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ANTIFUNGAL AND ANTIBACTERIAL ACTIVITY OF SYNTHESIZED 2-STYRYLCHROMONES

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ABSTRACT

*2-Styrylchromones have common structural features with flavones in containing the benzopyrone moiety, exhibiting very good biological activities. The 2-Styrylchromones have been synthesized in two steps by claisen condensation, characterized by spectral data in comparison with literature values and tested for their antifungal and antibacterial activity against Aspergillus niger & Penicillum chrysogenium fungal and Xanthomonas campesrtis & Agrobacterium tumafeciens bacterial strains. The 2-Styrylchromones **12** & **15** with hydroxyl and methoxy substitution at specific positions showed greater antifungal and antibacterial effects.*

KEYWORDS : *antifungal activity, antibacterial activity, 2-Styrylchromones, Aspergillus niger, Xanthomonas campestris etc.*

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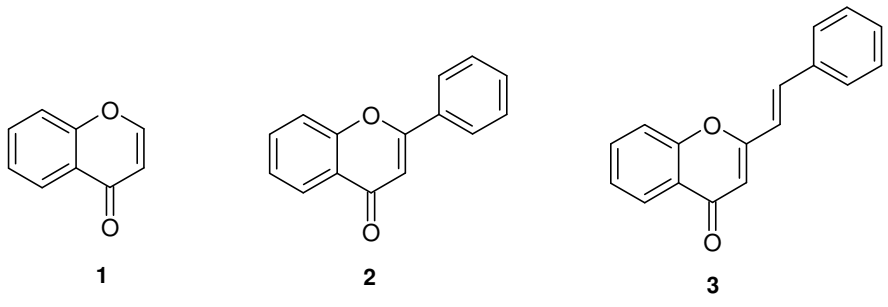
INTRODUCTION

Chromones are one of the most abundant classes of naturally occurring compounds that are ubiquitous in nature especially in plants[1]. 2-Styrylchromones are new class of flavonoids, structurally related to flavones (2-phenylchromones), and are one of the scarcest classes of natural chromones. Hormothamnione and 6-desmethoxyhormothamnione are the first known natural compounds, have been isolated from the marine blue green algae, cryptophyte, *chrysophaeum taylori*[2,3], by W.H.Gerwick. Before and after the isolation of natural 2-styrylchromones, several analogues of these compounds have been synthesized and tested in different biological system. The natural derivatives demonstrated cytotoxic activity against Leukemia cells[2,3] and those obtained by synthesis[4] exhibited antiallergic, antitumor[5], antagonism of A_3 adenosine receptor and xanthine oxidase inhibitor[6] properties.



1 Chromone

2 2-Phenylchromone

3 2-Styrylchromone

Figure-1

Both natural and synthetic compounds possess considerable biological activities[7-10] including anti-allergic activity[4] cytotoxicity[11], anti-tumor activity[4] and anti-rhinovirus activity[12]. Recently, 2-styrylchromonols and 2-styrylfuranochromones have been described as A_3 adenosine receptor antagonists (Karton *et al.* 1996). Taking into account the important properties and the similarity of 2-styrylchromones with flavones, several 2-styrylchromones have been synthesized by most promising laboratory methods like Aldol condensation / Oxidative cyclization[13-15] and Baker-Venkataraman rearrangement[16,17] to demonstrate their potential biological applications experimentally[6,18-21]. For these reasons, the report on the synthesis of new 2-styrylchromo-nes[22] include their biological activities[22, 23] was given recently. In this activity, it was concluded that the 2-stylylchromones with –OH substitution at particular position exhibited good antioxidant activity, whereas the compounds with cholro and methoxy substitution showed better antifungal and antibacterial activities.

An antifungal drug is a medicine used to treat fungal infection such as athlete's foot, ringworm, candidacies (thrush) etc. Antifungal work by exploiting differences between mammalian and fungal cells to kill the pathogenic fungal organism without causing any effect to the host. There are several classes of antifungal drugs, for example, polyene (nystatin), imidazole (miconazole, clotrimazole), triazoles (fluconazole), allylamines (amorolfine, butenafine), echinocandins (capsofungin), and others like griseofulvin, flucytosine are antifungals[24]. Some of essential oils[25] are also used as antifungal drugs.

