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SYNTHESIS, CHARACTERIZATIONS AND ANTIMICROBIAL ACTIVITY OF NEW AURONE DERIVATIVES

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ABSTRACT: Aurones constitute a less studied sub-class of flavonoides which occur rarely in nature. Aurones are responsible for the golden yellow colour of some flowers. Substituted 2-benzylidene-1-benzofuran-3 (2H)-ones are commonly known as aurones that belong to the naturally occurring flavonoids. In the present investigation, a series of some novel substituted 2-benzylidene-1-benzofuran-3 (2H)-ones (2a-j) have been synthesized by cyclization of 1-(substituted-2-hydroxy-phenyl)-3-(4'-dimethylamino-phenyl)-prop-2-en-1-one (2-hydroxychalcone) (1a-j) with mercuric acetate in DMSO. The products were tested for purity by TLC and structures of newly synthesized compounds were confirmed by physical data, IR, ¹H NMR and Mass spectral analysis. All these newly synthesized compounds were evaluated for their antibacterial activity against four different pathogens such as *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Bacillus subtilis* and antifungal activity against *Aspergillus niger*, *Penicillium chrysogenum*, *Fusarium moneliforme* and *Aspergillus flavus*, using Penicilline and Griseofulvin as standard drugs by agar cup method and Poison plate method, respectively.

INTRODUCTION: Substituted 2-benzylidene-1-benzofuran-3(2H)-ones are commonly known as aurones that belong to the naturally occurring flavonoids¹⁻² and are structurally isomeric to flavones. They play significant role in the pigmentation of flowers in which they are found. It also has reported antibacterial, antifungal and antiviral activities³⁻⁴. The chalcones, flavones, flavanols, flavanones and isoflavones have been well studied for preventing various diseases from past several years but the biological activity of aurones has not been extensively studied.

Aurones are mainly found in the use of anti-cancer⁵⁻⁶, antihyperglycemic activity⁷ and antioxidant activities⁸⁻⁹. Chalcone is the main source for the synthesis of aurone by oxidation, cyclization and rearrangement. Aurones exhibit a wide variety of biological activities such as antibacterial, antifungal¹⁰⁻¹¹, potential anti-influenza agents¹², *in-vitro* neuraminidase¹³, anti-inflammatory¹⁴ and antimalarial¹⁵⁻¹⁶.

Therefore, aurone is an important class of potentially Useful pharmacologically active compounds, their synthesis has wide spread applications in organic chemistry¹⁷⁻¹⁸. Because of these exciting biological activities, number of aurone compounds have been synthesized and studied their biological activities¹⁹⁻²⁰. In view of these observations, we report in the present investigation, the synthesis of some novel substituted 2-benzylidene-1-benzofuran-3 (2H)-

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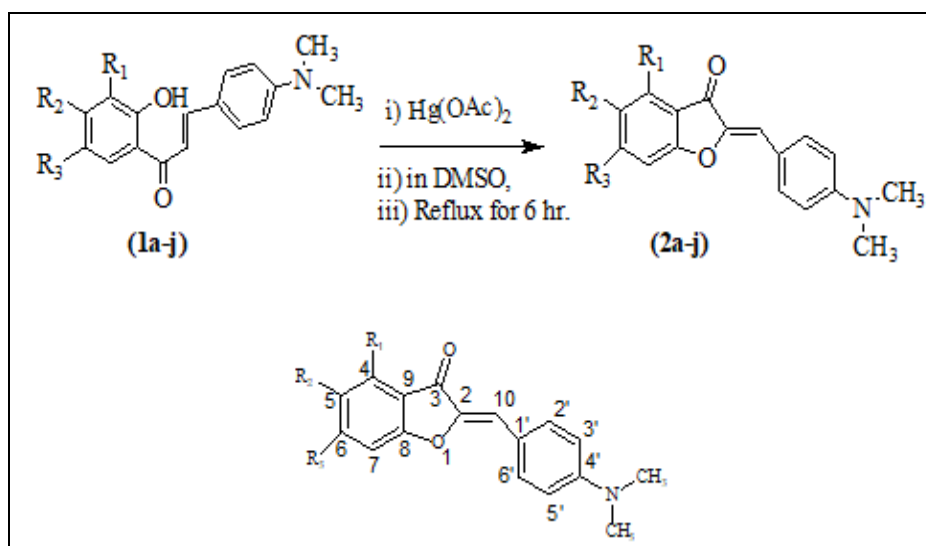
ones (2a-j), having halogen and dimethylamino groups with an aim to find new, significantly most active antimicrobial agents.

