Indian Journal of Chemistry
Vol. 46B, August 2007, pp. 1322-1327

# Synthesis and antimicrobial evaluation of some new 3-alkyl-, 3-(2′-naphthyl)isocoumarins and their (*dl*)-3,4 dihydroderivatives

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*Received 25 May 2006; accepted (revised) 7 May 2007*

3-(Substituted)isocoumarins **3a-c** are synthesized by the condensation of homophthalic acid **1** with acid chlorides **2a-c**, which on alkaline hydrolysis give keto-acids **4a-c**. (*dl*)-3-(Substituted)-3,4-dihydroisocoumarins **7a-c** are obtained by the reduction of keto-acids **4a-c** to racemic hydroxy-acids **6a-c** followed by cyclodehydration using acetic anhydride. The compounds **3a-c, 4a-c, 5c** and **7a-c** are assayed for antifungal activity against *T. longifusus, C. albicans, A. flavus, M. canis, F. solani* and *C. glaberata*. Structure activity relationship reveals that the antifungal activity of naphthyl substituted isocoumarin is better than that of alkyl substituted isocomarins. Same compounds are also evaluated for *in vitro* antibacterial activity against different strains of gram-negative and gram-positive bacteria. Antifungal activities of **3c, 5c** and **7c** are found to be quite higher than the standard drugs against *T. longifusus* and *A. flavus.*

**Keywords**: Isocoumrains, dihydroisocoumarins, synthesis, antimicrobial activity

Isocoumarins and 3,4-dihydroisocoumarins are the secondary metabolites of a wide variety of fungi, lichens, molds, bacteria, higher plants and insects. These compounds have shown a wide range of applications and biological activities such as antifungal, antitumor or cytotoxic, anti-inflammatory, anti allergic and enzyme inhibitory activity[1-3]. 3-Substituted-3,4-dihydroisocoumarins occur as mycotoxins, fungal metabolites[4] and are principal sweetener components of Japanese sweet tea[5,6]. These isocoumarins also have significant physiological activities[7,8].

In continuation of our previous studies[9-12], we have synthesized some new compounds **3a-c, 4a-c, 5c** and **7a-c**, which are evaluated for antifungal activity against *Trichophyton longifusus, Candida albicans, Aspergillus flavus, Microsporum canis, Fusarium solani* and *Candids glaberata*. These compounds are also evaluated for their antibacterial activity against *Escherchia coli, Bacillus subtilis, Shigella flexenari, Staphylococcus aureus, Pseudomonas aeruginosa* and *Salmonella typhi*. However the synthesis of compound **7c** is also reported by different routes[13-16].

## Chemistry

Synthesis of the compounds **3a-c, 4a-c, 5c** and **7a-c** is carried out as shown in **Scheme I**. Commercially available homophthalic acid **1** served as the starting material which is condensed with acid chlorides **2a-c** at 200°C to afford the 3-(substituted)isocoumarins **3a-c** according to the literature procedure[10,11]. In this reaction, acid chlorides are taken in excess, which worked as solvent also. These isocoumarins **3a-c** have shown characteristic 1H singlet at δ 6.22-7.09 for $C_4$–H in $^1$H NMR. The aromatic protons appeared in the acceptable region *i.e.* δ 7.31-8.69. In IR spectra, the lactonic carbonyl absorptions are observed at 1738, 1720 and 1709 $cm^{-1}$ respectively. Mass spectra of these isocoumarins **3a-c** showed molecular ion peaks [$M^+$] at different intensities, which confirmed their molecular masses.

Alkaline hydrolysis of **3a-c** has yielded the keto-acids **4a-c** in good yield. Small amount of keto-acids **4a-c** are treated with acetic anhydride to get back the isocoumarins **3a-c** by cyclodehydration. A characteristic 2H singlet at δ 4.08 for **4a** and at 4.03 for **4b** is observed for benzylic –$CH_2$ in $^1$H NMR. In IR spectra of **3a-c**, two absorptions, one for ketonic and other for carboxylic carbonyl, are observed in the region 1707, 1708, 1738 & 1683, 1685, 1662 $cm^{-1}$ respectively. Appearance of second absorption for carboxylic carbonyl indicated the conversion of isocoumarins to keto-acids respectively. In EIMS

