Synthesis and Evaluation of Anti-inflammatory and Analgesic Activity of Isoxazoline Bearing Tris (heterocycles)

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ABSTRACT

Series of tris(heterocycle) bearing isoxazoline ring in combination with imidazole ring has been synthesized via 1,3-dipolar cycloaddition reactions of N-(substituted)methyl-imidazole nitrile oxides with different dipolarophiles. All the newly synthesized compounds were characterized by infrared spectroscopy (IR), $^1$H and $^{13}$C nuclear magnetic resonance (NMR) and elemental analysis. Compounds were screened for their anti-inflammatory and analgesic activity. Compounds 4d, 4e and 4f were discovered as significant anti-inflammatory and analgesic agents showing activity comparable to that of standard drugs.

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**Introduction**

The [3+2] cycloaddition reaction of olefin and nitrile oxide to give isoxazoline has long been valued as an important transformation in chemical synthesis. These heterocyclic products can be readily elaborated to a variety of highly functionalized compounds.[1,2] Isoxazoline possess broad spectrum of biological activities like anti-tuberculosis, antifungal, anticancer, antiviral, insecticidal, antibiotic activities and precursors for different natural products.[3] In fact, Valdecoxx, an isoxazole derivative is now widely used in the market as anti-inflammatory drug.[4] The chemistry of the imidazole ring occupies an extremely important niche possessing diverse pharmacological activity within the family of five-membered ring heterocycles; imidazole moiety possess biological activity like anti-inflammatory.[5] analgesic,[6] antibacterial,[7] antifungal,[8] antituberculosis,[9] antiinfluenzaant[10] and potential anticytokine agents.[11] Compounds possessing imidazole moiety acts as new potent and selective 20-HETE synthase inhibitors,[12] 2-n-butyl-4-chloro-5-farnyl-imidazole is a key intermediate for the synthesis of Losartan a non-peptide angiotensin antagonist, which is an orally active anti hypertensive drug.[13]

1,3-dipolar cycloaddition reactions are useful tools for constructing isoxazoline,[14] nitrile oxides serves as excellent 1,3-dipoles. Cycloaddition of nitrile oxide to olefinic compounds are of synthetic interest, to prepare isoxazoline as versatile intermediates for the synthesis of bifuctional compounds.[15,16] There are two well-established, widely used methods for the in situ formation of nitrile oxides. The most common approach is base-induced elimination of hydrochloride from hydroximinoyl chlorides. The requisite hydroximinoyl chlorides are prepared from the corresponding oxime, derived from an aldehyde and an electrophilic chloride source (NCS, NaOCl and Cl2). This method is not amenable, however for substrates highly sensitive to oxidation or halogenation including electron-rich aromatics, olefins and sulfides.[17-21] The second approach, known as the Mukaiyama method which is widely used involves the dehydration of nitroalkanes by the action of phenyl isocyanate, using DCC or similar reagents in the presence of base.[22] Chloramine-T is used extensively for the generation of nitrile oxide and nitrile imine from aldoxime and aldehyde hydrazone for the synthesis of isoxazolines and pyrazoles respectively.[23-26] Ether-linked bis(isoxazoline) via 1,3-dipolar cycloaddition reactions of nitrile oxides with allyl alcohol and allyl ethers.[27] Isoxazoline bearing bis(heterocycles) has been synthesized by the reaction of bischalcones and bis sulfones as dipolarophiles with nitrile oxides, generated using chloramine-T as 1,3-dipole has been reported.[28,29] With this contextual information, it was considered worthwhile to synthesize hitherto unknown tris(heterocycle). Synthesis was carried out by taking N-(isoxazolyl)methyl-imidazole aldehyde as key starting material to obtain tris(heterocycle) derivatives of isoxazoline with another isoxazole and imidazole ring system. Upon biological evaluation combined and enhanced anti-inflammatory and analgesic activity were achieved. Significant activities were observed in the isoxazoline derivatives possessing halo and cyano substitution.

