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RESEARCH ARTICLE

Synthesis, Characterization and Antimicrobial Activity of 2-hydroxy-5-bromo-4-methoxy-N-(substituted phenyl) chalconeimine

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ABSTRACT

A series of 2-hydroxy-5-bromo-4-methoxy-N-(substituted phenyl) chalconeimine was synthesized, characterized and tested for their antimicrobial activity. These new derivative was achieved by treating 2-hydroxy-5-bromo-4-methoxy chalcone with substituted aniline at reflux temperature using ethanol as solvent in presence of H_2SO_4. Structures of the synthesized compounds were characterized using IR, ^{1}H-NMR and mass spectroscopy. The synthesized compounds were screened for their *in vitro* antibacterial activity against bacteria *S. aureus*, *E. coli*, *P. aeruginosa* and *S. Pyogenes*. And antifungal activity against *C. Albicans* and *A.Clavatus* some of these compounds exhibited moderate to good activity.

KEYWORDS

Chalconeimines, antimicrobial activity, substituted aniline.

INTRODUCTION

Chalcones are the important constituent of natural sources. They are firstly named by Kostanecki and Tambor[1]. Chalcones are medicinally important class of compounds. Chalcones are used as an intermediate for the synthesis of many heterocyclic compounds such as pyrimidines[2], pyrazolines[3], benzodiazepines[4], flavonone[5], isoxazoline[6], benzoxazolone[7], quinolines[8], indolinones[9] etc. The compounds with backbone of Chalcones have been reported to possess various biological activities such as antimicrobial[10], anti-inflammatory[11], analgesic[12], antimalarial[13], anticancer[14], antiviral[15], antioxidant[16], antitubercular[17], anti-HIV[18], insecticidal[19,20], bactericidal[21,22], fungicidal[23-24], activities.

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The presence of a reactive α-β unsaturated keto function in Chalcones is found to be responsible for their antimicrobial activity.

In the present work we report 2-Hydroxy-5-bromo-4-methoxy- N- (substituted phenyl) chalconeimines. Chalcone condenses with substituted aniline in ethanol in presence of 2, 3 drops of concentrated H_2SO_4 to give chalconeimine. The structures of the various synthesized compounds were assigned on the basis of Mass, IR, ^{1}H & ^{13}C-NMR spectroscopy. These compounds were screened for their antimicrobial activity.

EXPERIMENTAL

All chemicals were purchased from commercial suppliers and used without further purification. Melting points were determined on Vigo melting point apparatus and are uncorrected. All the compounds were routinely checked for their homogeneity by TLC on silica gel plate, IR

spectra were recorded in KBr pellets on Perkin-Elmer FT-IR spectrophotometer, ^{1}H NMR spectra were recorded on BRUKER spectrometer on 300 MHz in $CDCl_3$ using TMS as an internal standard. The mass spectra were recorded on FAB mass spectrometer to confirm their structure, The micro-organism *Staphylococcus aureus MTCC 96 (S. aureus), Escherichia coli MTCC 443 (E. coli), Pseudomonas aeruginosa MTCC 424 (P. aeruginosa), Streptococcus pyogenes MTCC 442 (S. Pyogenes), Candida albicans MTCC 227 (C. Albicans) and Aspergillus clavatus MTCC 1323 (A.Clavatus)* were purchased from the National Chemical Laboratory (NCL), Pune, India.

Biological Evaluation

Anti-bacterial activity of 3a-j:

The study has been conducted according to the method adopted by Cruickshank et al. Nutrient agar broth was melted in a water bath and cooked to 45 °C with gentle shaking to bring about uniform cooling. It was inoculated with 0.5-0.6 ml of 24 hour old culture especially and mixed well by gentle shaking before pouring on the sterilized Petri dish (25 ml each). The poured material was allowed to set (1.5 hour) and there after the "cups" was made by punching into the agar surface with a sterile cork borer and soaping out the punched part of agar. Into this "cups" 0.1 ml of test solution (prepared by dissolving 10 mg of sample in 1 ml DMF) was added by sterile micropipette. The plates were noted. The antibacterial activities of all compounds are compared against Ampicilin as a standard drug.

