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ORIENTAL JOURNAL OF CHEMISTRY
An International Open Access, Peer Reviewed Research Journal

www.orientjchem.org

ISSN: 0970-020 X
CODEN: OJCHEG
2023, Vol. 39, No.(3):
Pg. 675-683

Molecular Docking Studies, Synthesis of Novel Isoxazole Derivatives from 3-Methoxy Substituted Chalcone and Evaluation of their Anti-Inflammatory Activity

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http://dx.doi.org/10.13005/ojc/390318

(Received: April 10, 2023; Accepted: June 07, 2023)

ABSTRACT

Synthesis of 3-methoxy acetophenone with substituted benzaldehydes resulted in a number of novel chalcones. The chalcones were then treated to a cyclization reaction with hydroxylamine hydrochloride in ethanol to enable the synthesis of 3-methoxy acetophenone isoxazole derivatives. After purification, the structures of the synthesized compounds were identified using TLC, FTIR, ^{1}H NMR, ^{13}C NMR and a Mass spectroscopy. The carrageenan-induced paw edema method was used to test the compounds for anti-inflammatory activity. Based on the findings, the three compounds appeared to be moderate to extremely active.

Keywords: Chalcones, Isoxazole, Hydroxylamine hydrochloride, Molecular Docking, Anti-inflammatory activity.

INTRODUCTION

An essential defense mechanism for the body's protection against physical or chemical injury or illness threats harm is inflammation. Often fatal illnesses including autoimmune conditions, Crohn's disease, rheumatoid arthritis, and inflammatory syndrome bowel utilize this defense mechanism[1]. The essential regulator of the inflammatory process is a prostaglandin. When prostaglandin E2 is unstable, an enzyme known as isomerase that is specialized to this intermediate transforms it into a variety of prostanoids[2]. Arachidonic acid is transformed into prostaglandin E2 by the enzyme cyclooxygenase (COX). A number of produces prostaglandins, the main mediators of inflammation, pain, and increased body temperature, important enzymes are cyclooxygenases (COX) or prostaglandin endoperoxide synthases (hyperpyrexia). Two primary COX protein isoforms that the body processes are cyclooxygenases-2 (COX-2) and cyclooxygenases-1 (COX-1). and crucial biological mediators like prostanoids, prostacyclin, thromboxane, and prostaglandins are produced by COX-1, which is also

involved in the production of pain, blood clotting, and stomach protection[3]. While COX-2 has an essential function in the production of prostaglandins by inflammatory cells and the central nervous system and is engaged in pain through inflammation4. Inflammation has reduced the inhibition of COX-1, although the stomach's inner protective properties are also lost. This may cause stomach aches, ulcers, and including gastrointestinal and intestinal hemorrhage. COX-2, however, normally only affects inflammatory tissue[5]. Benzylidene acetophenones or benzal acetophenones are other names for chalcones[6]. Several heterocyclic ring systems, such as isoxazoles, pyrazolines, pyrimidines, and cyano pyridines, may be synthesized using chalcones[7]. Isoxazole is one of the five-membered heterocycles. In the molecule isoxazole, two heteroatoms, oxygen, and nitrogen, are situated next to one another. The molecular structure of the molecule is unsaturated due to two double bonds between carbon atoms[8–10]. Isoxazoles have a wide range of pharmacological and biological activities. which include antibacterial[11], antifungal[12], GABA antagonist[13], anti-inflammatory[14] antidiabetic[15] and anticancer[16-17]. Inspired by the above facts, The current study's objective was to synthesize novel isoxazole derivatives and assess their ability to reduce inflammation.

EXPERIMENTAL

MATERIALS AND METHOD

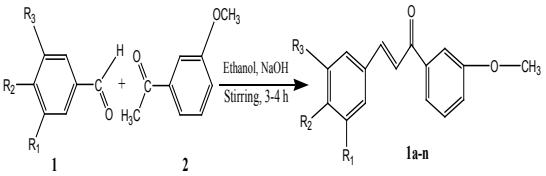
3-methoxyacetophenon, Hydroxylamine hydrochloride and different substituted benzaldehydes were acquired from AB Fine Chemicals for the current study project (Nasik, India). Parecoxib, which was employed as a reference medication, was purchased from Local Market. All of the additional chemicals and solvents were provided by CDH. All of the chemicals were analytical grade and had been cleaned up before usage.

The open capillary method was employed to identify the uncorrected melting points of the produced compounds. With a Perkin Elmer 2400 analyzer, the FTIR spectra of synthetic substances were captured on potassium bromide discs. Using a Bruker Advance Neo spectrophotometer and ^{1}H NMR uses tetramethylsilane (TMS) as an internal standard and $^{13}CNMR$ spectra of the produced compounds were captured in $CDCl_3$. In ppm, all chemical shift values are recorded. In SAIF, P. U., Punjab, India, synthetic substances underwent spectroscopic analysis.

To track the development of the reactions, silica gel G thin-layer chromatography (TLC) was employyed, and spots were seen in an iodine chamber. The carrageenan-induced paw edema method, will be used to pharmacologically evaluate the target drugs for anti-inflammatory activity.

