Microwave supported synthesis and antimicrobial evaluation of bis-pyrazole derivatives from N-phenyl glutarimides

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Abstract: A simple ecofriendly microwave supported solvent free synthesis of bis-chalcones was carried out by the reaction of di-substituted 4-hydroxy-3-methoxy benzaldehyde with different substituted N-phenylpiperidine-2, 6-diones in presence of neutral corundum. By the same way the novel bis-pyrazoles were developed from bis-chalcones and hydrazine hydrate with neutral corundum. All the derivatives were characterized and screened their antimicrobial potencies.

1. Introduction:

Chalcone and its bis-pyrazole derivatives play a vital role in a theoretical development of heterocyclic chemistry along with being used extensively in organic synthesis. The present work is dealing with the ecofriendly synthesis of novel theories of (3Z,4Z)-3,4-bis-(4-hydroxy-3-methoxybenzylidene)-7-(1-phenyl)-3,3a,3b,4,5,7-hexahydro-2H-piperidine-[2,3-c,5,4-c] dipyrazole (5a-j) developed from chalcones of N-phenyl glutarimides. Chalcones are the versatile forerunners of heterocycle family having a carbon bridge between α - β -unsaturated aromatic rings and carbonyl carbons. These are prepared by the condensation [1] of the substituted ketones, aldehyde and cyclic imide groups [2-7]. The chalcones are synthesized by using the several types of synthetic routes like solid phase claisen schmidt, cross aldol condensation, acid catalyst, coupling reaction [8], knoevenagel condensation [9] and microwave assisted synthesis [10-12]. On the other hand, the chalcone showed significant cytotoxic activities against cell line, human hepatocellular and lung carcinoma and breast cancer [13] and also potent microbial agents [14]. The chalcone based five membered pyrazoles are prepared by hydrazine hydrate or aromatic hydrazines in presence of sodium acetate [15], acetic acid [16,17] catalysts by conventional and microwave [18-20], facile grinding [21], chromine ring opening [22], solvent free [23], one pot tandem [24] and regio-selective [25] methods so on. The pyrazoline derivatives states the reasonable antibacterial [26], antifungal [27-29] activities. Some novel bioactive pyrazoles [30,31], microwave assisted synthesized pyrazoles [32-35] and newer techniques of pyrazole analogous synthesized by using eco-friendly single pot multicomponent, solvent free, solid support, microwave [36] and ultrasound synthetic methods are more beneficial than that of the conventional reflux methods [37]. The chalconic pyrazoles and pyrazolones are superior anticancer [38,39], anti-breast cancer [40], antipyretic [41], anti-oxidant [42], anti-inflammatory [43-45], anti-mycobacterial activity against tuberculosis H37Rv [46] and good antimicrobial [47-50], antifungal [51-53] agents. Some pyrazoline ring centered moffat oxidizing agents are found 5 α -reductase inhibitors [54].

2. Experimental:

2.1 Material method and Reagents

All the melting points of synthesized compounds were recorded and uncorrected by using open glass capillaries. IR spectra in (KBr pallets) were verified by Shimadzu FTIR-8400S and ATR Bruker alpha FT-IR spectrophotometer. ¹HNMR spectra were recorded on 400 MHz and 500.13 MHz by Bruker spectrophotometer. The reaction was monitored by TLC which was undertaken by

using precoated silica-gel aluminium sheets with the mixture of diethyl ether and ethyl acetate 7:3 proportion. Commercially purchased 4-hydroxy-3-methoxy benzaldehyde i.e. vanillin, hydrazine hydrate, neutral corundum (Al_2O_3) and ethanol were used for the preparation.

3. General Procedure for Synthesis of Bis-chalcones by using N-Phenyl Glutarimides:

The bis-chalcones (**3a-j**) are synthesized by the mixture of 5 mmole of previously prepared phenyl substituted glutarimides (**1a-j**) and 10 mmole of 4-hydroxy-3-methoxy-benzaldehyde (**2**) in 2 gm of neutral corundum (Al_2O_3) under microwave assisted solvent free conditions take place on 640W powers for 3-6 minutes. The afforded coloured flavonoids were recovered and recrystallized by ethanol as shown in the **scheme – I**.