Antifungal activity of 3a-j:

The fungicidal activity of all the compounds was studied at 1000 ppm concentration in vitro. Plant pathogenic organisms used were C. albicans and A. clavatus. The antifungal activity of all the compounds was measured on each of these plant pathogenic strains on a potato dextrose agar (PDA) medium such a PDA medium contained potato 200 gm., dextrose 20 gm., agar 20 gm., and water 1 liter. Five days old cultures were employed. The compounds to be tested were suspended (1000 ppm) in a PDA medium and autoclaved at 120°C for 15 min and at 15 atm. pressure. These media were poured into sterile Petri plates and the organisms were inoculated after cooling the Petri plates. The percentage inhibition for fungi was calculated after five days using the formula given below.

$$\text{Percentage of inhibition} = \frac{\mathbf{100\ (X - Y)}}{\mathbf{X}}$$

Where, X = Area of colony in control plate.

Y = Area of colony in test plate.

General procedure for Synthesis of 2-hydroxy-5-bromo-4-methoxychalcone [1]

2-hydroxy-5-bromoacetophenone (0.01 moles) and 4-anisaldehyde (0.01 mol) were dissolved in ethanol (10 ml) and aqueous NaOH (40%) was added drop wise under stirring. Reaction mass turn orange in colour was stirred at room temperature for overnight. The reaction mixture was acidified with dilute HCl and solid was filtered off, wash with sodium bicarbonate solution again followed by water. Product was purified by crystallization in ethanol and glacial acetic acid mixture.

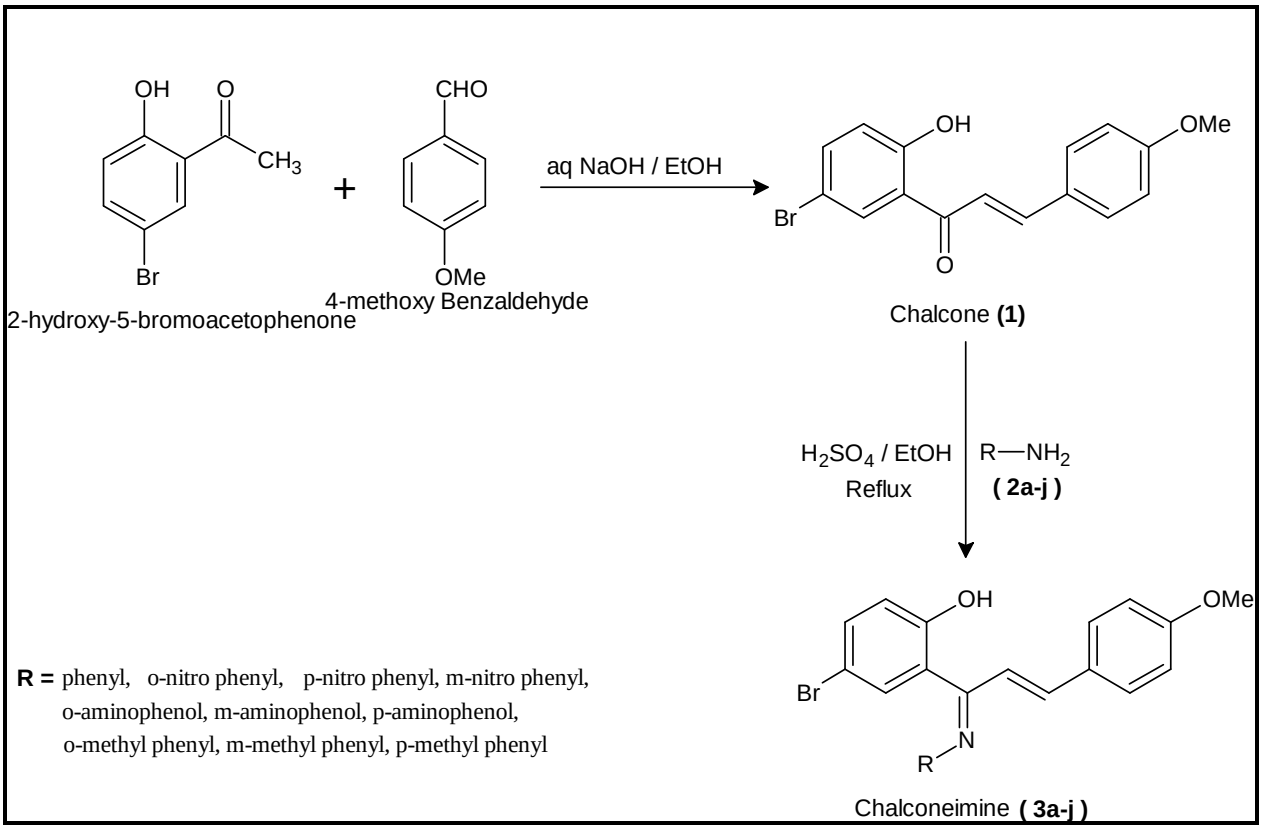
Yield: 75%; colour: yellow solid; m.p: 58-60°C; MF: $C_{16}H_{13}O_3Br$; MW: 333.17; IR (KBr, cm^{-1}):

