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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and analgesic and anti-inflammatory activities of 7-azaindazole chalcone derivatives

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Abstract A series of 7-azaindazole-chalcone (**6a–j**) derivatives were synthesized from 5-bromo-1H-pyrazolo[3,4-b] pyridine-3-carbaldehyde (**4**). The 5-bromo-1H-pyrazolo [3,4-b]pyridine (**3**) was subjected to Sommelet reaction to afford 5-bromo-1H-pyrazolo[3,4-b]pyridine-3-carbaldehyde (**4**), which underwent Claisen–Schmidt condensation with different substituted acetophenones (**5a–j**) in basic media to afford 7-azaindazole-chalcone (**6a–j**) derivatives. These compounds were characterized by their infrared, proton nuclear magnetic resonance, ^{13}C nuclear magnetic resonance, and mass spectral data. All these derivatives (**6a–j**) were evaluated for their anti-inflammatory and analgesic activities. Among the synthesized compounds **6j** and **6i** with CF_3 group and **6d** with Br group exhibited excellent anti-inflammatory activity, and the compound **6f** with benzyloxyphenyl showed less anti-inflammatory activity when compared with the activity of standard reference, indomethacin. Further, these derivatives (**6a–j**) were evaluated for their analgesic activity. Among them, compounds **6e**, **6d**, **6j**, and **6i** exhibited excellent analgesic activity when compared with the activity of standard drug diclofenac sodium.

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Introduction

In recent years, microbial infections have been associated with rates of morbidity and mortality. Antimicrobials are one of most significant weapons in fighting against bacterial infections. Growing drug resistance among bacterial pathogens drives the need for new agents effective against them. Heterocycles are important structural units found in a wide range of biologically active compounds (Quin and Tyrell, 2010). Indazoles are nitrogen containing heterocyclic compounds, and these were found to have numerous pharmaceutical applications (Peruncheralathan et al., 2004). These heterocyclic compounds are having a wide range of biological activities such as antimicrobial (Upadhyay et al., 2011), anticancer (Maggio et al., 2011), anti-inflammatory (Salvatore et al., 2010), antiviral (Rodgers et al., 1996), antiplatelet (Lee et al., 2001), antispermatogenic (Giorgio et al., 1976), and 5-HT6 antagonists (Kevin et al., 2011).

Similarly, chalcones (**1**, Fig. 1) are the aromatic ketones constituting an important group of natural products. The presence of a reactive α, β-unsaturated keto group in chalcones is found to be responsible for their antimicrobial activity (Prasad et al., 2008). Synthesis of chalcones and their derivatives have significant biological activity (Nowakowska, 2007). Licochalcone A (**2**), an oxygenated chalcone and isolated from the roots of Chinese licorice, was reported to have antimicrobial (Haraguchi et al., 1998 and Okada et al., 1989), antiprotozoal (Chen et al., 2001),

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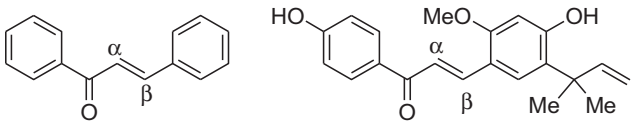


Chalcone (1) Licochalcone A (2)

Fig. 1 Structures of the chalcone compounds contaning reactive α, β-unsaturated chromophore

anti-inflammatory, antitumor (Shibata, 2000), and anti-oxidative (Haraguchi et al., 1998) activities.

In view of pharmacological importance of indazole and chalcone moieties, and to know the combined biological effect of both these moieties, it was considered worthwhile to synthesize certain new chemical entities having indazole and chalcone pharmacophores in a single molecular framework. Hence a series of novel azaindazole–chalcone derivatives were synthesized as analgesic and anti-inflammatory agents.

Materials and methods

Chemistry

All chemicals and reagents were obtained from Aldrich (Sigma–Aldrich, St. Louis, MO, USA), Lancaster (Alfa Aesar, Johnson Matthey Company, Ward Hill, MA, USA) and used. Reactions were monitored by thin layer chromatography (TLC) using silica gel glass plates containing 60 F-254 and visualization on TLC was achieved by ultra violet light. Melting points were determined with an electrothermal melting point apparatus, and are uncorrected. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on Bruker (400 and 100 MHz) instrument. Chemical shifts (δ) were reported in ppm downfield from internal tetramethylsilane standard. Mass spectra were recorded on Micro mass, Quattro LC using ESI+ software with capillary voltage 3.98 kV and electrospray ionization mode positive ion trap detector.

