Synthesis and Antimicrobial Activities of Some New 3-(Chlorophenyl-ethyl)-, 3-(Chlorophenylethenyl)-Isocoumarins and their Dihydro Derivatives

Gul S. Khan[a], G. Qadeer[a], Nasim H. Rama[a] and Khalid Mohammed Khan*[,b]

[a]*Department of Chemistry, Quaid-i-Azam University, Islamabad 45320, Pakistan.*

[b]*H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan*

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Abstract: The bioactive 3-(chlorophenylethyl)- and 3-(chlorophenylethenyl)-isocoumarins were synthesized by the condensation of simple homophthalic acid with 3-chlorophenylpropionoyl chlorides and chlorocinnamoyl chlorides, respectively. Alkaline hydrolysis of these isocoumarins gave the respective keto-acids. (*dl*)-3,4-Dihydroisocoumarins were obtained by the reduction of the keto-acids to racemic hydroxyl-acids**,** followed by cyclodehydration using acetic anhydride. These compounds were tested for their antimicrobial and cytotoxicity (Brine shrimp lethality) bioassay. Bioassay indicated that some compounds show good antimicrobial activities and positive lethality.

Keywords: Isocoumarins, dihydroisocoumarins, synthesis, antimicrobial screening.

The isocoumarin nucleus is an abundant structural motif in natural products [1]. Many constituents of the steadily growing class of known isocoumarins exhibit valuable biological properties, such as antifungal [2], antitumor or cytotoxic [3], antiinflammatory, antiallergic, and enzyme inhibitory activities [4]. Naturally occurring haloisocoumarins and halo-3,4-dihydroisocomuarins are very rare, however, a few examples of naturally occurring chlorine containing isocoumarins are known [5]. Naturally occurring isocoumarins having chlorine are bactabolins A, B, and C isolated from *Pseudomonas yoshitomiensis* [6], whereas LLZ 1640-5 was isolated from laderale culture of unidentified fungus [7]. Bactabolin A has been reported to be active against bacteria and viruses [8]. Some chloroisocoumarins are strong inhibitors of numerous serine proteases [9, 10].

Biological activities associated with chloroisocoumarins prompted us to synthesize more halogenated 3-substituted isocoumarins as a continuation of our efforts [11-17] towards the synthesis of bioactive isocoumarins, herein, we wish to report the synthesis of a number of 3-(chlorophenylethyl)-, 3-(chlorophenylethenyl)isocoumarins **3a-f** and (*dl*)-3-(chlorophenylethyl)-3,4-dihydroisocoumarins **6a-c**. The synthesized compounds were also screened for antimicrobial and cytotoxicity (Brineshrimp lethality) activities.

RESULTS AND DISCUSSION

Condensation of the acid chloride with homophthalic acid is useful for the preparation of 3-substituted isocoumarins skeleton [18]. Herein, a short and efficient synthesis of 3-(chlorophenylethyl)- and 3-(chlorophenylethenyl) isocoumarins **3a-f** and conversion of the later into corresponding (*dl*)-3-(chlorophenylethyl)-3,4-dihydroisocoumarins **6a-c** are achieved by using this method. Antimicrobial, cytotoxicity activities of the synthesized compounds are also described. Reaction of commercial homophthalic acid with chlorophenylpropionyl and chlorophenylcinnamoyl chlorides **2a-f** at elevated temperature afforded 3-(chlorophenylethyl)- and 3-(chlorophenylethenyl)isocoumarins **3a-f** in 70-78% yield . The isocoumarins showed the characteristic ^{1}H singlet of the isocoumarins moiety (H-4) at 6.48, 6.77, 6.75, 6.40, 6.42, and 6.41 ppm, respectively, in ^{1}H NMR. IR spectra showed the lactonic carbonyl absorption at 1721, 1719, 1722, 1720, 1727, 1726 cm,$^{-1}$ respectively. In IR spectrum of **3d-f**, double bond appeared at 1611, 1612, and 1616 cm,$^{-1}$ respectively, while these peaks are absent in the isocoumarins **3a-c**. Alkaline hydrolysis of **3a-c** furnished 2-[4'-(chlorophenyl)-2'-oxobutyl]benzoic acids **4a-c** were accomplished in 81-85% yield. The keto-acids **4a-c** showed 2H singlets at δ 3.30, 3.25, and 3.30 ppm (H-4, $ArCH_2$) in the ^{1}H NMR spectrum. The ketonic and carboxylic carbonyl absorptions of these keto-acids in IR spectrum were observed at 1700-1720, respectively. Sodium borohydride reduction of the keto-acids **4a-c** afforded the corresponding racemic hydroxyacids **5a-c**, which underwent cyclodehydration on refluxing with acetic anhydride to (*dl*)-3-(chlorophenyl-ethyl)-3,4-dihydroisocoumarins **6a-c**. The methylene protons (H-4) adjacent to the newly generated chiral center (C_3) in dihydroisocoumarins **6a-c** appeared as multiplets at δ 3.0-3.3, 3.0-3.3, 3.0-3.3 ppm, respectively. The H-3 protons appeared as a multiplet at δ 4.56, 4.54, and 4.54 ppm, respectively. The δ-lactonic carbonyl absorption appeared at 1703, 1717, 1703 cm,$^{-1}$ respectively, in the IR spectrum. HREI-MS of M^+ ions of all the synthesized compounds were in good agreement with the calculated

