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Synthesis, characterization and antimicrobial activity of 7-Amino-2-Styrylchromones

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ABSTRACT

*Nine derivatives of 7-Amino-2-Styrylchromones (3a–i) were synthesized and characterized. These compounds were examined for their antibacterial activity against the bacterial strains Klebsiella pneumonia, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and antifungal activity against Candida albicans. Klebsiella pneumoniae was found to be resistant to multiple antibiotics such as ampicillin, amoxicillin/ clavulanic acid , gentamycin, amikacin, levofloxacin, imipenem, cefazolin and cefepime. However all the test compounds**(3a-i)** showed good activity against Klebsiella pneumoniae at 12.5µg /mL concentration. Compound **3d**at12.5µg /mL concentration displayed a zone of inhibition of 25mm against Staphylococcus aureus.*

Key words: 7-Amino-2-Styrylchromone derivatives, Antibacterial, Antifungal

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INTRODUCTION

The need for new antimicrobials is increasing due to the emergence of multidrug resistant microorganisms, the re-emergence of previously deadly infectious diseases and the emergence of new infectious diseases[1]. Heterocycles play an important role in the design of therapeutic molecules. Chromones , oxygen-containing heterocycles exhibit a wide range of biological activities such as anti-HIV[2],anti-cancer[3],anti-microbial[4,5],anti-inflammatory[6],and anti-ulcer[7]. 2-styryl chromones are vinylogues of flavones. Only nine naturally occurring 2-styrylchromones have been reported [8-12].They have shown significant biological activities[13-16].Several 2-styrylchromone-6-carboxylic acids displayed anti-allergic activity when administered orally[17]. Hormothamnione showed potent cytotoxicity against P388 lymphocitic leukaemia and HL-60 human promyelotic leukaemia cell lines. These compounds have demonstrated strong protective effects against pro-oxidant agents observed in cellular and in non-cellular systems [11,12], making them good antioxidant compound candidates. However, reports on the antimicrobial activity of 2-styrylchromones are scarce.

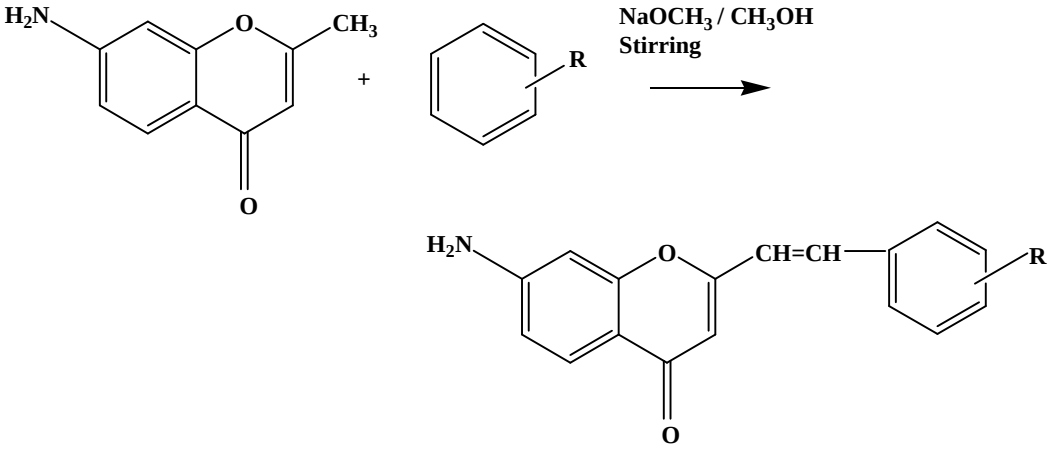
MATERIALS AND METHODS

All purchased chemicals were of analytical grade and used without further purification. Melting points were determined by open capillary method and are uncorrected. Purity of synthesized compounds was checked by thin layer chromatography and visualization of spots was done in UV chamber. The IR spectra in KBr pellets were recorded using Shimadzu FTIR 8400S spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded by Bruker AV400 (400MHz) spectrometer in deuterated dimethyl sulphoxide using tetramethylsilane as internal standard. Mass spectra were scanned on a Shimadzu LCMS (ESI) 2010A spectrometer.

