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SYNTHESIS AND ANTIBACTERIAL SCREENING OF IMIDAZOLE CONTAINING SUBSTITUTED FLAVONES

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ABSTRACT
Imidazole anchored chromones (2) and chlorochromones (3) were synthesized from substituted chalcones (1) in Scheme I. Formation and structures of all the compounds were confirmed by spectroscopic techniques and elemental analytical data. All the synthesized compounds were screened for antibacterial activity against four bacterial strains (*Escherichia coli*, *Pseudomonas fluorescens*, *Staphylococcus aureus*, *Bacillus subtilis*) using Ampicillin as a standard drug.
KEYWORDS: Antibacterial activity, Chromones, Imidazole

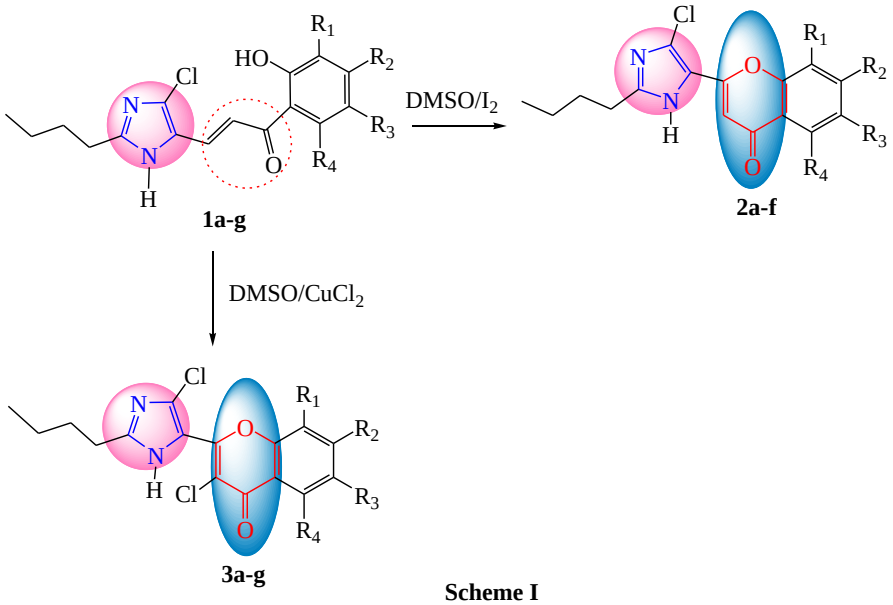
INTRODUCTION
Due to biological importance of flavones chemists take good efforts for synthesis of their novel derivatives. Position and nature of substituents decides potential of biological activities associated with flavones.

Imidazole derivatives have occupied a predominant role in the field of medicinal chemistry. Incorporation of imidazole motif is significant synthetic strategy in drug chemistry. Imidazole containing compounds have shown activities like anticancer [1], antibacterial [1], antimicrobial [2], antioxidant [3], antitubercular [4], antirheumatoid arthritis [5] and antiviral [6].

Chromones are heterobicyclic compound occurred in nature as well as synthetic flavones are also reported by researchers. Both natural and synthetic flavone is an important building block in drug discovery. They are known for their diverse biological activities like antimicrobial [7], antiglycation [8], antioxidant [9-10], anti-inflammatory [11], analgesic [11], selective agonists for neuromedin U 2 receptor [12], etc.
Keeping in mind the biological activities associated with chromones, in present study we have synthesized biologically important imidazole containing substituted chromones with the evaluation of antibacterial potential.

RESULTS AND DISCUSSION
Dawane B S [13] have reported the synthesis of (*E*)-3-(2-butyl-4-chloro-1*H*-imidazol-5-yl)-1-phenylprop-2-en-1-ones (**1a-g**). Chalcones (**1**) in presence of DMSO/I_2 gave chromones (**2a-f**). IR spectrum of compound **2a** showed band at 1622 cm^{-1} due to carbonyl. The ^{1}H NMR spectrum of **2a** showed singlet for chromone proton at δ 6.77 ppm. Chalcones (**1**) in presence of copper chloride in DMSO gave 3-chlorochromones (**3a-g**). IR spectrum of compound **3a** showed a band at 1622 cm^{-1} due to carbonyl functional group.

