SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF CHALCONE DERIVATIVES OF INDOLE NUCLEUS

R. Chauhan[2*], J. Dwivedi[3], A. A. Siddiqi Anees[1], and D. Kishore[2]

A series of previously unreported (2*Z*)-2-(1H-indol-1-yl)-3-(4-substituted phenyl)-1-phenylprop-2-en-1-one **(5a – d)** have been synthesized from easily accessible 2-(1H-indol-1-yl)-1-phenylethanone **(3),** which was obtained via a reaction of indole **(1)** with chloromethylphenyl ketone **(2)**. The structures of the synthesized products have been elucidated using IR, ^{1}H NMR, and mass-spectroscopic data and elemental analyses. The final products were screened for their antimicrobial activity. Excellent results were obtained against both bacteria and fungi. In conclusion, we have developed a novel, convenient and simple method for the preparation of indole – chalcone hybrid compounds via the reaction of indole derivative with carbonyl compounds in the presence of a strong base. The rapid conversion, excellent yield, utilization of a base, and operational simplicity are great advantages of the proposed method.

Key words: Mannich reaction, chalcone, indole derivatives, antimicrobial activity

INTRODUCTION

It is well known that the indole nucleus is associated with a large number of pharmacological properties including antibacterial [1, 2], anticancer [1, 2], antibiotic [3], central nervous system modulating [4], etc. By the same token, chalcone is an aromatic ketone that forms the central core for a variety of important biologically active compounds known as chalcones. They show antibacterial, antifungal, antitumor, and anti-inflammatory properties. Some chalcones demonstrated the ability to block voltage-dependent potassium channels [5].

There are several methods for synthesis of chalcone derivatives of indole [6, 7]. Indole can be substituted at nitrogen so as to obtain its salts. Indole is a week acid (pKa = 16.97) of comparable strength with respect to pyrrole and aliphatic alcohols. It can be converted into the N-sodio derivative by reaction with sodamide in liquid ammonia or by reaction with sodium hydride in an organic solvent. Salts of other metals can be made by using appropriate strong bases, such as potassium t-butoxide, Grignard reagent, and butyllithium.

The N-metalloid indoles are ambient nucleophiles and can react with electrophiles either at nitrogen or at C-3. The more ionic sodium and potassium salt tend to react preferentially at nitrogen, particularly with hard electrophiles. Substitution at nitrogen is also favored by the use of dipolar aprotic solvents [8]. Thus, the sodium salt of indole can be N-substituted by chloroacetophenone. In this way, 2-(1H-indol-1-yl)-1-phenylethanone (compound **3**) was prepared. This compound was used to synthesize desired chalcones, i.e. (2*Z*)-2-(1H-indol-1-yl)-3-(4-substituted)-1-phenylprop-2-en-1-ones **5a – 5d** (Scheme 1), by the Claisen – Schmidt condensation [9–12] of 2(1H-indol-1-yl)-1-phenylethanone **3** and 4-substituted benzaldehydes (**4a – 4d**). The goal of this study was to combine the two main structures, i.e. indole and chalcone, so as to obtain a hybrid molecule.

Indole was reacted with 1-phenyl-2-chloroacetophenone in the presence of sodium hydride which is a strong base. Dimethylformamide was used as a solvent, as it provides a better medium for the reaction. The reaction was allowed to proceed on stirring for 10 – 12 h at room temperature to yield desired indolyl acetophenone (compound **3**). This was reacted with 4-substituted benzaldehyde by stirring at room temperature, followed by the Claisen – Schmidt condensations in the presence of sodium hydroxide as a strong base. Finally, we obtained (2*Z*)-2-(1-H-indol-1-yl)-3-(4-methylphenyl)-1-phenylprop-2-en-1-ones (compounds **5a – 5d**).

The reactants were stirred for 8 – 10 h. (Scheme 1). This result clearly indicates the scope of the reaction of indolyl acetophenone (compound **3**) with respect to different aldehydes and ketones. The structures of the products were established from IR, ^{1}H NMR, and mass-spectroscopic data.

EXPERIMENTAL CHEMICAL PART

Melting points were measured in Buchi Model 510 apparatus and are uncorrected. The IR spectra were recorded on a Shimadzu 8400S FT-IR spectrophotometer, ^{1}H NMR spectra were measured on a Bruker 300-MHz Spectrophotometer us-

[1] Department of Pharmacy, Banasthali University, Tonk, Rajasthan, India.
[2] Department of Chemistry, Banasthali University, Tonk, Rajasthan, India.
[3] Department of Pharmacy, Jamia Hamdard University, New Delhi, India
[*] e-mail: rajanichauhan pharma@yahoo.com.

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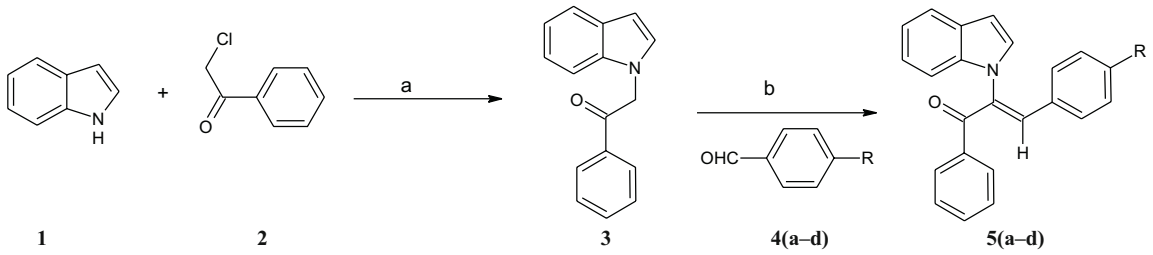


Scheme 1: (a) base NaH, DMF; (b) NaOH, ethanol, R = $–OCH_3$, $–NO_2$, –Cl, –Br.