*5-Bromo-1H-pyrazolo[3,4-b]pyridine-3-carbaldehyde (**4**)*

The 5-bromo-1H-pyrazolo [3,4-b]pyridine (**3**) (15 g, 75.7 mmol), hexamethylenetetramine (HMTA) (14.8 g, 106 mmol), 50 mL acetic acid, and 100 mL H_2O were taken and heated to reflux for 12 h. After the reaction solution was cooled to room temperature, the precipitate obtained was filtered, washed with water, and dried to get white solid. The crude compound was recrystallized from hexane to get the pure compound **4**, 15.7 g with 92 % in yield. IR (KBr) υ_{max} 3433, 3316, 3093, 2870, 1671, 1416, 1250, 1068, 783, 517; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 12.9$ (s, 1H, NH), 9.92 (s, 1H, CHO), 8.53 (d, 1H, $J = 6.10$ Hz, ArH), 8.47 (d, 1H, $J = 2.44$ Hz, ArH); EIMS *m/z* 224.7 [M–H]; $C_7H_4BrN_3O$.

*General procedure for the compounds **(6a–6f)***

The 5-bromo-1H-pyrazolo[3,4-b]pyridine-3-carbaldehyde (**4**) (400 mg, 1.76 mmol) was dissolved in 5 mL of ethanol, followed by addition of substituted acetophenones (**5a–j**) (1.76 mmol) and 3 drops of piperidine. The reaction mixture was heated under reflux for 6 h. After cooling, water (20 mL) was added slowly. The crystalline precipitate was separated by filtration, washed with water, dried, and purified by recrystallization from ethanol to afford compounds (**6a–j**).

*(E)-3-(5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)-1-phenylprop-2-en-1-one (**6a**)*

Yellow solid, **6a**, 491 mg with 85 % in yield; m.p.: 190–192 °C; IR (KBr) υ_{max} 3433, 2889, 1766, 1654, 1513, 1315, 1139, 976, 765; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta =$ 12.62 (s, 1H, NH), 8.83 (d, 1H, $J = 1.22$ Hz, ArH), 8.41 (d, 1H, $J = 1.22$ Hz, ArH), 8.17 (d, 2H, $J = 7.32$ Hz, ArH), 7.98 (d, 1H, $J = 15.2$ Hz, CH=), 7.72 (d, 1H, $J = 15.2$ Hz, CH=), 7.58 (t, 2H), 7.64–7.67 (m, 1H, ArH), ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 189.0$ (C=O), 148.0 (Ar-C), 144.0 (Ar-C), 138.1 (Ar-C), 137.5 (Ar-C), 134.4 (Ar-C), 132.6 (Ar-C), 130.8 (Ar-C), 128.6 (Ar-C), 128.4 (Ar-C), 119.1 (CH=), 117.3 (Ar-C), 112.6 (Ar-C), 111.1 (CH=); EIMS *m/z* 328.8 [M–H]; $C_{15}H_{10}BrN_3O$.

*(E)-3-(5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)-1-(3-chlorophenyl)prop-2-en-1-one (**6b**)*

Yellow solid, 507 mg with 79 % in yield; m.p.: 271–272 °C; IR (KBr) υ_{max} 3444, 3116, 2874, 1565, 1337, 1203, 1070, 827, 670, 498; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 12.6$ (s, 1H, NH), 8.86 (d, 1H, $J = 1.83$ Hz, ArH), 8.41 (d, 1H, $J = 2.44$ Hz, ArH), 8.16–8.14 (m, 2H, ArH), 8.00 (d, 1H, $J = 15.2$ Hz, CH=), 7.73–7.69 (m, 2H, CH=, ArH), 7.61 (t, 1H, ArH); ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 187.7$ (C=O), 148.0 (Ar-C), 144.1 (Ar-C), 140.0 (Ar-C), 138.4 (Ar-C), 134.8 (Ar-C), 133.6 (Ar-C), 132.3 (Ar-C), 130.9 (Ar-C), 130.6 (Ar-C), 127.9 (Ar-C), 127.1 (Ar-C), 119.1 (CH=), 116.8 (Ar-C), 112.8 (Ar-C), 111.2 (CH=); EIMS *m/z* 362.7 $[M]^+$; $C_{15}H_9BrClN_3O$.

