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Synthesis of some newer analogues of substituted dibenzoyl phenol as potent anti-inflammatory agents

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Abstract—Benzoylation of hydroxybenzophenones **1a**–**f** affords substituted benzoyl phenyl benzoates **3a**–**f**, which on Fries rearrangement using microwave irradiation led to a facile synthesis of solely dibenzoyl phenols **4a**–**f** in excellent yield. The newly synthesized compounds were screened for their anti-inflammatory activity and were compared with standard drugs. Out of the compounds studied, the compound **4e** showed more potent activity than the standard drugs at all doses tested.

1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are therapeutic agents useful in the treatment of inflammation, pain and pyresis.[1,2] Inflammatory responses are considered to be mediated in part by the prostaglandins (PGs). PGs are produced by the action of cyclooxygenase (COX) enzyme, which is also referred as prostaglandin H synthase on arachidonic acid.[3,4] 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.[5–7] COX isoforms are almost identical in structure but have important differences in substrate and inhibitor selectivity and in their intracellular locations.[8] NSAIDs block the formation of PGs and have anti-inflammatory, analgesic and anti-pyretic activities.[1,2] The discovery of COX-2[9] isoform has made possible the design of drugs that reduce inflammation without removing the protective PGs in the stomach and kidney made by COX-1. In addition, its discovery has opened the possibility of developing COX-2 selective inhibitors to act as an effective NSAIDs without the gastrotoxic effect.[6]

The competence of benzophenone analogues as chemotherapeutic agent especially as anti-inflammatory is well recognized.[1,2] Some of these analogues were synthesized by several scientists of the world and have been reported as potent anti-inflammatory agents.[10–12] Recently Ottosen et al. have reported synthesis and structural activity relationship of benzophenones as novel class of p38 MAP kinase inhibitors with high anti-inflammatory activity.[13] Our search for new molecules with anti-inflammatory activity[14] encouraged us to synthesize some newer more potent analogues of hydroxybenzophenones by modifying the aromatic moiety with the introduction of second benzoyl group at 2- and 6-positions. We have focused our interest on the synthesis and biological evaluation of substituted dibenzoyl phenols for a rational study of the structural activity relationships.

2. Chemistry

The synthetic sequence is outlined in Scheme 1. Benzoylation of substituted hydroxybenzophenones **1a**–**f** with respective benzoyl chlorides **2a**–**d** affords substituted benzoyl phenyl benzoate **3a**–**f**.[15] Compounds **3a**–**f** on thoroughly mixing with an equal amount of montmorrillonite k 10 clay in the solid state using vortex mixer and on subjecting to microwave irradiation for 10–13 min afforded substituted dibenzoyl phenols **4a**–**f** in excellent yield compared to one pot conventional method.[16] The newly synthesized compounds **3a**–**f**[17] and

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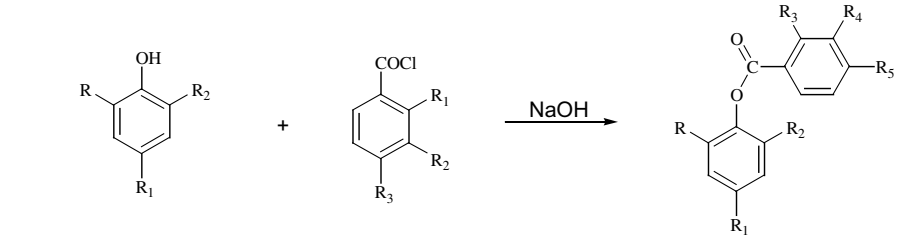


1a-f

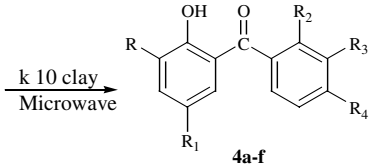
a: R=H, R_1=CH_3, R_2=4-CH_3COPh
b: R=H, R_1=CH_3, R_2=COPh
c: R=CH_3, R_1= 2-CH_3COPh, R_2=H
d: R=CH_3, R_1=COPh, R_2=H
e: R=H, R_1=CH_3, R_2=3-ClCOPh
f: R=H, R_1=CH_3, R_2=2-CH_3COPh

2a-d

a: R_1=R_2=H, R_3=CH_3
b: R_1=R_2=R_3=H
c: R_1=R_3=H, R_2=Cl
d: R_1=CH_3, R_2=R_3=H

3a-f

a: $R=R_3=R_4$=H, R_1=R_5=CH_3, R_2=4-CH_3COPh
b: $R=R_3=R_4=R_5$=H, R_1=CH_3, R_2=COPh
c: R=CH_3, R_1= 2-CH_3COPh, $R_2=R_3=R_4=R_5$=H
d: $R=R_5$=CH_3, R_1=COPh, $R_2=R_3=R_4$=H
e: $R=R_3=R_5$=H, R_1=CH_3, R_2=3-ClCOPh, R_4=Cl
f: $R=R_4=R_5$=H, $R_1=R_3$=CH_3, R_2=2-CH_3COPh



4a-f

a: $R_1=R_4$=CH_3, R=4-CH_3COPh, $R_2=R_3$=H
b: $R_2=R_3=R_4$=H, R_1=CH_3, R=COPh
c: R=CH_3, R_1= 2-CH_3COPh, $R_2=R_3=R_4$=H
d: $R=R_4$=CH_3, R_1=COPh, $R_2=R_3$=H
e: $R_2=R_4$=H, R_1=CH_3, R=-3-ClCOPh, R_3=Cl
f: $R_3=R_4$=H, $R_1=R_2$=CH_3, R=2-CH_3COPh

Scheme 1.