*Address correspondence to this author at the H.E.J. Research Institute of Chemistry, International center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan; Tel: 0092214824910; Fax: 0092214819018; E-mail: khalid.khan@iccs.edu; hassaan2@super.net.pk

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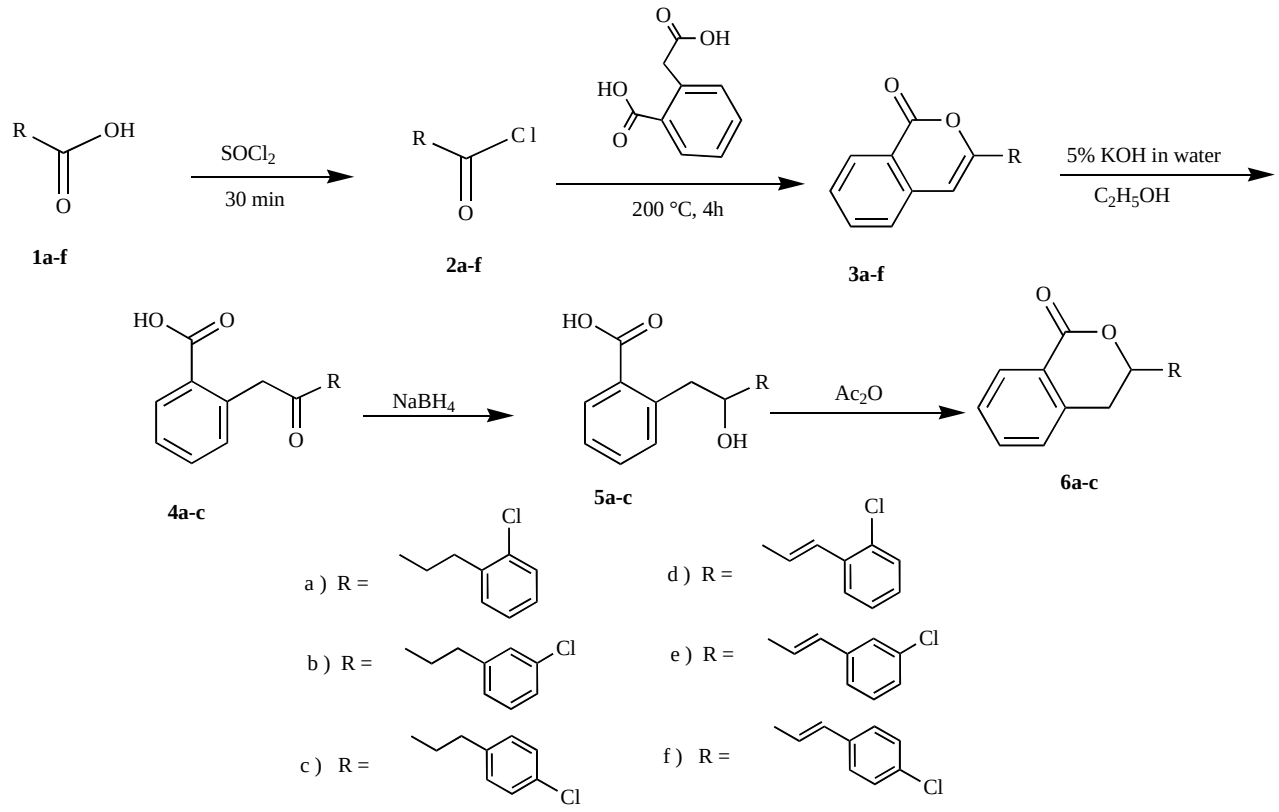

Scheme 1.

values providing further confirmation for their structures as shown Scheme **1**.

Isocoumarins, dihydroisocoumarins, and related compounds were tested by agar tube dilution method [19] for their *in vitro* fungicidal bioassay. Results were reported as linear growth inhibition (LGI) against some human pathogens (e.g. *Trichophyton longifusus, Candida albicans, Aspergillus flavus, Microsporum canis, Fusarium solani, Candida glabrata*). Linear Growth Inhibition results of compounds are given in Table **1**.

