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SYNTHESIS, CHARACTERISATION AND BIOLOGICAL EVALUATION OF 2-ARYL-4-PHENL-2H-CHROMENE-3-BENZIMADAZOLES

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ABSTARCT:
A new series of 2-aryl-4-phenyl-2H-chromene-3-benzimadazoles **(9a-j)** were synthesized by the condensation of 2,4-diary-2H-chromene carbaldehyde and *o*-phenylene diamines. The products were purified by column chromatography and structures of these compounds are established by IR, ^{1}H-NMR, ^{13}C-NMR and mass spectral data. All the synthesized compounds were screened for their anti-microbial activity and the results were compared with ciprofloxacin. Compound **9f** was found to be most potent compound of this series and with activities better than ciprofloxacin under the tested conditions.

KEYWORDS: Chalcone, flavanone, benzimidazole, heterocycles, condensation.

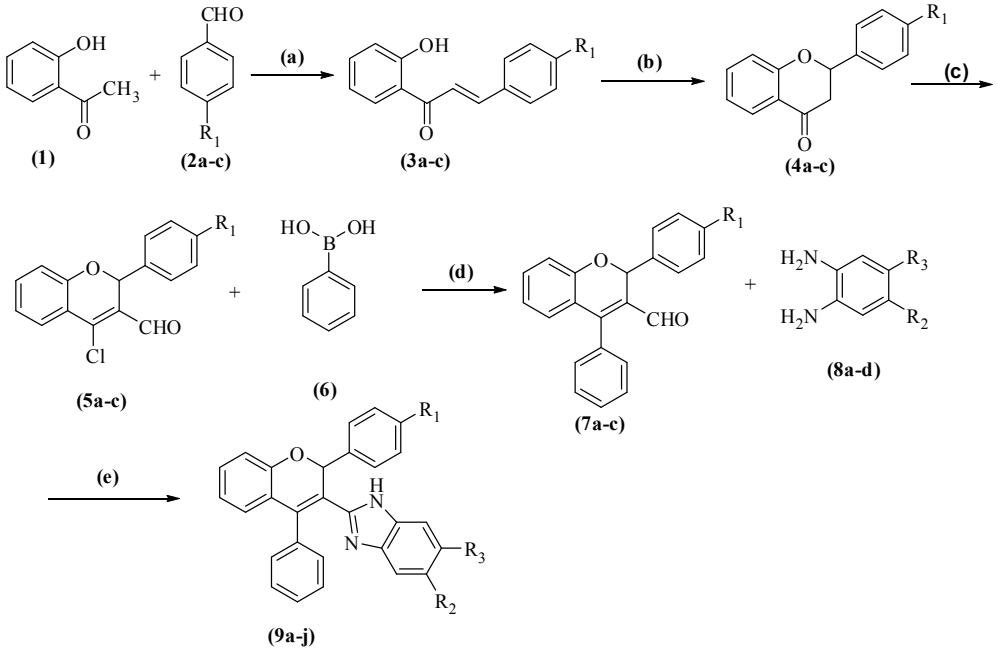
INTRODUCTION:

Benzimadazoles are important class of heterocycles that are frequently used in drug and agrochemical discovery programs. For examples, the benzimidazole core structure is found in a variety of commercial drugs such as Atacand, Nexium, Micardis, Protonix, and Vermox. Recent medicinal chemistry applications of benzimidazole analogs include antibacterial and antifungal
Agents[i-iii], anthelmintic agents[iv], HIV-1-induced cytopathic inhibitor[v], anti-inflammatory and antiulcer agents[vi], cytotoxic and antitumor agents[vii,viii], DNA binding agents[ix], enzyme and receptor agonists or antagonists[x]. Some benzimadazole based analogues were found to be promising in reducing plasma lipids in hamsters and cynomolgus monkeys[xi]. Other applications of benzimidazoles include their use as organic ligands[xii,xiii], fluorescent whitening agent dyes[xiv] and functional materials[xv,xvi]. Therefore, the construction of benzimadazole based heterocycles has always been of great interest to organic and medicinal chemists and has consequently received much attention[xvii]. In the present article, we report the synthesis and antibacterial activity of new 2,4-diaryl-2H-chromene-3-benzimadazoles derivatives.

Results and discussions:

Synthesis of 2,4-diaryl-2H-Chromene-3-benzimadazoles (9a-j): The reaction of 2-hydroxy acetohenone **(1)** with benzaldehydes **(2a-c)** in the presence of KOH/EtOH resulted in chalcones **(3a-c)**. These chalcones were converted into flavan-4-ones **(4a-c)** in EtOH/HCl

medium. These flavan-4-ones **(4a-c)** on reaction with DMF/$POCl_3$ gave 4-chloro-2-aryl-2H-3-chromene carbaldehydes **(5a-c)**. Equimolar quantities of 4-chloro-2-aryl-2H-3-chromene carbaldehydes **(5a-c)** and phenyl boronic acid **(6)** in DMF were stirred at 80°C for 4 hours to give 4-phenyl-2-aryl-2H-3-chromene carbaldehydes in good yields **(7a-c)**. The 4-phenyl-2-aryl-2H-3-chromene carbaldehydes **(7a-c)** on reaction with *o*-phenylene diamines **(8a-d)** in DMF solvent and sodium metabisulphate as a catalyst afforded title compounds **(9a-j)** in good yields.



