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Synthesis and anti-inflammatory activity of benzophenone analogues

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Abstract

A series of substituted benzophenone analogues has been synthesized and evaluated as orally active anti-inflammatory agents with reduced side effects. The anti-inflammatory and ulcerogenic activities of the compounds were compared with naproxen, indomethacin, and phenylbutazone. In carrageenan-induced foot pad edema assay, benzophenone analogues showed an interesting anti-inflammatory activity. In the air-pouch test, some of the analogues reduced the total number of leukocytes of the exudate, which indicates inhibition of prostaglandin production. Side effects of the compounds were examined on gastric mucosa, in the liver and stomach. None of the compounds showed significant side effects compared with nonsteroidal anti-inflammatory drugs such as indomethacin and naproxen.

Keywords: Benzphenone analogues; Anti-inflammatory activity.

1. Introduction

Inflammatory responses are thought to be mediated in part by the prostaglandins (PGs) derived from arachidonic acid by the action of prostaglandin H synthase, which is also referred to as cyclooxygenase (COX) [1,2]. Recent studies have shown that COX exists in two isoforms COX-1 and COX-2. Both COX are constitutively

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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]. Protective PGs, which preserve the integrity of the stomach lining and maintain normal renal function in a compromised kidney, are synthesized by COX-1. In addition to the induction of COX-2 in inflammatory lesions, it is present constitutively in the brain and spinal cord, where it may be involved in nerve transmission, particularly those for pain and fever. COX is the principal target of nonsteroidal anti-inflammatory drugs (NSAIDs) and metabolites of the COX pathway are widely accepted as mediators of the inflammatory response. NSAIDs block the formation of PGs and have anti-inflammatory, analgesic, and antipyretic activity [7]. The discovery of COX-2 has made possible the design of drugs that reduce inflammation without removing the protective PGs in the stomach and kidney made by COX-1.

Recently, benzophenone analogues were identified as potent anti-inflammatory agents [7,8]. Welstead et al. [9] and Branacaccio et al. [10] have reported the anti-inflammatory activity of benzoylphenylacetic acid. In addition Vigorita et al. [11] have identified polyaromatic trifluoroacetamides as anti-inflammatory agents. Based on these observations we synthesized hydroxybenzophenones (**2a–c**, Scheme 1), aroyl aryloxyacetic acid (**4a–c**, Scheme 1), and 2-(2-aroyl aryloxy)-*N*-phenyl acetamide analogues (**6a–c** and **7a–c**, Scheme 1), and evaluated them for their anti-inflammatory activity and side effects.

