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Synthesis of 2-(2-aroylaryloxy)methyl-oxazolines as potent analgesic and anti-inflammatory agents

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ABSTRACT

*The synthetic strategies and characterization of some novel benzophenone derivatives carrying oxazoline ring are described using microwave irradiation technique. The compounds **2a-h**, **3a-h** and **4a-h** were screened for their analgesic, anti-inflammatory, ulcerogenic, cyclooxygenase activities and acute toxicity. The results revealed that halo compounds **4a** (48.3%), **4c** (45.4%) and **4f** (40.2%) displayed significant anti-inflammatory activity with low ulcerogenic activity in comparison with that of the standard drugs, aspirin (35.3%) and phenyl butazone (35.5%).*

Keywords Oxazolines; Analgesic; Anti-inflammatory; Ulcerogenic; Cyclooxygenase; acute toxicity.

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most commonly prescribed medicines for the management and treatment of various inflammatory conditions. These drugs interfere with the production of lipid autacoids known as prostaglandins (PGs), which play an important role in eliciting inflammatory reactions and its sign and symptoms [1,2]. NSAIDs block the biosynthesis of PGs, primarily by inhibiting the arachidonic acid metabolism via inhibition of several enzymes involved in their synthesis including cyclooxygenase enzyme (COX-1 and COX-2). Both COX are constitutively expressed in most tissues, but COX-2, in contrast to COX-1, is the mitogen inducible isoform. The inducing stimuli for COX-2 include pro-inflammatory cytokines and growth factors, implying a role for COX-2 in both inflammation and control of cell growth [3-5]. COX isoforms are almost identical in structure but have important differences in substrate and inhibitor selectivity and in their intracellular locations [6]. However, long term use of some selective COX-2 inhibitors has shown potential limitations including cardiovascular complications, aggravation of ulcers among high-risk patients, delay in healing process of gastroduodenal ulcers, prostacyclin deficiency leading to thrombosis and kidney toxicity [7-8]. Hence, selective COX-2 inhibitors because of their high cost and undesirable side effects are not the ideal candidates for the treatment/management of various chronic inflammatory disorders and therefore, efforts should be made for the development of new orally active, potent, improved and safer NSAIDs with low or no gastrointestinal side effects.

Benzophenone analogues possess a high analgesic efficacy [9,10] and are also endowed with anti-inflammatory property [11-13]. Moreover the efficiency of oxazoline analogues as chemotherapeutic agents especially as analgesic [14] and anti-inflammatroy [15,16] agents is well documented. The search for new molecules with anti-inflammatory activity [17] encouraged us to synthesize some newer, more potent oxazoline analogues using microwave technique by modifying the benzophenone moiety with the integration of oxazoline ring to verify the importance of this moiety on the pharmacological activity. In continuation of our ongoing program to develop environmentally benign [18], and due to the growing interest in the application of microwave irradiation in chemical reaction enhancement [19]. We have focused our interest on the design, synthesis of substituted 2-(2-aroyl

aryloxy)methyl-oxazolines for their possible *in vivo* anti-inflammatory plus analgesic actions including gastrointestinal safety (acute ulcerogenicity).

MATERIALS AND METHODS

Chemistry

Chemicals were purchased from Aldrich Chemical Co. TLC was performed on aluminium-backed silica plated with visualization by UV-light. Melting points were determined on a Thomas Hoover capillary melting point apparatus with a digital thermometer. A simple household microwave oven operating at 2450 MHz (power 900W), equipped with a turntable was used. IR spectra were recorded in Nujol on FT-IR Shimadzu 8300 spectrophotometer, ^{1}H NMR spectra were recorded on a Bruker 300 MHz NMR spectrophotometer in $CDCl_3$ and chemical shifts were recorded in parts per million down field from tetramethylsilane. Mass spectra were obtained with a VG70-70H spectrophotometer and important fragments are given with the relative intensities in the brackets. Elemental analysis results are within 0.4% of the calculated value.

Preparation of (2-hydroxy-5-methylphenyl)-(2-bromophenyl)methanone (2a):

In a typical procedure, **1a** (5 g, 1.7 mmol) was thoroughly mixed with montmorillonite K 10 clay (1:3 w/w) in the solid state using a vortex mixer and subjected to microwave irradiation at its 40% power for 5 min. After the completion of the reaction the product was extracted with dichloromethane and the solvent was evaporated. The crude product, on recrystallization with ethanol furnished **2a.** Yield 85%. mp. 78-81^{o}C; IR (Nujol): 3510-3610 (OH), 1650 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$): δ 2.3 (s, 3H, CH_3), 6.8-7.75 (m, 7H, Ar-H), 12.0 (bs, 1H, OH); MS: m/z (rel. int. %): 291 (M^+, 85%). Anal. Calcd. for $C_{14}H_{11}BrO_2$: C, 57.73; H, 3.78; Br, 27.49. Found: C, 57.71; H, 3.79; Br, 27.46.

