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Synthesis and crystallographic analysis of benzophenone derivatives—The potential anti-inflammatory agents

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Abstract—Fries rearrangement of substituted phenyl benzoates **1a**–**j** to substituted hydroxy benzophenones **2a**–**j** was achieved in excellent yield. Further benzoylation of **2a**–**j** to benzoyloxy benzophenones **4a**–**n**, a benzophenone analogue was achieved in good yield. All the newly synthesized compounds were evaluated for their anti-inflammatory activity and were compared with standard drugs. Out of the compounds studied, the compounds **4c**, **4e**, **4g**, **4h and 4k** with chloro and methyl substituents at para position showed more potent activity than the standard drugs at all doses tested.

1. Introduction

Inflammatory responses are considered to be intervened 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 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.

The proficiency of benzophenone analogues as chemotherapeutic agent especially as anti-inflammatory is well documented.[7] Benzophenone analogues synthesized by several scientists have been reported as effective anti-inflammatory agents.[8–10] Recently synthesis and structure-activity relationship of benzophenones as novel class of p38 MAP kinase inhibitors with high anti-inflammatory activity have been reported.[11] In light of these observations and our exploration for new molecules with anti-inflammatory activity[12] encouraged us to synthesize benzoyloxy benzophenones, potent analogues of benzophenones, by modifying the hydroxyl group of hydroxy benzophenones with the incorporation of benzoyloxy group. We have focused our interest on the synthesis and biological evaluation of substituted benzoyloxy benzophenones for a rational study of the structure-activity relationships.

Keywords: Synthesis; Crystal structure; Benzoyloxy benzophenone; Anti-inflammatory activity.
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Table 1. Anti-inflammatroy, ulcerogenic, cyclooxygenase and toxicity data of compounds **4a–n**

Compound	Dose (mg/kg po)	Anti-inflammatory activity % oedema inhibition relative to control	±SEM[a]	Dose (mg/kg po)	Ulcerogenic activity		Cyclooxygenase activity assay inhibitory action of some selected compounds % inhibition 10 μM	ED_{50} (mg/kg po)	ALD_{50} (mg/kg po)
					% of animal with hyperaemia	% of animal with ulcer			
4a	20	155	±0.0050	100	20	45	Ni	75.5	>1000
	40	29.4	±0.1002	200	35	55			
	80	46.5	±0.0673	400	55	80			
4b	20	20.6	±0.0232	100	40	10	65	76.2	>1000
	40	30.5	±0.0311	200	60	15			
	80	60.1	±0.0055	400	80	20			
4c	20	31.4	±0.0180	100	25	15	Ni	57.3	>1000
	40	46.5	±0.0088	200	50	25			
	80	85.5	±0.0065	400	75	18			
4d	20	29.2	±0.0016	100	50	10	85	60.5	>1000
	40	30.4	±0.0034	200	70	15			
	80	77.1	±0.0013	400	100	20			
4e	20	20.5	±0.0015	100	30	20	30	70.1	>1000
	40	35.5	±0.0001	200	55	25			
	80	50.5	±0.0009	400	90	45			
4f	20	18.5	±0.0005	100	25	50	Ni	75.5	>1000
	40	30.2	±0.0002	200	40	65			
	80	40.5	±0.0002	400	50	75			
4g	20	35.5	±0.1002	100	60	05	60	65.5	>1000
	40	48.5	±0.0707	200	80	10			
	80	60.1	±0.0208	400	100	15			
4h	20	16.6	±0.0352	100	50	15	70	76.2	>1000
	40	44.6	±0.0101	200	70	20			
	80	64.1	±0.0177	400	90	25			
4i	20	15.1	±0.0004	100	40	10	20	78.6	>1000
	40	29.9	±0.0011	200	60	20			
	80	56.4	±0.0004	400	100	40			
4j	20	14.7	±0.0044	100	20	40	Ni	77.9	>1000
	40	24.2	±0.0014	200	40	50			
	80	47.5	±0.0021	400	60	80			
4k	20	23.4	±0.0053	100	50	05	87	61.5	>1000
	40	46.5	±0.0067	200	70	10			
	80	77.1	±0.0024	400	100	25			
4l	20	21.1	±0.0004	100	70	10	40	63.6	>1000

	40	29.8	±0.1002	200	90	20				
	80	63.8	±0.0705	400	100	40				
4m	20	15.1	±0.0004	100	25	50	Ni	55.7		>1000
	40	29.9	±0.0011	200	40	65				
	80	56.4	±0.0004	400	50	75				
4n	20	17.5	±0.0177	100	50	20	70	78.9		>1000
	40	33.9	±0.0055	200	70	30				
	80	64.8	±0.0086	400	90	40				
Aspirin	20	29.5	±0.1002	100	25	75	95	95.5		—
	40	30.5	±0.0311	200	55	90				
	80	55.1	±0.0003	400	85	85				
Phenyl butazone	20	30.1	±0.0002	100	25	35	85	—		—
	40	33.0	±0.0012	200	50	65				
	80	55.1	±0.0003	400	80	90				
Control	20	—		30	—	—	Ni	—		—
	40			60						
	80			90						

Ni = no inhibition.
[a] Values are means of three different determinates and the values are <5%.