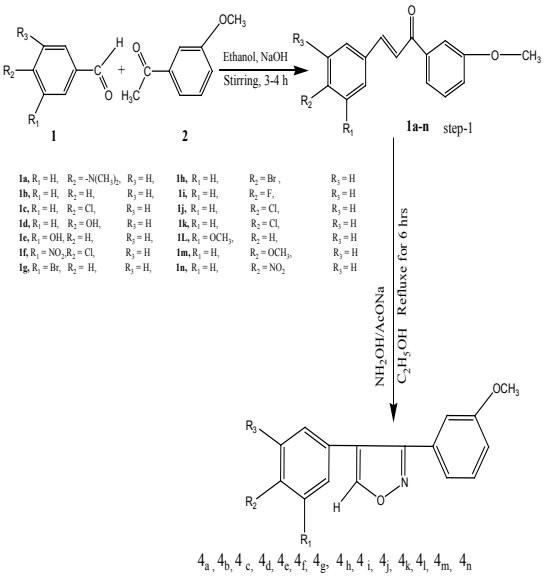
Synthesis of substituted chalcones for (4a-4n)

Equimolar amounts substituted benzaldehyde and 3-methoxy acetophenone were mixed in pure alcohol (0.02 mol), and 10% NaOH solution (25 mL) was poured in while being stirred dropwise. The action was then permitted to continue for 3–4 h before being stopped overnight. To obtain the outcome, the reaction mixture was then submerged in cold water for a whole night. The final product was created by recrystallizing the crude product from ethanol.



1a, R_1 = H, R_2 = -N(CH_3)$_2$, R_3 = H, **1h,** R_1 = H, R_2 = Br , R_3 = H
1b, R_1 = H, R_2 = H, R_3 = H, **1i,** R_1 = H, R_2 = F, R_3 = H
1c, R_1 = H, R_2 = Cl, R_3 = H **1j,** R_1 = H, R_2 = Cl, R_3 = H
1d, R_1 = H, R_2 = OH, R_3 = H **1k,** R_1 = H, R_2 = Cl, R_3 = H
1e, R_1 = OH, R_2 = H, R_3 = H, **1L,** R_1 = OCH_3, R_2 = H, R_3 = H
1f, R_1 = NO_2,R_2 = Cl, R_3 = H **1m,** R_1 = H, R_2 = OCH_3, R_3 = H
1g, R_1 = Br, R_2 = H, R_3 = H, **1n,** R_1 = H, R_2 = NO_2 R_3 = H

RESEARCH SCHEME



Scheme 1. Synthesis of Isoxazole derivatives (4a-4n)

Basic fundamentals of Isoxazole Derivative Synthesis (4a-4n)

(0.01 mole) chalcones (1a-1n), (0.01 mole) hydroxylamine hydrochloride were added to and Sodium acetate ethanol (25 mL), and the mixture was mixed and then refluxed in an oil bath for six hours before being put in the cold water (50 mL). The precipitate obtained from ethanol was cleaned, filtered, and recrystallized. (Scheme 1).

{4-[4-(3-Methoxy-phenyl)-isoxazol-3-yl]-phenyl}-dimethyl-amine (4a)

Characterization Brown solid; Yield: 70%; m. p. 290-291°C. Elemental analysis of $C_{18}H_{18}N_2O_2$ calcd. (found)%: C, 73.45; H, 6.16; N, 9.52; O, 10.87. IR (KBr, νmax, cm^{-1}): 2861 (O-CH_3), 1617 (Ar-H, stretching), 3551 (str, NH amide), 1636 (C=N, stretching in ring), 1026 (CO, stretching in ring), 832 (CH=CH p-disub-Aromatic). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.97 (s, 1H, CH=CH), 6.76-7.57 (m, 8H, Ar-H), 3.83 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ : 130.69 (C1), 113.76 (C2), 110.85 (C4), 121.67 (C6), 155.33 (C8), 155.35 (C13), 130.27 (C15), 112.62 (C16,C18), 130.69 (C19), 40.309 (OCH_3), Mass: m/z: (M+1) 295.10.

4-(3-Methoxy-phenyl)-3-phenyl-isoxazole (4b)

Characterization White solid; Yield: 85%; m. p. 223-224°C. Elemental analysis of $C_{16}H_{13}NO_2$ calcd. (found)%: C, 76.48; H, 5.21; N, 5.57; O, 12.73. IR (KBr, νmax, cm^{-1}): 2861 (O-CH_3), 1617 (Ar-H, stretching), 3551 (str, NH amide), 1636 (C=N, stretching in ring), 1026 (CO, stretching in ring), 832 (CH=CH p-disub-Aromatic). ^{1}H NMR (500 MHz, $CDCl_3$) δ : 8.96 (s, 1H, CH=CH), 6.92-7.57 (m, 8H, Ar-H), 3.83 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 130.69 (C1), 113.76 (C2), 110.85 (C4), 121.67 (C6), 155.57 (C8), 130.27 (C15), 129.69 (C16, C18), 55.34 (OCH_3), Mass: m/z: (M+1) 252.94.

