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

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Abstract: A simple eco-friendly 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 schemdit, 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], chromium 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 mojffat oxidizing agents are found 5α-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 Brucker alpha FT-IR spectrophotometer. ¹HNMR spectra were recorded on 400 MHz and 500.13 MHz by Brucker 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$_2$O$_3$) 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$_2$O$_3$) 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.

\[ \text{Scheme - I: (3Z,5Z)-3,5-bis-(4-hydroxy-3-methoxy-benzylidene)-N-phenyl-piperidine-2,6-dione (3a-j)} \]

3.1 (3Z,5Z)-3,5-bis-(4-hydroxy-3-methoxy-benzylidene)-1-phenylpiperidine-2,6-dione (3a):
Molecular Formula: C$_{27}$H$_{32}$NO$_6$, Yellowish White Amorphous Solid, Yield: 79.91%, M. W.: 457.47, Melting Point (°C): 78-80 °C, C, H, N Anal Observed: C, 71.06; H, 6.98; N, 3.24, FTIR (KBr): >C=O (2-Peaks): 1595 cm$^{-1}$ and 1672 cm$^{-1}$, =C-H: 2968 cm$^{-1}$, aromatic ring (3-Peaks): 1455 cm$^{-1}$, 1513 cm$^{-1}$ and 1541 cm$^{-1}$, Ar-OCH$_3$: 1157 cm$^{-1}$, Ar-OH: 3320 cm$^{-1}$, $^1$H NMR (200.13 MHz; CDCl$_3$; δ ppm): 7.40-6.26 (m, 8H, Ar-H and =CH), 9.87 (s, 1H, -OH), 3.70 (s, 3H, -OCH$_3$), 2.46 (s, 2H, -CH$_2$)

3.2 (3Z,5Z)-3,5-bis-(4-hydroxy-3-methoxy-benzylidene)-1-(4-bromophenyl)-piperidine-2,6-dione (3b):
Molecular Formula: C$_{27}$H$_{32}$BrNO$_6$, Dark Yellow Granular Crystals, Yield: 94.37%, M. W.: 536.37, Melting Point (°C): 94-96 °C, C, H, N Anal Observed: C, 60.88; H, 4.25; N, 2.75, FTIR (KBr): >C=O (2-Peaks): 1667 cm$^{-1}$ and 1689 cm$^{-1}$, =C-H: 3032 cm$^{-1}$, aromatic ring (3-Peaks): 1462 cm$^{-1}$, 1514 cm$^{-1}$ and 1590 cm$^{-1}$, Ar-OCH$_3$: 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: C$_{27}$H$_{32}$ClNO$_6$, Yellow Solid, Yield: 82.25%, M. W.: 491.92, Melting Point (°C): 76-78 °C, C, H, N Anal Observed: C, 66.42; H, 4.68; N, 2.99, FTIR (KBr): >C=O (2-Peaks): 1667 cm$^{-1}$ and 1742 cm$^{-1}$, =C-H: 3030 cm$^{-1}$, aromatic ring (3-Peaks): 1462 cm$^{-1}$, 1512 cm$^{-1}$ and 1590 cm$^{-1}$, Ar-OCH$_3$: 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-tolypiperidine-2,6-dione (3d):
Molecular Formula: C$_{28}$H$_{34}$NO$_6$, Yellow Granular Crystals, Yield: 83.98%, M. W.: 471.5, Melting Point (°C): 95-97 °C, C, H, N Anal Observed: C, 71.86; H, 5.73; N, 3.55, FTIR (KBr): >C=O (2-Peaks): 1594 cm$^{-1}$ and 1675 cm$^{-1}$, =C-H: 2946 cm$^{-1}$, aromatic ring (3-Peaks): 1459 cm$^{-1}$, 1514 cm$^{-1}$ and 1596 cm$^{-1}$, Ar-OCH$_3$: 1171 cm$^{-1}$, Ar-OH: 3280 cm$^{-1}$, $^1$H NMR-(500.13 MHz; 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: C₃₈H₃₄NO₈, Yellow Granular Crystals, Yield: 69.41%, M. W.: 487.5, Melting Point (°C): 68-70 °C, C, H, N Anal Observed: C₅, 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-methoxybenzylidene)-1-(4-fluorophenyl)piperidine-2,6-dione (3f): Molecular Formula: C₃₈H₃₄FNO₈, 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-methoxybenzylidene)-1-(4-nitrophenyl)piperidine-2,6-dione (3g): Molecular Formula: C₃₈H₃₄N₂O₈, 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-N: 1512 cm⁻¹