From the biological assay results in Table **1**, which summarize the antifungal activity of the target compounds, some showed significant activity against the three fungi *Trichphyton Longifusus, Aspergillus Flavus,* and *Microsporum canis*. Compounds **4a**, **4b**, **6a**, and **6b** exhibited considerable activities against *Trichphyton longifusus, Aspergillus flavus,* and *Microsporum canis*. Even the activities of compounds **3b**, **4b**, and **6b** reach to 90% at 200 µg/ml. The other synthesized compounds also show antifungal activity but not too good. None of the synthesized compounds show antifungal activity against *Candida albicans* and *Candida glabrata*.

All the synthesized compounds were screened for antibacterial activity against four Gram-negative and two Gram-positive bacterial strains i.e. Gram negative are *Escherichia coli, Shigella flexnari, Pseudomonas aeruginosa,* and *Salmonella typhi* and two Gram-positive bacterial stains are *Bacillus subtilis and Staphyloccus aureus.* Using agar well diffusion method [20] as a literature protocol. The results of antibacterial activity are displayed in Table **2**.

The activity was determined *via* the growth inhibition of microorganism, i.e. the zone inhibition, measured in millimeters. The results indicate that our compounds are

Table 1. Anti-Fungal Activity of Compounds (3b-c, 4a-c, 6a-c) as Linear Growth Inhibition (%) at 200 µg/ml of Media SDA

Pathogens	Compounds								Std. Drugs	Inhibition (%)
	3b	3c	4a	4b	4c	6a	6b	6c		
Trichphyton longifusus	50	30	40	90	0	20	70	50	Miconazole	100
Candida albicans	0	0	0	0	0	0	0	0	Miconazole	100
Aspergillus flavus	0	0	85	90	85	85	85	0	Amphotericin B	100
Microsporum canis	90	75	50	90	0	85	90	45	Miconazole	100
Fusarium solani	0	0	50	0	15	0	0	0	Miconazole	100
Candida glabrata	0	0	0	0	0	0	0	0	Miconazole	100

Table 2. Antibacterial Activity (*In Vitro*) of all Synthesized Compounds (3a-f, 4a-c, 6a-c)

Bacteria	Compounds												Imipenem
	3a	3b	3c	3d	3e	3f	4a	4b	4c	6a	6b	6c	
Escherichia coli	0	0	12	0	0	0	0	0	0	0	9	0	30
Shigella flexeneri	0	0	15	0	0	0	0	0	0	0	0	0	27
Pseudomonas aeruginosa	12	0	16	0	0	20	0	16	18	15	20	0	24
Salmonella typhi	12	0	0	0	0	19	16	16	10	14	15	15	25
Bacillus subtilis	0	0	0	0	0	0	0	0	0	0	0	0	33
Staphyloccus aureus	0	0	0	0	0	12	16	0	0	0	13	10	33

generally weekly or moderately active and only **3f** and **6b** out of twelve compounds tested, demonstrated significant antibacterial activity. It is evident from Table **2**, that compounds **3f** and **6c** exhibit significant activity against *Pseudomonas aeruginosa* with a zone of inhibition of 20 mm as compared to the standard drug (imipenem) having zone of inhibition of 24 mm. Compounds **3b**, **3d**, and **3e** were completely inactive against all bacterial strains. None of the compounds were active or weekly active against *Escherichia coli*, *Shigella flexeneri*, and *Bacillus subtilis.*

Compounds **3b**, **3c**, **3e**, **4a**, and **6a-c** were tested for cytotoxicity by the brine shrimp lethality bioassay. From the data collected in Table **3**, it is evident that the majority of the compounds in this study displayed promising cytotoxicity (LD_{50} = 1.226 - 7.562 μg/ml). It may be noted that compound 2-[4'-(chlorophenyl)-2'-oxobutyl]benzoic acid **4c** showed highest degree of cytotoxicity (LD_{50} = 7.562 μg/ml) than the standard drug and its dihydroisocoumarin derivative also showed cytotoxicity (2.0978). Interestingly, the 2-[2'-(chloropheny)-2'-oxobutyl]benzoic acid **4a** was completely inactive but its dihydroisocoumarin showed cytotoxicity (1.226). We have, however, no information about the mode of action of these compounds. Further research on the modification of structure and the mode of action is in progress.

The findings of this research suggest that isocoumarins and related compounds may be used as potent bioactive antibacterial and antifungal agents. Furthermore, the methods used in this research to synthesize isocoumarins and related compounds may be useful to enhance the fungicidal potential for agricultural as well as pharmaceutical purposes. These studies may serve as a basis for the further chemical modification on the isocoumarin moiety, which may be the incorporation of an NH_2 or SH group or an amidic functionality into the isocoumarin residue. These functionalities may help to enhance the activity of isocoumarins and dihydroisocoumarins and may lead to the development of a potential bioactive derivative of clinical interest without having severe effects attributed to β-lactam ring.