3-(4-Chloro-phenyl)-4-(3-methoxy-phenyl)-isoxazole (4c)

Characterization White solid; Yield: 80%; m. p. 265-267°C. Elemental analysis of $C_{16}H_{12}ClNO_2$ calcd. (found)%: C, 67.26; H, 4.23; Cl, 12.41; N, 4.90; O, 11.20. IR (KBr, νmax, cm^{-1}): 2861 (O-CH_3), 1617 (Ar-H, stretching), 3551 (str, NH amide), 1636 (C=N, stretching in ring), 1026 (CO, stretching in ring), 832 (CH=CH p-disub-Aromatic). ^{1}H NMR (500 MHz, $CDCl_3$) δ : 8.97 (s, 1H, CH=CH), 6.92-7.56 (m, 8H, Ar-H), 3.83 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, CDCl3) δ : 130.96 (C1), 113.76 (C2), 110.85 (C4), 121.67 (C6), 155.58 (C8), 130.91 (C15), 129.25 (C16, C18), 130.91 (C19), 55.33 (OCH_3), Mass: m/z: (M+1) 286.02.

4-[4-(3-Methoxy-phenyl)-isoxazol-3-yl]-phenol (4d)

Characterization Black solid; Yield: 80%; m. p. 334-335°C. Elemental analysis of $C_{16}H_{13}NO_3$ calcd. (found)%: C, 71.90; H, 4.90; N, 5.24; O, 17.96. IR (KBr, νmax, cm^{-1}): 2861 (O-CH_3), 1617, 3432 (OH), (Ar-H, stretching), 3551 (str, NH amide), 1636 (C=N, stretching in ring), 1026 (CO, stretching in ring), 832 (CH=CH p-disub-Aromatic). ^{1}H NMR (500 MHz, $CDCl_3$) δ : 8.97 (s, 1H, CH=CH), 6.73-7.42 (m, 8H, Ar-H), 5.021 (s,1H, OH), ^{13}C NMR (100 MHz, $CDCl_3$) δ : 130.96 (C1), 113.76 (C2), 110.85 (C4), 121.67 (C6), 155.37 (C8), 130.69 (C15), 116.40 (C16, C18), 121.67 (C19), 55.34 (OCH_3), Mass: m/z: (M+1) 268.07.

3-[4-(3-Methoxy-phenyl)-isoxazol-3-yl]-phenol (4e)

Characterization Black solid; Yield: 80%; m. p. 413-415°C. Elemental analysis of $C_{16}H_{13}NO_3$ calcd. (found)%: C, 71.90; H, 4.90; N, 5.24; O, 17.96. IR (KBr, νmax, cm^{-1}): 2861 (O-CH_3), 1617, 3432 (OH), (Ar-H, stretching), 3551 (str, NH amide), 1636 (C=N, stretching in ring), 1026 (CO, stretching in ring), 832 (CH=CH p-disub-Aromatic). ^{1}H NMR (500 MHz, $CDCl_3$) δ : 8.87 (s, 1H, CH=CH), 6.85-7.42 (m, 8H, Ar-H), 5.021 (s,1H, OH), 3.83 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ : 130.96 (C1), 113.76 (C2), 110.85 (C4), 121.67 (C6), 155.36 (C8), 130.69 (C15), 118.40 (C16), 122.56 C18), 121.67 (C19), 55.34 (OCH_3), Mass: m/z: (M+1) 268.04.

4-(3-Methoxy-phenyl)-3-(3-nitro-phenyl)-isoxazole (4f)

Characterization Brown solid; Yield: 85%; m. p. 421-422°C. Elemental analysis of $C_{16}H_{12}N_2O_4$ calcd. (found)%: C, 64.86; H, 4.08; N, 9.46; O, 21.60. IR (KBr, νmax, cm^{-1}): 2861 (O-CH_3), 1617 (Ar-H, stretching), 3551 (str, NH amide), 1636 (C=N, stretching in ring), 1026 (CO, stretching in ring), 832 (CH=CH p-disub-Aromatic). ^{1}H NMR (500 MHz, $CDCl_3$) δ : 9.03 (s, 1H, CH=CH), 6.92-8.74 (m, 8H, Ar-H), 3.83 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ : 130.69 (C1), 113.76 (C2), 110.85 (C4), 121.67 (C6), 155.68 (C8), 125.26 (C15), 126.80 (C17), 130.29 C18), 136.52 (C19), 55.34 (OCH_3), Mass: m/z: (M+1) 297.01.

3-(3-Bromo-phenyl)-4-(3-methoxy-phenyl)-isoxazole (4g)

Characterization Yellow solid; Yield: 85%; m. p. 294-295°C. Elemental analysis of $C_{16}H_{12}BrNO_2$ calcd. (found)%: C, 58.20; Br, 24.20; N, 4.24; H, 3.66; O, 9.69. IR (KBr, νmax, cm^{-1}): 2861 (O-CH_3), 1617 (Ar-H, stretching), 3551 (str, NH amide), 1636 (C=N, stretching in ring), 1026 (CO, stretching in ring), 832 (CH=CH p-disub-Aromatic). ^{1}H NMR (500 MHz, $CDCl_3$) δ : 8.96 (s, 1H, CH=CH), 6.92-7.77 (m, 8H, Ar-H), 3.83 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ : 130.69 (C1), 113.76 (C2), 110.85 (C4), 121.67 (C6), 155.34 (C8), 132.24 (C15), 133.80 (C17), 130.83 C18), 128.59 (C19), 55.34 (OCH_3), Mass: m/z: (M+1) 330.00.