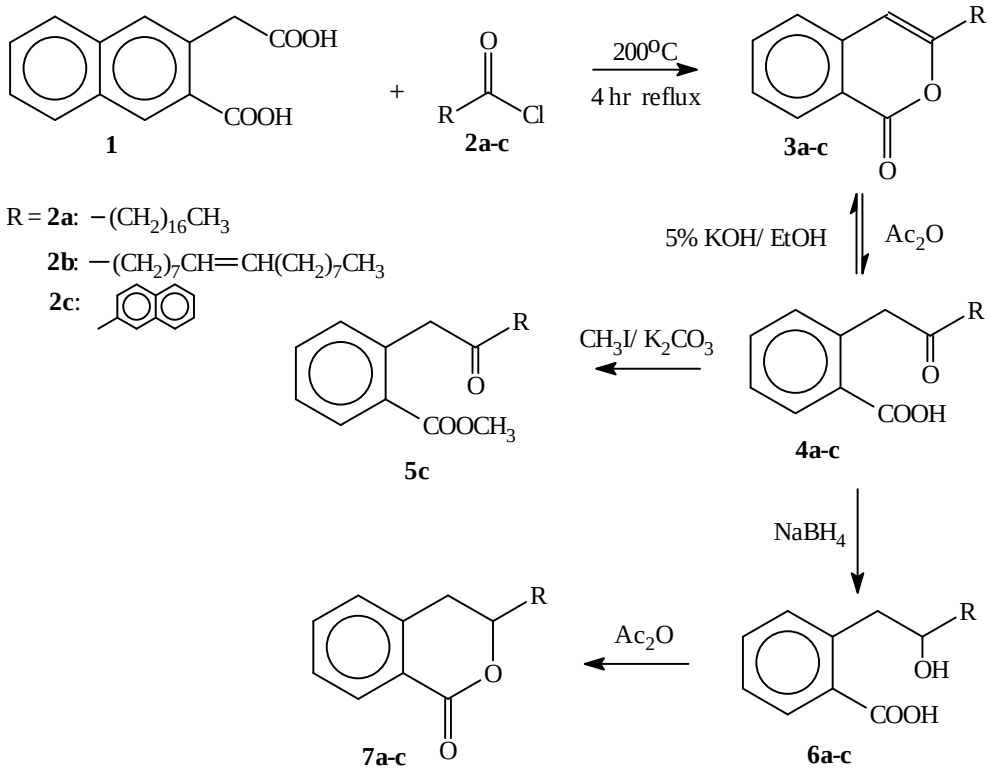
spectra, characteristic $M^+–H_2O$ peaks are observed whose intensity is much higher than that of $M^+$ peaks. This may be attributed to instability of keto-acids at higher temperature. The isocoumarins, which are obtained back on refluxing the keto-acids **4a-c** with acetic anhydride, showed similar melting points and spectral data as of already synthesized isocoumarins **3a-c**.

Methylation of **4c** with excess of methyl iodide has yielded the methyl keto-ester **5c** which is confirmed by 3H singlet for $OCH_3$ at δ 3.96 in $^1H$ NMR while ketonic and ester carbonyl absorptions in IR spectrum appeared at 1695 & 1705 $cm^{-1}$. EIMS spectrum of **5c** exhibited a typical $M^+–CH_3OH$ peak.

Sodium borohydride reduction of the keto-acids **4a-c** furnished corresponding racemic hydroxy-acids **6a-c** which are cyclodehydrated with acetic anhydride in crude form to produce (*dl*)-3-(substituted)-3,4-dihydroisocoumarins **7a-c** which exhibited the carbonyl absorptions at 1705, 1725 and 1707 $cm^{-1}$ respectively in IR spectra. A typical AB pattern for $C_3–H$ and ABX pattern for $C_4–H$ protons is observed in $^1H$ NMR spectra of these compounds **7a-c**. Thus each proton of $C_4–H$ has shown double doublet at δ 2.43 for **7a** and at 2.31 & 2.44 for **7b**. The other double doublet is observed at δ 4.47-4.52 due to $C_3–H$ for **7a** and at 4.19-4.24 for **7b**. In case of the dihydroisocoumarin **7c** two double doublets are observed for $C_4–H$ at δ 3.19-3.45 and another double doublet at δ 5.72 for $C_3–H$. The molecular masses of these compounds **7a-c** are confirmed by the appearance of molecular ion peaks [$M^+$] in their EIMS spectra. The corresponding IR, $^1H$ NMR and mass spectral data for all the synthesized compounds are presented in experimental section.