An antibacterial is an agent that inhibits bacterial growth or kills bacteria[26] which are responsible for various diseases like tuberculosis, pneumonia, and foodborne illnesses including some infections such as tetanus, typhoid fever, diphtheria, syphilis, and leprosy. Antibacterial antibiotics are commonly classified based on their mechanism of action, chemical structure, or spectrum of activity. Those that target the bacterial cell wall (penicillins and cephalosporins) or the cell membrane (polymixins), or interfere with essential bacterial enzymes (rifamycins, lipiarmycins, quinolones, and sulphonamides) have bactericidal activities. Those that target protein synthesis (aminoglycosides, macrolides, and tetracyclins) are usually bacteriostatic[27]. Further categorization is based on their target specificity. "Narrow-spectrum" antibacterial antibiotics target specific types of bacteria, such as gram-negative or gram-positive bacteria, whereas broad-spectrum antibiotics affect a wide range of bacteria.

MATERIALS AND METHODS:

All the compounds screened for their antifungal and antibacterial activities have been synthesized in good yields from *o*-hydoxyacetophenones and cinnamic acid esters through β-diketones as intermediates by following general procedure[28] in two steps according scheme-1.

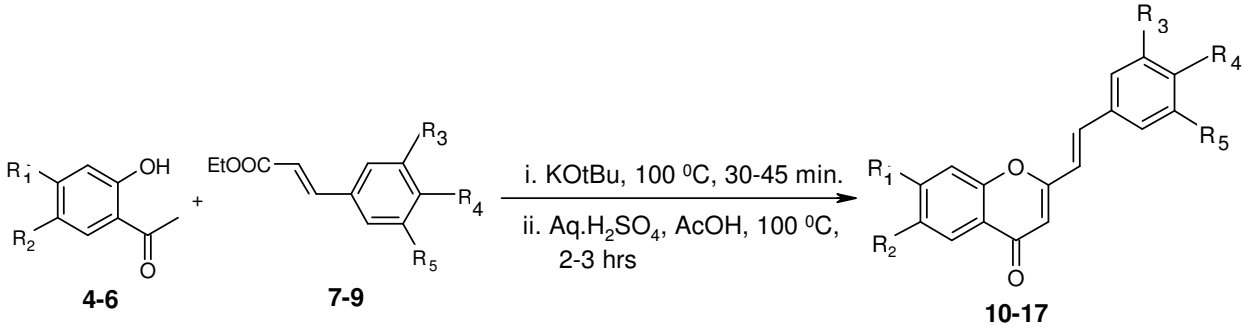
Synthesis of β-diketones:

To a mixture of 2-hydroxyacetophenone (0.01 mole) and cinnamic acid ester (0.0125 mole), add approximately 4g of powdered potassium t-butoxide. The reaction mixture was refluxed at 100

^{0}C for about 30-45 min with stirring in water both using calcium chloride guard tube. Then the reaction mixture was cooled to room temperature and poured into crushed ice containing dilute hydrochloric acid. The diketone thus obtained was filtered, washed with cold water until it became free from mineral acid and dried. It was recrystallized from hexane - ethylacetate to get pure compound.

Synthesis of 2-styrylchromones:

2-Styrylchromones were prepared by refluxing β-diketones (5 mmol) in nearly 6 mL of acetic acid in the presence of dilute sulphuric acid (0.5 mL) on a boiling water bath for about 3 hours. The crude product obtained from reaction mixture was died well and recrystallized from ethyl alcohol. The detailed of all synthesized 2-styrylchromones with their yields and melting points are represented in table-1.



Scheme-1

For compounds **4-9**

4 R_1 =H; R_2=OAc
5 R_1 =OAc; R_2= H
6 R_1= H; R_2= OMe
7 R_3=R_5=H; R_4= OAc
8 R_3=OMe; R_4=OAc; R_5=H
9 R_3=R_4=R_5=OMe

For compounds **10-17**

10 R_1=R_3=R_5=H; R_2=R_4= OH
11 R_1=R_3=R_5=H; R_2= OMe; R_4= OH
12 R_2=R_5=H; R_1=R_4= OH; R_3= OMe
13 R_1=R_5=H; R_2=R_4= OH; R_3= OMe
14 R_1=R_5=H; R_2=R_3= OMe; R_4=OH
15 R_1=OH; R_2=H; R_3=R_4=R_5=OMe
16 R_1=H; R_2=OH; R_3=R_4=R_5=OMe
17 R_1=H; R_2= R_3=R_4=R_5=OMe

Table-1:

