SHORT PAPER

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Synthesis and evaluation of antifungal and antibacterial activity of ethyl 3,5-diarylisoxazole-4-carboxylates†

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Nitrile oxides generated *in situ* by the oxidative dehydrogenation of aldoximes (**2**) react with α-cyanocinnamate esters (**1**) to afford ethyl 3,5-diarylisoxazole-4-carboxylates (**4**) in good yield. The esters (**4**) were tested for their antimicrobial activity.

Keywords: nitrile oxides, cycloaddition, isoxazoles, antifungals, antibacterials

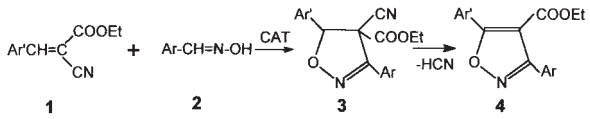
The importance of heterocyclic chemistry in biological systems is well established. Compounds possessing isoxazole and isoxazoline ring system show a variety of biological activities (insecticidal, antibacterial, antibiotic, antitumour, antifungal, *etc*.)[1,2] and they also serve as prodrugs for an antiarthritic agent.[3] Isoxazolines also serve as important building blocks for the synthesis of biologically active molecules.[2] This has prompted us to synthesise new heterocycles and to study their biological activities.

1,3-Dipolar cycloaddition reactions are useful tools for constructing biologically potent five membered heterocycles[2,4] and nitrile oxides serve as excellent 1,3-dipoles. Apart from the usual synthesis of nitrile oxides,[5] Rai *et al*. have developed new methods for generating nitrile oxides involving oxidative dehydrogenation of aldoximes using oxidants such as chloramine-T (CAT)[6] and mercuric acetate.[7] Since nitrile oxides dimerise readily, they are usually generated *in situ* and trapped by dipolarophiles.

The classical method employed for the synthesis of isoxazoles involves 1,3-dipolar cycloaddition reactions of nitrile oxides to alkynes. For instance, the reaction of nitrile oxides with diethyl acetylenedicarboxylate affords diethyl 3-arylisoxazole-4,5-dicarboxylates. In continuation of our work on 1,3-dipoar cycloaddition reactions of nitrile oxides with various dipolarophiles,[8] here we have adopted our own method[7] for converting aldoximes into nitrile oxides and used ethyl α-cyanocinnamates as the alkene part of the dipolarophile with a hope to get more biologically potent heterocycles. The precursor cyanocinnamates were prepared by the reaction of aldehydes with ethyl cyanoacetate.[9]

This paper also describes the synthesis and results of the biological testing of ethyl 3,5-diarylisoxazole-4-carboxylates **4** for their antifungal and antibacterial activities against the Gram negative bacterium *Escherichia coli*, Gram positive bacterium *Bacillus cirroflagellosus*, and the fungi *Aspergillus niger* and *Fusarium poa*. *E. coli*, *B. cirroflagellosus* and *A. niger* cells were found to be resistant to the compounds at the 20μg and 100μg concentrations, while *F. poa* cells were found to be resistant to 100μg concentrations. All compounds showed noticeable antimicrobial activity, which was indicated by the clearing zones present around the discs in comparision with the positive control. The results of the antibacterial and antifungal activity of the synthesised compounds were given in Tables 1 and 2.

In a typical reaction of nitrile oxide with ethyl α-cyanocinnamate, an equimolar mixture of 3,4-dimethoxybenzaldehyde oxime **1**, the α,β-unsaturated compound **2** and chloramine-T trihydrate in ethanol was refluxed on a water bath for 3 hours. The reaction was followed by TLC and continued until the disappearance of starting materials was observed. After the usual work up, the product **4a** was obtained in 68% yield. The ethyl 3,5-diarylisoxazole-4-carboxylate (**4**) may be formed by elimination of HCN from the cycloadduct **3**. Although nitrile oxide addition to a CN group is known to yield a 1,2,4-oxadiazoline with tetracyanoethylene, here the CN group does not undergo cycloaddition, because of its poorer dipolarophile nature compared to the C=C group. This is supported by the cycloaddition of nitrile oxides to acrylonitrile, which form cyano-substituted 2-isoxazolines.[10]



1 **2** **3** **4**

4	Ar	Ar′
a	3,4-$(MeO)_2C_6H_3$	Ph
b	3,4-$(MeO)_2C_6H_3$	4-$MeOC_6H_4$
c	3,4,5-$(MeO)_3C_6H_2$	Ph
d	4-$MeOC_6H_4$	4-ClC_6H_4
e	4-$MeOC_6H_4$	4-$MeOC_6H_4$

IR, ^{1}H NMR, ^{13}C NMR, MS studies and elemental analysis provided the proof of the cycloadduct structures. For instance, in the IR spectra, ethyl 3,5-diarylisoxazole-4-carboxylates **4** showed the bands in the region 1650–1675 cm^{-1} and 1715–1725 cm^{-1} for C=N group and ester group respectively, while the band in the region 2220–2240 cm^{-1} for a nitrile group was absent, which is a clear indication of the loss of the CN group as HCN from the intermediate **3** to form the cycloadduct **4**.