3-(4-Bromo-phenyl)-4-(3-methoxy-phenyl)-isoxazole (4h)

Characterization Yellow solid; Yield: 85%; m. p. 294-295°C. Elemental analysis of $C_{16}H_{12}BrNO_2$ calcd. (found)%: C, 58.20; H, 3.66; Br, 24.20; N, 4.24; O, 9.69. IR (KBr, νmax, cm^{-1}): 2861 (O-CH_3), 1617 (Ar-H, stretching), 3551 (str, NH amide), 1636 (C=N, stretching in ring), 1026 (CO, stretching in ring), 832 (CH=CH p-disub-Aromatic). ^{1}H NMR (500 MHz, $CDCl_3$) δ : 8.97 (s, 1H, CH=CH), 6.92-7.64 (m, 8H, Ar-H), 3.83 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ : 130.69 (C1), 113.76 (C2), 110.85 (C4), 121.67 (C6), 155.58 (C8), 131.87 (C15), 130.56 (C16), 55.34 (OCH_3), Mass: m/z: (M+1) 330.01.

3-(4-Fluoro-phenyl)-4-(3-methoxy-phenyl)-isoxazole (4i)

Characterization Brown solid; Yield: 80%; m. p. 237-238°C. Elemental analysis of $C_{16}H_{12}FNO_2$ calcd. (found) %: C, 71.37; H, 4.49; F, 7.06; N, 5.20; O, 11.88. IR (KBr, νmax, cm^{-1}): 2861 (O-CH_3), 1617 (Ar-H, stretching), 3551 (str, NH amide), 1636 (C=N, stretching in ring), 1026 (CO, stretching in ring), 832 (CH=CH p-disub-Aromatic). ^{1}H NMR (500 MHz, $CDCl_3$) δ : 8.97 (s, 1H, CH=CH), 6.92-7.97 (m, 8H, Ar-H), 3.83 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ : 130.69 (C1), 113.76 (C2), 110.85 (C4), 121.67 (C6), 155.34 (C8), 131.07 (C15,C19), 116.87 (C16), 55.34 (OCH3), Mass: m/z: (M+1) 270.07.

3-(3-Chloro-phenyl)-4-(3-methoxy-phenyl)-isoxazole (4j)

Characterization White solid; Yield: 80%; m. p. 266-267°C. Elemental analysis of $C_{16}H_{12}ClNO_2$ calcd. (found)%: C, 67.26; H, 4.23; Cl, 12.41; N, 4.90; O, 11.20. H, 5.34 (5.32). IR (KBr, νmax, cm^{-1}): 2861 (O-CH_3), 1617 (Ar-H, stretching), 3551 (str, NH amide), 1636 (C=N, stretching in ring), 1026 (CO, stretching in ring), 832 (CH=CH p-disub-Aromatic). ^{1}H NMR (500 MHz, $CDCl_3$) δ : 8.97 (s, 1H, CH=CH), 6.92-7.52 (m, 8H, Ar-H), 3.83 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ : 130.67 (C1), 113.76 (C2), 110.85 (C4), 121.67 (C6), 155.42 (C8), 130.69 (C15), 129.16 (C16 C18), 128.91 (C19), 55.34 (OCH3), Mass: m/z: (M+1) 286.02.

3-(2-Methoxy-phenyl)-4-(3-methoxy-phenyl)-isoxazole (4k)

Characterization Brown solid; Yield: 84%; m. p. 269-270°C. Elemental analysis of $C_{17}H_{15}NO_3$ calcd. (found)%: C, 72.58; H, 5.37; N, 4.98; O, 17.06. IR (KBr, νmax, cm^{-1}): 2861 (O-CH_3), 1617 (Ar-H, stretching), 3551 (str, NH amide), 1636 (C=N, stretching in ring), 1026 (CO, stretching in ring), 832 (CH=CH p-disub-Aromatic). ^{1}H NMR (500 MHz, $CDCl_3$) δ : 8.86 (s, 1H, CH=CH), 6.88-7.57 (m, 8H, Ar-H), 3.92 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ : 130.69 (C1), 113.76 (C2), 110.86 (C4), 121.65 (C6), 155.77 (C8), 111.96 (C16), 130.92 (C17), 122.62 (C18), 131.39 (C19) 55.33 (OCH_3), 56.40 (OCH_3), Mass: m/z: (M+1) 282.02.