EXPERIMENTAL SECTION
Physical constants were recorded in liquid paraffin bath with the help of capillaries and are uncorrected. IR spectra were recorded on *Shimadzu* IR Affinity-1S fourier transform infrared spectrophotometer. ^{1}H NMR spectra were recorded on Bruker Avance II 400 MHz NMR spectrometer with DMSO-d_6 as a solvent and TMS as an internal standard. Peak values are shown in δ (ppm). Mass spectra were recorded on Water acquity TQD mass spectrometer.



Scheme I

ANTIBACTERIAL SCREENING

All the synthesized compounds were screened for antibacterial activity against four bacterial strains using Ampicillin as a standard drug.

All bacterial cultures were first grown in Luria Burtony media at 37°C at 180 rpm. Once the culture reaches 1 O D it is used for antibacterial assay. Gram negative bacterial strains *Escherichia coli* (NCIM 2576), *Pseudomonas fluorescens* (NCIM 2059) and Gram positive bacterial strains *Staphylococcus aureus* (NCIM 2602), *Bacillus subtilis* (NCIM 2162) were obtained from NCIM (NCL, Pune) and grown in Luria Burtony medium from Hi Media, India. 0.1 % of 1 O D culture at 620 nm was used for screening inoculated culture was added into each well of 96 well plate containing the compounds to be tested. Optical density for each plate was measured at 620 nm after 8 h for Gram negative bacteria and after 12 h for Gram positive bacteria. The results are summarized in **Table 1**. The results showed that Compound **2b** shows moderate activity against *E. coli* and *B. subtilis* at concentration 100 µg/mL. Compound **3a** shows moderate activity against *E. coli* while compound **3b** shows moderate activity towards *B. subtilis.* Remaining compounds were weakly active or inactive against all bacterial strains. None of the compounds showed activity against *P. fluorescens* and *S. aureus*.

Table- 1 Antibacterial screening of synthesized compounds (% inhibition)

	Concentration in µg/mL											
	Gram Negative Bacteria						Gram Positive Bacteria					
	P. fluorescens			*E. coli*			*S. aureus*			*B. subtilis*		
Comp	100	30	10	100	30	10	100	30	10	100	30	10
2a	-	-	-	10.9	9.7	-	-	-	-	29.1	23.4	1.6
2b	-	-	-	63.6	22.3	14.9	-	-	-	65.4	18.3	-
2c	-	-	-	-	-	-	-	-	-	27.4	-	-
2d	-	-	-	47.8	47.0	22.4	-	-	-	23.9	-	-
2e	-	-	-	34.9	16.0	12.0	-	-	-	30.0	0.7	-
2f	-	-	-	35.9	8.8	-	-	-	-	38.8	19.4	-
3a	-	-	-	52.8	-	-	-	-	-	41.6	-	-
3b	-	-	-	-	-	-	-	-	-	61.8	29.3	17.4
3c	-	-	-	38.0	34.5	29.7	-	-	-	15.9	8.4	8.2
3d	-	-	-	35.7	18.2	15.6	-	-	-	0.5	-	-
3e	-	-	-	31.4	26.4	17.0	-	-	-	0.2	-	-

3f	-	-	-	29.9	22.4	2.20	-	-	-	17.8	-	-
3g	-	-	-	35.5	22.2	15.1	-	-	-	25.6	-	-
AMP	97.0	95.2	92.2	96.6	92.1	92.0	95.0	93.8	91.1	98.5	95.0	90.5

AMP - Ampicillin

2-(2-Butyl-4-chloro-1*H*-imidazol-5-yl)-4*H*-chromen-4-ones, 2a-f:

Compound **1** (3 mmol) was dissolved in 15 mL of DMSO. Catalytic amount of iodine (0.01 g) was added to it. The reaction mixture was heated in between 120 to 130ºC for 2 h in oil bath and left overnight, then poured over crushed ice. The solid obtained was filtered and washed with dil. sodium thiosulphate followed by water. The product was purified by recrystallization from ethanol to afford compound **2**.