EXPERIMENTAL SECTION

General Experimental Procedures

Chlorocinnamic acids were purchased from Aldrich. Melting points of the compounds were determined in open capillaries using Gallenkemp melting point apparatus and are uncorrected. IR spectra were recorded on a Hitachi model 270-50 spectrophotometer as KBr discs, ^{1}H NMR (400 & 300 MHz) spectra in $CDCl_3$ on a Bruker AM-500 using TMS as internal standard and HRMS on a MAT-112-S machine at 70ev.

General Procedure for the Synthesis of 3-(Chlorophenyl-ethyl)isocoumarins (3a-c)

Mixtures of homophthalic acid (2.06 g, 11 mmol) and substituted chlorophenylpropionyl chlorides **2a-c** (9.5 g, 47 mmol) were heated at 200 °C under reflux for four h. The reaction mixtures were dissolved in ethyl acetate and aqueous solution of sodium carbonate was added in order to remove the unreacted homophthalic acid. The organic layers were separated, concentrated, and chromatographed on silica gel using petroleum ether (40-80 °C) as eluent to afford 3-(chlorophenylethyl)isocoumarins **3a-c** as solids, which were further purified by recrystallization from methanol.

3-(2-Chlorophenethyl)-1H-isochromen-1-one (3a)

Yield 77%; m.p 70 °C; IR ν_{max} (KBr) 2930, 1720, 1072 cm^{-1}, ^{1}H NMR (acetone-d_6) δ 2.57 (2H, t, *J* = 7.52, 8.1 Hz, H-1′), 2.98 (2H, t, *J* = 8.0 Hz, H-2′), 6.40 (1H, s, H-4), 7.15-7.23 (2H, m, H-4″,6″), 7.3-7.34 (2H, m, H-3″,5″), 7.50 (1H, dd, *J* = 1.9, 8.2 Hz, H-7), 7.60 (1H, d, *J* = 7.5 Hz, H-5), 7.71 (1H, ddd, *J* = 7.3, 0.9 Hz, H-6), 8.12 (1H, dd, *J* = 7.3, 0.9 Hz, H-8) ppm; EIMS (70ev): *m/z* (%) 284 (M^+, 37.5), 286 (M^+ +2, 11.85), 159 (60.4), 125 (100), 103 (32.3); HRMS: *m/z* Found; 284.0602 (Cl^{35}) (Calcd for $C_{17}H_{13}O_2Cl^{35}$; 284.0604) & Found; 286.0572 (Cl^{37}), (Calcd for $C_{17}H_{13}O_2Cl^{37}$; 286.0575).

3-(3-Chlorophenethyl)-1H-isochromen-1-one (3b)

Yield 75%; m.p 85 °C; IR ν_{max} (KBr) 2935, 1727, 1072 cm^{-1}; ^{1}H NMR (acetone- d_6) δ 2.83 (2H, t, *J* = 7.2,8.1 Hz, H-1′), 2.99 (2H, t, *J* = 7.2, 8.1 Hz, H-2′), 6.42 (1H, s, H-4), 7.14-7.18 (2H, m, H-4″, 5″), 7.19 (1H, dd, *J* = 7.6, 1.4 Hz, H-6″), 7.25 (1H, bs, H-2″), 7.44 (1H, dd, *J* = 7.0, 7.8 Hz, H-7), 7.5 (1H, ddd, *J* = 7.5, 1.2 Hz, H-6), 8.11 (1H, dd, *J* = 8.6, 1.2 Hz, H-8) ppm; EIMS (70ev): *m/z* (%) 284 (M^+, 37.5), 286 (M^+ +2, 11.8), 159 (3.4), 125 (100), 103 (11.8); HRMS: *m/z* Found; 284.0609 (Cl^{35}) (Calcd for $C_{17}H_{13}O_2Cl^{35}$; 284.0604) & Found; 286.0576 (Cl^{37}) (Calcd for $C_{17}H_{13}O_2Cl^{37}$; 286.0575).

Table 3. Brine Shrimp (*Artemia salina*) Lethality Bioassay

Code No	Dose (μg/ml)	No of Shrimps	No. of Survivors	LD_{50} (μg/ml)	STD. DRUG	LD_{50} (μg/ml)	Remarks
3b	100	30	24	--------	Etoposide	7.4625	No Cytotoxicity
	10	30	30				
	1	30	30				
3c	100	30	1	1.6963	Etoposide	7.4625	Positive Lethality
	10	30	6				
	1	30	18				
3e	100	30	3	3.9484	Etoposide	7.4625	Positive Lethality
	10	30	11				
	1	30	21				
4a	100	30	24	-------	Etoposide	7.4625	No Cytotoxicity
	10	30	30				
	1	30	30				
4c	100	30	7	7.562	Etoposide	7.4625	Positive Lethality
	10	30	17				
	1	30	19				
6a	100	30	0	1.226	Etoposide	7.4625	Positive Lethality
	10	30	2				
	1	30	17				
6b	100	30	10	--------	Etoposide	7.4625	No Cytotoxicity
	10	30	30				
	1	30	30				
6c	100	30	0	2.0978	Etoposide	7.4625	Postive Lethality
	10	30	9				
	1	30	18				

