

Synthesis and evaluation of antimicrobial activity of a new series of bis(isoxazoline) derivatives

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Abstract

A series of ether-linked bis(isoxazoline) derivatives **4** were prepared by 1,3-dipolar cycloaddition reactions of nitrile oxides with allyl alcohol and allyl ethers. Products **4** were characterized by IR, NMR, and elemental analysis and were evaluated for their antimicrobial activity.

Keywords: Bis-isoxazoline, 1,3-dipolar cycloaddition, chloramine-T, antimicrobial activity

Introduction

Among five-membered heterocycles, isoxazolines represent a class of compounds of great biological importance. For instance, isoxazolines possess a broad spectrum of biological activity^{1,2} (insecticidal, antibacterial, antibiotic, antitumour, antifungal, etc). Isoxazoline also serves as an important building block for the synthesis of biologically active molecules² and serves as a prodrug for an antiarthritic agent.³ In fact, Valdecoxib is an isoxazoline derivative, now widely used in the market as an anti-inflammatory drug.⁴ Literature studies reveal that bis-heterocycles bearing isoxazoline⁵ or pyrazoline⁶ were synthesized via 1,3-dipolar cycloaddition of aldoxime / aldehyde hydrazone to divinyl ketone / sulfone using chloramine-T as a dehydrating agent.

1,3-Dipolar cycloaddition reactions are useful tools for the construction of biologically potent five-membered heterocycles,² and nitrile oxides serve as excellent 1,3-dipoles. Cycloaddition of nitrile oxide to olefinic compounds are of synthetic interest, since the resulting isoxazolines are versatile intermediates for the synthesis of bifunctional compounds.⁷ Nitrile oxides can be generated by dehydrogenation of aryl aldoximes with mercuric acetate,⁸ manganese dioxide,⁹ *tert*-butyl hypochlorite,¹⁰ chloramine-T¹¹ etc. In our laboratory,¹¹ we used chloramine-T extensively for the generation of nitrile oxides and nitrile imines from aldoximes and aldehyde hydrazones, respectively. Recently, Rai *et al.* used chloramine-T for the generation and cycloaddition of α -nitrosoolefin¹² and α -azoalkenes¹³ from ketoximes and ketone hydrazones,

respectively. With this background, it was considered worthwhile to prepare bis(isoxazoline) derivatives starting from simple allyl alcohols and screen them for antimicrobial activity. The present communication reports on the synthesis of ether-linked bis(isoxazoline) derivatives via 1,3-dipolar cycloaddition reactions and the evaluation of their antimicrobial activity.

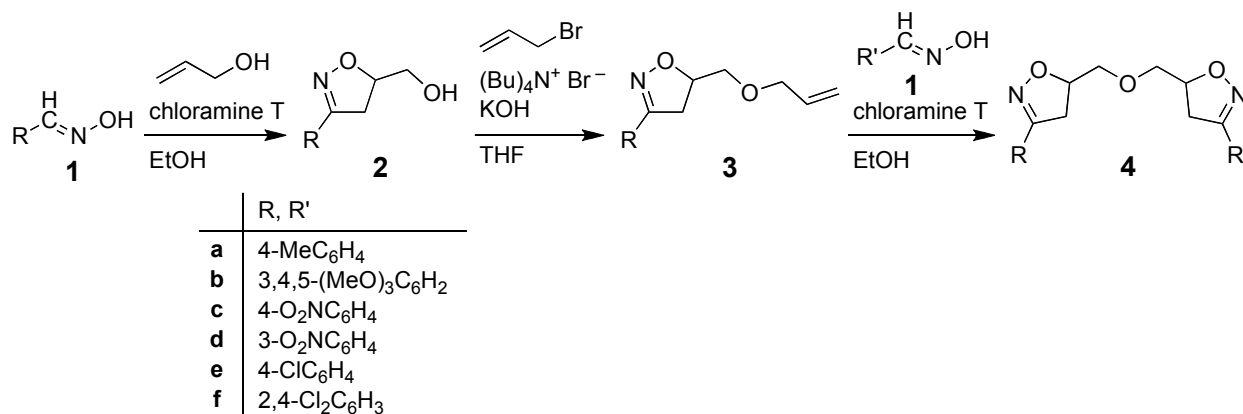
Results and Discussion

Synthesis

The starting materials, aldoximes **1** were prepared from the corresponding aldehydes employing known methods.¹⁴ Oxidative dehydrogenation of aromatic aldoximes **1a–d** by chloramine-T afforded nitrile oxides, which were intercepted *in situ* by allyl alcohol in refluxing ethanol. The pale yellow oils obtained were identified by NMR spectroscopy as (3-aryl-4,5-dihydroisoxazol-5-yl)methanols **2a–d** (Scheme 1).

Compound **2a** exhibits a multiplet at δ 4.92–5.10 assigned to 5-H of the isoxazoline ring. Two doublets of doublet at δ 3.25 and δ 3.57 correspond to 4-CH_AH_B. A multiplet at δ 3.88–3.98 and a broad signal at δ 3.12 correspond to the CH₂OH moiety. Thus, the formation of **2** indicates a regioselective reaction.

