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RESEARCH ARTICLE

3-Arylidene-5-(4-isobutylphenyl)-2(3H)-furanones: a new series of anti-inflammatory and analgesic compounds having antimicrobial activity

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Abstract

An ideal anti-inflammatory drug should have the desired effect in minimum dose with minimum side effects. Antimicrobial actions associated with such agents will be an added advantage as they broaden the spectrum of the compounds. Promising anti-inflammatory and antimicrobial activity together with low ulcerogenic properties of some 2(3*H*)-furanones, synthesized in our previous study, prompted us to investigate the effect of the isobutyl group on their pharmacological profile. Since compounds **3**, **9**, **13**, and **14** have both anti-inflammatory and analgesic effects in addition to low ulcerogenic incidence, they were selected for investigation of their inhibitory effects on various cyclo-oxygenase enzymes. It was found that they were more selective toward COX-2 enzymes. An MIC of 6.25 μg/mL was recorded for compounds **3**, **13**, and **14** against *S. aureus*, *E. coli*, *R. oryza*, and *P. citrum*. The study supports the development of furanone derivatives as potential anti-inflammatory agents with antimicrobial activity.

Keywords: *Furanones; anti-inflammatory; ulcerogenic; lipid peroxidation; antimicrobial activity*

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) have been recognized as a vital class of therapeutic agents for the alleviation of pain and inflammation associated with numerous pathological conditions conditions, viz. arthritis, bursitis, and tendinitis. However, chronic administration of NSAIDs has been associated with clinically significant complications such as gastrointestinal (GI) symptoms including mucosal damage, bleeding, nausea, heartburn, dyspepsia, and abdominal pain; and renal toxicity[1–2]. Polytherapy, which is considered to be tailored to patients' needs, increases the risk for developing NSAID-related complications especially in the elderly, patients with a prior history of peptic ulcer disease, patients with impaired liver or kidney functions, and patients taking anticoagulants, corticosteroids, etc. concurrently. These observations place new emphasis on the need as well as the search for new alternative and more effective agents that will take care of inflammation and infection together, from both the pharmacoeconomic and the patient compliance points of view[3].

Among a wide variety of compounds that have been explored for developing pharmaceutically important antimicrobial agents, unsaturated γ-lactones have played an important role. Moreover, furanone ring derivatives (α,β-unsaturated lactones) acquire a special place in natural chemistry and in heterocyclic chemistry, as the furanone system is a frequently encountered structural motif in many pharmacologically relevant compounds. They are active constituents of many natural and synthetic compounds exhibiting pronounced biological activities, such as antioxidant[4], cytotoxic[5,6], antifungal[7,8], antibacterial[9–11], anti-inflammatory[10,11], cardiotonic[12], analgesic[11], cyclo-oxygenase-2 (COX-2) inhibitory[13,14], and antiviral[15].

In view of these observations and as part of our ongoing research program[11,16–19] on anti-inflammatory and analgesic compounds with antimicrobial activity, we report

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the synthesis and preliminary biological evaluation with emphasis on the biological target of a series of 3-arylidene-5-(4-isobutylphenyl)-2(3*H*)-furanones (**2–18**). The newly synthesized compounds have been found to possess potential anti-inflammatory and analgesic activities with lesser ulcerogenic effect and lipid peroxidation along with an antimicrobial effect.

Materials and methods

Chemistry

Chemicals were purchased from Merck and Sigma-Aldrich as "synthesis grade" and used without further purification. Melting points were determined by the open tube capillary method and are uncorrected. Purity of the compounds was checked by thin layer chromatography (TLC) on silica gel G plates (Merck No. 5544) using toluene:ethyl acetate:formic acid (5:4:1) as the solvent system, and the spots were located either under ultraviolet light or through exposure to iodine vapors. Infrared (IR) spectra were measured on potassium bromide pellets using a PerkinElmer 1725X spectrophotometer. ^{1}H-nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Spectrospin DPX-300 MHz apparatus in $CDCl_3$ with tetramethylsilane (TMS) as an internal standard; chemical shifts (δ) are reported in parts per million (ppm) downfield from TMS. The splitting pattern abbreviations are as follows: s, singlet; d, doublet; dd, double doublet; t, triplet; q, quartet; m, multiplet. Mass spectra were recorded on a Jeol JMS-D 300 instrument fitted with a JMS 2000 data system at 70 eV. Spectral data are consistent with assigned structures. Elemental analyses were performed on a PerkinElmer model 240 analyzer (C, H, N) and found to be within a range of ±0.4% of theoretical values.

The synthesis of 2(3*H*)-furanone derivatives involved two steps.

Synthesis of 3-(4-isobutylbenzoyl)propionic acid (1)

3-(4-Isobutylbenzoyl)propionic acid was synthesized according to the earlier reported method[10],[11] for the synthesis of 3-(substituted-benzoyl)propionic acid using dry isobutylbenzene (50 mL), under anhydrous conditions in the presence of anhydrous aluminum chloride (0.15 mol) and succinic anhydride (0.1 mol). It was crystallized from aqueous ethanol to give a colorless compound which gave effervescence with sodium bicarbonate, yield 62%, mp 109–110°C, R_f 0.64; ^{1}H-NMR (δ, ppm): 0.87 (d, 6H, 2 × CH_3), 1.86 (m, 1H, CH), 2.53 (d, 2H, CH_2), 2.62 (t, 2H, CH_2), 3.22 (t, 2H, CH_2), 7.21 and 7.52 (d, each, 4H, 2 × A_2B_2, phenyl), 12.18 (s, 1H, COOH).

General procedure for the synthesis of 3-arylidene-5-(4-isobutylphenyl)-2(3H)-furanones (2–18)

Compound **1** (3 mmol) and aromatic aldehydes (equimolar, 3 mmol) were fused together in the presence of acetic anhydride (5–8 drops) in a round-bottomed flask for half an hour. To this fused mixture, triethylamine (two drops) was added and was further heated on a heating mantle for another 15 min. After the completion of reaction a solid mass was obtained, which, on crystallization with methanol, gave the desired products.