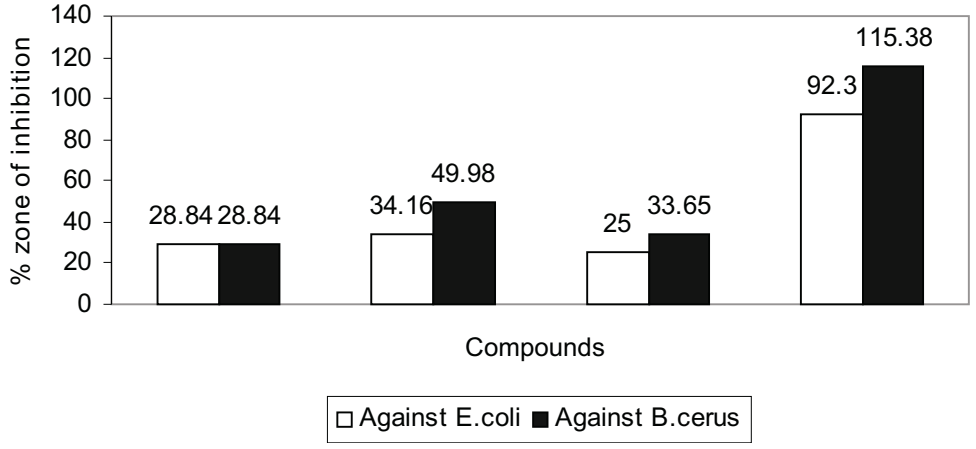


Fig. 1. Comparison of percentage zone of inhibition against bacteria.

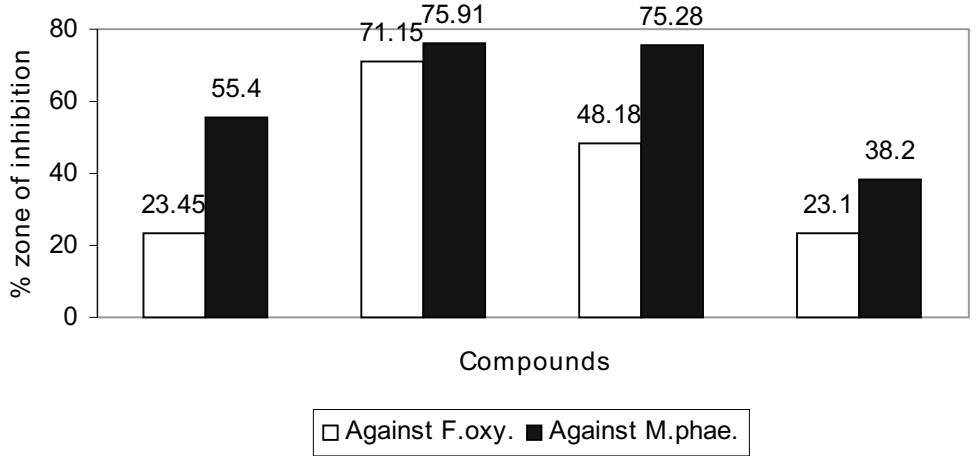
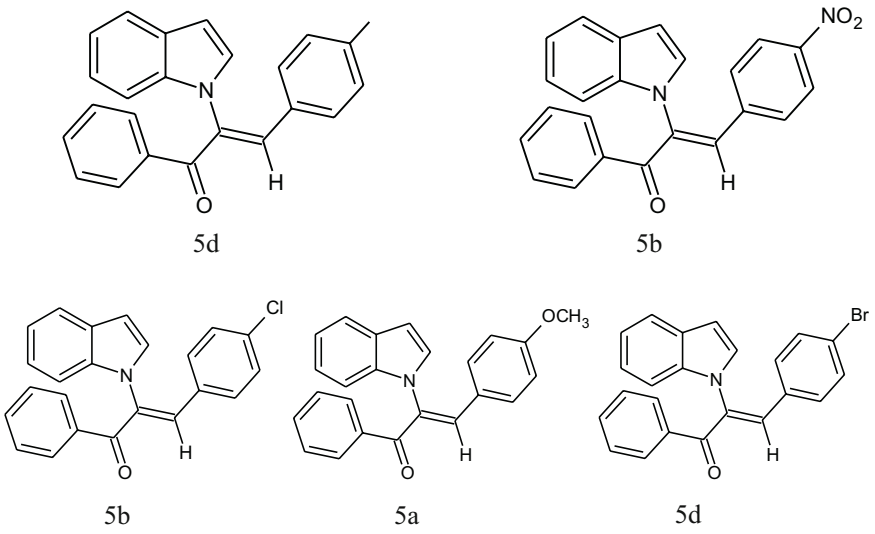


Fig. 2. Comparison of percentage zone of inhibition against fungi.

ing TMS as an internal standard, and the mass spectra were obtained in Autospec EI instrument. Column chromatography was performed with silica gel (100 – 200 mesh) and TLC, with silica gel GF_{254}. Elemental analyses were carried out on a Heraeus CNH rapid analyzer. Analytical results are presented below and in Table 1.

