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

Mustafa Ceylan[1*], İsa Karaman[2] and Meryem Keçeci Sarıkaya[1]

[1]*Department of Chemistry, Faculty of Arts and Science, Gaziosmanpasa University, 60250, Tokat, Türkiye*

[2]*Department of Bioengineering, Faculty of Engineering and Natural Sciences, Gaziosmanpasa University, 60250, Tokat, Türkiye*

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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-cyclehexadine (**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 Et_3N in toluene at 110 ^oC for 24 hours afforded 2-(4-acetylphenyl)-3a,4,7,7a-tetrahydro-1*H*-4,7-ethanoisoindole-1,3-dione (**8**) in high yield. Piperidine-catalyzed addition of benzaldehyde derivatives (**9a-i**) to the compound **8** in CH_2Cl_2 at 55 ^oC gave the expected chalcone derivatives (**10-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,[3-5] anticancer,[6,7] antitumor,[8] antimicrobial,[9,10] antibacterial,[11] antidiabetic,[12] anti-inflammatory,[12] anti-tuberculosis, anti-fungal,[13] and antileishmanyal.[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 photosensitive[17] and cytotoxic.[18] It was reported that chalcones have potential of inhibiting HIV1 virus[19] 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 technologies[21] and medical treatments, development of new chalcone derivatives has been the interest of research groups.

* Corresponding author: Email: mustafac.ceylan@gop.edu.tr ; Tel: +90-3562521616.

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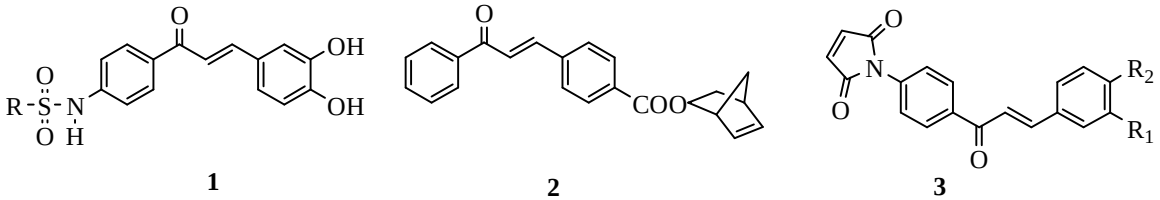

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In this study, syntheses and antibacterial activities of novel chalcone derivatives, containing 4,7-ethanoisoindole-1,3-dione unit has been reported.

2. Results and Discussion

Addition of maleic anhydride (**5**) to 1,3-cyclohexadine (**4**) gave the *endo*-adduct (**6**) in 90 % yield (Figure 1).[22] Heating mixture of the adduct (**6**) and 1-(4-aminophenyl)ethanone (**7**) in the presence of Et_3N in toluene at 110 °C for 24 hours gave 2-(4-acetylphenyl)-3a,4,7,7a-tetrahydro-1*H*-4,7-ethanoisoindole-1,3-dione (**8**) in high yield (say percent? or say quantitatively) (Scheme).[23] 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_2Cl_2 at 55 °C (Scheme, Table 1). Although the yields of calcones 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).

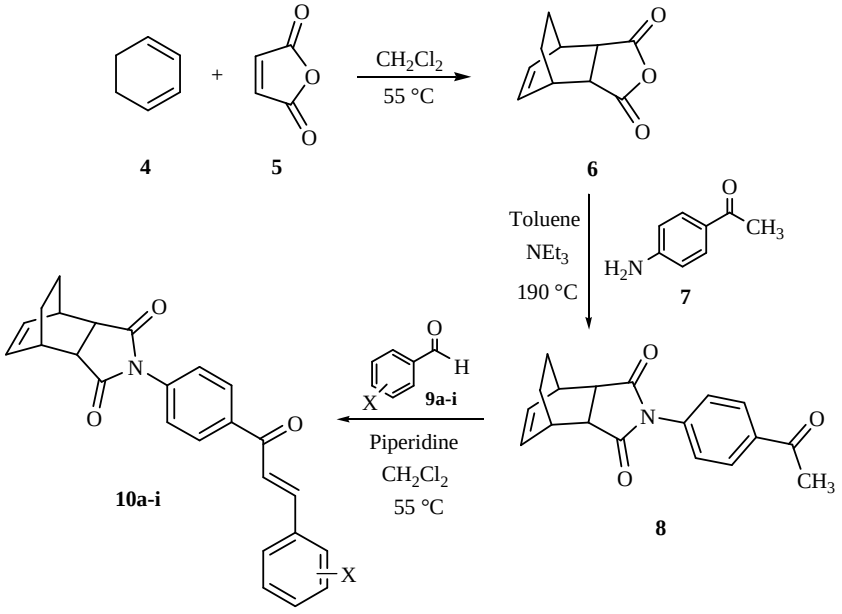

Figure 1. Synthesis of the chalcone derivatives (**10a-i)**, containing 4,7-ethanoisoindole-1,3-dione unit.