By stirring the hydroxymethyl compounds **2** with excess of allyl bromide in the presence of tetrabutylammonium bromide (used as phase transfer catalyst) and potassium hydroxide, 5-allyloxymethyl-4,5-dihydroisoxazoles **3a–d** were prepared as yellow oils. Compound **3a** exhibits two multiplets at δ 5.26–5.37 and at δ 5.65–5.81 corresponding to the vinylic CH₂ and CH groups, respectively. Two multiplets at δ 3.71–3.82 and 4.16–4.28 correspond to the CH₂O and allylic CH₂O groups, respectively.



Scheme 1

The olefinic group in **3** was used to form another isoxazoline ring. Refluxing of **3a–d** with **1a–f** in the presence of chloramine-T for 3 h gave yellow oils of 3-aryl-substituted 5-[[[4,5-

dihydroisoxazol-5-yl)methoxy]methyl]-4,5-dihydroisoxazoles **4**, characterized by IR, NMR, and elemental analyses. In addition to the aromatic and substituent signals, the ^1H NMR spectrum of the cycloadduct **4ab** exhibits signals of the isoxazoline CH_2 and methine groups at δ 3.26–3.55 and at δ 4.93–5.10, respectively; the OCH_2 signals are displayed at δ 3.8–4.1. The ^{13}C NMR of **4ab** exhibits the signals of the CH and CH_2 groups in the isoxazoline ring at δ 37.7 and at δ 75.5, respectively; the OCH_2 carbons resonate at 78.9. The IR confirms the absence of $\text{C}=\text{C}$ and OH groups.

Antimicrobial activity

Compounds **4** were tested for antimicrobial activity against various strains. As standards the following were used: streptomycin and tetracycline against bacteria, and Nystatin against fungi. All tests were performed in triplicate, and the average is reported. Five bacteria and five fungal species were used as the antimicrobial test strains namely: *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas fluorescens*, *Xanthomonas campestris pvs*, *Xanthomonas oryzae*, *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporium*, *Trichoderma species*, *Fusarium and monaliforme*. The bacterial strains were maintained on the LB agar medium and the filamentous fungi were maintained on potato dextrose agar (PDA) medium at 28 °C. The agar disk diffusion method¹⁵ was used to test antimicrobial activity using potato dextrose agar medium.

Conclusions

Ether-linked bis(isoxazoline) derivatives **4** were synthesized and tested for antimicrobial activity by disc diffusion and micro dilution methods, showing moderate to potent inhibition.

Experimental Section

Chemistry

General Procedures. ^1H NMR spectra were recorded on a Bruker AM 300 MHz spectrometer using CDCl_3 as solvent and tetramethylsilane as internal standard. ^{13}C NMR spectra were measured on Jeol 400 (100 MHz) instrument. Elemental analyses were obtained on a Vaio-EL instrument. Thinlayer chromatography (TLC) was carried out with pre-coated silica gel G plates.

[4,5-Dihydro-3-(*p*-tolyl)isoxazol-5-yl]methanol (**2a**). Typical procedure

A mixture of **1a** (1.0 g, 7.40 mmol) and chloramine-T trihydrate (2.08 g, 7.41 mmol) in ethanol (20 mL) was stirred at room temperature for 5 min. To this mixture, allyl alcohol (0.43 g, 7.41 mmol) in ethanol (5 mL) was added and the reaction mixture was heated on a water bath for 3 h. After completion of the reaction (monitored by TLC) the reaction mixture was cooled to room temperature. Sodium chloride formed was filtered off and washed with ethanol (15 mL). Filtrate

and washing were combined and evaporated in vacuum. The residue was extracted with ether (25 mL), the ether extract was washed successively with water (2 × 15 mL), 5% NaOH (2 × 15 mL), and saturated brine solution (10 mL). The organic layer was dried over anhydrous sodium sulphate. After evaporation of the solvent the product was purified by the column chromatography using the chloroform/acetone (9:1) as eluent, and a yellow oil **2a** was obtained (1.07 g, 76%). ¹H NMR (300 MHz, CDCl₃): δ 2.34 (s, 3H, CH₃), 3.12 (br, 1H, OH), 3.25 (dd, *J* = 8.0, 2.0 Hz, 1H, 4-CH_A), 3.57 (dd, *J* = 8.0, 2.0 Hz, 1H, 4-CH_B), 3.88–3.98 (m, 2H, OCH₂), 4.92–5.10 (m, 1H, 5-CH), 7.28–7.69 (m 4H, H_{Ar}). ¹³C NMR (100 MHz, CDCl₃): δ 24.7 (CH₃), 37.4 (CH₂), 70.9 (CH₂), 77.5 (CH), 129.2–129.4 (4 CH), 131.4 (C), 141.3 (C), 156.8 (C). Anal. Calcd. for C₁₁H₁₃NO₂: C, 69.09; H, 6.85; N, 7.32. Found: C, 69.15, H, 6.93, N, 7.30.