3521 (O-H), 1663 (C=O), 1574 and 1448 (C=C, aromatic), 1607 (C=C, olefinic);^{1}H-NMR (500 MHz, $CDCl_3$, δ / ppm): 12.09 (s, 1H, -OH), 8.02 (d, 1H, Ar-CH), 7.9 (d, 1H, Ar-CH), 7.68-7.66 (dd, 2H, Ar-CH), 7.58-7.55 (dd, 1H, Ar-CH), 7.47-7..44 (d, 1H, Ar-CH), 6.99-6.93 (m, 2H, Ar-CH), 3.89 (s, 3H, $-CH_3$); MS (*m*/*z*): 334.06 (M+1).

General procedure for synthesis of 2'-hydroxy-5'-bromo-4-methoxy-N-(substituted phenyl)-chalconeimine **(3a-j):**

2-hydroxy-5-bromo-4-methoxy-chalcone **[1]** (0.01mol) and substituted aniline (0.01 mol) was dissolved in ethanol (20 ml). 2-3 drops of conc. H_2SO_4 was added and refluxed for 3 hrs. On cooling and dilution with ice cold water separated solid was filtered off and was purified

REACTION SCHEME



Scheme 1: Synthesis of 2-hydroxy-5-bromo-4-methoxy-N-(substituted phenyl) chalconeimine **(3a-j)**

Table 1: Physical parameters of chalconeimine derivatives (3a-j)

Sr. No	R	Product code	MP (^{0}C)	Molecular Formula	Molecular Weight	Yield (%)
1	Phenyl	3a	82	$C_{22}H_{18}O_2NBr$	408	78
2	o-nitro phenyl	3b	48	$C_{22}H_{17}O_4N_2Br$	453	56
3	m-nitro phenyl	3c	70	$C_{22}H_{17}O_4N_2Br$	453	60
4	p-nitro phenyl	3d	72	$C_{22}H_{17}O_4N_2Br$	453	65
5	o-aminophenol	3e	82	$C_{22}H_{18}O_3NBr$	424	51
6	m-aminophenol	3f	98	$C_{22}H_{18}O_3NBr$	424	70
7	p-aminophenol	3g	84	$C_{22}H_{18}O_3NBr$	424	75
8	o-methyl phenyl	3h	86	$C_{23}H_{20}O_2NBr$	422	72
9	m-methyl phenyl	3i	80	$C_{23}H_{20}O_2NBr$	422	78
10	p-methyl phenyl	3j	78	$C_{23}H_{20}O_2NBr$	422	65

by recrystallized from ethanol. All the physical parameter of synthesized compound summarized in **Table-I.**

2-hydroxy-5-bromo-4-methoxy-N-(phenyl)-chalconeimine **(3a)**

Yield: 78%; m.p.82°C; IR (KBr, cm^{-1}): 3295 (-OH), 1632 (-C=N), 1607 (C=C, olefinic), 1574, 1448 (C=C, aromatic) ; ^{1}H-NMR (300 MHz, $CDCl_3$, δ / ppm): 12.05 (s, 1H, -OH), 7.1 (d, 1H, -CHβ), 6.67 (d, 1H, -CHα), 8.2-7.3 (m, 12H, Ar-CH), 3.75 (s, 3H, $-CH_3$); MS (*m*/*z*): 409.3 (M+1).

2-hydroxy-5-bromo-4-methoxy -N-(o-nitro phenyl)-chalconeimine **(3b)**

Yield: 56%; m.p. 48°C; IR (KBr, cm^{-1}): 3291 (-OH), 1642 (-C=N), 1608 (C=C, olefinic), 1587, 1455 (C=C, aromatic) ;^{1}H-NMR (300 MHz, $CDCl_3$, δ / ppm): 12.1 (s, 1H, -OH), 7.12 (d, 1H, -CHβ), 6.62 (d, 1H, -CHα), 7.99.2-7.27 (m, 11H, Ar-CH), 3.72 (s, 3H, $-CH_3$); MS (*m*/*z*): 454.06 (M+1).