3-(4-Chlorophenethyl)-1H-isochromen-1-one (3c)

Yield 74%; m.p 109 °C; IR ν_{max} (KBr) 2940, 1726, 1080 cm^{-1}; ^1H NMR (acetone- d_6) δ 2.83 (2H, t, *J* = 7.8 Hz, H-1′), 3.00 (2H, t, *J* = 7.9 Hz, H-2′), 6.40 (1H, s, H-4), 7.2-7.3 (4H, m, H-2″, 3″, 5″, 6″), 7.44 (1H, d, *J* = 7.8 Hz, H-7), 7.5 (1H, dd, *J* = 7.6 Hz, H-5), 7.72 (1H, ddd, *J* = 7.5, 1.2 Hz, H-6), 8.12 (1H, dd, *J* = 7.8, 1.2 Hz, H-8) ppm; EIMS (70ev): *m/z* (%) 284 (M^+, 11.4), 286 (M^+ +2, 3.56), 159 (3.4), 125 (100), 103 (11.8); HRMS: *m/z* Found; 284.0601 (Cl^{35}) (Calcd for $C_{17}H_{13}O_2Cl^{35}$; 284.0604) & Found; 286.0571 (Cl^{37}) (Calcd for $C_{17}H_{13}O_2Cl^{37}$; 286.0575).

General Procedure for the Synthesis of 3-(Chlorophenyl-ethenyl)isocoumarins (3d-f)

Mixtures of homophthalic acid (2.27 g, 12 mmol) and substituted chlorocinnamoyl chlorides **2d-f** (10.4 g, 51 mmol) were heated at 200 °C under reflux for four h. The mixtures were dissolved in ethyl acetate and aqueous solution of sodium carbonate was added in order to remove the unreacted homophthalic acid. The organic layers were separated, concentrated, and chromatographed on silica gel using petroleum ether (40-80 °C fractions) as eluent to afford 3-(chlorophenylethenyl)]isocoumarins **3d-f** as solids, which were further purified by recrystallization from methanol.

3-[-2-(2-Chlorophenyl)ethenyl]-1H-isochromen-1-one (3d)

Yield 73%; m.p 162 °C; IR ν_{max} (KBr) 3030, 1721, 1611, 1059 cm^{-1}; ^1H NMR (acetone-d_6) δ 6.48 (1H, s, H -4), 6.67 (1H, d, *J* = 15.9 Hz, H-2′), 7.39 (1H, d, *J* = 15.4 Hz, H-1′), 7.19-7.27 (2H, m, H-3″, 5″), 7.37-7.42 (2H, m, H-4″, 6″), 7.46 (1H, dd, *J* = 7.5 Hz, H-7), 7.59 (1H, dd, *J* = 5.9, 1.6 Hz, H-5), 7.67 (1H, ddd, *J* = 7.0, 1.2 Hz, H-6), 8.27 (1H, dd, *J* = 7.8, 0.9 Hz, H-8) ppm; EIMS (70ev): *m/z* (%) 282 (M^+, 29.4), 247 (21.2), 191 (61.4), 165 (6.6), 89 (100); HRMS: *m/z* Found; 282.0442 (Cl^{35}) (Calcd for $C_{17}H_{11}O_2Cl^{35}$; 282.0448) & Found; 284.0420 (Cl^{37}) (Calcd for $C_{17}H_{11}O_2Cl^{37}$; 284.0418).

3-[-2-(3-Chlorophenyl)ethenyl]-1H-isochromen-1-one (3e)

Yield 70%; m.p 178 °C; IR ν_{max} (KBr) 3026, 1721, 1612, 1061 cm^{-1}; ^1H NMR (acetone-d_6) δ 6.77 (1H, s, H-4), 7.05 (1H, d, *J* = 16.0 Hz, H-2′), 7.29 (1H, d, *J* = 16.0 Hz, H-1′), 7.32 (1H, m, H-3″), 7.54 (1H, dd, *J* = 7.78, 7.9 Hz, H-4″), 7.52-7.55 (2H, m, H-7, 5″), 7.58 (1H, dd, *J* = 8.1, 3.2 Hz, H-5), 7.67 (1H, bs, H-2″), 7.77 (1H, ddd, *J* = 7.0, 1.2 Hz, H-6), 8.17 (1H, d, *J* = 7.8, 0.9 Hz, H-8) ppm; EIMS (70ev): *m/z*

(%) 282 (M^+, 84.8), 284 (M^+ +2, 25.1), 219 (100), 191 (77.7), 165 (12.3); HRMS: *m/z* Found; 282.0431 (Cl^{35}) (Calcd for $C_{17}H_{11}O_2Cl^{35}$; 282.0448) & Found; 284.0424 (Cl^{37}) (Calcd for $C_{17}H_{11}O_2Cl^{37}$; 284.0418).