Antibiotic disc of ampicillin (10µg), amoxicillin/ clavulanic acid (20/10 µg), gentamycin (10 µg), amikacin (30 µg), levofloxacin (5 µg), imipenem (10 µg), cefazolin (30 µg), cefepime (30 µg) were purchased from HI-Media, Mumbai, India.

General method for the synthesis of 7-Amino-2-Styrylchromones (3a-i)

To a solution of sodium methoxide (0.02 mol) in 20 mL of methanol was added 7-amino- 2-methylchromone (0.01 mol) and 0.012 mol of aromatic aldehyde. The solution was stirred for 12-18 hours. The reaction progress was monitored using TLC(dichloromethane : ethyl acetate 4:1). After the reaction was complete the reaction mixture was cooled and poured into water containing ice and filtered. The product was washed with water and recrystallized from methanol to obtain 7-Amino-2-Styrylchromones.



Scheme for the synthesis of 7-Amino-2-Styrylchromones(3a-i)

Table 1: Substituents in ring B of Styrylchromones

Compound code	R	Compound code	R
3a	H	3f	3,4-dichloro
3b	4-chloro	3g	3,4-methylenedioxy
3c	4-nitro	3h	4-benzyloxy
3d	3,4-dimethoxy	3i	4-methyl
3e	2-Hydroxy		

***E*)-7-Amino-2-styryl-4*H*-chromen-4-one (3a)**

Yield: 81 %; m.p. 146-148 °C, IR (KBr) (cm^{-1}):1680(C=O), 3320, 3401(NH_2), 3090 (CH str Ar); ^{1}H NMR (400 MHz, DMSO-d6) δ ppm: 5.18(s,2H,NH_2), 6.38 (s, H-3), 6.27 – 7.72 (10 H, Ar-H and vinylic H); LC-MS*m/z* 263.00 (M^+)

(*E*)-7-Amino-2-(4-chlorostyryl)-4*H*-chromen-4-one (3b)

Yield: 75%; m.p. 158-160 °C, IR (KBr) (cm^{-1}):1672 (C=O), 3336, 3400(NH_2), 3062(CH str Ar); ^{1}H NMR (400 MHz, DMSO-d6) δ ppm: 5.08(s,2H,NH_2), 6.30 (s, H-3), 6.10 – 7.94 (9 H, Ar-H and vinylic H); LC-MS*m/z* 298.50 (M+1)

(*E*)-7-Amino-2-(4-nitrostyryl)-4*H*-chromen-4-one (3c)Yield: 71%; m.p. 150-152 °C, IR (KBr) (cm^{-1}):1680(C=O), 3336, 3405(NH_2), 3082 (CH str Ar); ^{1}H NMR (400 MHz, DMSO-d6) δ ppm: 5.21(s,2H, NH_2), 6.41 (s, H-3), 6.35 – 7.71 (9 H, Ar-H and vinylic H); LC-MS*m/z* 309.00 (M+1)

(*E*)-7-Amino-2-(3,4-dimethoxystyryl)-4*H*-chromen-4-one (3d)

Yield: 80 %; m.p. 168-170 °C, IR (KBr) (cm^{-1}):1672 (C=O), 3328, 3400(NH_2), 3072 (CH str Ar); ^{1}H NMR (400 MHz, DMSO-d6) δ ppm:3.80(s,3H, OCH_3), 3.83 (s,3H, OCH_3), 5.12 (s,2H, NH_2), 6.31 (s, H-3), 6.09 – 7.79 (8 H, Ar-H and vinylic H); LC-MS*m/z* 324.08 (M+1)