2-hydroxy-5-bromo-4-methoxy -N-(m-nitro phenyl)-chalconeimine **(3c)**

Yield: 60%; m.p: 70 °C, IR (KBr, cm^{-1}): 3292 (-OH), 1641 (-C=N), 1607 (C=C, olefinic), 1584, 1446 (C=C, aromatic); ^{1}H-NMR (300 MHz, $CDCl_3$, δ / ppm): 12.11 (s, 1H, -OH), 7.10 (d, 1H, -CHβ), 6.64 (d, 1H, -CHα), 8.04-7.22 (m, 11H, Ar-CH), 3.72 (s, 3H, $-CH_3$); MS (*m*/*z*): 454.36 (M+1).

2-hydroxy-5-bromo-4-methoxy -N-(p-nitro phenyl)-chalconeimine **(3d)**

Yield: 65%; m.p: 72°C; IR (KBr, cm^{-1}): 3296 (-OH), 1647 (-C=N), 1600 (C=C, olefinic), 1581, 1445 (C=C, aromatic) ; ^{1}H-NMR (300 MHz, $CDCl_3$, δ / ppm): 12.11 (s, 1H, -OH), 7.11 (d, 1H, -CHβ), 6.62 (d, 1H, -CHα), 7.95-7.22 (m, 11H, Ar-CH), 3.76 (s, 3H, $-CH_3$); MS (*m*/*z*): 454.6 (M+1).

2-hydroxy-5'-bromo-4-methoxy -N-(o-aminophenol)-chalconeimine **(3e)**

Yield: 51%; m.p.; 82 °C; IR (KBr, cm^{-1}): 3299 (-OH), 1645 (-C=N), 1600 (C=C, olefinic), 1581, 1438 (C=C, aromatic) ; ^{1}H-NMR (300 MHz, $CDCl_3$, δ / ppm): 12.1 (s, 1H, -OH), 7.18 (d, 1H, -CHβ), 6.74 (d, 1H, -CHα), 8.07-7.35 (m, 11H, Ar-CH), 3.76 (s, 3H, $-CH_3$); MS (*m*/*z*): 425.46 (M+1).

2-hydroxy-5-bromo-4-methoxy -N-(m-aminophenol)-chalconeimine **(3f)**

Yield: 70%; m.p.; 98 °C; IR (KBr, cm^{-1}): 3295 (-OH), 1650 (-C=N), 1611 (C=C, olefinic), 1588, 1442 (C=C, aromatic) ; ^{1}H-NMR (300 MHz, $CDCl_3$, δ / ppm): 12.12 (s, 1H, -OH), 7.17 (d, 1H, -CHβ), 6.67 (d, 1H, -CHα), 8.1-7.3 (m, 11H, Ar-CH), 3.75 (s, 3H, $-CH_3$); MS (*m*/*z*): 425.40 (M+1).

2-hydroxy-5-bromo-4-methoxy -N-(p-aminophenol)-chalconeimine **(3g)**

Yield: 75%; m.p. 84°C; IR (KBr, cm^{-1}): 3290 (-OH), 1649 (-C=N), 1600 (C=C, olefinic), 1584, 1448 (C=C, aromatic) ; ^{1}H-NMR (300 MHz, $CDCl_3$, δ / ppm): 12.11 (s, 1H, -OH), 7.19 (d, 1H, -CHβ), 6.67 (d, 1H, -CHα), 8.15-7.35 (m, 11H, Ar-CH), 3.71 (s, 3H, $-CH_3$); MS (*m*/*z*): 425.58 (M+1).

2-hydroxy-5-bromo-4-methoxy -N-(o-methyl phenyl)-chalconeimine **(3h)**

Yield: 72%; m.p: 86°C; IR (KBr, cm^{-1}): 3288 (-OH), 1652 (-C=N), 1610 (C=C, olefinic), 1586, 1444 (C=C, aromatic) ;^{1}H-NMR (300 MHz, $CDCl_3$, δ / ppm): 12.05 (s, 1H, -OH), 7.17 (d, 1H, -CHβ), 6.67 (d, 1H, -CHα), 8.1-7.3 (m, 11H, Ar-CH), 3.77 (s, 3H, $-CH_3$), 2.35 (s, 3H,-CH_3); MS (*m*/*z*): 423.6 (M+1).