3-[-2-(4-Chlorophenyl)ethenyl]-1H-isochromen-1-one (3f)

Yield 78%; m.p 210 °C; IR ν_{max} (KBr) 3063, 1721, 1616, 1068 cm^{-1}; ^1H NMR (acetone-d_6) δ 6.75 (1H, s, H-4), 6.98 (1H, d, *J* = 16.0 Hz, H-2′), 7.30 (1H, d, *J* = 16.0 Hz, H-1′), 7.39 (2H, d, *J* = 9.2 Hz, H-2″, 6″), 7.54 (1H, d, *J* = 7.2 Hz, H-7), 7.57 (1H, dd, *J* = 7.8, 3.4 Hz, H-5), 7.63 (1H, dd, *J* = 8.5 Hz, H-3″,5″), 7.76 (1H, ddd, *J* = 6.6, 1.5 Hz, H-6), 8.16 (1H, dd, *J* = 7.9, 0.9 Hz, H-8) ppm;. EIMS (70ev): *m/z* (%) 282 (M^+, 95.5), 284 (M^+ +2, 30.28), 254 (20), 219 (56), 191 (100), 165 (10.2), 89 (75); HRMS: *m/z* Found; 282.0430 (Cl^{35}) (Calcd for $C_{17}H_{11}O_2Cl^{35}$; 282.0448) & Found; 284.0403 (Cl^{37}) (Calcd for $C_{17}H_{11}O_2Cl^{37}$; 284.0418).

General Procedure for the Synthesis of 2-[4′-(Chlorophenyl)-2′-oxobutyl]benzoic acid (4a-c)

Solutions of 3-(chlorophenylethyl)isocoumarins **3a-c (**2.2 g, 8 mmol**)** in ethanol (29 ml) and potassium hydroxide (5% in water, 34 ml) were refluxed for 5 h. After cooling, the reaction mixtures were evaporated under reduced pressure to remove ethanol. Cold water (20 ml) was then added and the reaction mixtures were acidified with dilute hydrochloric acid and extracted with dichloromethane (2 × 25 ml). The solvent was rotary evaporated to afford crude solids, which were recrystallized from ethyl acetate to give keto-acids **4a-c.**

2-[4-(2-Chlorophenyl)-2-oxobutyl]benzoic acid (4a)

Yield 81%; m.p. 80 °C; IR ν_{max} (KBr) 3300, 2923, 1700, 1082 cm^{-1}; ^1H NMR (acetone-d_6) δ 2.60 (2H, t, *J* = 7.4, 8.0 Hz, H-3), 3.02 (2H, t, *J* = 8.0 Hz, H-4), 3.30 (2H, s, CH_2), 7.16-7.26 (2H, m, H-4″, 6″), 7.32-7.37 (2H, m, H-3″, 5″), 7.48 (1H, dd, *J* = 8.0 Hz, H-7), 7.51 (1H, dd, *J* = 7.8 Hz, H-5), 7.82 (1H, ddd, *J* = 8.0, 1.2 Hz, H-6), 8.03 (1H, dd, *J* = 8.8, 1.2 Hz, H-8), 11.2 (1H, s, exchangeable with D_2O) ppm; EIMS (70ev): *m/z* (%) 284 (M^+-H_2O, 10.7), 138 (13.8), 125 (100), 103 (40.5); HRMS: *m/z* Found; 284.0715 (M^+-H_2O) (Calcd for $C_{17}H_{13}O_2Cl$; 284.0710).

2-[4-(3-Chlorophenyl)-2-oxobutyl]benzoic acid (4b)

Yield 85%; m.p. 58 °C; IR ν_{max} (KBr) 3300, 2920, 1699, 1080 cm^{-1}; ^1H NMR (acetone-d_6) δ 2.58 (2H, t, *J* = 7.5 Hz., H-3'), 2.88 (2H, t, *J* = 7.6 Hz, H-4'), 3.25 (2H, s, CH_2), 7.16 (1H, dd, *J* = 7.7 Hz, H-5″), 7.23 (IH, s, H-2″), 7.26 (1H, d, *J* = 7.1 Hz, H-4″), 7.28 (1H, d, *J* = 7.5 Hz, H-6″), 7.35 (1H, dd, *J* = 8.0 Hz, H-7), 7.5 (1H, dd, *J* = 7.1, 1.5 Hz, H-5), 7.7 (1H, ddd, *J* = 7.9, 1.3 Hz, H-6), 8.01 (1H, dd, *J* = 8.9, 1.1 Hz, H-8), 11.2 (COOH, s, exchangeable with D_2O) ppm; EIMS (70ev): *m/z* (%) 284 (M^+-H_2O, 32.4), 286 (15.8), 184 (95), 167 (42), 139 (74.9), 125 (100); HRMS: *m/z* Found; 284.0711 (M^+-H_2O) (Calcd for $C_{17}H_{13}O_2Cl$; 284.0710).