2-(1H-Indol-1-yl)-1-phenylethanone (3). To a solution of indole **1** (1.0g, 0.010 mole) in DMF (30 ml) were added chloromethylphenyl ketone **2** (2.31g, .015 mole) and sodium hydride as a strong base. The mixture was stirred at room temperature for 10 – 12 h. The target compound was crystallized by pouring the reaction mixture into ice-cold water and standing for 24 h. Crystals were separated by filtration and recrystallized from suitable solvent: yield, 70%; m.p., 213°C; IR spectrum in KBr (ν_{max}, cm^{-1}): 1700 (keto, CO), 1240 (C-N), 780 – 670 (=C–C arom.), 1575(C=C stre),



5d 5b 5b 5a 5d

Scheme 2: Structures of compounds **5a – 5d**

1460(C-H, CH_3); ^{1}H NMR spectrum in $CDCl_3$/TMS (δ, ppm): 7.17 (s, 5H, CH indole ring, J = 0.125), 6.978 – 7.86 (s, CH, aryl, J = 0.050 & 0.450), 5.11 (s, CH_2); mass spectrum (*m*/*z*): 235 (M^++1), 130, 128, 105, 85.

(2*Z*)-2-(1H-Indol-1-yl)-3-(4-methoxyphenyl)-1-phenyl prop-2-en-1-one (5a). To a solution of 2-(1H-indol-1-yl)-1-phenylethanone **3** (3.55g, 0.010 mole) in ethanol (30 ml) was added p-methoxybenzaldehyde **4a** (1.36g, 0.010 mole) and sodium hydroxide as a base and the mixture was stirred for about 8 h. The target compound was crystallized by pouring the reaction mixture into ice-cold water and standing for 24 h. Crystals were separated by filtration and recrystallized from suitable solvent: yield, 70%, m.p., 213°C; IR spectrum in KBr (ν_{max}, cm^{-1}): 1680 (keto, C=O), 1600 (conj. C=C), 1360 (ter. C-N), 1240 (O-CH_3), 1444 (C-H, CH_3); ^{1}H NMR spectrum in $CDCl_3$/TMS (δ, ppm): 7.3 (s, CH indole ring, J = 0.525), 7.45 – 7.81 (s, CH aryl, J = 0.550), 7.9 (s, aryl CH=CH), 3.73 (s, 3H, OCH_3); mass spectrum (*m*/*z*): 353 (M^++1), 322, 276, 246, 236, 237, 233, 221.

TABLE 1. Physical and analytical data for compounds 3 and 5a – 5d

No.	Compound No.	Molecular formula	M. W.	M.p. (°C)	Yield (%)	Elemental analysis (Calcd/ Found) N/Cl/Br
1.	**3**	$C_{16}H_{13}NO$	235	213	70	5.95/5.60
2	**5a**	$C_{24}H_{19}NO_2$	353	360	66	3.96/3.96
3	**5b**	$C_{23}H_{16}N_2O_3$	368	82	64	7.60/7.62
4	**5c**	$C_{23}H_{16}NOCl$	358	270	63	3.91/3.88, 9.91/9.63
5	**5d**	$C_{23}H_{16}NOBr$	402	121	66	3.48/3.50, 19.86/19.92

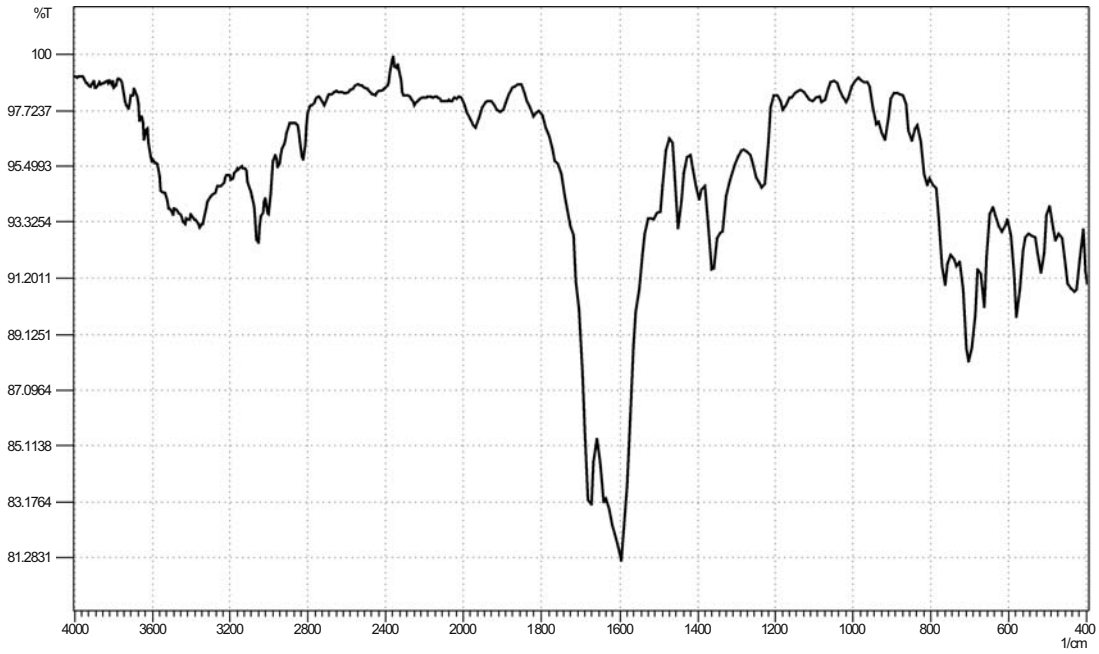
(2*Z*)-2-(1H-indol-1-yl)-3-(4-nitrophenyl)-1-phenylpro p-2-en-1-one (5b): To a solution of 2-(1H-indol-1-yl)-1-phenyl ethanone **3** (3.55g, 0.010 mole) in ethanol (30 ml) was added p-nitrobenzaldehyde **4b** (1.36g, 0.010 mole) and sodium hydroxide as a base and the mixture was stirred for about 8 h. The target compound was crystallized by pouring the reaction mixture into ice-cold water and standing for 24 h. Crystals were separated by filtration and recrystallized from suitable solvent: yield, 70%; m.p. 213°C; IR spectrum in KBr (ν_{max}, cm^{-1}): 1760 (keto, CO), 1600 (conj. C=C), 1340 (ter. C-N), 1350 (sym nitro); ^{1}H NMR spectrum in $CDCl_3$/TMS (δ, ppm): 7.3 (s, CH indole ring, J = 0.525), 7.45 – 7.81 (s, CH aryl, J = 0.550), 7.9(s, aryl CH=CH); mass spectrum (*m*/*z*): 357 (M^++1), 103, 122, 135, 85.