**Materials and Methods**

Melting points were determined on Thomas Hoover melting point apparatus and were uncorrected. 1H NMR spectra were recorded on a Bruker AM 300 MHz spectrometer using CDCl3 as solvent and tetramethylsilane as internal standard. 13C NMR spectra were measured on Jeol 400 (100 MHz) instrument. The chemical shifts are expressed in δ and following abbreviations were used. s=singlet, d=doublet, t=triplet and m=multiplet. Infrared (IR) spectra were recorded on Shimadzu 8300 IR spectrometer. Elemental analyses results were obtained on a Vario-EL instrument and thin layer chromatography was carried out with BDH silica gel G on glass slides.

**Experimental**

**General procedure for the synthesis of tris(heterocycle) 4(a-h)**

A mixture of 2 (1.61 mmol) and chloramine-T trihydrate (0.47 g, 1.63 mmol) in ethanol (15 mL) was stirred at room temperature for 5 min. To this mixture, 3a-h (1.69 mmol) in ethanol (5 mL) was added and the reaction mixture was heated on 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 purified by the column chromatography using the chloroform/acetone (8:2) as eluent to get pure compounds 4a-h with good yield.

3-(2-butyl-4-chloro-1-((3,5-dimethylisoxazol-4-yl)methyl)-1H-imidazol-5-yl)-4,5-dihydroisoxazol-5-carbonitrile [4a]: 1H NMR (300 MHz, CDCl3): δ 0.97 (t, 3H, CH3), 1.34 (m, 2H, CH2), 1.62 (m, 2H, CH2), 2.35 (s, 6H, 2CH3), 2.56 (t, 2H, CH2), 3.34 (dd, J = 7.8, 2.0 Hz, 1H, 4-CHA), 3.62 (dd, J = 7.8, 2.0 Hz, 1H, 4-CHB), 4.98 (s, 2H, CH2), 5.28 (dd, J = 6.2 Hz, 2.0 Hz, 1H, 5-CH); 13C NMR (100 MHz, CDCl3): δ 6.5 (CH), 11.2 (CH2), 14.0 (CH3), 22.3 (CH2), 22.6 (CH2), 25.6 (CH3), 33.6 (CH2), 41.1 (CH2), 68.8 (CH), 100.7 (C), 118.8 (C), 122.3 (C), 126.1 (C), 148.3 (C), 158.8 (C), 159.9 (C), 164.5 (C); IR (KBr pellets cm⁻¹) 2942, 2339, 1659, 1329, 1117; CHN C17H26ClN2O2 Calculated: C, 56.43; H, 5.57; N, 19.36%; Found: C, 56.47, H, 5.53, N, 19.38%; Yield 67%; Thick oil.

2-butyl-4-chloro-5-(4,5-dihydro-5-phenylisoxazol-3-yl)-1-((3,5-dimethylisoxazol-4-yl)methyl)-1H-imidazole [4b]: 1H NMR (300 MHz, CDCl3): δ 0.93 (t, 3H, CH3), 1.31 (m, 2H, CH2), 1.60 (m, 2H, CH2), 2.33 (s, 6H, 2CH3), 2.52 (t, 2H, CH2), 3.36 (dd, J = 8.0, 2.4 Hz, 1H, 4-CHA), 3.65 (dd, J = 8.0, 2.4 Hz, 1H, 4-CHB), 4.98 (s, 2H, CH2), 5.33 (dd, J = 6.2Hz, 2.2 Hz, 1H, 5-CH); 13C NMR (100 MHz, CDCl3): δ 6.8 (CH3), 11.6 (CH2), 14.3 (CH3), 22.4 (CH2), 22.7 (CH2), 25.7 (CH3), 33.8 (CH2), 41.8 (CH2), 80.8 (CH), 100.7 (C), 122.4 (C), 126.6 (C), 127.3 (2CH), 127.8 (CH), 129.2 (2CH), 140.9 (C), 148.3 (C), 158.7 (C), 159.9 (C), 164.8 (C); IR (KBr pellets cm⁻¹) 2960, 1670, 1661, 1332, 1215; CHN C25H25N2O2 Calculated: C, 63.99; H, 6.10; N, 13.57; Found: C, 63.95, H, 6.11, N, 13.59; Yield 71%; Thick oil.

