Research Article ISSN: 2349 – 7106

Asian Journal of Research in Chemistry and Pharmaceutical Sciences

Journal home page: www.ajrcps.com

DESIGN AND SYNTHESIS OF NOVEL 5-(4-FLUORO-3-METHYLPHENYL)-3-(SUBSTITUTED ARYL) ISOXAZOLE DERIVATIVES AS POTENT ANTI-INFLAMMATORY AND ANALGESIC AGENTS

S. Raja*[1], V. Muralidharan[1], C. Asha Deepti[1]

[1]*Department of Pharmaceutical Chemistry, GITAM Institute of Pharmacy, GITAM (Deemed to be University), Gandhi Nagar, Rushikonda, Visakhapatnam-530 045, Andhra Pradesh, India.

ABSTRACT

A new series of 5-(4-fluoro-3-methylphenyl)-3-(substituted aryl) isoxazoles (2a-2h) were designed and synthesized by the treatment of substituted chalcones (1a-1h) with phenylhydrazine. Reaction of 4-fluoro-3-methyl-acetophenone (1) with different aryl aldehydes (2) in ethanol was carried out to gain 1a-1h derivatives. The synthesized compounds were characterized by FT-IR, ^{1}H-NMR, Mass spectroscopy and bases of elemental analysis. The *in vitro* and *in vivo* anti-inflammatory screening of the newly synthesized compounds was performed using Human Red Blood Cell (HRBC) membrane stabilization method and carrageenan-induced paw edema standard technique, respectively. The analgesic activity was also performed using the acetic acid writhing test. From the screening results, it was found that the compound 2h showed increased potency with 74.09 % inhibition of edema and 93.4% of HRBC membrane stabilization. From the results, it was revealed that the synthesized compounds with groups like hydroxyl, chloro and bromo showed the most potent activity compared to that of standard drug, diclofenac.

KEYWORDS

Isoxazoles, Chalcones, Anti-inflammatory, Analgesic activity, Carrageenan, HRBC method and Acetic acid writhing test.

Author for Correspondence:

S. Raja,

Department of Pharmaceutical Chemistry,

GITAM Institute Pharmacy,

Gandhi Nagar, Rushikonda, Andhra Pradesh, India.

Email: sraja61@gmail.com

INTRODUCTON

Inflammation is a complex process attempted by the body that may vary from a localized to a generalized response at self protection to remove harmful stimuli[1]. It was characterized by the accumulation of local fluids and leucocytes with the objective of healing the damaged tissue. Commencement of the inflammatory process was manifested by the signals of the inflammation which includes Dolor (pain), Rubor (Redness), Calor (Heat), Tumor (swelling) and Functio laesa (loss of function). The most commonly used treatment for inflammations is non-steroidal anti-inflammatory drugs (NSAID) medication which prevents the formation of prostaglandins by inhibiting the cyclooxygenase enzyme, which are the major players of the inflammatory process. Many of the anti-inflammatory drugs modulate the signaling pathways which are activated by the cytokines including NF-kB, STAT, Smad, Mtor and so on[2].

Heterocyclic chemistry is a branch which is inseparable from mankind because they are ubiquitous in nature. Human beings are totally dependent on the drugs derives from heterocyclic rings. Much attention has paid to the synthesis of nitrogen containing heterocyclic units viz., pyrazoles, pyrimidines and isoxazole derivatives mainly due to their broad spectrum of biological and pharmacological activities. These heterocycles were derived from the key intermediate chalcone. Derivatives of isoxazole[3-5] have played a decisive role in the record of heterocyclic chemistry. It has been used extensively important pharmacophores and synthons in the field of synthetic and medicinal chemistry. Isoxazoles are the indispensable for a number of drugs like cox-2 inhibitor, nitric oxide donor–furaxan etc. Owing to their versatile chemotherapeutic importance, a momentous amount of research effort has been focused on these nuclei to derive the compounds with potent activity and negligible adverse effects.

Isoxazole is an azole. It is a five membered heterocyclic ring with three carbon atoms, one oxygen atom and one nitrogen atom adjacent to each other. Isoxazole derivatives possess significant medicinal properties[6] and commercial utilities viz., cycloserine with antibiotic properties, isocarboxazid, a monoamine oxidase inhibitor with antipsychotic activity and denazol (Figure No.1), a steroid isoxazole with anabolic activity[7]. Naturally occurring isoxazoles, ibotenic acid, muscimol and muscazone were isolated from *Amanita species* possess CNS activity. Isoxazole derivatives possess different properties such as adenosine antagonist[8], lipoxygenase[9], anti-helmentic[10], anti-leukemic[11], antibacterial[12], anti-inflammatory[13] and anti-HIV[14]. In addition, 2134 Spiroisoxazolines[15] and benzofuroisoxazoles[16] were used as anti-convergents. Based on the above literature review and considering the wide applications of isoxazole molecule in medicinal chemistry, an attempt has been made to synthesize different substituted novel isoxazole derivatives as anti-inflammatory and analgesic agents.

MATERIAL AND METHODS

The chemicals and reagents used were obtained from various chemical units Avra, Sigma Aldrich, SRL and SD Fine Chem. The solvents used were purified before their use. The silica gel G used for thin layer chromatography (TLC) was obtained from E. Merck India Ltd. Solvent systems used were n-hexane: acetone (7:3). All the melting points were taken in open glass capillary and are uncorrected. ^{1}H NMR spectra were taken on a Bruker ultra-shield (400 MHz) NMR spectrometer in $CDCl_3$ using tetramethylsilane [$(CH3)_4Si$] as the internal standard. Chemical shift (δ) are expressed in ppm. Mass spectra were obtained on a JEOL-SX-102 instrument using electron impact ionization. All the IR spectra were recorded in KBr pellets on a Jasco FT-IR 410 spectrometer. Elemental analysis were performed on a Perkin Elmer model 240c analyzer and were within ±0.4% of the theoretical values.

