



## Eco-friendly Syntheses of Bioactive Iodoflavone from Chalcone under Microwave Condition for the Screening of Their Antibacterial Activity

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### Abstract

Flavonoids possess potent biological activity due to the presence of heterocyclic ring moiety and have been established as one of the biologically important scaffolds. Iodinated-flavones were synthesized from corresponding chalcones. Both conventional and microwave techniques were used to synthesize these compounds to make a comparative study. A significant reduction in reaction time and increase in % yield has been observed in the microwave technique. Spectroscopic techniques such as UV, IR, and NMR have been used to confirm the structures of all compounds. Agar disk diffusion method was employed to evaluate the antibacterial activities of the newly synthesized compounds against some bacterial strains such as *Salmonella typhi*, *Streptococcus spp*, *Shigella spp*. etc. Iodoflavone was found to be higher potency against most the bacteria, while others were moderately active.

Received: 17 January 2021

Revised: 14 June 2022

Accepted: 10 July 2022

DOI: <https://doi.org/10.3329/JSciTR.v3i1.62808>

**Keywords:** Iodoflavones; Chalcone; Flavonoid; Microwave Irradiation; Antibacterial activity

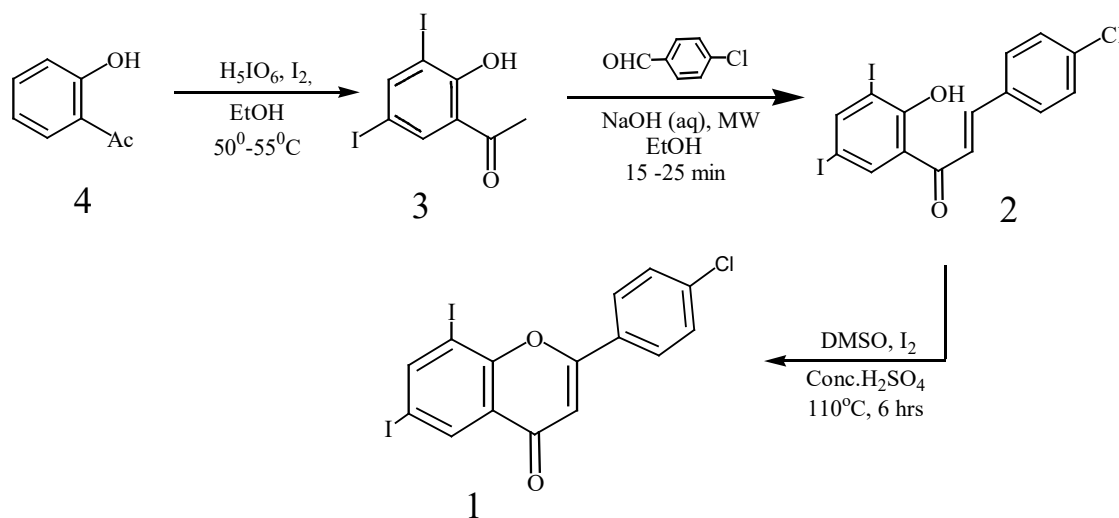
### Introduction

Flavones are dietary supplement produced almost exclusively by the Leguminosae or bean family. The hetero-aromatic component having coumarine nucleus with additional aromatic moiety gives a nice structural pattern for flavonoid molecules. These phytochemicals have enormous biological properties. (Hossain *et al.*, 2006). A distinctive feature of the prenylated polyphenoles is the possession of biological activity such as insecticidal, anti-fungal, antioxidant, and anticancer properties. Prenyl- as well as soyisoflavones also has potential for the complementary and/or alternative chemoprevention therapies against long-term health problems associated with menopause (Tsukayama *et al.*, 2007; Hossain *et al.*, 2006). Due to their diversified activities flavones have been receiving considerable attention in the fields of medicine, food supplements, agrochemicals (Varady, 1965) and cosmetics in recent years (Dewic, 1992; Tsukayama *et al.*, 2004; Moriarty *et al.*, 1998). Soy isoflavones, mainly including daidzein and genistein, together with their respective glycosidic conjugates of daidzin and genistin, have received considerable attention for their potential role in reducing the risk of head and neck, lung, breast and prostate cancers.

