International Journal of Pharmaceutical Chemistry
ISSN: 2249-734X (Online)
Journal DOI: 10.7439/ijpc
CODEN: IJPCH3 (American Chemical Society)
Research Article

Synthesis, characterization, anti-inflammatory and in vitro antibacterial activity of some (±)-1-Aryl-6,7-dichloroisochromans

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Abstract

The present work reports the synthesis of some novel isochromans as antibacterial which additionally possess anti-inflammatory potential to find a therapeutic agent which can be employed to cure bacterial infection as well as the inflammation caused by bacterial infection. Synthesis of some 1-aryl-6,7-dichloroisochromans **(3a-g)** was carried out by condensation of 2-(3,4-dimethoxyphenyl)ethanol with a variety of aromatic aldehydes via an acid catalyzed oxa-Pictet-Spengler reaction under microwave irradiation. The structures of the synthesized compounds were assigned on the basis of FT-IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopic data. The isochromans **(3a-g)** were screened for antibacterial activity against ten different bacterial strains using three Gram-positive and seven Gram-negative bacteria. The isochromans **(3a-g)** were found to exhibit good to excellent antimicrobial activity compared to levofloxacin used as positive control. Antibacterial activity results shows that most of these isochromans are more active against gram negative bacteria as compared to gram positive bacteria. The *in vivo* anti-inflammatory activity of the synthesized compounds was also evaluated using the carrageenan-induced hind paw edema method and was compared with that of ibuprofen. The newly synthesized isochromans **(3c)**, **(3e)** and **(3f)** exhibited promising anti-inflammatory activity.

Keywords: isochromans; microwave-accelerated; oxa-Pictet-Spengler; *In vitro* antibacterial activity; In vivo Antiinflammatory activity

1. Introduction

Isochroman (3,4-dihydro-1*H*-benzo[*c*]pyran) is a common oxa-heterocycle structural motif in a variety of bioactive natural products such as 1,6,8-trihydroxy-3-heptyl-7-carboxyisochroman [1,2], an antibiotic and topoisomerase II inhibitor from the *Penicillum* sp., pseudodeflectusin [3], a selective human cancer cytotoxin from *Aspergillus pseudodeflectus*, isochromans from softwood lignin [4], and the male wing gland pheromone of bumble-bee wax moth, *Aphomia sociella*[5]. Hydroxy-1-aryl-isochromans such as 1-phenyl-6,7-dihydroxyisochroman and 1-(3-methoxy-4-hydroxy)phenyl-6,7-dihydroxyisochroman have been identified in extra-virgin olive oil [6]. These natural isochromans or their synthetic derivatives have been shown to exhibit beneficial antioxidant effects [7]. The antiplatelet activity and antioxidant power of these isochromans were also evaluated, and were found to be effective free radical scavengers and inhibited platelet aggregation and thromboxane release evoked by agonists [8]. 3,7-Dimethoxy-8-hydroxy-6-methoxyisochroman isolated from *Penicillium corylophilium* and its synthetic analogues exhibit plant growth regulatory and herbicidal activity [9].

Various synthetic isochromans have been shown to act as estrogen receptor ligands [10-11], dopamine receptor ligands [12], and as fragrances, such as the commercial musk odorant galaxolide [13]. Simple 1-substitued isochromans have been shown to exhibit a wide variety of physiological activities such as antihistaminic, anticholinergic, diuretic, sympathomimetic, and antihypertensive [14]. In the last century drug discovery has greatly been based on the idea of "one molecule–one target–one disease" but now a days there has been an increasing recognition that molecules that modulate multiple targets simultaneously can be beneficial for treating several diseases [15]. The inflammatory mediator's prostaglandins and thromboxanes, are generated by oxidation of the

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arachidonic acid through cyclooxygenase pathway. The arachidonic acid oxygenation products mediate diverse effects that induce acute inflammation caused by bacterial infection [16].

The present work designed to synthesize antibacterial compounds which possess anti-inflammatory potential additionally. In this way a single compound can be employed to cure bacterial infection as well as the inflammation caused by bacterial infection. The fused phenyl ring of isochroman scaffold possesses halogens (chloro) groups to evaluate their role in antibacterial as well as anti-inflammatory activity. Synthesis of title compounds was carried out by oxa-Pictet–Spengler reaction, a variation of the Pictet–Spengler reaction in which a phenethyl alcohol reacts with a carbonyl compound to give a 1-substitued isochroman derivative. Typically, aqueous HCl, zinc chloride-HCl gas, *p*-toluenesulfonic acid, titanium tetrachloride or stannic chloride have been used as Friedel–Crafts catalysts alongwith high reaction temperatures [17]. The activated substrates such as 2-(3,4-dihydroxy)phenylethanol undergo the oxa-Pictet–Spengler reaction under mild conditions [18]. However, the reaction time required to obtain the satisfactory yields varies from 1 day for aldehydes to 2 days to *ca* 1 week for ketones nonetheless the yields were lower. Eco friendly syntheses without organic solvents using microwave irradiation yielded reduce reaction times, increase product purity and yields.