[4,5-Dihydro-3-(3,4,5-trimethoxyphenyl)isoxazol-5-yl]methanol (2b). From **1b** (1.0 g, 4.74 mmol), chloramine-T (1.33 g, 4.74 mmol) and allyl alcohol (0.275, 4.74 mmol): Yellow oil **2b** (0.97 g, 76%), *R_f* 0.32 (chloroform/acetone, 9:1). ¹H NMR (300 MHz, CDCl₃): δ 3.14 (br, 1H, OH), 3.23 (dd, *J* = 8.0, 4.0 Hz, 1H, 4-CH_A), 3.56 (dd, *J* = 8.0, 4.0 Hz, 1H, 4-CH_B), 3.85–3.96 (m, 2H, OCH₂), 3.90 (s, 6H, OCH₃), 3.93 (s, 3H, OCH₃), 4.98–5.10 (m, 1H, 5-CH), 6.83 (s, 2H, H_{Ar}). ¹³C NMR (100 MHz, CDCl₃): δ 36.7 (CH₂), 56.4 (2 OCH₃), 56.6 (OCH₃), 70.6 (CH₂), 77.2 (CH), 106.7 (2 CH), 128.2 (C), 141.3 (C), 150.8 (2C) 156.1 (C). Anal. Calcd. for C₁₃H₁₇NO₅: C, 58.42; H, 6.41; N, 5.24. Found: C, 58.44; H, 6.45, N, 5.22.

[4,5-Dihydro-3-(4-nitrophenyl)isoxazol-5-yl]methanol (2c). From **1c** (1.0 g, 6.02 mmol), chloramine-T (1.70 g, 6.04 mmol) and allyl alcohol (0.35 g, 6.03 mmol): Yellow oil **2c** (0.95 g, 71%) *R_f* 0.32 (chloroform/acetone, 9:1). ¹H NMR (300 MHz, CDCl₃): δ 3.19 (br, 1H, OH), 3.29 (dd, *J* = 8.0, 3.0 Hz, 1H, 4-CH_A), 3.60 (dd, *J* = 8.0, 3.0 Hz, 1H, 4-CH_B), 3.87–3.95 (m, 2H, OCH₂), 5.09–5.21 (m, 1H, 5-CH), 7.80–8.19 (4H, H_{Ar}). ¹³C NMR (100 MHz, CDCl₃): δ 37.2 (CH₂), 70.6 (CH₂), 77.4 (CH), 121.3 (2 CH), 130.2 (2 CH), 140.3 (C), 150.9 (C), 156.4 (C). Anal. Calcd. for C₁₀H₁₀N₂O₄: C, 54.05; H, 4.54; N, 12.61. Found: C, 54.02; H, 4.55, N, 12.62.

[4,5-Dihydro-3-(3-nitrophenyl)isoxazol-5-yl]methanol (2d). From **1d** (1.0 g, 6.02 mmol), chloramine-T (1.70 g, 6.04 mmol) and allyl alcohol (0.35 g, 6.03 mmol): yellow oil **2d** (0.9 g, 67%), *R_f* 0.36 (chloroform/acetone, 9:1). ¹H NMR (300 MHz, CDCl₃): δ 3.18 (br, 1H, OH), 3.28 (dd, *J* = 8.0, 4.0 Hz, 1H, 4-CH_A), 3.61 (dd, *J* = 8.0, 3.0 Hz, 1H, 4-CH_B), 3.89–3.99 (m, 2H, OCH₂), 5.11–5.20 (m, 1H, 5-CH), 7.67–8.60 (m, 4H, H_{Ar}). ¹³C NMR (100 MHz, CDCl₃): δ 37.1 (CH₂), 70.4 (CH₂), 77.2 (CH), 123.3 (CH), 124.1 (CH), 129.9 (CH), 134.7 (C), 135.3 (CH), 148.6 (C), 156.5 (C). Anal. Calcd. for C₁₀H₁₀N₂O₄: C, 54.05; H, 4.54; N, 12.61. Found: C, 54.02; H, 4.55, N, 12.62.

5-[(Allyloxy)methyl]-4,5-dihydro-3-(*p*-tolyl)isoxazole (3a). Typical procedure

A mixture of **2a** (1.0 g, 5.23 mmol), allyl bromide (0.63 g, 5.25 mmol), tetrabutylammonium bromide (0.17 g, 0.53 mmol) and potassium hydroxide (0.29 g, 5.18) was stirred overnight in THF (15 mL). After completion of the reaction (monitored by TLC), the solvent was evaporated under vacuum, the residue was extracted with ethyl acetate (2 x 15 mL), washed with water (2 x 10 mL) and dried (Na₂SO₄). The solvent was evaporated, and the residue was subjected to

column chromatography (chloroform/benzene, 8:2) to give a yellow oil **3a** (0.98 g, 81%). ¹H NMR (300 MHz, CDCl₃): δ 2.38 (s, 3H, CH₃), 3.23 (dd, *J* = 8.0, 4.0 Hz, 1H, 4-CH_A), 3.55 (dd, *J* = 8.0, 3.0 Hz, 1H, 4-CH_B), 3.71–3.82 (m, 2H, OCH₂), 4.16–4.28 (m, 2H, OCH₂), 4.98–5.10 (m, 1H, 5-CH), 5.26–5.37 (m, 2H, =CH₂), 5.65–5.81 (m, 1H, =CH), 7.12–7.45 (m, 4H, H_{Ar}). ¹³C NMR (100 MHz, CDCl₃): δ 24.7 (CH₃), 37.7 (CH₂), 74.9 (CH₂), 75.5 (CH), 78.9 (CH₂), 115.8 (CH₂), 129.1–129.3 (4 CH), 131.2 (C), 141.2 (C), 151.7 (CH), 156.6 (C). Anal. Calcd. for C₁₄H₁₇NO₂: C, 72.70; H, 7.41; N, 6.06. Found: C, 72.72, H, 7.40, N, 6.07.