2. Results and discussion

2.1. Pharmacological evaluation

The characteristic feature of the title compounds is due to the presence of benzoate group. All the esters **4a**–**n** have shown good anti-inflammatory activity in the range 24.2–48.9% at a dose of 40 mg/kg po. Among **4a**–**n**, the compound **4g** with three chloro groups at the para position elicited maximum inhibition of oedema (48.5%). Compounds **4c** (46.5%), with two methyl and one chloro groups at para position and **4k** (46.5%) with two chloro and one bromo groups at para position elicited same activity. On the other hand, compound **4h** (44.6%) with three different groups, methyl, chloro and fluoro at para position, exhibited less activity compared to **4c** and **4k**. Compound **4j** with one chloro and one methyl groups at para position of benzophenone ring exhibited least activity (24.2%). In addition compounds **4a** with two methyl groups at the para position in the phenyl and benzoate ring and **4m** with two chloro groups at meta position exhibited little higher activity (29.4% and 29.9%) compared to compound **4j**. Compounds **4i** with one methyl group in phenyl ring and **4l** with three chloro groups at ortho, meta and para position exhibited almost same activity (29.9% and 29.6 %). Compounds **4b** (30.5%) in which the three methyl groups are at para position in different benzene rings, **4d** (30.4%) with methyl group in benzoate ring and two chloro groups at para position in the other two rings and **4f** (30.2%) without any substituent in benzoate ring have shown comparable activity. Compounds **4e** (35.5%) with two methyl and one fluoro groups at para position and **4n** (33.9%) with three methyl groups, two at ortho and one at para position, have exhibited near activity. Based on the above results, title compounds have been tested at three graded doses (20, 40 and 80 mg/kg po) and compared with standard drugs aspirin and phenyl butazone. The comparison results with standard drugs are listed in Table 1.

2.1.1. Ulcerogenic activity. The title compounds **4a**–**n** exhibited low degree of ulcer production activity (10–65%) at 200 mg/kg po. Among **4a**–**n**, compounds **4g** with three chloro groups at the para position in aromatic ring and **4k** with two chloro groups at para position exhibited lesser ulcerogenic activity (10%) compared to standard drugs, aspirin and phenyl butazone.

2.1.2. Cyclooxygenase assay activity. Compounds **4b**, **4d**, **4e**, **4g** **4h** and **4k** showed good cyclooxygenase activity indicating that these compounds reduce inflammatory response by inhibition of prostaglandins. The other compounds **4a**, **4c** **4f**, **4j** and **4m**, which do not inhibit the cyclooxygenase activity, seem to act through some other mechanism rather than inhibiting prostaglandin synthesis. It appears that compounds **4a**, **4c** **4f**, **4j** and **4m** inhibit the Phospholipase A_2 (PLA_2) enzyme. The PLA_2 class of enzymes catalyze hydrolysis of the 2-acyl ester of 3-Sn phosphoglycerides to yield arachidonic acid which is responsible for the production of pro-inflammatory lipid mediators such as PGs.[13–15]

Table 2. Crystal data and structure refinement table

Compound	**4b**	**4d**
CCDC number	CCDC 617402	CCDC 617403
Empirical formula	$C_{23}H_{20}O_3$	$C_{21}H_{14}Cl_2O_3$
Formula weight	344.39	385.22
Temperature	293(2) K	293(2) K
Wavelength	0.71073 Å	0.71073 Å
Crystal system	Monoclinic	Monoclinic
Space group	*P*21/*c*	*P*21/*c*
Cell dimensions	a = 13.565(10) Å	a = 10.359(7) Å
	b = 10.076(9) Å	b = 7.761(6) Å
	c = 13.519(12) Å	c = 22.965(15) Å
	β = 98.730(4)_	β = 104.156(3)_
Volume	1826(3) Å^3	1790(2) Å^3
Z	4	4
Density(calculated)	1.252 Mg/m^3	1.429 Mg/m^3
Absorption coefficient	0.082 mm^{-1}	0.381 mm^{-1}
F(000)	728	792
Crystal size	0.3 × 0.27 × 0.25 mm	0.27 × 0.25 × 0.2 mm
ıTheta$_{max}$	25.03	25.02
Index ranges	$-16 \leqslant h \leqslant 16$	$-12 \leqslant h \leqslant 12$
	$-11 \leqslant k \leqslant 11$	$-8 \leqslant k \leqslant 9$
	$-15 \leqslant l \leqslant 16$	$-27 \leqslant l \leqslant 27$
Reflections collected	5477	4706
Independent reflections	3185 [R(int) = 0.0211]	2715 [R(int) = 0.0201]
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data/restraints/parameters	3185/0/238	2715/0/237
Goodness-of-fit on F^2	1.093	1.152
Final R indices [I > 2_(I)]	R_1 = 0.0442, wR_2 = 0.1317	R_1 = 0.0402, wR_2 = 0.1266
R indices (all data)	R_1 = 0.0596, wR_2 = 0.1474	R_1 = 0.0467, wR_2 = 0.1328
Largest diff. peak and hole	0.413 and −0.360 e Å^{-3}	0.299 and −0.282 Å^{-3}

