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Synthesis and Antimicrobial activity of Novel Isoxazolines by 1, 3-Dipolar Cycloaddition Reactions

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Abstract: Nitrile oxides were generated by the catalytic dehydrogenation of aromatic aldehyde oximes **2a-g** with chloramine-T as oxidizing agent. The 1,3-dipolar cycloaddition of *in situ* generated nitrile oxides with ethyl oleate **1** produced a series of new ethyl 8-(3-aryl-4-octyl-4,5-dihydroisoxazol-5-yl)octanoate **3a-g** in 65-84% yield. Some of the synthesized cycloadducts have exhibited moderate to good antifungal and antibacterial activity.
Key words: Cycloaddition, dipolar, isoxazolines, antifungal, antibacterial, MIC's.

Introduction

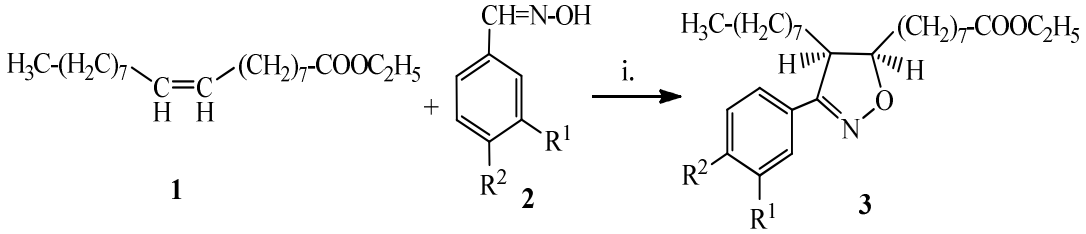
Five membered heterocycle such as isoxazolines are considered as useful intermediates in organic synthesis, they serve as building blocks for the construction of various biologically active molecules.[1] Isoxazoline and their derivatives are known to exhibit enormous biological applications such as hypoglycemic, analgesic, anti-inflammatory and HIV-inhibitory activity,[2] also found to exhibit antimicrobial,[3] potent selective agonists at human cloned dopamine D4 receptors.[4] $GABA_A$ antagonist,[5] COX-2 inhibitory,[6] antioxidant,[7] anticancer,[8] and antibiotic, antitumour, insecticidal, tubercular, antinociceptive activities.[1-2]

The most convenient synthesis of isoxazoline and isoxazole ring system has been executed in the literature via 1, 3-dipolar cycloaddition reactions of alkenes and alkynes with nitrile oxides generated *in situ* from aldoximes.[9-10] Chloramine-T is considered as useful reagent in organic synthesis, particularly in the synthesis of biologically active heterocycles.[11-12] In view of broad spectrum of synthetic and biological applications of isoxazolines, and in search of efficient antimicrobial agents, this project was undertaken. Here in we report the use of biomolecule ethyl oleate as dipolarophile moiety in 1, 3-dipolar cycloaddition reactions in the synthesis of new isoxazolines and their biological evaluation for antibacterial and antioxidant activity.

Materials and Methods

The chemicals/reagents used were purchased from sigma-aldrich chemicals (India) and Merck Chemicals (India). The ^{1}H NMR 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 · ppm. Mass spectra were obtained on Shimadzu LCMS-2010A spectrophotometer (positive 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 analyzer. Chromatographic separations were carried out on silica gel (70-230 mesh, Merck) column using hexane: ethyl acetate (8:1) as eluent.

In a general 1,3-dipolar cycloaddition reaction, a mixture of ethyl oleate **1**, aromatic aldoximes **2,** and Chloramine-T in ethyl alcohol was refluxed on a water bath conditions for 2-3 hours. The progress of the reaction was monitored by TLC. After the completion of the reaction and usual work up, the reaction gave the cycloadducts **3** in 65-84% yield (scheme-1).



Reagents and conditions**:** i. Chloramine-T, EtOH, 100^{o}C, 3h

3 a) R^1= H, R^2= H; b) R^1= H, R^2= OCH_3; c) R^1= OCH_3, R^2= OCH_3; d) R^1= H, R^2= CH_3;
e) R^1= H, R^2= F; f) R^1= H, R^2= Cl; g) R^1= H, R^2= NO_2.

