Synthesis, characterization and biological activities of novel chalcone derivatives, containing 4,7-ethanoisoindole-1,3-dione units

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Abstract: Novel chalcone derivatives, containing 4,7-ethanoisoindole-1,3-dione units were synthesized starting from 1,3-cyclohexadine (4) and maleic anhydride (5). Addition of maleic anhydride (5) to 1,3-cyclohexadine (4) gave an endo-adduct, 3a,4,7,7a-tetrahydro-4,7-ethano-2-benzofuran-1,3-dione (6), in 90% yield. Heating the solution of the adduct dione (6) and 1-(4-aminophenyl)ethanone (7) in the presence of Et₃N in toluene at 110 °C for 24 hours afforded 2-(4-acetylphenyl)-3a,4,7,7a-tetrahydro-1H-4,7-ethanoisoindole-1,3-dione (8) in high yield. Piperidine-catalyzed addition of benzaldehyde derivatives (9a-i) to the compound 8 in CH₂Cl₂ at 55 °C gave the expected chalcone derivatives (10a-i) in the range of 42% - 96% yields. The antibacterial activities of the chalcone derivatives (10a-i) were evaluated against human pathogenic microorganism and the compounds showed low activity compared to the standard, name of the standard.

Key Words: 4,7-Ethanoisoindole-1,3-dione; chalcone; antibacterial activity; SCF.

1. Introduction

Chalcone is a general name for the compounds containing 1,3-diaryl-2-propane-1-one unit, which are members of flavonoids. Natural or synthetic chalcones are known to have various biological activities,¹ such as antioxidant,² antimalarial,³⁵ anticancer,⁶,⁷ antitumor,⁸ antimicrobial,⁹,¹⁰ antibacterial,¹¹ antidiabetic,¹² anti-inflammatory,¹² anti-tuberculosis, anti-fungal,¹³ and antileishmanial.¹ Moreover, the derivatives containing sulfonamide (1), ester (2) and pyrrole-2,5-dione units (3) have important biological activities such as antimalarial,¹⁴ antitumor,¹⁵ anti-pigment,¹⁶ and photosensitive¹⁷ and cytotoxic.¹⁸ It was reported that chalcones have potential of inhibiting HIV virus¹⁹ and active toward leukemia.²⁰ As chalcones have also had various applications such as in optical materials as UV-absorbing filters, food industry and holographic paper technologies²¹ and medical treatments, development of new chalcone derivatives has been the interest of research groups.

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In this study, syntheses and antibacterial activities of novel chalcone derivatives, containing 4,7-ethanoisoindole-1,3-dione unit has been reported.

2. Results and Discussion

Addition of maleic anhydride (5) to 1,3-cyclohexadine (4) gave the endo-adduct (6) in 90 % yield (Figure 1). Heating mixture of the adduct (6) and 1-(4-aminophenyl)ethanone (7) in the presence of Et$_3$N in toluene at 110 °C for 24 hours gave 2-(4-acetylphenyl)-3a,4,7,7a-tetrahydro-1H-4,7-ethanoisoindole-1,3-dione (8) in high yield (say percent? or say quantitatively) (Scheme).

Structure of the compound 8 was explained on the basis of spectral data, in the $^1$H NMR spectrum of which, disappearance of –NH$_2$ at ??? and appearance of appearing an AA’XX’ system at 8.05 (brd, $J = 8.4$ Hz) and 7.36 (brd, $J = 8.4$ Hz) ppm from aryl protons indicated the proposed structure. Furthermore, the two signals of the carbonyl carbons at 197.0 and 177.6 (2 C=O) ppm due to the symmetry structure of the compound 8, totaling to 11 signals in the $^{13}$C NMR spectrum and the molecular-ion peak appeared at $m/z$ 295 (M$^+$) are in accordance with the structure of 8.

A conventional method was applied for synthesis of the chalcone derivatives (10a-I) As NaOH-catalyzed reaction of 8 with the benzaldehyde derivatives (9a-i) in EtOH produced a mixture of hydrolyzed and semi-hydrolized products along with chalcone derivatives (10a-i), piperidine was replaced with NaOH, which yielded the expected chalcone derivatives (10a-i) in the range of 42 - 96 % yields in CH$_2$Cl$_2$ at 55 °C (Scheme, Table 1). Although the yields of calcone in the case of the 4-substituted benzaldehydes were high (Table 1, entry 2 and 3), the 2-substituted benzaldehyde gave lower yield (Table 1, entry 7).

