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Research Article

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Synthesis, characterization and antimicrobial activity of 6-bromo-4-methoxy-4-(substituted phenyl) iminoflavone

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ABSTRACT

Chalcone and their heterocycalic analogues are known to posses a broad spectram of biological effects. The present study is devoted to the synthesis of 6-bromo-4-methoxy-4-(substituted phenyl) imino flavones. The newly synthesized compounds were screened for their antimicrobial and antifungal activities. 2-hydroxy-5-bromo-4-methoxy-chalconeimine on refluxing in DMSO for two hrs in presence of catalytic amount of iodine in presence of conc. H_2SO_4 affort a corresponding imino flavones in high yield. The structures were established on the basis of spectral data (IR, NMR) and chemical reactions.

Keywords: Flavones, 2-hydroxy chalcone, spectroscopy, Antimicrobial activity.

INTRODUCTION

Flavonoids are ubiquitous in photosynthesizing cells and therefore occur widely in the plant kingdom [1]. They are found in fruit, vegetables, nuts, seeds, stems and flowers as well as tea, wine [2], propolis and honey [3], and represent a common constituent of the human diet [4]. The function of flavonoids in flowers is to provide colours attractive to plant pollinators [2, 5]. In leaves, these compounds are increasingly believed to promote physiological survival of the plant, protecting it from, for example, fungal pathogens and UV-B radiation [4, 5]. In addition, flavonoids are involved in photosensitization, energy transfer, the actions of plant growth hormones and growth regulators, control of respiration and photosynthesis, morphogenesis and sex determination [2, 4]. Synthesis of flavones and their derivatives has attracted considerable attention due to their significant biocidal [6-8], pharmaceutical [9-12], antioxidant [13-16], anti-anxiolytic [17], anticancer[18], and anti-inflammatory[19,20] effects. In the light of these results a number of flavones derivatives have been synthesized and their biological activities [21-26] studied. The use of dimethyl sulphoxide (DMSO) as oxidizing agents for affecting this conversion has been reported by several workers [27-29]. However the DMSO-I_2 for the oxidation of 2'-hydroxy chalcones to flavones has not been used so far, though resently Iodine-DMSO-Sulphuric acid system has been applied [30] for dehydration of flavonides and flavones derivatives are synthesized resently by using DDQ/DMSO-I_2/Diphenyl disulfide by oxidative cyclization of 2-hydroxy chalcone [31,32], and also flavanol derivatives are synthesized by using H_2O_2 & NaOH [33]. Resently Chalconeimine is converted in flavoneimine by oxidative cyclisation in presence of DMSO-I_2 [34-39].

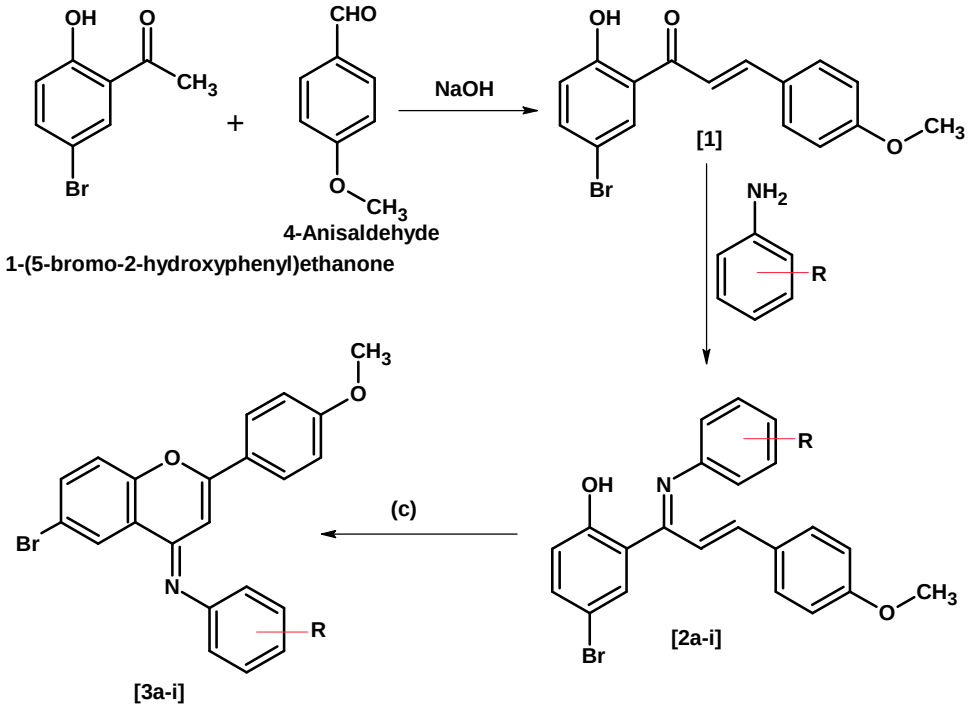
In this communication synthesis of flavoneimine by oxidative cyclisation of chalconeimines is prerformed in DMSO medium in presense of catalytic amount of Iodine and 2-3drops of conc. H_2SO_4.