2a: IR: 3196 (N-H), 1622 (C=O), 1595 (C=N), 1240 (C-O-C) cm^{-1}; ^1H NMR (DMSO-d_6): δ 0.93 (t, 3H, CH_3, *J*= 7.4 Hz), 1.33-1.38 (m, 2H, CH_2), 1.67-1.71 (m, 2H, CH_2), 2.70 (t, 2H, CH_2, *J*= 7.4 Hz), 6.77 (s, 1H, Ar-H), 7.60 (d, 1H, Ar-H, *J*= 8.8 Hz), 7.93 (dd, 1H, Ar-H, *J*= 8.8 & 2.4 Hz), 8.10 (d, 1H, Ar-H, *J*= 2.4 Hz), 13.03 (s, 1H, N-H); MS: *m/z* (M+1) 383. Ana. Calcd for $C_{16}H_{14}BrClN_2O_2$: C, 50.35; H, 3.70; N, 7.34. Found: C, 50.38; H, 3.73; N, 7.36%.

2b: IR: 3195 (N-H), 1623 (C=O), 1597 (C=N), 1242 (C-O-C) cm^{-1}; ^1H NMR (DMSO-d_6): δ 0.94 (t, 3H, CH_3, *J*= 7.2 Hz), 1.34-1.39 (m, 2H, CH_2), 1.68-1.72 (m, 2H, CH_2), 2.35 (s, 3H, CH_3), 2.71 (t, 2H, CH_2, *J*= 7.2 Hz), 6.75 (s, 1H, Ar-H), 7.20 (s, 1H, Ar-H), 7.54 (s, 1H, Ar-H), 13.01 (s, 1H, N-H); MS: *m/z* (M+1) 351. Anal. Calcd for $C_{17}H_{16}Cl_2N_2O_2$: C, 58.13; H, 4.59; N, 7.98. Found: C, 58.16; H, 4.61; N, 8.01%.

2c: IR: 3196 (N-H), 1620 (C=O), 1595 (C=N), 1239 (C-O-C) cm^{-1}; ^1H NMR (DMSO-d_6): δ 0.94 (t, 3H, CH_3, *J*= 7.3 Hz), 1.32-1.35 (m, 2H, CH_2), 1.66-1.69 (m, 2H, CH_2), 2.35 (s, 3H, CH_3), 2.71 (t, 2H, CH_2, *J*= 7.3 Hz), 6.75 (s, 1H, Ar-H), 6.76-7.79 (m, 3H, Ar-H), 13.04 (s, 1H, N-H); MS: *m/z* (M+1) 317. Anal. Calcd for $C_{17}H_{17}ClN_2O_2$: C, 64.46; H, 5.41; N, 8.84. Found: C, 64.49; H, 5.43; N, 8.86%.

2d: IR: 3198 (N-H), 1626 (C=O), 1596 (C=N), 1241 (C-O-C) cm^{-1}; ^1H NMR (DMSO-d_6): δ 0.92 (t, 3H, CH_3, *J*= 7.4 Hz), 1.33-1.36 (m, 2H, CH_2), 1.67-1.73 (m, 2H, CH_2), 2.72 (t, 2H, CH_2, *J*= 7.4 Hz), 6.79 (s, 1H, Ar-H), 7.62-7.92 (m, 2H, Ar-H), 13.02 (s, 1H, N-H); MS: *m/z* (M+1) 371. Anal. Calcd for $C_{16}H_{13}Cl_3N_2O_2$: C, 51.71; H, 3.53; N, 7.54. Found: C, 51.75; H, 3.56; N, 7.56%.