Figure 1. Synthesis of the chalcone derivatives (10a-i), containing 4,7-ethanoisoindole-1,3-dione unit.
Structures of the synthesized chalcone derivatives (10a-i) were characterized on the basis of the spectral data (\(^1\)H-NMR, \(^{13}\)C NMR, IR and Mass). Olefinic H atoms of \(\alpha,\beta\)-unsaturated moiety of 10a-i produced an AB system (A part of AB system, doublet, \(J = 15.8\text{-}15.2\) Hz and B part of AB system, doublet \(J = 15.8\text{-}15.2\) Hz) in the range of 7.96\text{-}7.73 and 7.51\text{-}7.39 ppm, respectively. The coupling constants, \(J = 15.8\text{-}15.2\) Hz, confirms a trans configuration of the compounds 10a-i. The \(^{13}\)C-NMR spectra of 10a-i showed the characteristic carbon atom of the amide (O=C-N) with a chemical shift at 177 ppm.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compounds</th>
<th>M.p. (°C)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10a</td>
<td>192</td>
<td>70</td>
</tr>
<tr>
<td>2</td>
<td>10b</td>
<td>226</td>
<td>89</td>
</tr>
<tr>
<td>3</td>
<td>10c</td>
<td>220</td>
<td>96</td>
</tr>
<tr>
<td>4</td>
<td>10d</td>
<td>225</td>
<td>52</td>
</tr>
<tr>
<td>5</td>
<td>10e</td>
<td>217</td>
<td>87</td>
</tr>
<tr>
<td>6</td>
<td>10f</td>
<td>244</td>
<td>67</td>
</tr>
<tr>
<td>7</td>
<td>10g</td>
<td>179</td>
<td>42</td>
</tr>
<tr>
<td>8</td>
<td>10h</td>
<td>185</td>
<td>85</td>
</tr>
<tr>
<td>9</td>
<td>10i</td>
<td>210</td>
<td>71</td>
</tr>
</tbody>
</table>
Antibacterial activities of the chalcones (10a-i) against 7 microorganisms (Staphylococcus aureus ATCC 29213 (gram +), Escherichia coli 111, Pseudomonas aeruginosa ATCC 9027, Salmonella enteridis ATCC 13076 (gram -), Candida albicans ATCC 1213 and Candida utilis KUEN 1031 (yeast)) were determined with the disc diffusion method. SCF (30 µg sulbactam, 75 µg cefoperazone) and DMSO were used as positive and negative controls, respectively. Antibacterial activities were evaluated measuring the zone of inhibition against the test microorganisms (Table 2).

Table 2. Antibacterial activities of the chalcone derivatives (10a-i) (105 µg/disc) against the bacterial strains.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Compounds and inhibition zones (mm)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>10a</td>
</tr>
<tr>
<td>S. aureus ATCC 29213</td>
<td>9</td>
</tr>
<tr>
<td>E. coli 111</td>
<td>-</td>
</tr>
<tr>
<td>P. aeruginosa ATCC 9027</td>
<td>7</td>
</tr>
<tr>
<td>S. enteridis ATCC 13076</td>
<td>7</td>
</tr>
<tr>
<td>C. albicans ATCC 1213</td>
<td>9</td>
</tr>
<tr>
<td>C. utilis KUEN 1031</td>
<td>9</td>
</tr>
</tbody>
</table>

SCF: (Sulbactam (30 µg)+cefoperazone (75 µg)) = positive control
DMSO: = Negative control
NT: Not tested
- : Inactive

The tested compounds (10a-i) showed low antibacterial activity compared to the positive control (SCF) (Table 2). All the compounds (10a-i) had a moderate activity against S. aureus ATCC 29213 and S. enteridis ATCC 13076 with 7, 8 and 9 mm inhibition zone, and while the compounds 10c, f and h showed activity against E. coli 111 (with 8 mm inhibition zone), the other compounds were inactive.