EXPERIMENTAL SECTION

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,

Antibacterial and anti-fungal activity (anti-microbial activity) was carried out by Agar cup method. The bacterial strains are identified strains and obtained from National chemical Laboratory (NCL), Pune, India

Reaction Scheme:



Scheme 1

(a). NaOH/Ethanol / RT, (b) Ethanol /H_2SO_4/ Reflux, (c) I_2/ DMSO / Reflux

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

A solution of 2-hydroxy-5-bromoacetophenone (0.01 moles) and 4-anisaldehyde (0.01 mol) were dissolved in ethanol (10 ml), under stirring and aqueous NaOH (40%) was added drop wise. Orange colour solid was separate out. The reaction mixture was stirred at room temperature and kept overnight.The reaction mixture was acidified with HCl (50%). The separated solid was filtered and wash with 1% sodium bicarbonate solution again followed by water. Product was crystallized from ethanol and glacial acetic acid (1:1) to give yellow colour solid [1]. Yellow solid; Yield: 75%; m.p: 58-60°C. Resently Chalcone derivatives are synthesized by using $SOCl_2$/EtOH [40] and NaOH/ rectified spirit also used for the synthesis of chalcone. [41]

General procedure for the Synthesis of 2'-hydroxy-5'-bromo-4-methoxy-N-(substituted phenyl)-chalconeimine (2a-i):

2-hydroxy-5-bromo-4-methoxychalcone [1] (0.01mol) and substituted aniline (0.01 mol) was dissolved in ethanol (20 ml). To this mixture 2-3 drops of conc. H_2SO_4 was added and it was refluxed for 3 hrs. On cooling and dilution

with ice cold water, a solid mass separated out. It was recrystallized from ethanol. Dark yellow solid; Yield: 78 %; m.p: 80-82 °C

General procedure for the Synthesis of 6-Bromo-4-methoxy-4-(substituted phenyl) iminoflavone (3a-i):
2-hydroxy-5-bromo-4-methoxy- N-(substituted phenyl) chalcone imine [2a-i] (0.01mole) was dissolved in dimethyl sulphoxide (DMSO) (40ml) and was treated dropwise with 2-3 drops of conc. H_2SO_4.The mixture was refluxed for 10 min it was then cooled and little catalytic amount of iodine was added. The reaction mixture was again heated for 3 hr on water bath, Cooled and diluted with ice cold water. The resulting solid was treated with 10% sodium thiosulphate solution to remove unreacted iodine and finally with water and crystallized from ethanol to give brown crystalline compound [42]. Dark brown solid; Yield: 82%; m.p: 140-142 °C

6-Bromo-4-methoxy-4-(phenyl) iminoflavone (3a)
Yield: 82%, Colour: dark brown solid, m.p: 140-142°C, Molecular formula: $C_{22}H_{16}O_2NBr$, Molecular weight: 405.9

IR (KBr) ν max cm^{-1}:
3076.46 cm^{-1} (CH aromatic stretching), 1604.77 cm^{-1} (C=C aromatic stretching), 1654.92 cm^{-1} (C=N stretching), 1255.66 cm^{-1} (C-O stretching), 1022.27 cm^{-1} (C-Br stretching), 1217.08 & 1122.57cm^{-1}(C-O,Stretching in –OCH_3), 833.25cm^{-1}(trisubstituted), 815.89cm^{-1}(p-disubstituted), 765.74 cm^{-1},657.73 cm^{-1} (monosubstituted)

^{1}H NMR: [δ $CDCl_3$]:
6.84-8.39 (m 12H, Ar-H), 2.33 (s, 1H, C=CH), 3.88 (s, 3H, -OCH_3)