MATERIALS AND METHODS: All the solvents and reagents were obtained from commercial sources and were used without further purification. The melting points were determined by Open Capillary method and are uncorrected. The mass spectra were obtained with a Shimadzu GC-MS spectrophotometer. The IR spectra in KBr were recorded on Shimadzu Spectrophotometer and ¹H NMR spectra were recorded in DMSO on Avance 300 MHz Spectrometer using TMS as internal standard. The chemical shift values are expressed in part per million (ppm) downfield from the internal standard and signals are quoted as *s* (singlet), *d* (doublet), *t* (triplet) and *m* (multiplet). Thin-layer chromatography (TLC) was used to monitor the progress of all reactions and to check the purity of the compounds by using ethyl acetate and petroleum ether as an eluent in the ratio of (3:7

v/v). All the newly synthesized substituted 2-benzylidene-1-benzofuran-3 (2*H*)-one compounds were tested for their antimicrobial activities by agar cup method and Poison plate method, respectively.

General Method for the Synthesis of Substituted 2-benzylidene-1-benzofuran-3 (2*H*)-one (Aurones) Derivatives:

The substituted 1-(substituted - 2 - hydroxy-phenyl) - 3 - (4'-dimethylamino-phenyl) - prop - 2 - en - 1 - one (2-hydroxy-chalcone) (0.002mol) was dissolved in dimethyl sulfoxide (DMSO) (20 ml) and after adding mercuric acetate (0.003mol); the reaction mixture was refluxed for 6 hours. The progress of the reaction was monitored by using TLC [eluent: ethyl acetate; petroleum ether (3:7)]. After completion of the reaction, the product was poured on crushed, ice-cold water. The solid crude product obtained was filtered, washed with cold water, dried and recrystallized by using ethanol to get corresponding substituted 2-benzylidene-1-benzofuran-3 (2*H*)-ones.



SCHEME 1: SYNTHESIS OF SUBSTITUTED 2-BENZYLIDENE-1-BENZOFURAN-3 (2*H*)-ONE (AURONES) DERIVATIVES (2A-J)

TABLE 1: PHYSICAL DATA OF SYNTHESIZED SUBSTITUTED 2-BENZYLIDENE-1-BENZOFURAN-3 (2*H*)-ONE (AURONES) DERIVATIVES (2A-J)

| Sr. no. | Entry | R ₁ | R ₂ | R ₃ | Molecular formula | Molecular weight | Yield in (%) | Melting Point °C |
|---------|-------|----------------|-----------------|-----------------|--|------------------|--------------|------------------|
| 1 | 2a | Cl | H | Cl | C ₁₇ H ₁₃ Cl ₂ O ₂ N | 336 | 55 | 115-117 |
| 2 | 2b | I | H | Cl | C ₁₇ H ₁₃ IClO ₂ N | 426 | 50 | 108-110 |
| 3 | 2c | Br | H | Cl | C ₁₇ H ₁₃ BrClO ₂ N | 379 | 55 | 105-107 |
| 4 | 2d | Br | H | Br | C ₁₇ H ₁₃ Br ₂ O ₂ N | 423 | 50 | 116-118 |
| 5 | 2e | H | H | Br | C ₁₇ H ₁₄ BrO ₂ N | 344 | 56 | 137-139 |
| 6 | 2f | H | H | Cl | C ₁₇ H ₁₄ ClO ₂ N | 300 | 60 | 127-129 |
| 7 | 2g | Br | CH ₃ | Cl | C ₁₈ H ₁₅ BrClO ₂ N | 393 | 56 | 142-144 |
| 8 | 2h | I | CH ₃ | Cl | C ₁₈ H ₁₅ IClO ₂ N | 440 | 50 | 170-172 |
| 9 | 2i | Cl | H | CH ₃ | C ₁₈ H ₁₆ ClO ₂ N | 314 | 60 | 139-141 |
| 10 | 2j | Cl | H | Br | C ₁₇ H ₁₃ BrClO ₂ N | 379 | 58 | 111-113 |