Scheme I: (3Z,5Z)-3,5-bis-(4-hydroxy-3-methoxy-benzylidene)-N-phenyl-piperidine-2,6-dione (**3a-j**)

3.1 (3Z,5Z)-3,5-bis-(4-hydroxy-3-methoxy-benzylidene)-1-phenylpiperidine-2,6-dione (**3a**):

Molecular Formula: $\text{C}_{27}\text{H}_{23}\text{NO}_6$, Yellowish White Amorphous Solid, Yield: 79.91%, M. W.: 457.47, Melting Point ($^{\circ}\text{C}$): 78-80 $^{\circ}\text{C}$, C, H, N Anal Observed: C, 71.06; H, 6.98; N, 3.24, FTIR (KBr): $>\text{C}=\text{O}$ (2-Peaks): 1595 cm^{-1} and 1672 cm^{-1} , $=\text{C}-\text{H}$: 2968 cm^{-1} , aromatic ring (3-Peaks): 1455 cm^{-1} , 1513 cm^{-1} and 1541 cm^{-1} , Ar-OCH₃: 1157 cm^{-1} , Ar-OH: 3320 cm^{-1} , ^1H NMR-(200.13 MHz; CDCl_3 ; δ ppm): 7.40-6.26 (m, 8H, Ar-H and $=\text{CH}$), 9.87 (s, 1H, -OH), 3.70 (s, 3H, -OCH₃), 2.46 (s, 2H, -CH₂)

3.2 (3Z,5Z)-3,5-bis-(4-hydroxy-3-methoxy-benzylidene)-1-(4-bromophenyl)-piperidine-2,6-dione (3b**):** Molecular Formula: $\text{C}_{27}\text{H}_{22}\text{BrNO}_6$, Dark Yellow Granular Crystals, Yield: 94.37%, M. W.: 536.37, Melting Point ($^{\circ}\text{C}$): 94-96 $^{\circ}\text{C}$, C, H, N Anal Observed: C, 60.88; H, 4.25; N, 2.75, FTIR (KBr): $>\text{C}=\text{O}$ (2-Peaks): 1667 cm^{-1} and 1689 cm^{-1} , $=\text{C}-\text{H}$: 3032 cm^{-1} , aromatic ring (3-Peaks): 1462 cm^{-1} , 1514 cm^{-1} and 1590 cm^{-1} , Ar-OCH₃: 1172 cm^{-1} , Ar-OH: 3294 cm^{-1} , Ar-Br: 1072 cm^{-1}

3.3 (3Z,5Z)-3,5-bis-(4-hydroxy-3-methoxy-benzylidene)-1-(4-chlorophenyl)-piperidine-2,6-dione (3c**):** Molecular Formula: $\text{C}_{27}\text{H}_{22}\text{ClNO}_6$, Yellow Solid, Yield: 82.25%, M. W.: 491.92, Melting Point ($^{\circ}\text{C}$): 76-78 $^{\circ}\text{C}$, C, H, N Anal Observed: C, 66.42; H, 4.68; N, 2.99, FTIR (KBr): $>\text{C}=\text{O}$ (2-Peaks): 1667 cm^{-1} and 1742 cm^{-1} , $=\text{C}-\text{H}$: 3030 cm^{-1} , aromatic ring (3-Peaks): 1462 cm^{-1} , 1512 cm^{-1} and 1590 cm^{-1} , Ar-OCH₃: 1170 cm^{-1} , Ar-OH: 3287 cm^{-1} , Ar-Cl: 1026 cm^{-1}

3.4 (3Z,5Z)-3,5-bis-(4-hydroxy-3-methoxy-benzylidene)-1-p-tolylpiperidine-2,6-dione (**3d**):