5-[(Allyloxy)methyl]-4,5-dihydro-3-(3,4,5-trimethoxyphenyl)isoxazole (3b). From **2b** (1.0 g, 3.74 mmol), allyl bromide (0.45 g, 3.75 mmol) and tetrabutylammonium bromide (0.12 g, 0.37 mmol) and potassium hydroxide (0.21 g, 3.75 mmol): Pale yellow oil (0.84 g, 73%) *R_f* 0.47 (chloroform/benzene, 8:2). ¹H NMR (300 MHz, CDCl₃): δ 3.22 (dd, *J* = 8.0, 3.0 Hz, 1H, 4-CH_A), 3.54 (dd, *J* = 9.0, 3.0 Hz, 1H, 4-CH_B), 3.68–3.81 (m, 2H, OCH₂), 3.88 (s, 6H, OCH₃), 3.93 (s, 3H, OCH₃), 4.10–4.26 (m, 2H, OCH₂), 4.97–5.08 (m, 1H, 5-CH), 5.21–5.34 (m, 2H, =CH₂), 5.61–5.77 (m, 1H, =CH), 6.84 (s, 2H, H_{Ar}). ¹³C NMR (100 MHz, CDCl₃): δ 37.5 (CH₂), 56.3 (2 OCH₃), 56.6 (OCH₃), 74.8 (CH₂), 75.5 (CH), 78.7 (CH₂), 115.7 (CH₂), 106.6 (2 CH), 128.2 (C), 131.4 (C), 141.4 (C), 151.3 (2C), 156.4 (C). Anal. Calcd. for C₁₆H₂₁NO₅: C, 62.53; H, 6.89; N, 4.56. Found: C, 62.52, H, 6.84, N, 4.57.

5-[(Allyloxy)methyl]-4,5-dihydro-3-(4-nitrophenyl)isoxazole (3c). From **2c** (1.0 g, 4.5 mmol), allyl bromide (0.54 g, 4.5 mmol) and tetrabutylammonium bromide (0.15 g, 0.45 mmol) and potassium hydroxide (0.25 g, 4.55 mmol): Pale yellow oil **3c** (0.82 g, 70%) *R_f* 0.46 (chloroform-benzene, 8:2). ¹H NMR (300 MHz, CDCl₃): δ 3.27 (dd, *J* = 8.0–2.0 Hz, 1H, 4-CH_A), 3.56 (dd, *J* = 8.0, 2.0 Hz, 1H, 4-CH_B), 3.70–3.83 (m, 2H, OCH₂), 4.13–4.28 (m, 2H, OCH₂), 5.08–5.18 (m, 1H, 5-CH), 5.25–5.35 (m, 2H, =CH₂), 5.64–5.79 (m, 1H, =CH), 7.78–8.21 (m, 4H, H_{Ar}). ¹³C NMR (100 MHz, CDCl₃): δ 37.7 (CH₂), 74.9 (CH₂), 75.6 (CH), 78.8 (CH₂), 115.9 (CH₂), 121.3 (2 CH), 130.2 (2C), 131.8 (C), 140.4 (C), 151.5 (C), 156.5 (C). Anal. Calcd. for C₁₃H₁₄N₂O₄: C, 59.54; H, 5.38; N, 10.68. Found: C, 59.56, H, 5.36, N, 10.69.

5-[(Allyloxy)methyl]-4,5-dihydro-3-(3-nitrophenyl)isoxazole (3d). From **2d** (1.0 g, 4.5 mmol), allyl bromide (0.54 g, 4.5 mmol) and tetrabutylammonium bromide (0.15 g, 0.45 mmol) and potassium hydroxide (0.25 g, 4.55 mmol) as pale yellow oil in (0.8 g, 68% yield) *R_f* 0.50 (chloroform-benzene, 8:2). ¹H NMR (300 MHz, CDCl₃): δ 3.27 (dd, *J* = 7.0, 2.0 Hz, 1H, 4-CH_A), 3.56 (dd, *J* = 8.0, 2.0, 1H, 4-CH_B), 3.70–3.83 (m, 2H, OCH₂), 4.13–4.28 (m, 2H, OCH₂), 5.07–5.18 (m, 1H, CH), 5.25–5.35 (m, 2H, =CH₂), 5.64–5.79 (m, 1H, =CH), 7.68–8.31 (m, 4H, H_{Ar}). ¹³C NMR (100 MHz, CDCl₃): δ 37.7 (CH₂), 74.9 (CH₂), 75.6 (CH), 78.8 (CH₂), 115.9 (CH₂), 121.3 (2 CH), 130.2 (2C), 131.8 (C), 140.4 (C), 151.5 (C), 156.5 (C); Anal. Calcd. for C₁₃H₁₄N₂O₄: C, 59.54; H, 5.38; N, 10.68. Found: C, 59.56, H, 5.36, N, 10.69.