Antimicrobial Activities:

Antibacterial Activity: All the newly synthesized substituted 2-benzylidene-1-benzofuran-3 (2H)-one derivatives (2a-j) were assessed for their antibacterial and antifungal activities against four different strains of bacteria such as *E. coli*, *S typhi*, *S. aureus* and *B. subtilis* and four fungi like *Aspergillus niger*, *Penicillium chrysogenum*, *Fusarium moneliforme* and *Aspergillus flavus*. The test for antibacterial activity was carried out by agar cup method²¹ (cup size 8 mm) with nutrient agar as a medium where as antifungal activity was carried out by using potato-dextrose agar (PDA) medium by same agar cup plate method. All newly synthesized compounds were dissolved in DMSO and used as controlled concentration for each test compound which was 100 µg/ml. The experiments were performed in triplicate, in order to minimize the errors. Zone of inhibition was recorded after incubation at 37 °C for 24 hrs, zone of inhibition produced by each compound was measured in mm.

After incubation plates were observed for the zone of inhibition of bacterial growth around the agar cup, results were recorded by measuring the zone of inhibition in millimeter (mm) by using the zone reader. All the newly synthesized substituted 2-benzylidene - 1 - benzofuran - 3 (2H) - one (2a-j) compounds were evaluated for their antibacterial activity against the selected four different pathogens such as *E. coli*, *S. typhi*, *S. aureus* and *B. subtilis*. The compounds 2a, 2c and 2i show good activity and 2b, 2e, 2g and 2h compounds does not show activity against *E. coli*. The compounds 2b, 2h, and 2j showed weak activity against *S. typhi*, while 2a, 2c, 2f and 2g showed stronger activity in comparison with standard (Penicilin) drug. All the synthesized compounds of aurone except 2b, 2e, 2h and 2i showed moderate activity against *S. aureus*. The bromine substituted compound 2c showed significant activity against *B. subtilis* as compared to standard drug.

TABLE 2: ANTIBACTERIAL ACTIVITY DATA OF SUBSTITUTED 2-BENZYLIDENE-1-BENZOFURAN-3 (2H)-ONE (AURONES) DERIVATIVES (2A-J)

| Sr. no. | Entry | molecular formula | Antibacterial activity (Zone of Inhibition in mm) | | | |
|---------|------------------|--|---|-------------------------|------------------------------|--------------------------|
| | | | <i>Escherichia coli</i> | <i>Salmonella typhi</i> | <i>Staphylococcus aureus</i> | <i>Bacillus subtilis</i> |
| 1 | 2a | C ₁₇ H ₁₃ Cl ₂ O ₂ N | 13 | 17 | 23 | 12 |
| 2 | 2b | C ₁₇ H ₁₃ IClO ₂ N | -- | 13 | -- | 11 |
| 3 | 2c | C ₁₇ H ₁₃ BrClO ₂ N | 13 | 15 | 21 | 17 |
| 4 | 2d | C ₁₇ H ₁₃ Br ₂ O ₂ N | 10 | -- | 18 | -- |
| 5 | 2e | C ₁₇ H ₁₄ BrO ₂ N | -- | -- | -- | -- |
| 6 | 2f | C ₁₇ H ₁₄ ClO ₂ N | 11 | 16 | 17 | 12 |
| 7 | 2g | C ₁₈ H ₁₅ BrClO ₂ N | -- | 17 | 21 | 13 |
| 8 | 2h | C ₁₈ H ₁₅ IClO ₂ N | -- | 12 | -- | -- |
| 9 | 2i | C ₁₈ H ₁₆ ClO ₂ N | 12 | -- | -- | -- |
| 10 | 2j | C ₁₇ H ₁₃ BrClO ₂ N | 10 | 13 | 22 | 14 |
| | +ve Control DMSO | | -ve | -ve | -ve | -ve |
| | Penicilline | | 12 | 20 | 34 | 22 |