General procedure for the synthesis of substituted chalcones (1a-1h)

The key intermediates (E)-1-(4-fluoro-3-methylphenyl)-3-(substituted aryl) prop-2-en-1-one (1a-1h) were prepared according to the reported literature[17]. The starting material 4-fluoro-3-methyl acetophenone (2mmol) was treated with aromatic aldehydes (2mmol) in presence of catalytic amount of lithium hydroxide. Ethanol (20ml) was used as a solvent. The reaction mixture was kept for constant stirring using a multistage magnetic stirrer at room temperature until the solution turns turbid. The reaction was monitored by TLC (n-hexane: acetone - 7:3). Then the reaction mixture was poured into crushed ice and neutralized with the help of dil.

HCl. The precipitate was filtered under vacuum, washed with cold ethanol and distilled water. The obtained substituted chalcones were purified by recrystalization and column chromatography. The synthetic reaction was illustrated in Scheme No.1.

(E)-1-(4-fluoro-3-methylphenyl)-3-(3,4,5-trimethoxyphenyl) prop-2-en-1-one (1a)

Yield 86%, Mp 96ºC; FT-IR (KBr) cm^{-1}: 1651 (C=O, Chalcone); 1585 (C=C); 1244 (C-O-CH_3); 2924 (C-CH_3); 1122 (C-F); ^{1}HNMR (400 MHz, $CDCl_3$, δ ppm): 2.38(s, 3H, -CH_3); 3.952 (d, 9H, -OCH_3); 7.758 (d, 1H, α-H); 7.926 (d, 1H, β-H), 7.719 (s, 1H, Ar-H), 7.14(d, 1H, Ar-H); 6.889 (d, 1H, Ar-H); 7.12(s, 1H, Ar-H); 7.28(s, 1H, Ar-H); MS (EI) m/z: 331 [M^+]; Anal. Calcd for $C_{19}H_{19}FO_4$; C, 69.08; H, 80; F, 5.75; O, 19.37.

(E)-3-(2-bromophenyl)-1-(4-fluoro-3-methylphenyl) prop-2-en-1-one (1b)

Yellow crystals (EtOH), Yield = 76%; mp 100-102ºC. FT-IR (KBr) cm^{-1}: 1658 (C=O, Chalcone), 1588 (C=C), 2924 (C-CH_3), 1153(C-F), 752, 9 (C-Br). ^{1}HNMR (400 MHz, $CDCl_3$, δ ppm): 2.384 (s, 3H, -CH_3), 7.374 (d, 1H, α-H), 8.155 (d, 1H, β-H), 7.119-7.93 (d, 7H, Ar-H). MS (EI) m/z: 319 (M^+). Anal. Calcd for $C_{16}H_{12}$BrFO: C, 60.21; H, 3.79; F, 5.95; O, 5.01; Br, 25.04.

(E)-1-(4-fluoro-3-methylphenyl)-3-(2-nitrophenyl) prop-2-en-1-one (1c)

Orange crystals (EtOH), Yield = 77%; mp 94-96ºC. FT-IR (KBr) cm^{-1}: 1674 (C=O, Chalcone), 1513 (C=C), 2878 (C-CH_3), 1292 (C-F), 1341 (C-NO_2). ^{1}HNMR (400 MHz, $CDCl_3$, δ ppm): 2.39 (s, 3H, CH_3), 7.86 (d, 1H, α-H), 8.09 (d, 1H, β-H), 7.585 (s, 1H, Ar-H), 7.173 (d, 1H, Ar-H), 7.71 (d, 1H, Ar-H) 8.15 (s, 1H, Ar-H), 8.112 (d, 1H, Ar-H), 7.582 (d, 1H, Ar-H), 7.928 (d, Ar-H). MS (EI) m/z: 284 (M^+). Anal. Calcd for $C_{16}H_{12}FNO_3$: C, 67.36; H, 4.24; F, 6.66; N, 4.91; O, 16.83.

(E)-1-(4-fluoro-3-methylphenyl)-3-(4-hydroxy-3-methoxy-5-nitrophenyl) prop-2-en-1-one (1d)

Pale yellow crystals (EtOH), Yield = 78%; mp 91-93ºC. FT-IR (KBr) cm^{-1}: 1684 (C=O, Chalcone), 1546 (C=C), 2944 (C-CH_3), 1103(C-F), 1230 (C-OCH_3), 1366 (NO_2), 3200 (OH). ^{1}HNMR (400 MHz, $CDCl_3$, δ ppm) CH_3: 2.186 (s, 3H, CH_3), 1.727 (s, 1H, OH) 4.043 (s, 3H, -OCH_3), 7.664 (d, 1H, α-H), 8.245 (d, 1H, β-H), 7.285-8.245(m, 5H, Ar-H). MS (EI) m/z: 331, 09 (M^+). Anal. Calcd for $C_{17}H_{14}NO_5$: C, 61.63; H, 4.26; F, 5.73; N, 4.23; O, 24.15.