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For example, Kato *et al.* reported that both genistin and daidzin possess anticancer effects at relatively early stages of prostate cancer development. Several studies indicated that genistein exerted antiproliferative influences on prostate cancer through the suppression of telomerase activity, which represses telomerase activity in prostate cancer cells not only by repressing hTERT transcriptional activity but also by posttranslational modification of hTERT via Akt (Micki *et al.*, 1998; Varvoglis, 2002; Martin-Cordero *et al.*, 2000)

In the recent years, several natural polyphenolics were shown to exhibit high activity against Gram-positive and Gram-negative bacteria, suggesting that a suitably substituted coumarine ring might perhaps be the minimum requirement for biological activity. In view of high activity of such derivatives, synthesis of suitably prenylflavones is an ongoing challenge and continues to attract the attention of the synthetic chemistry community. Accordingly, well-documented methods of synthesis molecules were adopted. A plethora of new compounds have been synthesized along with reported compounds for the purpose of screening of antimicrobial activity. In this study, iodoflavone was synthesized using synthetic route which exhibited better antimicrobial activity compared to precursors.



**Scheme 1: Synthesis of iodoflavone**

## Materials and methods

Synthesis of 2'-hydroxy-3', 5'-diiodoacetophenone (**3**) (Hossain *et al.* 2006).

### *Conventional heating (CH) method*

2-Hydroxyacetophenone (1.48 g, 10.88 mmol) was dissolved in EtOH (12 mL) followed by the successive addition of I<sub>2</sub> (2 g, 7.88 mmol) and orthoperiodic acid (0.82 g, 4.02mmol in 7 mL water). The reaction mixture was stirred at 55° to 60 °C temperature for about 6 hours. The

completion of the reaction was monitored using thin layer chromatography (TLC) (EtOAc: n-hexane = 1:5). Cooling and diluting the reaction mixture with H<sub>2</sub>O gave a powdered solid. The crude mass was purified by recrystallization from EtOH. Finally, the titled compound was obtained as pale yellow crystalline needles.

#### *Microwave irradiation (MWI) method*

2-Hydroxyacetophenone (1.48 g, 10.88mmol) was dissolved in EtOH (8mL) in a two necked round bottomed flask followed by the successive addition of I<sub>2</sub> (2 g, 7.88mmol) and orthoperiodic acid (0.82 g, 4.02 mmol in 7 mL water). Then the mixture was irradiated under MW for 8 min (5 sec × 32 times, 1min interval/irradiation). The reaction was monitored using the same mobile phase as the CH method in TLC (EtOAc: n-hexane = 1:5). Cooling and diluting the reaction mixture with H<sub>2</sub>O gave a powdered solid. The crude mass was purified by recrystallization from EtOH. Finally, the titled compound was obtained as pale yellow crystalline needles.

**Table 1. Comparative data of CH and MWI method**

Method	Time	Solvent (mL)	%Yield
MWI	8 min	8	78
CH	6 h	12	65

Melting point: 120 – 121 °C

UV λ max (log ε) EtOAc: 365.5 nm (1.145)

IR: KBr (cm<sup>-1</sup>): 3442 (w, -OH str.), 3040(s, Ar C-H, str.), 1571(s, Ar C=C, str.), and 1647 (s, C=O, str.), <sup>1</sup>H-NMR in CDCl<sub>3</sub>; δ(ppm): 13.10 (s, 1H, ArOH) 8.23-8.22 (d, 1H, H-4, J<sub>o</sub>= 2Hz), 8.02-8.01(d, 1H, H-6, J<sub>m</sub>= 2Hz) and 2.67 (s, 3H, COCH<sub>3</sub>).

*Synthesis of 1-(2'-hydroxy-3', 5'-diiodophenyl)-3-(4''-chlorophenyl)-propenone (2)*(Hossain et al. 2006).

#### *MWI method*

The mixture of 4'-chlorobenzaldehyde (0.4215 g, 3 mmol) and 2'-hydroxy-3', 5'-diiodoacetophenone (1.634 g, 3 mmol) was dissolved in alc. NaOH (0.35 g, 7 mmol in 60 mL EtOH). Then the mixture was irradiated under MW for 12 min (10 sec × 72 times, 1 min interval/irradiation). The reaction was monitored using the same mobile phase as the CH method in TLC (EtOAc: n-hexane = 1:5). After completion of the reaction, the mixture was neutralized with a 5% dil. HCl and extracted with EtOAc. The solvent was removed under reduced pressure to give a semi-solid mass which was recrystallized from EtOH.