Antifungal Activity: The antifungal activity of synthesized substituted 2-benzylidene-1-benzofuran-3 (2H)-one (aurones) derivatives (2a-j) were screened against four plant pathogenic and mold fungi, such as *Aspergillus niger*, *penicillium chrysogenum*, *Fusarium moneliforme* and *Aspergillus flavus*. The antifungal activities of the synthesized substituted 2-benzylidene-1-benzofuran-3 (2H)-one (2a-J) compounds were assessed by poisoned plate method²². Griseofulvin (100 µg/disc) was used as a standard drug for the antifungal test. Potato Dextrose Agar (PDA) was used as a basal medium to test fungi. The compounds of 100 µg were mixed with sterilized

potato dextrose agar (PDA) medium at 40 °C at the rate of 100 mg/mL PDA. The medium was poured in sterilized Petri-plates and allowed solidified PDA media and then incubated at 30 °C for 72 hours. The growth of fungal area was measured in mm after 4 days of incubation at 30 °C. The control set was maintained using only PDA with DMSO as a growth medium. Results were measured as the growth of fungi (does not show antifungal activity), reduced growth of fungi (to observed moderate antifungal activity), and no growth of fungi (antifungal activity observed in the area). All the newly synthesized compounds were evaluated for their antifungal activity against the four different

pathogens *Aspergillus niger*, *Penicillium chrysogenum*, *Fusarium moneliforme* and *Aspergillus flavus*. The antifungal activity of some aurone compounds showed good activity against

four pathogens. The presence of more electronegative substituted halogen atoms and dimethylamino groups were found to responsible for increasing antimicrobial activity.

TABLE 3: ANTIFUNGAL ACTIVITY DATA OF SUBSTITUTED 2-BENZYLIDENE-1-BENZOFURAN-3 (2H)-ONE (AURONES) DERIVATIVES (2A-J)

| Sr. no. | Entry | molecular formula | Antifungal activity (Zone of Inhibition in mm) | | | |
|---------|----------------------------|--|--|--------------------------------|-----------------------------|---------------------------|
| | | | <i>Aspergillus niger</i> | <i>Penicillium chrysogenum</i> | <i>Fusarium moneliforme</i> | <i>Aspergillus flavus</i> |
| 1 | 2a | C ₁₇ H ₁₃ Cl ₂ O ₂ N | -ve | -ve | -ve | -ve |
| 2 | 2b | C ₁₇ H ₁₃ IClO ₂ N | RG | -ve | RG | -ve |
| 3 | 2c | C ₁₇ H ₁₃ BrClO ₂ N | -ve | RG | -ve | RG |
| 4 | 2d | C ₁₇ H ₁₃ Br ₂ O ₂ N | -ve | -ve | RG | -ve |
| 5 | 2e | C ₁₇ H ₁₄ BrO ₂ N | RG | -ve | -ve | -ve |
| 6 | 2f | C ₁₇ H ₁₄ ClO ₂ N | -ve | RG | -ve | RG |
| 7 | 2g | C ₁₈ H ₁₅ BrClO ₂ N | -ve | -ve | RG | -ve |
| 8 | 2h | C ₁₈ H ₁₅ IClO ₂ N | -ve | RG | -ve | RG |
| 9 | 2i | C ₁₈ H ₁₆ ClO ₂ N | RG | RG | RG | -ve |
| 10 | 2j | C ₁₇ H ₁₃ BrClO ₂ N | -ve | -ve | RG | -ve |
| | +ve Control DMSO | | +ve | +ve | +ve | +ve |
| | -ve Control (Griseofulvin) | | -ve | -ve | -ve | -ve |