Reagents and reaction conditions: (a) KOH, EtOH, RT, 8h. (b) H_2SO_4, EtOH, Reflux, 24h. (c) DMF, $POCl_3$, 0°C-RT, 12h. (d) DMF, Na_2CO_3, 80°C, 4h. (e) DMF, $Na_2S_2O_5$, 80°C, 4h

In the IR spectrum of **9a**, peaks were observed at 3063cm^{-1}(NH), 1597cm^{-1}(C=N) and 1443cm^{-1}(C=C). In the ^{1}H-NMR of spectrum of **9a**, the newly formed benzimadazole protons of H-4''',7''', H-5''',6'''appeared as a multiplet at δ 7.59, 7.27 and NH proton appeared at δ 7.42 as broad singlet. The other peaks appeared at δ 7.64 (brs, H-2",3",4",5",6"), 7.20 (m, H-,4'), 7.15 (m, H-2',3',5',6',2), 6.97 (dd, J=8.2, 1.0Hz, H-7), 6.78 (ddd, J=8.5, 7.5, 1.0Hz, H-6), 6.67 (dd, J=7.7, 1.5Hz, H-8). In the ^{13}C-NMR spectrum of **9a**, the benzimadazole carbon (C-1''') appeared at δ 138.4, C-3''', 8''' at δ 135.8 and C-4''',5''',6''',7''' at 122.0. The other signal assignments are as follows : 151.7 ((C-8a), 147.9 (C-4), 135.5 (C-3), 129.6 (C-5), 129.3 (C-2",3",5",6"), 128.5 (C-1"), 128.7 (C-7), 127.1 (C-2',6'), 126.9 (C-4"), 126..5 (C-3',5'), 125.9 (C-4'), 123.4 (C-1'), 120.2 (C-6), 119.9 (C-4a), 116.1 (C-8), 74.1 (C-2). In the mass spectrum of **9a**, molecular ion peak was observed at m/z 401 [M+H].

ANTIBACTERIAL ACTIVITY

The synthesized compounds were screened for their antibacterial potential against two organisms namely *E.coli* and *P.aeruginosa* and the results were compared with ciprofloxacin as standard drug. It was found that all the synthesized compounds showed antibacterial action against the tested strains with varying degree of activity and the MIC values are shown in table 1.

Table 1: Antibacterial activity of the prepared benzimadazole derivatives

Compound Name	MIC Value (µg/ml)	
	Escherichia coli	*Pseudomonas aeruginosa*
9a	20.5	25.6
9b	30	37.6
9c	13.8	15.2
9d	21.8	22.5
9e	35	37.8
9f	6.2	3.5
9g	25	15.5
9h	35.8	32.1
9i	22.5	27.8
9j	24.7	20.3
Ciprofloxacin	19.3	20.4

Among all the compounds, derivative **9f** with two chlorine substituents showed excellent activity with MIC values of 6.2 and 3.5 µg/ml against *E.coli* and *P.aeruginosa* respectively which is higher compared to ciprofloxacin which showed MIC values of 19.3 and 20.4 µg/ml respectively. In addition, compound **9c** also showed excellent activity higher than ciprofloxacin with MIC values of 13.8 and 15.2 µg/ml but lower than compound **9f**. Compound **9d** which has two chlorines showed similar activity as ciprofloxacin with MIC values of 21.8 and 22.5 µg/ml against E.coli and P.aeruginosa respectively. The difference between compound **9f** and **9d** is the presence of methyl substituent on the phenyl ring substituent on the chromanol moiety which is absent in compound **9d**. Compounds **9j** and **9g** were showing slightly higher activity towards P.aeruginosa compared to ciprofloxacin with MIC values of 15.5 and 20.3 µg/ml respectively. Other compounds namely **9a**, **9e**, **9h** and **9i** were showing lower activity compared to ciprofloxacin.

EXPERIMENTAL SECTION:

Chemistry

All reactions were carried out under nitrogen atmosphere in oven-dried glassware with magnetic stirring**.** All the chemicals and solvents were purchased from Sd fine chemicals, Bombay, India. Solvents were purified and dried according to standard procedures. Silica gel (60–120 mesh) for column chromatography was purchased from M/s Acme Synthetic Chemicals (Mumbai, India) and pre-coated TLC plates (Silica gel 60F254) were purchased from Merck (Darmstadt, Germany). The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker 400 and 100 MHz, respectively, and TMS was used as an internal standard. Chemical shifts relative to TMS as internal standards were given as δ values in ppm. Mass spectra were recorded using electron spray ionization on Waters e2695 Seperators module (Waters, Milford, MA, USA) mass spectrometer. IR spectra were recorded on a Fourier transform (FT-IR), USA (Perkin-Elmer model 337) instrument. The melting points were determined on a Barnstead Electro Thermal 9200 Instrument.

1). General procedure for synthesis of chalcones (3a-c):

2-Hydroxyacetophenone **(1)** (36.76mmoles) benzaldehyde **(2a)** (38 mmoles) were dissolved in 100 mL of ethanol. KOH (60%) was added drop wise, until the solution was alkaline. The reaction mixture was stirred for 8 hrs at room temperature. Neutralization with dilute HCl yielded a solid, which was chromatographed over silica gel by eluting with pet.ether : chloroform (8:2) to give yellow solid **3a** (50%yield). Similar procedure was adopted for **3b** and **3c**.

i) Synthesis of chalcone (3a): IR (KBr): 1637 cm^{-1} (C=O). ^{1}H-NMR (200MHz, $CDCl_3$): δ 12.72 (s, 2′-OH), 7.95 (m, H-3, H-6′), 7.77 (m, H-4,2″,6″), 7.65 (m, 4H, H-2, H-3",4",5") and 7.20 (m, H-3′,5′). MS: m/z 225[M+H].