(E)-3-(3,4-dimethoxyphenyl)-1-(4-fluoro-3-methylphenyl) prop-2-en-1-one (1e)

Lemon yellow crystals (EtOH), Yield = 81%; mp 85-87ºC. FT-IR (KBr) cm^{-1}: 1656 (C=O, Chalcone), 1583 (C=C), 2930 (C-CH_3), 1254(C-F), 1142 (C-OCH_3). ^{1}HNMR (400 MHz, $CDCl_3$, δ ppm): 2.35 (s, 3H, CH_3), 3.936 (d, 6H, -OCH_3), 7.739 (d, 1H, α-H), 7.905 (d, 1H, β-H), 7.38 (s, 1H, Ar-H), 7.25 (d, 1H, Ar-H), 7.864 (d, 1H, Ar-H), 7.23 (s, 1H, Ar-H), 7.080 (d, 1H, Ar-H), 6.913 (d, 1H, Ar-H). MS (EI) m/z: 301 (M^+). Anal. Calcd for $C_{18}H_{17}FO_3$: C, 71.99; H, 5.71; F, 6.33; O, 15.98.

(E)-3-(4-(dimethylamino) phenyl)-1-(4-fluoro-3-methylphenyl) prop-2-en-1-one (1f)

Bright red crystals (EtOH), Yield = 83%; mp 93-95ºC. FT-IR (KBr) cm^{-1}: 1651 (C=O, Chalcone), 1593 (C=C), 2923 (C-CH_3), 1243(C-F). ^{1}HNMR (400 MHz, $CDCl_3$, δ ppm): 2.37 (s, 3H, CH_3), 3.06 (s, 6H, N(CH_3), 7.913 (d, 1H, α-H), 7.577 (d, 1H, β-H), 7.34 (s, 1H, Ar-H), 7.03 (d, 1H, Ar-H), 7.849 (d, 1H, Ar-H), 7.78 (s, 2H, Ar-H), 6.734 (d, 2H, Ar-H). MS (EI) m/z: 284 (M^+). Anal. Calcd for $C_{18}H_{18}$FNO: C, 76.30; H, 6.40; F, 6.71; O, 5.65; N, 4.94.

(E)-3-(4-chlorophenyl)-1-(4-fluoro-3-methylphenyl) prop-2-en-1-one (1g)

Lemon yellow crystals (EtOH), Yield = 77%; mp 101-103ºC. FT-IR (KBr) cm^{-1}: 1663 (C=O, Chalcone), 1591 (C=C), 2960 (C-CH_3), 1243(C-F), 819 (C-Cl). ^{1}HNMR (400 MHz, $CDCl_3$, δ ppm): 2, 38 (s, 3H, CH_3), 7,591 (d, 1H, α-H), 7,93 (d, 1H, β-H), 7,58 (s, 1H, Ar-H), 7, 433 (d, 1H, Ar-H), 7, 61 (d, 1H, Ar-H), 7, 60 (s, 1H, Ar-H), 7, 75 (d, 1H, Ar-H), 7, 47 (d, 1H, Ar-H). MS (EI) m/z: 275 (M^+). Anal. Calcd for $C_{16}H_{12}$ClFO: C, 69.95; H, 4.40; Cl, 12.91; F, 6.92; O, 5.82.

(E)-3-(5-bromo-2-hydroxy-3-methoxyphenyl)-1-(4-fluoro-3-methylphenyl) prop-2-en-1-one (1h)

Orange crystals (EtOH), Yield = 69%; mp 93-95ºC, FT-IR (KBr) cm^{-1}: 1656 (C=O, Chalcone), 1581 (C=C), 2923 (C-CH_3), 1195 (C-F), C-OH (3243), C-OCH_3 (1255), C-Br (705): ^{1}HNMR (400 MHz, $CDCl_3$, δ ppm): 1,617 (s, 3H, CH_3), 7,34 (d, 1H, α-

H), 9,879 (s, 1H, β-H), 7, 203 (d, 5H, Ar-H), 7, 285(s, 1H, OH), 3, 943(s, 3H, C-OCH_3): MS (EI) m/z: 365 (M^+): Anal. Calcd for $C_{17}H_{14}FBrO_3$: C, 55.91; H, 3.83; Br, 21.88; F, 5.20; O, 13.14.

Synthesis of substituted isoxazoles (2a–2h)

The substituted chalcones (1a-1h) were treated with hydroxylamine hydrochloride in presence of 5ml glacial acetic acid. Catalytic amount of sodium acetate was added to the reaction mixture. The above reaction mixture was refluxed in 20ml of ethanol for 6 to 8 hrs. After completion of the reaction (monitored by TLC) the reaction mixture was poured in to crushed ice. The precipitate was filtered under vacuum. The crude product obtained was recrystallized using suitable solvent.

5-(4-fluoro-3-methylphenyl)-3-(3,4,5-trimethoxyphenyl) isoxazole (2a)

Yield 66 %, Mp 114ºC , FT-IR (KBr) cm^{-1}: 1051 (C-F); 2928 (C-CH_3 Str); 1496 (Ar C=C); 1613 (C=N str); 848 (N-O str); 1240 (OCH_3 str): ^{1}HNMR (400 MHz, $CDCl_3$, δ ppm) 2.356 (s, 3H, CH_3); 6.67 (s, 1H, CH-Isoxazole); 7.86 (s, 1H, C_2-H); 6.99 (s, 1H, C_5-H); 8.71 (m, 1H, C_6-H); 4.16 (s, 6H, O-CH_3); 3.75 (s, 3H, O-CH_3); 7.12 (m, 2H, $C_{2''}$ and $C_{6''}$-H): MS (EI) m/z: 343 [M^+]; Anal. Calcd for $C_{19}H_{18}FNO_4$; C, 66.46; H, 5.28; F, 5.53; N, 4.08; O, 18.64.