*3-Benzylidene-5-(4-isobutylphenyl)-2(3H)-furanone (**2**)* Yellow solid, yield 52%, mp 130–131°C, R_f 0.72; IR (cm^{-1}): 1765, 1600, 830; ^{1}H-NMR (ppm): 0.85 (d, 6H, J = 6.5 Hz, 2 × CH_3), 1.89 (m, 1H, CH), 2.48 (d, 2H, J = 7.0 Hz, CH_2), 6.70 (s, 1H, furanone ring), 7.05 (m, 2H, H-2, 6, arylidene ring), 7.18 and 7.42 (d, each, J = 8.67 Hz, 2 × A_2B_2, phenyl), 7.31 (t, 1H, H-4, arylidene ring), 7.38 (m, 2H, H-3, 5, arylidene ring), 7.48 (s, 1H, olefinic H); ^{13}C-NMR ($CDCl_3$; ppm) 166.6 (C-1), 124.3 (C-2), 104.6 (C-3), 156.7 (C-4), 134.2 (C-5), 132.6 (C-6), 128.4 (C-7, 11), 130.2 (C-8, 10), 131.9 (C-9), 137.4 (C-12), 127.3 (C-13, 17), 128.1 (C-14, 16), 133.2 (C-15), 48.2 (C-18), 27.8 (C-19), 23.5 (C-20, 21); Mass (*m/z*) 303 (M^+), 160, 133. Anal. Calcd. for $C_{21}H_{20}O_2$: C, 82.86; H, 6.62. Found: C, 82.63; H, 6.61%.

*3-(2-Chlorobenzylidene)-5-(4-isobutylphenyl)-2(3H)-furanone (**3**)* Pale yellow solid, yield 64%, mp 175–176°C, R_f 0.80; IR (cm^{-1}): 1760, 1605, 835; ^{1}H-NMR (ppm): 0.86 (d, 6H, J = 6.5 Hz, 2 × CH_3), 1.87 (m, 1H, CH), 2.55 (d, 2H, J = 7.0 Hz, CH_2), 6.85 (s, 1H, furanone ring), 7.28 and 7.52 (d, each, J = 8.1 Hz, 2 × A_2B_2, phenyl), 7.31 (t, 1H, H-4, arylidene ring), 7.36 (s, 1H, olefinic H), 7.46 (dd, 1H, H-6, arylidene ring), 7.59 (d, 2H, J = 7.2 Hz, H-3, 5, arylidene ring); ^{13}C-NMR ($CDCl_3$; ppm) 167.4 (C-1), 124.3 (C-2), 104.6 (C-3), 157.8 (C-4), 132.7 (C-5), 131.8 (C-6), 133.7 (C-7), 129.8 (C-8, 10), 130.3 (C-9), 125.9 (C-11), 136.3 (C-12), 127.5 (C-13, 17), 126.8 (C-14, 16), 134.1 (C-15), 48.7 (C-18), 26.2 (C-19), 22.9 (C-20, 21); Mass (*m/z*) 338 (M^+), 160, 133. Anal. Calcd. for $C_{21}H_{19}ClO_2$: C, 74.44; H, 5.65. Found: C, 74.58; H, 5.64%.

*3-(3-Chlorobenzylidene)-5-(4-isobutylphenyl)-2(3H)-furanone (**4**)* Dark yellow solid, yield 56%, mp 173–174°C, R_f 0.74; IR (cm^{-1}): 1762, 1602, 832; ^{1}H-NMR (ppm): 0.86 (d, 6H, J = 6.5 Hz, 2 × CH_3), 1.82 (m, 1H, CH), 2.53 (d, 2H, J = 7.0 Hz, CH_2), 6.81 (s, 1H, furanone ring), 7.19 and 7.48 (d, each, J = 7.8 Hz, 2 × A_2B_2, phenyl), 7.30 (m, 3H, H-4, 5, 6, arylidene ring), 7.31 (s, 1H, olefinic H), 7.55 (s, 1H, H-2, arylidene ring); ^{13}C-NMR ($CDCl_3$; ppm) 167.8 (C-1), 125.1 (C-2), 108.1 (C-3), 157.8 (C-4), 135.7 (C-5), 133.5 (C-6), 127.8 (C-7), 132.9 (C-8), 132.4 (C-9), 131.1 (C-10), 126.7 (C-11), 136.8 (C-12), 126.3 (C-13, 17), 125.7 (C-14, 16), 133.6 (C-15), 47.5 (C-18), 27.1 (C-19), 23.3 (C-20, 21); Mass (*m/z*) 338 (M^+), 160, 133. Anal. Calcd. for $C_{21}H_{19}ClO_2$: C, 74.44; H, 5.65. Found: C, 74.62; H, 5.66%.

*3-(4-Chlorobenzylidene)-5-(4-isobutylphenyl)-2(3H)-furanone (**5**)* Yellow solid, yield 64%, mp 183–184°C, R_f 0.82; IR (cm^{-1}): 1755, 1608, 836; ^{1}H-NMR (ppm): 0.90 (d, 6H, J = 6.5 Hz, 2 × CH_3), 1.85 (m, 1H, CH), 2.51 (d, 2H, J = 7.0 Hz, CH_2), 6.83 (s, 1H, furanone ring), 7.21 and 7.54 (d, each, J = 8.4 Hz, 2 × A_2B_2, phenyl), 7.33 (s, 1H, olefinic H), 7.44 and 7.66 (d, each, J = 8.7 Hz, 2 × A_2B_2, arylidene ring); ^{13}C-NMR ($CDCl_3$; ppm) 166.3 (C-1), 123.6 (C-2), 106.8 (C-3), 157.4 (C-4), 134.5 (C-5), 131.5 (C-6), 131.9 (C-7, 11), 132.3 (C-8, 10), 126.6 (C-9), 136.4 (C-12), 127.5 (C-13, 17), 126.1 (C-14, 16), 133.2 (C-15), 48.2 (C-18), 27.7 (C-19), 23.6 (C-20, 21);

Mass (*m/z*) 338 (M^+), 160, 133. Anal. Calcd. for $C_{21}H_{19}ClO_2$: C, 74.44; H, 5.65. Found: C, 74.38; H, 5.67%.