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 α,β-unsaturated moiety of **10a-i** produced an AB system (A part of AB system, doublet, J = 15.8-15.2 Hz and B part of AB system, doublet J = 15.8-15.2 Hz) in the range of 7.96-7.73 and 7.51-7.39 ppm, respectively. The coupling constants, J = 15.8-15.2 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 1. Synthesized chalcone derivatives (**10a-i).**

Entry	Compounds	M.p. (°C)	Yield (%)
1	**10a**	192	70
2	**10b**	226	89
3	**10c**	220	96
4	**10d**	225	52
5	**10e**	217	87
6	**10f**	244	67
7	**10g**	179	42
8	**10h**	185	85
9	**10i**	210	71

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.

Microorganisms	Compounds and inhibition zones (mm)										
	10a	**10b**	**10c**	**10d**	**10e**	**10f**	**10g**	**10h**	**10i**	**SCF**	**DMSO**
S. aureus ATCC 29213	9	8	8	NT	9	9	8	8	8	28	-
E. coli 111	-	-	8	NT	-	8	-	8	-	24	-
P. aeruginosa ATCC 9027	7	8	7	NT	-	-	-	8	9	19	-
S. enteridis ATCC 13076	7	8	8	NT	8	7	7	7	8	23	-
C. albicans ATCC 1213	9	9	9	NT	-	-	8	-	8	19	-
C. utilis KUEN 1031	9	9	10	NT	-	9	9	8	9	20	-

SCF: (Sulbactam (30 µg)+cefoperazona (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 are 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-1*H*-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. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were measured, using a Bruker Avance 400 MHz with tetramethylsilane as an internal standard in deuterochloroform. 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. Quadrapole, 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 ^{o}C. The mixture was heated to 55 ^{o}C and stirred for 4 hours. The reaction mixture was washed with water (50 ml) and the organic layer was dried on $NaSO_4$. Removal of the

solvent at reduced pressure afforded the title compound (**6**) as a white solid in 90%, mp.: 145-147 °C (lit.[22] m.p. 147 °C). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ = 6.33 (dd, *J* = 4.4, 3.2 Hz, 2H), 3.24 (m, 2H), 3.15 (m, 2H) 1.64-1.41 (AA'BB' system, 4H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ = 172.8, 133.1, 44.7, 31.6, 22.9. IR (KCl, cm^{-1}): 3052, 2977, 2958, 2867, 1770, 1375, 1238, 1220, 1178, 941, 904, 781, 728, 685, 578. GC-MS (CH_2Cl_2, M^+): 178 (2.6%), 150 (0.5%), 134 (1.2%), 106 (18.8%), 91 (10.7%), 78 (100%), 51 (11.9%).

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-4,7-ethano-2-benzofuran-1,3-dione (2 g, 10 mmol) and 4 ml of Et_3N 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 CH_2Cl_2 to obtain the pure product in quantitative yield as a yellow solid, mp.: 175 °C. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ = 8.04, (d, *J* = 8.4 Hz, 2H; AA' part of AA'XX' system), 7.36, (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), 3.06 (m, 2H), 2.63 (s,3H, -CH_3), 1.71-1.45 (AA'BB' system, 4H, -CH_2CH_2-). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ = 197.0, 177.6 (2C), 136.6, 136.0, 132.5 (2C), 129,0 (2C), 126.4 (2C), 44.2 (2C), 32.0 (2C), 26.7 (2C), 23.6. IR (KCl, cm^{-1}): 3045, 2950, 2935, 2861, 1706, 1683, 1598, 1508, 1390, 1263, 1189, 956, 933, 842, 800, 698, 599, 499,478. GC-MS (CH_2Cl_2, M^+): 295 (32.0%), 280 (100%), 200 (67.9%), 146 (29.7%), 90 (23.0%), 78 (63.3%), 54 (5.2%).