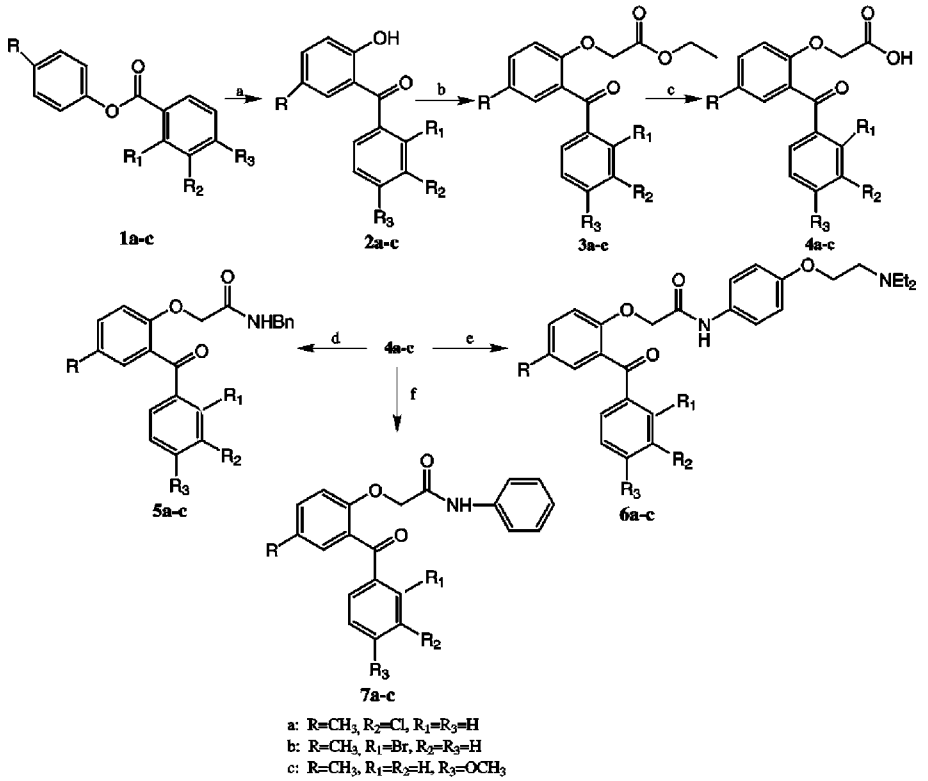
2. Experimental synthesis

2.1. General

Chemicals were purchased from Aldrich Chemical 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. IR spectra were recorded in Nujol on FT-IR Shimadzu 8300 spectrometer and ^{1}H NMR spectra were recorded on a Bruker 300 MHz spectrometer in $CDCl_3$. Chemical shifts were recorded in parts per million downfield from tetramethylsilane. Mass spectra were obtained with a VG70-70H mass spectrometer and elemental analysis results are within 0.4% of the calculated value.

*2.1.1. (2-Hydroxy-5-methylphenyl)-(3-chlorophenyl)methanone (**2a**)*

A solution of anhydrous aluminum chloride (3.2 g, 0.02 mol) in dry nitrobenzene (25 mL) was added to 4-methyl phenyl chloro benzoate (**1a**, 5 g, 0.02 mol) dissolved in nitrobenzene (10 mL). The mixture was protected from moisture by a calcium chloride guard tube and refluxed with stirring for 30 min. At the end of this period the solution was cooled and treated with acidic ice-cold water. The nitrobenzene was



Scheme 1. (a) Anhydrous $AlCl_3$, (b) K_2CO_3, $ClCH_2CO_2Et$, Acetone, (c) NaOH, EtOH, H_2O, (d) BF_3 etherate, $NH_2CH_2C_6H_5$, Dry benzene, (e) BF_3 etherate, $H_2NC_6H_4O(CH_2)_2NEt_2$, Dry benzene, (f) BF_3 etherate, $NH_2C_6H_5$, Dry benzene. *Note.* Bn = –CH_2–C$_6$H$_5$.

removed by steam distillation. The residual solid was crushed into powder, extracted with 10% sodium hydroxide (3 × 50 mL), and the basic aqueous solution was neutralized with 10% hydrochloric acid. The product was extracted into ether and the ether layer washed well with a saturated sodium chloride solution. Evaporation of the ether after drying over anhydrous sodium sulphate followed by recrystallization from alcohol gave (2-hydroxy-5-methyl phenyl) chloro phenyl methanone **2a** in 85% yield. Compounds **2b** and **2c** were synthesized by analogous procedures using **1b** and **1c**, respectively, and obtained in 70 and 75% yield, respectively.

2a: mp 71–73 °C; IR (Nujol): 1673 (C═O), 3550–3640 cm^{-1} (OH); ^1H NMR ($CDCl_3$): 2.2 (s, 3H, CH_3), 7.0–7.65 (m, 7H, Ar-H), 12.15 (bs, 1H, OH); MS (EI) *m*/*z*: 246 (M^+, 88); *Anal.* Calcd for $C_{14}H_{11}ClO_2$ (246.5): C, 68.15; H, 4.46; Cl, 14.40. Found: C, 68.17; H, 4.44; Cl, 14.42%.

2b: mp 78–81 °C; IR (Nujol): 1650 (C═O), 3510–3610 cm^{-1} (OH); ^1H NMR ($CDCl_3$): 2.3 (s, 3H, CH_3), 6.8–7.75 (m, 7H, Ar-H), 12.0 (bs, 1H, OH); MS (EI) *m*/*z*: 291 (M^+, 85); *Anal.* Calcd for $C_{14}H_{11}BrO_2$ (291): C, 57.73; H, 3.78; Br, 27.49. Found: C, 57.71; H, 3.79; Br, 27.46%.

2c: mp 75–78 °C; IR (Nujol): 1668 (C═O), 3540–3650 cm^{-1} (OH); ^1H NMR ($CDCl_3$): 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 (EI) *m/z*: 242 (M^+, 83); *Anal.* Calcd for $C_{15}H_{14}O_3$ (242): C, 73.38; H, 5.78. Found: C, 73.35; H, 5.76%.