In ^{1}H NMR spectra, all ethyl 3,5-diarylisoxazole-4-carboxylates showed the peaks due to aromatic and substituent protons in the expected regions. The peak expected in the region δ 5.0–5.5 ppm[10] for a CH proton was absent. This supports the proton loss in the form of HCN. It may be supposed that the sulfonamide base formed in the reaction abstracts the proton to form a carbanion, which in turn helps in the loss of CN ion to form the more stable isoxazoles rather than the expected isoxazolines.

In ^{13}C NMR, all isoxazoles gave the signals due to aromatic and substituent carbons at the expected region. The signals

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† This is a Short Paper, there is therefore no corresponding material in *J Chem. Research (M)*.

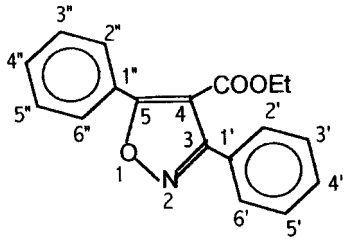

Fig. 1. Compounds **4**, showing numbering

due to newly formed C_3-carbon appeared in the region δ_c 155.4–157.6 ppm, while, C_4 and C_5 carbon showed the signals in the region δ_c 140.6–143.1 and 151.1–153.0 ppm respectively (Fig. 1). All isoxazoles gave significantly stable MH^+ ion peaks with a relative abundance ranging from 16 to 52% and base peak at (MH^+-73) most probably by the loss of $C_2H_5^+$ ion and CO_2 molecule, which strongly favours the formation of the products. The heteroatoms containing compounds have been known to have a high cross section for the formation of MH^+ (M+1) peaks particularly when the parent compound fails to register a molecular ion.[10,11]

Experimental

Melting points were taken in open capillaries using a Thomas Hoover melting point apparatus and are uncorrected. The compounds were routinely checked for their purity by TLC using silica gel G. IR spectra were recorded in nujol mulls on a Shimadzu 8300 spectrometer. ^{1}H NMR spectra were recorded either on a Bruker 300 MHz or Jeol 60 MHz Hitachi Perkin Elmer spectrometer in $CDCl_3$ solution. Tetramethylsilane (TMS) was used as an internal standard and the chemical shifts are expressed in ppm (δ). The ^{13}C NMR spectra were measured on Jeol GSX 400 (100 MHz) instrument and the values are in parts per million downfield from the tetramethyl silane. For assignment numbering, see Fig. 1. Mass spectra were obtained on an electron impact Maspec MSW 9629 spectrometer and the important fragments are given with the relative intensities in brackets. Thin layer chromatograms were obtained on silica gel (HF, 254, SD Fine chem.) coated on glass slides. Chromatographic separations were carried out on a silica gel (70–230 mesh, Merck.) column using benzene:ethyl acetate::8:2 as eluant.

The antimicrobial activity of the synthesised compounds was determined by the paper disc diffusion assay,[12,13] Briefly, a lawn of the organism was prepared by spreading 50 ml of overnight cultures (conc. 10^6~10^7 CFU/ml) onto agar set in petri dishes. LB agar medium was used for the bacteria made by adding 1.5 g of agar, 1 g of NaCl, 1 g of tryptone and 0.5 g of yeast extract to 100 ml of water (pH = 7.4). Sterile filter paper discs containing the required concentration of the compounds were placed at equal distances on the agar plate. Control experiments were performed with equivalent volumes of the solvents without the test compounds. Standard materials at a concentration of 2 μg was used as the positive control. The plates were incubated at 37°C for 24 h to check clearing zones around the discs.