2-hydroxy-5-bromo-4-methoxy -N-(m-methylphenyl)-chalconeimine **(3i)**

Yield: 78%; m.p: 80 °C; IR (KBr, cm^{-1}): 3288 (-OH), 1640 (-C=N), 1609 (C=C, olefinic), 1583, 1451 (C=C, aromatic) ; ^{1}H-NMR (300 MHz, $CDCl_3$, δ / ppm): 12.1 (s, 1H, -OH), 7.19 (d, 1H, -CHβ), 6.67 (d, 1H, -CHα), 8.1-7.23 (m, 11H, Ar-CH), 3.75 (s, 3H, $-CH_3$), 2.37 (s, 3H,-CH_3); MS (*m*/*z*): 423.58 (M+1).

2-hydroxy-5-bromo-4-methoxy -N-(p-methylphenyl)-chalconeimine **(3j)**

Yield: 65% ; m.p: 78°C; IR (KBr, cm^{-1}): 3285 (-OH), 1655 (-C=N), 1613 (C=C, olefinic), 1586,

1446 (C=C, aromatic) ; ^{1}H-NMR (300 MHz, $CDCl_3$, δ / ppm): 12.1 (s, 1H, -OH), 7.2 (d, 1H, -CHβ), 6.69 (d, 1H, -CHα), 8.08-7.34 (m, 11H, Ar-CH), 3.72 (s, 3H, $-CH_3$), 2.36 (s, 3H,$-CH_3$); MS (*m*/*z*): 423.48 (M+1).

RESULTS AND DISCUSSION

New 2-hydroxy-5-bromo-4-methoxy-N-(substituted phenyl) chalconeimine have been synthesized by the reaction of 2-hydroxy-5-bromo-4-methoxy Chalcone (1) and substituted aniline at reflux temperature using ethanol as solvent in presence of conc. H_2SO_4 in 51-78 %yield.

All the synthesized compounds were characterized using different spectroscopic techniques. IR spectrum showed characteristic band of hydroxide at 3295 cm^{-1}, imine group (-C=N) at 1650 and olefinic stretching at 1611. ^{1}H NMR spectrum was carried out at 300 MHz and showed some characteristics pattern of peaks. Hydroxide came at 12.1, olefinic α and β at 6.6 and 7.7 resp. Whereas aromatic protons appeared at 7.1-8.2 ppm. Electron ionization mass spectrometric fragmentation pattern of all the compounds were same.

Antimicrobial Activity

All the compounds **(3a-j)** were tested for their antimicrobial activity against the microorganism *S. aureus, E. coli, P. aeruginosa, S. Pyogenes, C. Albicans* and *A. Clavatus.* The results were compared with the standard antibacterial drug Amplicilline, Chloramphenicol, Ciprofloxacin, Norfloxacin and standard antifungal drug Greseofulvin and Nystatin. The zones of inhibition values (mm) for antibacterial activity were shown in **Fig.1** and summarized in **Table II**, while antifungal activity was shown in **Fig.2** and summarized in **Table III**. It shows that chalconeimine having methyl group at ortho and meta position of aniline i.e. **3h** & **3i** have higher antibacterial activity against the bacterial stain *P. aeruginosa* compare to all other chalconeimine**. 3b** and **3g** having moderate activity against *S. pyrogenes* while **3g** and **3h** having higher activity against *E. coli.* The entire compound show lower activity against *S. aurous*. **3g, 3h** and **3i** have higher antifungal activity against both the antifungal agent while all other have moderate activity**.**

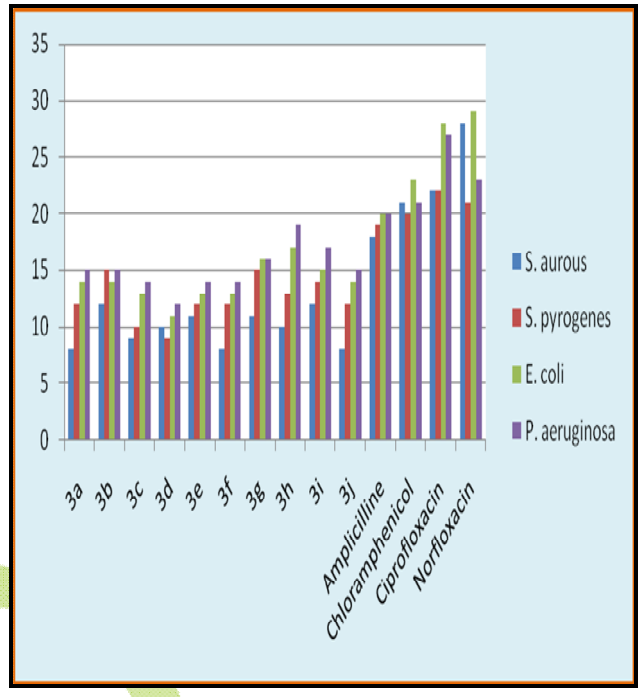


Figure 1: Antibacterial activity of halconeimine (3a-j)