Compound	R_1	R_2	R_3	R_4	R_5	Yield (%)	M.P.(^{0}C)
10	H	OH	H	OH	H	83	270-72
11	H	OMe	H	OH	H	85	258-60
12	OH	H	OMe	OH	H	77	260-62
13	H	OH	OMe	OH	H	78	268-70
14	H	OMe	OMe	OH	H	78	232-34
15	OH	H	OMe	OMe	OMe	76	250-52
16	H	OH	OMe	OMe	OMe	80	249-51
17	H	OMe	OMe	OMe	OMe	84	173-75

BIOLOGICAL ACTIVITY:

Growth and maintenance of Test Microorganism for Antimicrobial Studies:

Bacterial cultures of Bacillus subtilis (B. subtilis), Escherichia coli (E. coli), Agrobacterium tumafeciens and Xanthomonas campestris and fungal culture of Aspergillus niger and Penicillum chrysogenium were obtained from the culture collection centre, Department of the Applied Botany and Biotechnology, University of Mysore, India, were used for antimicrobial test organisms. The bacteria were maintained on nutrient broth (NB) at 37 ^{0}C and fungi were maintained on potato dextrose agar (PDA) at 28 ^{0}C.

Preparation of Inoculum:

The gram positive (*Bacillus subtils* and *Agrobacterium tumafeciens*) and gram negative bacteria (*Xanthomonas Campestris* and *Escherichia coli*) were pre-cultured in nutrient broth overnight in a rotary shaker at 37 ^{0}C, centrifuged at 10,000 rpm for 5 min, pellet was suspended in double distilled water and the cell density was standardized spectrophotometrically (A nm). The fungal inoculums (*Aspergillus niger* and Penicillum chrysogenium) was prepared from a 5 to 10 day old culture grown on potato dextrose agar medium. The petri dishes were flooded with 8 to 10 ml of distilled water and the conidia were scraped using sterile spatula. The spore density of each fungus was adjusted with spectrophotometer (A nm) to obtain a final concentration of approximately 10^5 spores/ml.

ANTIFUNGAL ACTIVITY

Determination by Agar cup method:

The antifungal activity of 2-styrylchromones was studied by agar cup method[29]. Sterilized glass Petri dishes were used and potato dextrose agar was used as basal medium for test fungi. The saboroudes broth medium was prepared by taking peptone (1.0 g) and dextrose (4.0 g) in warm distilled water (100 mL). Single colony of the selected fungal culture was inoculated in to broth medium and kept for incubation for overnight at 25 ^{0}C. The saboroudes agar medium was prepared by taking peptone (1.0 g), dextrose (4.0 g) and agar (2.0 g) in warm distilled water (100 mL) and plated into Petri dishes let for solidification for about ten minutes. The overnight fungal culture was spread evenly over the entire surface and left undisturbed for few minutes to percolate the culture. Wells (4 mm) were created using a sterile borer into the solidified agar medium. The selected compounds were added to each well (100 µL & 200 µL) at peripheral and the reference compound (Fluconazole) added at the centre. Thus the prepared plates were incubated at room temperature (at about 25 ^{0}C) for about 3-5 days. After incubation period the plates were collected and recorded the inhibition zone in mm (from the margin of the well to surface of inhibition).

Dimethyl sulphoxide (DMSO) was used as solvent to prepare the stock solutions (5 mg in 5 mL) of the compounds initially and also to maintain proper control. A control well was also placed on the test plates to compare the effect of the test samples and to nullify the effect of solvent (DMSO) respectively.

ANTIBACTERIAL ACTIVITY

Determination by disc diffusion method

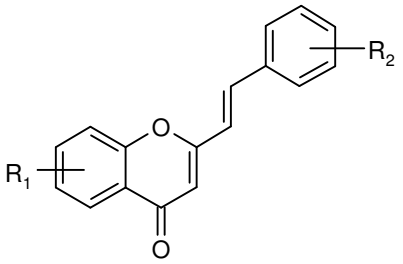
The antibacterial activity of synthesized chromones was tested by disc diffusion method[30]. The potato dextrose agar was used as basal medium for testing bacteria, which was prepared by taking yeast extract (3 gm/lit), peptone (10 gm/lit), dextrose (20 gm/ lit), agar (15 gm/lit), distilled water (1 lit) and with pH (6.0) and plated into Petri dishes, let for solidification for about ten minutes. The potato dextrose agar plates were inoculated with each bacterial culture (10 days in old) by point inoculation. The filter paper discs (5mm in diameter) impregnated with 100 µL and 200 µL concentrations of the extracts were placed on test organism seeded plates. DMSO was used to dissolve the tested compounds and was completely evaporated before application on test organism-seeded plates. The blank disk impregnated with solvent DMSO followed by drying off, was used as negative control and Ciprofloxacin used as positive control. The activity was determined after 72 hrs of incubation at 28 ^{0}C. The diameters of the inhibition zones were measured in mm.