*(E)-3-(5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)-1-(4-chlorophenyl)prop-2-en-1-one (**6c**)*

Yellow solid, 487 mg with 76 % in yield; m.p.: 274–276 °C; IR (KBr) υ_{max} 3428, 1748, 1486, 1334, 1143, 972, 784,

619; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 12.6$ (s, 1H, NH), 8.85 (bs, 1H, ArH), 8.41 (bs, 1H, ArH), 8.19 (d, 2H, $J = 7.93$ Hz, ArH), 7.98 (d, 1H, $J = 15.8$ Hz, CH=), 7.68 (d, 1H, $J = 15.2$ Hz, CH=), 7.63 (d, 2H, $J = 8.54$ Hz, ArH); ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 187.9$ (C=O), 148.0 (Ar-C), 144.1 (Ar-C), 138.1 (Ar-C), 137.5 (Ar-C), 136.7 (Ar-C), 134.8 (Ar-C), 130.9 (Ar-C), 130.3 (Ar-C), 128.7 (Ar-C), 119.1 (CH=), 116.9 (Ar-C), 112.7 (Ar-C), 111.2 (CH=); EIMS *m/z* 362.7 $[M]^+$; $C_{15}H_9BrClN_3O$.

*(E)-3-(5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)-1-(3-bromophenyl)prop-2-en-1-one (**6d**)*

Yellow solid, 511 mg with 71 % in yield; m.p.: 278–280 °C; IR (KBr) υ_{max} 3429, 2611, 1652, 1459, 1326, 1139, 970, 772, 631; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 12.6$ (s, 1H, NH), 8.86 (bs, 1H, ArH), 8.41 (bs, 1H, ArH), 8.28 (s, 1H, ArH), 8.19 (d, 1H, $J = 7.32$ Hz, ArH), 8.00 (d, 1H, $J = 15.8$ Hz, CH=), 7.84 (d, 1H, $J = 7.93$ Hz, ArH), 7.69 (d, 1H, $J = 15.8$ Hz, CH=), 7.55 (t, 1H, ArH); ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 187.7$ (C=O), 148.0 (Ar-C), 144.1 (Ar-C), 140.2 (Ar-C), 138.4 (Ar-C), 135.2 (Ar-C), 134.8 (Ar-C), 130.9 (Ar-C), 130.7 (Ar-C), 127.5 (Ar-C), 122.2 (Ar-C), 119.1 (CH=), 116.8 (Ar-C), 112.8 (Ar-C), 111.2 (CH=); EIMS *m/z* 406.6 [M–H]; $C_{15}H_9Br_2N_3O$.

*(E)-3-(5-Bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)-1-(4-bromophenyl)prop-2-en-1-one (**6e**)*

Yellow solid, 534 mg with 74 % in yield; m.p.: 281–283 °C; IR (KBr) υ_{max} 3417, 2613, 1568, 1654, 1393, 1234, 1072, 902, 773, 619; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 12.6$ (s, 1H, NH), 8.85 (d, 1H, $J = 2.44$ Hz, ArH), 8.40 (d, 1H, $J = 1.83$ Hz, ArH), 8.11 (d, 1H, $J = 8.54$ Hz, ArH), 7.98 (d, 1H, $J = 15.8$ Hz, CH=), 7.77 (d, 2H, $J = 8.54$ Hz, ArH), 7.67 (d, 1H, $J = 15.2$ Hz, CH=); ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 188.4$ (C=O), 148.0 (Ar-C), 144.3 (Ar-C), 138.3 (Ar-C), 137.2 (Ar-C), 134.7 (Ar-C), 131.8 (Ar-C), 131.0 (Ar-C), 130.6 (Ar-C), 126.8 (Ar-C), 119.2 (CH=), 117.1 (Ar-C), 112.9 (Ar-C), 111.3 (CH=); EIMS *m/z* 406.7 [M–H]; $C_{15}H_9Br_2N_3O$.