*2.1.2. Ethyl [2-(3-chlorobenzoyl)-4-methylphenoxy]acetate (**3a**)*

A mixture of **2a** (5 g, 0.02 mol) and ethyl chloroacetate (2.4 g, 0.02 mol) in dry acetone (60 mL) and anhydrous potassium carbonate (2.8 g, 0.02 mol) was refluxed for 8 h. Subsequently, the reaction mixture was cooled and the solvent removed under reduced pressure. The residual mass was triturated with ice water to remove potassium carbonate and extracted with ether (3 × 50 mL). The ether layer was washed with 10% sodium hydroxide solution (3 × 30 mL), followed by water (3 × 30 mL), and then dried over anhydrous sodium sulphate and evaporated to dryness to yield crude solid. Recrystallization with ethanol gave **3a** in 80% yield. Compounds **3b** and **3c** were synthesized analogously starting with **2b** and **2c**, respectively. Compounds **3b** and **3c** were obtained in 71 and 72% yield, respectively.

3a: mp 60–62 °C; IR (Nujol): 1670 (C═O), 1735 cm^{-1} (ester, C═O); ^1H NMR ($CDCl_3$): 1.2 (t, $J = 7$ Hz, 3H, CH_3 of ester), 2.3 (s, 3H, CH_3), 4.2 (q, $J = 6$ Hz, 2H, CH_2 of ester), 4.45 (s, 2H, OCH_2), 7.2–7.6 (m, 7H, Ar-H); MS (EI) *m/z*: 332.5 (M^+, 62); *Anal.* Calcd for $C_{18}H_{17}ClO_4$ (332.5): C, 64.96; H, 5.11; Cl, 10.67. Found: C, 64.94; H, 5.07; Cl, 10.64%.

3b: mp 65–67 °C; IR (Nujol): 1665 (C═O), 1730 cm^{-1} (ester, C═O); ^1H NMR ($CDCl_3$): 1.21 (t, $J = 7$ Hz, 3H, CH_3 of ester), 2.3 (s, 3H, CH_3), 4.22 (q, $J = 6$ Hz, 2H, CH_2 of ester), 4.46 (s, 2H, OCH_2), 7.2–7.6 (m, 7H, Ar-H); MS (EI) *m/z*: 377 (M^+, 61); *Anal.* Calcd for $C_{18}H_{17}BrO_4$ (377): C, 57.29; H, 4.50; Br, 21.22. Found: C, 57.26; H, 4.53; Br, 21.25%.

3c: mp 58–60 °C; IR (Nujol): 1660 (C═O), 1730 cm^{-1} (ester, C═O); ^1H NMR ($CDCl_3$): 1.2 (t, $J = 7$ Hz, 3H, CH_3 of ester), 2.25 (s, 3H, CH_3), 3.8 (s, 3H, OCH_3), 4.2 (q, $J = 6$ Hz, 2H, CH_2 of ester), 4.42 (s, 2H, OCH_2), 7.0–7.6 (m, 7H, Ar-H); MS (EI) *m/z*: 328 (M^+, 59); *Anal.* Calcd for $C_{19}H_{20}O_5$ (328): C, 69.51; H, 6.09. Found: C, 69.49; H, 6.05%.

*2.1.3. 2-[(2-(3-Chlorobenzoyl)-4-methylphenoxy)ethanoic acid (**4a**)*

Compound **3a** (2.0 g, 6.0 mmol) was dissolved in ethanol (10 mL) and treated with a solution of sodium hydroxide (0.6 g, 15 mmol) in water (10 mL). The mixture was heated under refluxed for 3 h, cooled and acidified with 1 N hydrochloric acid. The oily precipitate was extracted with dichloromethane (3 × 30 mL) and the solution washed with water (3 × 25), dried and evaporated to give an oil. Crystallization from hexane afforded **4a** as a white solid in 75% yield. Compounds **4b** and **4c** were synthesized analogously starting with **3b** and **3c**, respectively. Compounds **4b** and **4c** were obtained in 74 and 70% yield, respectively.

4a: mp 120–22 °C; IR (Nujol): 3400–3500 (acid OH), 1730 (acid C═O), 1675 cm^{-1} (C═O); ^1H NMR ($CDCl_3$): 2.3 (s, 3H, CH_3), 4.46 (s, 2H, OCH_2), 7.2–7.7 (m, 7H, Ar-H), 9.5 (s, 1H, COOH); MS (EI) *m/z*: 304.5 (M^+, 60); *Anal.* Calcd for $C_{16}H_{13}ClO_4$ (304.5): C, 63.06; H, 4.30; Cl, 11.63. Found: C, 63.04; H, 4.26; Cl, 11.60%.