2. Experimental

Melting points were recorded using a MEL TEMP MP-D apparatus and are uncorrected. ^{1}H NMR spectra were recorded at 300 MHz using a Bruker AM-300 machine. FTIR spectra were recorded on an FTS 3000 MX spectrophotometer. Mass Spectra (EI, 70eV) on a MAT 312 instrument, and elemental analyses were conducted using a LECO-183 CHNS analyzer. The reaction was carried out in an unmodified domestic microwave oven (MW 900 W, frequency 2450 MHz, Power level 1, Dawlance, Pakistan). 2-(3,4-Dichlorophenyl)ethanol and aldehydes were the commercial products from Aldrich or Fluka. The purity of the compounds was checked on silica gel coated Al plates (Merck).

2.1 General procedure for the synthesis of (±)-1-Aryl-6,7-Dichloro-3,4-dihydro-1*H*-isochromenes (3a-g)

To a mixture of 2-(3,4-dichlorophenyl)ethanol **(1)** (0.182 g, 1 mmol) and substituted benzaldehydes **(2a-g)** (1 mmol), a catalytic amount of *p*-toluenesulfonic acid monohydrate was added. The reaction mixture was homogenized and irradiated for 2–3 min at room temperature. On completion of reaction, as monitored by TLC (every 30 s) and product was purified by thick layer chromatography using petroleum ether and ethyl acetate (7:2) as eluent and silica gel HF-254 as stationary phase. The product obtained was recrystallized from ethyl acetate.

2.3 Antibacterial Activity

Antibacterial activity of the synthesized isochromanes **(3a-g)** was determined against various gram positive and gram negative bacterial strains by using agar well diffusion method [19]. The purified samples were dissolved in DMSO 5mg/ml. DMSO is the negative control and antibiotic levofloxacin is the positive control in this *in vitro* antibacterial study.

Ten bacterial strains *Escherichia coli* (Gram -) (ATCC 25922), *Klebsiella pneumonae* (Gram -) (ATCC 6633), *Lactobacillus bulgaricus* (Gram -) (ATCC 25929), *Micrococcus luteus* (Gram +) (ATTC 9341), *Pasteurella multocida* (Gram -) (ATCC 9150), *Proteus vulgaris* (Gram -) (ATCC 49565), *Pseudomonas aeruginosa* (Gram -) (ATCC 33347), *Salmonella typhi* (Gram -) (ATTC 19430), *Staphylococcus aureus* (Gram +) (ATCC 29213) and *Staphylococcus epidermidis* (Gram +) (ATCC 29232) were selected in this antibacterial assay. All of the tested microorganisms were maintained on nutrient agar at 4°C and sub-cultured before use. The bacteria studied are clinically important ones causing several infections and it is essential to overcome them through some active therapeutic agents.

The antibacterial assay was performed by agar well diffusion method against different bacterial strains. Each tested bacterium was sub-cultured in nutrient broth at 37°C for 24h. One hundred micro liters of each bacterium was spread with the help of sterile spreader on to a sterile Muller-Hinton agar plate so as to achieve a confluent growth. The plates were allowed to dry and wells (6mm diameter) were punched in the agar with the help of cork borer. 0.1mL of the each compound solution (5mg/mL) in DMSO was introduced in to the well and the plates were incubated overnight at 37°C.

The antimicrobial spectrum of the compounds was determined for the bacterial species in terms of diameter of the zones around each well. The diameters of the zone of inhibition produced by the compounds were compared with those produced by the commercial antibiotic levofloxacin (5mg/mL). This is the common antibiotic used for

the treatment of infections caused by gram positive and gram negative bacteria. The control activity was deducted from the test and the results obtained were plotted. The experiment was performed three times to minimize the error and the mean values are presented in Table 1.

3. Pharmacology

3.1 Animals

Adult healthy mice of either sex having weight of 20-30g were used in this study. These mice were placed in polypropylene cages in animal house of Faculty of Pharmacy, University of Sargodha. Humidity and temperature of animal house were maintained. Animals were allowed to free access of water and were given palatable clean food. Dark and light cycle of 12/12 hours was preserved. The animals were treated according to guidelines of National Institute of Health (NIH). The study protocols ware approved from the local ethical committee of the University of Sargodha.