2e: IR: 3193 (N-H), 1625 (C=O), 1594 (C=N), 1243 (C-O-C) cm^{-1}; ^1H NMR (DMSO-d_6): δ 0.92 (t, 3H, CH_3, *J*= 7.3 Hz), 1.35-1.39 (m, 2H, CH_2), 1.66-1.70 (m, 2H, CH_2), 2.35 (s, 6H, CH_3), 2.72 (t, 2H, CH_2, *J*= 7.4 Hz), 6.77 (s, 1H, Ar-H), 6.60-7.32 (m, 2H, Ar-H), 13.09 (s, 1H, N-H); MS: *m/z* (M+1) 331. Anal. Calcd for $C_{18}H_{19}ClN_2O_2$: C, 65.35; H, 5.79; N, 8.47. Found: C, 65.37; H, 5.82; N, 8.50%.

2f: IR: 3197 (N-H), 1623 (C=O), 1597 (C=N), 1246 (C-O-C) cm^{-1}; ^1H NMR (DMSO-d_6): δ 0.94 (t, 3H, CH_3, *J*= 7.5 Hz), 1.38-1.41 (m, 2H, CH_2), 1.69-1.72 (m, 2H, CH_2), 2.73 (t, 2H, CH_2, *J*= 7.5 Hz), 6.78 (s, 1H, Ar-H), 7.10-7.78 (m, 3H, Ar-H), 13.15 (s, 1H, N-H); MS: *m/z* (M+1) 321. Anal. Calcd for $C_{16}H_{14}ClFN_2O_2$: C, 59.91; H, 4.40; N, 8.73. Found: C, 59.95; H, 4.43; N, 8.77%.

2-(2-Butyl-4-chloro-1*H*-imidazol-5-yl)-3-chloro-4*H*-chromen-4-ones, 3a-g:

Compound **1** (3 mmol) was dissolved in 15 mL DMSO. To it excess of copper chloride (2 g) was added. The reaction mixture was heated in between 120 to 130ºC for 2 h in oil bath and left overnight, then poured over crushed ice. The solid thus obtained was filtered and washed with dil. HCl followed by water. The product was purified by recrystallization from ethanol to afford compound **3**.

3a: IR: 3190 (N-H), 1622 (C=O), 1595 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6): δ 0.85-0.92 (m, 3H, CH_3), 1.29-1.33 (m, 2H, CH_2), 1.63-1.68 (m, 2H, CH_2), 2.70 (t, 2H, CH_3, *J*= 7.6 Hz), 7.69

(d, 1H, Ar-H, *J*= 8.0 Hz), 8.03 (d, 1H, Ar-H, *J*= 8.0 Hz), 8.18 (s, 1H, Ar-H), 13.04 (s, 1H, N-H); MS: *m/z* (M-1) 413. Anal. Calcd for $C_{16}H_{13}BrCl_2N_2O_2$: C, 46.18; H, 3.15; N, 6.73. Found: C, 46.21; H, 3.17; N, 6.74%.

3b: IR: 3192 (N-H), 1620 (C=O), 1592 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6): δ 0.86-0.93 (m, 3H, CH_3), 1.30-1.34 (m, 2H, CH_2), 1.64-1.69 (m, 2H, CH_2), 2.35 (s, 3H, CH_3), 2.68 (t, 2H, CH_2, *J*= 7.5 Hz), 6.76-7.52 (m, 2H, Ar-H), 13.11 (s, 1H, N-H); MS: *m/z* (M-1) 383. Anal. Calcd for $C_{17}H_{15}Cl_3N_2O_2$: C, 52.94; H, 3.92; N, 7.26. Found: C, 52.96; H, 3.95; N, 7.30%.

3c: IR: 3190 (N-H), 1621 (C=O), 1593 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6): δ 0.87-0.95 (m, 3H, CH_3), 1.32-1.35 (m, 2H, CH_2), 1.66-1.70 (m, 2H, CH_2), 2.35 (s, 3H, CH_3), 2.71 (t, 2H, CH_2, *J*= 7.7 Hz), 6.76-7.79 (m, 3H, Ar-H), 13.04 (s, 1H, N-H); MS: *m/z* (M-1) 349. Anal. Calcd for $C_{17}H_{16}Cl_2N_2O_2$: C, 58.13; H, 4.59; N, 7.98. Found: C, 58.16; H, 4.61; N, 8.01%.