3-(2-bromophenyl)-5-(4-fluoro-3-methylphenyl) isoxazole (2b)

Yield 69 %, Mp 119ºC , FT-IR (KBr) cm^{-1}: 1062 (C-F); 2942 (C-CH_3 Str); 1533 (Ar C=C); 1481 (C=N str); 852 (N-O str); 693 (C-Brstr): ^{1}HNMR (400 MHz, $CDCl_3$, δ ppm) 2.388 (s, 3H, CH_3); 7.48 (s, 1H, CH-Isoxazole); 7.34 (s, 1H, C_2-H); 7.65 (s, 1H, C_5-H); 7.71 (s, 1H, C_6-H); 7.48 (s, 2H, $C_{3''}$ and $C_{5''}$-H); 7.66 (s, 2H, $C_{4''}$ and $C_{6''}$-H): MS (EI) m/z: 332 [M^+]; Anal. Calcd for $C_{16}H_{11}BrFNO$; C, 57.85; H, 3.34; Br, 24.06; F, 5.72; N, 4.22; O, 4.82.

5-(4-fluoro-3-methylphenyl)-3-(2-nitrophenyl) isoxazole (2c)

Yield 72%, Mp 98ºC, FT-IR (KBr) cm^{-1}: 1177.21 (C-F); 2920.25 (C-CH_3 Str); 1600.54 (Ar C=C); 1518.73 (C=N str); 820.82 (N-O str); 1677 (NO_2 str): ^{1}HNMR (400 MHz, $CDCl_3$, δ ppm) 2.369 (s, 3H, CH_3); 7.081 (s, 1H, CH-Isoxazole); 7.560 (s, 1H, C_2-H); 7.428 (d, J=2.4Hz, 1H, C_5-H) 7.820 (d, J=7.6Hz, 1H, C_6-H); 7.304 (m, 1H, $C_{5''}$-H); 7.701 (m, 1H, $C_{4''}$-H); 8.053 (d, 2H, $C_{3''}$ and $C_{6''}$-H): MS (EI) m/z: 298 [M^+]; Anal. Calcd for $C_{16}H_{11}FN_2O_3$; C, 64.43; H, 3.72; F, 6.37; N, 9.39; O, 16.09.

4-(5-(4-fluoro-3-methylphenyl)isoxazol-3-yl)-2-methoxy-6-nitrophenol (2d)

Yield 69%, Mp 149ºC, FT-IR (KBr) cm^{-1}: 1057.28 (C-F); 2930.13 (C-CH_3 Str); 1619.56 (Ar C=C); 1545.83 (C=N str); 1008.21 (N-O str); 1020.30 (OCH_3): ^{1}HNMR (400 MHz, DMSO, δ ppm) 2.509 (s, 3H, CH_3); 3.905 (s, 3H, OCH_3); (s, 1H, CH-Isoxazole); 8.126 (s, 1H, C_2-H); 7.476 (d, J=1.6Hz 1H, C_5-H); 7.660 (d, J=1.6Hz, 1H, C_6-H); 10.786 (s, 1H, O-H): MS (EI) m/z: 344 [M^+]; Anal. Calcd for $C_{17}H_{13}FN_2O_5$; C, 59.30; H, 3.81; F, 5.52; N, 8.14; O, 23.24.

3-(3,4-dimethoxyphenyl)-5-(4-fluoro-3-methylphenyl) isoxazole (2e)

Yield 83%, Mp 99ºC, FT-IR (KBr) cm^{-1}: 1019.15 (C-F); 2972.47 (C-CH_3 Str); 1635.55 (Ar C=C); 1510.73 (C=N str); 1008.24 (N-O str); 1057.30 (OCH_3): ^{1}HNMR (400 MHz, $CDCl_3$, δ ppm) 2.344 (s, 3H, CH_3); 3.943 (d, J=11.6Hz, 6H, OCH_3); 6.727 (s, 1H, CH-Isoxazole); 7.160 (s, 1H, C_2-H); 7.057 (d, J=2.4Hz, 1H, C_5-H) 7.102 (m, 1H, C_6-H); 6.873 (m, 1H, $C_{5''}$-H); 7.561 (m, 1H, $C_{2''}$-H);7.369 (m, 1H, $C_{6''}$-H): MS (EI) m/z: 313 [M^+]; Anal. Calcd for $C_{18}H_{16}FNO_3$; C, 69.00; H, 5.15; F, 6.06; N, 4.47; O, 15.32.

4-(5-(4-fluoro-3-methylphenyl)isoxazol-3-yl)-N, N-dimethylaniline (2f)

Yield 78%, Mp 100ºC, FT-IR (KBr) cm^{-1}: 1194.54 (C-F); 2923.12 (C-CH_3 Str); 3078.31 (Ar C-H str); 1609.61 (Ar C=C); 1519 (C=N str); 820.82 (N-O str); 1224.15 (N-CH_3): ^{1}HNMR (400 MHz, $CDCl_3$, δ ppm) 2.326 (s, 3H, CH_3); 2.974 (d, J=11.6Hz, 6H, N-$(CH_3)_2$); 5.688 (s, 1H, CH-Isoxazole); 7.591 (m, 1H, C_2-H); 7.073 (m, 1H, C_5-H) 7.290 (m, 1H, C_6-H); 6.760 (m, 2H, C_3 and $C_{5''}$-H); 7.609 (m, 1H, $C_{2''}$-H);7.505 (m, 1H, $C_{6''}$-H): MS (EI) m/z: 296 [M^+]; Anal. Calcd for $C_{18}H_{17}FN_2O$; C, 72.95; H, 5.78; F, 6.41; N, 9.45 O, 5.40.