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3-(2-butylyl-4-chloro-1-(3,5-dimethylisoxazol-4-yl)methyl)-4,5-dihydroisoxazol-5-yl acetate [4d]: $^1$H NMR (300 MHz, CDCl$_3$): δ 0.98 (t, 3H, CH$_3$), 1.36 (m, 2H, CH$_2$), 1.65 (m, 2H, CH$_2$), 2.11 (s, 3H, CH$_3$), 2.34 (s, 6H, 2CH$_3$), 2.59 (t, 2H, CH$_2$), 2.39 (dd, $J = 7.4, 2.0$ Hz, 1H, 4-CHA), 3.60 (dd, $J = 7.4, 2.0$ Hz, 1H, 4-CHB), 4.98 (s, 2H, CH$_2$), 5.78 (dd, $J = 6.0$ Hz, 2.0 Hz, 1H, 5-CH); $^1$C NMR (100 MHz, CDCl$_3$): δ 6.9 (CH$_3$), 11.3 (CH$_3$), 14.3 (CH$_3$), 21.3 (CH$_3$), 22.4 (CH$_2$), 22.6 (CH$_2$), 25.7 (CH$_3$), 33.8 (CH$_3$), 70.1 (CH$_3$), 96.7 (CH), 100.8 (C), 122.9 (C), 126.2 (C), 148.3 (C), 159.8 (C), 164.8 (C); IR (KBr pellets cm$^{-1}$) 2947, 1756, 1658, 1384, 1126; CHN C$_{18}$H$_{22}$Cl$_n$O$_m$ Calculated: C, 54.75; H, 5.87; N, 14.19; Found: C, 54.79, H, 5.83, N, 14.16; Yield 72 %; Thick oil.

5-(5(bromomethyl)-4,5-dihydroisoxazol-3-yl)-2-butylyl-4-chloro-1-(3,5-dimethylisoxazol-4-yl)methyl)-1H-imidazole [4f]: $^1$H NMR (300 MHz, CDCl$_3$): δ 0.94 (t, 3H, CH$_3$), 1.31 (m, 2H, CH$_2$), 1.60 (m, 2H, CH$_2$), 2.34 (s, 6H, 2CH$_3$), 2.53 (t, 2H, CH$_2$), 3.20-3.64 (m, 4H, 2CH$_2$), 4.94 (s, 2H, CH$_2$), 4.94-5.10 (m, 1H, CH); $^1$C NMR (100 MHz, CDCl$_3$): δ 6.8 (CH$_3$), 11.6 (CH$_3$), 14.2 (CH$_2$), 22.3 (CH$_2$), 22.5 (CH$_2$), 25.6 (CH$_3$), 33.5 (CH$_3$), 37.8 (CH$_3$), 51.8 (CH$_3$), 69.8 (CH), 100.7 (C), 123.5 (C), 126.3 (C), 148.3 (C), 158.7 (C), 159.8 (C), 164.6 (C); IR (KBr pellets cm$^{-1}$) 2955, 1673, 1392, 1136, 1110; CHN C$_{17}$H$_{22}$BrCl$_n$O$_m$ Calculated: C, 52.99; H, 5.76; N, 14.54; Found: C, 64.74, H, 6.33, N, 13.10; Yield 74 %; Thick oil.