*(E)-1-(3-(benzyloxy)phenyl)-3-(5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)prop-2-en-1-one (**6f**)*

Yellow solid, 609 mg with 79 % in yield; m.p.: 195–197 °C; IR (KBr) υ_{max} 3434, 2912, 1588, 1445, 1268, 1158, 1019, 870, 756, 632; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 12.6$ (s, 1H, NH), 8.82 (d, 1H, $J = 2.44$ Hz, ArH), 8.40 (d, 1H, $J = 1.83$ Hz, ArH), 7.95 (d, 1H, $J = 15.9$ Hz, CH=), 7.79 (d, 1H, $J = 7.93$ Hz, ArH), 7.70–7.66 (m, 2H, CH=, ArH), 7.50 (t, 3H, ArH), 7.41 (t, 2H, ArH), 7.36–7.29 (m, 2H, ArH), 5.23 (s, 2H, benzy-H); ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 188.7$ (C=O), 158.5 (Ar-C), 147.9 (Ar-C), 144.0 (Ar-C), 139.5 (Ar-C), 137.6 (Ar-C), 136.8 (Ar-C), 134.3 (Ar-C), 130.8 (Ar-C), 129.8 (ArH), 128.4 (Ar-C), 127.9 (Ar-C), 127.7 (Ar-C), 121.1 (Ar-C), 119.3 (Ar-C), 119.1 (CH=), 117.4 (Ar-C), 114.0 (Ar-C), 112.6 (Ar-C), 111.1 (CH=), 69.3 (CH2-, benzyl); EIMS *m/z* 432.9 [M–H]; $C_{22}H_{16}BrN_3O_2$.

*(E)-3-(5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)-1-(pyridin-3-yl)prop-2-en-1-one (**6g**)*

Yellow solid, 521 mg with 90 % in yield; m.p.: 263–264 °C; IR (KBr) υ_{max} 3431, 1655, 1412, 1235, 1019, 803, 637; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 12.6$ (s, 1H, NH), 9.37 (d, 1H, $J = 1.22$ Hz, ArH), 8.91 (d, 1H, $J = 1.83$ Hz, ArH), 8.83–8.81 (q, 1H, ArH), 8.48 (d, 1H, $J = 7.93$ Hz, ArH), 8.41 (d, 1H, $J = 1.83$ Hz, ArH), 8.01 (d, 1H, $J = 15.8$ Hz, CH=), 7.71 (d, 1H, $J = 15.2$ Hz, CH=), 7.62–7.59 (m, 1H, ArH); ^{13}CNMR (DMSO-d_6, 100 MHz): $\delta = 188.1$ (C=O), 152.8 (Ar-C), 149.6 (Ar-C), 148.0 (Ar-C), 144.1 (Ar-C), 138.4 (Ar-C), 135.8 (Ar-C), 135.2 (Ar-C), 133.2 (Ar-C), 131.0 (Ar-C), 123.8 (Ar-C), 119.0 (CH=), 116.9 (Ar-C), 112.8 (Ar-C), 111.1 (CH=); EIMS *m/z* 329.8 $[M]^+$; $C_{14}H_9BrN_4O$.

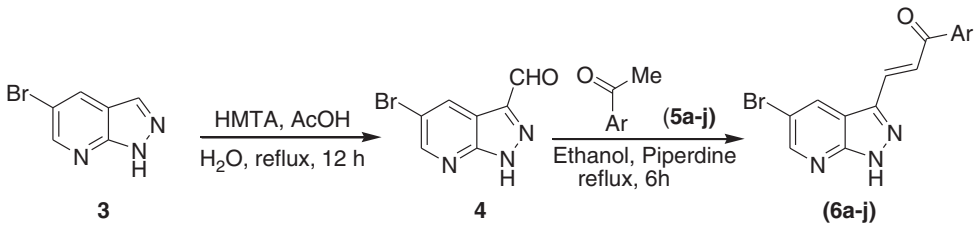
*(E)-3-(5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)-1-(pyridin-4-yl)prop-2-en-1-one (**6h**)*