2b: Yield 85%. mp. 75-78 °C; IR (Nujol): 3540-3650 (OH), 1668 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$): δ 2.32 (s, 3H, CH_3), 3.75 (s, 3H, OCH_3) 7.1-7.75 (m, 7H, Ar-H), 12.0 (bs, 1H, OH); MS: m/z (rel. int. %): 242 (M^+, 83%). Anal. Calcd. for $C_{15}H_{14}O_3$: C, 74.38; H, 5.78. Found: C, 74.35; H, 5.76.

2c: Yield 92%. mp. 71-73^{o}C; IR (Nujol): 3550-3640 (OH), 1673 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$): δ 2.2 (s, 3H, CH_3), 7.0-7.65 (m, 7H, Ar-H), 12.15 (bs, 1H, OH); MS: m/z (rel. int. %): 246.5 (M^+, 88%). Anal. Calcd. for $C_{14}H_{11}ClO_2$: C, 68.15; H, 4.46; Cl, 14.40. Found: C, 68.17; H, 4.44; Cl, 14.42.

2d: Yield 91%. mp. 81-83^{o}C; IR (Nujol): 3545-3649 (OH), 1670 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$): δ 2.3 (s, 3H, CH_3), 6.85-7.75 (m, 8H, Ar-H), 12.05 (bs, 1H, OH); MS: m/z (rel. int. %): 212 (M^+, 87%). Anal. Calcd. for $C_{14}H_{12}O_2$: C, 79.24; H, 5.66. Found: C, 79.26; H, 5.64.

2e: Yield 85% mp.78-80^{o}C; IR (Nujol): 3555-3645 (OH), 1675 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$): δ 2.15 (s, 3H, CH_3), 7.2-7.8 (m, 7H, Ar-H), 12.1 (bs, 1H, OH); MS: m/z (rel. int. %): 246.5 (M^+, 85%). Anal. Calcd for $C_{14}H_{11}ClO_2$: C, 68.15; H, 4.46; Cl, 14.40. Found: C, 68.14; H, 4.44; Cl, 14.39.

2f: Yield 89%. mp. 74-76 ^{o}C; IR (Nujol): 3545-3635 (OH), 1671 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$): δ 2.22 (s, 3H, CH_3), 6.95-7.63 (m, 7H, Ar-H), 12.2 (bs, 1H, OH); MS: m/z (rel. int. %): 246.5 (M^+, 86.5%). Anal. Calcd. for $C_{14}H_{11}ClO_2$: C, 68.15; H, 4.46; Cl, 14.40. Found: C, 68.15; H, 4.45; Cl, 14.38.

2g: Yield 90%. mp. 82-83^{o}C; IR (Nujol): 3535-3641 (OH), 1658 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$): δ 2.3-2.35 (d, *J*=6Hz, 6H, $2CH_3$), 7.0-7.7 (m, 7H, Ar-H), 12.1 (bs, 1H, OH); MS: m/z (rel. int. %): 226 (M^+, 85%). Anal. Calcd. for $C_{15}H_{14}O_2$: C, 79.64; H, 6.19. Found: C, 79.63; H, 6.16.

2h: Yield 84%. mp. 72-74^{o}C; IR (Nujol): 3500-3635 (OH), 1660 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$): δ 2.3 (s, 3H, CH_3), 3.75 (s, 9H, OCH_3), 6.8-7.5 (m, 5H, Ar-H), 11.8 (bs, 1H, OH); MS: m/z (rel. int. %): 302 (M^+, 83%). Anal. Calcd. for: $C_{17}H_{18}O_5$: C, 67.54; H, 6.0. Found: C, 67.55; H, 6.03.

Preparation of 2-[(2-(2-bromobenzoyl)-4-methylphenoxy)ethanoic acid (3a):

In a typical procedure a mixture of **2a** (2.91 g, 0.01 mol) ethyl chloroacetate (1.22 g, 0.01 mol) and potassium carbonate (2.76 g, 0.02 mol) was thoroughly mixed with k 10 clay (1:3 w/w) in the solid state using a vortex mixer and subjected to microwave irradiation operating at its 40% power for 7 min. After separating clay from reaction mixture a white solid of **3a** (2.1 g) was obtained. This was dissolved in ethanol (10 ml) and treated with a solution of sodium hydroxide (0.3 g, 7.8 mmol) in water (10 ml). The mixture was stirred for 2 h, cooled and acidified with 1 N hydrochloric acid. The pasty mass was extracted with dichloromethane (3×25 ml) and the solution was washed with water (3×25 ml), dried and evaporated to give crude solid, which recrystallized from hexane afforded **3a**. Yield.