*3-(2-Nitrobenzylidene)-5-(4-isobutylphenyl)-2(3H)-furanone (**6**)* Brown solid, yield 68%, mp 109–110°C, R_f 0.80; IR (cm^{-1}): 1753, 1604, 830; ^{1}H-NMR (ppm): 0.86 (d, 6H, J = 6.5 Hz, 2 × CH_3), 1.85 (m, 1H, CH), 2.50 (d, 2H, J = 7.0 Hz, CH_2), 6.76 (s, 1H, furanone ring), 7.30 and 7.71 (d, each, J = 8.67 Hz, 2 × A_2B_2, phenyl), 7.64 (s, 1H, olefinic H), 7.86 (m, 3H, H-4, 5, 6, arylidene ring), 8.18 (d, 1H, J = 8.1 Hz, H-3, arylidene ring); ^{13}C-NMR ($CDCl_3$; ppm) 167.9 (C-1), 126.8 (C-2), 91.2 (C-3), 156.3 (C-4), 132.7 (C-5), 130.5 (C-6), 146.8 (C-7), 125.8 (C-8), 128.6 (C-9, 11), 131.8 (C-10), 135.9 (C-12), 127.3 (C-13, 17), 126.8 (C-14, 16), 133.6 (C-15), 48.5 (C-18), 27.2 (C-19), 22.9 (C-20, 21); Mass (*m/z*) 349 (M^+), 160, 133. Anal. Calcd. for $C_{21}H_{19}NO_4$: C, 72.19; H, 5.48; N, 4.01. Found: C, 72.33; H, 5.50; N, 4.03%.

*3-(3-Nitrobenzylidene)-5-(4-isobutylphenyl)-2(3H)-furanone (**7**)* Buff color solid, yield 68%, mp 139–140°C, R_f 0.78; IR (cm^{-1}): 1745, 1608, 827; ^{1}H-NMR (ppm): 0.87 (d, 6H, J = 6.5 Hz, 2 × CH_3), 1.88 (m, 1H, CH), 2.50 (d, 2H, J = 7.0 Hz, CH_2), 7.54 (s, 1H, furanone ring), 7.33 and 7.77 (d, each, J = 7.5 Hz, 2 × A_2B_2, phenyl), 7.60 (s, 1H, olefinic H), 7.77 (m, 1H, H-5, arylidene ring), 8.26 (d, 1H, J = 8.1 Hz, H-6, arylidene ring), 8.34 (d, 1H, J = 8.1 Hz, H-4, arylidene ring), 8.58 (s, 1H, H-2, arylidene ring); ^{13}C-NMR ($CDCl_3$; ppm) 167.4 (C-1), 124.1 (C-2), 108.1 (C-3), 157.8 (C-4), 132.7 (C-5), 133.5 (C-6), 124.8 (C-7), 165.8 (C-8), 122.6 (C-9), 128.4 (C-10), 126.7 (C-11), 136.7 (C-12), 126.8 (C-13, 17), 126.4 (C-14, 16), 133.2 (C-15), 47.3 (C-18), 27.8 (C-19), 23.5 (C-20, 21); Mass (*m/z*) 349 (M^+), 160, 133. Anal. Calcd. for $C_{21}H_{19}NO_4$: C, 72.19; H, 5.48; N, 4.01. Found: C, 72.35; H, 5.45; N, 4.02%.

*3-(4-Nitrobenzylidene)-5-(4-isobutylphenyl)-2(3H)-furanone (**8**)* Light yellow solid, yield 78%, mp 200–202°C, R_f 0.80; IR (cm^{-1}): 1755, 1611, 832; ^{1}H-NMR (ppm): 0.87 (d, 6H, J = 6.5 Hz, 2 × CH_3), 1.87 (m, 1H, CH), 2.50 (d, 2H, J = 7.0 Hz, CH_2), 7.35 and 8.12 (d, each, J = 7.8 Hz, 2 × A_2B_2, phenyl), 7.47 (s, 1H, furanone ring), 7.66 (s, 1H, olefinic H), 7.83 and 8.28 (d, each, J = 8.7 Hz, 2 × A_2B_2, arylidene ring); ^{13}C-NMR ($CDCl_3$; ppm) 166.7 (C-1), 125.2 (C-2), 107.8 (C-3), 156.1 (C-4), 134.6 (C-5), 135.2 (C-6), 128.1 (C-7, 11), 122.3 (C-8, 10), 162.6 (C-9), 136.4 (C-12), 127.3 (C-13, 17), 126.8 (C-14, 16), 132.5 (C-15), 48.2 (C-18), 28.3 (C-19), 22.9 (C-20, 21); Mass (*m/z*) 349 (M^+), 160, 133. Anal. Calcd. for $C_{21}H_{19}NO_4$: C, 72.19; H, 5.48; N, 4.01. Found: C, 72.28; H, 5.51; N, 4.03%.

*3-(4-Fluorobenzylidene)-5-(4-isobutylphenyl)-2(3H)-furanone (**9**)* Yellowish brown solid, yield 54%, mp 211–212°C, R_f 0.78; IR (cm^{-1}): 1755, 1600, 830; ^{1}H-NMR (ppm): 0.88 (d, 6H, J = 6.5 Hz, 2 × CH_3), 1.88 (m, 1H, CH), 2.50 (d, 2H, J = 7.0 Hz, CH_2), 6.72 (s, 1H, furanone ring), 7.25 and 7.52 (d, each, J = 8.7 Hz, 2 × A_2B_2, phenyl), 7.30 and 7.68 (d, each, J = 8.88 Hz, 2 × A_2B_2, arylidene ring), 7.38 (s, 1H, olefinic H); ^{13}C-NMR ($CDCl_3$; ppm) 166.1 (C-1), 123.9 (C-2), 107.4 (C-3), 157.6 (C-4), 135.5 (C-5), 131.8 (C-6), 128.6 (C-7, 11), 118.3 (C-8, 10), 164.6 (C-9), 135.9 (C-12), 126.2 (C-13, 17), 127.4 (C-14, 16), 134.1 (C-15), 47.6 (C-18), 27.8 (C-19), 23.5 (C-20, 21); Mass (*m/z*) 322 (M^+), 160, 133. Anal. Calcd. for $C_{21}H_{19}FO_2$: C, 78.24; H, 5.94. Found: C, 78.42; H, 5.90%.

*3-(4-Methoxybenzylidene)-5-(4-isobutylphenyl)-2(3H)-furanone (**10**)* Yellow shining crystals, yield 64%, mp 175–176°C, R_f 0.78; IR (cm^{-1}): 1760, 1607, 830; ^{1}H-NMR (ppm): 0.86 (d, 6H, J = 6.5 Hz, 2 × CH_3), 1.87 (m, 1H, CH), 2.48 (d, 2H, J = 7.0 Hz, CH_2), 3.82 (s, 3H, OCH_3), 6.76 (s, 1H, furanone ring), 6.98 and 7.58 (d, each, J = 8.7 Hz, 2 × A_2B_2, arylidene ring), 7.15 and 7.42 (d, each, J = 8.4 Hz, 2 × A_2B_2, phenyl), 7.36 (s, 1H, olefinic H); ^{13}C-NMR ($CDCl_3$; ppm) 167.8 (C-1), 122.7 (C-2), 108.1 (C-3), 156.5 (C-4), 134.9 (C-5), 125.7 (C-6), 130.6 (C-7, 11), 116.3 (C-8, 10), 162.8 (C-9), 136.4 (C-12), 126.3 (C-13, 17), 126.8 (C-14, 16), 133.2 (C-15), 48.3 (C-18), 27.9 (C-19), 24.2 (C-20, 21), 53.7 (C-22); Mass (*m/z*) 334 (M^+), 160, 133. Anal. Calcd. for $C_{22}H_{22}O_3$: C, 79.02; H, 6.63. Found: C, 79.18; H, 6.62%.