3.8 (3Z,5Z)-3,5-bis-(4-hydroxy–3-methoxybenzylidene)-1-(naphthalen-1-yl)-piperidine-2,6-dione (3h): Molecular Formula: C₃₄H₃₄N₂O₈, Whits 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-methoxybenzylidene)-1-(3-chloro-4-fluorophenyl)-piperidine-2,6-dione (3i): Molecular Formula: C₃₄H₃₄ClFNO₈, 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-methoxybenzylidene)-1-(2,4,5-trichlorophenyl)-piperidine-2,6-dione (3j): Molecular Formula: C₃₄H₂₉Cl₃NO₈, 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-pyrazolo 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 g 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-methoxybenzylidine)-7-(1-phenyl)-3,3a,3b,4,5,7-hexahydro-2H-piperidine-[2,3-c,5,4-c] dipyrazole (5a): Molecular Formula: C_{28}H_{29}N_{5}O_{5}, Brown Solid, Yield: 82.57%, M. W.: 515.56, 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-methoxybenzylidine)-7-(4-bromophenyl)-3,3a,3b,4,5,7-hexahydro-2H-piperidine-[2,3-c,5,4-c] dipyrazole (5b): Molecular Formula: C_{29}H_{30}BrN_{5}O_{5}, 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, -OH), 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-methoxybenzylidine)-7-(4-chlorophenyl)-3,3a,3b,4,5,7-hexahydro-2H-piperidine-[2,3-c,5,4-c] dipyrazole (5c): Molecular Formula: C_{29}H_{30}ClN_{5}O_{5}, 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, -OH), 10.06 (s, 1H, -OH), 3.45 (d, 1H, -CH₂), 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-methoxybenzylidine)-7-(p-tolyl)-3,3a,3b,4,5,7-hexahydro-2H-piperidine-[2,3-c,5,4-c] dipyrazole (5d): Molecular Formula: C_{29}H_{30}N_{5}O_{5}, 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-methoxybenzylidine)-7-(4-methoxyphenyl)-3,3a,3b,4,5,7-hexahydro-2H-piperidine-[2,3-c,5,4-c] dipyrazole (5e): Molecular Formula: C_{29}H_{30}N_{5}O_{5}, 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)-3a,3b,4,5,7-hexahydro-2H-piperidine-[2,3-c,5,4-c] dipyrazole (5f): Molecular Formula: C₂₇H₂₄FN₅O₄, 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, 1602 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)-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 (s, 3H, Ar-OH), 3.77 (s, 3H, -OCH₃), 3.40 (d, 1H, =CH), 8.58 (s, 1H, -OH), 10.53 (s, 1H, -OH), 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)-3a,3b,4,5,7-hexahydro-2H-piperidine-[2,3-c,5,4-c] dipyrazole (5h): Molecular Formula: C₂₁H₂₉N₂O₄, Dark Brown Solid, Yield: 84.96%, M. W.: 553.59, 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)-3a,3b,4,5,7-hexahydro-2H-piperidine-[2,3-c,5,4-c] dipyrazole (5i): Molecular Formula: C₂₇H₂₅ClF₃N₄O₄, 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)-3a,3b,4,5,7-hexahydro-2H-piperidine-[2,3-c,5,4-c] dipyrazole (5j): Molecular Formula: C₂₇H₂₃Cl₃N₄O₄, 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-2,4,5Cl₃: 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 | Bacillus subtilis (100 μg/ml) | Escherichia coli (100 μg/ml) | Candida albicans (100 μg/ml) | Aspergillus niger (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                       |
| 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 | Bacillus subtilis (100 μg/ml) | Escherichia coli (100 μg/ml) | Candida albicans (100 μg/ml) | Aspergillus niger (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                       |
| 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.

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:
Inmediate 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 synhtesized 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.