ii) 4"-Methyl chalcone (3b): m.p. 114^{0}C. Yield (50%). IR.(KBr): 1635 cm^{-1} (C=O).
^{1}H-NMR (400MHz, $CDCl_3$): δ 12.90 (s, 2'-OH), 7.91 (dd, J=8.0, J=1.5Hz, H-6'), 7.87 (d, J=15.5Hz, H-3), 7.58 (d, J=15.3Hz, H-2), 7.53 (d, J=8.0Hz, H-2",6"), 7.47 (ddd, J=8.5, J=7.7, J=1.5Hz, H-4'), 7.21 (d, J=8.0Hz, H-3",5"), 7.00 (dd, J=8.5, J=1.0Hz, H-3'), 6.90 (ddd, J=8.2, J=7.7, 1.2Hz, H-5'), 2.3 (s, 4"-CH_3).^{13}C-NMR (100.6 MHz, $CDCl_3$): δ 193.7 (C=O), 163.6 (C-2'), 145.5 (C-3), 141.6 (C-4"), 136.2 (C-4'), 131.8 (C-1"), 129.8 (C-3",5"), 129.6 (C-6'), 128.7 (C-2", 6"), 120.0 (C-1'), 118.9 (C-2), 118.6 (C-3'), 21.6 (4"-CH_3). MS: m/z 239[M+H].

iii) 4"-Methoxy chalcone (3c): m.p. 87^{0}C. IR (KBr): 1637 cm^{-1} (C=O).
^{1}H-NMR (200MHz, $CDCl_3$): δ 12.85 (s, 2′-OH), 7.95 (dd, J=9.0,2.0Hz, H-6′), 7.90 (d, J=16.0Hz, H-3), 7.58 (d, J=9,0Hz, H-2″,6″), 7.50 (m, H-4,2), 6.96 (d, J=9.0Hz, H-3″,5″), 6.90 (m, H-3′,5′) and 3.85 (s, 4″-OCH_3). ^{13}C-NMR (50.3 MHz, $CDCl_3$): δ 193.4 (C=O), 163.4 (C-2′-OH), 161.8 (C-4″), 145.1 (C-3 olefinic), 135.9 (C-4′), 130.3 (C-2″,6″), 129.3 (C-6′), 127.8 (C-1″), 119.9 (C-1′), 118.5 (C-5′), 118.3 (C-3′), 117.4 (C-2 olefinic), 114.3 (C-3″,5″), 55.2 (4″-OCH_3). MS: m/z 245[M+H].

2). Synthesis of flavan-4-ones (4a-c):

i) Flavan-4-one (4a): Chalcone **(3a)** (10 mmoles) was dissolved in ethanol (100 ml). To the resulting solution conc. HCl (10 ml) was added and the mixture was refluxed for 24 hr on a water bath. At the end of the reaction ethanol was distilled off and water added. The solid that separated was filtered and chromatography over silica gel by eluting with pet ether: chloroform (8:2) which was recrystallised from methanol as white crystals to give **4a** (90%yield). Similar procedure was adopted for **4b** and **4c**.
IR (KBr): 1689 cm^{-1} (C=O). ^{1}H-NMR (200MHz, $CDCl_3$): δ 7.93 (dd, J=10.0,2.0Hz, H-5), 7.45 (m, H-7, and H-2′,6′), 7.05 (m, H-6,8), 5.50 (dd, J=17.0, 4.0Hz, H-2), 3.08 (dd, J=17.0, 14.0Hz, H_a-3) and 2.85 (dd, J=17.0, 4.0Hz, H_e-3).MS: m/z 225[M+H].

ii) 4′-Methylflavan-4-one (4b): m.p. 114^{0}C. Yield (85%). IR(KBr): 1692 cm^{-1} (C=O).
^{1}H-NMR (200MHz, $CDCl_3$): δ 7.95 (dd, J=10.0, 2.0Hz, H-5), 7.45 (dd, J=10.0, 2.0Hz, H-7), 7.35 (d, J=9.0Hz, H-2',6'), 7.25 (d, J=9.0Hz, H-3',5'), 7.00 (dd, J=10.0, 10.0Hz, H-6,8), 5.45 (dd, J=17.0, 4.0Hz, H-2), 3.08 (dd, J=17.0, 14.0Hz, H_a-3), 2.82 (dd, J=17.0, 4.0Hz, H_e-3) and 2.35 (s, 4'-CH_3). ^{13}C-NMR (50.3 MHz, $CDCl_3$): δ 191.8 (C-4), 161.5 (C-8a), 138.5 (C-4'), 135.9 (C-7), 135.7 (C-1'), 129.3 (C-2',6'), 126.9 (C-5), 126.0 (C-3',5'), 121.4 (C-6), 120.8 (C-4a), 118.0 (C-8), 79.4 (C-2), 44.4 (C-3) and 21.1 (C-4'-CH_3). MS: m/z 239[M+H]**.**

iii) 4'-Methoxyflavan-one (4c): m.p. 78^{0}C. Yield (88%). IR (KBr): 1683 cm^{-1} (C=O).
^{1}H-NMR (200MHz, $CDCl_3$): δ 7.95 (dd, J=10.0,2.0Hz, H-5), 7.50 (m, H-7), 7.40 (d, J=9.0Hz, H-2′,6′), 7.00 (m, H-6,8), 6.95 (d, J=9.0Hz, H-3′,5′), 5.45 (dd, J=17.0, 4.0Hz, H-2), 3.95 (s, 4′-OCH_3), 3.09 (dd, J=17.0, 14.0Hz, H_a-3) and 2.80 (dd, J=17.0, 4.0Hz, H_e-3). ^{13}C-NMR (50.3 MHz, $CDCl_3$): δ 191.8 (C-4), 161.4 (C-8a), 159.7 (C-4′), 135.8 (C-7), 130.6 (C-1′), 127.5 (C-2′,6′), 126.7 (C-5), 121.2 (C-6), 120.7 (C-4a), 117.9 (C-8), 114.0 (C-3',5'), 79.1 (C-2), 55.0 (C-4′-OCH_3) and 44.2 (C-3). MS: m/z 245[M+H].