The compounds 10e-g were inactive against P. aeruginosa ATCC 9027. On the other hand the rest of the chalcones possessed low activity. The compounds 10a-c, g and i had remarkable activity (with 8 and 9 mm inhibition zone) against C. albicans ATCC 1213. All compounds except 10e showed remarkable activity against C. utilis KUEN 1031 (with 8, 9 and 10 mm inhibition zone). The compound 10c, bearing chlorine atom on phenyl ring, displayed activity (what low high??) against all the microorganisms. The MIC (minimum inhibition concentration) of the compounds was not determined due to their low activity. (Some were remarkable???)

In conclusion, to our best knowledge, for the first time, a series of the novel 2-{4-[(2 E)-3-(aryl)prop-2-enoyl]phenyl}-3a,4,7,7a-tetrahydro-1H-4,7-ethanoisoindole-1,3-dione (10a-i) were prepared in three steps, starting from 1,3-cyclohexadiene and maleic anhydride. The antibacterial activities of the chalcones were evaluated against human pathogenic microorganism using the SCF as positive control, showed lower antibacterial activity compared to the positive control (SCF).

3. Experimental

3.1. General: Melting points were measured on Electrothermal 9100 apparatus and were not corrected. 1H NMR (400 MHz) and 13C NMR (100 MHz) spectra were measured, using a Bruker Avance 400 MHz with tetramethylsilane as an internal standard in deuterchloroform. IR spectra were recorded on a Jasco FTIR-430 spectrophotometer with NaCl optics. Mass spectra were recorded on a ThermoFinnigan Trace GC/Trace DSQ/A1300 (E.I. Quadrupole, 70 eV) equipped with a SGE-BPX5 MS capillary column (30 m × 0.25 mm i.d., 0.25 µm). Elemental analyses were obtained from a LECO CHNS 932 Elemental Analyzer.

3.2.1. Synthesis of 3a,4,7,7a-tetrahydro-4,7-ethano-2-benzofuran-1,3-dione (6): To a stirred solution of 1,3-cyclohexadiene (4) (2 g, 30 mmol) in 30 ml dichloromethane was added maleic anhydride (5) (2.5 g, 30 mmol) at 5 °C. The mixture was heated to 55 °C and stirred for 4 hours. The reaction mixture was washed with water (50 ml) and the organic layer was dried on Na2SO4. Removal of the
3.2.2. Synthesis of 2-(4-acetylphenyl)-3a,4,7,7a-tetrahydro-1H-4,7-ethanoisoindole-1,3-dione (8): To a stirred solution of 3a,4,7,7a-tetrahydro-1H-4,7-ethanoisoindole-1,3-dione (2 g, 10 mmol) and 4 ml of Et₂N in 25 ml toluene was added 1-(4-aminophenyl)ethanone (1.52 g, 10 mmol). The reaction mixture was heated at 110 °C for 24 hours. After removing the solvent under reduced pressure, the crude product was crystallized from CH₂Cl₂ to obtain the pure product as a yellow solid, mp.: 175 °C. ¹H NMR (400 MHz, CDCl₃, ppm): δ = 8.04, (d, J = 8.4 Hz, 2H; AA’ part of AA’XX’ system), 7.40 (d, J = 8.4 Hz, 2H, XX’ part of AA’XX’ system), 6.33 (brt, J = 4.4 Hz, 2H, olefinic), 3.29 (m, 2H, 1H, olefinic), 2.63 (s, 3H, -CH₃). IR (KCl, cm⁻¹): 177.6(2C), 145.5, 141.3, 138.0, 135.5, 132.5 (2C), 131.9 (2C), 129,7 (2C), 129.2, 128.5 (2C), 126.4 (2C), 119.5, 114.4, 55.4, 44.3 (2C), 32.0 (2C), 23.6 (2C). GC-MS (CH₂Cl₂): 207 (29.49%), 200 (37.33%), 145 (4.03%), 115 (3.50%), 108 (43.97%), 91 (40%), 78 (80%), 65 (10%).