5-[[[(4,5-Dihydro-3-(3,4,5-trimethoxyphenyl)isoxazol-5-yl)methoxy]methyl]-4,5-dihydro-3-(*p*-tolyl)isoxazole (4ab). Typical procedure

A mixture of **3a** (1.0 g, 4.33 mmol), **1b** (0.91g, 4.31 mmol) and chloramine-T trihydrate (1.22 g, 4.34 mmol) in ethanol (20 mL) was heated on a water bath. After 3 h the reaction was

completed, and the mixture was cooled to room temperature. Sodium chloride formed was filtered off and washed with ethanol (15 mL). Filtrate and washing were combined and the solvent was evaporated in vacuum. The residue was extracted with ether (25 mL), the extract was washed successively with water (2 × 15 mL), 10% NaOH (2 × 15 mL), and saturated brine solution (10 mL). The organic layer was dried over anhydrous sodium sulphate. Evaporation of the solvent yielded the product, which was subjected to column chromatography using chloroform as eluent to give a yellow oil **4ab** (1.34 g, 68%), R_f 0.65 (chloroform). $^1\text{H NMR}$ (300 MHz, CDCl_3): δ 2.37 (s, 3H, CH_3), 3.26–3.55 (m, 4H, 4-, 4'- CH_2), 3.8–4.1 (m, 4H, OCH_2), 3.90 (s, 6H, OCH_3), 3.95 (s, 3H, OCH_3), 4.93–5.10 (m, 2H, 5-, 5'-CH), 6.90 (s, 2H, H_{Ar}), 7.26–7.67 (m, 4H, H_{Ar}). $^{13}\text{C NMR}$ (100 MHz, CDCl_3): δ 24.7 (CH_3), 37.7 (2 CH_2), 56.2 (2 OCH_3), 56.5 (OCH_3), 75.5 (2 CH), 78.9 (2 CH_2), 106.9 (2 CH), 128.6 (C), 129.2–129.4 (4 CH), 131.2 (C), 141.1 (C), 141.7 (C), 151.3 (2 C), 156.9 (2 C). Anal. Calcd. for $\text{C}_{24}\text{H}_{28}\text{N}_2\text{O}_6$: C, 65.44; H, 6.41; N, 6.36. Found: C, 65.45, H, 6.43, N, 6.33.

5-[[[4,5-Dihydro-3-(4-nitrophenyl)isoxazol-5-yl]methoxy]methyl]-4,5-dihydro-3-(*p*-tolyl)-isoxazole (4ac). From **3a** (0.5 g, 2.16 mmol), **1c** (0.36 g, 2.16 mmol) and chloramine-T trihydrate (0.62 g, 2.20 mmol): Yellow oil **4ac** (0.61 g, 69%), R_f 0.60 (chloroform). $^1\text{H NMR}$ (300 MHz, CDCl_3): δ 2.37 (s, 3H, CH_3), 3.20–3.55 (m, 4H, 4-, 4'- CH_2), 3.8–4.1 (m, 4H, OCH_2), 5.0 (m, 2H, 5-, 5'-CH), 7.24–8.11 (m, 8H, H_{Ar}). $^{13}\text{C NMR}$ (100 MHz, CDCl_3): δ 24.5 (CH_3), 37.5 (2 CH_2), 75.3 (2 CH), 78.7 (2 CH_2), 121.7 (2 CH), 129.2–129.3 (4 CH), 129.6 (2 CH), 131.1 (C), 140.3 (C), 140.9 (C), 151.1 (C), 156.8 (2C). Anal. Calcd. for $\text{C}_{21}\text{H}_{21}\text{N}_3\text{O}_5$: C, 63.79; H, 5.35; N, 10.63. Found: C, 63.73, H, 5.39, N, 10.53.

5-[[[4,5-dihydro-3-(*p*-tolyl)isoxazol-5-yl]methoxy]methyl]-3-(4-chlorophenyl)-4,5-dihydroisoxazole (4ae). From **3a** (0.5 g, 2.16 mmol), **1e** (0.34 g, 2.19 mmol) and chloramine-T trihydrate (0.62 g, 2.20 mmol): Yellow oil **4ae** (0.66 g, 76%), R_f 0.67 (chloroform). $^1\text{H NMR}$ (300 MHz, CDCl_3): δ 2.36 (s, 3H, CH_3), 3.3–3.65 (m, 4H, 4-, 4'- CH_2), 3.75–4.05 (m, 4H, OCH_2), 5.0 (m, 2H, 5-, 5'-CH), 7.22–7.97 (m, 8H, H_{Ar}). $^{13}\text{C NMR}$ (100 MHz, CDCl_3): δ 24.4 (CH_3), 37.4 (2 CH_2), 75.2 (2 CH), 78.7 (2 CH_2), 129.0–129.2 (4 CH), 129.3–130.7 (4 CH), 131.2 (C), 132.3 (C), 136.8 (C), 140.8 (C), 156.6 (2C). Anal. Calcd. for $\text{C}_{21}\text{H}_{21}\text{ClN}_2\text{O}_3$: C, 65.54; H, 5.50; N, 7.28. Found: C, 65.53, H, 5.49, N, 7.34.

5-[[[4,5-Dihydro-3-(*p*-tolyl)isoxazol-5-yl]methoxy]methyl]-3-(2,4-dichlorophenyl)-4,5-dihydroisoxazole (4af). From **3a** (0.5 g, 2.16 mmol), **1f** (0.41 g, 2.16 mmol) and chloramine-T trihydrate (0.62 g, 2.20 mmol): Yellow oil **4af** (0.76 g, 72%), R_f 0.64 (chloroform). $^1\text{H NMR}$ (300 MHz, CDCl_3): δ 2.34 (s, 3H, CH_3), 3.35–3.65 (m, 4H, 4-, 4'- CH_2), 3.72–3.97 (m, 4H, OCH_2), 5.1 (m, 2H, 5-, 5'-CH), 7.20–8.13 (m, 7H, H_{Ar}). $^{13}\text{C NMR}$ (100 MHz, CDCl_3): δ 24.4 (CH_3), 37.7 (2 CH_2), 75.5 (2 CH), 78.9 (2 CH_2), 127.1 (CH), 129.1–129.3 (4 CH), 130.7 (CH), 131.1 (C), 132.3 (CH), 135.5 (C), 135.7 (C), 137.8 (C), 140.7 (C), 156.5 (2C). Anal. Calcd. for $\text{C}_{21}\text{H}_{20}\text{Cl}_2\text{N}_2\text{O}_3$: C, 60.15; H, 4.81; N, 6.68. Found: C, 60.16, H, 4.79, N, 6.71.