2-[4-(4-Chlorophenyl)-2-oxobutyl]benzoic acid (4c)

Yield 81%; m.p 102 °C; IR ν_{max} (KBr) 3300, 2930, 1695, 1092 cm^{-1}; ^1H NMR (acetone-d_6) δ 2.58 (2H, t, *J* = 7.6 Hz, H-3'), 2.88 (2H, t, *J* = 7.5 Hz, H-4'), 3.30 (2H, s, CH_2), 7.2-7.3 (4H, m, H-2″, 3″, 5″, 6″), 7.35 (1H, dd, *J* = 8.0 Hz, H-7), 7.5 (1H, dd, *J* = 7.1, 1.5 Hz, H-5), 7.7 (1H, ddd, *J* = 7.9, 1.3 Hz, H-6), 8.02 (1H, dd, *J* = 8.6, 0.9 Hz, H-8), 11.2 (COOH, s, exchangeable with D_2O) ppm; EIMS (70ev): *m/z* (%) 302 (M^+, 4.4), 248 (3.8), 184 (6.7), 167 (11), 169 (3.1), 139 (22.8), 141 (7.8), 127 (26.9), 118 (100); HRMS: *m/z* Found; 302.0701 (Calcd for $C_{17}H_{15}O_3Cl$; 302.0710).

General Procedure for the Synthesis of (*dl*)-3-(Chlorophenylethyl)-3,4-dihydroisocoumarin (6a-c)

Keto-acids **4a-c** (1.2 g, 4 mmol) were stirred over night at room temperature with sodium borohydride (0.26 g, 6.87 mmol) in sodium hydroxide (1% in water, 40 ml). The reaction mixtures were chilled and acidified with dilute hydrochloric acid to yield hydroxy-acids **5a-c,** which were cyclodehydrated on refluxing with acetic anhydride for 1 h. The reaction mixtures were diluted with chilled water (30 ml) and extracted with dichloromethane (100 ml). The solvent was evaporated to afford *(dl)*-3- (chlorophenylethyl)-3,4-dihydroisocoumarins **6a-c.**

(dl)-3-(2-Chlorophenethyl)-3,4-dihydro-1H-isochromen-1-one (6a)

Yield 77%; m.p 89-90 °C; IR ν_{max} (KBr) 2926, 1703, 1090 cm^{-1}; ^1H NMR (acetone-d_6) δ 2.59 (2H, t, *J* = 7.4, 8.0 Hz, H-2′), 2.9 (2H, m, H-1′), 3.00 (1H, m, H-4a), 3.3 (1H, m, H-4b), 4.56 (1H, m, H-3), 7.15-7.24 (2H, m, H-4″, 6″), 7.3-7.37 (2H, m, H-3″, 5″), 7.56 (1H, dd, *J* = 8.1 Hz, H-7), 7.6 (1H, d, *J* = 7.3 Hz, H-5), 7.75 (1H, ddd, *J* = 7.5, 1.2 Hz, H-6), 7.99 (1H, dd, *J* = 7.6, 0.9 Hz, H-8) ppm; EIMS (70ev): *m/z* (%) 286 (M^+, 78.7), 288 (M^+ +2,28.8), 251 (12.6), 233 (11.6), 174 (2.4), 167 (7.4), 151 (100), 139 (8.7), 125 (57), 103 (15.6); HRMS: *m/z* Found; 286.0770 (Cl^{35}) (Calcd for $C_{17}H_{13}O_2Cl^{35}$; 286.0761) & Found; 288.0723 (Cl^{37}) (Calcd for $C_{17}H_{13}O_2Cl^{37}$; 288.0731).

(dl)-3-(3-Chlorophenethyl)-3,4-dihydro-1H-isochromen-1-one (6b)

Yield 71%; m.p 71-73 °C; IR ν_{max} (KBr) 2927, 1717, 1080 cm^{-1}; ^1H NMR (acetone-d_6) δ 2.6 (2H, m, H-1′), 2.86 (2H, *J* = 7.5 Hz, H-2′), 3.0 (1H, m, H-4a), 3.3 (1H, m, H-4b), 4.54 (1H, m, H-3), 7.16-7.20 (1H, m, H-4″, 5″), 7.23-7.28 (1H, m, H-2″, 6″), 7.57 (1H, dd, *J* = 8.7 Hz, H-7) 7.60 (1H,dd, *J* = 7.5, 1.4 Hz, H-5), 7.62 (1H, ddd, *J* = 7.6, 1.2 Hz, H-6), 8.00 (1H, dd, *J* = 7.7, 0.9 Hz, H-8) ppm; EIMS (70ev): *m/z* (%) 286 (M^+, 100), 288 (M^+ +2, 36.5), 139 (9.4), 125 (12.5), 119 (45.9), 103 (12.4); HRMS: *m/z* Found; 286.0770 (Cl^{35}) (Calcd for $C_{17}H_{13}O_2Cl^{35}$; 286.0761) & Found; 288.0741 (Cl^{37}) (Calcd for $C_{17}H_{13}O_2Cl^{37}$; 288.0731).