RESULTS & DISCUSSIONS:

2-Styrylchromones are oxygen heterocyclic compounds, possessing very good biological active properties. These compounds were prepared by claisen condensation in two steps, starting from simple molecules like *o*-hydoxyacetophenones and cinnamic acid ester, and were characterized[22] by their IR, NMR & LCMS spectral data. For example in ^{1}H-NMR spectra, the resonance signal at δ 6.20-6.38 corresponding to H at 3-position. The peaks for β-hydrogens (δ 7.3-7.6) appeared at higher

chemical shift values than α-hydrogens (δ 6.9-7.19) due to conjugation of double bond with carbonyl group. The trance configuration of these hydrogens was established from large J values at 16.0-16.1 Hz. The phnolic protons appear at δ 8.8-10.0 whereas methoxyl protons at δ 3.70-3.83 and aromatic protons at δ 6.82-7.65.

The present work represents mainly the antimicrobial activity, i.e. antifungal and antibacterial activity of totally eight synthesized compounds (**10-17**).



10-17

Figure-2

10). 6-Hydroxy-2-(4-hydroxystyryl)chromone
11). 6-Methoxy-2-(4-hydroxystyryl)chromone
12).7-Hydroxy-2-(4-hydoxy-3-methoxystyryl)chromone
13). 6-Hydroxy-2-(4-hydoxy-3-methoxystyryl)chromone
14). 6-Methoxy-2-(4-hydoxy-3-methoxystyryl)chromone
15). 7-Hydroxy-2-(3,4,5-trimethoxystyryl)chromone
16). 6-Hydroxy-2-(3,4,5-trimethoxystyryl)chromone
17). 6-Methoxy-2-(3,4,5-trimethoxystyryl)chromone

All of the synthesized compounds **10-17** were evaluated *in vitro* against two pathogenic fungi, *Aspergillus Niger* & *Penicillum chrysogenium* using agar cup method at the concentration of 100 & 200 µg. The results provided in table-2 indicate that most of the prepared compounds have weak to good antifungal activity against the tested fungi. The results showed that most of the synthesized compounds displayed lower activities against *A. Niger* & *P. chrysogenium*, only two 2-styrylchromones (**12** & **15**) showed better inhibitory activity against both fungi compared to the remaining compounds.

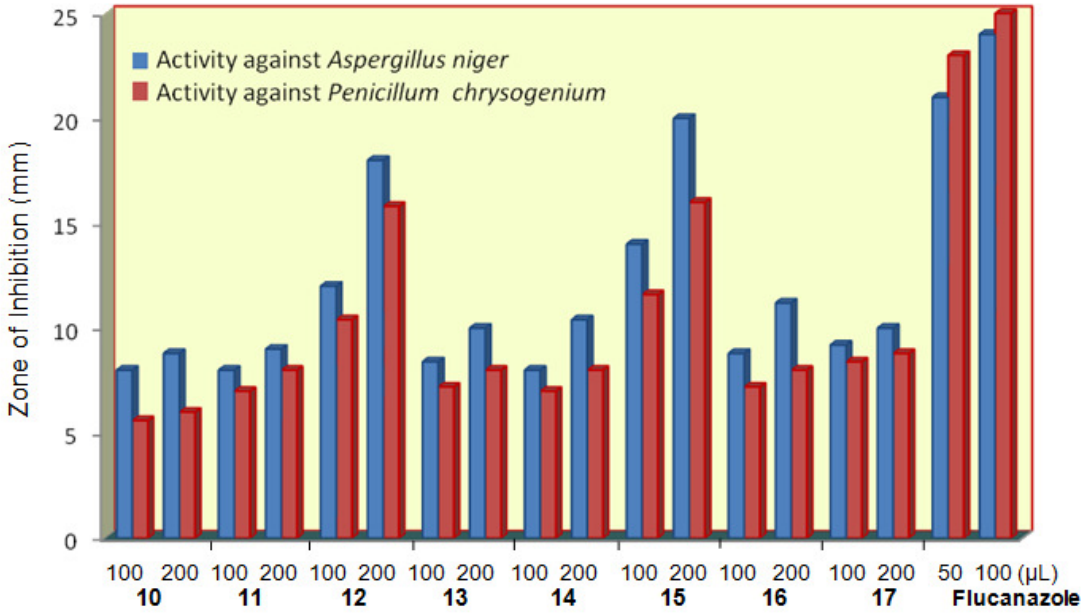
We can conclude that changing the substituent on chromone benzene could lead to a little change in activity, for instance, the compounds (**10** &**11, 16** & **17**) displayed almost all similar activities with hydroxyl and methoxy substitution at the 6-position. Furthermore, the compounds with the same substituent but at different position on chromone ring exhibited different activity. For instance, the activity of the compounds **12** and **15** with 7-OH substituent was 18 & 20 mm, but the activity of the compounds **13** and **16** with 6- OH substituent was 10 and 11.2 mm of zone inhibition at 200 µg concentration against *A. Niger.* The experimental results indicated that the 2-styrylchromones with oxygenation at 7,4' positions exhibited significant antifungal activity. The results of diameter of zone inhibition (in mm) of synthetic compounds have been incorporated in Table-2 and a graphical representation of the activity is represented in Figure-3**.**

