4-C), 114.1-114.3 (2C, Ar-C), 115.4-115.7 (2C, Ar-C), 116.8 (1C, CN), 128.2-128.4 (2C, Ar-C), 129.0-129.2 (2C, Ar-C), 130.1 (1C, Ar-C), 130.5 (1C, Ar-C), 149.8 (1C, Ar-C), 156.8 (1C, 5-C), 161.5 (1C, 3-C), 161.9 (1C, Ar-C). MS (relative abundance) m/z: 295 (MH^+, 100), 270 (32), 238 (20), 172 (16), 155 (44). Anal. Cacld. for $C_{17}H_{11}FN_2O_2$, C, 69.38, H, 3.77, N, 9.52%; Found: C, 69.25, H, 3.66, N, 9.47%.

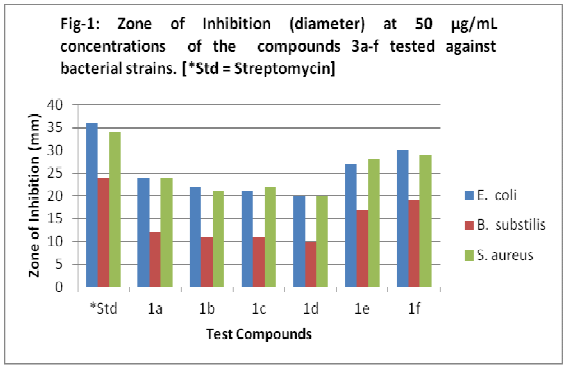
3-(4-Chlorophenyl)-5-(4-methoxyphenyl)-isoxazole-4-carbonitrile **3f:** Obtained as a pale yellow oil in 54% yield. IR (Nujol): 1650 cm^{-1} C=N (str), 2220 cm^{-1} CN (str). ^1H NMR ($CDCl_3$): δ 3.84 (s, 3H, OCH_3), 6.91-6.92 (dd, 2H, Ar-H), 6.93-6.94 (dd, 2H, Ar-H), 7.38-7.40 (dd, 2H, Ar-H), 7.78-7.79 (dd, 2H, Ar-H). ^{13}C NMR ($CDCl_3$): δ 55.6 (1C, OCH_3), 100.5 (1C, 4-C), 114.4-114.6 (2C, Ar-C), 115.7-115.9 (2C, Ar-C), 116.8 (1C, CN), 128.3-128.5 (2C, Ar-C), 129.0-129.2 (2C, Ar-C), 130.5 (1C, Ar-C), 130.8 (1C, Ar-C), 150.2 (1C, Ar-C), 156.4 (1C, 5-C), 161.7 (1C, 3-C), 162.2 (1C, Ar-C). Anal. Cacld. for $C_{17}H_{11}ClN_2O_2$, C, 65.71, H, 3.57, N, 9.02%; Found: C, 65.69, H, 3.48, N, 8.93%.

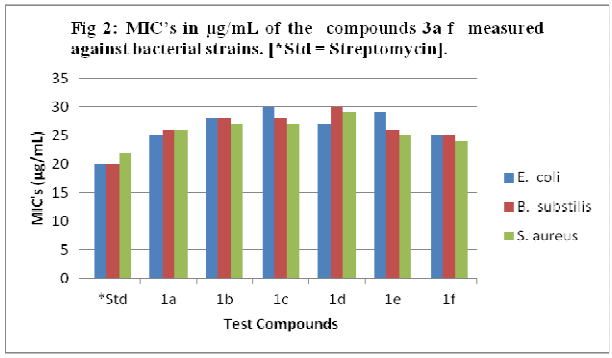
RESULTS AND DISCUSSION

The structures of the cycloadducts were provided by IR, ^1H NMR, ^{13}C NMR, MS studies and elemental analysis. For instance, in IR spectra, the cycloadducts **3** gave the absorptions bands in the region 1655-1670 cm^{-1} for C=N (str) group which is a clear indication of the formation of cycloadducts, a strong and sharp absorption bands in the region 2230-2240 cm^{-1} for CN (str) which supports the fact that the triple bond of CN group is unaffected during the cycloaddition reaction.

