Bioorganic & Medicinal Chemistry Letters 18 (2008) 4409–4412

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and anti-inflammatory activity of 2-(2-aroylaroxy)-4,6-dimethoxy pyrimidines

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ARTICLE INFO

Article history:
Received 1 December 2007
Revised 13 May 2008
Accepted 17 June 2008
Available online 20 June 2008

Keywords:
Synthesis
Pyrimidines
Anti-inflammatory activity

ABSTRACT

Abstract-Reaction of **6a–f** individually with 2-methylsulfonyl-4,6-dimethoxypyrimidine yielded **7a–f** in excellent yield. The newly synthesized heterocycles were characterized by IR, ^{1}H NMR, and mass spectral data. Compounds **7a–f** was screened for their anti-inflammatory activity and were compared with standard drugs. Of the compounds studied, the compound **7e** showed more potent activity than the standard drugs at all doses tested.

The competence of benzophenone analogues as chemotherapeutic agents, 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.[3–5] Recently Ottosen et al. have reported synthesis and structure–activity relationship of benzophenones, which are novel class of p38 MAP kinase inhibitors with high anti-inflammatory activity.[6]

In our continuing work on benzophenones,[7,8] we are interested to incorporate a pyrimidine group in one of the phenyl rings. The reason for this is that pyrimidine derivatives comprise a diverse and interesting group of drugs.[9] The subject has been discussed recently.[10] Earlier, a comprehensive review concerning pyrimidines had been published by Brown.[11] Pyrimidines in general are extremely important for their biological activities. For example, some are antiviral agents,[12] the others are selective cholecystokinin subtype 1 (CCK1) receptor antagonists,[13] and a few are anti-inflammatory.[14–16] In fact, there are so many pyrimidine derivatives with pharmacological activities.

Since many benzophenone analogues have presented anti-inflammatory activities,[14] we perceived that when two moieties, such as benzophenone and pyrimidine are joined the new molecules might exhibit superior anti-inflammatory activity. With this idea in mind the present work was undertaken.

Condensation of thiourea **1** with diethylmalonate **2** generated the starting material, 2-mercapto-pyrimidine-4,6-diol **3**. Alkylation with methyl iodide gave 2-methylmercapto-4,6-dimethoxypyrimidine **4**, which further on perborate oxidation converted the methylmercapto group to a sulfone **5**, a better leaving group for displacement by nucleophiles. Reaction of **6a–f** individually with 2-methylsulfonyl-4,6-dimethoxypyrimidine yielded **7a–f** in good to excellent yield. The newly synthesized compounds **7a–f**[17] were characterized by IR, ^{1}H NMR and mass spectrophotometer. The synthetic maneuvering leading to **7a–f** has been represented in Scheme 1.

Anti-inflammatory activity: Albino rats were used to perform paw edema inhibition test adopting Winter et al.[18] 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 control groups, respectively.

Ulcerogenic activity: Group of 10 rats (body weight 200–230 g), fasted for 24 h. were treated with an oral dose of test compound,

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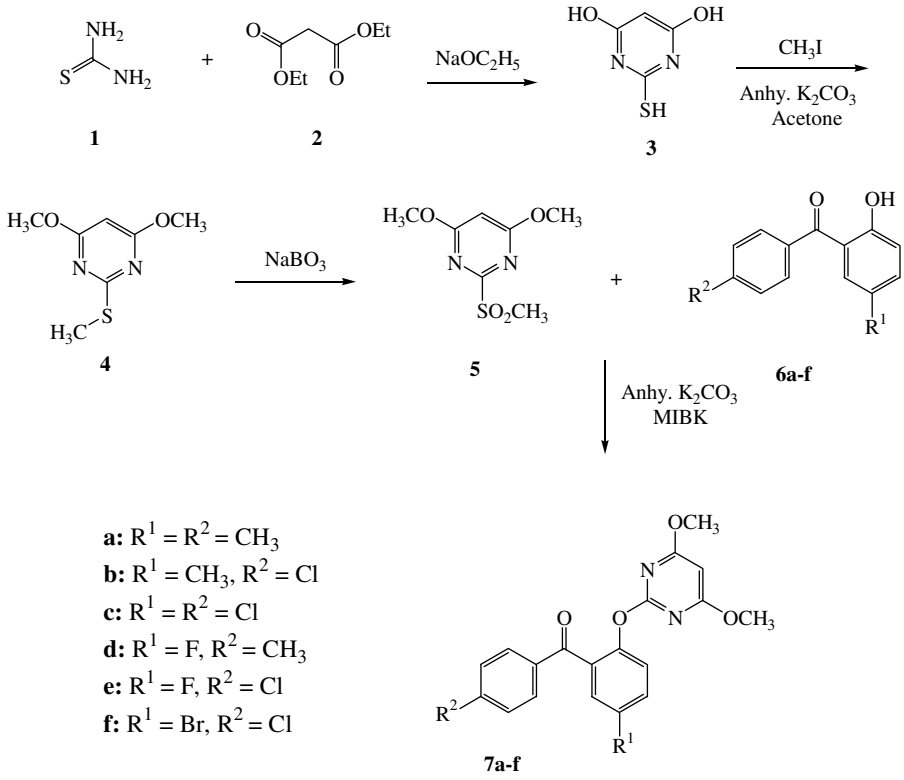
doi:10.1016/j.bmcl.2008.06.061