**Pharmacology**

The compounds **3a-c, 4a-c, 5c** and **7a-c** are evaluated *in vitro* for antibacterial activity by standard agar well diffusion method[13] against various gram-negative and gram-positive bacteria. Antifungal activity of aforementioned compounds is examined by agar tube dilution method against six different fungi: *Trichophyton longifusus, Candida albicans, Aspergillus flavus, Microsporum canis, Fusarium solani* and *Candida glaberata*. Imipenem was used as standard drug to compare the antibacterial activity of the tested compounds. Miconazole and amphotericin-




**Scheme I**

B were used as reference standards for antifungal activity **Table I**. The compounds have shown much greater antifungal activity than reference standards against *Trichophyton longifusus* and *Aspergillus flavus*, whereas the activity of **3c, 4c, 5c** and **7c** against *Microsporum canis* and *Fusarium solani* was almost equal to the standards. However, all the tested compounds have shown poor antibacterial activity.

## Results and Discussion

In the present study, three isocoumarins **3a-c** and three 3,4-dihydroisocoumarins **7a-c** and four related compounds **4a-c** and **5c** are synthesized and evaluated for their antifungal and antibacterial activities. Synthesis of these compounds has been carried out as depicted in the synthetic scheme. All the synthesized compounds are purified by column chromatography and characterized by their physical, analytical and spectral data.

Antifungal activities of the tested compounds are summarized in **Table I**. Results reveal that the compounds have shown good antifungal activities but antibacterial activity is either very low or nil. All the tested compounds are inactive against fungi *Candida albicans* and *Candida glaberata*. Antifungal activity of compounds **3c, 5c** and **7c** against *Trichophyton longifusus* and *Aspergillus flavus* is higher than the reference standard drugs miconazole and amphotericin-B. Moreover, their activity against *Fusarium solani* is almost equal to the standards.

Structure activity relationship reveals that the antifungal activity of naphthyl substituted isocoumarins is better than that of alkyl substituted isocoumarins.

## Experimental Section

Melting points of the compounds are determined in open capillaries using Gallenkemp melting point apparatus and are uncorrected. Purity of synthesized compounds is checked by precoated TLC plates (E-Merck Kiesel gel 60-$F_{254}$). The infrared (IR) spectra are recorded on a Hitachi model 270-50 spectrophotometer as KBr discs or as neat liquids. $^1$H NMR (500 MHz) spectra are recorded on a Brucker AM-400 as $CDCl_3$ solutions using TMS as internal standard. The chemical shifts ($\delta$) are reported in parts per million (ppm) relative to TMS in $CDCl_3$ solutions. Mass spectra (EIMS) are recorded at 70 eV on a MAT-112-S spectrometer. All chemicals used for the synthesis are purchased from Aldrich Company Ltd., Dorset (UK).

**Preparation of 3-Alkyl- and 3-(2′-Naphthyl)isocoumarins, 3a-c**. A mixture of homophthalic acid **1** (11.2 mmole) and acid chlorides **2a-c** (17 mmole) are heated under reflux at 200°C for 3 hr using an oil-bath. The residue after concentration is chromatographed on silica gel column using pet. ether (60-80° fraction) to give isocoumarins **3a-c** as colorless solids. These are further purified by crystallization from methanol.