4b: mp 125–27 °C; IR (Nujol): 3410–3510 (acid OH), 1735 (acid C═O), 1670 cm^{-1} (C═O); ^1H NMR ($CDCl_3$): 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 (EI) *m/z*: 349 (M^+, 58); *Anal.* Calcd for $C_{16}H_{13}BrO_4$ (349): C, 55.01; H, 3.72; Br, 22.92. Found: C, 55.04; H, 3.75; Br, 22.94%.

4c: mp 130–32 °C; IR (Nujol): 3470–3575 (acid OH), 1738 (acid C═O), 1660 cm^{-1} (C═O); ^1H NMR ($CDCl_3$): 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 (EI) *m/z*: 300 (M^+, 55); *Anal.* Calcd for $C_{17}H_{16}O_5$ (300): C, 68.0; H, 5.33. Found: C, 68.03; H, 5.35%.

*2.1.4. N-benzyl-2-[2-(3-chloro-benzoyl)-4-methyl-phenoxy]-acetamide (**5a**)*

A mixture of **4a** (1.0 g, 3.2 mmol), benzyl amine (0.43 g, 4.0 mmol) and boron trifluoride etherate (0.83 g, 6.0 mmol) in dry benzene (15 mL) was refluxed for 6 h. The refluxing solvent was dried by circulation over anhydrous sodium sulphate in a Soxhlet's apparatus. The reaction mixture was washed with 10% aqueous sodium hydroxide (3 × 20 mL), 10% hydrochloric acid (3 × 20 mL) and then with water (3 × 25 mL). After being dried with anhydrous sodium sulphate, the organic layer was concentrated under reduced pressure. The amide was purified by crystallization from ethanol. Further purification was carried out by chromatography on silica gel using hexane and ethanol (7:2) as eluent to give **5a** as white solid in 70% yield. Compounds **5b** and **5c** were synthesized by analogous procedures using **4b** and **4c**, respectively, and obtained in 72 and 75% yield, respectively.

5a: mp 116–18 °C; IR (Nujol): 1705 (amide C═O), 1665 cm^{-1} (C═O); ^1H NMR ($CDCl_3$): 2.25 (s, 3 H, CH_3), 4.0 (s, 2H, NCH_2), 4.4 (s, 2H, OCH_2), 4.8 (bs, 1H, CONH), 7.1–7.7 (bm, 12H, Ar-H); MS (EI) *m/z*: 394 ($M^+ + 1$, 08); *Anal.* Calcd for $C_{23}H_{20}ClNO_3$ (393): C, 70.13; H, 5.08; Cl, 9.02. Found: C, 70.15; H, 5.10; Cl, 9.05%.

5b: mp 124–26 °C; IR (Nujol): 1710 (C═O of amide), 1670 cm^{-1} (C═O); ^1H NMR ($CDCl_3$): 2.3 (s, 3H, CH_3), 4.2 (s, 2H, NCH_2), 4.45 (s, 2H, OCH_2), 4.9 (bs, 1H, CONH), 7.2–7.8 (bm, 12H, Ar-H); MS (EI) *m/z*: 440 ($M^+ + 1$, 07); *Anal.* Calcd for $C_{23}H_{20}BrNO_3$ (439): C, 62.87; H, 4.55; Br, 18.22; N, 3.18. Found: C, 62.88; H, 4.52; Br, 18.25; N, 3.15%.

5c: mp109–111 °C; IR (Nujol): 1700 (C═O of amide), 1660 cm^{-1} (C═O); ^1H NMR ($CDCl_3$): 2.22 (s, 3H, CH_3), 3.75 (s, 3H, OCH_3), 3.95 (s, 2H, NCH_2), 4.38 (s, 2H, OCH_2), 4.75 (bs, 1H, CONH), 7.0–7.9 (bm, 12H, Ar-H); MS (EI): *m/z* 390 ($M^+ + 1$, 08); *Anal.* Calcd for $C_{24}H_{23}NO_4$ (389): C, 74.03; H, 5.91; N, 3.59. Found: C, 74.05; H, 5.93; N, 3.56%.