Scheme-1: Synthesis of isoxazolines by 1,3-dipolar cycloaddition

Minimum inhibitory concentrations (MICs) of the synthesised compounds **3a-g** against different fungal and bacterial strains were determined by broth dilution technique.[13-14]

General procedure for cycloaddition:

A mixture of oxime **2** (2.2 mmol), ethyl oleate **1** (2.0 mmol) and chloramine-T trihydrate (3.0 mmol) in ethyl alcohol (30 ml) was refluxed on water bath for 3 hours. The progress of the reaction was monitored by TLC. After the completion of the reaction, the sodium chloride formed was filtered off, and the filtrate was evaporated in vacuo. The residual mass was extracted into ether (30 mL), washed successively with water (2 X 20 mL), 10% sodium hydroxide (2 X 20 mL) and saturated brine solution (1 X 15 mL). The organic layer was dried over anhydrous sodium sulphate and the solvent was evaporated to dryness to get the cycloadducts. The products were purified by column chromatography using hexane: ethyl acetate (9:2 v/v) as eluent.

Results and Discussion:

Chemistry:

The general synthetic pathway employed is depicted in the scheme-1.

Ethyl 8-(4-octyl-3-phenyl-4,5-dihydroisoxazol-5-yl)octanoate **3a**: Obtained from ethyl oleate **1** (2.0 mmol) and benzaldehyde oxime **2** (2.2 mmol), as light yellow oil in 68% yield. ^{1}H NMR ($CDCl_3$): δ 0.88 (q, 3H, CH_3), 1.02 (t, 3H, CH_3), 1.24-1.48 (m, 14H, CH_2), 1.88-1.94 (m, 8H, CH_2), 2.22-2.28 (m, 4H, CH_2), 3.60 (t, 2H, $COCH_2$), 3.98-4.00 (q, 2H, OCH_2), 4.14 (d, 1H, *J=8.6Hz*, C_4-H), 5.20 (d, 1H, *J=8.2Hz*, C_5-H), 7.50-7.72 (m, 5H, Ar-H). MS (relative abundance) m/z: 430 (M+1, 24), 429 (M^+, 33), 400 (16), 311 (M-118, 100), 297 (34), 265 (54), 247 (20). Anal. Calcd. for $C_{27}H_{43}NO_3$, C, 75.48; H, 10.09; N, 3.26%; Found: C, 75.40; H, 10.01; N, 3.30%.

***Ethyl 8-(3-(4-methoxyphenyl)-4-octyl-4,5-dihydroisoxazol-5-yl)octanoate* 3b:** Obtained from ethyl oleate **1** (2.0 mmol) and 4-methoxybenzaldehyde oxime **2** (2.2 mmol), as a light yellow oil in 66% yield. ^{1}H NMR ($CDCl_3$): δ 0.86 (q, 3H, CH_3), 1.08 (t, 3H, CH_3), 1.16-1.46 (m, 14H, CH_2), 1.88-1.93 (m, 8H, CH_2), 2.20-2.30 (m, 4H, CH_2), 3.64 (t, 2H, $COCH_2$), 3.86 (s, 3H, OCH_3), 3.98-4.02 (q, 2H, OCH_2), 4.16 (d, 1H, *J=8.8Hz,* C_4-H), 5.18 (d, 1H, *J=9.0Hz,* C_5-H), 7.20 (dd, 2H, Ar-H), 7.56 (dd, 2H, Ar-H). MS (relative abundance) m/z: 460 (M+1, 16), 459 (M^+, 26), 429 (26), 444 (22), 311 (M-148, 100), 297 (30), 265 (50), 247 (26). Anal. Calcd. for $C_{28}H_{45}NO_4$, C, 73.16; H, 9.87; N, 3.05%; Found: C, 73.20; H, 9.98; N, 3.00%.