3.2 Subcutaneous administration

The skin of the animals between the shoulder blades was lifted up and a triangle was formed. The needle of the syringe was then introduced in the folds of the skin into the base of the triangle and drug was advanced into subcutaneous tissue. The drug was ejected by pressing the plunger of the syringe.

3.3. Antiinflammatory activity

The newly synthesized compounds were evaluated for their *in vivo* antiinflammatory activity using the carrageenan-induced hind paw edema method [20]. Adult Sprague-Dawley rats, weighing 150–200 g, were used. The animals were allowed food and water *ad libitum*, except during the experiment. They were housed in a room at 23 ± 2 °C with a 12 h light/dark cycle. The animals were randomly allocated into groups of six animals each at the beginning of the experiment and were fasted for 24 h before the experiment with free access to water. All of the compounds and the reference drug were suspended in 0.5% carboxymethyl cellulose (CMC) solution. The standard drug ibuprofen was administered orally at a dose of 20 mg/kg.

The tested compounds were administered orally at an equimolar oral dose relative to 20 mg/kg of ibuprofen. The control group received a 0.5% CMC solution. Into the subplantar region of the right hind paw of each rat, 0.1 mL of 1% carrageenan solution in saline was injected subcutaneously, 1 h after the administration of the test compounds and standard drug. The right hind paw volume was measured after 3 h of carrageenan treatment by means of a plethysmometer. The percent edema inhibition was calculated from the mean effect in the control and treated animals according to the following equation:

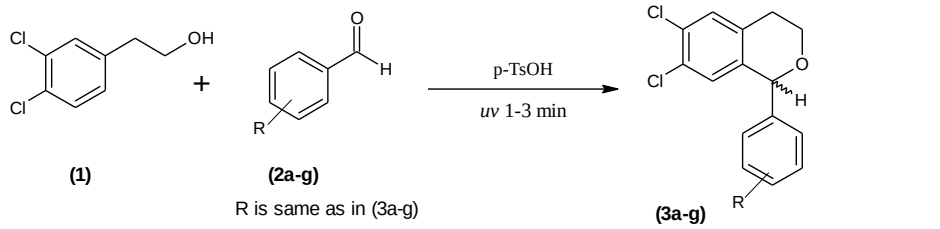
$$\text{Percent edema inhibition} = (v_c - v_t / v_c) \times 100$$

Where v_t represents the mean increase in paw volume in rats treated with tested compounds and v_c represents the mean increase in paw volume in the control group of rats. The potency was calculated as regards the percentage of the change of the standard and tested compounds, as depict in the Table 2.

4. Results and discussion

4.1 Chemistry

Synthesis of 1-substitued isochromans by an acid catalyzed oxa-Pictet Spengler reaction is normally carried out in methanol at reflux temperature. The reaction time varies from 1-day to several days and despite this the reaction is not complete in some cases [21]. Commercial 2-(3,4-dichlorophenyl)acetic acid was reduced using sodium boron hydride in THF/methanol to afford 2-(3,4-dichlorophenyl)ethanol [22]. Suitably substituted benzaldehydes were condensed with 2-(3,4-dimethoxyphenyl)ethanol in presence of a catalytic amount of *p*-toluenesulfonic acid by microwave irradiation (Scheme **1**). The homogenized reaction mixture was irradiated at frequency 2450MHz, wavelength 12cm and the progress of reaction was monitored by TLC every 30 s to establish the minimum time required to complete the reaction. Thus isochromans **3a-g** was obtained during *ca* 1-3 min. in good to high yields. The isochromans were characterized by the C_1-H singlet at δ5.58-6.69 in NMR. The non-planar nature of tetrahydropyran ring was indicated by separate 2H multiplets at δ 3.98 and 3.74 and at 2.56 and 2.94 for C-3 and C-4 methylene protons respectively.



3a: R = H
3b: R = 2-Cl
3c: R = 4-Cl
3d: R = 3-OCH_3
3e: R = 3-OCH_3, 4-OH
3f: R = 3,4,5-$(OCH_3)_3$
3g: R = 5-NO_2-3,4-CH_2O_2

Scheme I Synthesis of Isochromans

6,7-Dichloro-1-phenyl-3,4-dihydro-1*H*-isochromene (3a)