(*E*)-7-Amino-2-(2-hydroxystyryl)-4*H*-chromen-4-one (3e)Yield: 76%; m.p. 174-176 °C, IR (KBr) (cm^{-1}):1675(C=O), 3331, 3401(NH_2), 3080(CH str Ar); ^{1}H NMR (400 MHz, DMSO-d6) δ ppm: 5.18 (s, 2H, NH_2), 6.43 (s, H-3), 6.35 – 7.90 (9 H, Ar-H and vinylic H) , 9.35 (s,1H,OH); LC-MS*m/z* 280.08 (M+1)

(*E*)-7-Amino-2-(3,4-dichlorostyryl)-4*H*-chromen-4-one (3f)
Yield: 81 %; m.p. 156-158 °C, IR (KBr) (cm^{-1}):1676 (C=O), 3325, 3405(NH_2), 3084(CH str Ar); ^{1}H NMR (400 MHz, DMSO-d6) δ ppm: 5.10 (s,2H, NH_2), 6.30 (s, H-3), 6.12 – 7.90 (8 H, Ar-H and vinylic H); LC-MS*m/z* 333.08 (M+1)

(*E*)-7-Amino-2-(3,4-methylenedioxystyryl)-4*H*-chromen-4-one (3g)
Yield: 80%; m.p. 212-214 °C, IR (KBr) (cm^{-1}):1680(C=O), 3336, 3405(NH_2), 3092(CH str Ar); ^{1}H NMR (400 MHz, DMSO-d6) δ ppm: 5.24(s,2H,NH_2),6.09 (s, 2H, O CH_2 O); 6.32 (s, H-3), 6.13 – 7.66 (8 H, Ar-H and vinylic H); ^{13}C-NMR(DMSO-d_6) δ : 101.97 (O-CH_2-O), 102.42 (C-3) ,106.92 , 109.77,113.43,113.69,119.50, 124.33, 126.50, 127.28, 130.23,135.18, 148.56, 149.02,154.96, 158.24, 160.88 (15-C), 176.30(C=O);LC-MS*m/z* 308.17 (M+1)

(*E*)-7-Amino-2-(4-benzyloxystyryl)-4*H*-chromen-4-one (3h)
Yield: 75%; m.p. 218-220 °C, IR (KBr) (cm^{-1}):1682 C=O), 3340, 3403(NH_2), 3040(CH str Ar); ^{1}H NMR (400 MHz, DMSO-d6) δ ppm: 5.00 (s,2H, NH_2), 5.25(s, 2H, Ar-CH_2), 6.31 (s, H-3), 6.23 – 8.01 (14 H, Ar-H and vinylic H); LC-MS*m/z* 370.17 (M+1)
(*E*)-7-Amino-2-(4-methylstyryl)-4*H*-chromen-4-one (3i)
Yield: 79%; m.p. 165-167 °C, IR (KBr) (cm^{-1}):1670(C=O), 3330, 3405(NH_2), 3070 (CH str Ar); ^{1}H NMR (400 MHz, DMSO-d6) δ ppm: 2.30 (s, 3H, CH_3), 5.20(s, 2H, NH_2), 6.41 (s, H-3), 6.20 – 7.92 (9 H, Ar-H and vinylic H); LC-MS*m/z* 278 (M+1)

Antimicrobial activity
For antibacterial studies microorganisms employed were Gram-positive bacteria (*Staphylococcus aureus*ATCC 25923), Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922), clinical isolates *Klebsiella pneumonia*(multi drug resistant) and *Candida albicans* was used for the antifungal activity study. The antimicrobial activity was determined using the agar well diffusion method. The selected bacterial and fungal strains were revived by plating on nutrient agar medium and Sabouraud dextrose agar (SDA) medium respectively. After overnight incubation at 37°C, isolated colonies were selected and identities of the organisms were confirmed by standard procedure. Isolated bacterial colonies were then transferred to sterile Muller-Hinton Broth (MHB) and Candida albicans was transferred to Sabouraud Dextrose Broth (SDB)and incubated overnight. The growth concentration was adjustedto 10^5CFU/mL by using 0.5 McFarland's turbidity standard. DMSO was used as negative control. Drugs used as positive control were imipenum 10µg, ampicillin 10µg and ketaconazole 10µg.