2.1.3. ALD$_{50}$ studies. The toxicity study of these compounds indicates their good safety margin.

2.2. X-ray data collection, structure, solution and refinement

Single crystals of suitable size were chosen for X-ray diffraction studies. The data were collected at room temperature on a DIPLabo Image Plate system with graphite monochromated radiation (MoK). Each exposure of the image plate was set to a period of 400 s. Thirty-six frames of data were collected in the oscillation mode with an oscillation range of 5° and processed using Denzo.[23] The reflections were merged with Scalepack. All of the frames in A and B could be indexed using a primitive monoclinic lattice. The structures were solved by direct methods using SHELXS-97.[24] Least-squares refinement using SHELXL-97[24] with isotropic temperature factors for all the nonhydrogen atoms converged the residuals R_1 to **4b** = 1639 and **4d** = 0.1729, respectively. Subsequent refinements were carried out with anisotropic thermal parameters for non-hydrogen atoms and isotropic temperature factors for the hydrogen atoms which were placed at chemically acceptable positions. The hydrogen atoms were allowed to ride on their parent atoms. The residuals finally converged to **4b** = 0.442 and **4d** = 0.402, respectively. The comparative details of the crystal data and refinement are given in Table 2. Table 3 gives the list of selected bond lengths and bond angles which are in good agreement with the standard values. The ORTEP of the molecules with thermal ellipsoids drawn at 50% probability are shown in Figures 1 and 3, respectively.

The compound **4b** crystallizes in the monoclinic space group *P*21/*c* with Z = 4. The dihedral angles between

Table 3. Bond lengths and bond angles (Å) (°)

4b		**4d**	
Atoms	Length	Atoms	Length
C2–O17	1.400(2)	C2–O17	1.400(3)
C5–C7	1.506(2)	C5–Cl7	1.743(2)
C8–O9	1.215(2)	C8–O9	1.221(3)
C8–C10	1.484(2)	C8–C10	1.487(3)
C10–C15	1.391(2)	C10–C15	1.395(3)
C13–C16	1.504(3)	C13–Cl16	1.741(2)
O17–C18	1.371(2)	O17–C18	1.368(3)
C18–O19	1.198(2)	C18–O19	1.197(3)
C18–C20	1.477(2)	C18–C20	1.478(3)
C23–C26	1.509(3)	C23–C26	1.513(3)
C1–C2–O17	121.8(2)	C1–C2–O17	115.6(2)
C4–C5–C7	121.6(2)	C4–C5–Cl7	119.5(2)
C4–C3–C8	119.3(2)	C4–C3–C8	117.1(2)
O9–C8–C3	118.8(2)	O9–C8–C3	118.8(2)
C10–C8–C3	120.1(2)	C10–C8–C3	120.4(2)
C11–C10–C8	122.5(2)	C11–C10–C8	122.0(2)
C15–C10–C8	119.3(2)	C15–C10–C8	118.9(2)
C14–C13–C16	121.5(2)	C14–C13–Cl16	118.9(2)
O19–C18–O17	123.0(2)	O19–C18–O17	122.6(2)
O19–C18–C20	126.8(2)	O19–C18–C20	126.3(2)
C22–C23–C26	121.7(2)	C22–C23–C26	120.6(2)
C25–C20–C18	122.9(2)	C25–C20–C18	118.7(2)

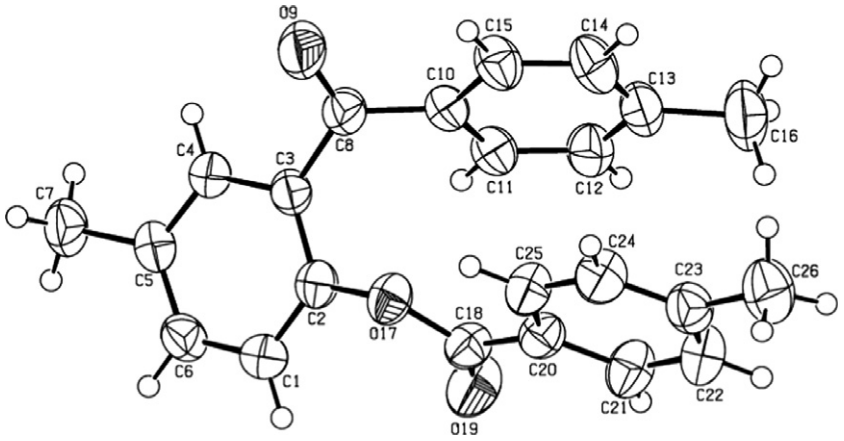


Figure 1. Ortep of 4b at 50% probability.

Table 4. Selected dihedral angles between the least squares planes (°)

	4b	4d
Plane 1 and 2	63.28(12)	61.97(12)
Plane 1 and 3	59.75(12)	51.95(12)
Plane 2 and 3	13.63(13)	17.10(13)

Plane 1, the phenyl ring C1/C6; Plane 2: the phenyl ring C10/C15; Plane 3, the phenyl ring C20/C25.

the least squares planes of the two phenyl rings bridged by the keto carbonyl group and the phenyl rings bridged by the ester group are tabulated in Table 4. The torsion angle values of 44.10(34)_ and −178.77(30)_ for C4–C3–C8–O9 and O19–C18–C20–C25, respectively, indicate that the carbonyl groups are oriented in −synclinal and +antiperiplanar conformations, respectively. The structure exihibits an intermolecular hydrogen bond of the type C–H···O which holds the molecule together in the crystal lattice resulting in a hydrogen bonded dimer as shown in Figure 2. The intermolecular hydrogen bond between the central methylphenyl ring and the keto carbonyl moiety has a length of 3.458 (5) Å and an angle of 172° with symmetry code x, 3/2 − y, 1/2 + z.