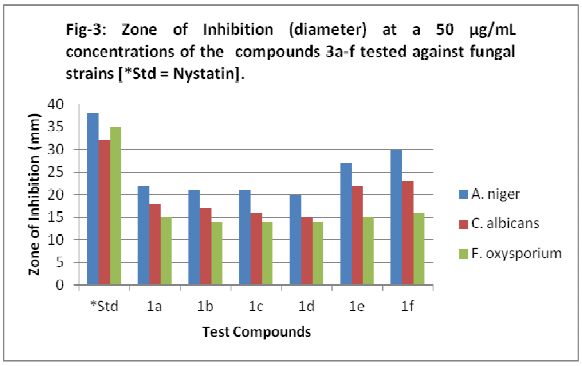
In ^{1}H NMR spectra, all cycloadducts **3** showed the peaks due to aromatic and substituent protons at the expected region. In ^{13}C NMR, all products gave the signals due to aromatic and substituent carbons at the expected region. The signals due to newly formed C_4-carbon appeared in the region δ_c 99.9-100.5 ppm, while, C_5-carbon showed the signals in the region δ_c 156.3-157.1 ppm and C_3-carbon showed the signals in the region δ_c 161.0-164.6 ppm. The signals due to CN group carbon appear in the region δ_c 116.2-117.0 ppm; which shows that the CN triple bond is unaffected during cycloaddition. All cycloadducts showed significantly stable molecular ion peaks with a relative abundance ranging from 10-45% and base peak at (MH^+). Further, they gave satisfactorily CHN analysis with a deviation of ±0.10% from the theoretical values. All these observations strongly favor the formation of the cycloadducts.

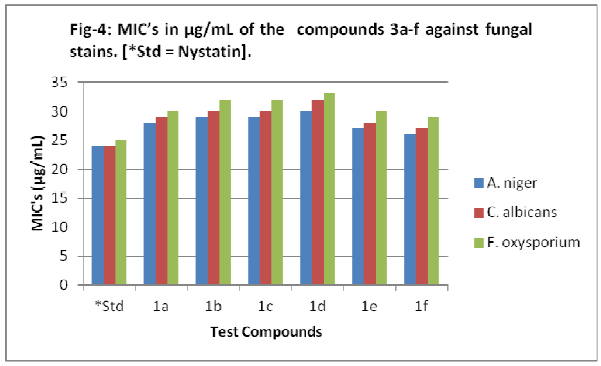
The results of the antibacterial evaluation of the synthesized compounds revealed that all these compounds exhibit remarkable activity against *E.coli* and *S.* aureus; moderate activity against *Bacillus substilis* [Fig-1]. The results of MIC's determination show that some of these compounds can be used as control measures at lower concentrations [Fig-2].





The results of the antifungal activity evaluation of the synthesized compounds show that; the compounds were highly active against *A. niger* and *C. albicans*; moderately active against *Fusarium oxysporium* [Fig-3]. The results of MIC's determination show that some of these compounds can be used as control measures at lower concentrations [Fig-4]. The presence of halogen substituents in the aromatic ring enhanced the activity of the compounds against all the bacterial and fungal organisms tested.





CONCLUSION

The divergence in their biological activity of the new compounds validates the significance of this study. The study revealed that the most of the compounds tested showed moderate to good antimicrobial. However, the effect of compounds on the host cell and their mode of action remain to be studied.

Acknowledgements
The authors are grateful to M.A. Paneendra, Biocon India Pvt. Ltd, Bangalore for providing 3-(4-methoxyphenyl)propiolonitrile and to the University Grants Commission, New Delhi, for the financial support.

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