8. References:

[1] Voskiene A., Mickevicius V. and Mikulskiene G., Synthesis and structural characterization of products condensation 4-carboxy-1-(4-styrylcarmonylphenyl)-2-pyrrolidinones with hydrazines, ARKIVOC, (xv), (2007), 303-314
[2] Dhivare R. S. and Rajput Shankarsingh S., Synthesis, characterization and antimicrobial evolution of six membered cyclic imides, International Journal of Chemistry and Pharmaceutical Sciences, 3(8), (2015), 1877-1880
[3] Dhivare Ravindra S. and Rajput Shankarsingh S., Synthesis and antimicrobial activity of five membered cyclic imide derivatives of mono, di and tri substituted aromatic amines and napthyl amine, World Journal of Pharmaceutical Research, 4(6), (2015), 1650-1658
[4] Rajput S. S., Synthesis and Characterization of Bis-Heterocyclic derivatives of 1-(3-Chlorophenyl)-Pyrrrolidine-2, 5-Dione, International Journal of Advances in Pharmacy, Biology and Chemistry, 1(2), (2012), 242-246
[5] Patil M. M. and Rajput S. S., Succinimides: Synthesis, reaction and biological activity, International Journal of Pharmacy and Pharmaceutical Sciences, 6(11), 2014, 8-14
[6] Patil C. B., Mahajan S. K. and Katti S. A., Chalcone: A versatile molecule, Journal of Pharmaceutical Science and Research, 1(3), (2009), 11-22
[7] Ghosh R. and Das A., Synthesis and biological activities of chalcones and their heterocyclic derivatives: a review, world journal of pharmacy and pharmaceutical sciences, 3(3), (2014),578-595
[8] Suwito H., Jumina, Mustofa, Kristanti A. N. and Puspaningsih N. N. T., Chalcones: Synthesis, structure diversity and pharmacological aspects, Journal of Chemical and Pharmaceutical Research, 6(5), (2014), 1076-1088
[9] Khalaf M. I., Abdel-Wahab A. and Kandil F., Knoevenagel condensation of 5-substituted furan-2-carboxaldehyde with indan-1,3-dione, Iraqi Journal of Science, 53(1), (2012), 17-24
[10] Dhivare Ravindra S. and Rajput S. S., Synthesis and antimicrobial evaluation of some novel bis-heterocyclic chalcones from cyclic imides under microwave irradiation, Chemical Science Review and Letters, 4(15), (2015), 937-944
[11] Dhivare Ravindra S. and Rajput S. S., Microwave Assisted Synthesis and Antimicrobial Activities of 3,4-bis-(4-hydroxy-3-methoxybenzylidene)-7-(N-phenyl)-3,3a,3b,4,5,7-hexahydro-2H pyrrolo-[2,3-c,5,4-c]–dipyrazole, International Journal of Current Trends in Pharmaceutical Research (IJCTPR), 3(6), (2015), 1106-1109,
[12] Dhivare R. S. and Rajput S. S., Microwave assisted synthesis and antimicrobial screening of novel 9-(N-phenyl)-4,5-(2*,-"-methoxyphenyl)-9H-1,3,6,8,9-hexa-azo-fluorene-2,7-diamine derivatives using bis-heterocyclic chalcones, *International Journal of Advances in Pharmacy, Biology and Chemistry*, 4(4), (2015), 863-870

[13] Hieu B. T., Thuy L. T., Thuy V. T., Tien H. X., Chinh L. V., Hoang V. D. and Vu T. K., Design, Synthesis and In Vitro Cytotoxic Activity Evaluation of New Mannich Bases, *Bull. Korean Chem. Soc.*, 33(5), (2012),1586-1592

[14] Sharma B. K., Ameta S. C. and Dwivedi V. K., Ecofriendly synthesis of chalcones and their 2-pyrazolone and isoxazolines derivatives as potential microbial agents, *International Journal of Chemical Sciences*, 12(4), (2014), 1121-1134

[15] Ibrahim M. M., Al-Refai M., Abu-El-Halawa R., Tashtoush H., Alsohaili S. and Masad M., Synthesis of some new Chalcone and 4,5-Dihydro-1H-pyrazole Derivatives as Potential Antimicrobial Agents, *Journal of Molecular Liquid*, 7(2), (2012), 115-123

[16] Tala S. D., Vekariya P. B., Ghetiya R. M. and Joshi S. H., Synthesis and biological study of some new chalcone and pyrazole derivatives, *Indian Journal of Chemistry, 52B*, (2013), 807-809

[17] Ehsan S., Ghafoor S. and Khan B., Microwave assisted synthesis of nitrogen and hydroxyl containing bioactive compounds as drug motif, *International Journal of Science and Research*, 3(9), (2014), 195-199

[18] Kalirajan R., Rathore L., Jubie S., Gowramma B., Gomathy S. and Sankar S., Microwave assisted synthesis of some novel pyrazole substituted benzimidazoles and evaluation of their biological activities, *Indian Journal of Chemistry, 50B*, (2011), 1794-1799

[19] Chandak H. S., Microwave assisted synthesis of n- [4-(5-aryl-1H/phenazol -3-y1)-phenyl]-benzenesulfonylamides, *RASAYAN J. Chem.*, 5(2), (2012), 177-182