74%. mp.125-127 ^{o}C; IR (Nujol): 3410-3510 (acid OH), 1735 (acid C=O), 1670 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$): δ 2.3 (s, 3H, CH_3), 4.45 (s, 2H, OCH_2), 7.1-7.6 (m, 7H, Ar-H), 9.4 (s, 1H, COOH); MS: m/z (rel. int. %): 349 (M^+, 58%). Anal. Calcd. For $C_{16}H_{13}BrO_4$: C, 55.01; H, 3.72; Br, 22.92. Found: C, 55.04; H, 3.75; Br, 22.94.

3b: Yield 70%. mp.130-132 ^{o}C; IR (Nujol): 3470-3570 (acid OH), 1738 (acid C=O), 1660 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$): δ 2.25 (s, 3H, CH_3), 3.8 (s, 3H, OCH_3), 4.42 (s, 2H, OCH_2), 7.0-7.6 (m, 7H, Ar-H), 9.1 (s, 1H, COOH); MS: m/z (rel. int. %): 300 (M^+, 55%). Anal. Calcd. For $C_{17}H_{16}O_5$: C, 68.0; H, 5.33. Found: C, 68.03; H, 5.35.

3c: Yield 75% mp.120-122^{o}C; IR (Nujol): 3400-3500 (acid OH), 1730 (acid C=O), 1675 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$): δ 2.3 (s, 3H, CH_3), 4.45 (s, 2H, OCH_2), 7.2-7.7 (m, 7H, Ar-H), 9.5 (s, 1H, COOH); MS: m/z (rel. int. %): 304.5 (M^+, 60%). Anal. Calcd. For $C_{16}H_{13}ClO_4$: C, 63.06; H, 4.30; Cl, 11.63. Found: C, 63.04; H, 4.26; Cl, 11.60.

3d: Yield 75%. mp. 112-115^{o}C; IR (Nujol): 3405-3540 (acid OH), 1733 (acid C=O), 1655 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$): δ 2.2 (s, 3H, CH_3), 4.44 (s, 2H, OCH_2), 6.9-7.55 (m, 8H, Ar-H), 9.2 (s, 1H, COOH); MS: m/z (rel. int. %): 270 (M^+, 58%). Anal. Calcd. For $C_{16}H_{14}O_4$: C, 71.11; H, 5.11. Found: C, 71.11; H, 5.11.

3e: Yield 72%. mp.108-110^{o}C; IR (Nujol): 3405-3550 (acid OH), 1731 (acid C=O), 1662 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$): δ 2.2 (s, 3H, CH_3), 4.45 (s, 2H, OCH_2), 6.88-7.6 (m, 7H, Ar-H), 9.3 (s, 1H, COOH); MS: m/z (rel. int. %): 304.5 (M^+, 59%). Anal. Calcd. For $C_{16}H_{13}ClO_4$: C, 63.06; H, 4.30; Cl, 11.63. Found: C, 63.03; H, 4.25; Cl, 11.61.

3f: Yield 69%. mp. 125-127^{o}C; IR (Nujol): 3410-3555 (acid OH), 1730 (acid C=O), 1655 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$): δ 2.2 (s, 3H, CH_3), 4.45 (s, 2H, OCH_2), 6.9-7.6 (m, 7H, Ar-H), 9.3 (s, 1H, COOH); MS: m/z (rel. int. %): 304.5 (M^+, 58%). Anal. Calcd. For $C_{16}H_{13}ClO_4$: C, 63.06; H, 4.30; Cl, 11.63. Found: C, 63.08; H, 4.29; Cl, 11.65.

3g: Yield 71%. mp. 131-133 ^{o}C; IR (Nujol): 3403-3535 (acid OH), 1730 (acid C=O), 1655 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$): δ 2.2 (d, *J*=6Hz, 6H, $2CH_3$), 4.45 (s, 2H, OCH_2), 6.85-7.55 (m, 7H, Ar-H), 9.25 (s, 1H, COOH); MS: m/z (rel. int. %): 284 (M^+, 57%). Anal. Calcd. For $C_{17}H_{16}O_4$: C, 71.83; H, 5.63. Found: C, 71.82; H, 5.67.

3h: Yield 68%. mp. 120-122^{o}C; IR (Nujol): 3400-3500 (acid OH), 1730 (acid C=O), 1645 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$): δ 2.1 (s, 3H, CH_3), 3.78 (s, 9H, $3OCH_3$), 4.4 (s, 2H, OCH_2), 6.75-7.5 (m, 5H, Ar-H), 9.1 (s, 1H, COOH); MS: m/z (rel. int. %): 360 (M^+, 56.5%). Anal. Calcd. For $C_{19}H_{20}O_7$: C, 63.33; H, 5.55. Found: C, 63.30; H, 5.59.