Scheme 1.

except the 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.[19]

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[20] 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 Calderano et al.[21] 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 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 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, 1 μM hematin. The substrate, 20 μM arachidonic acid with trace amount of [1- ^{14}C] arachidonic acid [approximately 200 (xx) cpm] was 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 radiolabeled arachidonic acid was separated, and from this radiolabeled PGE_3 was separated by RP-HBLC with 2 nmol unlabeled PGE_2 as an internal 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

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

Phospholipase A2 (PLA_2) activity: Assayed with [^{14}C] oleate-labeled autoclaved *Escherichia coli* as the substrate.[22] The reaction mixture, 350 μl, contained 100 mM Tris/HCl, pH 8.0, 5 mM Ca^{2+} and 3.15×10^9 autoclaved *E. coli* cells (corresponding to 10,000 c.p.m. and 60 nmol of lipid phosphorous). The amount of enzyme protein was chosen such that 10–15% hydrolysis of substrate was obtained when incubated at 37 °C for 60 min. The reaction components were mixed in the following order: buffer, calcium, water, and benzoyl phenyl benzoates. Adding labeled *E. coli* substrate started the reaction. The reaction was terminated by adding 100 μl of 2.0 N HCl and 100 μl of fatty acid free BSA (100 mg/ml). The tubes were vortex-mixed and centrifuged at 20,000*g* for 5 min. Aliquot (140 μl) of the supernatant containing released [^{14}C] Oleic acid was mixed with scintillation cocktail and counted in a Hewlett Packard liquid Scintillation Analyzer TRI CARB 2100 TR.

All the 2-(2-aroylaroxy)-4,6-dimethoxy pyrimidines **7a**–**f** have shown superior anti-inflammatory activity in the range 21.5–48.6% at a dose of 40 mg/kg po. Among **7a**–**f**, the compound **7e** with chloro and fluoro groups at para position in benzoyl and phenoxy moieties of benzophenone elicited maximum inhibition of edema (48.6%). Compound **7a** with two methyl groups at the para position in benzoyl and phenoxy moieties of benzophenone displayed more activity (45.8%) compared to **7f** (33.2%) in which para position of benzoyl and phenoxy moiety of benzophenone carries chloro and bromo groups, respectively. Nevertheless compound **7b** having methyl and chloro substituents at para position

Table 1
Anti-inflammatroy, ulcerogenic, cyclooxygenase, PLA2, and toxicity data of compounds **7a–f**

Compound	Dose (mg/kg po)	Anti-inflammatory activity % edema inhibition relative to control	Dose (mg/kg po)	Ulcerogenic % of animal with hyperemia	Activity % of animal with ulcer	Cyclooxygenase activity assay inhibitory action of some selected compound % inhibition 10 μM	ED_{50} (mg/kg po)	PLA2 activity assay inhibitory action % inhibition 10 μg	ALD_{50} (mg/kg po)
7a	20	20.2	100	50	20				>1000
	40	45.8	200	70	30	87	60.2	10	
	80	55.3	400	100	40				
7b	20	11.5	100	20	40				>1000
	40	21.5	200	40	50	ni	77.5	80	
	80	45.5	400	60	80				
7c	20	13.4	100	40	10				>1000
	40	29.5	200	60	20	20	78.3	40	
	80	58.7	400	100	40				
7d	20	19.4	100	70	10				>1000
	40	29.1	200	90	20	40	62.5	20	
	80	58.2	400	100	40				
7e	20	29.9	100	30	10				>1000
	40	48.6	200	60	20	ni	51.2	60	
	80	88.6	400	90	12				
7f	20	13.7	100	50	5				>1000
	40	33.2	200	70	10	70	77.2	30	
	80	57.7	400	90	15				
Aspirin	20	29.9	100	30	80				—
	40	38.8	200	60	90	99	98.3	90	
	80	61.4	400	90	90				
Phenyl butazone	20	29.6	100	30	30				—
	40	34.1	200	60	60	89	—	85	
	80	51.9	400	90	90				
Control	20	–	30	—	—				—
	40		60			ni	—	ni	
	80		90						

ni, no inhibition.

of phenoxy and benzoyl moieties, respectively, of benzophenone displayed least activity (22.2%) at a dose of 40 mg/kg po. On the contrary compounds **7c** (29.5%) and **7d** (29.1%) in which benzoyl moiety carries chloro and methyl groups, respectively, and phenoxy moiety carries chloro and fluoro groups, respectively, have shown less activity compared to compounds **7a**, **7e** and **7f**. 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 such as are listed in Table 1.