3-(4-chlorophenyl)-5-(4-fluoro-3-methylphenyl) isoxazole (2g)

Yield 84%, Mp 90ºC, FT-IR (KBr) cm^{-1}: 1057.31 (C-F); 2972.45 (C-CH_3 Str); 1508 (C=N str);

1008.01 (N-O str); 1019.03 (C-Cl): ^{1}HNMR (400 MHz, $CDCl_3$, δ ppm) 2.349 (s, 3H, CH_3); 6.953 (s, 1H, CH-Isoxazole); 7.642 (s, 1H, C_2-H); 7.089 (d, J=2.4Hz, 1H, C_5-H); 7.386 (m, 1H, C_6-H); 7.425 (m, 2H, $C_{3''}$ and $C_{5''}$-H); 7.785 (m, 1H, $C_{2''}$-H);7.766 (m, 1H, $C_{6''}$-H): MS (EI) m/z: 287 [M^+]; Anal. Calcd for $C_{16}H_{11}$ ClFNO; C, 66.79; H, 3.85; Cl, 12.32; F, 6.60; N, 4.87; O, 5.56.

4-bromo-2-(5-(4-fluoro-3-methylphenyl) isoxazol-3-yl)-6-methoxyphenol (2h)

Yield 68%, Mp 131ºC, FT-IR (KBr) cm^{-1}: 1018 (C-F); 2924 (C-CH_3 Str); 1587 (Ar C=C); 1518 (C=N str); 1124 (N-O str); 1263 (OCH_3); ; 732 (C-Br); 3358 (OH): ^{1}HNMR (400 MHz, DMSO, δ ppm) 2.32 (s, 3H, CH_3); 7.03 (s, 1H, CH-Isoxazole); 6.87 (m, 1H, C_2-H); 7.31 (m, 1H, C_5-H); 7.63(m, 1H, C_6-H); 3.85 (s, 3H, O-CH_3); 9.14 (s, 1H, OH); 7.21 (1H, $C_{4''}$-H); 7.61 (1H, $C_{5''}$-H): MS (EI) m/z: 377 [M^+]: Anal. Calcd for $C_{17}H_{13}$ Br FNO_3; C, 53.99; H, 3.46; Br, 21.13; F, 5.02; N, 3.70; O, 12.69.

Pharmacological Activities

The synthesized compounds were evaluated for their anti-inflammatory and analgesic activities. The test compounds (2a - 2h) and the standard drug were administered in the form of a suspension (1% carboxy methyl cellulose as a vehicle) by oral route for analgesic and anti-inflammatory activities. Six animals were taken in each group. The animals were maintained in colony cages at 25 ± 2ºC, relative humidity of 45–55%, under a 12 h light and dark cycle and were fed standard animal feed[18]. All the animals were acclimatized for a week before use.

***In-vitro* anti-inflammatory screening**

HRBC membrane stabilization method as used for the estimation of anti-inflammatory activity *in vitro*[19]. The human blood was collected and mixed with equal volume of Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid, 0.42% NaCl) and centrifuged at 3000 rpm for 10 min. The packed cells were washed with iso-saline (0.36%) and a 10% suspension was made. Various concentrations of the isoxazole derivatives (2a – 2h) were prepared (75, 150, 200 µg/ml) using distilled water and to each concentration 1ml of phosphate buffer, 2ml hyposaline and 0.5 ml of Human Red Blood Cell (HRBC) suspension were added. It was incubated at 37ºC for 30 min and centrifuged at 3000 rpm for 20 min and the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Diclofenac (75, 150, 200 µg/ml) was used as the reference standard and the control was prepared by omitting the compounds under examination.

The percentage of HRBC membrane stabilization or protection was calculated by using the following formula

$$\text{Formula} = 100 - \frac{(\text{Absorbanceoftestsolution} - \text{Absorbanceoftestcontrol})}{\text{Absorptionoftestcontrol}} \times 100$$

***In-vivo* anti-inflammatory screening**

Carrageenan induced rat paw edema method was used to screen in vivo anti-inflammatory screening[20]. Wister albino rats of either sex, weighing between 150-200 gms were divided into 10 groups of six animals each. They were allowed for fasting overnight and given water adlibitum. The test drugs (100mg/kg body weight) and the standard drug (100mg/kg body weight) were administered orally with the help of the oral catheter. After 30mins, 0.05ml of 1% carrageenan suspension was slowly injected subcutaneously into the subplantar region of the left hind paw to all the groups to produce inflammation. Group I was given only 1% sodium CMC suspension (1ml/kg) and was used as carrageenan treated control. Group II was treated with the standard drug diclofenac (100mg/kg). Similarly, the rest of the groups were administered with test drugs (2a – 2h) respectively. After the administration of carrageenan, the volume of its displacement was measured volumetrically by comparing it with zero minute reading and again after every 1, 2, 3 and 4 hours of induction with plethysmometer apparatus and compared. The percentage increase of paw thickness was determined at 0, 1, 2, 3 and 4 hrs after induction of inflammation.

The anti-inflammatory activity was expressed as a percentage inhibition**.**

$$\%\text{Inhibition} = \frac{(\text{control} - \text{test})}{\text{control}} \times 100$$

Analgesic activity

Acetic acid writhing test was performed on Wister albino rats by following the method of Berkowitz *et al*[21]. Test compounds were given to the animals at the dose of 50 mg/kg, 30 min later the animals were injected interperitoneally with 0.25 ml/rat of 0.5% acetic acid. The mean number of writhes for each experimental group and the percentage decrease compared with the control group was calculated after 60 min.