6-Bromo-4 methoxy- 4-(o-nitrophenyl) iminoflavone (3b):
Yield: 81%, Colour: dark yellow solid, m.p: 128-130°C, Molecular formula: $C_{22}H_{15}O_4N_2Br$, Molecular weight: 450.9

IR (KBr) ν max cm^{-1}:
3078.39 cm^{-1}(CH aromatic stretching), 1562.34 cm^{-1} (C=C aromatic stretching), 1604.77 cm^{-1} (C=N stretching), 1255.66 cm^{-1} (C-O stretching), 1355.96 cm^{-1} (C-NO2 sym), 1512.19 cm^{-1} (C-NO2 asym), 1024.20 cm^{-1}(C-Br stretching), 1122.57 cm^{-1}(C-O Stretching in –OCH_3), 833.25 cm^{-1},(trisubstituted), 794.67cm^{-1}(p-disubstituted), 732.95cm^{-1} (o-disubstituted)

^{1}H NMR: [δ $CDCl_3$]:
6.80-8.38 (m 11H, Ar-H), 1.68 (s, 1H, C=CH), 3.88 (s, 3H, -OCH_3)

6-Bromo-4-methoxy-4-(m-nitrophenyl) iminoflavone (3c):
Yield: 84%, Colour: dark brown solid, m.p: 130-134°C, Molecular formula: $C_{22}H_{15}O_4N_2Br$, Molecular weight: 450.9

^{1}H NMR: [δ $CDCl_3$]:
6.79-8.36 (m 11H, Ar-H), 1.81 (s, 1H, C=CH), 3.90 (s, 3H, -OCH_3)

6-Bromo-4-methoxy-4-(p-nitrophenyl) iminoflavone (3d):
Yield: 80%, Colour: dark yellow solid, m.p 130-132°C, Molecular formula: $C_{22}H_{15}O_4N_2Br$, Molecular weight: 450.9

^{1}H NMR: [δ $CDCl_3$]:
6.81-8.35 (m 11H, Ar-H), 1.74 (s, 1H, C=CH), 3.90 (s, 3H, -OCH_3)
Synthesis of 6-Bromo-4-methoxy-4-(o-aminophenol) iminoflavone (3e):
Yield: 83%, Colour: Ash colour solid, m.p: 148-150°C, Molecular formula: $C_{22}H_{16}O_3NBr$, Molecular weight: 421.9

IR (KBr) ν max cm^{-1}:
3206.76 cm^{-1} (-OH aromatic stretching), 3076.46 cm^{-1} (CH aromatic stretching), 1564.27 cm^{-1} (C=C aromatic stretching), 1656.85 cm^{-1} (C=N stretching), 1255.66 cm^{-1} (C-O stretching), 1022.27 cm^{-1} (C-Br stretching), 1217.08 & 1122.27 cm^{-1}(C-O Stretching in –OCH_3), 833.25 cm^{-1}, (trisubstituted), 815.89 cm^{-1} (p-disubstituted), 765.74 cm^{-1} (o-disubstituted).

^{1}H NMR: [δ $CDCl_3$]:
6.88-8.41 (m 11H, Ar-H), 1.81 (s, 1H, C=CH), 3.91 (s, 3H, -OCH_3)

Synthesis of 6-Bromo-4-methoxy-4-(m-aminophenol) iminoflavone (3f):
Yield: 87%, Colour: Ash colour solid, m.p: 120-122°C, Molecular formula: $C_{22}H_{16}O_3NBr$, Molecular weight: 421.9

^{1}H NMR: [δ $CDCl_3$]:
6.84-8.02 (m 11H, Ar-H), 2.28 (s, 1H, C=CH), 3.90 (s, 3H, -OCH_3)

Synthesis of 6-Bromo-4-methoxy-4-(p-aminophenol) iminoflavone (3g):
Yield: 85%, Colour: Ash colour solid, m.p: 140-145°C, Molecular formula: $C_{22}H_{16}O_3NBr$, Molecular weight: 421.9

^{1}H NMR: [δ $CDCl_3$]:
6.81-8.39 (m 11H, Ar-H), 1.99 (s, 1H, C=CH), 3.90 (s, 3H, -OCH_3)

Synthesis of 6-Bromo-4-methoxy-4-(o-methylphenyl) iminoflavone (3h):
Yield: 80%, Colour: Greenish solid, m.p: 120-124°C, Molecular formula: $C_{23}H_{18}O_2NBr$, Molecular weight: 419.9

IR (KBr) ν max cm^{-1}:
3074.53 cm^{-1} (CH aromatic stretching), 2935.66 cm^{-1} (CH Aliphatic stretching), 1562.34 cm^{-1} (C=C aromatic stretching), 1641.42 cm^{-1} (C=N stretching), 1255.66 cm^{-1} (C-O stretching), 1024.20 cm^{-1} (C-Br stretching), 1217.08 & 1122.57 cm^{-1}(C-O Sretching in –OCH_3), 833.25 cm^{-1}, (trisubstituted), 817.82 cm^{-1} (p-disubstituted), 765.74 cm^{-1}(o-disubstituted).