[20] Thakrar S. and Shah A., A rapid and highly efficient microwave synthesis of highly functionalized chalcones derivatives, *International Journal of ChemTech Research, 4*(1), (2012), 394-402

[21] Poovan S., Murugan S., Sangaraiah N. and Alagusundaram P., An efficient and facile synthesis of novel 1,2,3-triazolyl-Nacylpyrazoline hybrids, *Chinese Chemical Letters, 25*, (2014), 146–148

[22] Khodairy A., Synthesis studies on the synthesis of some new fused heterocyclic compounds derived from 3,5-pyrazolidinedione, *Journal of Chinese Chemical Society*, 54, (2007), 93-102

[23] Thirunayanan G. and Guna Sekar K., Solvent-Free Synthesis of Some1-Acetyl Pyrazoles, *Journal of the Korean Chemical Society, 57*(5), (2013), 599-605

[24] Mohammadi B. and Adib M., Microwave assisted one-pot tandem three-component synthesis of 2,4,5-triaryl-2-4-dihydro-3H-1,2,4-triazol-3-one derivatives, *Chinese Chemical Letters, 25*, (2014), 553–556

[25] Lokhande P. D., Waghmare B. Y. and Sakate S. S., Regioselective one-pot synthesis of 3,5-diarylpzrazoles, *Indian Journal of Chemistry, 44*(B), (2005), 2338-2342

[26] Baluja S. and Chanda S., Pyrazoline derivatives: Synthesis and antibacterial studies, *World Research Journal of Biochemistry, 1*(1), (2012), 6-10

[27] Bhanat K., Parashar B. and Sharma V. K., Microwave Induced Synthesis and Antimicrobial activities of various substituted Pyrazolidines from Chalcones, *Research Journal of Chemical Sciences, 4*(2), (2014), 68-74

[28] Chauhan A., Sharma P. K., Kaushik N. And Kumar N., Synthesis of novel pyrazole analogues as efficacious antimicrobial agents, *International Journal of Pharmacy and Pharmaceutical Sciences, 3*(5), (2011), 166-176

[29] Kotla V. V., Dalavai V. K. and Chunduri V. R., Synthesis and biological activity studies of some novel pyrazoline derivatives, *Der Pharma Chemica, 4*(5), (2012), 2003-2008

[30] Yusuf M. and Jain P., Synthetic and biological studies of pyrazolines and related heterocyclic compounds, *Arabian Journal of Chemistry, B*, (2014), 553-596
[31] Kendre M. M. and Baseer M. A., Synthesis and Antimicrobial Evaluation of Some New Pyrazoline Derivatives, Indian Journal of Applied Research, 3(8), (2013), 72-73

[32] Bhagat P. R., Microwave assisted synthesis of 1-(3-chlorophenyl)-3, 5-diaryl – pyrazoles, Int. J. Chem. Sci., 6(1), (2008), 68-72

[33] Swarnkar D., Ameta R. and Vyas R., Microwave assisted synthesis of some pyrazole derivatives and their antibacterial and antifungal activity, The Pharma Innovation Journal, 3(10), (2014), 05-09

[34] Awad B. M., Abdallah S. M., Hefny H. A., Abdou M. H., Abd-Elmonem F. I. and Abd-Elmonem N. A., Synthesis of some medicinal and biological active (E)-2-arylidine-4-oxo-4-(4-Narylaminophenyl) phenylamino)butanoic acids and (E)-4-(3-aryliden)e-2,5-dioxopyrrolidin-1-yl)-Narylbenzenesulfonamides, Life Science Journal, 9(4), (2012), 567-577

[35] Dhivare R. S. and Rajput S. S., Microwave assisted synthesis and microbial screening of novel amino-pyrimidine derivatives using bis-chalcones, An International Research Journal of Chemistry and Chemical Sciences (JCCS), 5(10), (2015), 550-556.

[36] Dabholkar V. V. And Gavande R. P., A microwave-catalyzed rapid, efficient and ecofriendly synthesis of substituted pyrazol-5-ones, J.Serb.Chem.Soc., 68(10), (2003), 723-727

[37] Arora H. K. and Jain S., The synthetic development of pyrazole nucleus: From reflux to microwave, Der Pharmacia Lettre, 5(1), (2013), 340-354

[38] Goyal A., Jain N.and Jain S., Synthesis and antibacterial screening of some 1-phenyl-3-aryl-5-(4-(4-butanoloxo) phenyl)-1H-pyrazoles, Der Pharmacia Sinica, 4(4), (2013), 112-117