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-1*H*-4,7-ethanoisoindole-1,3-dione (**8**) and benzaldehyde derivatives (**9a-i**) in a ratio of (1:1) in 20 ml of CH_2Cl_2 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 $NaSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by crystallization from *n*-hexane-CH_2Cl_2 (9:1).

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%. ^{1}H 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), 7.81 (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 of –Ph-CH_3), 7.39 (d, *J* = 8.0 Hz, 2H BB' part of AA'BB' system of –Ph-CH_3), 6.33 (dd, *J* = 4.4, 3.2 Hz, 2H), 3.30 (m, 2H), 3.06 (m, 2H), 2.42 (s, 3H, -CH_3), 1.71-1.44 (AA'BB' system, 4H, -CH_2CH_2-). ^{13}C NMR (100 MHz, $CDCl_3$, 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), 21.5. IR (KCl, cm^{-1}): 3043, 2935, 2956, 2935, 2861, 1704, 1662, 1590, 1396, 1332, 1301, 1193, 1024, 1014, 1004, 798, 715. GC-MS (CH_2Cl_2, M^+): 397 (58.98%), 382 (100%), 317 (46.48%), 302 (32.13%), 289 (0.29%), 281 (16.20%), 221 (21.11%), 207 (31.53%), 200 (51.58%), 145 (73.35%), 115 (77.27%), 91 (60%), 78 (95%), 65 (23%).

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%. ^{1}H NMR (400 MHz, $CDCl_3$, 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, AA' part of AA'XX' system CH_3OPh), 6.96 (d, *J* = 8.8 Hz, 2H, XX' part of AA'XX' of CH_3OPh), 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, -CH_2CH_2-). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ = 189.7, 177.7(2C), 1.61.8, 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 (CH_2Cl_2, 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%).

2-{4-[(2E)-3-(4-chlorophenyl)prop-2-enoyl]phenyl}-3a,4,7,7a-tetrahydro-1H-4,7-ethanoisoindole-1,3-dione (10c): Yellow solid, mp., 220 °C, Yield: 96%. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ = 8.09 (d, *J* = 8.4 Hz, 2H, AA' part of AA'XX' system), 7.60 (d, *J* = 8.4 Hz, 2H, XX' part of AA'XX' system), 7.78 (d, *J* = 15.6 Hz, 1H, A part of AB system), 7.48 (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 ClPh)), 7.38 (d, *J* = 8.4 Hz, 2H, BB' part of AA'BB' system of ClPh), 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_2CH_2-). ^{13}C NMR (100 MHz, $CDCl_3$, 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^{-1}): 3056, 2946, 2865, 1704, 1666, 1606, 1556, 1392, 1313, 1189, 1072, 993, 800, 779. GC-MS (CH_2Cl_2, M^+): 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%), 78 (100%), 51 (15%).

2-{4-[(2E)-3-(3-bromophenyl)prop-2-enoyl]phenyl}-3a,4,7,7a-tetrahydro-1H-4,7-ethanoisoindole-1,3-dione (10d): Yellowish solid, mp.: 225 °C., Yield: 52%. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ = 8.10 (d, *J* = 8.8 Hz, 2H, AA' part of AA'XX' system), 7.41 (d, *J* = 8.8 Hz, 2H, XX' part of AA'XX' system), 7.74 (d, *J* = 15.6 Hz, 1H, A part of AB system), 7.49 (d, *J* = 15.6 Hz, 1H, B part of AB system), 7.81, (m, 1H), 7.56 (dd, *J* = 8.0, 1,2 Hz, 2H), 7.35 (t, *J* = 8.0 Hz, 1H), 6.33 (dd, *J* = 6.4, 4.4 Hz, 2H, olefinic), 3.30 (m, 2H) 3.07, (m, 2H), 1.71-1.47 (AA'BB' system, 4H, -CH_2CH_2-). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ = 189.3, 177.6(2C), 143.8, 137.6, 136.6, 135.8, 135.8 (2C), 133.2 (2C), 132,5 (2C), 129.6, 129.2, 129,0 (2C), 126.5 (2C), 126.4, 122.2, 44.3 (2C), 32.0 (2C), 26.7, 23.6 (2C). IR (KCl, cm^{-1}): 3083, 3072, 2948, 2863, 1706, 1660, 1606, 1596, 1563, 1380, 1297, 1214, 1174, 1027, 1012, 983, 823, 804. GC-MS (CH_2Cl_2, M^+): 463 (26.87%), 417 (0.73%), 382 (33.39%), 302 (24.66%), 281 (28.56%), 200 (56.44%), 146 (37.28%), 102 (60.74%), 90 (30.12%), 78 (100%), 65 (10.72%), 51(15.23%).