***Ethyl 8-(3-(3,4-dimethoxyphenyl)-4-octyl-4,5-dihydroisoxazol-5-yl)octanoate* 3c:** Obtained from ethyl oleate **1** (2.0 mmol) and 3,4-dimethoxybenzaldehyde oxime **2** (2.2 mmol), as a pale yellow oil in 78% yield. ^{1}H NMR ($CDCl_3$): δ 0.84 (q, 3H, CH_3), 1.05 (t, 3H, CH_3), 1.20-1.48 (m, 14H, CH_2), 1.86-1.92 (m, 8H, CH_2), 2.16-2.24 (m, 4H, CH_2), 3.64 (t, 2H, $COCH_2$), 3.85 (s, 6H, OCH_3), 3.94-4.00 (q, 2H, OCH_2), 4.06 (d, 1H, *J=8.0Hz,* C_4-H), 5.26 (d, 1H, *J=8.8Hz,* C_5-H), 6.98-7.28 (m, 3H, Ar-H). Anal. Calcd. for $C_{29}H_{47}NO_5$, C, 71.13; H, 9.67; N, 2.86%; Found: C, 71.18; H, 9.60; N, 2.89%.

***Ethyl 8-(3-(4-methylphenyl)-4-octyl-4,5-dihydroisoxazol-5-yl)octanoate* 3d:** Obtained from ethyl oleate **1** (2.0 mmol) and 4-methylbenzaldehyde oxime **2** (2.2 mmol), as a viscous mass in 72% yield. ^{1}H NMR ($CDCl_3$): δ 0.82 (q, 3H, CH_3), 1.12 (t, 3H, CH_3), 1.21-1.46 (m, 14H, CH_2), 1.90-1.92 (m, 8H, CH_2), 2.18-2.32 (m, 4H, CH_2), 2.44 (s, 3H, CH_3), 3.60 (t, 2H, $COCH_2$), 3.96-4.00 (q, 2H, OCH_2), 4.08 (d, 1H, *J=8.6Hz,* C_4-H), 5.20 (d, 1H, *J=8.0Hz,* C_5-H), 7.24 (dd, 2H, Ar-H), 7.62 (dd, 2H, Ar-H). Anal. Calcd. for $C_{28}H_{45}NO_{23}$, C, 75.80; H, 10.22; N, 3.16%; Found: C, 75.88; H, 10.20; N, 3.10%.

***Ethyl 8-(3-(4-fluorophenyl)-4-octyl-4,5-dihydroisoxazol-5-yl)octanoate* 3e:** Obtained from ethyl oleate **1** (2.0 mmol) and 4-fluorobenzaldehyde oxime **2** (2.2 mmol), as a colourless oil in 70% yield. ^{1}H NMR ($CDCl_3$): δ 0.78 (q, 3H, CH_3), 1.15 (t, 3H, CH_3), 1.20-1.52 (m, 14H, CH_2), 1.91-1.93 (m, 8H, CH_2), 2.20-2.32 (m, 4H, CH_2), 3.62 (t, 2H, $COCH_2$), 3.96-4.01(q, 2H, OCH_2), 4.05 (d, 1H, *J=8.0Hz,* C_4-H), 5.25 (d, 1H, *J=8.2Hz,* C_5-H), 7.21 (dd, 2H, Ar-H), 7.65 (dd, 2H, Ar-H). MS (relative abundance) m/z: 448 (M+1, 24), 447 (M^+, 12), 446 (M-1, 28), 432 (22), 311 (M-135, 100), 297 (20), 265 (58), 247 (20). Anal. Calcd. for $C_{27}H_{42}FNO_3$, C, 72.45; H, 9.46; N, 3.13%; Found: C, 72.36; H, 9.31; N, 3.18%.

***Ethyl 8-(3-(4-chlorophenyl)-4-octyl-4,5-dihydroisoxazol-5-yl)octanoate* 3f:** Obtained from ethyl oleate **1** (2.0 mmol) and 4-chlorobenzaldehyde oxime **2** (2.2 mmol), as a colorless oil in 80% yield. ^{1}H NMR ($CDCl_3$): δ 0.81 (q, 3H, CH_3), 1.12 (t, 3H, CH_3), 1.22-1.48 (m, 14H, CH_2), 1.90-1.94 (m, 8H, CH_2), 2.21-2.34 (m, 4H, CH_2), 3.60 (t, 2H, $COCH_2$), 3.96-4.03 (q, 2H, OCH_2), 4.14 (d, 1H, *J=7.8Hz,* C_4-H), 5.21 (d, 1H, *J=8.6Hz,* C_5-H), 7.25 (dd, 2H, Ar-H), 7.61 (dd, 2H, Ar-H). MS (relative abundance) m/z: 465 (M^+, ^{37}Cl, 20), 463 (M^+, ^{35}Cl, 60), 448 (20), 311 (M-152, 100), 297 (28), 265 (62), 247 (18). Anal. Calcd. for $C_{27}H_{42}ClNO_3$, C, 69.88; H, 9.12; N, 3.02%; Found: C, 69.76; H, 9.01; N, 3.12%.