Yellow solid, 546 mg with 94 % in yield; m.p.: 265–267 °C; IR (KBr) υ_{max} 3431, 1655, 1412, 1235, 1019, 803, 637; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 12.6$ (s, 1H, NH), 9.37 (d, 1H, $J = 1.22$ Hz, ArH), 8.91 (d, 1H, $J = 1.83$ Hz, ArH), 8.83–8.81 (q, 1H, ArH), 8.48 (d, 1H, $J = 7.93$ Hz, ArH), 8.41 (d, 1H, $J = 1.83$ Hz, ArH), 8.01 (d, 1H, $J = 15.8$ Hz, CH=), 7.71 (d, 1H, $J = 15.2$ Hz, CH=), 7.62–7.59 (m, 1H, ArH); ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 188.1$ (C=O), 152.8 (Ar-C), 149.6 (Ar-C), 148.0 (Ar-C), 144.1 (Ar-C), 138.4 (Ar-C), 135.8 (Ar-C), 135.2 (Ar-C), 133.2 (ArH), 131.0 (Ar-C), 123.8 (Ar-C), 119.0 (CH=), 116.9 (Ar-C), 112.8 (Ar-C), 111.1 (CH=); EIMS *m/z* 329.8 $[M]^+$; $C_{14}H_9BrN_4O$.

*(E)-3-(5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)-1-(2-fluoro-4-(trifluoromethyl)phenyl)prop-2-en-1-one (**6i**)*

Yellow solid, 612 mg with 84 % in yield; m.p.: 252–254 °C; IR (KBr) υ_{max} 3444, 2071, 1565, 1419, 1178, 975, 826, 672, 543; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 12.6$ (s, 1H, NH), 8.70 (d, 1H, $J = 1.83$ Hz, ArH), 8.41 (d, 1H, $J = 1.83$ Hz, ArH), 7.95–7.82 (m, 3H, CH=, ArH), 7.74 (d, 1H, $J = 7.93$ Hz, ArH), 7.31 (d, 1H, $J = 15.8$ Hz, CH=); ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 188.5$ (C=O), 160.9 (ArH), 158.4 (Ar-C), 148.3 (Ar-C), 144.4 (Ar-C), 140.0 (Ar-C),

Scheme 1 Synthesis of azaindazole-chalcone derivatives (**6a–j**)



5a; Ar = Phenyl
5b; Ar = 3-Chlorophenyl
5c; Ar = 4-Chlorophenyl
5d; Ar = 3-Bromophenyl
5e; Ar = 4-Bromophenyl
5f; Ar = 3-Benzyloxyphenyl
5g; Ar = 3-Pyridyl
5h; Ar = 4-Pyridyl
5i; Ar = 2-Fluoro-4-trifluoromethylphenyl
5j; Ar = 4-Trifluoromethylphenyl

6a; Ar = Phenyl
6b; Ar = 3-Chlorophenyl
6c; Ar = 4-Chlorophenyl
6d; Ar = 3-Bromophenyl
6e; Ar = 4-Bromophenyl
6f; Ar = 3-Benzyloxyphenyl
6g; Ar = 3-Pyridyl
6h; Ar = 4-Pyridyl
6i; Ar = 2-Fluoro-4-trifluoromethylphenyl
6j; Ar = 4-Trifluoromethylphenyl

135.7 (Ar-C), 131.6 (Ar-C), 130.8 (Ar-C), 121.7 (Ar-C), 121.2 (Ar-C), 119.1 (CH=), 114.5 (Ar-C), 114.2 (CF3), 113.0 (Ar-C), 110.9 (CH=); EIMS *m/z* 414.8 $[M]^+$; $C_{16}H_8BrF_4N_3O$.