Preparation of 2-[2-(2-bromobenzoyl)-4-methylphenoxy]methyl-oxazoline (4a):
In a typical procedure, a mixture of **3a** (0.5 g, 1.0 mmol) and ethanolamine (0.06 g, 1.0 mmol) was subjected to microwave irradiation operating at its 20% power for 7 min. The reaction mixture was extracted into ether, washed with water and dried over anhydrous sodium sulphate. After evaporation of ether layer the crude solid was recrystallized with ethanol to afford **4a.** Yield 80%. mp. 98-100 ^{o}C; IR (Nujol): 1690 (C=N), 1658 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$): δ 2.3 (s, 3H, CH_3), 3.5 (t, *J* = 7 Hz, 2H, NCH_2), 4.45 (t, *J* = 7 Hz, 2H, OCH_2), 4.72 (s, 2H, OCH_2), 6.95-7.7 (m, 7H, Ar-H); MS: m/z (rel. int. %): 374 (M^+, 30%). Anal. Calcd. For $C_{18}H_{16}BrNO_3$: C, 57.75; H, 4.27; Br, 21.39; N, 3.74. Found: C, 57.77; H, 4.31; Br, 21.35; N, 3.76.

4b: Yield 79%. mp. 104-106^{o}C; IR (Nujol): 1670 (C=N), 1648 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$): δ 2.2 (s, 3H, CH_3), 3.4 (t, *J* = 7 Hz, 2H, NCH_2), 3.78 (s, 3H, OCH_3), 4.35 (t, *J* = 7 Hz, 2H, OCH_2), 4.6 (s, 2H, OCH_2), 6.78-7.5 (m, 7H, Ar-H); MS: m/z (rel. int. %): 325 (M^+, 29%) Anal. Calcd. For $C_{19}H_{19}NO_4$: C, 70.15; H, 5.84; N, 4.30. Found: C, 70.13; H, 5.81; N, 4.32.

4c: Yield 81%. mp. 110-112^{o}C; IR (Nujol): 1680 (C=N), 1652 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$): δ 2.25 (s, 3H, CH_3), 3.45 (t, *J* = 7 Hz, 2H, NCH_2), 4.41 (t, *J* = 7 Hz, 2H, OCH_2), 4.7 (s, 2H, OCH_2), 6.85-7.6 (m, 7H, Ar-H); MS: m/z (rel. int. %): 329.5 (M^+, 30.5%). Anal. Calcd. For $C_{18}H_{16}ClNO_3$: C, 65.55; H, 4.85; Cl, 10.77; N, 4.24. Found: C, 65.57; H, 4.89; Cl, 10.75; N, 4.26.

4d: Yield 81%. mp. 104-106^{o}C; IR (Nujol): 1675 (C=N), 1650 cm^{-1} (C=O),; ^{1}H NMR ($CDCl_3$): δ 2.23 (s, 3H, CH_3), 3.4 (t, *J* = 7 Hz, 2H, NCH_2), 4.4 (t, *J* = 7 Hz, 2H, OCH_2), 4.65 (s, 2H, OCH_2), 6.8-7.5 (m, 8H, Ar-H); MS: m/z (rel. int. %): 295 (M^+, 31%). Anal. Calcd. For $C_{18}H_{17}NO_3$: C, 73.22; H, 5.76; N, 4.74. Found: C, 73.20; H, 5.80; N, 4.76%.

4e: Yield 81%. mp.110-112^{o}C; IR (Nujol): 1678 (C=N), 1650 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$): δ 2.25 (s, 3H, CH_3), 3.45 (t, *J* = 7 Hz, 2H, NCH_2), 4.41 (t, *J* = 7 Hz, 2H, OCH_2), 4.69 (s, 2H, OCH_2), 6.85-7.6 (m, 7H, Ar-H); MS: m/z

(rel. int. %): 329.5 (M^+, 30%). Anal. Calcd. For $C_{18}H_{16}ClNO_3$: C, 65.55; H, 4.85; Cl, 10.77; N, 4.24. Found: C, 65.53; H, 4.83; Cl, 10.79; N, 4.22.

4f: Yield 81%. mp.118-120^oC; IR (Nujol): 1685 (C=N), 1655 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 2.28 (s, 3H, CH_3), 3.48 (t, *J* = 7 Hz, 2H, NCH_2), 4.45 (t, *J* = 7 Hz, 2H, OCH_2), 4.7 (s, 2H, OCH_2), 6.88-7.65 (m, 7H, Ar-H); MS: m/z (rel. int. %): 329.5 (M^+, 30%). Anal. Calcd. For $C_{18}H_{16}ClNO_3$: C, 65.55; H, 4.85; Cl, 10.77; N, 4.24. Found: C, 65.57; H, 4.87; Cl, 10.79; N, 4.23.