***Ethyl 8-(3-(4-nitrophenyl)-4-octyl-4,5-dihydroisoxazol-5-yl)octanoate* 3g:** Obtained from ethyl oleate **1** (2.0 mmol) and 4-nitrobenzaldehyde oxime **2** (2.2 mmol), as a light yellow oil in 69% yield. ^{1}H NMR ($CDCl_3$): δ 0.88 (q, 3H, CH_3), 1.10 (t, 3H, CH_3), 1.18-1.49 (m, 14H, CH_2), 1.93-1.96 (m, 8H, CH_2), 2.16-2.30 (m, 4H, CH_2), 3.68 (t, 2H, $COCH_2$), 3.99-4.03 (q, 2H, OCH_2), 4.08 (d, 1H, *J=7.7Hz,* C_4-H), 5.28 (d, 1H, *J=8.3Hz,* C_5-H), 8.01 (dd, 2H, Ar-H), 8.28 (dd, 2H, Ar-H). Anal. Calcd. for $C_{27}H_{42}N_2O_5$, C, 68.32; H, 8.92; N, 5.90%; Found: C, 68.21; H, 8.86; N, 5.82%.

The structures of the cycloadducts were provided by spectral studies and elemental analysis. For instance, in ^{1}H NMR spectra, all cycloadducts **3a-g** showed the peaks due to aromatic and substituent protons at the expected region. The consistent pattern signals due to C_4-H appear as doublet in the region δ 4.05-4.16 ppm., while signals due to C_5-H appears as doublet in the region δ 5.18-5.28 ppm. The coupling constant (*J*) values were in range *J=7.7-8.8Hz,* and *J=8.0-9.0Hz* for C_4-H and C_5-H respectively; these values suggests that the reaction leads to form *cis* products.

All the synthesised cycloadducts **3a-g** gave significantly stable molecular ion peaks with a relative abundance ranging from 10-65% and base peak at m/z 311. Further, satisfactorily CHN analysis with a deviation of ±0.10% from the theoretically calculated values observed strongly favor the formation of the cycloadducts.

Antimicrobial activity:

Antifungal activity: Minimum inhibitory concentrations (MICs) of the synthesised compounds **3a-g** were determined against fungal species *C. neoformans, A. niger, A. flavus* and *C. albicans.* The experiments were performed in triplicate; the results are expressed as a mean of three determinations and were summarized in Table-1.

Table 1: MIC's of the test compounds 3a-g against different fungi species

Compound	Minimum inhibitory concentration (MIC)* in µg/mL			
	C. neoformans	*A. niger*	*A. flavus*	*C. albicans*
3a	50	100	-	-
3b	25	50	50	25
3c	25	100	100	50
3d	-	-	200	100
3e	25	50	-	-
3f	12.5	25	25	25
3g	-	-	-	-
Std**	25	50	50	25

*Values are expressed as mean of the three determinations (n=3),
**Amphotericin B was used as reference standard drug

All the synthesised compounds **3a-g** exerted a moderate to good *in vitro* antifungal activity against the tested organisms, except **3g** that contain a strong electron withdrawing nitro substituent failed to exhibit inhibition against all the organisms even at a higher concentration of 200 µg/mL. Similarly, the compounds **3a**, **3e** failed to inhibit the growth of *A. flavus* and *C. albicans* and **3d** failed to inhibit the growth of *C. neoformans* and *A. niger* organisms. The compound **3f** having chloro substitution exhibited remarkable activity against all the organisms tested in comparison with the standard, while **3b** showed good and the remaining compounds exhibited moderate activity.

Antibacterial activity:

The antibacterial activities of the synthesised compounds 3a-g were determined against Gram-negative bacteria species *Escherichia coli, Pseudomonas aeruginosa,* Gram-positive bacteria species *Staphylococcus aureus, Streptococcus pyogenes.* The experiments were performed in triplicate; the results are expressed as a mean of three determinations and were summarized in Table-2.