5-[[[4,5-Dihydro-3-(3,4,5-trimethoxyphenyl)isoxazol-5-yl]methoxy]methyl]-4,5-dihydro-3-(4-nitrophenyl)isoxazole (4bc): From **3b** (0.5 g, 1.62 mmol), **1c** (0.27 g, 1.62 mmol) and chloramine-T trihydrate (0.46 g, 1.63 mmol): Yellow oil **4bc** (0.56 g, 73%), R_f 0.53 (chloroform). $^1\text{H NMR}$ (300 MHz, CDCl_3): δ 3.15–3.51 (m, 4H, 4-, 4'- CH_2), 3.63–3.74 (m, 4H, OCH_2),

3.88 (s, 6H, OCH₃), 3.92 (s, 3H, OCH₃), 5.10 (m, 2H, 5-, 5'-CH), 6.8 (s, 2H, H_{Ar}), 7.70–8.3 (m, 4H, H_{Ar}). ¹³C NMR (100 MHz, CDCl₃): δ 37.3 (2 CH₂), 56.3 (2 OCH₃), 56.6 (OCH₃), 75.2 (2 CH), 78.5 (2 CH₂), 106.8 (2 CH), 121.3 (2 CH), 128.4 (C), 130.2 (2 CH), 140.2 (C), 141.6 (C), 150.7 (C), 150.9 (2C), 156.9 (2C). Anal. Calcd. for C₂₃H₂₅N₃O₈: C, 58.59; H, 5.34; N, 8.91. Found: C, 58.62, H, 5.35, N, 8.98.

5-[[[3-(4-Chlorophenyl)-4,5-dihydroisoxazol-5-yl]methoxy]methyl]-4,5-dihydro-3-(3,4,5-trimethoxyphenyl)isoxazole (4be): Obtained from **3b** (0.5 g, 1.62 mmol), **1e** (0.25 g, 1.61 mmol) and chloramine-T trihydrate (0.46 g, 1.63 mmol): Yellow oil **4be** (0.5 g, 67%), *R_f* 0.60 (chloroform). ¹H NMR (300 MHz, CDCl₃): δ 3.24–3.57 (m, 4H, 4-, 4'-CH₂), 3.68–3.78 (m, 4H, OCH₂), 3.89 (s, 6H, OCH₃), 3.93 (s, 3H, OCH₃), 5.20 (m, 2H, 5-, 5'-CH), 6.70 (s, 2H, H_{Ar}), 7.27–7.60 (m, 4H, H_{Ar}). ¹³C NMR (100 MHz, CDCl₃): δ 37.5 (2 CH₂), 56.3 (2 OCH₃), 56.6 (OCH₃), 75.3 (2 CH), 78.8 (2 CH₂), 106.8 (2 CH), 128.4 (C), 129.1 (2 CH), 130.4 (2 CH), 132.1 (C), 136.7 (C), 141.3 (C), 150.8 (2 C), 156.84 (2 C). Anal. Calcd. for C₂₃H₂₅ClN₂O₂: C, 59.94; H, 5.47; N, 6.08. Found: C, 59.95, H, 5.49, N, 6.03.

5-[[[3-(2,4-Dichlorophenyl)-4,5-dihydroisoxazol-5-yl]methoxy]methyl]-4,5-dihydro-3-(3,4,5-trimethoxyphenyl)isoxazole (4bf). From **3b** (0.5 g, 1.62 mmol), **1f** (0.31 g, 1.63 mmol), and chloramine-T trihydrate (0.46 g, 1.63 mmol): Yellow oil **4bf** (0.59 g, 73%), *R_f* 0.58 (chloroform). ¹H NMR (300 MHz, CDCl₃): δ 3.25–3.60 (m, 4H, 4-, 4'-CH₂), 3.63–3.75 (m, 4H, OCH₂), 3.86 (s, 6H, OCH₃), 3.90 (s, 3H, OCH₃), 5.0–5.3 (m, 2H, 5-, 5'-CH), 6.70 (s, 2H, H_{Ar}), 7.2–7.5 (m, 3H, H). ¹³C NMR (100 MHz, CDCl₃): δ 37.6 (2 CH₂), 56.3 (2 OCH₃), 56.6 (OCH₃), 75.2 (2 CH), 78.7 (2 CH₂), 106.7 (2 CH), 127.1 (CH), 128.3 (C), 130.5 (CH), 132.2 (CH), 135.2 (C), 135.4 (C), 138.7 (C), 141.4 (C), 150.8 (2 C), 156.4 (2 C). Anal. Calcd. for C₂₃H₂₄Cl₂N₂O₆: C, 55.77; H, 4.88; N, 5.66. Found: C, 55.75, H, 4.89, N, 5.63.