Molecular Formula: $\text{C}_{28}\text{H}_{25}\text{NO}_6$, Yellow Granular Crystals, Yield: 83.98%, M. W.: 471.5, Melting Point ($^{\circ}\text{C}$): 95-97 $^{\circ}\text{C}$, C, H, N Anal Observed: C, 71.86; H, 5.73; N, 3.55, FTIR (KBr): $>\text{C}=\text{O}$ (2-Peaks): 1594 cm^{-1} and 1675 cm^{-1} , $=\text{C}-\text{H}$: 2946 cm^{-1} , aromatic ring (3-Peaks): 1459 cm^{-1} , 1514 cm^{-1} and 1596 cm^{-1} , Ar-OCH₃: 1171 cm^{-1} , Ar-OH: 3280 cm^{-1} , ^1H NMR-(500.13 MHz; $\text{DMSO}-d_6$; δ

ppm): 7.48-6.97 (m, 5H, Ar-H and =CH), 9.78 (s, 1H, -OH), 3.70 (s, 3H, -OCH₃), 2.71 (s, 2H, -CH₂)

3.5 (3Z,5Z)-3,5-bis-(4-hydroxy-3-methoxybenzylidene)-1-(4-methoxyphenyl)piperidine-2,6-dione (3e): Molecular Formula: C₂₈H₂₅NO₇, Yellow Granular Crystals, Yield: 69.41%, M. W.: 487.5, Melting Point (°C): 68-70 °C, C, H, N Anal Observed: C, 69.09; H, 5.72; N, 3.20, FTIR (KBr): >C=O (2-Peaks): 1596 cm⁻¹ and 1677 cm⁻¹, =C-H: 2971 cm⁻¹, aromatic ring (3-Peaks): 1460 cm⁻¹, 1514 cm⁻¹ and 1596 cm⁻¹, Ar-OCH₃: 1171 cm⁻¹, Ar-OH: 3466 cm⁻¹

3.6 (3Z,5Z)-3,5-bis-(4-hydroxy-3-methoxy-benzylidene)-1-(4-fluorophenyl)-piperidine-2,6-dione (3f): Molecular Formula: C₂₇H₂₂FNO₆, Yellow Granular Crystals, Yield: 90.84%, M. W.: 475.47, Melting Point (°C): 85-87 °C, C, H, N Anal Observed: C, 68.81; H, 4.93; N, 3.33, FTIR (KBr): >C=O (2-Peaks): 1594 cm⁻¹ and 1672 cm⁻¹, =C-H: 3089 cm⁻¹, aromatic ring (3-Peaks): 1426 cm⁻¹, 1513 cm⁻¹ and 1594 cm⁻¹, Ar-OCH₃: 1158 cm⁻¹, Ar-OH: 3316 cm⁻¹, Ar-F: 1126 cm⁻¹

3.7 (3Z,5Z)-3,5-bis-(4-hydroxy-3-methoxy-benzylidene)-1-(4-nitrophenyl)-piperidine-2,6-dione (3g): Molecular Formula: C₂₇H₂₂N₂O₈, Pale Yellow Granular Crystals, Yield: 78.33%, M. W.: 502.47, Melting Point (°C): 93-95 °C, C, H, N Anal Observed: C, 64.76; H, 4.65; N, 5.85, FTIR (KBr): >C=O (2-Peaks): 1668 cm⁻¹ and 1711 cm⁻¹, =C-H: 3078 cm⁻¹, aromatic ring (3-Peaks): 1460 cm⁻¹, 1512 cm⁻¹ and 1590 cm⁻¹, Ar-OCH₃: 1170 cm⁻¹, Ar-OH: 3269 cm⁻¹, Ar-NO₂: 1512 cm⁻¹

3.8 (3Z,5Z)-3,5-bis-(4-hydroxy-3-methoxy-benzylidene)-1-(naphthalen-1-yl)-piperidine-2,6-dione (3h): Molecular Formula: C₃₁H₂₅NO₆, Whitish Brown Solid, Yield: 91.74%, M. W.: 507.53, Melting Point (°C): 88-90 °C, C, H, N Anal Observed: C, 73.79; H, 5.02; N, 2.98, FTIR (KBr): >C=O (2-Peaks): 1667 cm⁻¹ and 1704 cm⁻¹, =C-H: 3078 cm⁻¹, aromatic ring (3-Peaks): 1429 cm⁻¹, 1460 cm⁻¹, 1511 cm⁻¹, 1589 cm⁻¹ and 1667 cm⁻¹, Ar-OCH₃: 1172 cm⁻¹, Ar-OH: 3178 cm⁻¹