Table 2: MIC's of the test compounds 3a-g against different bacteria species

Compound	Minimum inhibitory concentration (MIC)* in µg/mL			
	S. aureus	*S. pyogenes*	*E. coli*	*P. aeruginosa*
3a	50	100	-	-
3b	25	100	100	50
3c	25	100	100	50
3d	100	200	100	50
3e	25	50	50	25
3f	12.5	25	25	25
3g	-	-	-	-
Std**	25	50	50	25

*Values are expressed as mean of the three determinations (n=3),
**Ciprofloxacin was used as reference standard drug.

The synthesized compounds **3a-f** showed moderate to good antibacterial activity against the organisms tested. However, the compound **3g** having strong electron withdrawing nitro substitution on the benzene ring failed to inhibit the growth against the all species tested even at a higher concentration of 200 µg/mL. The compound **3a** found inactive against *E. coli* and *P. aeruginosa*. The presence of chloro substitution in compound **3g** influenced it to exhibit inhibition to greater extent against the organisms tested.

Conclusion:

The use of biomolecule ethyl oleate as one of the precursor in organic synthesis validates the significance of this study. The utility of ethyl oleate in the synthesis of novel isoxazolines and their *in vitro* antifungal and antibacterial activity results reveals that the compound ethyl 8-(3-(4-chlorophenyl)-4-octyl-4,5-dihydroisoxazol-5-yl)octanoate **3f** may become an potential antifungal and antibacterial drug. We believe that the insights gained in this study would be useful for the development of potential drug candidates derived from isoxazoline derivatives in the development of novel antimicrobial agents.

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References:

1. Caramella P, Grunanger P. 1, 3-Dipolar Cycloaddition Chemistry, ed. A. Padwa, John Wiley and Sons, New York. 1984; Vol 1:337-345.
2. Pinho EM, Teresa MVD. *Curr. Org. Chem*. 2005; 9:925.
3. Ajay Kumar K, Rai KML, Umesha KB. *J. Chem. Res (S).* 2001:436.
4. Michael R, Howard BB, Ian C, Raymond B, Frances E, Rosemarie M, Shil P, Smita P, Ian R, Stephen BF, Paul DL. *J. Med. Chem*. 1996: 39:1943.
5. Bente F, Anne TJ, Lena T, Tine BS, Henrik TV, Christine E, Uffe K, Connie S, Povl K–L, Tommy L. *J. Med. Chem.* 2002; 45:2454.
6. John JT, David LB, Jeffery SC, Matthew JG, Carol MK, Jaime LM, William EP, Roland SR, Alexander FS, Yan YZ, Ben SZ, Karen S. *J. Med. Chem*. 2000; 43:775.
7. Jayaroopa P, Vasanth Kumar G, Renuka N, Akshatha KN, Mahadevamurthy S, Ajay Kumar K. *Der Pharmacia Lettre*, 2012; 4(6):1685-1691.
8. Li W-T, Hwang D-R, Chen C-P, Shen C-W, Huang C-L, Chen T-W, Lin C-H, Chang Y-L, Chang Y-Y, Lo Y-K, Tseng H-Y, Lin C-C, Song J-S, Chen H-C, Chen S-J, Wu S–H, Chen C-T. *J. Med. Chem.* 2003; 46:1706.
9. Vasanth Kumar G, Jayaroopa P, Bi Bi Ahmadi Khatoon, Mylarappa BN, Ajay Kumar K. *Der Pharma Chemica*, 2012; 4(6): 2283-2287.
10. Jayaroopa P, Vasanth Kumar G, Renuka N, Ajay Kumar K. *IOSR J of App Chem.*, 2012; 1(4): 20-23.
11. Ajay Kumar K, Govindaraju M, Vasanth Kumar G. *Indian J of Heterocycl Chem.*, 2010; 20:183-184.
12. Ajay Kumar K, Lokanatha Rai KM, Umesha KB. *Tetrahedron*, 2001; 57:6993-6996.
13. Vasanth Kumar G, Govindaraju M, Renuka N, Bi Bi Ahmadi Khatoon, Mylarappa BN, Ajay Kumar K. *Rasayan J Chem.*, 2012; 5(3):338-342.
14. Ajay Kumar K, Lokanatha Rai KM, Umesha KB. *Bulg. Chem. Commun.* 2004; 36:249-252.

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