*(E)-3-(5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)-1-(4-(trifluoromethyl)phenyl)prop-2-en-1-one (**6j**)*

Yellow solid, 626 mg with 89 % in yield; m.p.: 243–245 °C; IR (KBr) υ_{max} 3443, 1933, 1566, 1323, 1165, 1013, 860, 639, 460; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 12.6$ (s, 1H, ArH), 8.86 (d, 1H, $J = 1.83$ Hz, ArH), 8.41 (d, 1H, $J = 1.83$ Hz, ArH), 8.33 (d, 1H, $J = 7.93$ Hz, ArH), 8.01 (d, 1H, $J = 15.8$ Hz, CH=), 7.93 (d, 2H, $J = 7.93$ Hz, ArH), 7.69 (d, 1H, $J = 15.8$ Hz, CH=); ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 188.6$ (C=O), 148.2 (ArH), 144.3 (Ar-C), 141.6 (Ar-C), 139.0 (Ar-C), 135.3 (Ar-C), 132.2 (Ar-C), 131.9 (Ar-C), 131.1 (Ar-C), 129.3 (Ar-C), 125.7 (Ar-C), 125.4 (Ar-C), 122.7 (CF3), 119.2 (CH=), 117.2 (Ar-C), 113.0 (Ar-C), 111.3 (CH=); EIMS *m/z* 394.8 [M-2]; $C_{16}H_9BrF_3N_3O$.

Pharmacology

Carrageenan was purchased from Himedia Laboratories Pvt Ltd, Mumbai, India, and Indomethacin and Diclofenac sodium were procured from Oxford Laboratory, Mumbai, India.

Anti-inflammatory activity

Animals

Adult male wistar rats weighing 170–190 g were obtained from Albino research and training institute (Hyderabad, India). Rats were kept in wire-mesh cages and maintained under constant environmental conditions for 12 h at light–dark cycle (23 ± 2 °C) on standard feed (pellet diet) and water (ad libitum). Before the experiments, rats were fasted overnight with free access to water. The general behavior of animals was normal during the course of experiment. Laboratory conditions and in vivo experiments were approved, according to the guidelines established by the Institutional Animal Ethical Committee (IAEC), approved by committee for the purpose of control and supervision of experiments on animals (CPCSEA) (1722/Ro/Ere/S/13/CPCSEA), India.

Carrageenan-induced rat paw edema

The anti-inflammatory activity of compounds (**6a–j**) on Carrageenan-induced rat paw edema was carried out according to Winter et al. (1962). The animals were divided into 12 groups of 6 rats each. The disease control group received intraperitoneally 0.1 mL/kg Carrageenan along with the vehicle solution (1 % carboxymethylcellulose).

The standard reference group received indomethacin (10 mg/kg), and the test groups received compounds **6a–j** (10 mg/kg). After 30 min, 0.05 mL of 1 % Carrageenan suspension was injected into the left hind paw. The paw volume was measured by using a plethysmometer at 0 h (Vc) (before Carrageenan injection) and 1, 3, and 5 h later (Vt) (after Carrageenan injection). Paw swelling was determined for each rat and the difference between Vt and Vc was taken as the edema value. The percent inhibition of edema was calculated according to the following formula:

$$\frac{Vc\_Vt}{Vc} \times 100$$

Table 1 Anti-inflammatory effect of the intraperitoneal administration of compounds (**6a–j**) and of the reference standard drug (Std) (indomethacin) in carrageenan-induced rat paw edema

Treatment group	Dose (mg/kg)	edema (mean ± SEM)			edema Inhibition (%)		
		1 h	3 h	5 h	1 h	3 h	5 h
DC		0.17 ± 0.005	0.31 ± 0.005	0.40 ± 0.004	—	—	—
Std	10	0.16 ± 0.005^{ns}	$0.16 \pm 0.006^{***}$	$0.11 \pm 0.003^{***}$	5.9	48.3	72.5
6a	10	0.16 ± 0.006^{ns}	$0.20 \pm 0.005^{***}$	$0.15 \pm 0.002^{***}$	5.9	35.5	62.5
6b	10	0.15 ± 0.005^{ns}	$0.18 \pm 0.004^{***}$	$0.15 \pm 0.003^{***}$	11.7	42	62.5
6c	10	0.16 ± 0.006^{ns}	$0.18 \pm 0.003^{***}$	$0.14 \pm 0.003^{***}$	5.9	42	65
6d	10	0.16 ± 0.004^{ns}	$0.17 \pm 0.003^{***}$	$0.13 \pm 0.004^{***}$	5.9	45.1	67.5
6e	10	0.17 ± 0.006^{ns}	$0.18 \pm 0.004^{***}$	$0.14 \pm 0.006^{***}$	0	42	65
6f	10	0.17 ± 0.007^{ns}	$0.21 \pm 0.005^{***}$	$0.17 \pm 0.005^{***}$	0	32.2	57.5
6g	10	0.16 ± 0.005^{ns}	$0.19 \pm 0.002^{***}$	$0.15 \pm 0.004^{***}$	5.9	38.7	62.5
6h	10	0.16 ± 0.004^{ns}	$0.19 \pm 0.004^{***}$	$0.16 \pm 0.005^{***}$	5.9	38.7	60
6i	10	0.16 ± 0.006^{ns}	$0.17 \pm 0.006^{***}$	$0.13 \pm 0.005^{***}$	5.9	45.1	67.5
6j	10	0.15 ± 0.003^{ns}	$0.18 \pm 0.003^{***}$	$0.12 \pm 0.007^{***}$	1.7	42	70