**Table 2. Comparative data of CH and MWI method**

Method	Time	Solvent (mL)	%Yield
MWI	12 min	60	82
CH	60 h	180	71

Melting point: 184 – 185 °C

UV  $\lambda$  max (log  $\epsilon$ ) EtOAc: 336 nm (1.123)

IR: KBr ( $\text{cm}^{-1}$ ): 3404 (w, -OH, str.) 2997 (w, ArC-H, str.), 2933 (w, olefinic C-H, str.) 1691 (s, C=O, str.), 1635 (s, C=C, str.) and 1570 (s, Ar C C ring, str.)

$^1\text{H-NMR}$  in  $\text{CDCl}_3$ ;  $\delta$ (ppm): 13.70 (s, 1H, ArOH), 8.26 – 8.25 (d, 1H, H-4',  $J_m = 1.6\text{Hz}$ ), 8.16-8.16 (d, 1H, H-6',  $J_m = 1.6\text{Hz}$ ) 8.96-7.92 (d, 1H, H-2,  $J_{trans} = 15.2\text{Hz}$ ), 7.55-7.51 (d, 1H, H-3,  $J_{trans} = 15.2\text{Hz}$ ), 7.65-7.63 (d, 1H, H-3"/H-5",  $J_o = 8.4\text{Hz}$ ) and 7.47-7.45 (d, 1H, H-2"/H-6",  $J_o = 8.4\text{Hz}$ ).

The H-2 and H-3 protons of the titled chalcone 2 appeared as doublets ( $J_{trans} = 15.2\text{ Hz}$ ) in the ranges 8.96-7.92 ppm (H-2) and 7.55-7.51 ppm (H-3), respectively, which indicated the formation of  $\alpha$ ,  $\beta$ -unsaturated ketone in the chalcone molecule (2). The presence of doublet at 7.65-7.63 ppm corresponds to the two equivalent protons (H-3"/H-5") while the doublet at 7.47-7.45 ppm assign to two equivalent protons (H-2"/H-6") of the compound 2. These characteristic peaks were not observed in the case of the precursor compound 3.

*Synthesis of 2-(4'-chlorophenyl)(chromen-6,8-diiodo)-4-one (1)* (Hossain *et al.* 2006).

#### MWI method

The compound 2 (0.6 g, 1.18 mmol) was dissolved in 15 mL DMSO and was taken in a two necked round bottom flask. To this solution 2-3 drops of conc.  $\text{H}_2\text{SO}_4$  was added and then it was stirred for 5-10min. After that, a small crystal of  $\text{I}_2$  was added carefully to it. Then the mixture was irradiated under MW for 10 min (10-sec  $\times$  60 times, 1min interval/irradiation). The reaction was monitored using the same mobile phase as the CH method in TLC (EtOAc: n-hexane = 1:3) and extracted with EtOAc. The organic layer was washed with 20% NaOH and water which was dried over anhydrous  $\text{Na}_2\text{SO}_4$ . The crude mass obtained was purified by recrystallization from EtOH. As a result, an off-white colored fluffy crystal was obtained.

#### Comparative data of CH and MWI method

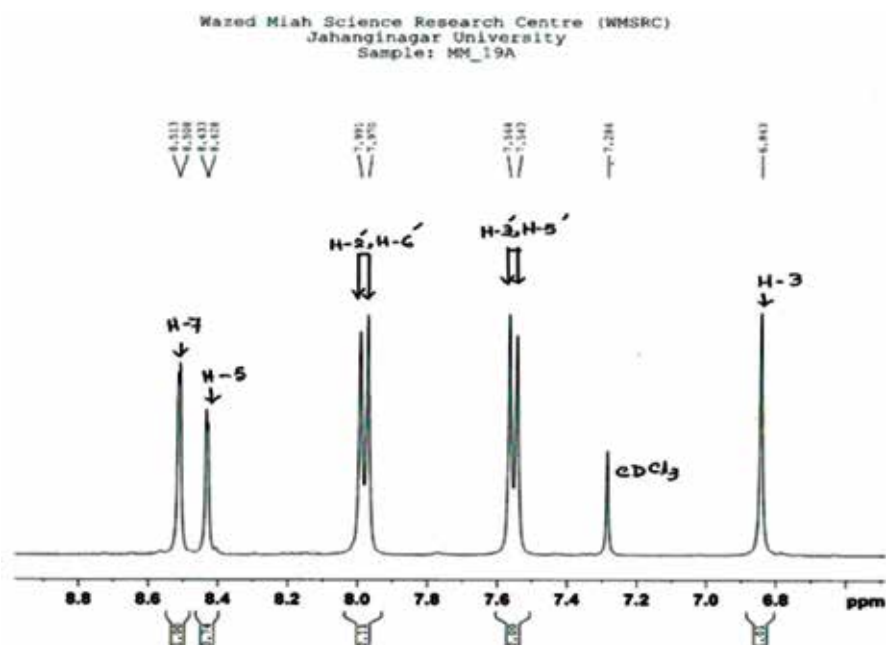
Method	Time	Solvent (mL)	%Yield
MWI	10 min	15	74
CH	6 h	20	69