5-[[[4,5-Dihydro-3-(4-nitrophenyl)isoxazol-5-yl]methoxy]methyl]-3-(4-chlorophenyl)-4,5-dihydroisoxazole (4ce). From **3c** (0.5 g, 1.90 mmol), **1e** (0.3 g, 1.93 mmol), and chloramine-T trihydrate (0.55 g, 1.96 mmol): Yellow oil **4ce** (0.5 g, 64%), *R_f* 0.62 (chloroform). ¹H NMR (300 MHz, CDCl₃): δ 3.38–3.58 (m, 4H, 4-, 4'-CH₂), 3.78–3.98 (m, 4H, OCH₂), 5.13–5.20 (m, 2H, 5-, 5'-CH), 7.34–8.16 (m, 8H, H_{Ar}). ¹³C NMR (100 MHz, CDCl₃): δ 37.4 (2 CH₂), 75.2 (2 CH), 78.7 (2 CH₂), 121.3 (2 CH), 129.2 (2 CH), 130.2 (2 CH), 130.7 (2 CH), 132.1 (C), 136.6 (C), 140.2 (C), 150.8 (C), 156.6 (2 C). Anal. Calcd. for C₂₀H₁₈ClN₃O₅: C, 57.77; H, 4.36; N, 10.11. Found: C, 57.79, H, 4.38, N, 10.13.

5-[[[4,5-Dihydro-3-(4-nitrophenyl)isoxazol-5-yl]methoxy]methyl]-3-(2,4-dichlorophenyl)-4,5-dihydroisoxazole (4cf). From **3c** (0.5 g, 1.90 mmol), **1f** (0.36 g, 1.89 mmol), and chloramine-T trihydrate (0.55 g, 1.96 mmol): Yellow oil **4cf** (0.56 g, 65%), *R_f* 0.67 (chloroform). ¹H NMR (300 MHz, CDCl₃): δ 3.41–3.59 (m, 4H, 4-, 4'-CH₂), 3.82–3.98 (m, 4H, OCH₂), 5.15–5.21 (m, 2H, 5-, 5'-CH), 7.21–7.73 (m, 4H, H_{Ar}), 7.92–8.16 (m, 3H, H_{Ar}). ¹³C NMR (100 MHz, CDCl₃): δ 36.4 (CH₂), 37.4 (CH), 75.2 (2 CH), 78.6 (2 CH₂), 121.2 (2 CH), 127.1 (CH), 130.2 (2 CH), 130.5 (CH), 132.1 (CH), 135.2 (C), 135.4 (C), 138.2 (C), 140.2 (C), 150.7 (C), 156.3 (C), 156.7 (C). Anal. Calcd. for C₂₀H₁₇Cl₂N₃O₅: C, 53.35; H, 3.81; N, 9.33. Found: C, 53.39, H, 3.80, N, 9.35.

5-[[[4,5-Dihydro-3-(*p*-tolyl)isoxazol-5-yl]methoxy]methyl]-3-(3-nitrophenyl)-4,5-dihydrois-

oxazole (4da). From **3d** (0.5 g, 1.90 mmol), **1a** (0.26 g, 1.92 mmol), and chloramine-T trihydrate (0.55 g, 1.96 mmol): Yellow oil **4da** (0.59 g, 78%), R_f 0.68 (chloroform). ^1H NMR (300 MHz, CDCl_3): δ 2.37 (s, 3H, CH_3), 3.23–3.49 (m, 4H, 4-, 4'- CH_2), 3.76–3.89 (m, 4H, OCH_2), 4.98–5.12 (m, 2H, 5-, 5'- CH), 7.12–7.56 (m, 4H, H_{Ar}), 7.62–8.33 (m, 4H, H_{Ar}). ^{13}C NMR (100 MHz, CDCl_3): δ 24.4 (CH_3), 37.4 (2 CH_2), 75.2 (2 CH), 78.8 (2 CH_2), 123.5 (CH), 124.2 (CH), 129.1–129.3 (4 CH), 129.8 (CH), 131.1 (C), 134.8 (C), 135.3 (CH), 140.5 (C), 148.7 (C), 156.9 (2 C). Anal. Calcd. for $\text{C}_{21}\text{H}_{21}\text{N}_3\text{O}_5$: C, 63.79; H, 5.35; N, 10.63. Found; C, 63.75, H, 5.37, N, 10.66.

5-[[[4,5-Dihydro-3-(3,4,5-trimethoxyphenyl)-5-yl]methoxy]methyl]-3-(3-nitrophenyl)-4,5-dihydroisoxazole (4db). From **3d** (0.5 g, 1.90 mmol), **1b** (0.4 g, 1.90 mmol), and chloramine-T trihydrate (0.54 g, 1.92 mmol): Yellow oil **4db** (0.63 g, 70%), R_f 0.65 (chloroform). ^1H NMR (300 MHz, CDCl_3): δ 3.23–3.55 (m, 4H, 4-, 4'- CH_2), 3.62–3.81 (m, 4H, OCH_2), 3.90 (s, 6H, OCH_3), 3.94 (s, 3H, OCH_3), 5.02–5.32 (m, 2H, 5-, 5'- CH), 6.80 (s, 2H, H_{Ar}), 7.63–8.46 (m, 4H, H_{Ar}). ^{13}C NMR (100 MHz, CDCl_3): δ 37.5 (2 CH_2), 56.2 (2 OCH_3), 56.5 (OCH_3), 75.4 (2 CH), 78.7 (2 CH_2), 106.9 (2 CH), 123.6 (CH), 124.3 (CH), 128.5 (C), 129.8 (CH), 134.9 (C), 135.3 (CH), 141.6 (C), 148.7 (C), 151.3 (2 C), 156.9 (2 C). Anal. Calcd. for $\text{C}_{23}\text{H}_{25}\text{N}_3\text{O}_8$: C, 58.59; H, 5.34; N, 8.91. Found; C, 58.45, H, 5.33, N, 8.93.