*2.1.5. 2-[2-(3-Chloro-benzoyl)-4-methyl-phenoxy]-N-[4-(2-diethylamino-ethoxy)-phenyl]-acetamide (**6a**)*

A mixture of **4a** (1.0 g, 3.2 mmol), 4-(2-diethylamino ethoxy) phenylamine (3.17 g, 4.0 mmol) and boron trifluoride etherate (0.83 g, 6.0 mmol) in dry benzene (15 mL) was refluxed for 6 h. The refluxing solvent was dried by circulation over anhydrous sodium sulphate in a Soxhlet's apparatus. The reaction mixture was washed with 10% aqueous sodium hydroxide (3 × 20 mL), 10% hydrochloric acid (3 × 20 mL) and then with water (3 × 25 mL). After being dried with anhydrous sodium sulphate, the organic layer was concentrated under reduced pressure. The amide was purified by

crystallization from ethanol. Further purification was carried out by chromatography on silica gel using hexane and ethanol (7:2) as eluent to give **6a** in 70% yield. Compounds **6b** and **6c** were synthesized by analogous procedures using **4b** and **4c**, respectively, and obtained in 71 and 73% yield, respectively.

6a: mp 142–44 °C; IR (Nujol): 1710 (amide C═O), 1670 cm^{-1} (C═O); ^1H NMR ($CDCl_3$): 1.02 (t, 6H, $2CH_3$), 2.2 (s, 3H, Ar-CH_3), 2.71 (q, 4H, $2CH_2$ of ethyl), 2.92 (t, 2H, N-CH_2), 4.05 (t, 2H, CH_2), 4.45 (s, 2H, OCH_2), 7.0–7.9 (bm, 11H, Ar-H), 9.6 (bs, 1H, CONH); MS (EI): *m/z* 495 (M^++1, 10); *Anal.* Calcd for $C_{28}H_{31}ClN_2O_4$ (494): C, 67.94; H, 6.47; Cl, 7.17; N, 5.66. Found: C, 67.96; H, 6.45; Cl, 7.19; N, 5.64%.

6b: mp 155–56 °C; IR (Nujol): 1715 (C═O of amide), 1675 cm^{-1} (C═O); ^1H NMR ($CDCl_3$): 1.1 (t, 6H, $2CH_3$), 2.25 (s, 3H, Ar-CH_3), 2.75 (q, 4H, $2CH_2$ of ethyl), 3.05 (t, 2H, N-CH_2), 4.1 (t, 2H, CH_2), 4.5 (s, 2H, OCH_2), 7.1–8.0 (bm, 11H, Ar-H), 9.7 (bs, 1H, CONH); MS (EI): *m/z* 540 (M^+ + 1, 09); *Anal.* Calcd for $C_{28}H_{31}BrN_2O_4$ (539): C, 62.33; H, 5.75; Br, 14.84; N, 5.19. Found: C, 62.35; H, 5.35; Br, 14.85; N, 5.17%.

6c: mp 134–36 °C; IR (Nujol): 1708 (C═O of amide), 1665 cm^{-1} (C═O); ^1H NMR ($CDCl_3$): 1.0 (t, 6H, $2CH_3$), 2.2 (s, 3H, Ar-CH_3), 2.7 (q, 4H, $2CH_2$ of ethyl), 2.91 (t, 2H, N-CH_2), 3.78 (s, 3H, OCH_3), 4.0 (t, 2H, CH_2), 4.4 (s, 2H, OCH_2), 6.95–7.9 (bm, 11H, Ar-H), 9.55 (bs, 1H, CONH); MS (EI): *m/z* 391 (M^+ + 1, 08); *Anal.* Calcd for $C_{28}H_{34}N_2O_5$ (390): C, 71.02; H, 6.93; N, 5.71. Found: C, 71.04; H, 6.95; N, 5.73%.

*2.1.6. 2-[2-(3-Chloro-benzoyl)-4-methyl-phenoxy]-N-phenyl-acetamide (**7a**)*

A mixture of **4a** (1.0 g, 3.2 mmol), aniline (0.37 g, 4.0 mmol) and boron trifluoride etherate (0.83 g, 6.0 mmol) in dry benzene (15 mL) was refluxed for 6 h. The refluxing solvent was dried by circulation over anhydrous sodium sulphate in a Soxhlet's apparatus. The reaction mixture was washed with 10% aqueous sodium hydroxide (3 × 20 mL), 10% hydrochloric acid (3 × 20 mL) and then with water (3 × 25 mL). After being dried with anhydrous sodium sulphate, the organic layer was concentrated under reduced pressure. The amide was purified by crystallization from ethanol. Further purification was carried out by chromatography on silica gel using hexane and ethanol (7:2) as eluent to give **7a** in 75% yield. Compounds **7b** and **7c** were synthesized by analogous procedures using **4b** and **4c**, respectively, and obtained in 72 and 73% yield, respectively.