**3-Heptadecylisocoumarin, 3a**: Yield 88%, m.p. 56°C, recrystallized from methanol. IR (KBr, $cm^{-1}$): 1738 (C=O, lactone), 1464 (C=C, aromatic), 3010 (C–H, aromatic), 2918 (C–H, aliphatic); $^1$H NMR ($CDCl_3$): 0.85 (3H, t, $J$ = 7.02, 6.51 Hz, 17′-$CH_3$), 1.21-1.25 (30H, m, $CH_2$ 2′-15′), 1.57-1.61 (2H, m, H-16′), 2.26 (2H, t, $J$ = 7.50 Hz, H-1′), 6.22 (1H, s, 4-H), 7.32 (1H, d, $J$ = 7.82 Hz, H-5), 7.63 (1H, dd, $J$ = 7.75, 7.40 Hz, H-6), 7.42 (1H, dd, $J$ = 7.97, 7.32 Hz, H-7), 8.23 (1H, d, $J$ = 7.89 Hz, H-8); EIMS (70 eV), *m/z* (%): 384 (34) [$M^+$], 173 (37), 159 (15), 145 (8), 118 (100), 117 (10).

**3-[(8*E*)-Heptadec-8-enyl]isocoumarin, 3b**: Yield 80%, oil. IR (KBr, $cm^{-1}$): 1720 (C=O, lactone), 1459 (C=C, aromatic), 3001 (C–H, aromatic), 2918 (aliphatic C–H stretching); $^1$H NMR ($CDCl_3$): 0.849 (3H, t, $J$ = 6.77, 5.44 Hz, 17′-$CH_3$), 1.22-1.27 (30H, m, H 2′-6′ & 11′-16′), 1.58-1.89 (2H, m, H-16′), 1.93-

**Table I —** Antifungal activity

| Compounds→ Fungi↓ | Linear Growth Inhibition (%) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **3a** | **3b** | **3c** | **4a** | **4b** | **4c** | **5c** | **7a** | **7b** | **7c** | **Std. Drug** | **Inhibition (%)** |
| *Trichophyton longifusus* | – | 40 | 80 | 50 | 55 | 65 | 75 | 30 | 50 | 80 | M | 70 |
| *Candida albicans* | – | – | – | – | – | – | – | – | – | – | M | 100 |
| *Aspergillus flavus* | – | 35 | 60 | – | – | – | 60 | – | 40 | 60 | A | 20 |
| *Microsporum canis* | 40 | 30 | 75 | 55 | 50 | 75 | 70 | 30 | – | 75 | M | 98 |
| *Fusarium solani* | 20 | – | 70 | – | 40 | 70 | 60 | – | – | 70 | M | 73 |
| *Candida glaberata* | – | – | – | – | – | – | – | – | – | – | M | 100 |

M = Miconazole, A = Amphotericin-B, Conc. used 400 μg/mL – = Inactive

1.98 (4H, m, H-7′,10′), 2.31 (2H, t, *J* = 7.42, 7.39 Hz, H-1′), 5.31 (2H, t, *J* = 13.91, 7.01 Hz, H-8′,9′), 6.22 (1H, s, 4-H), 7.31 (1H, d, *J* = 7.70 Hz, H-5), 7.41 (1H, dd, *J* = 7.54, 7.41 Hz, H-7), 7.63 (1H, dd, *J* = 7.59, 7.31 Hz, H-6), 8.22 (1H, d, *J* = 7.83 Hz, H-8); EIMS (70 eV), *m/z* (%): 382 (3.0) [$M^+$], 229 (13.5), 173 (21.3), 159 (8.2), 145 (7.1), 118 (100), 57 (17.2).

**3-(2′-Naphthyl)isocoumarin, 3c**: Yield 66%, m.p. 123°C, recrystallized from methanol. IR (KBr, $cm^{-1}$): 1709 (C=O, lactone); $^1$H NMR ($CDCl_3$): 7.09 (1H, s, H-4), 7.49-7.62 (4H, m, H-6,7,6′,7′), 7.89 (3H, d, *J* = 8.1, H-4′,5′,8′), 7.97 (1H, d, *J* = 7.98 Hz, H-5), 8.09 (2H, d, *J* = 8.1 Hz, H-8,3′), 8.69 (1H, s, H-1′); EIMS (70 eV), *m/z* (%): 272 (10) [$M^+$], 145 (2), 127 (100), 118 (4), 101 (11), 89 (7).