Melting point: 238 – 239 °C

UV  $\lambda$  max (log  $\epsilon$ ) EtOAc: 271 nm (1.877)

IR: KBr ( $\text{cm}^{-1}$ ): 3057 (s, ArC-H, str.), 2939 (s, olefinic C-H, str.) 1645 (s, C=O, str.), 1600 (s, C=C, str.) and 1543 (s, Ar C C ring, str.)

$^1\text{H-NMR}$  in  $\text{CDCl}_3$ ;  $\delta$ (ppm): 6.84 (s, 1H, H-3) 8.51-8.50 (d, 1H, H-7,  $J_m=2\text{Hz}$ ), 8.43-8.42 (d, 1H, H-5,  $J_m=2\text{Hz}$ ), 7.99-7.97 (d, 1H, H-2'/ H-6',  $J_o=8.4\text{Hz}$ ), and 7.564-7.543 (d, 1H,  $J_o=8.4\text{Hz}$ ).



**Fig. 1.** The expanded  $^1\text{H-NMR}$  spectrum of iodoflavone 1

The absence of characteristic peak at 13.70 ppm for phenolic type proton, Ar-OH and 7.55-7.51 ppm for proton (which is H-3 in the compound) designated the removal of these protons and formation of C-O bond in the carbonyl groups containing ring in iodoflavone (compound 1)

#### *Microbial Assay (Antibacterial Screening)*

Antibacterial susceptibility test was employed used to evaluate the efficacy of antimicrobials like the synthesized compounds against a number of microorganisms (Panda, 2012). The synthesized compounds were screened for their potency against a number of bacterial strains such as *Salmonella typhi*, *Streptococcus* spp. *Shigella* spp. etc. according to Agar disk diffusion method described previously.<sup>11</sup> The method is described here in brief. It is also known zone of inhibition method which is the most widely used method because of its simplicity and low cost. The method involves the preparation of a Petridish containing certain volume of agar, a known concentration of bacteria are then spread across the agar surface and allowed to establish. A paper disk (6 or 8 mm) containing a known volume of the test

substance is then placed in the centre of the agar and the dish incubated for 24 h or more period of time. At this time “cleared” zone (zone of inhibition) surrounding the disk is measured and compared with zones for standard antibiotics or literature values of isolated phytochemicals or similar extracts. The size of the zone is dependent on the rate of diffusion and growth of the applied organism.

## Results and discussion

The antibacterial screening was conducted for the all the precursors and the final compound named iodoflavone. Table 4 shows the diameter of the zone of inhibition (in mm) produced by the synthesized compounds against certain bacteria. The zone of inhibition for the standard (Cip-5) was between 21 to 23 mm while for iodoflavone was between 11 to 8 mm. the different values obtained for iodoflavone were due to various concentrations. Actually the higher concentration (1.0 mg/mL) showed better potency against *salmonella paratyphi*. Besides, statistical data of the compound **1** against different bacterial strain exhibits the highest potency among all precursors, which showed moderate activity at the said concentrations. The solvent DMSO exhibited no antimicrobial activity against these bacteria which demonstrated that the measurement of zone of inhibition of iodoflavone was unaffected by the solvent.