General procedure for cycloaddition

Preparation of ethyl 3-(3′,4′-dimethoxyphenyl)-5-phenylisoxazole-4-carboxylate (**4a**): A mixture of 3,4-dimethoxybenzaldehyde oxime (**1**) (0.72g, 4.0mmol), ethyl α-cyanocinnamate (**2**) (0.80g, 4.0mmol) and chloramine-T trihydrate (1.12g, 4.0mmol) was warmed on a water bath for 3 hours. After the reaction, it was cooled to room temperature. The sodium chloride formed in the reaction mixture was filtered off and washed with ethanol (1 × 15ml). The combined filtrate and washings were evaporated in vacuo. The residual part was extracted into ether (25ml), washed successively with water (2 × 15ml), 10% sodium hydroxide (2 × 15ml) and saturated brine solution (1 × 10ml). The organic layer was dried over anhydrous sodium sulfate. Evaporation of the solvent yielded the white solid, which gave one major spot corresponding to the product and three minor spots related to the unreacted starting materials and was purified by column chromatography using benzene : chloroform (8 : 2) as eluent. The prodct was recrystallised from chloroform : petroleum ether (1 : 5 v/v) as a white crystalline solid **4a** (0.96 g, 68%), m.p. 106–108 °C. ^{1}H NMR ($CDCl_3$): δ 1.25 (t, 3H, CH_3), 3.85 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 4.1 (q, 2H, OCH_2), 7.30–7.38 (m, 3H, Ar-H), 7.43–7.55 (m, 5H, Ar'-H). ^{13}C NMR ($CDCl_3$): δ 20.3 (CH_3), 55.4 (4′–OCH_3), 56.9 (3′-OCH_3), 62.6 (CH_2), 98.6 (3′-C), 105.3 (5′-C), 121.1 (1″-C), 122.0 (1′-C), 127.0 (3″,5″-C), 128.9 (2″,6″-C), 130.5 (6'-C), 131.3 (4″-C), 141.2 (4-C), 146.1 (2'C), 152.4 (5-C), 155.8 (4'-C) 156.7 (3-C), 167.9 (CO); MS (relative abundance) *m/z*: 354(MH^+, 32), 325(21), 281(100), 251(41), 221(52). Anal. Calc. for $C_{20}H_{19}NO_5$: C, 67.98; H, 5.38; N, 3.96%; Found: C, 67.92; H, 5.29; N, 3.90%.

Ethyl 3-(3′,4′-dimethoxyphenyl)-5-(4″-methoxyphenyl)isoxazole-4-carboxylate (**4b**)**:** this was obtained from 3,4-dimethoxybenzaldehyde oxime (0.72g, 4.0mmol) and the ethyl α-cyanocinnamate derivative (0.92g, 4.0mmol) as a white solid (1.07g, 71%). m.p. 74–78 °C. ^{1}H NMR ($CDCl_3$): δ 1.22 (t, 3H, CH_3), 3.89 (s, 6H, OCH_3), 3.91 (s, 3H, OCH_3), 4.14 (q, 2H, OCH_2), 6.98 (d, 2H, 3″,5″-H), 7.32–7.42 (m, 3H, Ar-H), 7.59 (d, 2H, 2″,6″-H). ^{13}C NMR ($CDCl_3$): δ 20.1 (CH_3), 55.4 (3′-OCH_3), 57.8 (4'-OCH_3), 58.1 (4″-OCH_3), 61.8 (CH_2), 97.9 (3′-C), 104.8 (5′-C), 114.3 (3″,5″-C), 121.6 (1″-C), 122.4 (1′-C), 128.8 (2″,6″-C), 130.3 (6′-C), 140.9 (4-C), 145.8 (2'C), 153.0 (5-C), 155.8 (4,4″-C) 156.8 (3-C), 168.0 (CO); MS (relative abundance) *m/z*: 384(MH^+, 28), 355(18), 311(100), 281(36), 251(40), 221(51). Anal. Calc. for $C_{21}H_{21}NO_6$: C, 65.79; H, 5.48; N, 3.65%; Found: C, 65.74; H, 5.45; N, 3.60%.

Ethyl 3-(3′,4′,5′-trimethoxyphenyl)-5-phenylisoxazole-4-carboxylate (**4c**)**:** Obtained from 3,4,5-trimethoxybenzaldehyde oxime (0.83g, 4.0mmol) and ethyl α-cyanocinnamate (0.80g, 4.0mmol) as a light brown gummy mass (0.79 g, 52%). ^{1}H NMR ($CDCl_3$): δ 1.36 (t, 3H, CH_3), 3.94 (s, 9H, OCH_3), 4.21 (q, 2H, OCH_2), 6.93 (s, 2H, 2',6'-H), 7.41–7.46 (m, 5H, Ar″-H). ^{13}C NMR ($CDCl_3$): δ 20.2 (CH_3), 56.1 (3″,5″,-OCH_3), 60.7 (4″-OCH_3), 62.3 (CH_2), 104.3 (2′,6′-C), 123.4 (1'-C), 127.1 (3″,5″-C), 128.6 (2″,6″-C), 140.5 (3′,5′-C), 140.6 (4-C), 151.1 (5-C), 153.2 (4′-C), 155.9 (3-C), 167.1 (CO); MS (relative abundance) *m/z*: 384(MH^+, 24), 355(16),

Table 1 Antibacterial activity of isoxazoles

Compound	*E. coli*				*B. cirroflagellosus*			
	Area of zone of inhibition mm²		Relative percentage inhibition		Area of zone of inhibition mm²		Relative percentage inhibition	
	Concentration		Concentration		Concentration		Concentration	
	5μg	100μg	5μg	100μg	20μg	100μg	20μg	100μg
4a	130.6	124.6	34.8	30.2	183.8	113.0	33.4	5.7
4b	124.6	122.7	30.2	28.7	206.1	203.5	42.3	41.2
4c	126.6	130.6	32.3	34.8	172.0	226.9	28.8	50.4
4d	138.9	147.4	41.0	47.5	153.9	158.3	21.7	23.4
4e	149.5	143.1	49.1	44.2	165.1	160.6	26.1	24.3
Standard	216.4[a]				353.3[b]			
Solvent	84.9[c]				98.5[d]			

[a]Ampicillin; [b]cotrimoxazole; [c]ethanol; [d]DMF.