**Preparation of 2-(Substituted)benzoic acids, 4a-c**. A solution of 3-(substituted)isocoumarins **3a-c** (5.3 mmole) in ethanol (50 mL) and potassium hydroxide (5% 100 mL) is refluxed for 4 hr. Ethanol is removed from the reaction mixture by distillation. Cold water (20 mL) is added followed by acidification with hydrochloric acid and then extracted with dichloromethane (3 × 20 mL), dried ($Na_2SO_4$) and solvent rotary evaporated to yield a crude solid, which is recrystallized to give **4a-c**.

The keto-acids **4a-c** are converted back into isocoumarins **3a-c** on refluxing with acetic anhydride for 1 hr. These isocoumarins also had the same $R_f$ values as of those synthesized earlier.

**2-(2′-Oxononadecyl)benzoic acid, 4a**: Yield 83%, m.p. 50°C, recrystallized from methanol. IR (KBr, $cm^{-1}$): 1707 (C=O, keto), 1683 (C=O, carboxylic), 1463 (C=C, aromatic), 3414 (OH); $^1$H NMR ($CDCl_3$): 0.85 (3H, t, *J* = 7.02, 6.51 Hz, 19′-$CH_3$), 1.23-1.27 (28H, m, $CH_2$ 4′-17′), 1.56-1.63 (2H, m, H-18′), 2.32 (2H, t, *J* = 7.51 Hz, H-3′), 4.08 (2H, s, H-1′), 7.16 (1H, d, *J* = 4.4 Hz, H-3), 7.35 (1H, ddd, *J* = 7.75, 7.45 Hz, H-4), 7.50 (1H, dd, *J* = 7.41, 6.31 Hz, H-5), 8.1 (1H, d, *J* = 6.74 Hz, H-6), 9.83 (1H, bs, –COOH, $D_2O$ exchangeable); EIMS (70 eV), m/z (%): 384 (2) [$M^+$–$H_2O$], 267 (3), 135 (4), 118 (100), 91 (2).

**2-[(10*E*)-2′-Oxononadec-10′-enyl)benzoic acid, 4b**: Yield 84%, oil. IR (KBr, $cm^{-1}$): 1708 (C=O, keto), 1685 (C=O, carboxylic), 1459 (C=C, aromatic), 3400 (OH); $^1$H NMR ($CDCl_3$): 0.849 (3H, t, *J* = 6.77, 5.44 Hz, 19′-$CH_3$), 1.22-1.27 (22H, m, H 4′-8′ & 13′-18′), 194-198 (4H, m, H-9′,12′), 2.31 (2H, t, *J* = 7.44, 7.42 Hz, H-3′), 4.03 (2H, s, H-1′), 5.33 (2H, t, *J* = 13.91, 7.01 Hz, H-10′,11′), 7.16 (1H, d, *J* = 7.48 Hz, H-3), 7.34 (1H, d, *J* = 7.36 Hz, H-4), 7.49 (1H, dd, *J* = 7.30, 7.29 Hz, H-5), 8.05 (1H, d, *J* = 7.70 Hz, H-6), 10.16 (1H, bs, –COOH, $D_2O$ exchangeable); EIMS (70 eV), *m/z* (%): 382 (3.3) [$M^+$–$H_2O$], 265 (2), 145 (7), 139 (3), 117 (6), 85 (6), 71 (12), 57 (100).

**2-(2′-Naphthoylmethyl)benzoic acid, 4c**: Yield 68%, m.p. 180°C, recrystallized from methanol. IR (KBr, $cm^{-1}$): 1738 (C=O, keto), 1662 (C=O, carboxylic), 3405 (OH); $^1$H NMR ($CDCl_3$): 3.3 (2H, s, $CH_2$), 7.53-7.61 (4H, m, H-4,5,6′,7′), 7.91 (3H, d, *J* = 8.1 Hz, H-4′,5′,8′), 7.98 (1H, d, *J* = 6.5 Hz, H-3), 8.02 (2H, d, *J* = 6.8 Hz, H-6,3′), 8.59 (1H, s, H-1′), 9.83 (1H, bs, –COOH, $D_2O$ exchangeable); EIMS (70 eV), *m/z* (%): 272 (10) [$M^+$–$H_2O$], 172 (100), 155 (50), 145 (2), 127 (64), 101 (7), 76 (5).