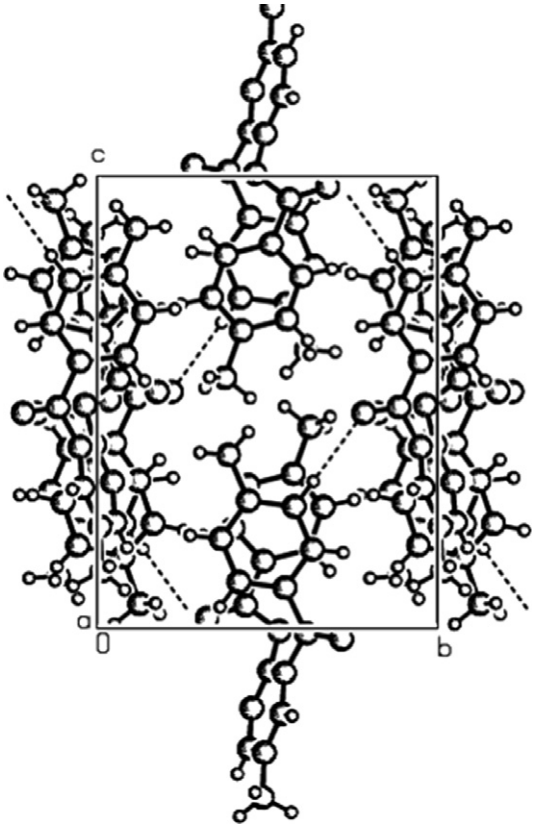


Figure 2. Packing of molecule **4b** down b axis.

The compound **4d** crystallizes in the monoclinic space group $P21/c$ with $Z = 4$. The dihedral angles between the least squares planes of the two phenyl rings bridged by the keto carbonyl group and the phenyl rings bridged by the ester group are tabulated in Table 4. The torsion angle values of −39.10(33)° and 12.56(36)° for C4—C3—C8–O9 and O19–C18–C20–C25, respectively, indicate that the carbonyl groups are oriented in −synclinal and +antisynperiplanar conformations, respectively. The structure exhibits an intermolecular hydrogen bond of the type C–H···O which holds the molecule together in the crystal lattice resulting in a hydrogen bonded dimer as shown in Figure 4. The intermolecular hydrogen bond between the central chlorophenyl ring and the ester carbonyl moiety has a length of 3.301(4) Å and an angle of 162° with symmetry code −x, −1/2 + y, 1/2 − z.

3. Conclusion

Biological activities, results indicate that introduction of benzoate group in **2a**–**j** is fruitful as it was found that, compounds with chloro and methyl substituents at para position in ring B and C (benzoate moiety) have shown good anti-inflammatory activity with reduced ulcer production activity.

4. Experimental

4.1. Chemistry

Chemicals were purchased from Aldrich Chemical Co. TLC was performed on aluminium-backed silica plates with visualization by UV-light. Melting points were

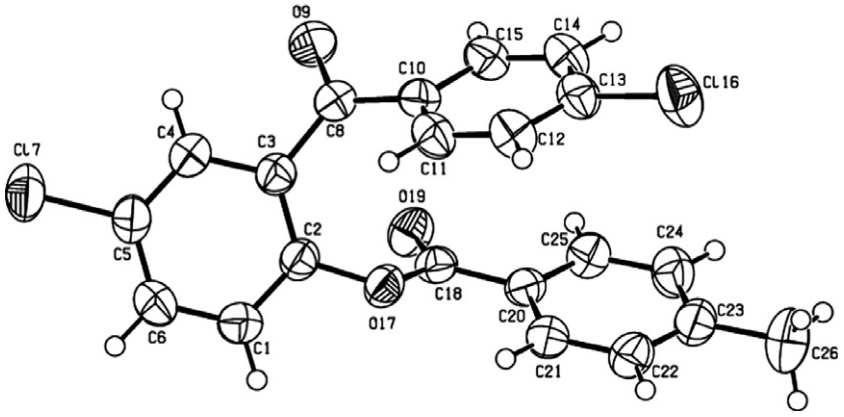


Figure 3. Ortep of **4d** at 50% probability.

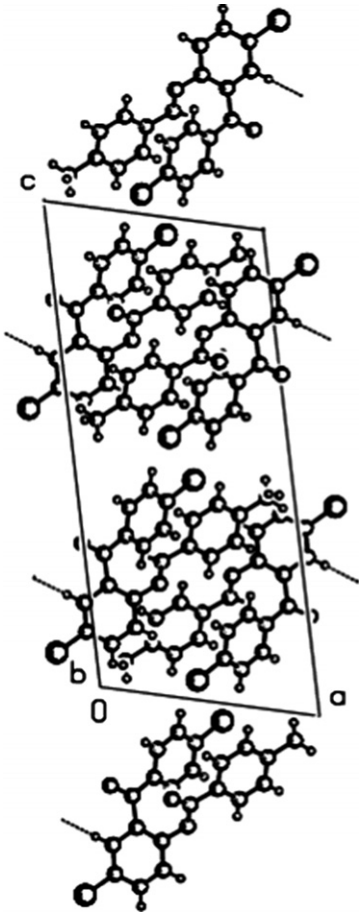


Figure 4. Packing of molecule **4d** down b axis.