Yield 77%; m. p. 68 °C; R_f 0.75; IR (KBr): 1257 (C-O), 1613 (C=C), 2941 (C-H), 3067 (Ar-H) cm^{-1}. ^1H NMR ($CDCl_3$, δ ppm) 7.99 (1H, s, H-8), 7.61 (1H, s, H-5), 7.3-7.4 (5H, m, H-2'-H-6'), 5.67 (1H, s, H-1), 4.52 (1H, td, *J*=4.6, 5.2, H-3), 4.22 (1H, td, *J*=3.8, 3.2, H-3), 3.05 (1H, td, *J*=4.6, 4.9, H-4), 2.95 (1H, td, *J*=4.1, 4.5, H-4). ^{13}C NMR ($CDCl_3$, δ ppm) 144.2 (C-8a), 140.3 (C-1'), 136.0 (C-4a), 131.1 (C-7), 129.7 (C-8), 129.0 (C-3',C-5'), 128.3 (C-2',C-6'), 127.8 (C-5), 127.7 (C-6), 126.3 (C-4'), 69.5 (C-1), 62.3 (C-3), 28.2 (C-4); MS (70eV): m/z (%) 279 [$M^{+\cdot}$] (45), 202 (100), 173 (37), 77 (51); Anal. Calcd for $C_{15}H_{12}Cl_2O$: C, 64.51 H, 4.30 Found, C, 64.48 H, 4.28.

6,7-Dichloro-1-(2-chlorophenyl)-3,4-dihydro-1*H*-isochromene (3b)

Yield 82%; oil; R_f 0.7; IR (KBr) 1250 (C-O), 1608 (C=C), 2934 (C-H), 3056 (Ar-H) cm^{-1}. ^1H NMR ($CDCl_3$, δ ppm) 7.45 (1H, s, H-8), 7.45 (1H, d, *J*=1.5, H-3'), 7.43 (1H, s, H-5), 7.3-7.4 (3H, m, H-4', H-5', H-6'), 5.74 (1H, s, H-1), 4.06 (1H, td, *J*=4.3, 3.8, H-3), 3.66 (1H, td, *J*=4.8, 4.1, H-3), 3.08 (1H, td, *J*=4.2, 5.1, H-4), 2.80 (1H, td, *J*=4.6, 5.1, H-4). ^{13}C NMR ($CDCl_3$, δ ppm) 144.2 (C-8a), 139.2 (C-1'), 136.0 (C-4a), 133.6 (C-2'), 131.1 (C-7), 129.7 (C-6'), 129.4 (C-3'), 129.0 (C-8), 127.8 (C-5), 127.7 (C-4', C-6), 127.4 (C-5'), 62.3 (C-3), 60.4 (C-1), 28.2 (C-4); MS (70eV): m/z (%) 313.5 [$M^{+\cdot}$] (56), 202 (100), 173 (32), 111.5 (39). Analysis calc. for $C_{15}H_{11}Cl_3O$: C, 57.41, H, 3.50 % found, C, 57.39, H, 3.48 %.

6,7-Dichloro-1-(4-chlorophenyl)-3,4-dihydro-1*H*-isochromene (3c)

Yield 72%; m. p. 79-81 °C; R_f 0.75; IR (KBr) 1215 (C-O), 1628 (C=C), 2974 (C-H), 3086 (Ar-H) cm^{-1}. ^1H NMR ($CDCl_3$, δ ppm) 7.41 (1H, s, H-8), 7.34 (2H, d, *J*=2.1, H-3',H-5'), 7.09 (1H, s, H-5), 7.05 (2H, d, *J*=2.1, H-2'-H-6'), 5.24 (1H, s, H-1), 4.23 (1H, td, *J*=4.5, 5.3, H-3), 3.84 (1H, td, *J*=4.3, 5.2, H-3), 3.51 (1H, td, *J*=4.1, 3.5, H-4), 2.81 (1H, td, *J*=4.3, 3.8, H-4). ^{13}C NMR ($CDCl_3$, δ ppm) 144.2 (C-8a), 138.4 (C-1'), 135.6 (C-4a), 131.8 (C-4'), 130.7 (C-7), 129.7 (C-2',C-6'), 129.4 (C-3',C-5'), 129.1 (C-8), 127.8 (C-5), 128.5 (C-6), 69.5 (C-1), 63.4 (C-3), 29.4 (C-4); MS (70eV): m/z (%) 313.5 [$M^{+\cdot}$] (56), 202 (100), 173 (32), 111.5 (39). Analysis calc. for $C_{15}H_{11}Cl_3O$: C, 57.41, H, 3.50 % found, C, 57.39, H, 3.48 %.