**Table 4. The diameter of the zone of inhibition (in mm) produced by the synthesized iodoflavone. 1.**

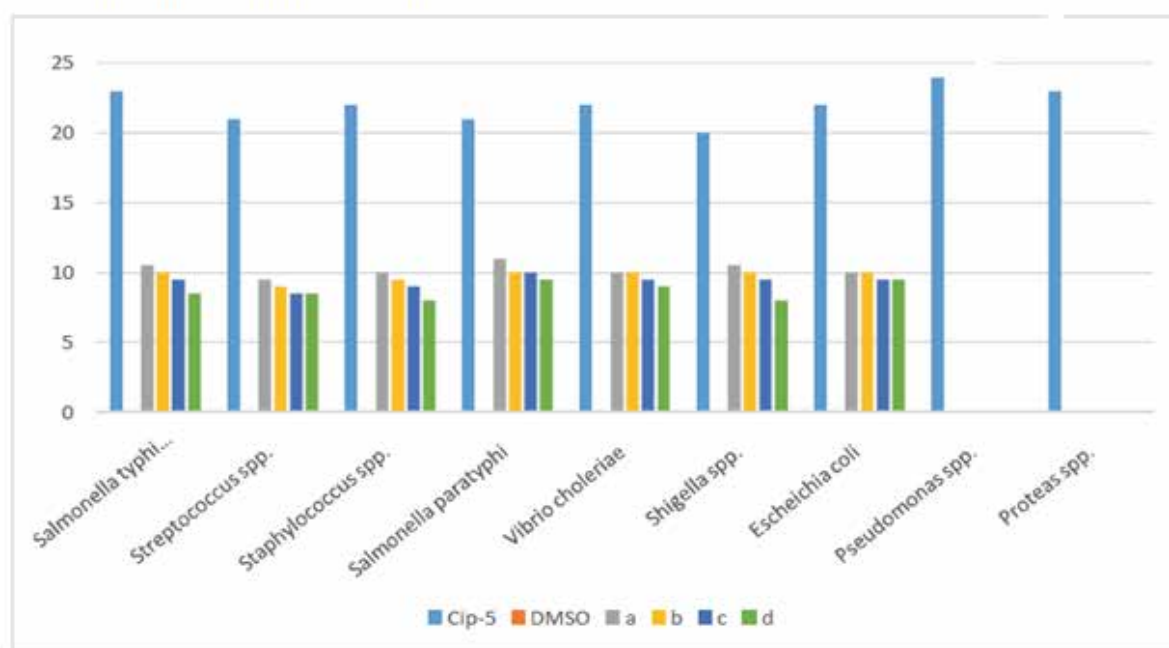
Statistical data for the zone of inhibition due to <b>1</b>									
Zone of inhibition in mm in diameter									
Sample ID	<i>Salmonella typhi</i>	<i>Streptococcus spp.</i>	<i>Staphylococcus spp.</i>	<i>Salmonella paratyphi</i>	<i>Vibrio cholerae</i>	<i>Shigella spp.</i>	<i>Escheichia coli</i>	<i>Pseudomonas spp.</i>	<i>Proteus spp.</i>
Cip-5	23	21	22	21	22	20	22	24	23
DMSO	NA	NA	NA	NA	NA	NA	NA	NA	NA
a	10.5	9.5	10	11	10	10.5	10	NA	NA
b	10	9	9.5	10	10	10	10	NA	NA
c	9.5	8.5	9.0	10	9.5	9.5	9.5	NA	NA
d	8.5	8.5	8	9.5	9	8	9.5	NA	NA

The bar graph showing the diameter of the zone of inhibition (in mm) produced by the synthesized iodoflavone. 1. Against certain bacterial strains is delineated in fig. 2. As per chemical structural features there were mainly two different types of compounds synthesized under the study area. It is obvious that structural variation brings about the bioactivity and, of course, structural modification of molecules alters the biological activity in a regular trend. Without exception this has been reflected in the case of the synthesized precursors of varying their structural moieties. Accordingly, coumarine part of the final

compound has the vital heterocyclic moiety so that it probably plays the vital role being work as the active site for the better potency. Panda et al. has investigated the antimicrobial activities of different plant parts of *Vitex negundo* L. at different solvent extracts. All different solvent extracts exhibited mark activity with zone of inhibitions 9.6-15.6 mm for bark and 9.3-14.3 mm for leaf (Panda *et al.* 2011). The present study showed comparable activity with that study.

**Fig. 2. The bar graph showing the diameter of the zone of inhibition (in mm) produced by the synthesized iodoflavone 1 (in different concentrations) against certain bacteria.**

The result can be manifested graphically as -



Cip-5: Ciprofloxacin (oral antibiotic) as a standard And  
'a', 'b', 'c', 'd' denote the different concentrations of the test sample.

## Conclusion

This study reported the antibacterial activity of iodoflavone at various concentrations and the precursors. The target molecule showed marked potency against almost all the bacterial strains as expected. Therefore, the strategies in designing and synthesis of iodo-based flavones to facilitate the incorporation of prenyl- or alkynyl- group and other related compounds and the consequent *in vivo* screening of those should be the main objective of further investigations. This would lead to the synthesis of short listed target molecules of the active metabolites with potent antibacterial activity.



## Acknowledgement

The authors are grateful to The Ministry of Science and Technology, The People's Republic of Bangladesh for providing the funding. The authors are also cordially thankful to Wazed Miah Science Research Center, Jahangirnagar University for providing all spectral support.

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