4g: Yield 70%. mp.116-118 ^oC; IR (Nujol): 1671 (C=N), 1650 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 2.24 (d, *J*=6Hz, 6H, $2CH_3$), 3.41 (t, *J* = 7 Hz, 2H, NCH_2), 4.36 (t, *J* = 7 Hz, 2H, OCH_2), 4.62 (s, 2H, OCH_2), 6.8-7.6 (m, 7H, Ar-H); MS: m/z (rel. int. %): 309 (M^+, 30%). Anal. Calcd. For $C_{19}H_{19}NO_3$: C, 73.78; H, 6.14; N, 4.53. Found: C, 73.76; H, 6.16; N, 4.51.

4h: Yield 75%. mp.100-102 ^oC; IR (Nujol): 1665 (C=N), 1645 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 2.1 (s, 3H, CH_3), 3.4 (t, *J* = 7 Hz, 2H, NCH_2), 3.77 (s, 9H, $3OCH_3$), 4.32 (t, *J* = 7 Hz, 2H, OCH_2), 4.6 (s, 2H, OCH_2), 6.75-7.5 (m, 5H, Ar-H); MS: m/z (rel. int. %): 385 (M^+, 29.5%). Anal. Calcd. For $C_{21}H_{23}NO_6$: C, 65.45; H, 5.97; N, 3.63. Found: C, 65.44;H, 6.0; N, 3.61.

Biology

Animals

All the animal experiments with albino rats and mice were carried out at Farooqia College of Pharmacy, Mysore and permission for conducting these experiments was obtained from institutional Animals Ethics Committee (CPCSEA Regd. No. 443/01/a).

Analgesic activity

To determine the analgesic activity acetic acid writhing test was performed on mice, adopting Davis et al method [20]. Groups of five mice body weight (25-30g) of both sexes were given dose of a test compound. After 30 min, *Intra-peritoneal* injection of 0.25 mL of 5% solution of aqueous acetic acid during the following 20 min was given to induce writhing in animals. The mean number of writhes for each experimental groups and percentage decrease compared with the control group (five mice not treated with test compounds) were calculated after 60 min. The test compounds were administrated orally at a dose of 20, 40 and 80 mg/kg for the evaluation of analgesic activity.

Anti-inflammatory activity

The synthesized compounds were evaluated for their anti-inflammatory activity by adopting carrageenan-induced paw edema method [21] in albino rats of either sex, weighing 125–160 g. Groups of five rats were given a dose of a test compound. After 30 min, an injection of 0.2 mL of 1% solution of carrageenan (in sterile 0.9% NaCl solution) was given by subcutaneous route into the sub-plantar region of the right hind paw of each rat. The volume of the animal's paw was determined using glass Plethysmometer at 3 h after administration of carrageenan injection. The mean increase of paw volume at each time interval was compared with that of control group (five rats treated with carrageenan, but not with test compounds) at the same time intervals. The percentage inhibition values were calculated using the formula:

$$\% \text{ anti-inflammatory activity} = 1 - G_t/G_c \times 100$$

where G_t and G_c represent tested and controls groups, respectively.

Ulcerogenic activity

Groups of 10 rats (body weight 200-230 g), fasted for 24 h. were treated with an oral dose of test compound, except control group. All animals were sacrificed 5 h after the completion of dosing. With the aid of a microscope the stomach and small intestine of the rats were examined to find incidence of hyperaemia, shedding of epithelium, petechial, frank haemorrhages and erosion or discrete ulceration with or without perforation. The presence of any of these criteria was considered to be an evidence of ulcerogenic activity [22].

Acute toxicity study

Nearly 50% lethal dose (ALD_{50}) of the compounds was determined in albino mice (body weight 250-300 g). The test compounds were injected intraperitoneally at different dose levels in groups of 10 animals. After 24 h of drug administration, percent mortality in each group was observed from the data obtained. ALD_{50} was calculated by adopting Boriollo [23] method.

Cyclooxygenase activity

The *in vitro* test on microsomal fraction of mucosal preparation of rabbit distal colon was carried out in order to search out the plausible mechanism of the compounds. By adopting Walker et al [24] procedure the preparation was carried out. About 2-3 g of stripped, colonic mucosa was minced and homogenized in 3 volumes of tris buffer 0.1M, pH 8.0 and the homogenized was centrifuged. The precipitate was suspended in tris buffer 0.1 M, pH 8.0, and recentrifuged. For enzyme assay cyclooxygenase activity, the microsomal pellet was used immediately. By measuring the rate of conversion of arachidonic acid to PGE_2, cyclooxygenase activity was assayed. About 50 ml of microsomal fractions were incubated with test agents for 10 min at 37^0C in 30µl tris-HCl, pH 8.0 containing 2 mM reduced glutathione, 5 mM L–trytophan, 1 µM hematin. The substrate 20 µM arachidonic acid with tracer amount of [1- ^{14}C] arachidonic acid [approximately 200 (xx) cpm] was then added and the reaction proceeded for 5 min at 37^0C. The reaction was stopped by addition of 0.2 ml of ether/methanol/citric acid 0.2 M (30:4:1 v/v), which was precooled at -25^0C PGE_2, was extracted twice into the same mixture. The solvent was evaporated under nitrogen stream and radiolabelled arachidonic acid was separated and from this radiolabelled PGE_3 were separated by RP-HBLC with 2 nmol unlabelled PGE_2 as an interval standard. PG chromatographic profile was obtained by isocratic elution with 150 mM H_3PO_4 in water, pH 3.5, containing 30% acetonitrile, a flow rate of 1 ml/min monitoring the UV absorption at 214 nm. Radioactivity that co-eluted with authentic PGE2 was quantified by liquid scintillation spectrometry. Test samples were compared to paired control incubations. The percentage of inhibition was calculated as follows.