3.9 (3Z,5Z)-3,5-bis-(4-hydroxy-3-methoxy-benzylidene)-1-(3-chloro-4-fluorophenyl)-piperidine-2,6-dione (3i): Molecular Formula: C₂₇H₂₁ClFNO₆, Dark Yellow Solid, Yield: 82.23%, M. W.: 509.91, Melting Point (°C): 74-76 °C, C, H, N Anal Observed: C, 63.91; H, 4.69; N, 2.86, FTIR (KBr): >C=O (2-Peaks): 1595 cm⁻¹ and 1674 cm⁻¹, =C-H: 3027 cm⁻¹, aromatic ring (3-Peaks): 1459 cm⁻¹, 1511 cm⁻¹ and 1544 cm⁻¹, Ar-OCH₃: 1172 cm⁻¹, Ar-OH: 3309 cm⁻¹, Ar-F: 1172 cm⁻¹, Ar-Cl: 1056 cm⁻¹

3.10 (3Z,5Z)-3,5-bis-(4-hydroxy-3-methoxy-benzylidene)-1-(2,4,5-trichlorophenyl)-piperidine-2,6-dione (3j): Molecular Formula: C₂₇H₂₀Cl₃NO₆, Pinkish White Granular Crystals, Yield: 83.03%, M. W.: 560.81, Melting Point (°C): 103-105 °C, C, H, N Anal Observed: C, 58.58; H, 3.84; N, 2.87, FTIR (KBr): >C=O (2-Peaks): 1669 cm⁻¹ and 1694 cm⁻¹, =C-H: 3027 cm⁻¹, aromatic ring (3-Peaks): 1459 cm⁻¹, 1512 cm⁻¹ and 1580 cm⁻¹, Ar-OCH₃: 1170 cm⁻¹, Ar-OH: 3276 cm⁻¹, Ar-2,4,5Cl: 1078 cm⁻¹, ¹H NMR-(500.13 MHz; DMSO-d₆; δ ppm): 8.07-6.96 (m, 5H, Ar-H and =CH), 9.77 (s, 1H, -OH), 3.70 (s, 3H, -OCH₃), 2.30 (s, 2H, -CH₂)

4. General Procedure for Synthesis of Bis-pyrazoles:

The bis-pyrazole derivatives (**5a-j**) are synthesized by the mixture of 5 mmole of 3,5-bis-(4-hydroxy-3-methoxy-benzylidene)-1-phenylpiperidine-2,6-diones (**3a-j**) and 10 mmole of hydrazine hydrate (**4**) in 2 gm of neutral corundum (Al₂O₃) under microwave supported solvent free conditions on 640W powers for 3-6 minutes. The afforded coloured synthones were recovered and recrystallized by ethanol as shown in the **scheme – II**.

4.1 (3Z,4Z)-3,4-bis-(4-hydroxy-3-methoxybenzylidene)-7-(1-phenyl)-3,3a,3b,4,5,7-hexahydro-2H- piperidine-[2,3-c,5,4-c] dipyrazole (5a): Molecular Formula: C₂₇H₂₇N₅O₄, Brown Solid, Yield: 82.57%, M. W.: 485.53, Melting Point (°C): 151-153 °C, C, H, N Anal Observed: C, 66.98; H, 5.76; N, 14.86, FTIR (KBr): -NH (1-Peak): 3218 cm⁻¹, aromatic ring (3-Peaks): 1511 cm⁻¹, 1600 cm⁻¹ and 1651 cm⁻¹, Ar-OCH₃: 1280 cm⁻¹, Ar-OH: 3482 cm⁻¹