3.2.3. General Procedure for Synthesis of 2-(4-(2E)-3-arylprop-2-enoyl)phenyl]-3a,4,7,7a-tetrahydro-1H-4,7-ethanoisoindole-1,3-dione (10a-i): To a solution of 3a,4,7,7a-tetrahydro-1H-4,7-ethanoisoindole-1,3-dione (8) and benzaldehyde derivatives (9a-i) in a ratio of (1:1) in 20 ml of CH₂Cl₂ was added piperidine (2 equiv.). The reaction mixture was heated at 55 °C for 7 hours. Then, the reaction mixture was washed with water (2 X 50 ml) and 5% solution of HCl. The organic layer was dried over Na₂SO₄ and the solvent was removed under reduced pressure. The crude product was purified by crystallization from n-hexane-CH₂Cl₂ (9:1).

3.2.3.1. Synthesis of 2-(4-(2E)-3-(4-methylphenyl)prop-2-enoyl)phenyl]-3a,4,7,7a-tetrahydro-1H-4,7-ethanoisoindole-1,3-dione (10a): White solid, mp.: 192 °C; yield: 70%. ¹H NMR (400 MHz, CDCl₃, ppm): δ = 8.10 (d, J = 8.4 Hz, 2H; AA’ part of AA’XX’ system), 7.40 (d, J = 8.4 Hz, 2H, XX’ part of AA’XX’ system), 7.81 (d, J = 15.6 Hz, 1H, A part of AB system), 7.46 (d, J = 15.6 Hz, 1H, B part of AB system), 7.56 (d, J = 8.0 Hz, 2H, AA’ part of AA’BB’ system, 4H, -CH₂CH₂-), 7.39 (d, J = 8.0 Hz, 2H BB’ part of AA’BB’ system of –Ph-CH₂), 6.33 (dd, J = 4.4, 3.2 Hz, 2H), 3.30 (m, 2H, 2H, XX’ part of AA’XX’ system), 6.33 (dd, J = 4, 4, Hz, 2H), 3.29 (m, 2H, 2H, XX’ part of AA’XX’ system, 4H, -CH₂CH₂-), 1.71-1.44 (AA’BB’ system, 4H, -CH₂CH₂-). ¹³C NMR (100 MHz, CDCl₃, ppm): δ = 189.7, 177.6(2C), 145.5, 141.3, 138.0, 135.5, 132.5 (2C), 131.9 (2C), 129.7 (2C), 129.2, 128.5 (2C), 126.4 (2C), 120.8, 44.3 (2C), 32.0 (2C), 23.6 (2C). IR (KCl, cm⁻¹): 3043, 2935, 2956, 2935, 2861, 1704, 1662, 1590, 1396, 1332, 1301, 1193, 1024, 1014, 1004, 798, 715. GC-MS (CH₂Cl₂ M⁺): 397 (58.98%), 382 (100%), 317 (46.48%), 302 (32.13%), 289 (0.29%), 281 (16.20%), 221 (8.48%), 207 (31.53%), 200 (51.58%), 145 (73.35%), 115 (77.27%), 91 (60%), 78 (95%), 65 (23%).

3.2.3.2. Synthesis of 2-(4-(2E)-3-(4-methoxyphenyl)prop-2-enoyl)phenyl]-3a,4,7,7a-tetrahydro-1H-4,7-ethanoisoindole-1,3-dione (10b): Yellow solid, mp.: 226 °C; yield: 89%. ¹H NMR (400 MHz, CDCl₃, ppm): δ = 8.08 (d, J = 8.4 Hz, 2H, AA’ part of AA’XX’ system), 7.37 (d, J = 8.4 Hz, 2H, XX’ part of AA’XX’ system), 7.80 (d, J = 15.6 Hz, 1H, A part of AB system), 7.39 (d, J = 15.6 Hz, 1H, B part of AB system), 7.62 (d, J = 8.8 Hz, 2H, A part of AA’XX’ system CH₂OPh), 6.96 (d, J = 8.8 Hz, 2H, XX’ part of AA’XX’ system of CH₂OPh), 6.33 (dd, J = 4, 4, Hz, 2H), 3.88 (s, 3H), 3.29 (m, 2H), 3.07 (m, 2H), 1.71-1.44 (AA’BB’ system, 4H, -CH₂CH₂-). ¹³C NMR (100 MHz, CDCl₃, ppm): δ = 189.7, 177.7(2C), 161.8, 145.2, 138.1, 135.4, 132.5 (2C), 130.3 (2C), 129.1(2C), 127.4 (2C), 126.4 (2C), 119.5, 114.4, 55.4, 44.3 (2C), 32.0 (2C), 23.6 (2C). IR (KCl, cm⁻¹): 3056, 2996, 2935, 2867, 2834, 1704, 1658, 1589, 1569, 1508, 1386, 1294, 1255, 1213, 1191, 1170, 1029, 798, 705. GC-MS (CH₂Cl₂ M⁺): 413 (100%), 397 (0.25%), 382 (7.58%), 302 (6.51%), 289 (0.79%), 281 (10.94%), 221 (8.48%), 207 (29.49%), 200 (37.33%), 145 (4.03%), 115 (3.50%),108 (43.97%), 91 (40%), 78 (80%), 65 (10%).