(2*Z*)-2-(1H-Indol-1-yl)-3-(4-clorophenyl)-1-phenylpro p-2-en-1-one (5c). To a solution of 2-(1H-indol-1-yl)-1-phenylethanone **3** (3.55g, 0.010 mole) in ethanol (30 ml) was

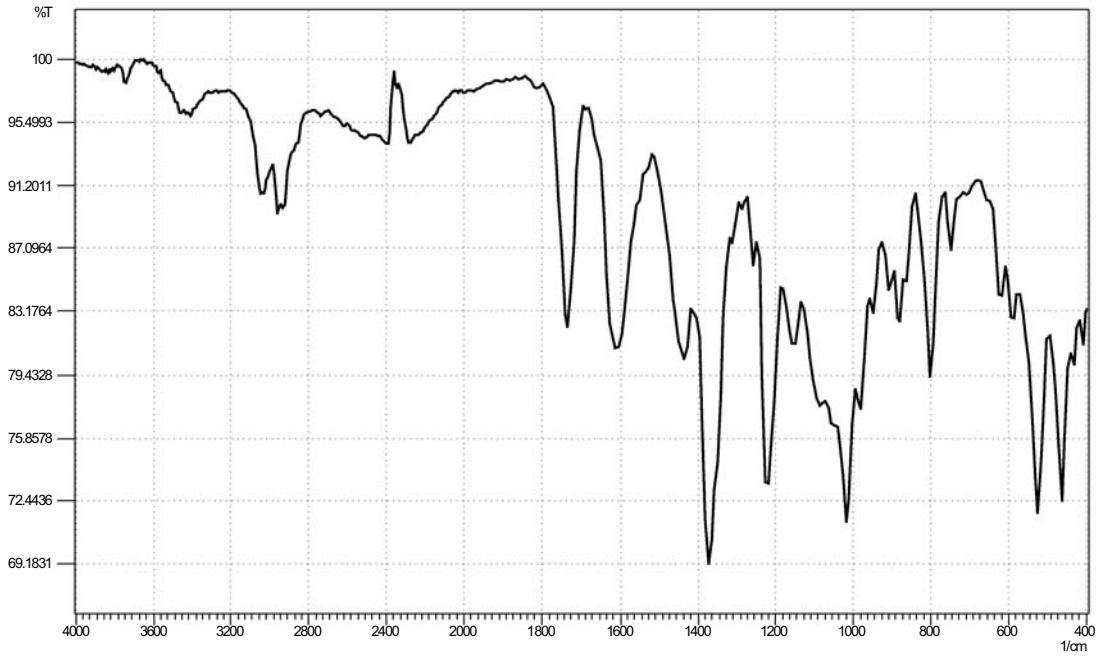
TABLE 2. Antibacterial activity of compounds 5a – 5d

Compound no.	Conc. (μg/disk)	*E.coli*		B. cereus	
		Zone of inhibition (mm)(test) (% activity)*	Zone of inhibition (mm), (std)	Zone of inhibition (mm) (test) (% activity)*	Zone of inhibition (mm), (std)
5a	166.66	7.5 (28.84)	26.00	7.5 (28.84)	26.00
5	166.66	9.00 (34.16)	26.00	12.66 (49.98)	25.33
5c	166.66	6.5 (25.00)	26.00	8.75 (33.65)	26.00
5d	166.66	24.00 (92.30)	26.00	30.00 (115.38)	26.00