3,4-Bis-(3-methoxy-phenyl)-isoxazole (4l)

Characterization Brown solid; Yield: 84%; m. p. 269-270°C. Elemental analysis of $C_{17}H_{15}NO_3$ calcd. (found)%: C, 72.58; H, 5.37; N, 4.98; O, 17.06. IR (KBr, νmax, cm^{-1}): 2861 (O-CH_3), 1617 (Ar-H, stretching), 3551 (str, NH amide), 1636 (C=N, stretching in ring), 1026 (CO, stretching in ring), 832 (CH=CH p-disub-Aromatic). ^{1}H NMR (500 MHz, $CDCl_3$) δ : 8.85 (s, 1H, CH=CH), 6.85-7.42 (m, 8H, Ar-H), 3.83 (s, 6H, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ : 129.96 (C1), 113.76 (C2), 110.85 (C4), 121.67 (C6), 155.36 (C8), 112.47 (C16), 130.69 (C17), 122.62 (C18), 125.19 (C19) 55.34 (OCH_3), 55.35 (OCH_3), Mass: m/z: (M+1) 286.04.

3-(4-Methoxy-phenyl)-4-(3-methoxy-phenyl)-isoxazole (4m)

Characterization Brown solid; Yield: 84%; m. p. 269-270°C. Elemental analysis of $C_{17}H_{15}NO_3$ calcd. (found) %: C, 72.58; H, 5.37; N, 4.98; O, 17.06. IR (KBr, νmax, cm^{-1}): 2861 (O-CH_3), 1617

(Ar-H, stretching), 3551 (str, NH amide), 1636 (C=N, stretching in ring), 1026 (CO, stretching in ring), 832 (CH=CH p-disub-Aromatic). ^{1}H NMR (500 MHz, $CDCl_3$) δ : 8.97 (s, 1H, CH=CH), 6.92-7.54 (m, 8H, Ar-H), 3.79 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ : 130.69 (C1), 113.76 (C2), 110.85 (C4), 121.67 (C6), 155.35 (C8), 114.64 (C16, C18), 130.69 (C17), 130.56 (C15, C19) 55.33 (OCH_3), Mass: m/z: (M+1) 286.02.

4-(3-Methoxy-phenyl)-3-(4-nitro-phenyl)-isoxazole 4n

Characterization Yellow solid; Yield: 80%; m. p. 270-271°C. Elemental analysis of $C_{16}H_{12}N_2O_4$ calcd. (found)%: C, 64.86; H, 4.08; N, 9.46; O, 21.60. IR (KBr, νmax, cm^{-1}): 2861 (O-CH_3), 1617 (Ar-H, stretching), 3551 (str, NH amide), 1636 (C=N, stretching in ring), 1026 (CO, stretching in ring), 832 (CH=CH p-disub-Aromatic). ^{1}H NMR (500 MHz, $CDCl_3$) δ : 8.97 (s, 1H, CH=C), 6.92-7.54 (m, 8H, Ar-H), 3.83 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ : 130.69 (C1), 113.76 (C2), 110.85 (C4), 121.67 (C6), 155.301 (C8), 125.54 (C16, C18), 129.67 (C15, C19) 55.33 (OCH_3), Mass: m/z: (M+1) 297.01.