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 was obtained with a VG70-70H mass spectrometer and elemental analysis results are within 0.4% of the calculated value.

The synthesis of the hitherto title compounds is as outlined in Scheme 1. Fries rearrangement of substituted phenyl benzoates **1a**–**j** with anhydrous aluminium chloride afforded substituted hydroxy benzophenones **2a**–**j** in excellent yield.[16,17] Benzoylation of **2a**–**j** with respective benzoyl chlorides **3a**–**e** affords substituted benzoyloxy benzophenones **4a**–**n**.[18] The compounds **4a**–**n** were characterized by IR, ^{1}H NMR and mass spectrophotometry.

4.2. (2-Hydroxy-5-methylphenyl) phenyl methanone (2a)

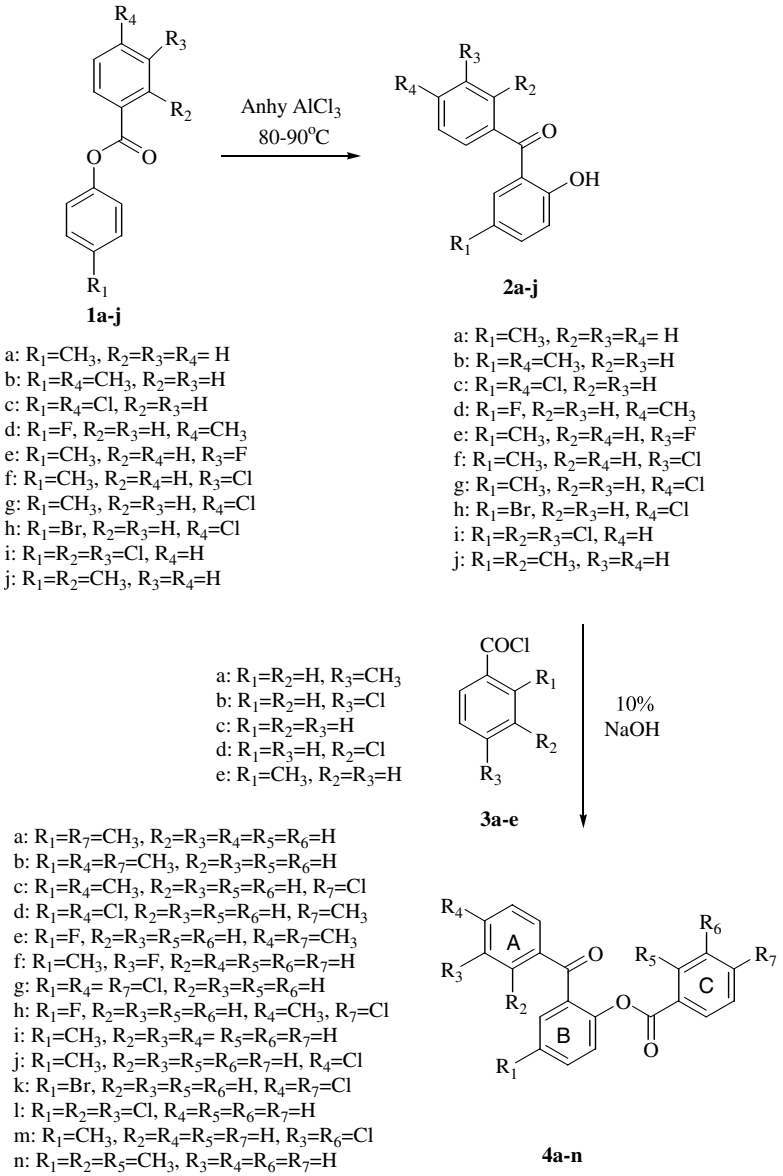
A mixture of anhydrous aluminium chloride (3.2 g, 0.02 mol) and **1a** (5 g, 0.02 mol) in dry nitrobenzene (40 mL) was protected from moisture by calcium chloride guard tube and refluxed at 80–90 °C with stirring for 45 min. At the end of this period the solution was cooled and decomposed by acidulated ice-cold water. Nitrobenzene was removed by steam distillation. The residual solid was crushed into powder, dissolved in ether and extracted with 10% sodium hydroxide. The basic aqueous solution was neutralized with 10% hydrochloric acid. The filtered solid was washed with distilled water and recrystallized from ethanol to afford pale yellow needles of **2a**.

Compound **2a**: Yield 85%, mp 81–83 °C; IR (Nujol): 1670 (C=O), 3545–3649 cm^{-1} (OH); ^{1}H NMR ($CDCl_3$) δ: 2.3 (s, 3H, CH_3), 6.85–7.75 (m, 8H, Ar-H), 12.05 (br s, 1H, OH); MS: *m*/*z* 212 (M^+, 87), 211 (100), 135 (60), 105 (35), 77 (56). Anal. Calcd for $C_{14}H_{12}O_2$ (212): C, 79.24; H, 5.66. Found: C, 79.26; H, 5.64%.