4a–f[18] were characterized by IR, ^{1}H NMR and mass spectrophotometer.

3. Pharmacological evaluation

3.1. Anti-inflammatory activity

Albino rats were used to perform paw oedema inhibition test adopting winter et al.[19] method. Groups of five rats (body weight 125–160 g), were given a dose of a test compound. 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 controls groups, respectively.

3.2. 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.[20]

3.3. 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.

3.4. 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.[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 homogenized was centrifuged. The precipitate as 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 °C in 30 μL Tris–HCl, pH 8.0 containing 2 mM reduced glutathione,

Table 1. Anti-inflammatory, ulcerogenic, cyclooxygenase and toxicity data of compounds **4a–f**

Compd	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 hyperaemia	% Of animal with ulcer			
4a	20	22.2	100	50	20	87	60.2	>1000
	40	45.4	200	70	30			
	80	77.1	400	100	40			
4b	20	13.7	100	20	40	NI	77.5	>1000
	40	22.2	200	40	50			
	80	45.5	400	60	80			
4c	20	14.1	100	40	10	20	78.3	>1000
	40	29.5	200	60	20			
	80	55.3	400	100	40			
4d	20	20.1	100	70	10	40	62.5	>1000
	40	29.1	200	90	20			
	80	62.8	400	100	40			
4e	20	30.3	100	30	10	NI	51.2	>1000
	40	48.2	200	60	20			
	80	94.1	400	90	12			
4f	20	16.6	100	50	05	70	77.2	>1000
	40	33.2	200	70	10			
	80	64.1	400	90	15			
Aspirin	20	30.4	100	30	80	99	98.3	—
	40	35.5	200	60	90			
	80	59.6	400	90	90			
Phenyl	20	31.3	100	30	30	89	—	—
Butazone	40	35.5	200	60	60			
	80	57.2	400	90	90			
Control	20	—	30	—	—	NI	—	—
	40		60					
	80		90					

NI = no inhibition.

5 mM L-trytophan, 1 μM haematin. The substrate 20 μM arachidonic acid with tracer amount of [1−^{14}C] arachidonic acid [approximately 200 (××) 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), which was precooled at −25 °C 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.

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

4. Results and discussion

The characteristic feature of the title compounds is the presence of two keto groups at the same phenyl ring. All the dibenzoyl phenols **4a**–**f** have shown good anti-inflammatory activity in the range 22.2–48.2% at a dose of 40 mg/kg po. Among **4a**–**f**, the compounds **4e** with two chloro groups at the *meta*-position in benzoyl moiety, elicited maximum inhibition of oedema (48.2%). Compound **4a** with three methyl groups, one at the *para*-position in the phenyl ring and the other two at *para*-position in benzoyl moiety, exhibited more activity (45.4%) compared to **4f** (33.2%) in which the two methyl groups in benzoyl moiety are at *ortho*-position. Whereas compound **4b** having only one methyl group at phenyl ring exhibited least activity (22.2%) at a dose of 40 mg/kg po. On the contrary compounds **4c** (29.5%) and **4d** (29.1%) in which benzoyl groups are at second and fourth position have shown less activity compared to compounds **4a**,**e** and **4f**, in which benzoyl groups are at second and sixth position. 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.

4.1. Ulcerogenic activity

The title compound **4a**–**f** exhibited low degree of ulcer production activity (10–50%) at 200 mg/kg po. Among **4a**–**f**, compound **4e** with two chloro groups at the *meta*-position in benzoyl moiety, exhibited lesser ulcerogenic activity (20%) compared to standard drugs, aspirin and phenylbutazone.

4.2. Cyclooxygenase assay activity

Compounds **4a**,**c**,**d** and **4f** showed good cyclooxygenase activity indicating that these compounds reduces inflammatory response by inhibition of prostaglandins. The other compounds **4b** and **4e**, which do not inhibit the cyclooxygenase activity, seems to act through some other mechanism rather than inhibiting prostaglandin synthesis. It appears that compounds **4b** and **4e** inhibits 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.[23–25]

4.3. ALD_{50} studies

The toxicity study of these compounds indicates their good safety margin.

5. Conclusion

From the results of the biological activities, it appears that introduction of benzoyl group in **1a**–**f** is fruitful as it was found that, **4a**–**f** showed good anti-inflammatory with reduced ulcer production activity. Moreover introduction of second benzoyl group using microwave oven is more beneficial with respect to yield compared to one pot conventional method.