3. Synthesis of 4-chloro-2-aryl-2H-3-chromene carbaldehydes (5a-c):

i) 4-Chloro-2-phenyl-2H-3-chromene carbaldehyde (5a): To flavan-4-one **(4a)** (10mmoles) in dry dimethyl formamide (50 mmoles) a freshly distilled dry phosphorus oxychloride (9 mmoles) was added with constant stirring at 0^o C. The reaction mixture was stirred overnight at room temperature and then poured on to crushed ice. The yellow compound **(5a)** that separated was filtered, washed with water and the product was purified on chromatography over silica gel by eluting with pet ether gave 4-chloro-2-phenyl-2H-3-

chromenecarbaldehydes **(5a)** 85% yield. This was recrystallised from methanol to give pale yellow needles. Similar procedure was adopted for **5b** and **5c.**

IR (KBr): 1601 (C=C) and 1661 cm^{-1} (CHO). ^{1}H-NMR (200MHz, $CDCl_3$): δ 10.27 (s, 3-CHO), 7.48 (dd, J=9.0, 2.0 Hz, H-5), 7.20 (m, H-2', 3', 4', 5', 6' and H-7), 6.70 (m, H-8, 6) and 6.30 (s, H-2). ^{13}C-NMR (50.3MHz, $CDCl_3$): δ 187.8 (C-3-CHO), 154.8 (C-8a), 143.5 (C-4), 137.9 (C-3), 134.3 (C-1'), 128.3 (C-2',6'), 128.5 (C-7), 126.6 (C-4'), 126.6 (C-3',5'), 126.3 (C-5), 121.7 (C-6), 119.7 (C-4a), 117.2 (C-8) and 74.8 (C-2). MS: m/z 271[M+H] and 273[M+H+2].

ii) 4-Chloro-2-(4'-methylphenyl)-2H-3-chromene carbaldehyde (5b): m.p. 98^{0}C, Yield (85%). IR (KBr): 1603 (C=C) and 1667 cm^{-1} (CHO). ^{1}H-NMR (200MHz, $CDCl_3$): δ 10.22 (s, 3-CHO), 7.65 (dd, J=10.0Hz, H-5), 7.30 (m, H-6), 7.10 (d, J=10.0Hz, H-2',6'), 7.00 (d, J=10.0Hz, H-3',5'), 6.95 (m, H-7), 6.68 (bd, J=10.0Hz, H-8), 6.25 (s, H-2) and 2.22 (4'-CH_3). ^{13}C-NMR (50.3 MHz, $CDCl_3$): δ 188.0 (C-3-CHO), 155.0 (C-8a), 143.5 (C-4), 138.5 (C-1'), 135.0 (C-4'), 134.0 (C-5), 129.2 (C-3',5'), 126.5 (C-2',6'), 126.2 (C-3-7), 122.0 (C-6), 120.0 (C-4a), 117.5 (C-8), 74.9 (C-2) and 21.0 (C-4'-CH_3). MS: m/z 285[M+H] and 287[M+H+2].

iii) 4-Chloro-2-(4'-methoxylphenyl)-2H-3-chromene carbaldehyde (5c): m.p. 85^{0}C. yield 88%. IR (KBr): 1600 (C=C) and 1660 cm^{-1} (CHO). ^{1}H-NMR (200MHz, $CDCl_3$): δ 10.30 (s, 3-CHO), 7.70 (dd, J=10.0, 2.0 Hz, H-5), 7.25 (m, 2',6' and H-7), 6.75 (m, H-3',5' and H-8), 6.30 (s, H-2) and 3.75 (s, 4'-OCH_3). ^{13}C-NMR (50.3MHz, $CDCl_3$): δ 187.8 (C-3-CHO), 159.8 (C-4'), 154.8 (C-8a), 143.3 (C-4), 134.2 (C-3), 130.0 (C-7), 128.2 (C-2',6'), 126.8 (C-1'), 126.2 (C-5), 121.7 (C-6), 119.8 (C-4a), 117.3 (C-8), 113.7 (C-3',5'), 74.7 (C-2) and 54.9 (C-4'-OCH_3). MS: m/z 301[M+H] and 303[M+H+2].

4. Synthesis of 4-phenyl-2-aryl-2H-3-chromene carbaldehydes (7a-c):

i) Synthesis of 2,4-dilphenyl-2H-3-chromene carbaldehydes (7a):

4-Chloro-2-phenyl-2H-3-chromene carbaldehyde **(5a)** (10mmol) was stirred in the presence of 5 mol% of tetrakiss (triphenylphosphine) palladium (0) at room temperature under nitrogen for 30min in DMF (20ml) and Na_2CO_3 (10mmol). Phenyl boronicacid (10mmol) **(6)** was added and the mixture was stirred under nitrogen for 30min. The reaction mixture was heated at 80^{o}C for about 4 hours, after completion of the reaction; the temperature of the reaction mixture was allowed to reach to room temperature and poured into crushed ice. The crude reaction product was extracted with chloroform, dried over sodium sulphate and the solvent was evaporated. The crude product was purified by column chromatography to give 2, 4-dilphenyl-2H-3-chromene carbaldehydes **7a**. similar procedure adopted for **7b** and **7c**.