*3-(3,4-Dimethoxybenzylidene)-5-(4-isobutylphenyl)-2(3H)-furanone (**11**)* Yellow shining crystals, yield 60%, mp 169–170°C, R_f 0.72; IR (cm^{-1}): 1768, 1605, 835; ^{1}H-NMR (ppm): 0.89 (d, 6H, J = 6.5 Hz, 2 × CH_3), 1.90 (m, 1H, CH), 2.46 (d, 2H, J = 7.0 Hz, CH_2), 3.96 (s, 6H, 2 × OCH_3), 6.89 (s, 1H, furanone ring), 6.96 (d, 1H, J = 8.42 Hz, H-5, arylidene ring), 7.18 (s, 1H, H-2, arylidene ring), 7.28 (d, 1H, J = 8.42 Hz, H-6, arylidene ring), 7.39 (s, 1H, olefinic H), 7.42 and 7.74 (d, each, J = 8.6 Hz, 2 × A_2B_2, phenyl); ^{13}C-NMR ($CDCl_3$; ppm) 167.3 (C-1), 123.7 (C-2), 108.6 (C-3), 157.8 (C-4), 135.4 (C-5), 125.3 (C-6), 110.6 (C-7), 151.3 (C-8), 147.6 (C-9), 113.8 (C-10), 123.5 (C-11), 136.3 (C-12), 126.6 (C-13, 17), 126.8 (C-14, 16), 133.2 (C-15), 47.6 (C-18), 27.3 (C-19), 23.5 (C-20, 21), 55.8 (C-22, 23); Mass (*m/z*) 364 (M^+), 160, 133. Anal. Calcd. for $C_{23}H_{24}O_4$: C, 75.80; H, 6.64. Found: C, 75.62; H, 6.67%.

*3-(3,4,5-Trimethoxybenzylidene)-5-(4-isobutylphenyl)-2-(3H)-furanone (**12**)* Light yellow crystals, yield 60%, mp 161–162°C, R_f 0.68; IR (cm^{-1}): 1770, 1611, 835; ^{1}H-NMR (ppm): 0.87 (d, 6H, J = 6.5 Hz, 2 × CH_3), 1.88 (m, 1H, CH), 2.52 (d, 2H, J = 7.0 Hz, CH_2), 3.92 (s, 3H, OCH_3), 3.93 (s, 6H, 2 × OCH_3), 6.84 (s, 2H, H-2, 6, arylidene ring), 6.87 (s, 1H, furanone ring), 7.33 and 7.70 (d, each, J = 8.67 Hz, 2 × A_2B_2, phenyl), 7.38 (s, 1H, olefinic H); ^{13}C-NMR ($CDCl_3$; ppm) 166.7 (C-1), 124.7 (C-2), 107.9 (C-3), 157.2 (C-4), 137.1 (C-5), 129.7 (C-6), 108.6 (C-7, 11), 152.3 (C-8, 10), 156.9 (C-9), 108.3 (C-11), 136.1 (C-12), 125.6 (C-13, 17), 126.5 (C-14, 16), 132.4 (C-15), 48.2 (C-18), 27.5 (C-19), 23.6 (C-20, 21), 54.9 (C-22, 24), 58.7 (C-23); Mass (*m/z*) 394 (M^+), 160, 133. Anal. Calcd. for $C_{24}H_{26}O_5$: C, 73.08; H, 6.64. Found: C, 73.12; H, 6.63%.

*3-(2,6-Dichlorobenzylidene)-5-(4-isobutylphenyl)-2(3H)-furanone (**13**)* Brown solid, yield 56%, mp 181–182°C, R_f 0.74; IR (cm^{-1}): 1758, 1605, 833; ^{1}H-NMR (ppm): 0.86 (d, 6H, J = 6.5 Hz, 2 × CH_3), 1.86 (m, 1H, CH), 2.49 (d, 2H, J = 7.0 Hz, CH_2), 6.32 (s, 1H, furanone ring), 7.38 and 7.52 (d, each, J = 8.4 Hz, 2 × A_2B_2, phenyl), 7.46 (s, 1H, olefinic H), 7.56 (m, 3H, H-3, 4, 5, arylidene ring); ^{13}C-NMR ($CDCl_3$; ppm) 168.4 (C-1), 126.1 (C-2), 105.1 (C-3), 157.8 (C-4), 127.4 (C-5), 126.7 (C-6), 133.6 (C-7, 11), 129.3 (C-8, 10), 130.9 (C-9), 136.4 (C-12), 126.3 (C-13, 17), 126.8 (C-14, 16), 133.2 (C-15), 47.6 (C-18), 27.8 (C-19), 22.9 (C-20, 21); Mass (*m/z*) 373 (M^+), 160,

133. Anal. Calcd. for $C_{21}H_{18}Cl_2O_2$: C, 67.57; H, 4.86. Found: C, 67.43; H, 4.87%.

*3-(2,4-Dichlorobenzylidene)-5-(4-isobutylphenyl)-2(3H)-furanone (**14**)* Dark yellow solid, yield 54%, mp 191–192°C, R_f 0.64; IR (cm^{-1}): 1760, 1609, 828; ^{1}H-NMR (ppm): 0.87 (d, 6H, J = 6.5 Hz, 2 × CH_3), 1.88 (m, 1H, CH), 2.52 (d, 2H, J = 7.0 Hz, CH_2), 6.17 (s, 1H, furanone ring), 7.36 (s, 1H, olefinic H), 7.48–7.98 (m, 7H, H-2, 3, 5, 6, phenyl and H-3, 5, 6, arylidene ring); ^{13}C-NMR ($CDCl_3$; ppm) 167.9 (C-1), 125.3 (C-2), 106.2 (C-3), 157.2 (C-4), 129.1 (C-5), 130.7 (C-6), 132.8 (C-7), 129.5 (C-8), 133.9 (C-9), 126.3 (C-10), 129.6 (C-11), 135.8 (C-12), 126.3 (C-13, 17), 126.3 (C-14, 16), 134.1 (C-15), 47.8 (C-18), 26.6 (C-19), 23.1 (C-20, 21); Mass (*m/z*) 373 (M^+), 160, 133. Anal. Calcd. for $C_{21}H_{18}Cl_2O_2$: C, 67.57; H, 4.86. Found: C, 67.63; H, 4.85%.