3d: IR: 3191 (N-H), 1622 (C=O), 1595 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6): δ 0.89-0.96 (m, 3H, CH_3), 1.30-1.36 (m, 2H, CH_2), 1.64-1.70 (m, 2H, CH_2), 2.70 (t, 2H, CH_2, *J*= 7.4 Hz), 7.61-7.68 (m, 2H, Ar-H), 13.02 (s, 1H, N-H); MS: *m/z* (M-1) 403. Anal. Calcd for $C_{16}H_{12}Cl_4N_2O_2$: C, 47.32; H, 2.98; N, 6.90. Found: C, 47.35; H, 3.00; N, 6.92%.

3e: IR: 3192 (N-H), 1623 (C=O), 1596 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6): δ 0.85-0.91 (m, 3H, CH_3), 1.30-1.37 (m, 2H, CH_2), 1.62-1.66 (m, 2H, CH_2), 2.33 (s, 6H, CH_3), 2.70 (t, 2H, CH_2, *J*= 7.3 Hz), 6.69-7.37 (m, 2H, Ar-H), 13.07 (s, 1H, N-H); MS: *m/z* (M-1) 363. Anal. Calcd for $C_{18}H_{18}Cl_2N_2O_2$: C, 59.19; H, 4.97; N, 7.67. Found: C, 59.22; H, 4.99; N, 7.70%.

3f: IR: 3193 (N-H), 1627 (C=O), 1597 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6): δ 0.90-0.96 (m, 3H, CH_3), 1.31-1.38 (m, 2H, CH_2), 1.63-1.70 (m, 2H, CH_2), 2.71 (t, 2H, CH_2, *J*= 7.6 Hz), 6.93-7.78 (m, 3H, Ar-H), 13.01 (s, 1H, N-H); MS: *m/z* (M-1) 353. Anal. Calcd for $C_{16}H_{13}Cl_2FN_2O_2$: C, 54.10; H, 3.69; N, 7.89. Found: C, 54.12; H, 3.72; N, 7.90%.

3g: IR: 3195 (N-H), 1627 (C=O), 1593 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6): δ 0.92-0.98 (m, 3H, CH_3), 1.32-1.38 (m, 2H, CH_2), 1.66-1.70 (m, 2H, CH_2), 2.71 (t, 2H, CH_2, *J*= 7.5 Hz), 6.79-7.89 (m, 3H, Ar-H), 13.06 (s, 1H, N-H); MS: *m/z* (M-1) 369. Anal. Calcd for $C_{16}H_{13}Cl_3N_2O_2$: C, 51.71; H, 3.53; N, 7.54. Found: C, 51.73; H, 3.55; N, 7.58%.

Table-2 Physical data of synthesized compounds (**Scheme I**)

Compd	R^1	R^2	R^3	R^4	M.P. (°C)	Yield (%)
2a	H	H	Br	H	240	39
2b	H	CH_3	Cl	H	172	32
2c	H	H	CH_3	H	220	34
2d	Cl	H	Cl	H	228	35
2e	CH_3	H	CH_3	H	138	36
2f	H	H	F	H	110	38
3a	H	H	Br	H	178	38
3b	H	CH_3	Cl	H	226	36
3c	H	H	CH_3	H	264	35
3d	Cl	H	Cl	H	172	33
3e	CH_3	H	CH_3	H	182	30
3f	H	H	F	H	218	38
3g	H	H	Cl	H	112	32

CONCLUSION

Imidazole containing flavones with bromo functional group shows moderate activity at concentration 100 µg/mL against bacterial strains *E. coli* and *B. subtilis*. While compounds containing alkyl (methyl) substituents shows moderate activity against *E. coli*.

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GRAPHICAL ABSTRACT