4.2 (3Z,4Z)-3,4-bis-(4-hydroxy-3-methoxybenzylidene)-7-(4-bromophenyl)-3,3a,3b,4,5,7-hexahydro-2H- piperidine-[2,3-c,5,4-c] dipyrazole (5b): Molecular Formula: C₂₇H₂₆BrN₅O₄, Yellowish Granular Crystals, Yield: 78.01%, M. W.: 564.43, Melting Point (°C): 157-159 °C, C, H, N Anal Observed: C, 57.86; H, 4.85; N, 12.82, FTIR (KBr): -NH (1-Peak): 3285 cm⁻¹, aromatic ring (3-Peaks): 1511 cm⁻¹, 1601 cm⁻¹ and 1659 cm⁻¹, Ar-OCH₃: 1280 cm⁻¹, Ar-OH: 3482 cm⁻¹, Ar-Br: 1033 cm⁻¹, ¹H NMR (500.13 MHz, DMSO-d₆, δ ppm): 7.62-6.74 (m, 5H, Ar-H and =CH), 8.58 (s, 1H, -NH), 10.05 (s, 1H, -OH), 3.39 (d, 1H, -CH₂), 2.36-2.28 (m, 1H, -CH), 1.89-1.81 (m, 2H, -CH₂), 3.77 (s, 3H, -OCH₃)

4.3 (3Z,4Z)-3,4-bis-(4-hydroxy-3-methoxybenzylidene)-7-(4-chlorophenyl)-3,3a,3b,4,5,7-hexahydro-2H- piperidine-[2,3-c,5,4-c] dipyrazole (5c): Molecular Formula: C₂₇H₂₆ClN₅O₄, Yellowish Granular Crystals, Yield: 75.37%, M. W.: 519.98, Melting Point (°C): 155-157 °C, C, H, N Anal Observed: C, 62.87; H, 4.98; N, 13.78, FTIR (KBr): -NH (1-Peak): 3292 cm⁻¹, aromatic ring (3-Peaks): 1512 cm⁻¹, 1599 cm⁻¹ and 1661 cm⁻¹, Ar-OCH₃: 1281 cm⁻¹, Ar-OH: 3484 cm⁻¹, Ar-Cl: 1033 cm⁻¹, ¹H NMR (500.13 MHz, DMSO-d₆, δ ppm): 7.64-6.74 (m, 5H, Ar-H and =CH), 8.58 (s, 1H, -NH), 10.06 (s, 1H, -OH), 3.45 (d, 1H, -CH), 2.38-2.27 (m, 1H, -CH), 2.05-1.82 (m, 2H, -CH₂), 3.77 (s, 3H, -OCH₃)

4.4 (3Z,4Z)-3,4-bis-(4-hydroxy-3-methoxybenzylidene)-7-(p-tolyl)-3,3a,3b,4,5,7-hexahydro-2H- piperidine-[2,3-c,5,4-c] dipyrazole (5d): Molecular Formula: C₂₈H₂₉N₅O₄, Yellowish Solid, Yield: 81.04%, M. W.: 499.56, Melting Point (°C): 158-160 °C, C, H, N Anal Observed: C, 67.89; H, 6.19; N, 13.95, FTIR (KBr): -NH (1-Peak): 3302 cm⁻¹, aromatic ring (3-Peaks): 1512 cm⁻¹, 1602 cm⁻¹ and 1656 cm⁻¹, Ar-OCH₃: 1281 cm⁻¹, Ar-OH: 3484 cm⁻¹

4.5 (3Z,4Z)-3,4-bis-(4-hydroxy-3-methoxybenzylidene)-7-(4-methoxyphenyl)-3,3a,3b,4,5,7-hexahydro-2H- piperidine-[2,3-c,5,4-c] dipyrazole (5e): Molecular Formula: C₂₈H₂₉N₅O₅, Umber Brown Solid Lumps, Yield: 82.81%, M. W.: 515.56, Melting Point (°C): 148-150 °C, C, H, N Anal