6,7-Dichloro-1-(3-methoxyphenyl)-3,4-dihydro-1*H*-isochromene(3d)

Yield 84%; m. p. 63 °C; R_f 0.7; IR (KBr) 1244 (C-O), 1618 (C=C), 2923 (C-H), 3063 (Ar-H) cm^{-1}. ^1H NMR ($CDCl_3$, δ ppm) 7.62 (1H, s, H-8), 7.36 (1H, s, H-5), 7.2-7.3 (3H, m, H-4', H-5', H-6'), 7.11 (1H, s, H-2'), 5.59 (1H, s, H-1), 4.51 (1H, td, *J*=4.6, 5.4, H-3), 4.21 (1H, td, *J*=4.3, 5.2, H-3), 3.90 (3H, s, 3'-OCH_3), 3.08 (1H, td, *J*=4.7, 5.1, H-4), 2.88 (1H, td, *J*=4.3, 3.7, H-4); ^{13}C NMR ($CDCl_3$, δ ppm) 161.2 (C-3'), 144.2 (C-8a), 141.3 (C-1'), 136.0 (C-4a), 131.1 (C-7), 129.0 (C-8), 127.8 (C-5), 127.7 (C-6), 126.1 (C-5'), 120.6 (C-6'), 112.3 (C-2'), 111.8 (C-4'), 69.8 (C-1), 62.3 (C-3), 55.7 (OCH_3), 27.9 (C-4); MS (70eV): m/z (%) 309 [$M^{+\cdot}$] (59), 202 (100), 173 (26), 107 (43); Analysis calc. for $C_{16}H_{14}Cl_2O_2$: C, 62.13, H, 4.53 % found, C, 62.10, H, 4.51 %.

6,7-Dichloro-1-(3-methoxy-4-hydroxyphenyl)-3,4-dihydro-1*H*-isochromene (3e)

Yield 74%; m. p. 53 °C; R_f 0.5; IR (KBr) 1236 (C-O), 1623 (C=C), 2913 (C-H), 3049 (Ar-H), 3365 (O-H) cm^{-1}. ^1H NMR ($CDCl_3$, δ ppm) 7.38 (1H, s, H-8), 7.36 (1H, s, H-5), 7.26 (1H, d, *J*=1.8, H-5'), 7.03 (1H, d, *J*=2.1, H-6'), 6.97 (1H, s, H-2'), 5.51 (1H, s, H-1), 3.88 (3H, s, 3'-OCH_3), 3.82 (1H, td, *J*=4.1, 3.8, H-3), 3.69 (1H, td, *J*=4.3, 5.2, H-3), 3.01 (1H, td, *J*=4.1, 3.5, H-4), 2.78 (1H, td, *J*=4.6, 3.7, H-4), 1.28 (1H, s, 4'-OH); ^{13}C NMR ($CDCl_3$, δ ppm) 151.9 (C-3'), 1.44.8 (C-8a), 143.3 (C-4'), 136.8 (C-4a), 133.9 (C-1'), 131.8 (C-7), 129.0 (C-8), 127.8 (C-5), 127.7 (C-6),

122.0 (C-6'), 117.4 (C-5'), 113.8 (C-2'), 62.7 (C-3), 69.3 (C-1), 56.2 (OCH_3), 28.6 (C-4),. MS (70eV): m/z (%) 325 [$M^{+\cdot}$] (47), 202 (100), 173 (36), 123 (24); Analysis calc. for $C_{16}H_{14}Cl_2O_3$: C, 59.07, H, 4.30 % found, C, 59.04, H, 4.27 %.

6,7-Dichloro-1-(3,4,5-trimethoxyphenyl)-3,4-dihydro-1*H*-isochromene (3f)

Yield 86%; m. p. 42-44 ^oC; R_f 0.75; IR (KBr) 1224 (C-O), 1638 (C=C), 2903 (C-H), 3033 (Ar-H) cm^{-1}. ^1H NMR ($CDCl_3$, δ ppm) 7.30 (1H, s, H-8), 7.28 (1H, s, H-5), 7.10 (2H, s, H-2', H-6'), 5.48 (1H, s, H-1), 3.91 (9H, s, 3',4',5'-OCH_3), 3.80 (1H, td, *J*=4.6, 5.1, H-3), 3.65 (1H, td, *J*=4.1, 5.5, H-3), 3.17 (1H, td, *J*=4.2, 5.1, H-4), 2.77 (1H, td, *J*=4.3, 3.8, H-4); ^{13}C NMR ($CDCl_3$, δ ppm) 151.3 (C-3',C-5'), 142.7 (C-8a), 138.5 (C-4a), 136.7 (C-4'), 134.6 (C-1'), 129.5 (C-8), 127.8 (C-5), 127.7 (C-6), 105.8 (C-2', C-6'), 70.8 (C-1), 60.3 (C-3), 56.7 (OCH_3), 29.7 (C-4); MS (70eV): m/z (%) 369 [$M^{+\cdot}$] (48), 202 (100), 173 (23), 167 (29), 136 (19); Analysis calc. for $C_{18}H_{18}Cl_2O_4$: C, 58.53, H, 4.87 % found, C, 58.51, H, 4.84 %.