[39] Kalirajan R., Rathore L., Gowramma B., Gomathy S. and Sankar S., Microwave assisted synthesis of some novel pyrazole substituted benzimidazoles and evaluation of their biological activities, Indian Journal of Chemistry, 50(B), (2011), 1794-1799

[40] Fouad S. A., Synthesis, Characterization and Anti-Breast Cancer Activity of Some new pyrazole, thiazole, chromene and pyridine derivatives, International Journal of Advanced Research, 2(12), (2014),442-453

[41] Pasin J.S.M., Ferreira A.P.O., Saraiva A.L.L., Ratzlaff V., Andrighetto R., Machado P., Marchesan S., Zanette R.A., Bonacorso H.G., Zanatta N., Martins M.A.P., Ferreira J. and Mello C.F., Antipyretic and antioxidant activities of 5-trifluoromethyl-4,5-dihydro-1H-pyrazoles in rats, Braz J Med Biol Res, 43(12), (2010), 1193-1202

[42] Kumar G. V., Govindraju M., Renuka N., Khatoon B. A., Mylarappa B. N. and Kumar K. A., Synthesis of 1,3,5-triaryl-4,6-dioxo-pyrolo[3,4-d]-7,8-dihydropyrazoles and their antimicrobial and antioxidant activity, RASAYAN J. Chem., 5(3), (2012), 338-342

[43] Malhotra P., Pattan S. And Nikalje A. P., Microwave assisted synthesis and anti-inflammatory activity of 3, 5diaryl substituted –2pyrazolines, International Journal of Pharmacy and Pharmaceutical Sciences, 2(2), (2010), 21-26

[44] Prasanna Raja P., Riyazulah M.S. and Kumar V.S., Synthesis and Biological Evaluation of some Chalcone Derivatives, International Journal of ChemTech Research, 2(4), (2010), 1998-2004

[45] Jadhav S. Y., Bhosale R. B., Shirame S. P., Sonawane V. D., Hublikar M. G., Sonawane K. D. and Shaikh R. U., Synthesis and biological evaluation of fluoro-hydroxy substituted pyrazole chalcones as anti-inflammatory, antioxidant and antibacterial agents, Int. J. Pharm Bio. Sci., 4(2), (2013), 390-397

[46] Ozdemir A., Tarun-Zitouni G. and Kaplancikli Z. A., Novel analogues of 2-pyrazoline: Synthesis, Characterization and anti-mycobacterial evaluation, Turk Journal of Chemistry, 32, (2008), 529-538

[47] Swarnkar D., Ameta R. and Vyas R., Microwave assisted synthesis, characterization and biological activity of some pyrazole derivatives, International Research Journal of Pharmacy, 5(6), (2014), 459-462

[48] Sharshira E. M. and Hamada N. M. M., Synthesis and Antimicrobial Evaluation of Some Pyrazole Derivatives, Molecules, 17, (2012), 4962-4971
[49] Azarifar D. and Shaebanzadeh M., Synthesis and Characterization of New 3,5-Dinaphthyl Substituted 2-Pyrazolines and Study of Their Antimicrobial Activity, *Molecules*, 7, (2002), 885-895

[50] MallikarjunaRao R., Sreeramulu J., Ravindranath L. K., NagarajaReddy G., Hanumantharayudu K., Nageswara Reddy G., Jayaraju A. and Madhusudhan P., *Journal of Chemical and Pharmaceutical Research*, 4(1), (2012), 272-278

[51] Shah S. H. and Patel P. S., Synthesis and Antimicrobial Activity of Azetidin-2-one containing Pyrazoline Derivatives, *Research Journal of Chemical Sciences*, 2(7), (2012), 62-68

[52] Yadav A. K., Singh V. And Singh D., Synthesis and Antifungal Activity of Some Novel 1-Phenyl-3-methyl-4-substituted phenyl-6-imino-4,7-dihydro-1,3-thiazino(5,4-d) pyrazoles, *Asian Journal of Chemistry*, 19(5), (2007), 3511-3514

[53] Kumar C., Reddy V.and Fasiulla, Synthesis, Characterization and Antimicrobial Screening on New 1,5-Disubstituted Pyrazoline Derivatives Bearing P-Methoxy-M-Chloro Phenyl Moiety, *International Journal of Scientific and Research Publications*, 3(5), (2013), 1-7

[54] Amr A. E., Abdel-Latif N. A. and Abdalla M. M., Synthesis of some new testosterone derivatives fused with substituted pyrazoline ring as promising 5α-reductase inhibitors, *Acta Pharm. 56*, (2006), 203-218