RESULTS AND DISCUSSION

Chemistry

The structures of the synthesized compounds were confirmed by elemental analysis and spectral (FTIR, ^{1}H NMR, and Mass) data. The formations of intermediate chalcones (1a-1h) were confirmed by the presence of IR bands in the region of 1700 to 1600 cm^{-1} range (CH=CH-C=O). The presence of fluoro group in the chalcones was characterized by a strong band in its IR spectrum at 1149 cm^{-1}. The presence of methyl group was confirmed by ^{1}H NMR peak at δ 2.3 ppm and IR absorption band in the region of 2924 cm^{-1}. The presence of the nitro group in the compounds 1cand 1dwas confirmed by the IR bands 1341 cm^{-1} and 1366 cm^{-1}. The IR band in the region of 820-1181 range corresponds to N-O stretching vibrations and the band in the region of 1481 to 1683 range corresponds to C=N stretching vibrations with medium intensity indicates the formation of isoxazoles (2a-2h). Its ^{1}H NMR spectrum showed a singlet peak at a range of δ 6.67 ppm due to the proton at the fourth position in the isoxazole ring confirms its formation. Further, their purity and molecular weight were confirmed by mass spectra.

Anti-inflammatory activity

For the identification of *in vivo* and *in vitro* anti-inflammatory activity in albino rats, test compounds were administered orally and challenged by carrageenan induced rat paw edema method and HRBC membrane stabilization method respectively. Compounds found to be active in these challenges are generally regarded to be significantly useful candidates in the treatment of inflammation. The data regarding the anti-inflammatory screening of all the compounds are reported in Table No.1 and No.2. In the anti-inflammatory investigation, five compounds 2h, 2g, 2b, 2d and 2f were found to be significantly active ($p<0.05$), as they showed similar percentage inhibition against the standard drug. The promising nature of the compounds may be attributed to the substitutions at the hydrophobic domain. These compounds had electron withdrawing groups at the para position of the hydrophobic aryl ring. In general, it was observed that the para substituted derivatives deliver more activity than the other derivatives. The para substituted derivatives get better fitted into the receptor site, thus shows supreme activity. Compounds that showed protection at 100 mg/kg after 4hrs, indicating the ability of these compounds to protect from inflammation at the relatively lower dose.

Analgesic activity

The acetic acid writhing test was performed to investigate analgesic activity. Compounds 2h, 2g and 2b revealed the protection in analgesic test, at a dose of 50mg/kg after 1h. It was comparable to results obtained for diclofenac which was recognized as reference analgesic drug for this screen. Among all compounds, 2h was found to be remarkably active 50 mg/kg dose after 1h. The other compounds 2g, 2b, and 2dshowed considerable analgesic activity 50 mg/kg after 1h. The remaining compounds 2a, 2c and 2e didn't show any activity. It was observed that in this method, the most active compound have the substitution at the para position of the aryl ring by electronegative group resulted in increased analgesic activity.

SAR of synthesized compounds

Carrageenan-induced paw edema test was performed to assess the anti-inflammatory activity of test compounds using Albino rats. The anti-inflammatory activity results (Table No.2) showed that all the test compounds protected rats from carrageenan-induced inflammation activity increased at 1 h and it reached a maximum level at 2 h. Declining in activity was observed at 3 h. The compounds possessing dimethoxy 2e and trimethoxy 2a phenyl ring exhibited least anti-inflammatory activity when compared to the reference standard diclofenac sodium. With increased lipophilicity, the compound with dimethylamino substituent 2f and nitrophenyl substituents 2c showed moderate activity. The presence of hydroxyl, methoxy, nitro substituents on the phenyl ring 2d showed equipotent activity with reference standard Diclofenac sodium. Similar, activity was observed with the derivative 2g which possesses para chloro phenyl group. Among all tested compounds hydroxy, methoxy and bromo phenyl analog 2h exhibited the better activity which was more potent than diclofenac. Like analgesic activity compounds 2h, 2g and 2b were found to be the most active agent which are moderately potent when compared to the reference standard drug diclofenac sodium.

Table No.1: Anti-inflammatory activity of Isoxazole (I-1 to I-6) HRBC Method

S.No	Compound	Concentration (µg/ml)	%Stabilization
1	2a	75	46.3%
		150	59.8%
		200	70.2%
2	2b	75	60.3%
		150	75.9%
		200	88.4%
3	2c	75	44.3%
		150	57.3%
		200	70.5%
4	2d	75	49.1%
		150	64.3%
		200	81.6%
5	2e	75	42.5%
		150	54.4%
		200	66.6%
6	2f	75	45.2%
		150	53.9%
		200	74.6%
7	2g	75	61.3%
		150	75.6%
		200	90.2%
8	2h	75	65.3%
		150	76.8%
		200	93.4%
9	Diclofenac	75	68.3%
		150	80.2%
		200	94.1 %

Table No.2: Effect of Isoxazole derivatives in carrageenan induced paw oedema in rats and analgesic activity