7a: mp 125–27 °C; IR (Nujol): 1710 (amide C═O), 1655 cm^{-1} (C═O); ^1H NMR ($CDCl_3$): 2.22 (s, 3H, Ar-CH_3), 4.43 (s, 2H, OCH_2), 7.1–7.8 (bm, 12H, Ar-H), 9.6 (bs, 1H, CONH); MS (EI): *m/z* 380 (M^+ + 1, 11); *Anal.* Calcd for $C_{22}H_{18}ClNO_3$ (379): C, 69.56; H, 4.74; Cl, 9.35; N, 3.68. Found: C, 69.54; H, 4.76; Cl, 9.33; N, 3.66%.

7b: mp 135–37 °C; IR (Nujol): 1705 (C═O of amide), 1675 cm^{-1} (C═O); ^1H NMR ($CDCl_3$): 2.3 (s, 3H, Ar-CH_3), 4.45 (s, 2H, OCH_2), 7.2–7.9 (bm, 12H, Ar-H), 9.7 (bs, 1H, CONH); MS (EI): *m/z* 425 (M^+ + 1, 10); *Anal.* Calcd for $C_{22}H_{18}BrNO_3$ (424): C, 62.26; H, 4.24; Br, 18.86; N, 3.30. Found: C, 62.28; H, 4.22; Br, 18.89; N, 3.33%.

7c: mp 118–20 °C; IR (Nujol): 1705 (C═O of amide), 1675 cm^{-1} (C═O); ^1H NMR ($CDCl_3$): 2.2 (s, 3H, Ar-CH_3), 4.41 (s, 2H, OCH_2), 7.0–7.75 (bm, 12H, Ar-H), 9.5 (bs, 1H, CONH); MS (EI): *m/z* 376 (M^+ + 1, 08); *Anal.* Calcd for $C_{23}H_{21}NO_4$ (375): C, 73.60; H, 5.60; N, 3.73. Found: C, 73.63; H, 5.62; N, 3.71%.

3. Biological evaluation of compounds

The animals were housed in groups of six and acclimatized to room conditions for at least 2 days before the experiments, with food and water at libitum. The food was withdrawn on the day before the experiment, but free access to water was allowed. The compounds (100 mg/kg) and the reference NSAIDs, phenylbutazone (100 mg/kg), indomethacin (10 mg/kg), and naproxen (30 mg/kg), were suspended in 0.5% carboxymethylcellulose (CMC) and administered orally by animal feeding needle. The control groups received appropriate volumes of the vehicle (0.5% CMC, oral) only.

3.1. Anti-inflammatory activity–carrageenan paw edema test (CPE) [12,13]

One hour after oral administration of the compounds, the thickness of right hind paw was measured by a peacock dial thickness gauge and 0.01 mL 2% carrageenan was injected subcutaneously into the plantar surface of the right hind paw. After 2 h, the volume of the edema was measured again and the anti-edematic effects of the drugs were estimated in terms of percent inhibition using following equation:

$$\textit{Anti}\text{-inflammatory activity}\,(\%) = [(m - m')/m] \times 100,$$

where m and m' indicate the difference in thickness between the first and second measurements of hind paws in control and test groups, respectively.

3.2. Air-pouch test [14–16]

The carrageenan powder was dissolved in saline to a concentration of 10 mg/mL. The solution was sterilized and homogenized by placing it in an oven at 90 °C for about 1 h. It was then maintained at 37 °C. Air pouches were formed by subcutaneous (sc) injection of 1 mL of air for 3 days. On the third day, after the initial injection of air, carrageenan solution (1 mL) was injected into the air pouch to induce inflammation. Compounds were administered orally 1 h before injection of the carrageenan into the pouch. After 4 h, mice were killed by ether exposure and pouches washed thoroughly with 3 mL of phosphate buffer solution (PBS) containing 50 μ/mL heparin. Lavage fluids were centrifuged at 2000 rpm for 15 min at 4 °C and the pellet was resuspended in 1 mL of PBS-heparin. The total number of polymorphonuclear leukocytes (PMNL) infiltration was measured using a Coulter Counter.

3.3. Histopathological examination

Mice were sacrificed 4 h after the paw edema experiments and their liver, stomachs, and kidneys were removed and put into 10% formalin solution. The sections taken from these specimens were stained with hematoxilen eosine and examined under the light microscope.