[(cpm control –cpm test /(cpm control)) ×100]

RESULTS AND DISCUSSION

The synthetic sequence is outlined in scheme 1. Hydroxybenzophenones **2a-h** were obtained from respective phenyl benzoate using montmorillonite K 10 clay and microwave radiations. Compounds **2a-h** on reaction with ethyl chloroacetate, followed by hydrolysis furnished 2-aroyl aryloxyacetic acids **3a-h**. [25] Condensation of **3a-h** with ethanolamine by microwave technique [26] afforded 2-(2-aroyl aryloxy) methyl-oxazolines **4a-h** in excellent yield. The hitherto reported methods for the conversion of carboxylic acids into corresponding oxazolines requires either heating to temperature of up to 200-220°C or the repeated use of thionyl chloride [27] to convert the carboxylic acids via the acid chloride to the corresponding amides followed finally by cyclization of the hydroxy amides with thionyl chloride to the desired oxazolines. Furthermore, Kwon et al [16] have converted carboxylic acids to oxazolines by elongated method. Since all these methods require either drastic or aggressive reagents such as thionyl chloride these procedures did not appear to be applicable to sensitive NSAID's. These findings prompted us to use microwave technique for the synthesis of 2-(2-aroyl aryloxy) methyl-oxazolines **4a-h**.

All the synthesized compounds were characterized by IR, NMR and mass spectral studies. Among the series of compounds **2a-h** and **3a-h**, compound **2a** and **3a** have been taken as a representative examples to discuss spectral characterization, In the IR spectrum of compound **2a,** the C=O band was observed at 1650 cm^{-1} and a broad peak for phenolic OH at 3510-3610 cm^{-1}. On the other hand, ^{1}H NMR of compound **2a** showed signal at 2.3 ppm as a singlet for methyl group and the signal at 6.8-7.75 ppm as a multiple for aromatic protons and singlet signal at 12.0 ppm for OH proton which indicate the formation of compound (2-hydroxy-5-methylphenyl)-(2-bromophenyl)methanone **2a**. Further, the appearance of a singlet peak at 1735 ppm for C=O group of acid and a broad peak for carboxylic OH at 3410-3510 cm^{-1} in the IR spectrum and the appearance of signal peak at 4.45 ppm for OCH_2 protons in ^{1}H NMR spectrum clearly indicate the formation of 2-[(2-(2-bromobenzoyl)-4-methylphenoxy)ethanoic acid **3a**.

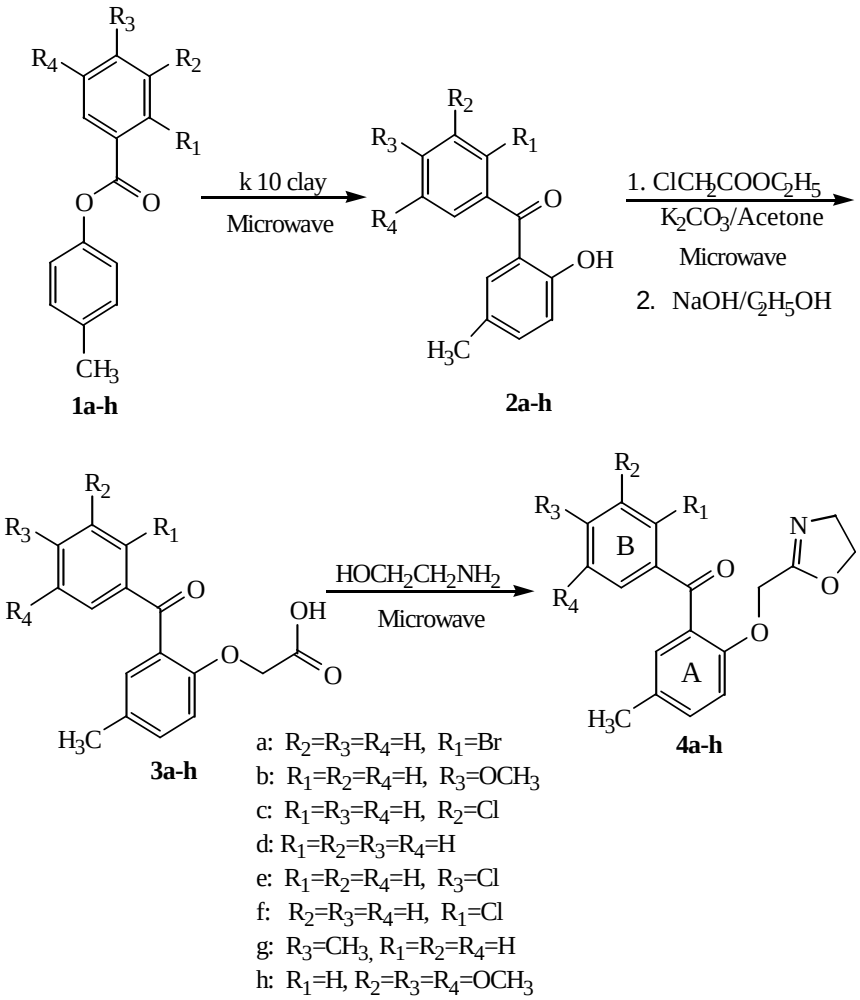
Similarly, among compounds **4a-h,** compound **4a** has been taken as a representative example to discuss spectral characterization, in the IR spectrum of compound **4a** disappearance of C=O and OH peaks at 1735 and 3410-3510 cm^{-1} respectively, and appearance of C=N peak at1690 and two new signals of protons NCH_2 and OCH_2 appeared as a triplet at 3.5 and 4.72 ppm respectively in ^{1}H NMR spectrum of compound **4a** indicate the cyclization and form oxazoline ring.