Methyl-3-(2-butylyl-4-chloro-1-(3,5-dimethylisoxazol-4-yl)methyl)-1H-imidazole-5-yl)-4,5-dihydroisoxazol-5-carboxylate [4g]: $^1$H NMR (300 MHz, CDCl$_3$): δ 0.97 (t, 3H, CH$_3$), 1.33 (m, 2H, CH$_2$), 1.62 (m, 2H, CH$_2$), 2.36 (s, 6H, 2CH$_3$), 2.57 (t, 2H, CH$_2$), 3.26 (dd, $J = 8.0, 2.2$ Hz, 1H, 4-CHA), 3.58 (dd, $J = 8.0, 2.2$ Hz, 1H, 4-CHB), 3.69 (s, 3H, CH$_3$), 4.98 (s, 2H, CH$_2$), 5.24 (dd, $J = 6.2$ Hz, 2.0 Hz, 1H, 5-CH); $^1$C NMR (100 MHz, CDCl$_3$): δ 6.8 (CH$_3$), 11.6 (CH$_3$), 14.1 (CH$_3$), 22.4 (CH$_2$), 22.6 (CH$_2$), 25.7 (CH$_3$), 33.8 (CH$_3$), 38.8 (CH$_3$), 52.4 (CH$_3$), 77.9 (CH), 100.8 (C), 122.9 (C), 126.2 (C), 148.3 (C), 158.9 (C), 159.8 (C), 164.8 (C); IR (KBr pellets cm$^{-1}$) 2941, 1750, 1662, 1385, 1130;CHN C$_{18}$H$_{22}$Cl$_n$O$_m$ Calculated: C, 54.75; H, 5.87; N, 14.19; Found: C, 54.72, H, 5.88, N, 14.18; Yield 78 %; Thick oil.

Methyl-3-(2-butylyl-4-chloro-1-(3,5-dimethylisoxazol-4-yl)methyl)-1H-imidazole-5-yl)-4,5-dihydro-5-methylisoxazol-5-carboxylate [4h]: $^1$H NMR (300 MHz, CDCl$_3$): δ 0.95 (t, 3H, CH$_3$), 1.32 (m, 2H, CH$_2$), 1.52 (s, 3H, CH$_3$), 1.61 (m, 2H, CH$_2$), 2.34 (s, 6H, 2CH$_3$), 2.53 (t, 2H, CH$_2$), 3.23 (s, 2H, CH$_2$), 3.69 (s, 3H, CH$_3$), 4.96 (s, 2H, CH$_2$); $^1$C NMR (100 MHz, CDCl$_3$): δ 6.9 (CH$_3$), 11.5 (CH$_3$), 14.3 (CH$_3$), 22.6 (CH$_2$), 22.7 (CH$_2$), 24.5 (CH$_3$), 25.5 (CH$_3$), 33.6 (CH$_3$), 46.8 (CH$_2$), 52.6 (CH$_3$), 85.9 (CH), 100.8 (C), 123.4 (C), 126.6 (C), 148.3 (C), 158.6 (C), 159.7 (C), 164.8 (C), 171.3 (C); IR (KBr pellets cm$^{-1}$) 2949, 1748, 1667, 1387, 1134; CHN C$_{18}$H$_{22}$Cl$_n$O$_m$ Calculated: C, 55.81; H, 6.16; N, 13.70; Found: C, 55.85, H, 6.18, N, 13.67; Yield 77 %; Thick oil.

Pharmacological Evaluation
Anti-inflammatory activity
Carrageenan-Induced Paw Edema
Anti-inflammatory activity of test compounds 4a-h was studied by carrageenan induced edema model method on test systems (albino Wistar rats) by either sex body weight between 145-175 g randomly dividing them into XI groups of four animals each and was fasted for overnight. Group I served as carrageenan inducer (control), 1% suspension of Type IV Lambda (Sigma) carrageenan was injected into the sub-planar region at the right hind paw one hour before administering test compounds, group II received 10 mL kg$^{-1}$ 0.5% sodium carboxymethyl cellulose (vehicle control)[30] and group III received ibuprofen (standard) of100 mg kg$^{-1}$ body weight through oral route. Group IV to group XI were administrated with test compounds (4a-h) in the dose of (100 mg kg$^{-1}$ body weight) and after administering samples, test systems were kept under clinical sign observation for 30 min and suspension of carrageenan (0.1 mL of 1% mg m$^{-1}$) was injected into the sub-planar region of right hind paw of each test system. The paw volume was measured by using digital plethysmometer (ITC Life science, USA), immediately after injection and again after3 hours. Edema volumes for test compounds treated and positivecontrol albino Wistar rats were compared statistically with those for the vehicle treated control albino Wistar rats; data are reported in Fig 1 and Fig 2.
Analgesic activity