6,7-Dichloro-1-(5-nitrobenzo[d] [1,3]dioxol-6-yl)-3,4-dihydro-1*H*-isochromene (3g)

Yield 42%; m. p. 59-61 ^oC; R_f 0.65; IR (KBr) 1262 (C-O), 1523 (C-NO_2), 1648 (C=C), 2954 (C-H), 3076 (Ar-H) cm^{-1}. ^1H NMR ($CDCl_3$, δ ppm) 7.41 (1H, s, H-8), 7.34 (1H, s, H-5), 7.15 (1H, s, H-6'), 7.07 (1H, d, *J*=1.8, H-3'), 5.90 (2H, s, O-CH_2-O), 5.38 (1H, s, H-1), 3.86 (1H, td, *J*=4.3, 5.1, H-3), 3.71 (1H, td, *J*=4.2, 3.8, H-3), 3.03 (1H, td, *J*=4.1, 3.9, H-4), 2.83 (1H, td, *J*=4.4, 5.1, H-4). ^{13}C NMR ($CDCl_3$, δ ppm) 155.4 (C-5'), 147.2 (C-4'), 144.4 (C-8a), 141.6 (C-2'), 135.8 (C-4a), 132.5 (C-1'), 131.7 (C-7), 130.4 (C-8), 128.1 (C-6), 126.8 (C-5), 114.2 (C-6'), 110.7 (C-3'), 101.2 (C-OCH_2O), 64.5 (C-3), 61.4 (C-1), 30.4 (C-4); MS (70eV): m/z (%) 368 [$M^{+\cdot}$] (25), 202 (100), 173 (42), 166 (19); Analysis calc. for $C_{16}H_{11}Cl_2NO_5$: C, 52.11, N, 5.22, H, 2.98 % found, C, 52.87, N, 5.14, H, 2.91 %.

4.2 Antibacterial activity

Antibacterial activity results of the isochromanes **(3a-g)** shows that most of these are more active against gram negative bacteria as compared to gram positive bacteria. The 7,8-Dichloro-1-(2-chlorophenyl)-3,4-dihydro-1*H*-isochromene **(3b)**, 7,8-Dichloro-1-(4-chlorophenyl)-3,4-dihydro-1*H*-isochromene **(3c)** and 7,8-Dichloro-1-(3-methoxy-4-hydroxyphenyl)-3,4-dihydro-1*H*-isochromene **(3e)** show moderate to potent activity against gram negative bacterial strains. The position and type of substituents at 1-phenyl ring play important role in the antibacterial activity of these compounds. The analogues which possess ortho or para chloro substituted 1-phenyl ring show higher antibacterial activity. The presence of electronegative substituent at ortho or para position of 1-phenyl ring plays vital role in the antibacterial activity of these compounds. But the electronegative is not parallel to the antibacterial activity because the chloro substituted derivative is more active as compared to flouro substituted.

The compound having 3-methoxy-4-hydroxy substituted 1-phenyl ring is more potent among all others which possess oxygenated substituted 1-phenyl ring. It reflects that antibacterial activity increases by the presence of polar hydroxyl group at para position because the compound which possess methoxy group at para position is inactive against the tested bacterial strains. It indicates that the nature of the substituent present at para position is important in antibacterial activity. The polarity of the para substituent is important in biological action. The polar hydroxyl group may participate in the receptor binding.

The compounds **(3c)** and **(3e)** show potent antibacterial activity against *Klebsiella pneumonae* and *Salmonella typhi* but the compound **(3a)** is inactive against these two bacteria. The first two derivatives possess the para substituted 1-phenyl ring but the last one does not. It is clear from these results that the substituent present at para position is critical for antibacterial activity.

4.3 Antiinflammatory activity

The synthesized compounds showed anti-inflammatory activity ranging from 15.30% to 66.37%, whereas the standard drug ibuprofen showed 72.88% inhibition of edema after three hours. The anti-inflammatory activity results revealed that the activity was dependant on the type and position of the substituent present on 1-phenyl ring. Compound **3c** showed 66.37% edema inhibition possessing electronegative chloro substituent at para position of the 1-phenyl ring. The electronegativity of the substituent is also important but position of the electronegative substituent at 1-phenyl ring plays key role in anti-inflammatory activity as the same substituent is present but at a different position in compound **3b** which showed less percent edema inhibition i.e 31.86%. Compound **3e** exhibited good inhibition of the paw inflammation than **3f** because of the presence of polar hydroxyl substituent at para position of 1-phenyl ring. Compound **3d** possesses 3,4,5-trimethoxy substituted 1-phenyl ring exhibited moderate edema inhibition compared to the standard drug. The figures 1 represented the comparison of percent edema inhibition of differently substituted isochromans **(3a-g)** and figure 2 showed potency comparison.