4. Results and discussion

Compounds **2a–c** were synthesized by a Fries rearrangement [17,18] of **1a–c** (Scheme 1). Condensation of **2a–c** with ethyl chloroacetate in the presence of anhydrous potassium carbonate in dry acetone gave ethyl (2-aroylaryloxy)acetates [19,20] **3a–c**. Alkaline hydrolysis of **3a–c** gave 2-aroyl aryloxyacetic acid **4a-c**. Condensation of **4a–c** with benzyl amine, 4-(2-diethylamino ethoxy) phenylamine or aniline in presence of boron trifluoride etherate and dry benzene gave 2-(2-aroyl aryloxy) acetamide analogues [21] **5a–c**, **6a–c**, and **7a–c**, respectively, in excellent yields.

The pharmacological results (Table 1) indicate that some of the compounds possess anti-inflammatory properties. In the CPE assay, compounds **2a**, **2b**, **4a**, **4b**, **6a**, **7a**, and **7b** showed anti-inflammatory activity. The most effective compounds were **2a**, **4a** and **7a**, which have a chloro group at the meta position. Compounds **2b**,**4b**, and **7b**, which have a bromo group at the ortho position, showed promising activity whereas compounds **2c**, **4c**, and **7c** showed only weak activity. The acetamide analogues **5a–c** also showed weak activity compared with phenylbutazone.

In addition to the synthesis and identification of new anti-inflammatory compounds, it is desirable to search for a new series of compounds with low ulceration potential. Our results show that the newly synthesized compounds reduce leukocytes, which suggests inhibition of PG synthesis. COX-1, is constitutively expressed in most

Table 1
Anti-inflammatory activity of benzophenone compounds

Compounds (100 mg/kg)	CPE (% inhibition SE)[a]	PMNL ($10^5/cm^3$) ($n = 6$, after 4 h)[b]
Control		121.50 (4.06)
2a	69.50 (9.55[c])	30.15 (0.77)
2b	60.60 (8.54[c])	32.41 (3.14)
2c	35.51 (1.21[d])	69.42 (0.96)
4a	67.32 (9.26[c])	31.75 (0.75)
4b	51.42 (3.97[c])	56.26 (0.37)
4c	n.a	n.t
5a	22.44 (3.08[d])	95.25 (0.80)
5b	15.45 (1.67[d])	104.76 (1.56)
5c	n.a	n.t
6a	51.41 (3.98[c])	56.25 (0.38)
6b	45.18 (9.89[c])	50.00 (1.32)
6c	n.a	n.t
7a	60.62 (8.55[c])	32.43 (3.15)
7b	51.41 (3.98[c])	56.25 (0.38)
7c	42.50 (3.82[c])	46.30 (0.68)
Naproxen (30 mg/kg)	67.50 (1.70[c])	50.20 (1.30)
Phenylbutazone (100 mg/kg)	53.27 (1.83[c])	53.27 (1.71)
Indomethacin (10 mg/kg)	32.10 (1.38[c])	68.80 (1.80)

PMNL, polymorphonuclear leucocytes; n.a.: no activity; n.t: not tested.
[a] Results are expressed as their mean values ($n = 6$).
[b] Mean SEM ($n = 6$).
[c] $p < 0.01$.
[d] $p < 0.05$.

tissues and appears to be relevant for tissue homeostatic functions of PGs, whereas COX-2 is an inducible isozyme and plays a role in many inflammatory reactions [5]. The enzymes are the primary targets of aspirin and other NSAIDs and thus are of major interest in pharmacology, pharmacogenetics, and epidemiology. NSAIDs have several potential pharmacologic effects. However, their anti-inflammatory action depends primarily on their ability to inhibit the COX enzymes [22]. This results in the decreased production of pro-inflammatory PGs. Macrophages is known to produce PGE_2 via the COX-2 dependent pathway in response to pro-inflammatory cytokines. Furthermore, rat peritoneal macrophages have been recently found to have the capacity to metabolize exogenous arachidonic acid to thromboxane via COX-1 and to PGE_2 via COX-2 [23]. This observation suggested that there is a preferential, phase-specific correlation between the two COX isoforms and the downstream respective terminal PG synthases.