Observed: C, 65.95; H, 5.83; N, 13.79, FTIR (KBr): -NH (1-Peak): 3309 cm^{-1} , aromatic ring (3-Peaks): 1512 cm^{-1} , 1602 cm^{-1} and 1653 cm^{-1} , Ar-OCH₃: 1280 cm^{-1} , Ar-OH: 3482 cm^{-1}

4.6 (3Z,4Z)-3,4-bis-(4-hydroxy-3-methoxybenzylidene)-7-(4-fluorophenyl)-3,3a,3b,4,5,7-hexahydro-2H- piperidine-[2,3-c,5,4-c] dipyrazole (5f): Molecular Formula: C₂₇H₂₆FN₅O₄, Yellowish Granular Crystals, Yield: 75.20%, M. W.: 503.52, Melting Point (°C): 152-154 °C, C, H, N Anal Observed: C, 64.81; H, 5.59; N, 14.15, FTIR (KBr): -NH (1-Peak): 3292 cm^{-1} , aromatic ring (3-Peaks): 1512 cm^{-1} , 1601 cm^{-1} and 1655 cm^{-1} , Ar-OCH₃: 1279 cm^{-1} , Ar-OH: 3481 cm^{-1} , Ar-F: 1225 cm^{-1}

4.7 (3Z,4Z)-3,4-bis-(4-hydroxy-3-methoxybenzylidene)-7-(4-nitrophenyl)-3,3a,3b,4,5,7-hexahydro-2H- piperidine-[2,3-c,5,4-c] dipyrazole (5g): Molecular Formula: C₂₇H₂₆N₆O₆, Yellow Granular Crystals, Yield: 83.01%, M. W.: 530.53, Melting Point (°C): 147-149 °C, C, H, N Anal Observed: C, 61.88; H, 5.14; N, 15.97, FTIR (KBr): -NH (1-Peak): 3286 cm^{-1} , aromatic ring (3-Peaks): 1547 cm^{-1} , 1601 cm^{-1} and 1649 cm^{-1} , Ar-OCH₃: 1264 cm^{-1} , Ar-OH: 3482 cm^{-1} , Ar-NO₂: 1507 cm^{-1} , ¹H NMR (500.13 MHz, DMSO-d₆, δ ppm): 8.20-6.74 (m, 5H, Ar-H and =CH), 8.58 (s, 1H, -NH), 10.53 (s, 1H, -OH), 3.77 (s, 3H, -OCH₃), 3.40 (d, 1H, -CH), 2.41-2.10 (m, 1H, -CH), 1.86-1.82 (m, 2H, CH₂)

4.8 (3Z,4Z)-3,4-bis-(4-hydroxy-3-methoxybenzylidene)-7-(naphthalen-4-yl)-3,3a,3b,4,5,7-hexahydro-2H- piperidine-[2,3-c,5,4-c] dipyrazole (5h): Molecular Formula: C₃₁H₂₉N₅O₄, Dark Brown Solid, Yield: 84.96%, M. W.: 535.59, Melting Point (°C): 149-151 °C, C, H, N Anal Observed: C, 69.84; H, 5.75; N, 12.91, FTIR (KBr): -NH (1-Peak): 3261 cm^{-1} , aromatic ring (3-Peaks): 1427 cm^{-1} , 1459 cm^{-1} , 1511 cm^{-1} , 1601 cm^{-1} and 1651 cm^{-1} , Ar-OCH₃: 1279 cm^{-1} , Ar-OH: 3482 cm^{-1}

4.9 (3Z,4Z)-3,4-bis-(4-hydroxy-3-methoxybenzylidene)-7-(3-chloro-4-fluorophenyl)-3,3a,3b,4,5,7-hexahydro-2H- piperidine-[2,3-c,5,4-c] dipyrazole (5i): Molecular Formula: C₂₇H₂₅ClFN₅O₄, Yellow Granular Crystals, Yield: 87.26%, M. W.: 537.97, Melting Point (°C): 148-150 °C, C, H, N Anal Observed: C, 60.19; H, 4.94; N, 12.99, FTIR (KBr): -NH (1-Peak): 3281 cm^{-1} , aromatic ring (3-Peaks): 1511 cm^{-1} , 1602 cm^{-1} and 1660 cm^{-1} , Ar-OCH₃: 1279 cm^{-1} , Ar-OH: 3484 cm^{-1} , Ar-F: 1164 cm^{-1} , Ar-Cl: 1033 cm^{-1}