Novel chalcone derivatives, containing 4,7-ethanoisoindole-1,3-dione units
2-{[2E]-3-(4-chlorophenyl)prop-2-enoyl]phenyl}-3a,4,7,7a-tetrahydro-1H-4,7-ethanoisoindole-1,3-dione (10c): Yellowish solid, mp.: 225 °C. Yield: 52%. \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}, ppm): δ = 8.10 (d, J = 8.8 Hz, 2H, AA' part of AA'XX' system), 7.61 (d, J = 8.8 Hz, 2H, XX' part of AA'XX' system), 7.78 (d, J = 15.6 Hz, 1H, A part of AB system), 7.49 (d, J = 15.6 Hz, 1H, B part of AB system), 7.42 (d, J = 8.4 Hz, 2H, AA' part of AA'BB' system of CIPh)), 7.38 (d, J = 8.4 Hz, 2H, BB' part of AA'BB' system of CIPh), 6.33 (dd, J = 7.8, 4.4 Hz, 2H, olefinic), 3.30 (m, 2H), 3.07 (m, 2H), 1.71-1.47 (AA'BB' system, 4H, -CH\textsubscript{2}CH\textsubscript{2}-). \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}, ppm): δ = 189.7, 177.6(2C), 143.5, 137.4, 136.8, 135.9, 133.4 (2C), 132.5 (2C), 130.9 (2C), 130.5, 129.2, 127.2 (2C), 126.5 (2C), 126.4, 123.1, 122.9, 44.3 (2C), 32.0 (2C), 23.6 (2C). IR (KCl, cm\textsuperscript{-1}): 3056, 2946, 2865, 1704, 1666, 1606, 1556, 1392, 1313, 1189, 1072, 993, 800, 779. GC-MS (CH\textsubscript{3}Cl\textsubscript{2}, M\textsuperscript{+}): 417 (61.33%), 337 (36.65%), 309 (0.27%), 302 (14.64%), 280 (10.72%), 240 (6.01%), 212 (8.47%), 207 (32.63%), 200 (51.23%), 165 (44.54%), 146 (24.46%), 137 (30.48%), 118 (14.99%), 102 (34.60%), 90 (30.3%), 78 (100%), 51 (15%).

2-{[2E]-3-(3-bromophenyl)prop-2-enoyl]phenyl}-3a,4,7,7a-tetrahydro-1H-4,7-ethanoisoindole-1,3-dione (10d): Yellowish solid, mp.: 217 °C. Yield: 87%. \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}, ppm): δ = 8.08 (d, J = 8.4 Hz, 2H, AA' part of AA'XX' system), 7.39 (d, J = 8.4 Hz, 2H, XX' part of AA'XX' system), 7.75 (d, J = 15.6 Hz, 1H, A part of AB system), 7.45 (d, J = 15.6 Hz, 1H, B part of AB system), 7.31 (d, J = 8.0 Hz, 1H), 7.21 (d, J = 7.6 Hz, 1H), 7.12 (m, 1H), 6.91 (dd, J = 8.0, 1.6 Hz, 1H), 6.33 (dd, J = 4.4, 3.2 Hz, 2H, olefinic), 3.31 (m, 2H) 3.08 (m, 2H), 1.72-1.47 (AA'BB' system, 4H, -CH\textsubscript{2}CH\textsubscript{2}-). \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}, ppm): δ = 189.6, 177.8(2C), 156.1, 145.1, 137.7, 136.2, 135.7, 132.5 (2C),130.2 (2C), 129.3 (2C), 126.5, 122.0 (2C), 121.2 (2C), 117.9, 114.8, 44.3 (2C), 32.0 (2C), 29.5, 23.6 (2C). IR (KCl, cm\textsuperscript{-1}): 3409, 3365, 2942, 2861, 1700, 1687, 1664, 1608, 1589, 1442, 1388, 1280, 1214, 1178, 1029, 987, 802. GC-MS (M\textsuperscript{+}): 399.14. 400.15