Compound **2b**: Yield 92%, mp 75–77 °C; IR (Nujol) ν (cm^{-1}): 1665 (C=O), 3510–3645 (OH); ^{1}H NMR ($CDCl_3$) δ: 2.3–2.35 (2s, 6H, CH_3), 6.85–7.7 (m, 8H, Ar-H), 12.1 (br s, 1H, OH); EI-MS: *m*/*z* (83%) M^+ 226. Anal. Calcd for $C_{15}H_{14}O_2$: C, 79.64; H, 6.19. Found: C, 79.63; H, 6.16.

Compound **2c**: Yield 94%, mp 85–87 °C; IR (Nujol) ν (cm^{-1}): 1668 (C=O), 3530–3632 (OH); ^{1}H NMR ($CDCl_3$) δ: 6.77–7.64 (m, 7H, Ar-H), 12.2 (br s, 1 H, OH); EI-MS: *m*/*z* (85%) M^+ 267. Anal. Calcd for $C_{13}H_8Cl_2O_2$: C, 58.46; H, 3.02; Cl, 26.55. Found: C, 58.45; H, 3.01; Cl, 26.54.

Compound **2d**: Yield 89%, mp 80–82 °C; IR (Nujol) ν (cm^{-1}): 1667 (C=O), 3540–3645 (OH); ^{1}H NMR



Scheme 1.

(CDCl$_3$) δ: 2.35 (s, 3H, CH$_3$), 6.81–7.58 (m, 7H, Ar-H), 12.3 (br s, 1H, OH); EI-MS: *m/z* (85%) M$^+$ 216. Anal. Calcd for $C_{14}H_{11}FO_2$: C, 73.03; H, 4.82; F, 8.25. Found: C, 73.04; H, 4.83; F, 8.26.

Compound **2e**: Yield 85%, mp 45–47 °C; IR (Nujol) ν (cm^{-1}): 1640 (C=O), 3320–3450 (OH); ^{1}H NMR (CDCl$_3$) δ: 2.3 (s, 3H, CH$_3$), 6.71–7.47 (m, 7H, Ar-H), 12.2 (br s, 1H, OH); EI-MS: *m/z* (85%) M$^+$ 216. Anal. Calcd for $C_{14}H_{11}FO_2$: C, 73.03; H, 4.82; F, 8.25. Found: C, 73.04; H, 4.83; F, 8.26.

Compound **2f**: Yield 90%, mp 71–73 °C; IR (Nujol) ν (cm^{-1}): 1673 (C=O), 3550–3640 (OH); ^{1}H NMR (CDCl$_3$) δ: 2.2 (s, 3H, CH$_3$), 7.0–7.65 (m, 7H, Ar-H), 12.15 (br s, 1H, OH); EI-MS: *m/z* (88%) M$^+$ 246.5. Anal. Calcd for $C_{14}H_{11}ClO_2$: C, 68.15; H, 4.46; Cl, 14.60. Found: C, 68.17; H, 4.44; Cl, 14.42.

Compound **2g**: Yield 87%, mp 85–87 °C; IR (Nujol) ν (cm^{-1}): 1670 (C=O), 3550–3635 (OH); ^{1}H NMR (CDCl$_3$) δ: 2.35 (s, 3H, CH$_3$), 6.71–7.64 (m, 7H, Ar-H), 12.15 (br s, 1H, OH); EI-MS: *m/z* (85%) M$^+$ 246. Anal. Calcd for $C_{14}H_{11}ClO_2$: C, 68.16; H, 4.49; Cl, 14.37. Found: C, 68.13; H, 4.48; Cl, 14.62.

Compound **2h**: Yield 81%, mp 82–84 °C; IR (Nujol) ν (cm^{-1}): 1660 (C=O), 3540–3630 (OH); ^{1}H NMR (CDCl$_3$) δ: 6.72–7.70 (m, 7H, Ar-H), 12.0 (br s, 1H, OH); EI-MS: *m/z* (M$^+$ 85%) 310, (M$^+$ 80%) 312.5. Anal. Calcd for $C_{13}H_8BrClO_2$: C, 50.13; H, 2.59; Br, 25.65; Cl, 11.38. Found: C, 50.14; H, 2.57; Br, 25.62; Cl, 11.36.

Compound **2i**: Yield 85%, mp 78–80 °C; IR (Nujol) ν (cm^{-1}): 1648 (C=O), 3305–3410 (OH); ^{1}H NMR (CDCl$_3$) δ: 6.9–7.65 (m, 6H, Ar-H), 11.9 (br s, 1H, OH); EI-MS: *m/z* (84%) M$^+$ 301.5. Anal. Calcd for

$C_{13}H_7Cl_3O_2$: C, 51.78; H, 2.34; Cl, 35.27. Found: C, 51.77; H, 2.33; Cl, 35.26.