4.10 (3Z,4Z)-3,4-bis-(4-hydroxy-3-methoxybenzylidene)-7-(2,4,5-trichlorophenyl)-3,3a,3b,4,5,7-hexahydro-2H- piperidine-[2,3-c,5,4-c] dipyrazole (5j): Molecular Formula: C₂₇H₂₄Cl₃N₅O₄, Brownish Granular Crystals, Yield: 76.53%, M. W.: 588.87, Melting Point (°C): 144-146 °C, C, H, N Anal Observed: C, 55.34; H, 4.31; N, 12.15, FTIR (KBr): -NH (1-Peak): 3284 cm^{-1} , aromatic ring (3-Peaks): 1511 cm^{-1} , 1602 cm^{-1} and 1674 cm^{-1} , Ar-OCH₃: 1279 cm^{-1} , Ar-OH: 3482 cm^{-1} , Ar-2,4,5Cl: 1078 cm^{-1}

5. Antimicrobial assay of bis-chalcones and bis-pyrazoles:

5.1 Antimicrobial activities of Bis-chalcones (3a-j):

The synthesized flavonoids **3a-j** were evaluated *in-vitro* for antibacterial activity against gram positive *Bacillus subtilis* and gram negative *Escherichia coli* bacterial strains. Also evaluated for antifungal potencies towards *Candida albicans* and *Aspergillus niger* fungal strains. The inhibition zone of diameter were measured in mm and tabulated in the table-1.

Table 1: ANTIMICROBIAL ACTIVITIES OF BIS-CHALCONES (3a-j)

Compd Code	Zone of diameter calculated in mm by (Mean±S.D.)			
	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>	<i>Candida albicans</i>	<i>Aspergillus niger</i>
	100 µg/ml	100 µg/ml	100 µg/ml	100 µg/ml
3a	7.33±0.57 **	7.33±0.57 **	---	---
3b	7.33±0.57 **	4.66±4.04 **	---	---
3c	8.66±0.57 **	8±0 **	---	10.15 ± 0.29 **
3d	7.66±1.52 **	7.66±0.57 **	---	---
3e	7±0 **	2.33±4.04 **	---	---
3f	8.33±1.15 **	8±1 **	---	---
3g	7.66±0.57 **	2.66±4.61 **	---	---
3h	7±1 **	2.66±4.61 **	---	---
3i	7.66±1.52 **	4.66±4.04 **	10.92 ± 0.09 **	---
3j	7.66±0.57 **	7.66±0.57 **	---	---
Ctrl	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Std	18.33±0.57	18.33±0.57	12.40 ± 0.43	10.45 ± 0.11
Keynote: Zone of inhibition measured in mm (Mean±S.D.) (N=3) ('---' means no zone)				

5.2 Antimicrobial activities of Bis-pyrazoles (5a-j):

The synthesized target compounds **5a-j** were evaluated *in-vitro* for antibacterial activity against gram positive *Bacillus subtilis* and gram negative *Escherichia coli* bacterial strains. Also assessed for antifungal influences against *Candida albicans* and *Aspergillus niger* fungal strains. The inhibition zone of diameter were calculated in mm and presented in the table–2.