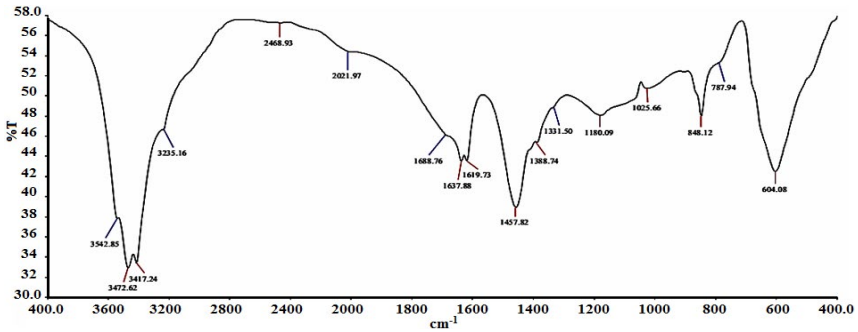


Fig. 1. FTIR spectrum of synthesized compound 4f

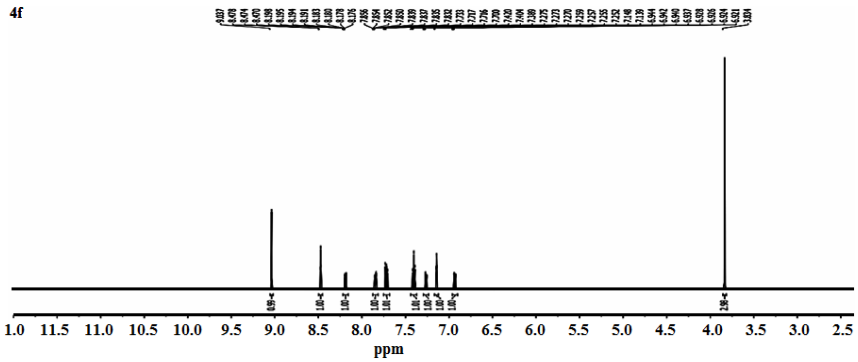


Fig. 2. ^{1}H NMR spectrum of synthesized compound 4f

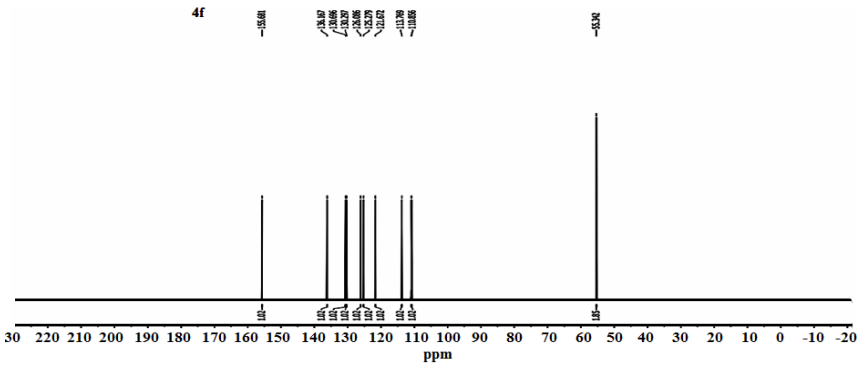


Fig. 3. ^{13}C NMR spectrum of synthesized compound 4f

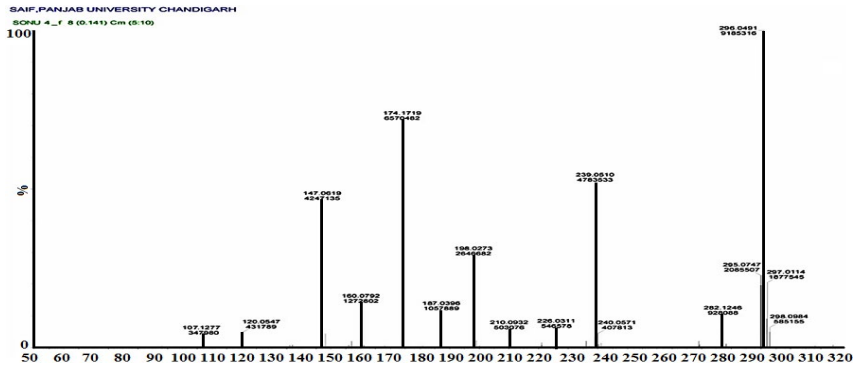


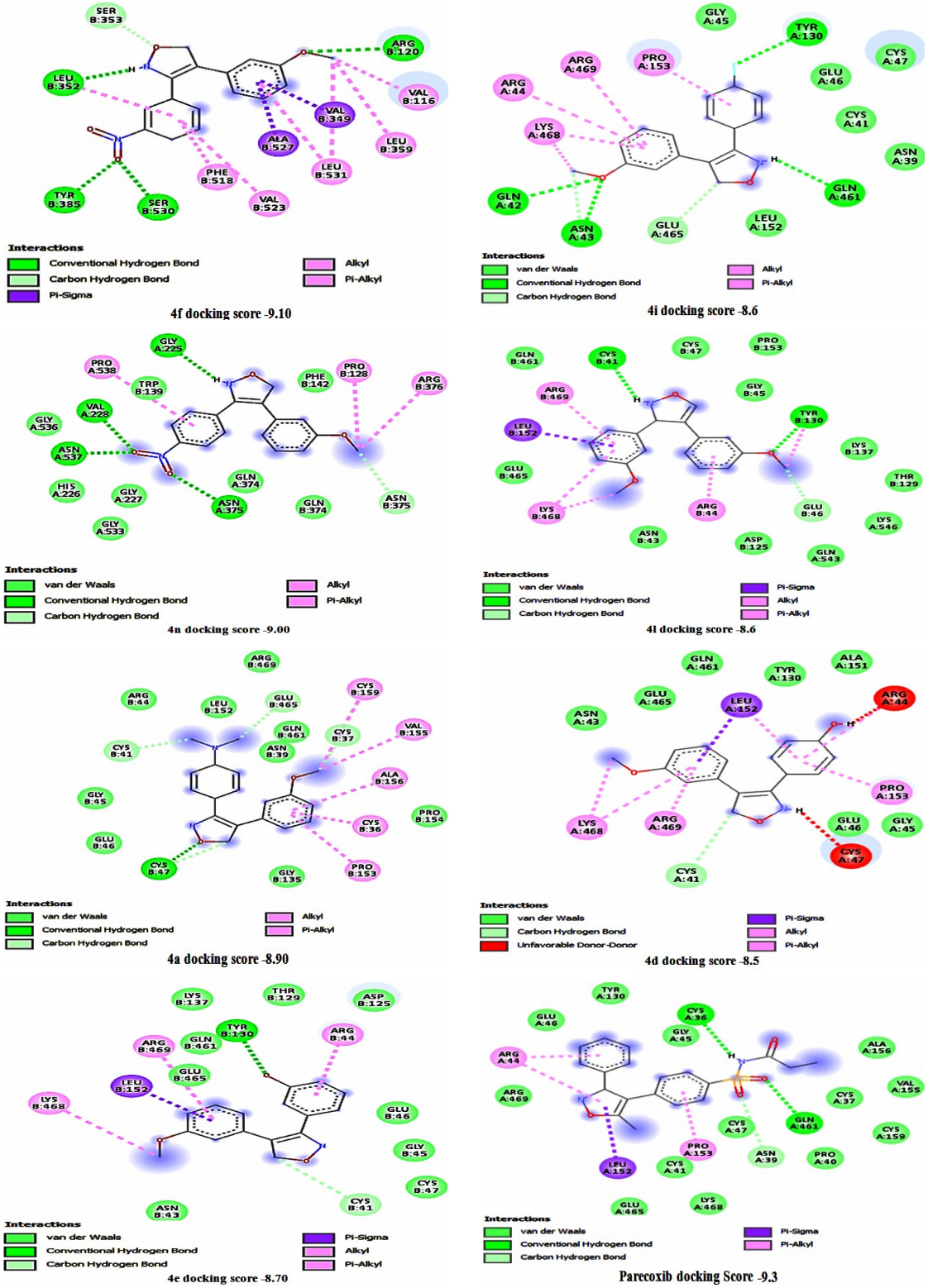
Fig. 4. Mass spectrum of synthesized compound 4f