*3-(4-Acetoxy-3-methoxybenzylidene)-5-(4-isobutylphenyl)-2(3H)-furanone (**15**)* Dark brown solid, yield 38%, mp 97–98°C, R_f 0.80; IR (cm^{-1}): 1762, 1602, 835; ^{1}H-NMR (ppm): 0.86 (d, 6H, J = 6.5 Hz, 2 × CH_3), 1.88 (m, 1H, CH), 2.29 (s, 3H, $OCOCH_3$), 2.50 (d, 2H, J = 7.0 Hz, CH_2), 3.88 (s, 3H, OCH_3), 6.92 (s, 1H, furanone ring), 7.22 (d, 1H, J = 8.1 Hz, H-5, arylidene ring), 7.31 and 7.81 (d, each, J = 7.8 Hz, 2 × A_2B_2, phenyl), 7.40 (s, 1H, H-2, arylidene ring), 7.52 (d, 1H, J = 8.1 Hz, H-6, arylidene ring), 7.57 (s, 1H, olefinic H); ^{13}C-NMR ($CDCl_3$; ppm) 167.6 (C-1), 124.7 (C-2), 108.1 (C-3), 157.8 (C-4), 137.4 (C-5), 129.7 (C-6), 109.7 (C-7), 151.3 (C-8), 140.9 (C-9), 122.1 (C-10), 123.2 (C-11), 136.4 (C-12), 125.7 (C-13, 17), 126.8 (C-14, 16), 133.2 (C-15), 48.2 (C-18), 27.8 (C-19), 23.5 (C-20, 21), 53.6 (C-22), 22.3 (C-23); Mass (*m/z*) 392 (M^+), 160, 133. Anal. Calcd. for $C_{24}H_{24}O_5$: C, 73.45; H, 6.16. Found: C, 73.53; H, 6.18%.

*3-(3,4-Methylenedioxybenzylidene)-5-(4-isobutylphenyl)-2-(3H)-furanone (**16**)* Yellow solid, yield 46%, mp 223–224°C, R_f 0.78; IR (cm^{-1}): 1764, 1608, 826; ^{1}H-NMR (ppm): 0.87 (d, 6H, J = 6.5 Hz, 2 × CH_3), 1.89 (m, 1H, CH), 2.54 (d, 2H, J = 7.0 Hz, CH_2), 5.97 (s, 2H, -OCH_2O-), 6.76 (s, 1H, furanone ring), 6.84 (s, 1H, H-5, arylidene ring), 7.08 (m, 2H, H-2, 6, arylidene ring), 7.16 (s, 1H, olefinic H), 7.23 and 7.61 (d, each, J = 7.91 Hz, 2 × A_2B_2, phenyl); ^{13}C-NMR ($CDCl_3$; ppm) 167.4 (C-1), 126.1 (C-2), 107.8 (C-3), 157.2 (C-4), 136.9 (C-5), 127.3 (C-6), 108.5 (C-7), 148.3 (C-8, 9), 108.1 (C-10), 125.2 (C-11), 136.9 (C-12), 126.3 (C-13, 17), 127.1 (C-14, 16), 133.6 (C-15), 48.7 (C-18), 27.5 (C-19), 24.3 (C-20, 21), 101.6 (C-22); Mass (*m/z*) 348 (M^+), 160, 133. Anal. Calcd. for $C_{22}H_{20}O_4$: C, 75.84; H, 5.79. Found: C, 75.72; H, 5.80%.

*3-(4-Hydroxybenzylidene)-5-(4-isobutylphenyl)-2(3H)-furanone (**17**)* Pale yellow solid, yield 52%, mp 125–126°C, R_f 0.82; IR (cm^{-1}): 3450, 1768, 1612, 832; ^{1}H-NMR (ppm): 0.88 (d, 6H, J = 6.5 Hz, 2 × CH_3), 1.90 (m, 1H, CH), 2.51 (d, 2H, J = 7.0 Hz, CH_2), 6.80 (s, 1H, furanone ring), 7.20 (m, 4H, H-2, 3, 5, 6, arylidene ring), 7.32 (s, 1H, olefinic H), 7.46 and 7.60 (d, each, J = 8.1 Hz, 2 × A_2B_2, phenyl), 10.38 (s, 1H, OH); ^{13}C-NMR ($CDCl_3$; ppm) 166.9 (C-1), 125.1 (C-2), 108.2 (C-3), 157.6 (C-4), 135.4 (C-5), 124.7 (C-6), 138.6 (C-7, 11), 116.3 (C-8, 10), 161.6 (C-9), 135.9 (C-12), 126.7 (C-13, 17), 126.8 (C-14, 16), 133.8 (C-15), 48.5 (C-18), 26.7 (C-19), 23.3 (C-20, 21); Mass (*m/z*) 320 (M^+), 160, 133. Anal. Calcd. for $C_{21}H_{20}O_3$: C, 78.73; H, 6.29. Found: C, 78.86; H, 6.28%.

*3-(2-Methylbenzylidene)-5-(4-isobutylphenyl)-2(3H)-furanone (**18**)* Yellow solid, yield 46%, mp 119–120°C, R_f 0.76; IR (cm^{-1}): 1768, 1610, 836; ^{1}H-NMR (ppm): 0.86 (d, 6H, J = 6.5 Hz, 2 × CH_3), 1.87 (m, 1H, CH), 2.32 (s, 3H, CH_3), 2.48 (d, 2H, J = 7.0 Hz, CH_2), 6.80 (s, 1H, furanone ring), 7.19 (m, 4H, H-3, 4, 5, 6, arylidene ring), 7.31 (s, 1H, olefinic H), 7.46 and 7.81 (d, each, J = 7.6 Hz, 2 × A_2B_2, phenyl); ^{13}C-NMR ($CDCl_3$; ppm) 167.7 (C-1), 124.3 (C-2), 104.6 (C-3), 157.8 (C-4), 135.2 (C-5), 130.5 (C-6), 136.3 (C-7), 129.2 (C-8, 10), 127.6 (C-9), 128.1 (C-11), 137.4 (C-12), 126.2 (C-13, 17), 127.1 (C-14, 16), 133.2 (C-15), 48.3 (C-18), 27.2 (C-19), 23.5 (C-20, 21), 21.8 (C-22); Mass (*m/z*) 318 (M^+), 160, 133. Anal. Calcd. for $C_{22}H_{22}O_2$: C, 82.99; H, 6.96. Found: C, 82.90; H, 6.97%.