(dl)-3-(4-Chlorophenethyl)-3,4-dihydro-1H-isochromen-1-one (6c)

Yield 66%; m.p 95-97 °C; IR ν_{max} (KBr) 2930, 1703, 1089 cm^{-1}; ^1H NMR (acetone-d_6) δ 2.58 (2H, m, H-1′), 2.88 (2H, t, *J* = 7.7 Hz, H-2′), 3.0 (1H, m, H-4a), 3.30 (1H, m, H-4b), 4.54 (1H, m, H-3), 7.20-7.24 (1H, m, H-4″, 6″), 7.27-7.31 (1H, m, H-3″, 5″), 7.58 (1H, *J* = 7.8 Hz, H-7), 7.6 (1H, dd, *J* = 7.6 Hz, H-5), 7.7 (1H, ddd, *J* = 7.5,1.2 Hz, H-6), 7.99 (1H, dd, *J* = 7.6, 0.9Hz, H-8) ppm; EIMS (70ev): *m/z* (%) 286 (M^+, 36.3), 288 (M^+ +2, 12.5), 167 (33.9), 125 (100), 103 (12.9); HRMS: *m/z* Found; 286.0761 (Cl^{35}) (Calcd for

$C_{17}H_{13}O_2Cl^{35}$; 286.0761) & Found; 288.0733 (Cl^{37}) (Calcd for $C_{17}H_{13}O_2Cl^{37}$; 288.0731).

Biological Studies

Antifungal Studies

Isocoumarins, dihydroisocoumarins, and related compounds were tested by agar tube dilution method [19] for their *in vitro* fungicidal bioassay. Results were reported as linear growth inhibition (LGI) against some human pathogens (e.g *Trichophyton longifusus, Candida albicans, Aspergillus flavus, Microsporum canis, Fusarium solani,* and *Candida glaberata*). Linear Growth Inhibition results of compounds are given in Table **1**.

Antibacterial Studies

Antibacterial activities of all compounds were determined *in vitro* by agar well diffusion method [20, 21]. The well were duged in the media with the help of a sterile metallic borer with centers at least 24 mm a part with two to eight hours old bacterial in culums contaminating approximately 10^4-10^6 colony forming units (CFU)/ml spread on the surface of nutrient agar with the help of a sterile cotton swab rotating firmly against the upper inside well of the lube to express excess fluid. Entire agar surface of the plate was streaked with the swab three times turning the plate 60 °C between each streaking. Recommended concentration of the test sample (3 mg/ml of DMSO) was then added in their respective wells. Other wells supplemented with DMSO and reference antibacterial drugs serving as negative and positive controls, respectively. The plates were incubated immediately at 37 °C for 14-19 hours or later if necessary. Activity was determined by measuring the diameter of zones showing complete inhibition (mm). Growth inhibition was calculated with reference to positive control. Imipenem was used as standard drugs against various strains of bacteria at a concentration of 3 mg/ml of DMSO solution. The results are reported in mm of zone diameter limits. Mostly compounds were active against bacteria *Staphyloccus aureus, Pseudomonas aeruginosa,* and *Salmonella typhi.* None of the compound was active against bacteria *Escherichia coli, Bacillus subtitis,* and *Shigella flexnari* except 5e, which showed low activity against *Escherichia coli.* Results are shown in Table **2**.

Cytotoxicity (Brine Shrimp Lethality) Studies

Brine shrimp (Artemia salin) eggs were hatched in a shallow rectangular plastic dish (22 x 32) filled with artificial seawater, which was prepared with commercial salt mixture (Instant Ocean, Aquarium Inc, Mentore Ohio, USA) and double distilled water. An unequal partition was made in the plastic dish with the help of a perforated device. Approximately 50 mg of eggs were sprinkled into the large compartment, which was darkened, while the smaller compartment was opened to ordinary light. After two days a pipette collected nauplii from the lighted side. A sample of the test compound was prepared by dissolving 20 mg of each compound into 2 ml of methanol; from the stock solution, 100, 10, and 1 microgram per ml was transferred to 9 vials, three of each dilution, and 1 vial was kept as control having 2 ml of methanol only. The solvent was allowed to evaporate overnight. After two days, when Shrimp larvae were ready 1 ml of seawater and 10 shrimp were added to each vial (30 shrimps/dilution) and the volume was adjusted with seawater to 5 ml per vial. After 24 h, the number of survivors was counted and data was analyzed by Finney computer program to determine LD_{50} [21-23] Table **3**.

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