Compound **2j**: Yield 90%, mp 71–73 °C; IR (Nujol) ν (cm^{-1}): 1665 (C=O), 3515–3645 (OH); ^1H NMR ($CDCl_3$) δ: 2.25–2.3 (2s, 6H, $2CH_3$), 6.9–7.7 (m, 7H, Ar-H), 12.05 (br s, 1H, OH); EI-MS: *m/z* (83%) M^+ 226. Anal. Calcd for $C_{15}H_{14}O_2$: C, 79.64; H, 6.19. Found: C, 79.62; H, 6.18.

4.3. 2-(4-Methylbenzoyloxy-5-methylphenyl)phenyl methanone (4a)

To a well-stirred ice-cold solution of **2a** (3 g, 0.014 mol) in 10% sodium hydroxide (20 mL), 4-methylbenzoyl chloride (**3a**, 1.96 g, 0.014 mol) was added dropwise and stirring was continued for about 15 min. The mixture was made alkaline by adding 10% sodium hydroxide. A white solid was separated, which was filtered, washed with water and on recrystallization with alcohol gave **4a.**

Compound **4a**: Yield 85%, mp 88–90 °C; IR (Nujol) ν (cm^{-1}): 1670 (C=O), 1760 (ester, C=O); ^1H NMR ($CDCl_3$) δ: 2.2–2.3 (2s, 6H, $2CH_3$), 7.13–8.02 (m, 12H, Ar-H); EI-MS: *m/z* (87%) M^+ 330. Anal. Calcd for $C_{22}H_{18}O_3$: C, 79.98; H, 5.49. Found: C, 79.97; H, 5.47.

Compound **4b**: Yield 88%, mp 97–99 °C; IR (Nujol) ν (cm^{-1}): 1668 (C=O), 1750 (ester, C=O); ^1H NMR ($CDCl_3$) δ: 2.3–2.5 (3s, 9H, $3CH_3$), 6.8–7.6 (m, 11H, Ar-H); EI-MS: *m/z* (87%) M^+ 334. Anal. Calcd for $C_{23}H_{20}O_3$: C, 80.21; H, 5.85. Found: C, 80.20; H, 5.7.

Compound **4c**: Yield 81%, mp 101–103 °C; IR (Nujol) ν (cm^{-1}): 1670 (C=O), 1755 (ester, C=O); ^1H NMR ($CDCl_3$) δ: 2.3–2.4 (3s, 9H, $3CH_3$), 6.75–7.7 (m, 11H, Ar-H); EI-MS: *m/z* (87%) M^+ 364. Anal. Calcd for $C_{22}H_{17}ClO_3$: C, 72.43; H, 4.70; Cl, 9.75. Found: C, 72.45; H, 4.71; Cl, 9.73.

Compound **4d**: Yield 78%, mp 138–140 °C; IR (Nujol) ν (cm^{-1}): 1665 (C=O), 1737 (ester, C=O); ^1H NMR ($CDCl_3$) δ: 2.3 (s, 3H, CH_3), 7.19–8.02 (m, 11H, Ar-H); EI-MS: *m/z* (87%) M^+ 385. Anal. Calcd for $C_{21}H_{14}Cl_2O_3$: C, 65.47; H, 3.66; Cl, 18.41. Found: C, 65.46; H, 3.65; Cl, 18.40.

Compound **4e**: Yield 81%, mp 90–92 °C; IR (Nujol) ν (cm^{-1}): 1665 (C=O), 1760 (ester, C=O); ^1H NMR ($CDCl_3$) δ: 2.3–2.4 (2s, 6H, $2CH_3$), 7.16–8.02 (m, 11H, Ar-H); EI-MS: *m/z* (86%) M^+ 348. Anal. Calcd for $C_{22}H_{17}FO_3$: C, 75.85; H, 4.92; F, 5.45. Found: C, 75.84; H, 4.91; F, 5.44.

Compound **4f**: Yield 74%, mp 65–67 °C; IR (Nujol) ν (cm^{-1}): 1672 (C=O), 1765 (ester, C=O); ^1H NMR ($CDCl_3$) δ: 2.3 (s, 3H, CH_3), 7.13–8.14 (m, 12H, Ar-H); EI-MS: *m/z* (86%) M^+ 334. Anal. Calcd for $C_{22}H_{15}FO_3$: C, 75.44; H, 4.52; F, 5.68. Found: C, 75.43; H, 4.51; F, 5.67.

Compound **4g**: Yield 79%, mp 122–124 °C; IR (Nujol) ν (cm^{-1}): 1662 (C=O), 1743 (ester, C=O); ^1H NMR ($CDCl_3$) δ: 7.37–8.08 (m, 11H, Ar-H); EI-MS: *m/z* (86%) M^+ 405.5. Anal. Calcd for $C_{20}H_{11}Cl_3O_3$: C, 59.22; H, 2.73; Cl, 26.22. Found: C, 59.20; H, 2.71; F, 26.20.

Compound **4h**: Yield 72%, mp 114–116 °C; IR (Nujol) ν (cm^{-1}): 1663 (C=O), 1745 (ester, C=O); ^1H NMR ($CDCl_3$) δ: 2.35 (s, 3H, CH_3), 7.16–8.08 (m, 11H, Ar-H); EI-MS: *m/z* (86%) M^+ 368. Anal. Calcd for $C_{21}H_{14}ClFO_3$: C, 68.39; H, 3.83; Cl, 9.61; F, 5.15. Found: C, 68.38; H, 3.82; Cl, 9.60; F, 5.14.