Molecular modelling studies

In this work, the isolated compounds (4a–4n) were docked with the target protein Cyclooxygenase-2 (PDB ID: 4COX-2) as part of studies on the molecular docking of the separated molecules. It was estimated through molecular docking studies how the test drugs would probably attach to the targeted Cyclooxygenase-2 proteins using Auto Dock Vina 4.2 (MGLtools 1.5.7).16 A graphic depicting possible docked positions and binding energy (kcal/mol) The compounds' structures (4a–4n) were shown in the proper 2D orientations using ChemDraw 16.0. In order to lower the energy of the compounds/ligands, ChemDraw3D extreme was employed. The docking simulation was carried out using AutoDock Vina when the ligand molecules' energies were lowered. The crystal structures of the receptor protein molecules were identified using the protein data database. The intricate coordinates of the structures, which contained water molecules and other atoms, are what gave rise to better resolution. A protein preparation method with default settings was used to prepare the proteins.[18-20]

Table 1: Total Binding Affinities Energy (kcal.mol^{-1}) comparison between Parecoxib and Isoxazole derivatives (4a-4n) against the Cyclooxygenase-2 (4COX-2) receptor

Compound Code	Involved amino acid residues within 5 Å	Interaction Hydrogen bond	Total Binding Affinity Energy (kcal.mol^{-1})	RMSD
4a	Cys36, Cys37, Cys41, Cys47, Cys159, Pro153, Val155, Ala156, Glu465	Cys41, Cys47	-8.9	3.395
4b	Asp 125, Ser126, Pro542, Gln543, Lys546	Ser126, Lys546	-8	34.136
4c	Asn39, Cys36, Cys37,Cys41, Cys47, Tyr136, Pro153, Ala156,	Asn39, Cys41	-7.9	32.611
4d	Cys41, Arg44, Cys47, Leu152, Pro153, Lys468, Arg469,	Cys41	-8.5	2.003
4e	Cys41, Arg44, Tyr130, Leu152, Lys468, Arg469,	Cys41 Tyr130	-8.7	1.773
4f	Val 116, Arg120, Val 349, Leu352,Ser353, Leu359, Tyr 385, Phe518, Val523, Alu527, Ser530, Leu531	Arg120,Leu352	-9.1	29.013
4g	Cys41, Tyr130, Leu152,Pro153, Lys468		-8.4	40.552
4h	Cys36, Arg41, Cys47, Gly135,Pro153,Ala156		-8	19.314
4i	Gln42, Asn43, Arg44, Tyr130, Pro153, Gln461, Glu465, Lys468, Arg469	Tyr130Gln461	-8.6	2.573
4j	Cys47, Tyr130, Leu152,Pro153, Lys468	Cys47-8.6		40.529
4k	Asn39, Cys36, Cys41, Cys47, Tyr136, Pro153, Ala156	Asn39 Cys41	-8.3	45.408
4l	Cys41, Arg44, Glu46,Tyr130, Leu152, Lys468,Arg469	Cys41Glu46	-8.6	2.559
4m	Pro153, Trp323,Ser548	Ser548	-8.4	29.66
4n	Pro128, Gly225,Val 228,Asn375, Arg376, Asn537, Pro538	Gly225 Asn375	-9	21.407
Parecoxib	Cys36,Asn39,Arg44,Leu152,Pro153Gln461	Cys36Gln461	-9.3	3.274



The separated molecules (4f, 4n, 4a, 4e, 4i, 4l, 4d) 2D docked images and Parecoxib binding affinities with the protein 4COX-2 receptor in the 2D images, respectively