Analgesic activity of test compounds 4a-h was carried out on test system (albino Wistar mice) of either sex with 10-25 g of body weight. The compounds were administered by body weight using a feeding tube as homogenized suspensions in 0.5% sodium carboxymethyl cellulose; was administered as the vehicle control. This method is based on acetic-acid-induced writhings in mice.[31, 32] Groups of six mice each were dosed with the test compounds and with aspirin (standard) at a dose of 100 mg kg\(^{-1}\) body weight, 1 h before the ip injection of 0.6% acetic acid (10 mL kg\(^{-1}\)). Test systems were injected with acetic acid, left alone for 5 min and total numbers of writhes were recorded for about 1.5 min after the 5 min interval. The mean value of writhes for each group was calculated and compared statistically with that for the vehicle-treated control group (n=6); data were reported as percent inhibition of the number of writhes. The test was repeated on additional groups of six mice, treated with compounds for which the reduction in writhes had been calculated to be > 10%; these results are shown in Fig 3 and Fig 4.

Results and Discussion

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Scheme: Reaction Condition; a) NH\(_2\)OH.HCl, CH\(_3\)COONa / EtOH, b) Chloramine-T / EtOH

The starting material N-(substituted)methyl-imidazole aldehyde 1 and its oxime 2 were prepared according to the literature procedure,[33,34] Oxidative dehydrogenation of N- (substituted)methyl-imidazole aldoxime 2 by chloramine-T trihydrate afforded nitrile oxides, which were intercepted in situ by different alkenes 3a-h in refluxing ethanol. The pale yellow thick oil obtained were characterized 1H NMR, 13C NMR and CHN analysis data successfully as3-[2-butyl-4-chloro-1-[(3,5-dimethylisoaxazol-4-yl)methyl]-1H-imidazol-5-yl]-4,5-dihydroisoaxazoline derivatives tris(heterocyle) 4(a-h) (Scheme). All the compounds were tested for anti-inflammatory activity in carrageenan-induced edema assay in rats at a dosage of 100 mg kg\(^{-1}\) body weight (Fig 1 and Fig 2). Three compounds (4a, 4e and 4f) have significant activity. Amongst these compounds, the two halogenated derivatives, 4e and 4f have more than 60% activity. At all of the doses they were less active than Ibuprofen. All of these compounds were tested for analgesic activity at 100 mg kg\(^{-1}\) in acetic-acid induced assay in mice (Fig 3 and Fig 4). Seven compounds had significant activity and the compound 4e exhibited the highest activity in the series. The percentage inhibition of carrageen induced paw edema volume was calculated using the formula

\[
\text{Percentage inhibition} = 100 \left(1 - \frac{V_t}{V_c}\right) \tag{1}
\]

Where, \(V_t\) = increase in paw volume in treatment group \(V_c\) = increase in paw volume in the control group

Fig 1: Anti-inflammatory activities of 4(a-h)

Fig 2: Analgesic activities of 4(a-h)
Acknowledgments
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Conclusion
Isoxazoline and imidazole bearing tris(heterocycles) were obtained in good yield via 1,3-dipolar cycloaddition reaction, and it is found that all the eight new molecules shown significant anti-inflammatory and analgesic activity. Compounds 4a, 4e and 4f possess cyano, chloro and bromo substitutions on one of the isoxazole ring shown good analgesic and anti-inflammatory activity. Further detailed in silico and cellular level studies will be take up for these biologically important molecules.
Competing Interests

The authors declare no conflict of interest.

References