Compound	1st Hr	2nd Hr	3rd Hr	4th Hr	% Inhibition of edema after 4Hrs	Analgesic activity % decrease of writhes in 60 min after treatment relative to control
2a	0.764±0.0549	0.925±0.0267*	0.836±0.0732	0.812±0.0234*	42.20	34
2b	0.534±0.0753	0.692±0.0472*	0.601±0.0142**	0.461±0.0256*	67.00	61
2c	0.672±0.0756	0.775±0.0325*	0.758±0.0357*	0.694±0.0257*	50.60	40
2d	0.612±0.0647	0.709±0.0213*	0.632±0.0364*	0.503±0.0256*	64.19	54
2e	0.706±0.0564	0.842±0.0143**	0.804±0.0674	0.762±0.0596	45.76	36
2f	0.641±0.0163**	0.846±0.0264*	0.712±0.0497*	0.593±0.0254*	57.79	45
2g	0.447±0.0921	0.635±0.416*	0.526±0.0123**	0.408±0.0249*	70.96	64
2h	0.321±0.0451*	0.529±0.0326*	0.406±0.0256*	0.364±0.0123**	74.09	66
Diclofenac	0.436±0.0126**	0.567±0.0441*	0.522±0.0463*	0.398±0.0176**	71.67	60
Control	0.863±0.0236	1.268±0.0129	1.339±0.0346	1.405±0.0561	--	--

Values are expressed as mean ± SD (n=6). Statistically significant (* = p<0.05, ** = p<0.01) difference in comparison to control.

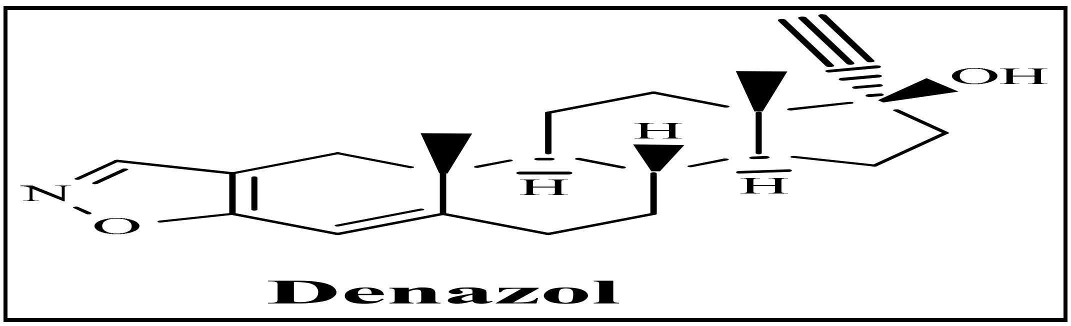
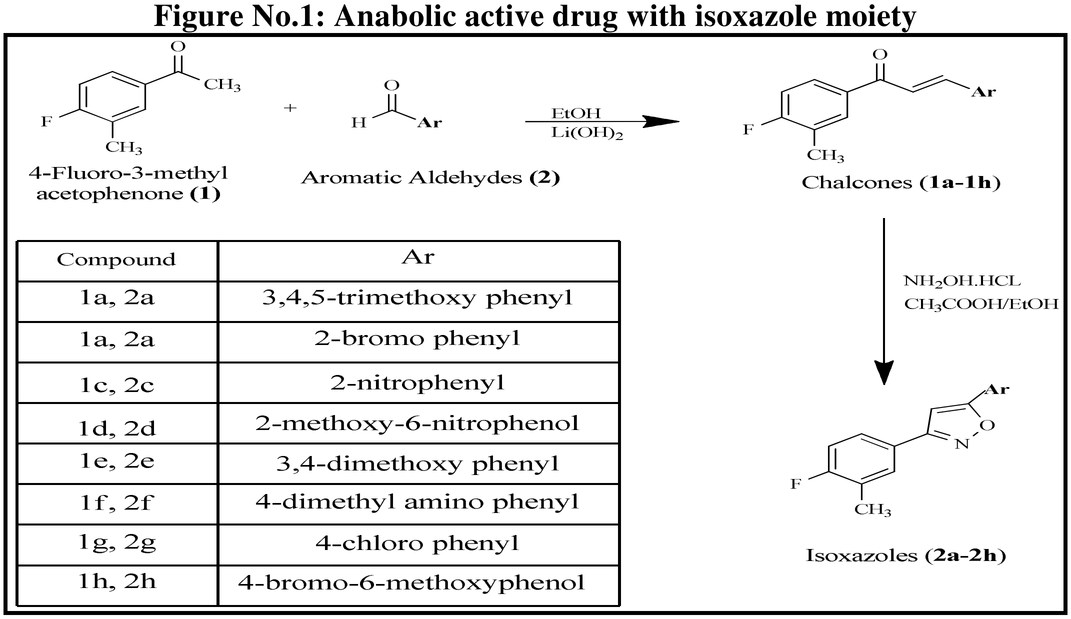


Figure No.1: Anabolic active drug with isoxazole moiety



Scheme No.1: Synthesis of 5-(4-fluoro-3-methylphenyl)-3-(Substituted aryl) isoxazole (2a - 2h)

CONCLUSION

In summary, a series of novel isoxazole derivatives 2a–2h were synthesized and characterized by FT-IR, ^{1}H-NMR, Mass spectroscopy and elemental analysis. These derivatives were evaluated for their analgesic and anti-inflammatory activities. In general, hydroxy phenyl substituted isoxazole compounds exhibited potent analgesic and anti-inflammatory activities. From the study, it was concluded that in this series nature of the substituent played a major role in analgesic and anti-inflammatory activity than its position. Among several tested compounds, 4-bromo-2-(5-(4-fluoro-3-methylphenyl)isoxazol-3-yl)-6-methoxyphenol 2h showed better analgesic and anti-inflammatory activities which was more potent than reference standard diclofenac. Hence, this analog could be developed as a new class of analgesic, and anti-inflammatory agents.