2-{4-[(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%. ^{1}H NMR (400 MHz, $CDCl_3$, 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_2CH_2-). ^{13}C NMR (100 MHz, $CDCl_3$, 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^{-1}): 3409, 3365, 2942, 2861, 1700, 1687, 1664, 1608, 1589, 1442, 1388, 1280, 1214, 1178, 1029, 987, 802**.** GC-MS (M^+): 399.14. 400.15

2-{4-[(2E)-3-(3-methylphenyl)prop-2-enoyl]phenyl}-3a,4,7,7a-tetrahydro-1H-4,7-ethanoisoindole-1,3-dione (10f): Yellowish solid, mp.: 244 °C, Yield: 67%. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ = 8.03 (d, *J* = 8.4 Hz, 2H, AA' part of AA'XX' system), 7.34 (d, *J* = 8.4 Hz, 2H, XX' part of AA'XX' system), 7.73 (d, *J* = 15,6 Hz, 1H, A part of AB system), 7.44 (d, *J* = 15.6 Hz, 1H, B part of AB system), 7.40 (m, 1H), 7.26 (t, *J* = 8.0, 1H), 7.18 (d, *J* = 7.2 Hz, 1H), 6.24 (brt, *J* = 3.6 Hz, 2H), 3.20 (m, 2H), 2.98 (m, 2H), 2.35 (s, 3H, -CH_3), 1.62-1.37 (AA'BB' system, 4H, -CH_2CH_2-). ^{13}C NMR (100 MHz, $CDCl_3$, 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), 21.5. IR (KCl, cm^{-1}): 3002, 2950, 2803, 1708, 1592, 1461, 1162, 1078, 1058, 943, 862, 555, 439. GC-MS (M^+): 397.16 and 398.17.

2-{4-[(2E)-3-(2-methylphenyl)prop-2-enoyl]phenyl}-3a,4,7,7a-tetrahydro-1H-4,7-ethanoisoindole-1,3-dione (10g): Yellowish soli, mp.: 179 °C, Yield: 42%. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ = 8.12 (d, *J* = 8.8 Hz, 2H, AA' part of AA'XX' system), 7.41 (d, *J* = 8.8 Hz, 2H, XX' part of AA'XX'), 8.15

(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, $-CH_2CH_2-$).^{13}C 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), 129.2 (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_2Cl_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-ethanoisoin-dole-1,3-dione (10h): Yellowish solid, mp.: 180 °C, Yield: 85%. ^{1}H 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), 7.83 (d, *J* = 15,8 Hz, 1H, A part of AB system), 7.51 (d, *J* = 15.8 Hz, 1H, B part of AB 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, $-CH_2CH_2-$). ^{13}C-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, 129.0, 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_2Cl_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-ethano-isoindole-1,3-dione (10i): Yellowish solid. mp.: 210 °C, Yield: 71%. ^{1}H 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), 7.46 (d, *J* = 5.4 Hz, 1H), 7.39 (m, 3H), 7.28 (d, *J* = 3.2 Hz, 1H), 7.12 (dd, *J* = 5.4, 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, $-CH_2CH_2-$). ^{13}C 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_2Cl_2, M^+): 389 (100%), 361 (10.58%), 309 (62.77%), 281 (41.64%), 225 (20.96%), 213 (20.62%), 200 (45.30%), 184 (28.62%), 172 (19.27%), 146 (22.99%), 137 (93.08%), 109 (63.88%), 90 (35%), 78 (95%), 65 (43%), 51(15%).

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 ^{o}C. *C. albicans* and *C. utilis* were grown in Sabouraud Dextrose Broth (Merck) by incubation for 24 h at 25 ^{o}C.

3.3.2. Disc-diffusion assay

Antibacterial activities were determined by disk-diffusion method,[9,24] 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 (Sulbactam (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 ^{o}C for 24 h for the bacterial strains and 48 h for the yeast strains.

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