* Data in brackets refer to percentage activity



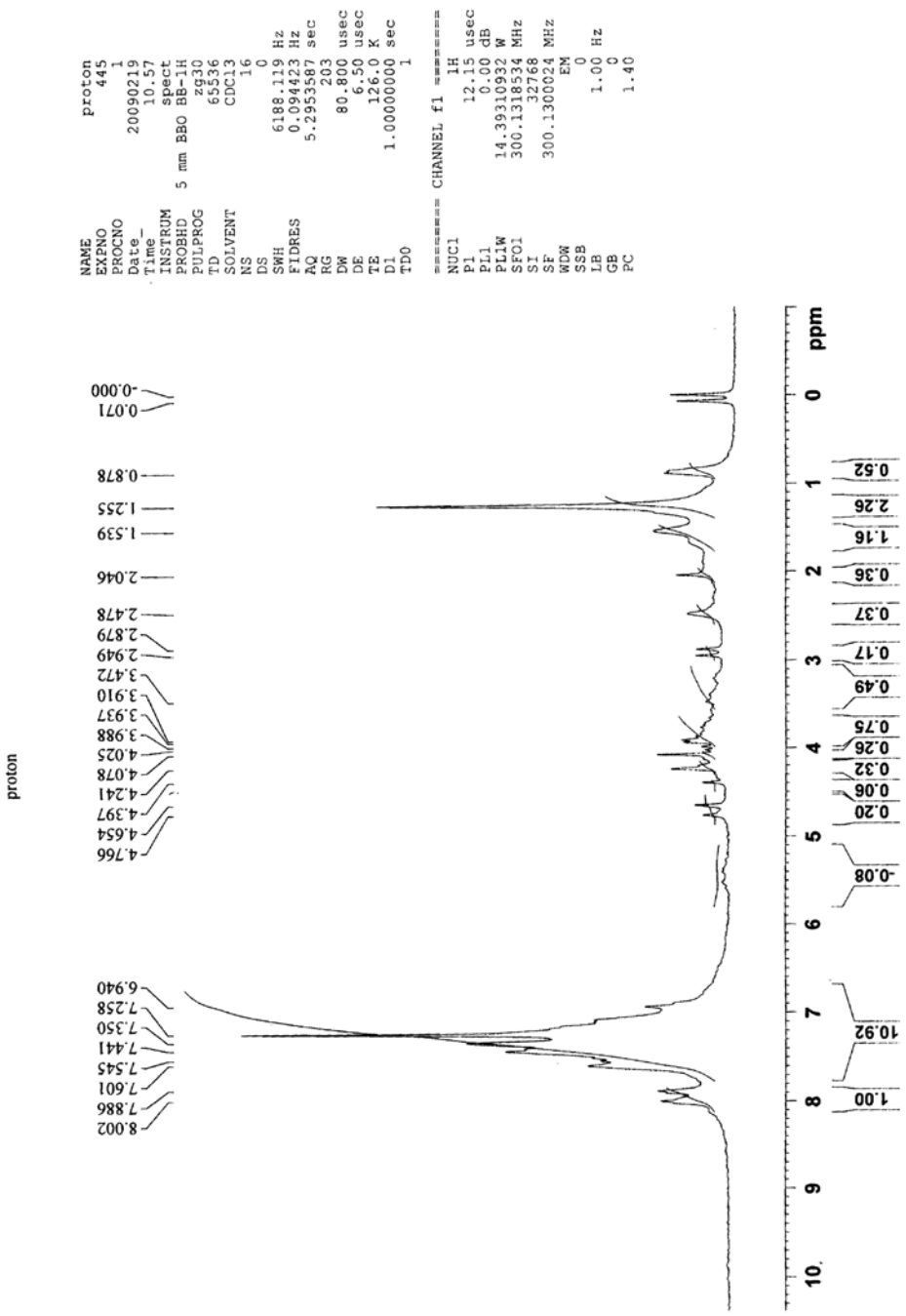
Spectrum 1: (2Z)-2-(1-H-indole-yl)-3-(4-methoxyphenyl)-1-phenylprop-2-en-1-one **(5a)**



Spectrum 2: (2Z)-2-(1-H-indole-yl)-3-(4-nitrophenyl)-1-phenylprop-2-en-1-one **(5b)**

added p-chlorobenzaldehyde **4c** (1.36g, 0.010 mole) and sodium hydroxide as a base and the mixture was stirred for about 8 h. The target compound was crystallized by pouring the reaction mixture into ice-cold water and standing for 24 h. Crystals were separated by filtration and recrystallized from suitable solvent: yield. 70%; m.p., 213°C; IR spectrum in KBr (ν_{max}, cm^{-1}): 1750 (keto, CO), 1600 (conj. C=C), 3220 (N-H), 785 (C-Cl); ^{1}H NMR spectrum in $CDCl_3$/TMS (δ, ppm): 7.3 (s, CH indole ring, J = 0.525), 7.45 – 7.81 (s, CH aryl, J = 0.550), 7.9 (s, aryl CH=CH); mass spectrum (*m*/*z*): 355.5 (M^++1), 244, 231, 239, 278.5, 219, 88.

(2*Z*)-2-(1H-Indol-1-yl)-3-(4-bromophenyl)-1-phenylprop-2-en-1-one (5d). To a solution of 2(1H-indol-1-yl)-1-phenylethanone **3** (3.55g, 0.010 mole) in ethanol (30 ml)