Analgesic activity

All the eight hydroxybenzophenones **2a-h** and 2-aroyl aryloxyacetic acids **3a-h** showed mild degree of analgesic activity. Among the title compounds **4a**, with a bromo group at ortho position and **4g** with two methyl groups at para position in ring A and B showed higher degree of activity compared to **2a-h** and **3a-h.** Analgesic activities of **2a-h**, **3a-h** and **4a-h** compounds and their comparison with standard drugs, aspirin and phenylbutazone are given in Table 1.

Anti-inflammatory activity

Hydroxybenzophenones **2a-h** has shown anti-inflammatory activity in the range 13.0 to 33.3%. Compound **2a** with a bromo group at the ortho position, elicited maximum inhibition of oedema at a dose of 40 mg/kg po. Based on it potent activity **2a** has been tested at three graded doses (20, 40 and 80 mg/kg po). Also **2a** was compared with standard drugs aspirin and phenyl butazone and it showed more potent activity than the standard drugs. Compounds **2c, 2e** and **2f** with a chloro group at meta, para and ortho position in ring B respectively, also showed potent anti-inflammatory activity. On the contrary compounds **2b** with a methoxy group at para position in ring B and **2h** with three methoxy groups at meta and para position in ring B showed lesser degree of activity. In addition, compounds **2d** with a methyl group at para position in ring A and **2g** with two methyl groups at para position in ring A and B showed lesser degree of activity. Among 2-aroyl aryloxyacetic acid **3a-h**, compound **3c** with a chloro group at the meta position in ring B has shown more degree of anti-inflammatory activity. Besides, compound **3a** with a bromo group at the ortho position in ring B exhibited lesser degree of activity compared to **3c** but more degree compared to **3b** and **3d-h**. Moreover the title compounds have shown more potent activities than their parent compounds, in the range 19.9-48.3%. The compound **4a** with a bromo group at ortho position in ring B is most active and compounds **4c, 4e** and **4f** with a chloro group at meta, para and ortho position in ring B respectively, showed promising activity. Compounds **4a** and **4c** were studied in detail at three graded doses and have shown dose dependent activity. Anti-inflammatory activity of **2a-h, 3a-h** and **4a-h** compounds and their comparison with standard drugs, aspirin and phenylbutazone are given in **Table 1**.



Scheme 1. Synthesis of oxazoline derivatives **4a-h**.

Table 1. Analgesic, anti-inflammatroy, ulcerogenic, cyclooxygenase and toxicity data of compounds 2a-h, 3a-h and 4a-h.