Microbiology

Table 1. Minimal inhibitory concentration X [$\mu\text{g mL}^{-1}$] and inhibitory zone diameter Y [mm] of compounds **4** against tested bacterial strains

Compound	<i>Bacillus subtilis</i>		<i>Escherichia coli</i>		<i>Pseudomonas fluorescens</i>		<i>Xanthomonas campestris pvs</i>		<i>Xanthomonas oryzae</i>	
	X	Y	X	Y	X	Y	X	Y	X	Y
4ab	32	7	35	7	31	9	34	6	28	7
4ac	28	6	32	6	28	6	31	6	27	6
4ae	27	9	31	6	28	4	29	5	26	9
4af	22	11	26	8	23	10	25	7	21	10
4bc	22	10	22	10	26	11	23	11	24	12
4be	25	7	22	9	28	13	27	10	23	11
4bf	21	7	26	13	23	16	25	12	23	12
4ce	22	10	21	11	25	14	22	11	24	11
4cf	19	13	17	14	20	17	19	14	23	12
4da	29	4	27	10	32	10	24	10	26	9
4db	24	9	22	9	29	15	25	12	23	10
Streptomycin	19	13	13	14	12	17	–	–	–	–
Tetracycline	–	–	–	–	–	–	9	12	13	12

Streptomycin sulfate (25 μg per disc) and Tetracycline (25 μg per disc) were used as positive reference standard antibacterial discs; compounds **4** (25 μg per disc).

The bacteria inoculum was prepared by suspending in 9 mL of sterile water for colonies from a 24 h culture on LB agar medium. For the filamentous fungi, the inoculum was prepared with the spores derived from 5–15 days cultures on PDA medium. The mycelia were covered with 10 mL of distilled water and the conidia were scraped using sterile pipette. The spores were recovered after filtration on sterile absorbent cotton and were resuspended in sterile distilled water. The cell density of each inoculum was adjusted with hemocytometer in order to obtain a final concentration of approximately 10^4 to 10^6 CFU mL⁻¹ for the bacteria and filamentous fungi, respectively.

Table 2. Minimal inhibitory concentration X [$\mu\text{g mL}^{-1}$] and inhibitory zone diameter Y [mm] of compounds **4** against tested fungal strains

Compound	<i>Aspergillus niger</i>		<i>Aspergillus flavus</i>		<i>Fusarium oxysporium</i>		<i>Fusarium monalifome</i>		<i>Trichoderma species</i>	
	X	Y	X	Y	X	Y	X	Y	X	Y
4ab	22	4	26	5	23	8	17	7	23	8
4ac	24	4	24	8	21	6	19	6	26	7
4ae	27	5	27	6	20	6	16	6	25	9
4af	19	6	21	8	18	9	14	9	19	11
4bc	18	8	18	9	14	12	14	11	15	14
4be	18	7	18	7	18	11	15	9	17	12
4bf	16	8	16	10	13	13	11	11	14	15
4ce	17	7	16	8	15	11	13	10	16	13
4cf	15	9	15	11	11	14	10	12	13	16
4da	18	6	19	6	18	7	15	7	19	10
4db	16	7	18	9	13	12	12	10	14	13
Nystatin	15	8	15	10	11	14	9	12	12	16

Nystatin (25 μg per disc) was used as positive reference standard antifungal discs; compounds **4** (25 μg per disc).

Nystatin (Himedia) was used as a positive control for fungi, and streptomycin and tetracycline for bacteria. Each disk contained 25 μg of standard drugs and 25 μg of compound **4**. Plates were first kept at 4 °C for at least 2 h to allow the diffusion of chemicals and then incubated at 28 °C. Inhibition zone were measured after 24 h of incubation of bacteria and after 48 h of incubation for fungi.

The micro dilution method¹⁶ was used to evaluate the minimum inhibitory concentration (MIC) of compounds **4**. The nutrient liquid medium and potato dextrose liquid medium were used as test media. Tests were performed in 96-well round bottom sterile culture plates. The suspensions of yeast and filamentous fungi were adjusted in sterile water to match the density of a 0.5 McFarland standard. The wells of a micro dilution plates were inoculated with 180 mL of

the culture medium containing a final inoculum of $0.5\text{--}2.5 \times 10^3$ CFU mL⁻¹. All compounds were dissolved in DMSO and were diluted twofold in the liquid medium to give a range of concentration from 640 to 0.1 µg mL⁻¹. 20 µL of each concentration were added to wells containing culture suspension except the growth control well. The final concentration ranged from 64 to 0.01 µg mL⁻¹. Plates were incubated at 35 °C for 48 h. Fungi growth was assessed at 494 nm by measuring the optical density in each well using an enzyme immunoassay multiwell reader (Sigma Diagnostic).

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