Table 2 Antifungal activity of isoxazoles

Compound	*A. niger*				*F. poa*	
	Area of zone of inhibition mm²		Relative percentage inhibition		Area of zone of inhibition mm²	Relative percentage inhibition
	Concentration		Concentration		Concentration	Concentration
	20µg	100µg	20µg	100µg	100µg	100µg
4a	265.9	265.9	47.7	47.7	224.3	58.3
4b	277.5	289.5	51.0	47.7	246.0	68.3
4c	311.0	304.8	60.4	58.6	181.4	38.4
4d	326.8	292.5	64.8	55.5	193.5	44.0
4e	323.6	326.8	63.9	64.8	274.6	81.6
Standard	452.3[a]				314.3[b]	
Solvent	95.3[c]				98.5[c]	

[a]Ampicillin; [b]cotrimoxazole; [c]DMF.

311(100), 281(26), 251(32), 221(48). Anal. Calc. for $C_{21}H_{21}NO_6$: C, 65.79; H, 5.48; N, 3.65%; Found: C, 65,72; H, 5.44; N, 3.61%.

Ethyl 3-(4′-methoxyphenyl)-5-(4″-chlorophenyl)isoxazole-4-carboxylate (**4d**): Obtained from 4-methoxybenzaldehyde oxime (0.60g, 4.0mmol) and the ethyl α-cyanocinnamate derivative **2** (0.93g, 4.0mmol) as a colourless crystalline solid (0.68g, 48%), m.p. 174–176 °C. ^{1}H NMR ($CDCl_3$): (δ) 1.30 (t, 3H, CH_3), 3.91 (s, 3H, OCH_3), 4.26 (q, 2H, OCH_2), 6.94 (d, 2H, 3′,5″-H), 7.00 (d, 2H, 3′,5′-H), 7.45 (d, 2H, 2″,6″-H), 7.56 (d, 2H, 2′,6’-H). ^{13}C NMR ($CDCl_3$): δ 20.6 (CH_3), 55.3 (4′-OCH_3), 62.1 (CH_2), 113.6 (3″,5″,-OCH_3), 114.8 (3′,5′-C), 121.8 (1′,1″-C), 128.6 (2’,6’-C), 128.9 (2″,6″-C), 143.1 (4-C), 152.6 (5-C), 155.3 (4′-C), 157.6 (3-C), 158.1 (4″-C), 168.1 (CO); MS (relative abundance) *m/z*: 360(MH^+, ^{37}Cl, 08), 358(^{35}Cl, 22), 329(14), 285(100), 255 (28). Anal. Calc. for $C_{19}H_{16}ClNO_4$: C, 63.86; H, 4.48; N, 3.59%; Found: C, 63.81; H, 4.43; N, 3.54%.

Ethyl 3-(4′-methoxyphenyl)-5-(4″-methoxyphenyl)isoxazole-4-carboxylate (**4e**): Obtained from 4-methoxybenzaldehyde oxime (0.60g, 4.0mmol) and the ethyl α-cyanocinnamate derivative **2** (0.92g, 4.0mmol) as a white crystalline solid (0.78g, 54%), m.p. 92–94 °C. ^{1}H NMR ($CDCl_3$): δ 1.32 (t, 3H, CH_3), 3.86 (s, 6H, OCH_3), 4.18 (q, 2H, OCH_2), 6.96 (dd, 4H, 3,5-Ar,Ar′-H), 7.52 (dd, 4H, 2,6-Ar,Ar′-H). ^{13}C NMR ($CDCl_3$): δ 20.9 (CH_3), 55.6 (4′,4″-OCH_3), 62.1 (CH_2), 114.2 (3′,5′-C), 114.8 (3″,5″-C), 121.0 (1’-C), 121.6 (1″-C), 128.4 (2′,6′-C), 128.8 (2″,6″-C), 142.6 (4-C), 151.9 (5-C), 155.4 (3-C), 167.9 (CO); MS (relative abundance) *m/z*: 354(MH^+, 28), 325(18), 281(100), 251(40), 221(54). Anal. Calc. for $C_{20}H_{19}NO_5$: C, 67.98; H, 5.38; N, 3.96%; Found: C, 67.94; H, 5.33; N, 3.94%.

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