2-{[2E]-3-(3-hydroxyphenyl)prop-2-enoyl]phenyl}-3a,4,7,7a-tetrahydro-1H-4,7-ethanoisoindole-1,3-dione (13e): Yellowish solid, mp.: 217 °C. Yield: 87%. \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}, ppm): δ = 8.08 (d, J = 8.4 Hz, 2H, AA' part of AA'XX' system), 7.39 (d, J = 8.4 Hz, 2H, XX' part of AA'XX' system), 7.75 (d, J = 15.6 Hz, 1H, A part of AB system), 7.45 (d, J = 15.6 Hz, 1H, B part of AB system), 7.31 (d, J = 8.0 Hz, 1H), 7.21 (d, J = 7.6 Hz, 1H), 7.12 (m, 1H), 6.91 (dd, J = 8.0, 1.6 Hz, 1H), 6.33 (dd, J = 4.4, 3.2 Hz, 2H, olefinic), 3.31 (m, 2H) 3.08 (m, 2H), 1.72-1.47 (AA'BB' system, 4H, -CH\textsubscript{2}CH\textsubscript{2}-). \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}, ppm): δ = 189.6, 177.8(2C), 156.1, 145.1, 137.7, 136.2, 135.7, 132.5 (2C),130.2 (2C), 129.3 (2C), 126.5, 122.0 (2C), 121.2 (2C), 117.9, 114.8, 44.3 (2C), 32.0 (2C), 29.5, 23.6 (2C). IR (KCl, cm\textsuperscript{-1}): 3409, 3365, 2942, 2861, 1700, 1687, 1664, 1608, 1589, 1442, 1388, 1280, 1214, 1178, 1029, 987, 802. GC-MS (M\textsuperscript{+}): 399.14. 400.15
Novel chalcone derivatives, containing 4,7-ethanoisooindole-1,3-dione units

(d, J = 15.6 Hz, 1H, A part of AB system), 7.44 (d, J = 15.6 Hz, 1H, B part of AB), 7.71 (d, J = 7.6 Hz, 1H), 7.33 (m, 1H), 7.27 (m, 2H), 6.33 (dd, J = 8.2, 4.8 Hz, 2H, olefinic), 3.30 (m, 2H), 3.07 (m, 2H), 2.51 (s, 3H, -CH$_3$), 1.72-1.45 (AA'BB' system, 4H, -CH$_2$CH$_2$-).

13C NMR (100 MHz, CDCl$_3$, ppm): δ = 189.4, 177.6(2C), 142.9, 141.3, 1 38.5, 137.8, 135.7 (2C), 133.7 (2C), 132.5 (2C),130.9, 130.4 (2C), 129.7 (2C), 126.4, 126.3, 122.7, 44.3 (2C), 32.0 (2C), 23.6(2C), 19.9. IR (KCl, cm$^{-1}$): 3052, 2938, 2863, 1708, 1664, 1596, 1481, 1375, 1309, 1213, 1016, 975, 800, 765, 709. GC-MS (CH$_2$Cl$_2$, M$^+$): 397 (10.16%), 382 (94.30%), 316 (10.17%), 302 (57.10%), 280 (17.01%), 266 (66.46%), 248 (18.62%), 221 (15.93%), 207 (22.70%), 200 (68.79%), 172 (24.76%), 146 (35.61%), 115 (93.37%), 91(65%), 78 (100%), 65 (22%), 51(15%).