ACKNOWLEDGEMENT

The authors are thankful to the UGC (New Delhi, India) for providing financial assistance to Department of Pharmacy, GITAM Institute of pharmacy, Gandhi Institute of Technology and Management (GITAM Deemed to be University), Visakhapatnam, Andhra Pradesh, India.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBILIOGRAPHY

1. Davies S P, Reddy H, Caivano M, Cohen P. Specificity and mechanism of action of some commonly used protein kinase inhibitors, *Biochem. J,* 351(Pt1), 2000, 95-105.
2. Filosa R, Kasi P D, Nabavi S M. New trends in anti-inflammatory drugs, *Eur. J. Med. Chem,* 153, 2018, 1-140.
3. Swamy P M G and Agasimundin Y S. Synthesis and antimicrobial screening of certain substituted chalcones and isoxazolines bearing hydroxy benzofuran, *Rasayan J. Chem,* 1(2), 2008, 421-428.
4. Kalirajan R, Sivakumar S U, Jubie S, Gowramma B, Suresh B. Synthesis and Biological evaluation of some heterocyclic derivatives of chalcones, *Int. J. Chem Tech Res,* 1(1), 2009, 27-34.
5. Gupta U, Sareen V, Khatri V, Chugh S. Synthesis and antifungal activity of new fluorine containing 4-(substitutedphenyl) hydrazono-3-methyl-2-pyrazolin-5-ones and 2-isoxazolin-5-ones, *Indian J. Heterocycl. Chem,* 13(4), 2004, 351-354.
6. Wilson C O, Gisvold O, Text book of Organic Medicinal and Pharmaceutical Chemistry, *Lippincott Williams and Wilkins Pitman, London,* 1966.
7. Kochetkov N K. Sokolov S D. Recent Developments in Isoxazole Chemistry, *Adv. Heterocycl. Chem,* 2, 1963, 365-422.
8. Shehata I A and Glannoh R A. Mesoionicisoxazolo [2, 3-a] pyrimidinediones and 1, 3, 4-oxadiazolo [3, 2-a] pyrimidinediones as potential adenosine antagonists, *J. Heterocycl. Chem,* 24(5), 1987, 1291-1295.
9. Rakesh K S, Jagadish S, Balaji K S, Zameer F, Swaroop T R, Mohan C D, Jayarama S, Rangappa K S. 3, 5-disubstituted isoxazole derivatives, potential inhibitors of inflammation and cancer, *Inflammation,* 39(1), 2016, 269-280.
10. Carr J B, Durham H G, Hass D K. Isoxazole anthelmintics, *J. Med. Chem,* 20(7), 1977, 934-939.
11. Raffa D, Daidone G, Maggio B, Cascioferro S, Plescia F, Schillaci D. Synthesis and antileukemic activity of new 3-(5-methylisoxazol-3-yl) and 3-(pyrimidin-2-yl)-2-styrylquinazolin-4(3H)-ones, *Farmaco,* 59(6), 2004, 451-455.
12. Modi S R, Naik H B. Synthesis and antibacterial activity of some chalcones and isoxazoles, *Oriental J. Chem,* 10(1), 1994, 85-86.
13. Habeeb A G, Praveen Rao P N, Edward E K. Design and synthesis of diarylisoxazoles, novel inhibitors of cyclooxygenase-2

(COX-2) with analgesic- anti-inflammatory activity, *Drug Dev. Res,* 51(40), 2000, 273-286.

14. Srirastara S, Bajpai L K, Batra S, Bhaduri A P, Maikhuri J P, Gupta G, Dhar J D. In search of new chemical entities with spermicidal and anti-HIV activities, *Bioorg. Med. Chem,* 7(11), 1999, 2607-2613.
15. Zen, Akinosuku, Hiroya, kyoko. *Jpn. Kokai Tokkyo Koho J P,* 1993, CA 120.
16. Zen, Akinosuki, Hiroya, kyoko. *Jpn. Kokai Tokkyo Koho J P,* 1994, CA 120.
17. Boeck P, Bandeira Falcao C A, Leal P C, Yunes R A, Filho V C, Torres-Santos EC, Rossi-Bergmann B. Synthesis of chalcone analogues with increased antileishmanial activity, *Bioorg. Med. Chem,* 14(5), 2006, 1538-1545.
18. Olfert E D, Cross B M, McWilliam A A. Canadian council of animal care guide to the care and use of experimental animals, 1, 2nd Edition, 1993, 1-193.
19. Gandhidasan R, Thamaraichelvan A, Baburaj. Anti-inflammatory action of Lanea coromondelica by HRBC membrane stabilization, *Fitotherapia,* 62, 1991, 82-83.
20. Azeem A K, Dilip C, Prashanth S S, Junise V, Hanan Shashima. Anti-inflammmtory activity of the glandular extracts of *Thunnus alalunga, Asia. Pac. J. For Med,* 3(10), 2010, 412-420.
21. Berkowitz B A, Finck S A D, Ngai S H. Nitrous oxide analgesia, reversal by naloxone and development of tolerance, *J. Pharmacol. Exp. Ther,* 203(3), 1997, 539-547.

Please cite this article in press as: Raja S *et al.* Design and synthesis of novel 5-(4-fluoro-3-methylphenyl)-3-(substituted aryl) isoxazole derivatives as potent anti-inflammatory and analgesic agents, *Asian Journal of Research in Chemistry and Pharmaceutical Sciences,* 7(1), 2019, 148-157.