m.p. 121-124^{o}C, Yield 70%, IR (KBr): 1740cm^{-1} (CHO). ^{1}H-NMR (400MHz, $CDCl_3$): δ 9.55 (s, CHO), 7.50 ((m, H-2", 3", 5", 6"), 7.43 (m, H-4"), 7.41 (m, H-7), 7.31 (m, H-2', 3', 4', 5', 6'), 6.96 (dd, J=8.2, 1.0Hz, H-5), 6.88 (dd, J=7.7, 1.7Hz, H-8), 6.84 ((ddd, J=8.0, 7.0, 1.2Hz, H-6), 6.44 (s, H-2'). ^{13}C-NMR (100.6MHz, $CDCl_3$): δ 190.7 (CHO), 154.7 (C-8a), 151.3 (C-4), 139.3 (C-3), 133.4 (C-5), 132.9 (C-1"), 130.8 (C-3",5"), 129.9 (C-2",6"), 129.4 (C-4"), 128.77 (C-1'), 128.71 (C-7), 128.5 (C-2',6'), 128.4 (C-4'), 126.9 (C-3',5'), 122.8 (C-4a), 121.5 (C-6), 117.5 (C-8), 73.5 (C-2). MS: m/z 313[M+H].

ii) 4-phenyl-2(4'-methylphenyl)-2H-3-chromene carbaldehydes (7b): light yellow, m.p. 125-128^{o}C, Yield 73%. IR (KBr): 1740cm^{-1}. ^{1}H-NMR (400MHz, $CDCl_3$): δ 9.53 (s, CHO), 7.50 (brs, H-2",3",5",6"), 7.30 (d, J=8.2Hz, H-2',6'), 7.24 (m, H-4",6), 7.08 (d, J=8.0Hz, H-3',5'), 6.91 (dd, J=8.2, J=1.0Hz, H-5), 6.85 (dd, J=7.7, J=1.7Hz, H-8), 6.80 (ddd, J=8.7, J=7.7, J=1.0Hz, H-7), 6.40 (s, H-2), 2.28 (s, 4'-CH_3). ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ 190.5 (CHO), 154.6 (C-8a), 151.0 (C-4), 138.1 (C-4'), 136.2 (C-1'), 133.2 (C-7), 132.9 (C-1"), 130.6 (C-3",5"), 130.0 (C-2",6"), 129.5 (C-4"), 129.1 (C-2',6'), 128.7 (C-3), 128.5 (C-5),

126.8 (C-3',5'), 122.8 (C-4a), 121.3 (C-6), 117.4 (C-8), 73.1 (C-2), 21.0 (4'-CH_3). MS: m/z 327[M+H].

iii) 4-phenyl-2(4'-methoxylphenyl)-2H-3-chromene carbaldehydes (7c): light yellow, m.p. 132-136^oC, Yield 76%. IR (KBr): 1740cm^{-1}. ^{1}H-NMR (400MHz, $CDCl_3$): δ 9.45 (s, CHO), 7.42 (brs, H-2",3",5",6"), 7.28 (d, J=8.5Hz, H-2',6'), 7.27 (m, H-6,7), 6.83 (dd, J=8.2, 7.0Hz, H-5), 6.81 (m, H-H-4"), 6.76 (m, H-3',5',8), 6.30 (s, H-2), 3.66 (s, OCH_3). ^{13}C-NMR (100.6MHz, $CDCl_3$): δ 189.6 (CHO), 158.6 (C-4'), 153.5 (C-8a), 150.0 (C-4), 132.2 (C-5), 131.8 (C-1"), 130.2 (C-1'), 129.66 (C-3",5"), 128.9 (C-2",6"), 128.2 (C-4"), 127.7 (C-3), 127.5 (C-7), 127.3 (C-2',6'), 121.8 (C-4a), 120.3 (C-6), 116.5 (C-8), 112.8 (C-3',5'), 72.0 (C-2), 54.1 (OCH_3). MS: m/z 343[M+H].

5. Synthesis of 4-phenyl-2-aryl-2H-3-chromene benzimidazoles (9a-j):

i) Synthesis of 2,4-diphenyl-2H-3-chromene-benzimadazole (9a):

To a solution of 2,4-diphenyl-2H-3-chromene carbaldehyde **(7a)** (10mmol), sodium metabisulphate (10mmol) in DMF (20ml) and O-phenylenediamine **(8a)** (11mmol) were added and stirred at room temperature for 15min. Then mixture was heated at 80^oC on oil bath for 5h. After completion of the reaction (by TLC), the crude reaction mixture was treated with crushed ice (100g). The solid was filtered and subjected to column chromatography with 60-120 mesh silica gel and eluted with petroleum ether: ethyl acetate to give 2,4-diphenyl-2H-3-chromene-benzimadazole **(9a)** (65% yield). Similar procedure adopted for **9b-j**.

mp 161-164^oC. IR (KBr): 3063cm^{-1}(NH), 1597cm^{-1}(C=N) and 1443cm^{-1}(C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 7.64 (brs,H-2",3",4",5",6"), 7.59 (m, H-4"',7"'), 7.42 (brs, NH), 7.27 (m, H-5"', 6"',5), 7.20 (m, H-,4'), 7.15 (m, H-2',3',5',6',2), 6.97 (dd, J=8.2, 1.0Hz, H-7), 6.78 (ddd, J=8.5, 7.5, 1.0Hz, H-6), 6.67 (dd, J=7.7, 1.5Hz, H-8). ^{13}C-NMR (100.6MHz, $CDCl_3$): δ 151.7 ((C-8a), 147.9 (C-4), 138.4 (C-1"'), 135.8 (C-3"',8"'), 135.5 (C-3), 129.6 (C-5), 129.3 (C-2",3",5",6"), 128.5 (C-1"), 128.7 (C-7), 127.1 (C-2',6'), 126.9 (C-4"), 126..5 (C-3',5'), 125.9 (C-4'), 123.4 (C-1'), 122.0 (C-4"',5"',6"',7"'), 120.2 (C-6), 119.9 (C-4a), 116.1 (C-8), 74.1 (C-2). MS: m/z 401[M+H].