Animals

Wistar rats and albino mice used in the present study were housed and kept in the Hamdard University Animal Care Unit (which follows the guidelines and rules laid down by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India). Animals of either sex, weighing and aged 180–200 g/12 weeks (rats) and 22–25 g/8 weeks (mice), were used. Before the experiment, the animals were housed in groups of six and acclimatized to room conditions for at least 2 days. Food and water were freely available. The food was withdrawn on the day before the experiment, but free access to water was allowed.

Anti-inflammatory activity

The synthesized compounds were evaluated for their anti-inflammatory activity using the carrageenan-induced paw edema method of Winter *et al.*[20]. The animals were randomly divided into groups of six. Group I was kept as control, and received only 0.5% carboxymethyl cellulose (CMC) solution. Group II was kept as standard and received ibuprofen (20 mg kg^{-1} p.o.). Carrageenan solution (0.1% in sterile 0.9% NaCl solution) in a volume of 0.1 mL was injected subcutaneously into the sub-plantar region of the right hind paw of each rat, 30 min after administration of the test compound (20 mg kg^{-1} p.o.) and standard drug. The paw volume was measured by saline displacement shown on the screen of a digital plethysmometer (Ugo Basile) at 2 and 3 h after carrageenan injection. The paw volume in the control group (V_c) and paw volume in groups treated with test compounds (V_t) were measured, and the percentage inhibition of edema was calculated using the formula:

$$\text{Anti-inflammatory activity (\% inhibition)} = [(V_c - V_t)/V_c] \times 100$$

Analgesic activity

Compounds which showed anti-inflammatory activity above 75% of ibuprofen inhibition were screened for analgesic activity. Analgesic activity was determined by the acetic acid-induced writhing method[21]. Mice were divided into groups of six. Group I was taken as control and received

CMC suspension only, and group II received the reference drug ibuprofen (20 mg kg^{-1} p.o.). The remaining groups were given the test drugs (20 mg kg^{-1}) suspended in 1.0% CMC orally. A 1% aqueous acetic acid solution (0.1 mL) was used as the writhing inducing agent. Acetic acid solution was injected intraperitoneally 3 h after treatment with the reference and test drugs to the various groups respectively and writhings were noted for 10–15 min after acetic acid administration:

$$\text{Analgesic activity (\% protection)} = [(n - n')/n] \times 100$$

where n is the mean number of writhes of the control group and n' is the mean number of writhes of the test group.

Acute ulcerogenesis

The acute ulcerogenesis test was done according to the method of Cioli *et al.*[22]. Wistar rats were divided into groups of six. Ulcerogenic activity was evaluated after oral (p.o.) administration of test compounds or ibuprofen at the dose of 60 mg kg^{-1}. Control rats received p.o. administration of vehicle (suspension of 1% CMC). Food but not water was removed 24 h before administration of the test compounds. After the drug treatment the rats were fed with a normal diet for 17 h and then sacrificed. The stomach was removed and opened along the greater curvature, washed with distilled water, and cleaned gently by dipping in normal saline. The mucosal damage was examined by means of a magnifying glass and compared with ibuprofen. For each stomach the mucosal damage was assessed according to the following scoring system: 0.5: redness, 1.0: spot ulcers, 1.5: hemorrhagic streaks, 2.0: ulcers >3 but <5, 3.0: ulcers >5.

The mean score of each treated group minus the mean score of the control group was regarded as the severity index of gastric mucosal damage.

Lipid peroxidation

Lipid peroxidation (LPO) in the gastric mucosa was determined according to the method of Ohkawa *et al.*[23]. After screening for ulcerogenic activity, the gastric mucosa was scraped with two glass slides and 10% of that tissue was homogenized at 10,000 rpm in 1.8 mL of 1.15% ice-cold KCl solution. Then, 1 mL of suspension medium was taken from the supernatant, and 0.5 mL of 30% trichloroacetic acid (TCA) followed by 0.5 mL of 0.8% thiobarbituric acid (TBA) was added to it. The tubes were covered with aluminum foil and kept in a shaking water bath for 30 min at 80°C. After 30 min, the tubes were taken out and kept in ice-cold water for 10 min; these were then centrifuged at 3000 rpm for 15 min. The absorbance of the supernatant was read at 540 nm at room temperature against the blank on an ultraviolet (UV) spectrophotometer.

The standard curve was used for estimating the concentration of malondialdehyde (MDA) prepared by using 1,1,3,3-tetraethoxypropane. The results are presented as nmol of MDA/mg of protein.

In vitro cyclo-oxygenase inhibition assays

Inhibition of the enzymes was determined using an enzyme immunoassay (EIA) kit (catalog no. 560101, Cayman Chemical, Ann Arbor, MI, USA) according to the methodology described by Jashim Uddin *et al.*[24]. The test compounds having good anti-inflammatory and analgesic activity were further evaluated for their ability to inhibit COX-1 and COX-2 enzymes.

Antimicrobial activity

The newly prepared compounds were screened for their antibacterial activity against *Escherichia coli* (ATCC-8739) and *Staphylococcus aureus* (ATCC-29737) bacterial strains at a concentration of 100 μg/mL by the turbidity method[25], using norfloxacin as standard. The antifungal activity of the compounds was also determined by the same method against *Penicillium citrum* and *Rhizopus oryza*, using fluconazole as standard. Compounds inhibiting growth of one or more of the above microorganisms were further tested for their minimum inhibitory concentration (MIC).

Statistical analysis

Data are expressed as mean ± standard error (SE) of the mean. For statistical analysis of the data, group means were compared by one-way analysis of variance (ANOVA) with *post hoc* analysis. The Tukey–Kramer test was applied *post hoc* to identify significance among groups; $p < 0.05$ was considered to be statistically significant.