Table-2 Antifungal activity of 2-styrylchromones:

Compound	**R_1**	**R_2**	**Conc.** (µL)	**Zone of inhibition** (mm)	
				Aspergillus niger	*Penicillum chrysogenium*
10	6-OH	4'-OH	100 200	8.0 8.8	5.6 6.0
11	6-OMe	4'-OH	100 200	8.0 9.0	7.0 8.0
12	7-OH	3'-OMe, 4'-OH	100 200	12.0 18.0	10.4 15.8
13	6-OH	3'-OMe,	100	8.4	7.2

		4’-OH	200	10.0	8.0
14	6-OMe	3’-OMe, 4’-OH	100 200	8.0 10.4	7.0 8.0
15	7-OH	3’,4’,5’-tri-OMe	100 200	14.0 20.0	11.6 16.0
16	6-OH	3’,4’,5’-tri-OMe	100 200	8.8 11.2	7.2 8.0
17	6-OMe	3’,4’,5’-tri-OMe	100 200	9.2 10.0	8.4 8.8
Fluconazole			50 100	21 24	23 25

Figure-3:



The antibacterial activity of 2-styrylchromones (**10**-**17**) was studied *in vitro* by disc difusion method at two different concentrations i.e. 100 µL & 200 µL, against gram positive bacteria, *Agrobacterium tumafeciens* and gram negative bacteria, *Xanthomonas campestris* stains. Initially, susceptibility test was carried out by measuring the inhibitory zone diameters on nutrient agar, with conventional disc diffusion method, and the inhibitory zone diameters were examined and noted for analysis. The screening results indicate that all the compounds exhibited better antibacterial activities against the tested bacteria. It was noticed that the 2-styrylchromones (**15, 16** & **17**) with methoxy substitution exhibited greater inhibitory activity against both bacteria along with compound **12,** than the remaining 2-styrylchromones.