Table 1: Antibacterial activity of 1-phenylsubstituted isochromanes (3a-g)

Comds.	*E. c*	*K. p*	*L. b*	*M. l*	*P. m.*	*P. v.*	*P. a.*	*S. t.*	*S. a.*	*S. e.*
3a	11.5	3	1	0	0	0	9	11	2	1
3b	3	1	0	0	0	0	5	2	0	0
3c	13	9	1	0	0.5	7	5	10	1	0
3d	1	2	0	0	2	0	0	2	0	0
3e	12	9	0	1	0	5	0	10	0	3
3f	1	0	2	0	0	3	0	0	0.5	0
3g	4	3	1.5	2	2.5	1.5	3	4	3.5	0
Standard	18	10	13	13	12	13	13	14	13	13

E. coli (***E. c***), *K. pneumonae* (***K. p***), *L. bulgaricus* (***L. b***), *M. luteus* (***M. l***), *P. multocida* (***P. m***), *P. vulgaricus* (***P. v***), *P. auriginosa* (***P. a***), *S. typhi* (***S. t***), *S. aureus* (***S. a***), *S. epidermidis* (***S. e***), Standard drug: levofloxacin

Table 2: Anti-inflammatory activity of 1-phenylsubstituted isochromanes (3a-g)

Compounds	Edema volume ± S.E after 3 h	% Inhibition of inflammation[a] after 3 h	Potency[b]
3a	1.652 ± 0.034	23.16	0.31
3b	1.465 ± 0.049	31.86	0.43
3c	0.723 ± 0.025*	66.37	0.91
3d	1.066 ± 0.021*	50.41	0.69
3e	0.800 ± 0.036*	62.79	0.86
3f	0.843 ± 0.030*	60.79	0.83
3g	1.821 ± 0.036	15.30	0.20
Ibuprofen	0.583 ± 0.060 *	72.88	1
Control	2.150 ± 0.056	-	-

* Significance from control, $P < 0.01$; [a]Percent edema inhibition was calculated with regards to the control group; [b]Potency was calculated with regards to the percentage inhibition of the ibuprofen-treated group.

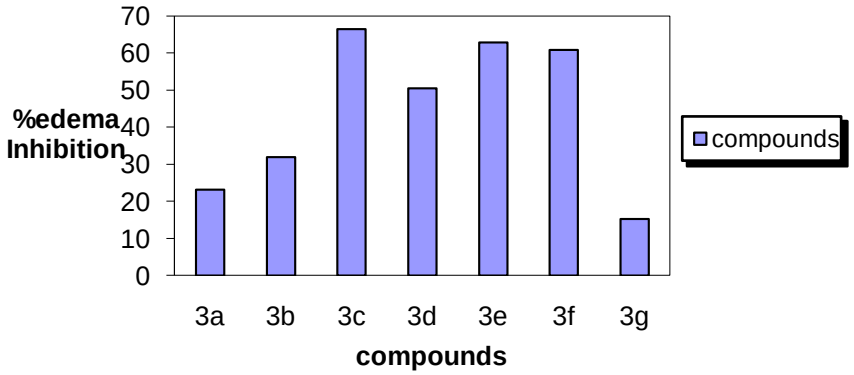


Figure 1: Percent edema inhibition of Isochromanes **(3a-g)** with regards to the Ibuprofen

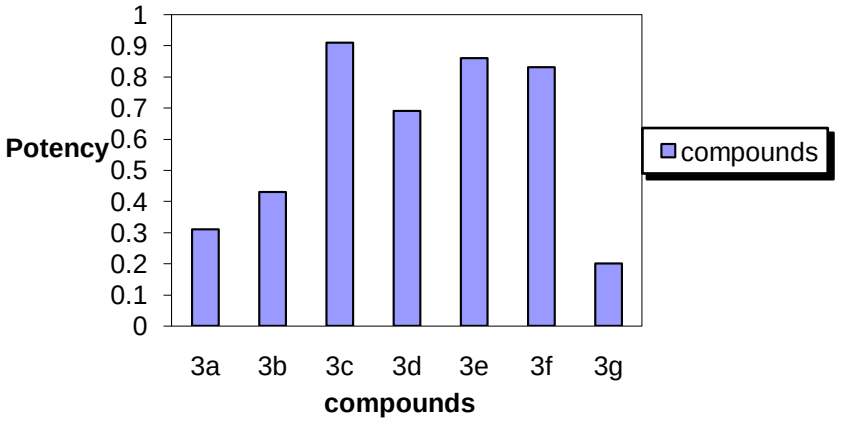


Figure 2: Potency of Isochromanes **(3a-g)** calculated with regards to the Ibuprofen

5. Conclusion

In conclusion, an eco-friendly one pot, microwave–assisted synthesis of 1-substitied isochromans is described. The solvent free synthesis displays numerous advantages such as shorter reaction times, high yields and inexpensive catalyst. The title compound **3c** exhibited excellent anti-inflammatory activity in vivo. The isochroman **3c** may serve as structural template for the design and development of new anti-inflammatory drugs.