Results and discussion

Chemistry

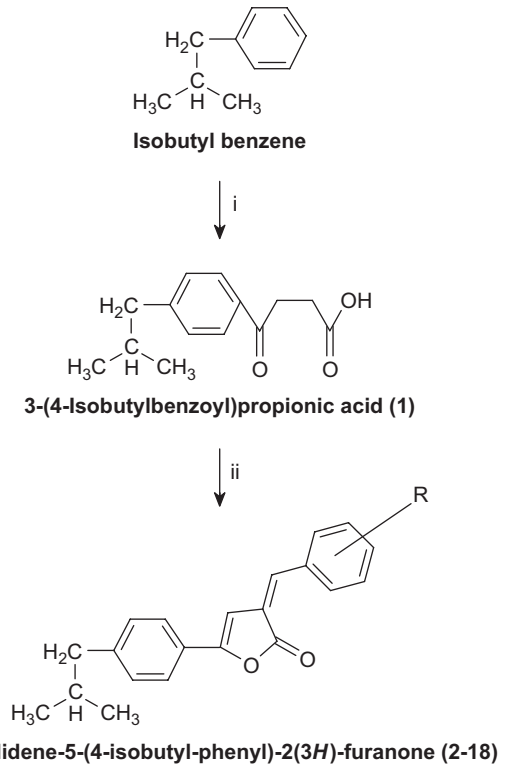
Eighteen compounds (**1–18**) were synthesized as outlined in Scheme 1. The required 3-(4-isobutylbenzoyl)propionic acid **1** was prepared by reacting isobutylbenzene with succinic anhydride in presence of anhydrous aluminum chloride followed by Friedel–Crafts acylation reaction conditions.

3-Arylidene-5-(4-isobutylphenyl)-2(3*H*)-furanones (**2–18**) were synthesized by condensing different aromatic aldehydes with **1** in the presence of triethylamine and acetic anhydride under anhydrous conditions following a modified Perkin reaction. Calculations of δ-values using incremental parameters for the hydrogen (semi-cyclic double bond) suggest (*E*)-configuration.

In the ^{1}H-NMR spectral data all the compounds showed two singlets of one proton each around δ 6.7 and δ 7.4, which could be assigned to the ring βH and the olefinic hydrogen of the arylidene substituents. Other peaks were observed at appropriate δ-values. The fragmentation pattern observed on the electron impact mass spectrum can be summarized as follows.

The 3-arylidene-5-(4-isobutylphenyl)-2(3*H*)-furanones gave an M^+ peak at reasonable intensity. The major fragment appears to be C_4H_9–C_6H_4–$C{\equiv}O^+$ arising from the heterocyclic oxygen and γ-carbon with its substituent; subsequently

it loses CO to give C_4H_9–$C_6H_4^+$. Occasionally, the aryl ring of the arylidene moiety also appeared as Ar^+. The molecular ion or other related ions produced the appropriate isotopic abundances due to the presence of chlorine atom(s).



Scheme 1. Synthesis of compounds **1–18**. (i) Anhydrous $AlCl_3$, succinic anhydride; (ii) aryl aldehyde, Ac_2O, triethylamine, fusion (30 min).

Anti-inflammatory activity

The results of *in vivo* anti-inflammatory activity of the synthesized compounds (**2–18)** are tabulated in Table 1. Among the compounds tested for anti-inflammatory activity, 3-(2,6-dichlorobenzylidene)-5-(4-isobutylphenyl)-2(3*H*)-furanone **13**, 3-(2,4-dichlorobenzylidene)-5-(4-isobutylphenyl)-2(3*H*)-furanone **14**, 3-(2-chlorobenzylidene)-5-(4-isobutylphenyl)-2-(3*H*)-furanone **3**, and 3-(4-fluorobenzylidene)-5-(4-isobutylphenyl)-2(3*H*)-furanone **9** showed 80.98%, 71.67%, 68.37%, and 66.13% inhibition of edema, respectively. The results indicate that the anti-inflammatory activity increases with an increase in electronegativity on the arylidene moiety.

The test compounds (**3**, **9**, **13**, and **14**) that exhibited approximately or above 75% anti-inflammatory activity in comparison with ibuprofen were further evaluated for their analgesic, ulcerogenic, and LPO effects.

Analgesic activity

The results of analgesic activity (Table 1) indicate that compounds **13** and **14** showed 59.03% and 57.83% protection against acetic acid-induced writhings, comparable to that of the standard ibuprofen (65.06%). Compounds **3** and **9** also showed good analgesic activity.

Ulcerogenic activity

The compounds that were screened for analgesic activity were further tested for their ulcerogenic activity. A severity index of 0.2 ± 0.12 and 0.1 ± 0.10 was observed with compounds **13** and **14**, respectively, much less than for the standard ibuprofen (0.9 ± 0.36). The results indicate that compounds

Table 1. Anti-inflammatory and analgesic activity along with ulcerogenic and lipid peroxidation effect of the synthesized compounds **2–18**.

	% Inhibition ± SEM[a]				Analgesic activity (writhing test)[b]	
Compound	After 2 h	After 3 h	Severity index[a]	Lipid peroxidation[†]	No. of writhes/30 min	% Protection
Control	—	—	0.00 ± 0.00	0.238 ± 0.002[b,***]	83 ± 1.317	—
Ibuprofen	80.88 ± 2.02	89.50 ± 2.56	0.9 ± 0.36*	0.608 ± 0.001[a,***]	29 ± 1.154***	65.06
2	4.12 ± 1.36***	19.54 ± 2.28***	0.6 ± 0.36	0.422 ± 0.001[ab,***]	—	—
3	39.33 ± 3.03***	68.37 ± 1.57***	0.4 ± 0.10	0.321 ± 0.001[ab,***]	39 ± 1.897***	53.01
4	28.48 ± 3.24***	56.49 ± 4.42***	0.2 ± 0.12	0.478 ± 0.001[ab,***]	—	—
5	24 ± 3.81***	37.03 ± 2.20***	0.3 ± 0.12*	0.587 ± 0.001[ab,**]	—	—
6	12 ± 3.07***	19.75 ± 4.54***	0.4 ± 0.10*	0.409 ± 0.002[ab,***]	—	—
7	8.52 ± 2.52***	25.00 ± 1.62***	0.4 ± 0.40	0.532 ± 0.001[ab,***]	—	—
8	4 ± 0.76***	31.60 ± 2.78***	0.5 ± 0.27	0.548 ± 0.001[ab,***]	—	—
9	36 ± 3.13***	66.13 ± 1.65***	0.2 ± 0.12	0.401 ± 0.002[ab,***]	41 ± 1.932***	50.60
10	4 ± 0.76***	32.36 ± 2.76***	0.5 ± 0.27	0.546 ± 0.001[ab,***]	—	—
11	18.82 ± 3.92***	34.00 ± 3.13***	0.9 ± 0.36*	0.526 ± 0.002[ab,***]	—	—
12	4 ± 0.91***	34.00 ± 1.36***	0.4 ± 0.40	0.548 ± 0.001[ab,***]	—	—
13	58.66 ± 1.50***	80.98 ± 0.63*	0.2 ± 0.12	0.489 ± 0.001[ab,***]	34 ± 1.290***	59.03
14	51.22 ± 2.34***	71.67 ± 2.18***	0.1 ± 0.10	0.382 ± 0.002[ab,***]	35 ± 1.238***	57.83
15	9.33 ± 2.54***	35.00 ± 1.68***	0.3 ± 0.12*	0.535 ± 0.001[ab,***]	—	—
16	32.22 ± 5.04***	51.02 ± 4.10***	0.4 ± 0.4	0.525 ± 0.017[ab,***]	—	—
17	33.33 ± 3.15***	49.38 ± 2.68***	0.1 ± 0.1	0.352 ± 0.014[ab,***]	—	—
18	29.77 ± 3.34***	38.68 ± 5.85***	0.4 ± 0.29	0.403 ± 0.005[ab,***]	—	—