All the values were expressed as mean ± SEM, $n = 6$

Statistically significant when compared to disease control (DC)

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, ns $p > 0.05$ followed by Tukey's test

Statistics

Results were expressed as the mean ± SEM of six animals per group. The data were analyzed using Tukey's test, $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ was considered significant and ns $p > 0.05$ was considered as non-significant.

Analgesic activity

Animals

Male albino mice weighing between 18–25 g were selected for the analgesic activity was housed under the uniform laboratory condition fed with commercial diet and provided with waterad-libitum, during the experiment. The animals were procured from Albino research and training institute (Hyderabad, India).

Hot plate reaction time in mice

The analgesic activity of compounds (**6a–j**) was obtained according to Oyedapo et al. (2008). The animals were placed individually on hot plate and regulated at temperature (54 ± 0.5 °C) before the treatment, and its reaction time was noted. The drug was given to each mice, after the initial reaction time was noted. Then the each animal was placed on the Eddy's hot plate at regulated temperature to obtain animal response, jump on the hot plate surface or licking of the forepaws and recorded as the hot-plate latency. The reaction time was noted by stop-watch and further re-determined after 30, 60, 90, 120 and 180 min after oral administration of standard and test drug.

Result and Discussion

Chemistry

The synthesis of these azaindole–chalcone (**6a–j**) derivatives was shown in Scheme 1. The intermediate **3** was mixed with HMTA in acetic acid and H_2O and refluxed for 12 h to afford pure aldehyde compound **4** in good yield. This aldehyde intermediate **4** underwent Claisen–Schmidt condensation with different substituted acetophenones (**5a–j**) in ethanol, by using piperidine as catalytic under reflux for 6 h to afford pure azaindole-chalcone (**6a–j**) derivatives.

Biological evaluation

The anti-inflammatory activity of synthesized (**6a–j**) compounds was done by using the Carrageenan induced rat paw edema method. The percentage protection of the compounds was calculated and presented in Table 1. The results revealed that the compounds (**6a–j**) tested at 10 mg/kg, produced a significant reduction of the edema during the entire period of observation. The compounds **6d**, **6j**, and **6i** exhibited the highest percentage inhibition of edema than other compounds. Compound **6i** shown 70 % inhibition of edema and compounds **6d** and **6j** produced 67.5 %

Table 2 Analgesic activity of the chalcone derivatives (**6a–j**) and of the reference standard drug (Std) (diclofenac sodium) on hot plate method in albino mice