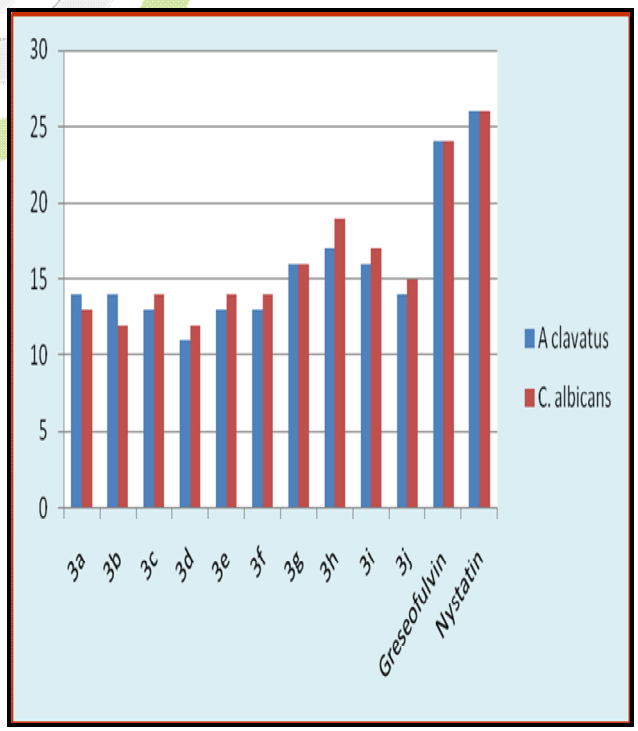


Figure 2: Antibacterial activity of halconeimine (3a-j)

Table 2: Antibacterial activity of chalconeimine (3a-j)

Sr. No.	Compound code	R	Zone of inhibition (in mm)			
			Antibacterial Activity			
			Gram +ve		Gram –ve	
			S. aurous	***S. pyrogenes***	***E. coli***	***P. aeruginosa***
1	3a	Phenyl	08	12	14	15
2	3b	o-nitro phenyl	**12**	**15**	14	15
3	3c	m-nitro phenyl	09	10	13	14
4	3d	p-nitro phenyl	10	09	11	12
5	3e	o-aminophenol	11	12	13	14
6	3f	m-aminophenol	08	12	13	14
7	3g	p-aminophenol	11	**15**	**16**	16
8	3h	o-methyl phenyl	10	13	**17**	**19**
9	3i	m-methyl phenyl	**12**	14	15	**17**
10	3j	p-methyl phenyl	08	12	14	15
11	Amplicilline	-	18	19	20	20
12	Chloramphenicol	-	21	20	23	21
13	Ciprofloxacin	-	22	22	28	27
14	Norfloxacin	-	28	21	29	23

Table 3: Antifungal activity of chalconeimine (3a-j)

Sr. No.	Compound code	R	Zone of inhibition (in mm)	
			Antifungal Activity	
			A clavatus	***C. albicans***
1	3a	Phenyl	14	13
2	3b	o-nitro phenyl	14	12
3	3c	m-nitro phenyl	13	14
4	3d	p-nitro phenyl	11	12
5	3e	o-aminophenol	13	14
6	3f	m-aminophenol	13	14
7	3g	p-aminophenol	**16**	**16**
8	3h	o-methyl phenyl	**17**	**19**
9	3i	m-methyl phenyl	**16**	**17**
10	3j	p-methyl phenyl	14	15
11	Greseofulvin	-	24	24
12	Nystatin	-	26	26

CONCLUSION

In summary, we have disclosed the rational design of a series of potent 2-hydroxy-5-bromo-4-methoxy-N-(substituted phenyl) chalconeimine derivatives. The biological data indicate that chalconeimine having methyl group are more active than the chalconeimine having amine group to aniline.

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