ii) 2,4-diphenyl-2H-3-chromene-5-chloro-benzimadazole (9b).

yellow solid. mp. 191-193^oC. yield 68%. IR (KBr): 3062cm^{-1}(NH), 1592cm^{-1}(C=N) and 1448 cm^{-1}(C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 7.91 (d, J=18.0Hz, H-5"'), 7.65 (brs, H-2",3",5",6"), 7.57 (d, J=7.0Hz, H-4",4"',7"'), 7.36 (brs, NH), 7.28 (m, H-3',4',5"), 7.20 (m, H-7), 7.11 (m, H-2,5), 7.01 (m, H-2',6'), 6.79 (ddd, J=8.2, 7.5, 1.0Hz, H-6), 6.67 (dd, J=7.7, 1.2Hz, H-8). ^{13}C-NMR (100.6MHz, $CDCl_3$): δ 152.8 ((C-8a), 149.8 (C-4), 139.3 (C-1"'), 137.1 (C-3), 136.7 (C-3"',8"'), 130.9 (C-5), 130.4 (C-2",3",5",6"), 129.5 (C-1"), 129.4 (C-7), 128.8 (C-3',5'), 128.1 (C-4"), 127..5 (C-2',6'), 127.0 (C-4'), 124.3 (C-1'), 123.9 (C-5"'), 123.1 ((C-6"'), 121.3 (C-6), 120.66 (C-7"'), 120.60(C-4a), 119.6 (C-4"'), 117.2 (C-8), 75.2 (C-2). MS: m/z 435[M+H] and 437[M+H+2].

iii) 2,4-diphenyl-2H-3-chromene-5-bromo-benzimadazole (9c).

brown solid. mp 179-181. yield 70%. IR (KBr): 3061cm^{-1}(NH), 1591cm^{-1}(C=N) and 1447 cm^{-1} (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 7.77 (d,J=18.8Hz, H-6"'), 7.55 (brs, H-2",3",5",6"), 7.48 (d, J=7.2Hz, H-4",4"',7"'), 7.26 (brs, NH), 7.16 (m, H-2',3',5',6'), 7.08 (m, H-4',7), 6.99 (s, H-2), 6.87 (d, J=7.7Hz, H-5), 6.69 (t, J=7.2Hz, H-6), 6.57 (dd, J=7.7, 1.2Hz, H-8). ^{13}C-NMR (100.6MHz, $CDCl_3$): δ 152.8(C-8a), 149.8 (C-4), 139.3 (C-1"'), 137.2 (C-3), 136.6 (C-3"',8"'), 131.0 (C-5), 130.4 (C-2",3",5",6"), 129.7 (C-1"), 129.5 (C-7), 128.3 (C-3',5'), 128.1 (C-4"), 127.5 (C-2',6'), 127.1 (C-4'), 125.7 (C-5"'), 124.3 (C-1'), 12.6 (C-6"'), 121.4 (C-6), 121.0 (C-7"'), 120.5 (C-4a), 117.9 (C-8), 111.0 (C-4"'), 75.2 (C-2). MS: m/z 479[M+H] and 481[M+H+2].

iv) 2,4-diphenyl-2H-3-chromene-5,6-dichloro-benzimadazole (9d).
dark yellow solid, mp. 209-211^{o}C, yield 68%. IR (KBr): 3015cm^{-1}(NH), 1592cm^{-1}(C=N) and 1431cm^{-1}(C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 7.86 (brs, H-6'), 7.73 (s, H-4'"), 7.65 (brs, H-3",4",5"), 7.56 (m, H-2",6",2'), 7.36 (brs, NH), 7.28 (m, H-3',4',5'), 7.19 (dd, J=8.2, 1.5Hz, H-5), 7.11 (s, H-3'"), 7.05 (s, H-2), 6.96 (d, J=7.5Hz, H-5), 6.80 (dd, J=7.2, 1.0Hz, H-6), 6.67 (dd, J=7.5, 1.2Hz, H-8). ^{13}C-NMR (100.6MHz, $CDCl_3$): δ 151.8 (C-8a), 149.8 (C-4), 141.0 (C-1'"), 138.1 (C-3), 136.7, 135.4 (C-3'",8'"), 131.5 (C-1"), 130.0 (C-5), 129.4 (C-2",3",5",6"), 128.5 (C-7), 127.2 (C-3',5'), 127.1 (C-4"), 126.4 (C-2',6'), 126.2 (C-1'), 126.1 (C-4'), 125.2, 123.1 (C-5'",6'"), 120.3 (C-6), 119.8 (C-7'"), 119.1 (C-4a), 116.2 (C-8), 110.5 (C-4'"), 7.1 (C-2). MS: m/z 469[M+H], 470[M+H+1] and 471[M+H+2].