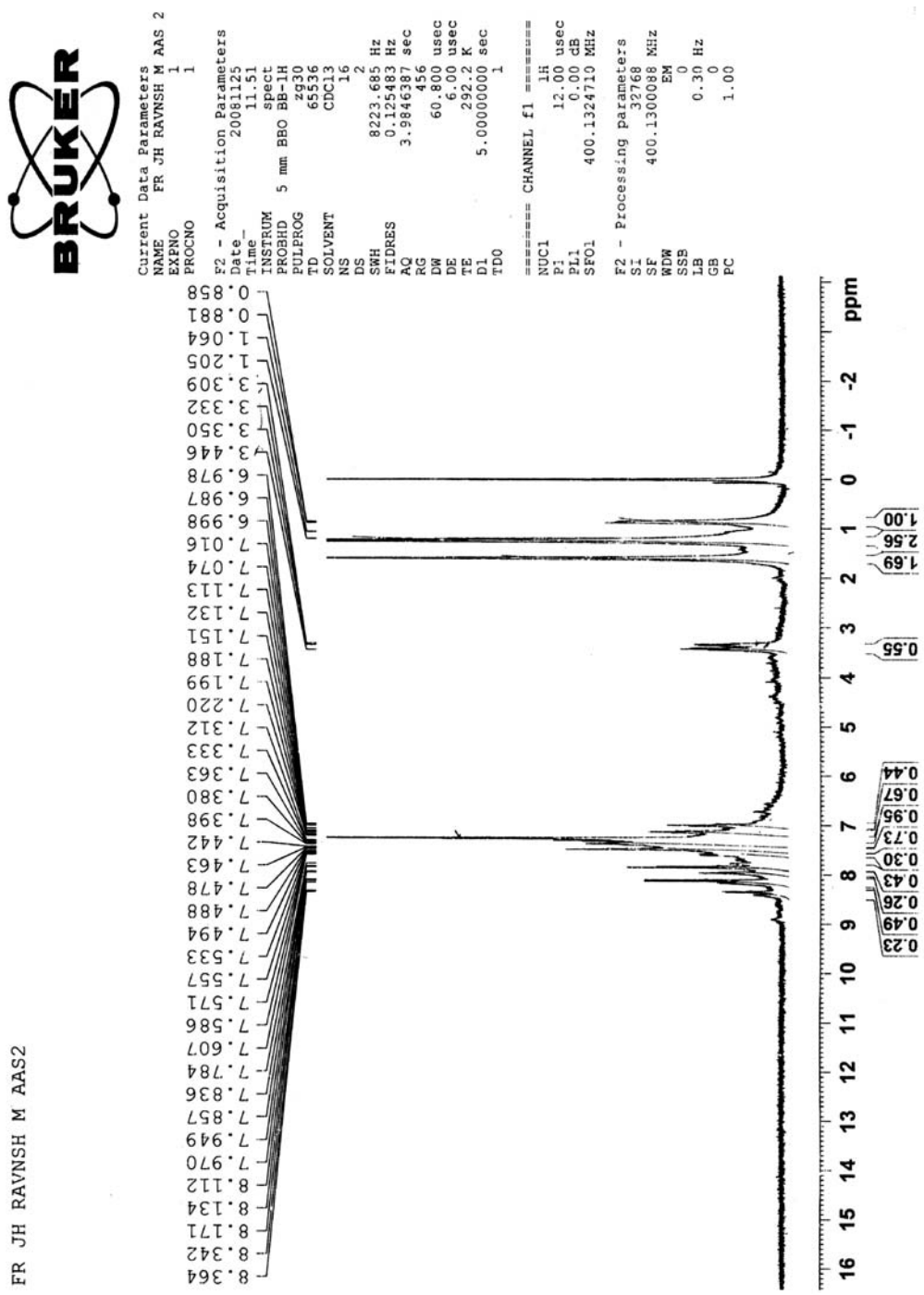


Spectrum 3: 1-phenyl-2-[-3-(substituted)-1-H-indole-1-yl] ethanone **(3)**

was added p-bromobenzaldehyde **4d** (1.36g, 0.010 mole) and sodium hydroxide as a base and the mixture was stirred for about 8 h. The target compound was crystallized by pouring the reaction mixture into ice cold water and standing for 24 h. Crystals were separated by filtration and recrystallized from suitable solvent: yield, 70%; m.p., 213°C; IR spectrum in KBr (ν_{max}, cm^{-1}): 1760 (keto, CO), 1600 (conj. C=C), 3225 (N-H), 580 (C-Br); ^1H NMR spectrum in $CDCl_3$/TMS (δ, ppm): 7.3 (s, CH indole ring, J = 0.525), 7.45 – 7.81 (s, CH aryl, J = 0.550), 7.9 (s, aryl CH=CH); mass spectrum (*m*/*z*): 481 (M^++1), 357, 303, 322.7, 221.6, 115.8.

EXPERIMENTAL PHARMACOLOGICAL PART

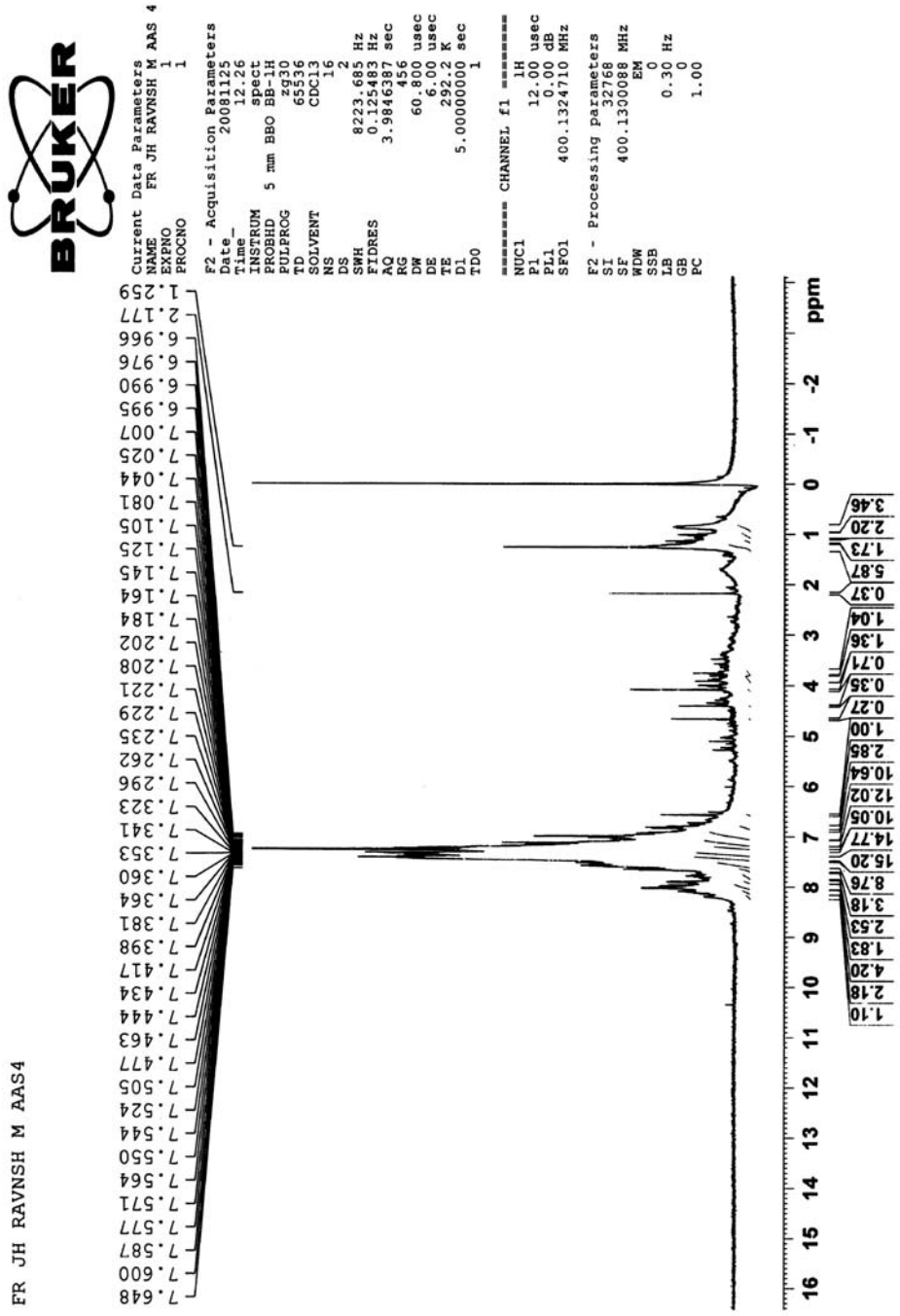
Antibacterial activity. Newly synthesized compounds **5a – 5d** were screened for their *in vitro* antifungal activity against *Escherichia coli* and *Bacillus cereus* according to the disk diffusion method. The percentage of inhibition was de-