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17. **3a**: Yield 78%, mp 97–99 °C; IR (Nujol): 1750 (ester, C=O); 1668 cm^{-1} (C=O), ^1H NMR ($CDCl_3$): δ 2.3–2.5 (3s, 9H, $3CH_3$), 6.8–7.6 (m, 11H, Ar–H); MS: *m/z* (75%) M^+ 344. Anal. Calcd for $C_{23}H_{20}O_3$: C, 80.21; H, 5.85. Found: C, 80.23; H, 5.83%.
3b: Yield 75%, mp 89–91 °C; IR (Nujol): 1760 (ester, C=O); 1670 cm^{-1} (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%.
3c: Yield 78%, mp 55–57 °C; IR (Nujol): 1740 (ester, C=O); 1645 cm^{-1} (C=O), ^1H NMR ($CDCl_3$): δ 2.3–2.4 (2s, 6H, $2CH_3$), 6.7–7.5 (m, 12H, Ar–H); MS: *m/z* (75.5%) M^+ 330. Anal. Calcd for $C_{22}H_{18}O_3$: C, 79.98; H, 5.49. Found: C, 79.97; H, 5.48%.
3d: Yield 79%, mp 50–52 °C; IR (Nujol): 1745 (ester, C=O); 1640 cm^{-1} (C=O), ^1H NMR ($CDCl_3$): δ 2.2–2.3 (2s, 6H, $2CH_3$), 6.75–7.7 (m, 12H, Ar–H); MS: *m/z* (75%) M^+ 330. Anal. Calcd for $C_{22}H_{18}O_3$: C, 79.98; H, 5.49. Found: C, 79.96; H, 5.47%.
3e: Yield 77%, mp 93–95 °C; IR (Nujol): 1755 (ester, C=O); 1670 cm^{-1} (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%.
3f: Yield 79%, mp 96–98 °C; IR (Nujol): 1735 (ester, C=O); 1655 cm^{-1} (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%.
18. **4a**: Yield 78%, mp 145–147 °C; IR (Nujol): 1650 and 1615 (C=O), 3405–3505 cm^{-1} (OH); ^1H NMR ($CDCl_3$): δ 2.32–2.5 (3s, 9H, $3CH_3$), 6.9–7.65 (m, 10H, Ar–H), 12.0 (br s, 1H, OH); MS: *m/z* (64%) M^+ 344. Anal. Calcd for $C_{23}H_{20}O_3$: C, 80.21; H, 5.85. Found: C, 80.20; H, 5.86%.
4b: Yield 80%, mp 159–161 °C [lit.: 160];[16] IR (Nujol): 1655 and 1620 (C=O), 3400–3500 cm^{-1} (OH); ^1H NMR ($CDCl_3$): δ 2.3 (s, 3H, CH_3), 6.8–7.6 (m, 12H, Ar–H); 12.02 (br s, 1H, OH); MS: *m/z* (63%) M^+ 316. Anal. Calcd for $C_{21}H_{16}O_3$: C, 79.73; H, 5.10. Found: C, 79.74; H, 5.11%.
4c: Yield 81%, mp 91–93 °C; IR (Nujol): 1645 and 1615 (C=O), 3360–3470 cm^{-1} (OH); ^1H NMR ($CDCl_3$): δ 2.3–2.41 (2s, 6H, $2CH_3$), 6.8–7.6 (m, 11H, Ar–H), 11.4 (br s, 1H, OH); MS: *m/z* (63.5%) M^+ 330. Anal. Calcd for $C_{22}H_{18}O_3$: C, 79.98; H, 5.49. Found: C, 79.99; H, 5.47%.
4d: Yield 80%, mp 96–98 °C; IR (Nujol): 1640 and 1612 cm^{-1} (C=O), 3350–3460 cm^{-1} (OH); ^1H NMR ($CDCl_3$): δ 2.3–2.42 (2s, 6H, $2CH_3$), 6.75–7.7 (m, 11H, Ar–H), 11.2 (br s, 1H, OH); MS: *m/z* (62.5%) M^+ 330. Anal. Calcd for $C_{22}H_{18}O_3$: C, 79.98; H, 5.49. Found: C, 79.95; H, 5.46%.
4e: Yield 77%, mp 148–150 °C; IR (Nujol): 1660 and 1620 cm^{-1} (C=O), 3420–3525 cm^{-1} (OH); ^1H NMR ($CDCl_3$): δ 2.3 (s, 3H, CH_3), 6.75–7.7 (m, 10H, Ar–H), 12.2 (br s, 1H, OH); MS: *m/z* (62%) M^+ 385. Anal. Calcd for $C_{21}H_{14}Cl_2O_3$: C, 65.47; H, 3.66; Cl, 18.41. Found: C, 65.48; H, 3.67; Cl, 18.43%.
4f: Yield 78%, mp 155–57 °C; IR (Nujol): 1652 and 1620 (C=O), 3415–3518 cm^{-1} (OH); ^1H NMR ($CDCl_3$): δ 2.1–2.3 (3s, 9H, $3CH_3$), 6.92–7.7 (m, 10H, Ar–H), 12.1 (br s, 1H, OH); MS: *m/z* (64.5%) M^+ 344. Anal. Calcd for $C_{23}H_{20}O_3$: C, 80.21; H, 5.85. Found: C, 80.23; H, 5.87%.
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