v) 2-(4'-Methylphenyl)-4-phenyl-2H-3-chromene-benzimadazole (9e):
light yellow solid, mp 173-176^{o}C, yield 71%. IR (KBr): 3058cm^{-1}(NH), 1590cm^{-1}(C=N) and 1437 cm^{-1} (C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 7.59 (brs, H-2",3",5",6"), 7.37 (d, J=7.8Hz, H-2',6'), 7.27 (m, H-4",7), 7.20 (brs, NH), 7.12 (d, J=7.6Hz, H-3',5'), 7.09 (m, H-4'",5'",6'",7'",2), 6.94 (dd, J=8.0, 1.2Hz, H-5), 6.83 (dd, J=7.8, 1.6Hz, H-6), 6.76 (dd, J=7.5, 1.6Hz, H-8), 2.23 (s, 4'-CH_3). ^{13}C-NMR (100.6MHz, $CDCl_3$): δ 153.4 (C-8a), 148.6 (C-4), 138.7 (C-1'"), 138.2 (C-4'), 136.4 (C-1'), 135.9 (C-3'",8'"), 135.4 (C-3), 130.1 (C-5), 129.7 (C-3',5'), 129.4 (C-2",3",5",6"), 128.7 (C-1"), 128.4 (C-7), 127.8 ((C-2',6'), 126.3 (C-4"), 122.4 (C-4'",5'",6'",7'"), 120.3 (C-6), 119.3 (C-4a), 116.2 (C-8), 74.0 (C-2), 21.3 (s, 4'-CH_3). MS: m/z 415[M+H].

vi) 2-(4'-Methylphenyl)-4-phenyl-2H-3-chromene-5,6-dichloro-benzimadazole (9f).
light yellow, mp 179-182^{o}C, yield 74%. IR (KBr): 3061cm^{-1}(NH), 1590cm^{-1}(C=N) and 1438 cm^{-1}(C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 7.74 (brs, H-5), 7.61 (brs, H-2",3",4",5",6"), 7.32 (d, J=8.2Hz, H-2',6'), 7.26 (brs, NH), 7.23 (m, H-4'",7'"), 7.19 (dd, J=7.8, 1.6Hz, H-7), 7.12 (d, J=7.8Hz, H-3',5'), 7.02 (s, H-2), 6.80 (dd, J=7.2, 1.2Hz, H-6), 6.72 (dd, J=7.8, 1.4Hz, H-8), 2.36 (s, 4'-CH_3). ^{13}C-NMR (100.6MHz, $CDCl_3$): δ 152.7 (C-8a), 149.2 (C-4), 139.2 (C-4'), 136.3 (C-1'"), 134.8 (C-3'",8'"), 133.2 (C-3), 131.4 (C-5), 130.2 (C-3',5'), 129.2 (C-2",3",5",6"), 128.2 (C-1"), 127.7 (C-7), 127.2 (C-2',6'), 126.7 (C-4"), 126.4, 125.2 (C-5'",6'"), 124.2 (C-1'), 122.3 (C-6), 121.6 (C-4'",7'"), 120.1 (C-4a), 117.8 (C-8), 74.6 (C-2), 21.6 (s, 4'-CH_3). MS: m/z 483[M+H], 484[M+H+1] and 485[M+H+2].

vii) 2-(4'-Methoxylphenyl)-4-phenyl-2H-3-chromene-benzimadazole (9g).
Off white solid, mp. 168-170^{o}C, yield 68%. IR (KBr): 3007cm^{-1}(NH), 1592cm^{-1}(C=N) and 1439 cm^{-1}(C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 7.84 (brs, H-5), 7.55 (brs, H-2",3",4",5",6"), 7.44 (d, J=8.5Hz, H-2',6'), 7.29 (brs, NH), 7.06 (m, H-4'",5'",6'",7'",2), 6.86 (d, J=7.7Hz, H-7), 6.70 (m, H-3',5',8), 6.60 (dd, J=7.7, 1.2Hz, H-6), 3.62 (s, OCH_3). ^{13}C-NMR (100.6MHz, $CDCl_3$): δ 158.3 (C-4'), 151.6 (C-8a), 147.8 (C-4), 135.8 (C-3'",8'"), 135.2 (C-1'"), 30.4 (C-3), 129.6 (C-5), 128.7 (C-2",3",5",6"), 128.6 (C-1"), 128.2 (C-7), 127.8 (C-2',6'), 125.8 (C-4"), 123.5 (C-1'), 122.6, 121.3 (C-4'",7'"), 120.17 (C-6), 120.11 (C-4a), 118.9 (C-5'",6'"), 116.2 (C-8), 112.5 (C-3',5'), 73.9 (C-2), 54.0 (OCH_3). MS: m/z 431[M+H].

viii) 2-(4'-Methoxylphenyl)-4-phenyl-2H-3-chromene-5-chloro-benzi madazole (9h):
brown solid, mp. 148-150^{o}C. yield 71%. IR (KBr): 3028cm^{-1}(NH), 1607cm^{-1}(C=N) and 1422 cm^{-1}(C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 7.80 (brs, H-5), 7.57 (brs, H-2",3",5",6"), 7.43 (d, J=8.5Hz, H-2',6',4"), 7.28 (brs, NH), 7.17 (m, H-4'",6'",7'"), 6.94 (s, H-2), 6.85 (d, J=7.5Hz, H-7), 6.72 (m, H-3',5',8), 6.60 (dd, J=7.7, 1.5Hz, H-6), 3.63 (s, OCH_3). ^{13}C-NMR (100.6MHz, $CDCl_3$): δ 158.4 (C-4'), 151.6 (C-8a), 150.0 (C-4), 135.9 (C-1'"), 135.6 (C-3'",8'"), 130.2 (C-3), 129.8 (C-5), 129.2 (C-2",3",5",6"), 128.5 (C-1"), 128.3 (C-7), 127.8 (C-