^{1}H NMR: [δ $CDCl_3$]:
6.84-8.39 (m 11H, Ar-H), 2.40 (s, 1H, C=CH), 3.91 (s, 3H, -OCH_3), 1.25 (d, 3-H, -CH_3)

Synthesis of 6-Bromo-4-methoxy-4-(p-methylphenyl) iminoflavone (3i):
Yield: 79%, Colour: Yellow solid, m.p: 126-128°C, Molecular formula: $C_{23}H_{18}O_2NBr$, Molecular weight: 419.9

^{1}H NMR: [δ $CDCl_3$]:
6.85-7.94 (m 11H, Ar-H), 1.93 (s, 1H, C=CH), 3.88 (s, 3H, -OCH_3), 1.26 (d, 3-H, -CH_3)

Biological Evaluation

Anti-bacterial activity of 3a-i:

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 100 ml of sample in 10 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-i:

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 ^{0}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.

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

Where, X = Area of colony in control plate.
Y = Area of colony in test plate

Table-.1: Antibacterial activity of compounds 3a-i

Compound No.	Zone of inhibition (in mm)			
	Gram +ve		Gram -ve	
	S. aureus	***S. pyrogenes***	***E. coli***	***P. aeruginosa***
3a	17	19	17	19
3b	17	17	17	18
3c	16	18	16	17
3d	16	17	14	15
3e	17	18	16	21
3f	18	18	16	18
3g	16	16	18	16
3h	18	19	21	15
3i	17	17	19	17
Amplicilline	**18**	**19**	**20**	**20**
Chloramphenicol	**21**	**20**	**23**	**21**
Ciprofloxacin	**22**	**22**	**28**	**27**
Norfloxacin	**28**	**21**	**29**	**23**

Table -2: Antifungal activity of compounds 3a-i

Compound No.	*C. albicans*	*A. clavatus*
3a	23	22
3b	21	22
3c	22	20
3d	22	19
3e	22	21
3f	23	21
3g	19	21
3h	21	22
3i	21	23
Greseofulvin	**24**	**24**
Nystatin	**26**	**26**

RESULTS AND DISSCUTION

In the present work we have decided to carry out the synthesis of Flavoneimines on refluxing 2-hydroxy-5-bromo-4-methoxy-N-(substituted)- chalconeimine in DMSO-I_2.This method quicker and appears to be of general applicability. The structures were established on the basis of spectral data (IR, NMR and Mass). All newly synthesized compounds 3a-i shown significant microbial activites.

Table 1 of antibacterial activity show that the compound 3a, 3c, 3d 3e, 3h more active in S. pyrogenes compare to S. aureus while 3b, 3f, 3g, and 3i having same activity in both in Gram +ve while 3a, 3b, 3c, 3d, 3e, 3f more active in P. aeruginosa compare to E-coli while 3g, 3h, 3i more active in E-coli compare to P. aeruginosa in Gram –ve. Table 2 of antifungal activity show that the compound 3b, 3g, 3h, 3i more active in A. clavatus compare to C. albicans while 3a, 3c, 3d, 3e, 3f, more active in C. aibicans compare to A. clavatus.

CONCLUSION

Newly synthesized iminoflavone 3a-i have been tested for their anti bacterial activity against gram positive bacteria S. aureus and S. pyrogenes while gram negative bacteria E. coli and P. aeruginosa. 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 100 ml of sample in 10 ml DMF. Amplicilline, Chloramphenicol, Ciprofloxacin Norfloxacin Greseofulvin and Nystatin were used as a reference compound. The entire compound shown good activity against gram positive and gram negative bacteria. Same compounds were tested for their anti fungal activity against A. clavatus and C. albicans using cup plate method. The compound 3c, 3d, 3g show moderate activity while 3a, 3b, 3e, and 3f, 3h,3i show good anti fungal activity.

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