Biological activity

An anti-inflammatory action

Regarding the anti-inflammatory effects, the best docking score compounds were selected (Table 1). Using the carrageenan-induced paw edema method, the synthetic compounds (4f, 4n, 4a, 4e, 4i, 4l, 4d, 4g,) for anti-inflammatory activity[21-24]. After two hours, the studied drugs' anti-inflammatory efficacy is reduced by 43.95% to 62.71%. In comparison to the reference medication Parecoxib, which showed 76.24% inhibition 2 h later and 75.93% inhibition 3 h later, the inhibition ranged from 44.32% to 63.71% after 3 hours. In comparison to the reference medicine parecoxib, the compounds (4f, 4n, and 4a) were discovered to be the series' most powerful compounds (Table 2). Compound 4-(3-Methoxy-phenyl)-3-(3-nitro-phenyl)-isoxazole 4f showed 61.99% inhibition 2 h later and 61.20% inhibition 3 h later, indicating a high anti-inflammatory effect of these compounds. Whereas substance 4-(3-Methoxy-phenyl)-3-(4-nitro-phenyl)-isoxazole 4n showed inhibition at 2 and 3 h, respectively, of 61.47% and 62.24%. After 2 and 3 h, compound 4-[4-(3-Methoxy-phenyl)-isoxazol-3-yl]-phenyl-dimethylamine (4a) inhibited the reaction by 62.69% and 63.69%, respectively. These results served as the foundation for additional research and compound development. The most potent anti-inflammatory effect was displayed by the isoxazole derivatives (4f, 4n, 4a, 4e, 4i, 4l, 4d, 4g) including 4f, 4n, and 4a. The chemical 4g had the least amount of anti-inflammatory action. More than 50% of the compounds 4e, 4i, and 4l exhibited inhibition.

Table 2: Compounds' anti-inflammatory activity (4f, 4n, 4a, 4e, 4i, 4l, 4d, 4g).

Compounds	Increased Paw Volume (mm)		%Inhibition		Potential
Treatments	after 2 h	after 3 h	after 2 h	after 3 h	
4f	0.35±0.02 ***	0.34±0.02 ***	62.71	63.71	0.82
4n	0.36±0.02 ***	0.36±02 ***	61.47	62.24	0.81
4a	0.36±0.04 ***	0.37±0.04 ***	61.99	61.2	0.79
4e	0.43±0.01 ***	0.43±0.02 ***	54.46	55	0.71
4i	0.45±0.02 ***	0.43±0.03 ***	52.18	55	0.69
4l	0.46±0.04 ***	0.46±0.05 ***	51.31	51.89	0.66
4d	0.49±0.02 ***	0.48±0.02 ***	47.81	50.34	0.63
4g	0.53±0.04	0.53±0.04 ***	43.95	44.32	0.57
Parecoxib	0.20±0.02 ***	0.22±0.02 ***	77.23	76.89	1
Tween 80	0.96±0.02	0.97±0.02	-	-	-

RESULTS AND DISCUSSION

Chemistry for synthesis

By following the steps in Scheme 1, all novel isoxazole derivatives (4a–4n) were successfully developed. Through the use of infrared spectroscopy, FTIR, ^{1}H NMR, ^{13}C NMR, Mass Spectra, and elemental analysis, the novel synthesized compounds were verified. substituted benzaldehyde and 3-methoxy acetophenone were combined in a Claisen-Schmidt Condensation reaction to synthesize substituted chalcones (4a-4n). Under constant stirring and substituted chalcones were cyclized with hydroxylamine hydrochloride and sodium acetate by refluxing on an oil bath for six hours to produce isoxazole derivatives (4a-4n).

Molecular model

Cyclooxygenase-2 (PDB ID: 4COX-2) was the subject of a molecular docking investigation of target compounds (4a-4n) in relation to the reference medication Parecoxib. The target molecules (4a-4n) had lower docking scores on Cyclooxygenase-2, indicating that they could have more effective anti-inflammatory. 4f, 4n, and 4a were among the produced compounds that were most effective because they had the lowest docking scores for Cyclooxygenase-2 (4COX-2) receptor.

DISCUSSION

Pharmacology

Albino rats of all groups were treated 0.3% sodium carboxymethyl cellulose, 100 mg per kg of the test chemicals and 10 mg per kg of parecoxib were suspended. Both the vehicle (control) and the test drugs were used. The injection is given intraperitoneally (i.p). The data showed that all tested substances significantly decreased edema caused by carrageenan, and the results are shown in

Table 2. Cyclooxygenase-2 was used as a common medication. 4f showed a significant amount of anti-inflammatory activity, whereas 4n showed the least amount. The findings indicated that the activity response was producing outcomes comparable to those of the docking research.

CONCLUSION

The synthesis of novel isoxazole derivatives was detailed in the current work. Mass Spectra, FTIR, ^{1}H NMR, and ^{13}C NMR Spectra were used to analyze the synthesized compounds. Using the carrageenan-induced paw edema method for anti-inflammatory activity, Due to the nito group's presence at the meta and para positions, the results showed that compound 4f and 4n was the most active molecule. Further research into the mechanisms of action of some of these compounds may result in the identification of novel compounds with potential anti-effects in a research investigation.

ACKNOWLEDGMENT

The management at IFTM University deserves praise for setting up the lab for the effective conduct of the intended study work's experiments.

Conflict of interest

There are no competing interests, according to the authors.

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