Previous work has shown that the inflammatory effect of carrageenan is due to an influx of predominantly neutrophilic leukocytes (predominantly PMNL) from blood circulation into the cavity. In addition, leukocyte migration is induced locally in the inflammatory process and leukocytes also intensify inflammation by releasing several inflammatory mediators. The method is used for measuring the edema induced by injection of carrageenan into the pouches in mice back. Air pouches were highly reactive to inflammatory stimulus. The enhanced inflammatory reactions in the sites correlated with formation of lining tissue, the type of cells, and/or the reactivity of newly formed blood cells [14,15,24,25]. Mean leukocyte numbers per milliliter of exudates for each drug compared with control values obtained from similar group of animals receiving vehicle alone and the degree of inflammatory response produced in the air-pouch cavity was assessed by measuring total cell number of the exudates. Compounds **2a**, **2b**, **4a**, **4b**, **6a**, **6b**, and **7a–c** reduced total number of leukocytes of the exudates. The reduction of leukocytes suggests inhibition of PG production. Compounds **2a**, **2b**, **4a**, and **7a** were inhibited PMNL production compared with control and reference compounds [4- to 4.5-fold and 1- to 1.4-fold greater, respectively (Table 1)].

The ulcerogenic potential of anti-inflammatory compounds can be demonstrated in animal models using positive (naproxen, idomethacin) and negative (phenylbutazone) controls. Upon microscopic examination, lesions seen in the stomach, kidney, and liver tissues are graded according to their severity. The grade of the scale is designed as (+) for mild (++) for moderate and (+++) for severe changes. Stomachs of the mice are thoroughly sectioned and both corpus and antrum are evaluated. Surface epithelium and the lamina propria of the gastric mucosa are all examined. Acute gastritis may exist in an earlier or milder nonerosive form with merely mucosal congestion, edema, and histological evidence of inflammation. These earlier changes are known to be transient and completely reversible within few days, but the development of erosions and hemorrhages is more serious and related to an increased risk of major upper gastrointestinal bleeding. In our study, the animals were sacrificed 4 h after ingestion of the drugs. None of the sections displayed the morphology of the ulceration, but the nonerosive form of acute gastritis was observed in various degrees of severity with compounds **5a–c** and **6b** (Table 2). Upon microscopic examination of

Table 2
Histopathological examination results benzophenone compounds

Compounds (100 mg/kg) p.o.	Kidney		Stomach	Liver		
	Edema	Infected cell (MNL)	Gastritis	Fatty change	Acute hepatitis/ spotty necrosis	Cholestasis
2a	–	–	–	–	–	–
2b	–	–	–	–	+	–
2c	–	–	–	++	++	++
4a	–	–	–	–	–	–
4b	–	–	–	–	++	–
4c	–	+	–	++	+	–
5a	–	–	+	–	+	–
5b	–	++	++	+	–	–
5c	–	–	++	+	+	+
6a	–	–	–	–	–	–
6b	–	++	++	++	+	–
6c	++	+	–	++	+	+
7a	–	–	–	–	+	–
7b	++	–	–	–	–	–
7c	++	–	–	–	–	–
Indomethacin (10 mg/kg)	++	+	+++	++	++	–
Naproxen (30 mg/kg)	+	+	+	+	+++	–

–, no; +, mild; ++, moderate; +++, severe side effects.

the kidney sections, the morphologic mononuclear cell infiltrations were characteristic for tubulointerstitial nephritis. The presence of some scattered eosinophil leukocytes also provided evidence for the drug effect. Focal areas displaying variable but generally mild degree of tubular regeneration were also present. Acute pyelonephritis should be considered in the differential diagnosis of the tubulointerstitial nephritis, but none of our samples showed interstitial suppurative (inflammation with polymorphonuclear leukocytes) inflammation with microabscesses. Therefore, the renal morphologic findings reflect the effects of the compounds. Liver tissues were also examined thoroughly and the integrity of the basic structure, degree of lobular and portal inflammation, and the presence or absence of necrosis, fatty change or cholestasis were evaluated. NSAIDs such as phenylbutazone [26] and naproxen [27] are known to cause acute or chronic hepatitis, confluent or spotty necrosis, cholestatic hepatitis and/or fatty change. This shows the importance of examining the liver tissues to better assess the safety of NSAIDs. In compounds **2c**, **4c**, **5b**, **5c**, **6b**, and **6c** (moderate, ++) showed macro and microvesicular fatty change and several scattered mild (+) focal spotty necrosis. Multiple foci of spotty necrosis (moderate ++) was seen with compounds **2c** and **4b**. Cholestatic hepatitis was observed for compounds **5c**, **2c**, and **6c** (Table 1).

In conclusion, a new series of benzophenone analogues showing anti-inflammatory activities was synthesized. Compounds with a chloro group at the meta position

(i.e., **2a**, **4a**, and **7a**) showed significant anti-inflammatory profile with low gastric ulceration incidence as compared with similar toxic profiles for reference NSAIDs in the liver.

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