References

[1] Imamura, N.; Ishikawa, T.; Ohtsuka, T.; Yamamoto, K.; Dekura, M.; Fukami, H.; Nishida, R. ; *Biosci. Biotech. Biochem.* 2000, *64*, 2216-2217.
[2] Inagaki, T.; Kaneda, K.; Suzuki, Y.; Hirai, H.; Nomura, E.; Sakakibara, T. Yamauchi, Y.; Huang, L. H.; Norcia, M.; Wondrack, L. M.; Sutclie, J.A.; Kojima, N. *J. Antibiot.* 1998, *51*, 112-116.
[3] Ogawa, A.; Murakami, C.; Kamisuki, S.; Kuriyama, I.; Yoshida, H.; Sugawara, F.; Mizushina, Y. *Bioorg. Med. Chem. Lett.* 2004, *14*, 3539-3543.
[4] Peng, J.; Lu, F.; Ralph, J. *Phytochemistry*, 1999, 50, 659-666; Ralph, J.; Peng, J.; Lu, F. *Tetrahedron Lett.*, 1998, *39*, 4963-4964.
[5] Kunesch, G.; Zagatti, P.; Pouverau, A.; Cassini, R. *Z. Naturforsch.*, 1987, *42c*, 657-659.
[6] Malstrom, J.; Christophersen, C.; Frisvad, J. *Phytochemistry.*, 2000, *54*, 301-309.
[7] Lorenz, P.; Zeh, M.; Lobenhoffer, J. M.; Schmidt, H.; Wolf, G.; Horn, T. F. W. *Free Radic. Res.* 2005, *39*, 535-545.
[8] Togna, G. I.; Togna, A. R.; Franconi, M.; Marra, C.; Guiso, M. *J. Nutr*. 2003, *133*, 2532-2536.
[9] Bianchi, D. A.; Blanco, N. E.; Carrillo, N.; Kaufman, T. S. *J. Agric. Food Chem.* 2004, *52*, 1923-1927.
[10] Cutler, H. G.; Majetich, G.; Tian, X.; Spearing, P. *J. Agric. Food Chem.* 1997, *45*, 1422-1429.
[11] Liu, J.; Birzin, E. T.; Chan, W.; Yang, Y. T.; Pai, L. Y.; DaSilva, C.; Hayes, E. C.; Mosley, R. T.; DiNinno, F.; Rohrer, S. P.; Schaeer, J. M.; Hammonda, M. L. *Bioorg. Med. Chem. Lett*. 2005, *15*, 715-718.
[12] TenBrink, R. E.; Bergh, C. L.; Duncan, J. N.; Harris, D. W.; Huff, R. M.; Lahti, R. A.; Lawson, C. F.; Lutzke, B. S.; Martin, I. J.; Rees, S. A.; Schlachter, S. K.; Sihr, J. C.; Smith, M. W. *J. Med. Chem*. 1996, *39*, 2435-2437.
[13] Frater, G.; Kraft, P. *Helv. Chim. Acta* 1999, *82*, 1656-1665.
[14] Yamato, M. *J. Synth. Org. Chem. Jpn.*, 1983, *41*, 958-971.
[15] Morphy, R. Rankovic, Z. *Curr Pharm Des.*, 2009, *15*, 587-600.
[16] Yoshikai, Y. *Curr Opin Infect Dis.*, 2001, *14*, 257-263.
[17] Bianchi, D. A.; Rua, F.; Kaufmann, T. S. *Tetrahedron Lett*. 2004, *45*, 411-415.
[18] Mohler, D. L.; Thompson, D. W. *Tetrahedron Lett.* 1987, *28*, 2567-2570.
[19] Ashraf, Z.; Muhammad, A.; Imran, M.; Tareq, A. H. *Int. J. Org. Chem.* 2011, *1*, 257-261.
[20] Nwafor, P. A.; Okwuasaba, F. K. *J. Ethanopharmacol*. 2003, *84*, 125-129.
[21] Guiso, M.; Marra, C.; Cavarischia, C. *Tetrahedron Lett*. 2001, *42*, 6531-6534.
[22] Saeed, A.; Ashraf, Z. *J. Chem. Sci*. 2006, *118*, 419-423.