2-{4-{[(2E)-3-phenylprop-2-enoyl]phenyl}-3a,4,7,7a-tetrahydro-1H-4,7-ethanoisooindole-1,3-dione (10h): Yellowish solid, mp.: 180 °C, Yield: 85%. 1H NMR (400 MHz, CDCl$_3$, ppm): δ = 8.10 (d, J = 8.4 Hz, 2H, AA' part of AA'XX' system), 7.40 (d, J = 8.4 Hz, 2H, XX' part of AA'XX' system), 7.66 (m, 2H, ArH), 7.45 (m, 3H, ArH), 6.34 (dd, J = 4.4, 3.2 Hz, 2H, olefinic), 3.30 (m, 2H), 3.07 (m, 2H), 1.71-1.48 (AA'BB' system, 4H, -CH$_2$CH$_2$-). 13C NMR (100 MHz, CDCl$_3$, ppm): δ = 189.7, 177.7(2C), 145.4, 121.9, 137.8, 135.7, 134.7, 132.5, 130.7, 129.3, 128.5, 126.5, 44.3, 32.1, 23.7. IR (KCl, cm$^{-1}$): 3062, 3048, 2956, 2942, 1708, 1656, 1602, 1373, 1241, 1220, 1168, 1037, 979, 806, 769, 711. GC-MS (CH$_2$Cl$_2$, M$^+$): 383 (100%), 303 (46.63%), 275 (18.11%), 248 (20.30%), 207 (32.49%), 200 (55.15%), 178 (25.86%), 146 (25.12%), 131 (70.89%), 103 (87.17%), 96 (32.05%), 90 (30.46%), 78 (89.36%), 65 (10.65%), 51 (16.97%).

2-{4-{[(2E)-3-(2-thienyl)prop-2-enoyl]phenyl}-3a,4,7,7a-tetrahydro-1H-4,7-ethanoisoindole-1,3-dione (10i): Yellowish solid. mp.: 210 °C, Yield: 71%. 1H NMR (400 MHz, CDCl$_3$, ppm): δ = 8.08 (d, J = 8.4 Hz, 2H, AA' part of AA'XX' system), 7.96 (d , J = 15.2 Hz, 1H, A part of AB system), 7.39 (m, 3H), 7.28 (d, J = 3.2 Hz, 1H), 6.32 (dd, J = 4.2, 3.6 Hz, 2H, olefinic), 3.29 (m, 2H), 3.06 (m, 2H), 1.72-1.45 (AA'BB' system, 4H, -CH$_2$CH$_2$-). 13C NMR (100 MHz, CDCl$_3$, ppm): δ = 188.9, 177.7(2C), 140.2, 137.7, 135.6, 132.5 (2C), 132.4, 129.1 (2C), 128.1, 126.4 (2C),120.5, 44.3 (2C), 32.0 (2C), 23.6 (2C), 19.9. IR (KCl, cm$^{-1}$): 3104, 3648, 2946, 2875, 1706, 1652, 1583, 1504, 1411, 1371, 1280, 1214, 1178, 1016, 1016, 981, 819, 792, 734, 705. GC-MS (CH$_2$Cl$_2$, M$^+$): 389 (100%), 361 (10.58%), 309 (46.63%), 275 (18.11%), 248 (20.30%), 207 (32.49%), 200 (55.15%), 178 (25.86%), 146 (25.12%), 131 (70.89%), 103 (87.17%), 96 (32.05%), 90 (30.46%), 78 (89.36%), 65 (10.65%), 51 (16.97%).

3.3.1. Preparation of Microorganisms

A total of 6 microbial cultures belonging to four bacterial and two fungal species were used (Table 2). The cultures were grown in Mueller-Hinton Broth (Merck) for all the bacterial strains by 24 h of incubation at 36 °C. C. albicans and C. utilis were grown in Sabouraud Dextrose Broth (Merck) by incubation for 24 h at 25 °C.

3.3.2. Disc-diffusion assay

Antibacterial activities were determined by disk-diffusion method, using 100 µL of suspension containing 10$^8$ CFU/mL of bacteria and 10$^6$ CFU/mL of yeast spread on Nutrient Agar (NA), Sabouraud Dextrose Agar (SDA) and Potato Dextrose Agar (PDA) medium, respectively. The blank discs (Oxoid = 6 mm in diameter) were impregnated with 20 µL of each substance (105 µg/disc) and placed on the inoculated agar. A mixture of SCF (Sublactam (30 µg) + Cefoperazona (75 µg)) (105 µg/disc) was used as positive reference standard to determine the sensitivity of a strain of each microbial species tested. The inoculated plates were incubated at 36 °C for 24 h for the bacterial strains and 48 h for the yeast strains.
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References