[+ve = No growth (Antifungal activity absent), RG = Reduced Growth (more than 50 % but less than 90 % i. e. Moderate Activity), -ve = No Growth (Antifungal Activity Observed 90 %)]

RESULTS AND DISCUSSION: In recent years, one of the most important conventional methods used for the synthesis of substituted 2-benzylidene-1-benzofuran-3 (2H)-one compounds has been the reaction of α , β -unsaturated carbonyl compounds, such as substituted 2'-hydroxychalcone with mercuric acetate²³⁻²⁴ in dimethyl sulfoxide (DMSO) was refluxed for 6 hour. The reaction mixture was distilled to remove the excess solvent and the reaction mixture was poured on Crushed, ice-cold water and recrystallized by using absolute ethanol to get substituted 2-benzylidene-1-benzofuran-3 (2H)-one compounds (2a-j) in 50-60 % yield. The newly synthesized compounds have been confirmed first by TLC and physical data of Products was different from that of corresponding reactants. The structures of newly synthesized substituted 2-benzylidene-1-benzofuran-3 (2H)-one (2a-j) derivatives were confirmed on the basis of IR, ¹HNMR and Mass spectral data. The IR spectrum of compound 2a exhibited peaks due to group -C=O at 1690-1697 cm⁻¹ and absorption peaks around 780-788 cm⁻¹ due to stretching -C-O-C- and also shows -C=CH- at 1652 cm⁻¹ which indicated formation of aurone ring. The mass spectra of all the compounds of aurones showed mass ion peaks which are also the base peaks of the compound by indicating the stability of the molecule. The structures of the substituted 2-

benzylidene-1-benzofuran-3 (2H)-one (aurones) (2a-j) derivatives were confirmed on the basis ¹HNMR spectra which shows δ 6.57-6.68 due to benzylidene proton. These observations are in agreement with the spectral data reported²⁵⁻²⁶. All the newly synthesized aurones were evaluated for their antibacterial activity against the selected four different pathogens, such as *E. coli*, *S. typhi*, *S. aureus* and *B. subtilis*. The compounds 2a, 2c and 2i show good activity and 2b, 2e, 2g and 2h compounds does not show activity against *E. coli*. The compounds 2b, 2h, and 2j showed weak activity against *S. typhi*, while 2a, 2c, 2f and 2g showed stronger activity in comparison with standard (Penicilin) drug. All the synthesized compounds of aurone except 2b, 2e, 2h and 2i showed moderate activity against *S. aureus*. The bromine substituted compound 2c showed significant activity against *B. subtilis* as compared with standard drug. All the newly synthesized compounds were evaluated for their antifungal activity against the four different pathogens *Aspergillus niger*, *Penicillium chrysogenum*, *Fusarium moneliforme* and *Aspergillus flavus*. The antifungal activity of some aurone compounds showed good activity against four pathogens. The presence of more electronegative substituted halogen atoms and dimethylamino groups were

found responsible for increasing antimicrobial activity.