Spectrum 4: (2Z)-2-(1-H-indole-yl)-3-(4-nitrophenyl)-1-phenylprop-2-en-1-one **(5b)**

termined at constant concentration of test (166.66 µl/disk) and standard (166.66 µl/disk) by dilution using dimethylsulfoxide as a solvent. Penicillin was used as a reference drug in these antibacterial screening studies. The results are presented in Table 2.

Antifungal activity. The antifungal activity studies on compounds **5a – 5d** were performed by the standard agar disk diffusion method. Seven-days-old cultures of *Fusarium oxysporium* and *Macrophomina phaeolina* were used as test organisms. They were grown on potato dextrose agar medium. The percentage of inhibition was determined at constant concentrations of test and reference by dilution technique using dimethylsulfoxide as solvent. The growth of microorganism was determined visually upon incubation for 24 h at 37°C. The reference drug used for the comparison in antifungal screening was fluconazole. The results are presented in Table 3.

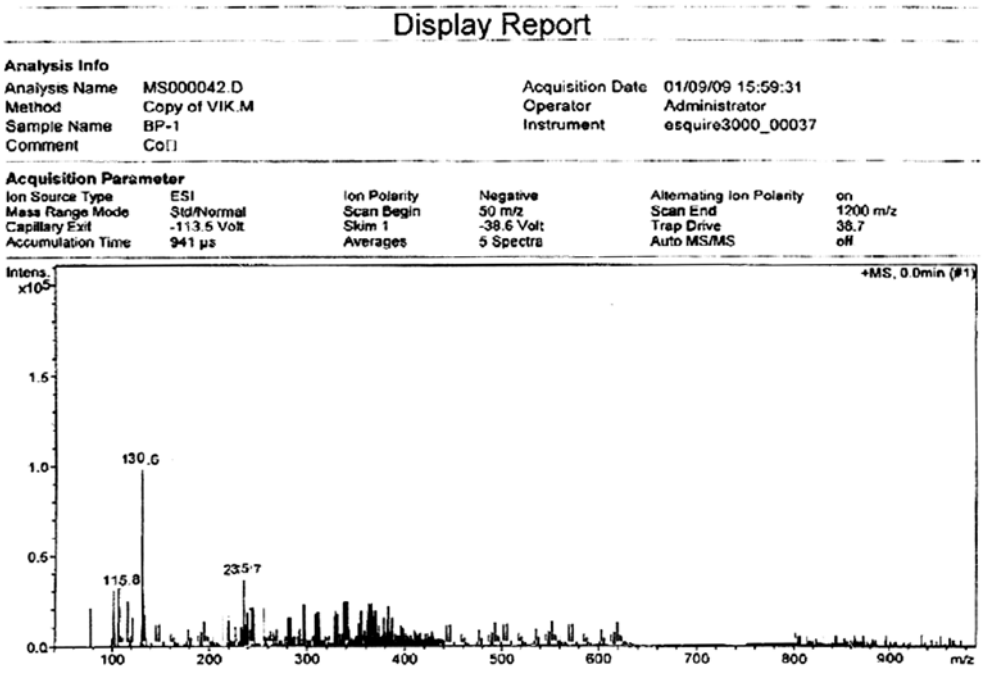


Spectrum 5: (2Z)-2-(1-H-indole-yl)-3-(4-clorophenyl)-1-phenylprop-2-en-1-one **(5c)**