Compound **4i**: Yield 75%, mp 89–91 °C; IR (Nujol): 1670 cm^{-1} (C=O1760), (ester, C=O); ^1H NMR ($CDCl_3$) δ: 2.2 (s, 3H, CH_3), 6.9–7.7 (m, 13H, Ar-H); MS: *m/z* (76%) M^+ 316. Anal. Calcd for $C_{21}H_{16}O_3$: C, 79.73; H, 5.10. Found: C, 79.75; H, 5.12%.

Compound **4j**: Yield 78%, mp 95–97 °C; IR (Nujol) ν (cm^{-1}): 1675 (C=O), 1760 (ester, C=O); ^1H NMR ($CDCl_3$) δ: 2.35 (s, 3H, CH_3), 7.13–8.14 (m, 12H, Ar-H); EI-MS: *m/z* (81%) M^+ 350. Anal. Calcd for $C_{21}H_{15}ClO_3$: C, 71.90; H, 4.31; Cl, 10.11. Found: C, 72.93; H, 4.30; Cl, 10.13.

Compound **4k**: Yield 72%, mp 126–128 °C; IR (Nujol): 1665 cm^{-1} (C=O), 1750 (ester, C=O); ^1H NMR ($CDCl_3$) δ: 7.14–8.08 (m, 11H, Ar-H); MS: *m/z* (77%) M^+ 449. Anal. Calcd for $C_{20}H_{11}Br\,Cl_2O_3$: C, 53.37; H, 2.46; Br, 17.75; Cl, 15.75. Found: C, 53.40; H, 2.44; Br, 17.73; Cl, 15.77.

Compound **4l**: Yield 77%, mp 90–92 °C; IR (Nujol) ν (cm^{-1}): 1660 (C=O), 1740 (ester, C=O); ^1H NMR ($CDCl_3$) δ: 6.9–7.65 (m, 11H, Ar-H); EI-MS: *m/z* (86%) M^+ 405.5. Anal. Calcd for $C_{20}H_{11}Cl_3O_3$: C, 59.22; H, 2.73; Cl, 26.22. Found: C, 59.23; H, 2.74; F, 26.23.

Compound **4m**: Yield 77%, mp 93–95 °C; IR (Nujol): 1670 cm^{-1} (C=O), 1755 (ester, C=O); ^1H NMR ($CDCl_3$) δ: 2.3 (s, 3H, CH_3), 6.75–7.7 (m, 11H, Ar-H); MS: *m/z* (77%) M^+ 385. Anal. Calcd for $C_{21}H_{14}\,Cl_2O_3$: C, 65.47; H, 3.66; Cl, 18.41. Found: C, 65.45; H, 3.65; Cl, 18.40%.

Compound **4n**: Yield 79%, mp 96–98 °C; IR (Nujol): 1655 cm^{-1} (C=O), 1735 (ester, C=O); ^1H NMR ($CDCl_3$) δ: 2.3–2.5 (3s, 9H, $3CH_3$), 6.92–7.7 (m, 11H, Ar-H); MS: *m/z* (74%) M^+ 344. Anal. Calcd for $C_{23}H_{20}O_3$: C, 80.21; H, 5.85. Found: C, 80.22; H, 5.84%.

4.4. Pharmacological evaluation

4.4.1. Anti-inflammatory activity. Adopting Winter et al.'s[19] method and using Albino rats paw oedema inhibition test was performed on compounds **4a**–**n**. To groups of five rats (body weight 125–160 g), a dose of test compounds **4a**–**n** was given. After 30 min, 0.2 mL of 1% carrageenan suspension in 0.9% sodium chloride

solution was injected subcutaneously, into planter aponeurosis of the hind paw and the paw volume was measured by a water plethysmometer socrel and then measured again after a time span of 3 h. 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 control groups, respectively.

4.4.2. Ulcerogenic activity. Groups of 10 rats (body weight 200–230 g), fasted for 24 h, were treated with an oral dose of test compounds **4a**–**n**, 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.[20]

4.4.3. Acute toxicity study. Nearly 50% lethal dose (ALD_{50}) of the compounds was determined in albino mice (body weight 25–30 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 Smith[21] method.

4.4.4. 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 Calderano et al.'s[22] procedure the preparation was carried out. About 2–3 g of stripped, colonic mucosa was minced and homogenized in 3 vols of Tris buffer 0.1, pH 8.0, and the homogenate was centrifuged. The precipitate was suspended in Tris buffer 0.1 M, pH 8.0, and recentrifuged. For enzyme assay of 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 was incubated with test agents for 10 min at 37 °C in 30 μL Tris–HCl, pH 8.0, containing 2 mM reduced glutathione, 5 mM L-trytophan and 1 μM haematin. The substrate 20 μM arachidonic acid with tracer amount of [1-^{14}C] arachidonic acid [approximately 200 (xx) cpm] then added and the reaction proceeded for 5 min at 37 °C. The reaction was stopped by addition of 0.2 mL of ether/methanol/citric acid 0.2 M (30:4:1), and the mixture was precooled at −25 °C PGE_2 and 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_2as 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 at 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.

$$[(\text{cpm control} - \text{cpm test}/(\text{cpm control})) \times 100]$$

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