Spectroscopic Data of Synthesized Compounds:
4,6-Dichloro-2-(4'-dimethylamino-benzylidene)-benzofuran-3-one (Aurone) 2a: Yield 55 % and M. P. 115-117 °C, IR (KBr): 1696 cm⁻¹ (-C=O), 1652 cm⁻¹ (-C=CH), 784 cm⁻¹ (-C-O-C-); 782 cm⁻¹ (C-Cl); ¹HNMR (CDCl₃): δ 3.0 (s, 6H, -N(CH₃)₂), δ 6.97 (s, 1H, =CH-Ar, H-10), δ 6.74 (s, 1H, H-7), δ 6.86 (d, 1H, H-5), δ 6.66 (d, 2H, H-3', H-5') δ 7.24 (d, 2H, H-2', H-6'), M.S. (m/z): = 336, Molecular formula: C₁₇H₁₃Cl₂O₂N.

4 - Bromo - 6 - Chloro - 2 - (4'-dimethylamino-benzylidene)-benzofuran-3-one (aurone) 2c: Yield 55 % and M. P. 105-107 °C, IR (KBr): 1695 cm⁻¹ (-C=O), 1645 cm⁻¹ (-C=CH), 787 cm⁻¹ (C-O-C-); 878 cm⁻¹ (C-Br); ¹HNMR (CDCl₃): δ 3.0 (s, 6H, -N(CH₃)₂), δ 6.90 (s, 1H, =CH-Ar, H-10), δ 6.58 (s, 1H, H-7), δ 6.92 (d, 1H, H-5), δ 6.52 (d, 2H, H-3', H-5') δ 7.27 (d, 2H, H-2', H-6'), M.S. (m/z): = 379 Molecular formula: C₁₇H₁₃BrClO₂N.

4 - Bromo - 6 - Chloro - 2 - (4'-dimethylamino-benzylidene) - 5 - methyl - benzofuran - 3-one (aurone) 2g: Yield 56 % and M. P. 139-141 °C, IR (KBr): 1697 cm⁻¹ (-C=O), 1636 cm⁻¹ (-C=CH), 781 cm⁻¹ (-C-O-C-); 884 cm⁻¹ (C-Br); ¹HNMR (CDCl₃): δ 3.0 (s, 6H, -N(CH₃)₂), δ 2.34 (s, 3H, -CH₃), δ 6.98 (s, 1H, =CH-Ar, H-10), δ 6.58 (s, 1H, H-7), δ 6.56 (d, 2H, H-3', H-5') δ 7.21 (d, 2H, H-2', H-6'), M.S. (m/z): = 393 Molecular formula: C₁₈H₁₅BrClO₂N.

4 - Chloro - 2 - (4' - dimethylamino - benzylidene) - 6 - methyl - benzofuran - 3 - one (aurone) 2i: Yield 60 % and M. P. 139-141 °C, IR (KBr): 1697 cm⁻¹ (-C=O), 1646 cm⁻¹ (-C=CH), 782 cm⁻¹ (-C-O-C-); 782 cm⁻¹ (C-Cl); ¹HNMR (CDCl₃): δ 2.99 (s, 6H, -N(CH₃)₂), δ 2.31 (s, 3H, -CH₃), δ 6.92 (s, 1H, =CH-Ar, H-10), δ 6.48 (s, 1H, H-7), δ 6.96 (d, 1H, H-5), δ 6.62 (d, 2H, H-3', H-5') δ 7.42 (d, 2H, H-2', H-6'), M.S. (m/z): = 314 Molecular formula: C₁₈H₁₆ClO₂N.

CONCLUSION: In this work, we have demonstrated the synthesis of substituted 2-benzylidene-1-benzofuran-3 (2H)-one derivatives using simple experimental procedure with good yields, relatively short reaction time, easily work up and low cost. From the result of antibacterial

and antifungal activities, it can be concluded that the substituted 2-benzylidene-1-benzofuran-3 (2H)-one derivatives and the ring system, presence of halogen and dimethylamino groups are responsible for the antibacterial and antifungal effects. The obtained results in all these assays during the study will be certainly useful to go for further research for drug designing. This might provide interesting and additional synthesizing of new derivatives.

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CONFLICTS OF INTEREST: The authors declare no conflict of interest.

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