Table 1: ANTIMICROBIAL ACTIVITIES OF BIS-PYRAZOLES (5a-j)

Compd Code	Zone of diameter calculated in mm by (Mean±S.D.)			
	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>	<i>Candida albicans</i>	<i>Aspergillus niger</i>
	100 µg/ml	100 µg/ml	100 µg/ml	100 µg/ml
5a	7.33±1.15 **	5±4.35 **	---	---
5b	7±0 **	5.33±4.61 **	---	---
5c	8.33±1.15 **	8.33±0.57 **	---	---
5d	7±1 **	8±0 **	---	---
5e	6.33±0.57 **	---	---	---
5f	7.33±1.52 **	8±1 **	---	---
5g	7.33±1.52 **	4.66±4.04 **	---	---
5h	7±1.73 **	7±0 **	---	---
5i	8.66±0.57 **	7.66±1.15 **	---	---
5j	6.33±0.57 **	2.33±4.04 **	7.42 ± 0.27 **	11.51 ± 0.41 **
Ctrl	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Std	18.33±0.57	18.33±0.57	12.40 ± 0.43	10.45 ± 0.11
Keynote: Zone of inhibition measured in mm (Mean±S.D.) (N=3) ('---' means no zone)				

6. Results and Discussion:

6.1 Chemistry:

The intermediate compounds of bis-chalcones **3a-j** were prepared by the reaction of substituted N-phenyl glutarimides using 4-hydroxy-3-methoxy benzaldehyde i.e. vanillin. The series of bis-pyrazoles **5a-j** were developed by the cyclization of bis-chalcones **3a-j** and hydrazine hydrate with the existence of neutral corundum (Al₂O₃) in microwave supported solvent free condition. The synthesized bis-pyrazoles were validated by ¹H NMR, ¹³C NMR, IR, and elemental anal.

6.2 Antimicrobial protocol (3a-j and 5a-j):

All the synthesized compounds **3a-j** and **5a-j** were evaluated *in-vitro* for antibacterial activity against gram positive *Bacillus subtilis* (MCMB-310) and gram negative *Escherichia coli* (MCMB-301) bacterial strains at the concentrations of 100µg/ml by bore plate method using DMF solvent and nutrient agar was employed as culture media. After 48 hrs of incubation at 37 °C, the results were obtained in the form of cleared zone and were noted after the period of incubation was over. Correspondingly these compounds were evaluated for antifungal potencies against fungal strains *Candida albicans* (NCIM-3471) and *Aspergillus niger* (NCIM- 545) at the concentration 100µg/ml per disc by paper disc diffusion method using DMSO solvent. The yeast *Candida albicans* cultured using a malt extract, glucose yeast extract peptone agar medium (MGYP medium) and for fungi *Aspergillus niger* potato dextrose agar medium was used. After 3-7 days of incubation at 30°C, the results of clear zones were noted. Ampicillin was used as a standard drug for antibacterial activities and Amphotericin-B used for antifungal activities as a standard.

Graph 1: Antibacterial activities of 3a-j Mean±SD

Graph 2: Antibacterial activities of 5a-j Mean±SD

6.3 Statistical Analysis:

All the results of the synthesized compounds **3a-j** and **5a-j** were carried out by the triplicate practice N=3 with Mean ± SD revealed by antimicrobial graph-1 and graph-2. The statistical tests were performed by using GraphPad prism-6 and GraphPad InStat 3.10 version software. The statistical

significance was accessed by one way ANOVA ensured by Dunnett Multiple Comparisons Test will performed by standard drug against synthesized compounds. P value < 0.05 were considered as statistically significant and stated by *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 compared to standard groups.

7. Conclusion:

Intermediate bis-chalcones (3a-j) has been prepared by the treatment of substituted N-phenyl glutarimides and vanillin with neutral corundum in microwave supported solvent free method. Then bis-pyrazoles (5a-j) were developed with the good yield by using the mediated bis-chalcones and hydrazine hydrate with neutral corundum by the same method. All the synthesized compounds were characterized by their physical, spectral as well as antimicrobial analysis. Almost all the synthesized compounds showed moderate to good antibacterial activities against gram positive *Bacillus subtilis* and gram negative *Escherichia coli* strains, but in case of antifungal assay, only 3i and 5j compound showed good activities against *Candida albicans* and 3c and 5j compound showed good potency towards *Aspergillus niger* fungal strains. The ecofriendly microwave method can be used for the preparation of different substituted heterocyclic syntheses.

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