Note. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. [†]Lipid peroxidation activity is expressed as nmol of MDA/mg of protein.
[a]Relative to the standard (ibuprofen) and data were analyzed by one-way ANOVA followed by Tukey test for $n = 6$.
[b]Relative to their respective control and data were analyzed by one-way ANOVA followed by Tukey test for $n = 6$.

were less toxic in terms of ulcerogenicity as compared to the standard (Table 1), also supported by the LPO studies.

Lipid peroxidation activity

It has been reported that compounds showing less ulcerogenic activity also show a reduced MDA content (Pohle et al). Therefore, an attempt was made to correlate the decrease in ulcerogenic activity of the compounds with that of LPO. Ibuprofen exhibited higher LPO (0.608 ± 0.001 nmol of MDA/mg of protein) in comparison to the control group (0.238 ± 0.002 nmol of MDA/mg of protein). It was found that all the furanone derivatives showed less ulcerogenic activity along with reduced LPO (Table 1).

In vitro cyclo-oxygenase inhibition assay

The test compounds (**3**, **9**, **13**, and **14**) that exhibited approximately or above 75% of anti-inflammatory activity in comparison with ibuprofen were further evaluated for identification of their biological targets. *In vitro* COX-1 and COX-2 enzyme inhibition data showed that the compounds tested were more selective toward COX-2 than COX-1, suggesting that the furanone ring helps the compounds in the orientation that favors COX-2 blocking (Table 2).

Table 2. *In vitro* COX inhibition data for compounds **3**, **9**, **13** and **14**.

Compound	COX-1 (IC_{50}, μM)[a]	COX-2 (IC_{50}, μM)[a]	COX-2 SI[b]
3	52.4	2.4	21.84
9	68.3	3.8	17.97
13	47.6	1.8	26.44
14	48.2	2.2	21.90
Celecoxib[24]	33.1	0.07	472
Rofecoxib[24]	>100	0.50	>200
Ibuprofen[27]	6.9	6.2	1.1

[a]Values are means of two determinations acquired using an ovine COX-1/COX-2 assay kit (catalog no. 560101, Cayman Chemical, Ann Arbor, MI, USA) and the deviation from the mean is <10% of the mean value.
[b]*In vitro* COX-2 selectivity index (COX-1/COX-2 IC_{50}).

Table 3. Antibacterial and antifungal activity: MIC (μg/mL) results for 2(3*H*)-furanones.

Compound	*Staphylococcus aureus*	*Escherichia coli*	*Rhizopus oryza*	*Penicillium citrum*
Norfloxacin	6.25	6.25	—	—
Fluconazole	—	—	6.25	6.25
2	>100	50	>100	>100
3	6.25	6.25	6.25	6.25
5	25	25	12.5	50
6	12.5	25	25	25
8	25	12.5	12.5	25
9	50	50	12.5	12.5
10	25	50	50	>100
11	12.5	25	50	50
12	12.5	12.5	50	50
13	6.25	6.25	6.25	6.25
14	6.25	6.25	6.25	6.25
15	50	>100	25	25
16	50	50	50	25
17	>100	>100	25	50
18	>100	>100	50	25

Antimicrobial activity

All the compounds tested for antimicrobial activity showed inhibition of growth. Compounds **3**, **13**, and **14** were most active against both bacterial and fungal strains with an MIC of 6.25 μg/mL. However, compound **9** was active only against fungal strains and compound **12** was active only against bacterial strains with an MIC of 12.5 μg/mL (Table 3).

These findings indicate that compounds **3**, **13**, and **14** having a chloro substitution at the 2-position and a disubstituted chloro group at the 2,6- and 2,4-position of the arylidene moiety are good antimicrobial agents. Compounds **13** and **14** have an added advantage of anti-inflammatory action, with a high analgesic effect equivalent to that of ibuprofen (standard).

Structure–activity relationship

1. The furanone ring is more specific toward COX-2 inhibition.
2. The presence of electron-withdrawing group(s) on the arylidene moiety of the furanone ring shows enhanced anti-inflammatory activity.
3. Anti-inflammatory activity increases with an increase in the number of electron-withdrawing groups on the arylidene moiety.
4. The presence of electron-withdrawing groups on the phenyl ring of the furanone ring shows better anti-inflammatory activity as compared to the isobutyl group.
5. The presence of electron-withdrawing groups also gives a lower ulcerogenic effect and LPO.
6. Antimicrobial activity increases with an increase in electronegativity.
7. An increase in the number of methoxyl groups also increases specificity toward the antibacterial effect.

Conclusions

Eighteen compounds were successfully synthesized. Biological evaluation showed that the compounds are promising anti-inflammatory and analgesic agents with low GI toxicity as indicated by the ulcerogenic effect and lipid peroxidation. Compounds containing halogen group(s) were more active as anti-inflammatory agents. *In vitro* COX-1 and COX-2 isozyme inhibition studies were also performed. Compounds were found to be more selective toward COX-2 as indicated by the COX-2 selectivity index. The furanone derivatives discovered in this study may provide valuable therapeutic intervention in monotherapy, having both anti-inflammatory and antimicrobial activities.

Among the compounds synthesized and tested, two compounds, **13** and **14**, emerged as lead compounds. It is conceivable that these derivatives could be further modified to develop potent and safer anti-inflammatory, antimicrobial, and analgesic agents.

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