2',6'), 125.9 (C-4"), 123.3 (C-1'), 122.1 (C-4"',5"',6"',7"'), 120.2 (C-6), 119.6 (C-4a), 116.2 (C-8), 112.5 (C-3',5'), 74.0 (C-2), 54.0 (OCH_3). MS: m/z 465[M+H] and 467[M+H+2].

ix) 2-(4'-Methoxylphenyl) -4-phenyl-2H-3-chromene -5-bromo benzimadazole (9i):
brown solid, mp 169-172^oC. yield 72%. IR (KBr): 3003cm^{-1}(NH), 1607cm^{-1}(C=N) and 1440 cm^{-1}(C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 7.90 (brs, H-5), 7.63 (brs, H-2",3",5",6"), 7.50 (d, J=8.5Hz, H-2',6',4"), 7.38 (brs, NH), 7.24 (m, H-4"',6"',7"'), 7.01 (s, H-2), 6.92 (d, J=8.5Hz, H-7), 6.78 (m, H-3',5',8), 6.77 (d, J=6.7Hz, H-6), 3.69 (s, OCH_3). ^{13}C-NMR (100.6MHz, $CDCl_3$): δ 159.5 (C-4'), 152.7 (C-8a), 150.0 (C-4), 137.1 (C-1"'), 136.7 (C-3"',8"'), 131.3 (C-3), 130.9 (C-5), 130.2 (C-2",3",5",6"), 129.5 (C-1"), 129.4 (C-7), 128.9 (C-2',6'), 127.0 (C-4"), 126.0 (C-4"',5"',6"',7"'), 124.4 (C-1'), 121.3 (C-6), 120.6 (C-4a), 117.3 (C-8), 113.7 (C-3',5'), 75.0 (C-2), 55.1 (OCH_3). MS: m/z 509[M+H] and 511[M+H+2].

x) 2-(4'-Methoxylphenyl)-4-phenyl-2H-3-chromene-5,6-dichloro- benzim ada zole (9j):
brown solid, mp. 193-196^oC, yield 74%. IR (KBr): 3006cm^{-1}(NH), 1613cm^{-1}(C=N) and 1445 cm^{-1}(C=C). ^{1}H-NMR (400MHz, $CDCl_3$): δ 7.86 (brs, H-5), 7.72 (brs, H-2",3",4",5",6"), 7.49 (d, J=8.5Hz, H-2',6'), 7.36 (brs, NH), 7.18 (m, H-4"',7"'), 6.98 (s, H-2), 6.92 (d, J=8.05Hz, H-7), 6.80 (m, H-3',5',8), 6.68 (dd, J=7.7, 1.5Hz, H-6), 3.71 (s, OCH_3). ^{13}C-NMR (100.6MHz, $CDCl_3$): δ 159.5 (C-4'), 152.8 (C-8a), 150.8 (C-4), 137.1 (C-1"'), 136.5 (C-3"',8"'), 131.2 (C-3), 131.1 (C-5), 130.2 (C-2",3",5",6"), 129.5 (C-7), 129.4 (C-1"), 128.9 (C-2',6'), 127.0 (C-4"), 127.3, 126.4 (C-3"',5"'), 124.2 (C-1'), 121.3 (C-6), 120.9 (C-4"',7"'), 120.3 (C-4a), 117.3 (C-8), 113.7 (C-3',5'), 75.0 (C-2), 55.1 (OCH_3). MS: m/z 499[M+H], 500[M+H+1] and 501[M+H+2].

Biology

Organisms Collection: Antibacterial activity and minimum inhibitory concentration (MIC) were determined against one gram-positive bacterium (Staphylococcus aureus) and one gram-negative bacterium (Pseudomonas aeruginosa). Both the organisms were collected from the Microbiology Research Laboratory of the Microbiology Department, Osmania University, Hyderabad.

Growth Media and Conditions: Nutrient agar media (HIMEDIA Laboratories) pH 7.2, nutrient broth media (HIMEDIA Laboratories) pH 6.8 were used for antibacterial screening, MIC determination.

Antibacterial Screening: Antibacterial screening is generally performed by disc diffusion method which is a qualitative to semi quantitative test. Briefly 15ml quantities of nutrient agar were plated in petri dish with 0.1 ml of a 10 dilutions of each bacterial culture. Filter paper discs (6 mm in diameter) impregnated with various concentrations of plant extracts were placed on test organism-seeded plates. Different compounds with different dilution were prepared range from 1µg/ml to 200µg/ml. The activity was determined after 18 h of incubation at 37°C. The diameters of zone of inhibition produced by the extract were then compared with the standard antibiotic Ampicillin 30 µm/disc. Each sample was used in triplicate for the determination of antibacterial activity.

Minimum Inhibitory Concentration Measurement: A current definition of the minimum inhibitory concentration (MIC) is the lowest concentration which resulted in maintenance or reduction of inoculums viability. Serial tube dilution technique was used to determine of MIC of the different compounds against gram-positive and gram-negative bacteria. The compounds (0 to 200µg) were dissolved in 1 ml DMSO to obtain stock solution. After preparation of suspensions of test organisms (1000 organism per ml), 1 drop of suspension (0.02 ml) was added to each broth dilution. After 18 h incubation at 37°C, the tubes were then examined for the growth. The MIC of the each compound was taken as the lowest

concentration that showed no growth. Growth was observed in those tubes where the concentration of the each compound was below the inhibitory level and the broth medium was observed turbid (cloudy). DMSO and Ampicillin were used as negative and positive control, respectively.

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