Ulcerogenic activity: The title compounds **7a–f** exhibited low degree of ulcer production activity (10–50%) at 200 mg/kg po. Among **7a–f**, compound **7e** with chloro and fluoro groups at para position in benzophenone moiety exhibited lesser ulcerogenic activity (20%) compared to standard drugs such as aspirin and phenylbutazone.

Cyclooxygenase assay activity: Compounds **7a**, **7c**, **7d** and **7f** showed good cyclooxygenase activity indicating that these compounds reduce inflammatory response by inhibition of Prostaglandins. The other compounds **7b** and **7e** do not inhibit the cyclooxygenase activity. *PLA2 assay activity:* In addition, compounds **7a–f** were tested for PLA2 activity. The PLA_2 class of enzymes catalyzes 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] Among compounds **7a–f**, **7b** and **7e** have exhibited more inhibition and compound **7c** has exhibited moderate activity.

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

From the results of the biological activities, it appears that introduction of pyrimidine moiety in **6a–f** is successful as it was found that **7a–f** showed good anti-inflammatory with reduced ulcer production activity.

Acknowledgment

The authors express their sincere gratitude to the University of Mysore, Mysore for providing laboratory facilities.

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17. Compound **7a**: Yield 0.59 g (82%). M.p. 124–126°C; IR (Nujol):) 1569 (pyrimidin-skeletal), 1663 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 2.36 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 3.7 (s, 6H, $2OCH_3$), 5.64 (s, 1H), 7.15–7.52 (m, 3H, Ar–H), 7.32 (d, J = 8.3 Hz, 2H, Ar–H), 7.58 (d, J = 8.5 Hz, 2H, Ar–H); LC-MS: m/z 365 (M^++1, 95); Anal. calcd. for $C_{21}H_{20}N_2O_4$: C, 69.21; H, 5.53; N, 7.69. Found: C, 69.17; H, 5.48; N, 7.65%.**7b**: Yield 0.6 g (79%). M.p. 114–116 °C; IR (Nujol):) 1560 (pyrimidin-skeletal), 1665 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 2.36 (s, 3H, CH_3), 3.7 (s, 6H, $2OCH_3$), 5.7 (s, 1H), 7.16–7.62 (m, 7H, Ar–H); LC-MS: m/z 385 (M^++1, 93); Anal. calcd for $C_{20}H_{17}ClN_2O_4$: C, 62.42; H, 4.45; Cl, 9.21; N, 7.25. Found: C, 62.37; H, 4.40; Cl, 9.17; N, 7.21%.**7c**: Yield 0.63 g (78%). M.p. 99–101 °C; IR (Nujol):) 1567 (pyrimidin-skeletal), 1670 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 3.72 (s, 6H, $2OCH_3$), 5.68 (s, 1H), 7.21–7.64 (m, 7H, Ar–H); LC-MS: m/z 406 (M^++1, 96); Anal. calcd for $C_{19}H_{14}Cl_2N_2O_4$: C, 56.30; H, 3.48; Cl, 17.50; N, 6.90. Found: C, 56.27; H, 3.42; Cl, 17.46; N, 6.86%.**7d**: Yield 0.6 g (82%). M.p. 105–107 °C; IR (Nujol):) 1559 (pyrimidin-skeletal), 1660 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 2.42 (s, 3H, CH_3), 3.71 (s, 6H, $2OCH_3$), 5.72 (s, 1H), 7.18–7.62 (m, 7H, Ar–H); LC-MS: m/z 369 (M^++1, 95); Anal. calcd for $C_{20}H_{17}FN_2O_4$: C, 65.20; H, 4.65; F, 5.16; N, 7.60. Found: C, 65.16; H, 4.60; F, 5.12; N, 7.54%.**7e**: Yield 0.61 g (79%). M.p. 89–91 °C; IR (Nujol):) 1565 (pyrimidin-skeletal), 1668 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 3.72 (s, 6H, $2OCH_3$), 5.75 (s, 1H), 7.2–7.65 (m, 7H, Ar–H); LC-MS: m/z 389 (M^++1, 96); Anal. calcd for $C_{19}H_{14}ClFN_2O_4$: C, 58.70; H, 3.62; Cl, 9.12; F, 4.89; N, 7.20. Found: C, 58.65; H, 3.57; Cl, 9.08; F, 4.82; N, 7.14%.**7f**: Yield 0.61 g (79%). M.p. 109–111 °C; IR (Nujol):) 1570 (pyrimidin-skeletal), 1661 cm^{-1}(C=O); ^1H NMR ($CDCl_3$): δ 3.74 (s, 6H, $2OCH_3$), 5.78 (s, 1H), 7.25–7.75 (m, 7H, Ar–H); LC-MS: m/z 450 (M^++1, 91); Anal. calcd for $C_{19}H_{14}BrClN_2O_4$: C, 50.75; H, 3.14; Br, 17.77; Cl, 7.88; N, 6.23. Found: C, 50.68; H, 3.07; Br, 17.69; Cl, 7.80; N, 6.18%.
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