**Methyl-2-(2′-naphthoylmethyl)benzoate, 5c**. The keto-acid **4c** (0.73 mmole), methyl iodide in excess and anhydrous potassium carbonate (1.5 g) in dry acetone (15 mL) is heated under reflux for 2 hr. The reaction mixture is filtered while hot. The cake is washed with warm dry acetone (10 mL) and solvent evaporated to give a crude solid, which is purified by column chromatography using silica gel and pet. ether to afford **5c**.

Yield 67%, m.p. 70°C, recrystallized from methanol. IR (KBr, $cm^{-1}$): 1705 (C=O, ester), 1695 (C=O, keto); $^1$H NMR ($CDCl_3$): 3.30 (2H, s, $CH_2$), 3.96 (3H, s, $OCH_3$), 7.53-7.61 (4H, m, H-4,5,6′,7′), 7.91 (3H, d, *J* = 8.1, H-4′,5′,8′), 7.98 (1H, d, *J* = 6.5 Hz, H-3), 8.02 (2H, d, *J* = 6.8 Hz, H-6,3′), 8.59 (1H, s, H-1′); EIMS (70 eV), *m/z* (%): 304 (5) [$M^+$],272 (10) [$M^+$–$CH_3OH$], 186 (59), 155 (99), 127 (100), 101 (10), 75 (13), 51 (24).

**(*dl*)-3-(Substituted)-3,4-dihydroisocoumarins, 7a-c**. The keto-acids **4a-c** (0.5 g, 2.07 mmole), prepared as described above, are dissolved in potassium hydroxide solution (1%, 25 mL) and sodium borohydride (0.25 g) is added. The mixture is stirred for 1 hr at room temp. After being acidified with HCl, the whole mixture is extracted twice with ethyl acetate (2 × 50 mL). Usual work-up give crude hydroxy acids **6a-c** (0.41 g), which are dissolved in acetic anhydride (1 mL) and heated under reflux for 2 hr. The mixture is cooled, water (25 mL) is added and the whole is stirred continuously overnight. The deposited crystals are collected by filtration and filtrate is extracted twice with dichloromethane (2 × 20 mL). After the removal of the solvent, the crude compound is purified by column chromatography on silica gel with pet. ether.

**(*dl*)-3-(Heptadecyl)-3,4-dihdyroisocoumarin, 7a**: Yield 80%, m.p. 37°C, recrystallized from methanol. IR (KBr, $cm^{-1}$): 2917 ($CH_2$, stretching), 1705 (C=O, lactone), 1464 (C=O, aromatic); $^1$H NMR ($CDCl_3$): 0.65 (3H, t, *J* = 6.84, 7.02 Hz, 17′-$CH_3$), 1.23-1.27 (30H, m, $CH_2$ 2′-16′), 1.59-1.65 (2H, m, 1′-$CH_2$), 2.30 (1Ha, d, *J* = 7.55, 2.0 Hz, H-4), 2.43 (1Hb, d, *J* = 7.44, 3.0 Hz, H-4), 4.47-4.52 (1H, ddd, *J* = 7.72, 5.21, 3.74, 1.5 Hz, H-3), 7.2 (1H, d, *J* = 7.5 Hz, H-5), 7.36 (1H, d, *J* = 7.6, 7.32 Hz, H-6), 7.5 (1H, dd, *J* = 7.52, 7.42 Hz, H-7), 8.1(1H, d, *J* = 7.79 Hz, H-8); EIMS (70 eV), m/z (%): 386 (82) [$M^+$], 189 (4), 175 (9), 161 (29), 147 (93), 119 (86), 118 (100), 90 (56).