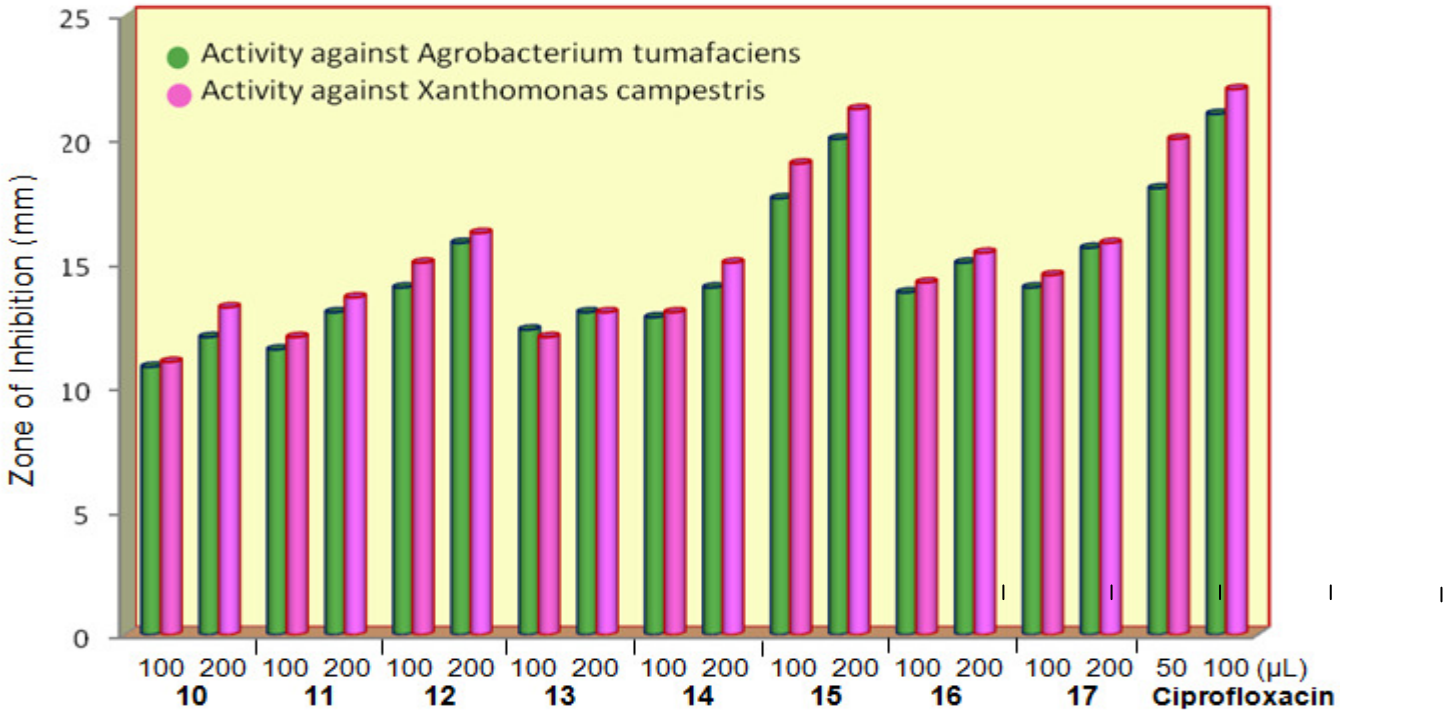
Moreover, different type of various substituents on the chomone benzene ring also affect the activity of the compounds, such as the compounds **11**, **14** & **17** with methoxy substituent at 6-position possessed good activity than the compounds **10**,**13** & **16** with hydroxyl substituent at same position. It was also observed that the 2-styrylchromones with oxygenation at 4', & 7-position exhibited highest bacterial effect than the other 2-styrylchromones against two tested bacteria. It was also noticed that as the number of electron releasing groups increased, the antibacterial activity of 2-styrylchromones also increased. The results of changes in diameter of zone of inhibition (in mm) of synthetic 2-

styrylchromones have been incorporated in Table-3
and fig.4

Table-3: Antibacterial activity of 2-styrylchromones:

Compound	R_1	R_2	Conc. (µL)	Zone of inhibition (mm)	
				Agrobacterium tumafeciens	*Xanthomonas campestris*
10	6-OH	4'-OH	100 200	10.8 12.0	11.0 13.2
11	6-OMe	4'-OH	100 200	11.5 13.0	12.0 13.6
12	7-OH	3'-OMe, 4'-OH	100 200	14.0 15.8	15.0 16.2
13	6-OH	3'-OMe, 4'-OH	100 200	12.3 13.0	12.0 13.0
14	6-OMe	3'-OMe, 4'-OH	100 200	12.8 14.0	13.0 15.0
15	7-OH	3',4',5'-tri-OMe	100 200	17.6 20.0	19.0 21.2
16	6-OH	3',4',5'-tri-OMe	100 200	13.8 15.0	14.2 15.4
17	6-OMe	3',4',5'-tri-OMe	100 200	14.0 15.6	14.5 15.8
Ciprofloxacin			50 100	18 21	20 22

Figure-4



CONCLUSION:

The synthesized compounds exhibit antifungal and antibacterial activities due to the presence of chromone structure with styryl group at 2-posiion. The antimicrobial activities of compounds depend on the nature, position and number of substituents. The electron releasing groups at specific positions provide impetus to 2-styrylchomones to exhibit better antifungal and antibacterial activities. The enhancement in the activities of 2-styrylchomones is observed with methoxy substitution over hydroxyl substitution.

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