Compd.	Dose (mg/kg po)	Analgesic activity % decrease of writhes in 25 min after treatment relative to control	Dose (mg/kg po)	Anti-inflammatory activity % oedema inhibition relative to control	Dose (mg/kg po)	Ulcerogenic activity		Cyclooxygenase activity assay inhibitory action of some selected compound % inhibition 10 µM	ED_{50} (mg/kg po)	ALD_{50} (mg/kg po)
						% of animal with hyperemia	% of animal with ulcer			
2a	20	10.5	20	17.1	100	50	05	70	78.8	>1000
	40	17.1	40	33.3	200	70	10			
	80	41.0	80	64.2	400	90	15			
2b	40	31.2	40	16.9	200	50	10	ni		
2c	20	9.5	20	14.2	100	40	10	20	78.8	>1000
	40	14.3	40	29.1	200	60	20			
	80	31.1	80	55.3	400	100	40			
2d	40	9.0	40	17.8	200	100	10	ni		>1000
2e	20	10.5	20	16.2	100	30	10	30	78.8	>1000
	40	16.1	40	30.0	200	70	20			
	80	31.0	80	62.9	400	100	30			
2f	40	16.4	40	25.9	200	80	20	ni		>1000
2g	40	11.6	40	18.8	200	60	20	ni		>1000
2h	40	7.9	40	13.0	200	30	20	ni		>1000
3a	20	10.3	20	20.1	100	70	10	40	63.8	>1000
	40	15.1	40	29.2	200	90	20			
	80	32.1	80	62.8	400	100	40			
3b	40	12.0	40	25.4	200	70	20	ni		>1000
3c	20	10.1	20	20.1	100	30	05	70	78.8	>1000
	40	16.0	40	30.1	200	60	10			
	80	30.2	80	59.9	400	90	20			
3d	40	17.3	40	22.3	200	10	40	ni		>1000

Compd.	Dose (mg/kg po)	Analgesic activity % decrease of writhes in 25 min after treatment relative to control	Dose (mg/kg po)	Anti-inflammatory Activity % oedema inhibition relative to control	Dose (mg/kg po)	Ulcerogenic activity		Cyclooxygenase activity assay inhibitory action of some selected compound % inhibition 10 µM	ED_{50} (mg/kg po)	ALD_{50} (mg/kg po)
						% of animal with hyperemia	% of animal with ulcer			
3e	20	10.8	20	13.3	100	20	15	20	78.8	>1000
	40	16.0	40	27.8	200	30	30			
	80	31.1	80	53.1	400	50	45			
3f	40	12.8	40	25.0	200	30	40	ni		>1000
3g	40	9.0	40	18.2	200	60	70	ni		
3h	40	9.0	40	21.0	200	60	50	ni		
4a	20	10.5	20	30.2	100	30	10	ni	51.2	>1000
	40	22.5	40	48.3	200	60	21			
	80	41.2	80	94.4	400	90	12			
4b	40	12.1	40	19.9	200	60	50	ni		>1000
4c	20	14.1	20	22.2	100	50	20	87	60.2	>1000
	40	18.0	40	45.4	200	70	30			
	80	35.3	80	77.1	400	100	40			
4d	40	12.1	40	24.1	200	60	40	ni		>1000
4e	40	16.1	40	32.2	200	90	50	ni		>1000
4f	20	8.9	20	29.3	100	30	10	ni	90.3	>1000
	40	14.8	40	40.2	200	60	22			
	80	29.3	80	68.8	400	90	15			
4g	40	21.8	40	22.4	200	100	04	ni		>1000
4h	40	15.2	40	29.3	200	100	10	ni		>1000
Aspirin	20	29.4	20	30.2	100	30	80	99	98.3	-
	40	44.2	40	35.3	200	60	90			
	80	54.5	80	59.3	400	90	90			
Phenyl	20	16.4	20	31.3	100	30	30	89	-	-
butazone	40	29.4	40	35.5	200	60	60			
	80	39.9	80	57.2	400	90	90			
Control	20	-	20	-	30	-	-	ni	-	-
	40		40		60					
	80		80		90					

Ulcerogenic activity
Compounds **2a-h** exhibited remarkable lesser degree of ulcer production activity (10 to 20%). The most active compound **2a** exhibited lesser ulcerogenic activity compared to standard drug, aspirin and phenylbutazone. Compounds **3a-h** also exhibited low ulcer production activity ranging between 10 and 50% at 200 mg/kg po. Nevertheless compounds **4a**, **4c** and **4f** showed very low degree of ulcerogenic activity.

Cyclooxygenase assay activity
Compounds **2a, 2c, 2e, 3a, 3c, 3e, 4a** and **4c** showed good cyclooxygenase activity indicating that these compounds reduce inflammatory response by inhibition of Prostaglandins. The other compounds do not inhibit the cyclooxygenase activity, therefore seems to act through some other mechanism rather than inhibiting prostaglandin synthesis.

ALD_{50} studies
The toxicity study of these compounds indicates their good safety margin.

CONCLUSION

From the result of pharmacological activity, we can conclude that integration of oxazoline ring into the benzophenone moiety is successful as the compounds **4a**, **4c, 4e** and **4f** were found to increase analgesic and anti-inflammatory activities compared to their parent compounds with decreased ulcer production activity. Most of the compounds were found to have no suppressive effect on cyclooxygenase, which is the prime mechanism of anti-inflammatory activity. It is quiet likely that the compounds which do not show cyclooxygenase inhibition may be acting for analgesic, anti-inflammatory and ulcerogenic activities by their conversion to metabolites which possess cyclooxygenase inhibition activity.

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