Determination of Antibacterial Activity: Petri dishes containing 20 mL of Muller-Hinton Agar (MHA) were used. The bacterial culture was spread over the surface of the MHA plate.4mm diameter wells were punched into the agar and filled with20µl solution of test compounds in various concentrations(200 µg/mL, 100 µg /mL, 50 µg /mL,25 µg/mL and 12.5 µg/ml).The plates were then incubated at 37°C for 18 hrs. Tests were done intriplicates and the mean was taken.

Determination of antifungal activity: Petri dishes containing 20 mL of SDA, were used. The fungal culture was spread over the surface of the SDA plate.4mm diameter wells were punched into the agar and filled with20µl solution of test compounds in various concentrations(200 µg/mL,100 µg /mL, 50 µg /mL,25 µg/mL and 12.5 µg/mL).The plateswere then incubated at 37°C for 18 hrs. Tests were done intriplicates and the mean was taken.

RESULTS AND DISCUSSION

The 7-Amino-2-Styrylchromones (3a–i) were obtained in good yields. The spectral data confirmed the structures of the compounds synthesized. The effect of synthesized compounds on bacterial and fungal strains are summarized in Table 2. The diameter of the clear zone of inhibition surrounding the well was measured in mm. The results indicated that all the test compounds displayed promising anti-bacterial activity against clinical strain of *Klebsiella pneumonia* which was multidrug resistant. The compound **3c** containing electron withdrawing nitro group showed very good activity against *Staphylococcus aureus*. The compounds **3g** and **3h** showed sensitivity towards *Candida albicans.*

Table 2: Zone of inhibition (mm) of synthesized compounds

Compound code	Anti-bacterial activity												Anti-fungal activity
	Gram negative						Gram positive						
	K. pneumoniae			*Ecoli*			*P.Arugenosa*			*S.aureus*			*C.albicans*
	50 µg /mL	25 µg /mL	12.5 µg/ mL	50 µg /mL	25 µg /mL	12.5 µg/mL	50 µg / mL	25 µg / mL	12.5 µg / mL	50 µg /mL	25 µg /mL	12.5 µg/mL	200 µg /mL
3a	12mm	10mm	8mm	-	-	-	8mm	-	-	-	-	-	-
3b	12mm	10mm	10mm	-	-	-	11mm	7mm	7mm	-	-	-	-
3c	12mm	13mm	10mm	-	-	-	8mm	8mm	-	20mm	25mm	25mm	-
3d	13mm	12mm	12mm	7mm	-	-	-	-	-	-	-	-	-
3e	11mm	11mm	11mm	-	-	-	-	-	-	7mm	7 mm	-	-
3f	12mm	11mm	10mm	-	-	-	-	-	-	-	-	-	-
3g	10mm	10mm	15mm	-	-	-	10mm	10mm	14mm	-	-	-	8mm
3h	11mm	13mm	14mm	-	-	-	-	-	-	-	-	-	10mm
3i	10mm	12mm	13mm	-	-	-	-	-	-	-	-	-	-
Imipenem (10µg)				17mm									
Ampicillin (10µg)							30mm			30mm			
Ketoconazole (10µg)													14mm
Klebsiella pneumoniae was resistant to ampicillin(10µg), amoxicillin/ clavulanic acid (20/10 µg), gentamycin (10 µg), amikacin (30 µg), levofloxacin (5 µg), imipenem (10 µg), cefazolin (30 µg) and cefepime (30 µg).													

(-) refers to no activity

CONCLUSION

A series of 7-Amino-2-Styrylchromones were synthesized and characterized. All the synthesized compounds exhibited significant antibacterial activity against MDR strain of *Klebsiella pneumoniae.* No specific effect on the activity either by electron donating or electron withdrawing groups could be established. However it is observed that electron withdrawing nitro group enhanced the activity against *S.aureus*.

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