Tret GP	0 min	30 min	60 min	90 min	120 min	180 min
NC	10.18 ± 0.47	10.50 ± 0.42	10.50 ± 0.42	10.66 ± 0.49	10.66 ± 0.33	10.50 ± 0.42
Std	10.67 ± 0.49^{ns}	$23.16 \pm 0.30^{**}$	$33.16 \pm 0.30^{**}$	$41.00 \pm 0.73^{**}$	$51.16 \pm 1.16^{**}$	$45.16 \pm 0.83^{**}$
6a	10.50 ± 0.76^{ns}	$17.83 \pm 0.30^{**}$	$23.83 \pm 0.47^{**}$	$30.66 \pm 0.88^{**}$	$39.16 \pm 0.94^{**}$	$34.50 \pm 0.76^{**}$
6b	10.50 ± 0.76^{ns}	$20.83 \pm 1.01^{**}$	$27.66 \pm 0.71^{**}$	$36.50 \pm 0.67^{**}$	$43.83 \pm 1.24^{**}$	$37.66 \pm 1.11^{**}$
6c	10.00 ± 0.57^{ns}	$19.00 \pm 0.57^{**}$	$28.33 \pm 1.68^{**}$	$36.83 \pm 1.40^{**}$	$44.66 \pm 1.33^{**}$	$37.00 \pm 0.96^{**}$
6d	11.17 ± 0.47^{ns}	$19.50 \pm 0.56^{**}$	$25.00 \pm 0.63^{**}$	$36.00 \pm 0.68^{**}$	$43.66 \pm 0.88^{**}$	$38.50 \pm 0.99^{**}$
6e	10.83 ± 0.60^{ns}	$19.83 \pm 0.94^{**}$	$28.16 \pm 0.70^{**}$	$35.50 \pm 0.76^{**}$	$44.50 \pm 0.99^{**}$	$39.00 \pm 1.15^{**}$
6f	10.50 ± 0.42^{ns}	$17.66 \pm 0.42^{**}$	$26.16 \pm 0.30^{**}$	$32.16 \pm 0.60^{**}$	$38.66 \pm 0.55^{**}$	$33.83 \pm 0.60^{**}$
6g	10.67 ± 0.66^{ns}	$14.83 \pm 0.30^{**}$	$24.16 \pm 0.47^{**}$	$31.83 \pm 0.74^{**}$	$41.83 \pm 0.94^{**}$	$36.50 \pm 0.76^{**}$
6h	10.33 ± 0.49^{ns}	$15.16 \pm 0.30^{**}$	$22.66 \pm 0.66^{**}$	$28.83 \pm 0.60^{**}$	$39.66 \pm 0.66^{**}$	$35.50 \pm 0.67^{**}$
6i	11.17 ± 0.47^{ns}	$20.16 \pm 0.60^{**}$	$31.33 \pm 0.33^{**}$	$39.50 \pm 0.76^{**}$	$46.33 \pm 0.84^{**}$	$41.16 \pm 0.94^{**}$
6j	11.00 ± 0.36^{ns}	$20.66 \pm 0.66^{**}$	$29.16 \pm 0.60^{**}$	$38.00 \pm 1.39^{**}$	$45.50 \pm 1.08^{**}$	$40.50 \pm 1.11^{**}$

All the values were represented as mean ± SEM, $n = 6$

Compare with normal control (NC), Treatment group (Tret GP)

p value: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, ns $p > 0.05$ followed by Dunnet's test

inhibition of edema. Compounds containing fluoro substitution exhibited more activity than compounds containing chloro and bromo substitutions. As all the tested compounds emerged as active against inflammation, it indicates that this basic moiety can be a promising scaffold for analgesic drugs.

Analgesic activity

The newly synthesized chalcone derivatives (**6a–j**) were screened for analgesic activity by using hot plate method. Among all tested compounds, **6d**, **6e**, **6j**, and **6i** exhibited maximum activity, while compounds **6a**, **6b**, **6c**, **6g**, **6h**, and **6f** showed moderate activity when compared with normal control. In general, it is observed from Table 2 that the compounds having 4-CF_3, 3-CF_3, 4-bromo, and 3-bromo exhibited excellent analgesic activity and remaining compounds showed moderate activity. As all the tested compounds emerged as active against analgesia and these results indicate that this basic moiety can be a promising scaffold for analgesic drugs. It may be suggested that the chalcone derivatives with suitable R group may lead to a good analgesic agent. However, this is a very promising preliminary study and further evaluation is needed to use them for clinical use.

Conclusion

A novel series of compounds (**6a–j**) was synthesized and the yield of each product was significantly influenced by the nature of substituent. The compounds **6j** and **6i** with CF_3 group and **6d** with Br substitution exhibited excellent anti-inflammatory activity and the compound **6f** with benzyloxyphenyl showed less anti-inflammatory activity. Remaining compounds **6a**, **6b**, **6c**, **6e**, **6g**, and **6h** exhibited moderate anti-inflammatory activity. Further, these derivatives (**6a–j**) were evaluated for their analgesic activity. Among them, compounds **6e** and **6d** with bromo and **6j** and **6i** with fluoro substitutions were exhibited excellent analgesic activity.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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