**(*dl*)-3-[(8*E*)-Heptadec-8enyl)-3,4-dihdyroisocoumarin, 7b**: Yield 81%, oil. IR (KBr, $cm^{-1}$): 2917 ($CH_2$, stretching), 1725 (C=O, lactone), 1456 (C=O, aromatic); $^1$H NMR ($CDCl_3$): 0.85 (3H, t, *J* = 6.82, 5.62 Hz, 17′-$CH_3$), 1.23-1.28 (22H, m, $CH_2$ 2′-6′ & 11′-16′), 1.58-1.60 (2H, m, H-′), 1.94-1.98 (4H, m, H-7′,10′), 2.31 (1Ha, dd, *J* = 7.55, 2.10 Hz, H-4), 2.44 (1Hb, d, *J* = 7.45, 3.10 Hz, H-4), 4.19-4.24 (1H, ddd, *J* = 7.46, 6.63, 5.64 Hz, H-3), 5.31 (2H, d, *J* = 13.91, 7.0 Hz, H-8′,9′), 7.31 (1H, d, *J* = 7.87 Hz, H-5), 7.41 (1H, dd, *J* = 7.56, 7.48 Hz, H-6), 7.63 (1H, dd, *J* = 7.51, 7.42 Hz, H-7), 7.88 (1H, d, *J* = 7.8 Hz, H-8); EIMS (70 eV), *m/z* (%), 384 (69) [$M^+$], 341 (3), 285 (4), 203 (2), 147 (92), 118 (100).

**(*dl*)-3-(2′-Naphthyl)-3,4-dihdyroisocoumarin, 7c**: Yield 72%, m.p. 136-37°C [lit.[14], 137-38°C], recrystallized from methanol. IR (KBr, $cm^{-1}$): 1707 (C=O, lactone), 1464 (C=O, aromatic); $^1$H NMR ($CDCl_3$): 3.21-3.40 (1Ha, dd, *J* = 11.9, 3.1 Hz, H-4), 3.19-3.45 (1Hb, d, *J* = 11.9, 3.0 Hz, H-4), 5.72 (1H, dd, *J* = 11.9, 2.9 Hz, H-3), 7.53-7.61 (4H, m, H-6,7,6′,7′), 7.91-7.92 (3H, d, *J* = 8.1, H-4′,5′,8′), 7.89 (1H, d, *J* = 7.98 Hz, H-5), 8.01-8.02 (2H, d, *J* = 8.05 Hz, H-8,3′), 8.59 (1H, s, H-1′); EIMS (70 eV), *m/z* (%): 274 (11) [$M^+$], 172 (100), 156 (6), 155 (49), 127 (68), 118 (53), 90 (19).

## Pharmacology

### Antifungal activity

The synthesized compounds **3a-c, 4a-c, 5c** and **7a-c** are tested by agar tube dilution method[17] for their antifungal activities at 400 μg/mL concentration. The results are reported (**Table I**) as linear growth inhibition (LGI) against some human, animal and plant pathogens. It is evident from these results that the compounds **3c, 5c** and **7c** have considerable growth inhibition against all the tested fungi except *Candida albicans* and *Candida glaberata*. Some compounds **3a, 4a** and **7a** have shown somewhat low activity against some pathogens as compared to standard drug miconazole. However, mutual comparison shows that the order of antifungal activity of these compounds is: isocoumarins > keto-acids > dihydro-isocoumarins > keto-esters. Activity of compounds **3c, 5c** and **7c** against *Trichophyton longifusus* and *Aspergillus flavus* is higher than the standard drugs (miconazole & amphotericin-B). Compounds **3b, 3c, 5c, 7b** and **7c** have also shown higher activity against *Aspergillus flavus* than the standard drug amphotericin-B.

### Antibacterial activity

Antibacterial activity of compounds **3a-c, 4a-c, 5c** and **7a-c** is determined *in vitro* by standard agar well diffusion method[18] against various gram-negative and gram-positive bacteria at a concentration of 200 μg/100 μL in DMSO solution. Imipenem was used as standard drug. 24 Hr old culture, containing approximately $10^4$–$10^6$ colony farming units (CFU) is spread on the surface of Muller Hinton Agar (MHA) plates. Wells are created in the medium with the help of a sterile metallic borer. Test samples are added in their respective wells. Experimental plates are incubated at 37°C for 24 hr and zones of inhibition are measured and compared with standard drug. Results indicates that all the tested compounds are either inactive or have very low activity.

## Acknowledgements

The authors gratefully acknowledge for the funds from URF project, Quaid-i-Azam University, Islamabad, Pakistan.

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