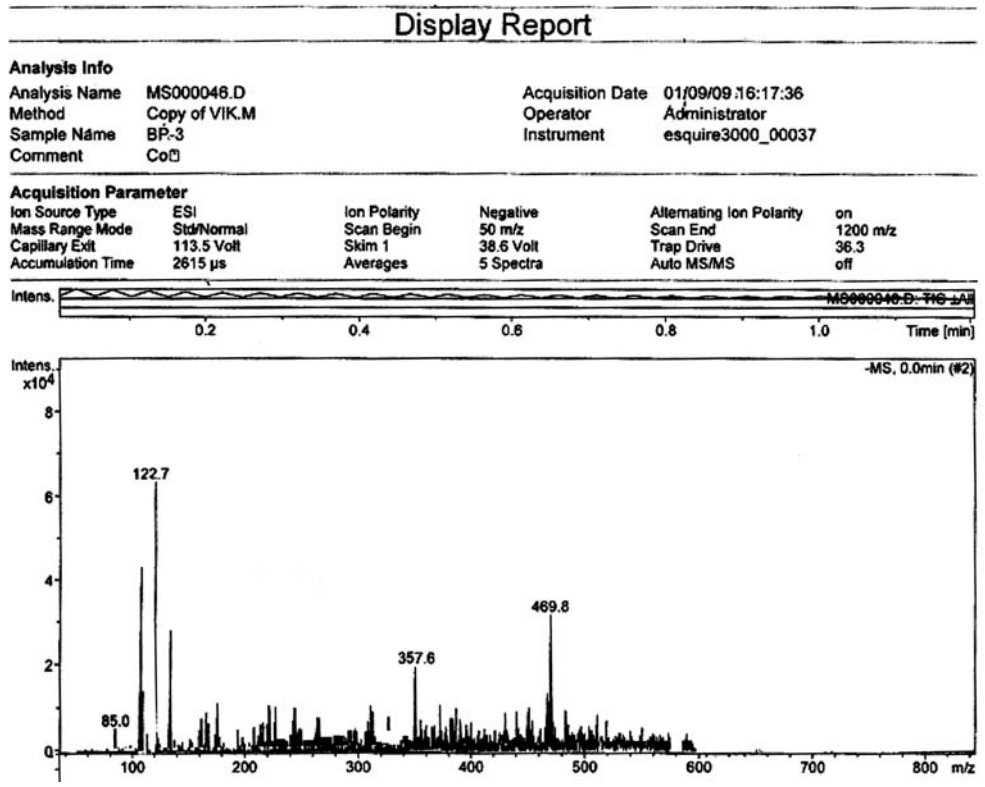
Blank control experiment with dimetylsulfoxide alone was done for both the antibacterial and antifungal studies.

RESULTS AND DISCUSSION

In the present study, N-alkylation of indole was used. The N-metalloid indoles are ambient nucleophiles and can react with electrophiles either at nitrogen or at C-3. The more ionic sodium and potassium salt tend to react preferentially at nitrogen, particularly with hard electrophiles. Substitution at nitrogen is also favored by the use of dipolar aprotic solvents [11 – 13]. Thus, the sodium salt of the indole can be N-substituted by chloroacetophenone. In this way, 2-(1H-indol-1-yl)-1-phenylethanone (compound **3**) was prepared. The IR spectra of the products were compared with the reactant spectra. The IR spectra of 2-(1H-indol-1-yl)-1-phenylethanone (**3**) displayed a sharp peak of C=O at 1700 cm^{-1}. The disappearance of -NH peak at 3500 cm^{-1} indicates that



Spectrum 6:1-phenyl-2-[-3-(substituted)-1-H-indole-1-yl] ethanone **(3)**



Spectrum 7: (2Z)-2-(1-H-indole-yl)-3-(4-nitrophenyl)-1-phenylprop-2-en-1-one **(5b)**

the indole -NH is converted to –NC group, which gives the peak of C–N at 1240 cm^{-1}. The peaks at 3066, 3022, 995.2, and 919.1 cm^{-1} (attributed to ν_{C-H} and $\nu_{C-H\ (def)}$) clearly indicated the presence of –CH=CH_2 group in this molecule. The peak at 1460 cm^{-1} gives a clear evidence that there is CH_2 group in the molecule.

The ^{1}H NMR spectra of compound **3** shows multiplet for Ar-H at δ = 6.97 – 7.36, 7.86 ppm (d, CH aryl). A singlet for

TABLE 3. Antifungal activity of compounds 5a – 5d

Compound no.	Conc. (µg/disk)	*F. oxysporium*		*M. phaeolina*	
		Zone of inhibition (mm)(test) (% activity)*	Zone of inhibition (mm), (std)	Zone of inhibition (mm)(test) (% activity)*	Zone of inhibition (mm), (std)
5a	166.66	9.23 (23.45)	39.35	12.56 (55.40)	22.67
5b	166.66	28.00 (71.15)	39.35	17.21 (75.91)	22.67
5c	166.66	22.00 (48.18)	45.66	17.33 (75.28)	23.02
5d	166.66	10.55 (23.10)	45.66	8.66 (38.20)	22.67

* Data in brackets refer to percentage activity

2H at δ = 3.309 – 3.446 ppm was assigned to CH_2-C=O moiety. There were also a multiplet for CH indole ring at δ = 7.17 ppm and a singlet for CH_2 at δ = 5.11 ppm.

The IR spectra of (2*Z*)-2-(1H-indol-1-yl)-3-(4-substituted phenyl)-1-phenylprop-2-en-1-ones (**5a – 5d)** conforms to a keto group and a conjugated double bond by showing the appropriate peaks. A difference in the spectra of **4a – 4d** and **5a – 5d** is due to substitution at *para* positions. It shows peaks at 1240 (O–CH_3), 1350 (sym nitro), 540 – 785 (C–Cl), <667 cm^{-1} (C-Br).

The antibacterial and antifungal activities of all newly prepared compounds against two bacteria and two fungi are presented in Table 2. The antibacterial activity of compound **5d** is quite good as it is as active as the reference drug against *E. coli* and shows greater percentage activity than reference against *B. cereus*. Compound **5c** have lowest percentage activity against *E. coli* among these four compounds and compound **5a** have least antibacterial percentage activity against *B. cereus.* On the other hand, compound **5a** a shows equal percentage activity against both *E. coli* and *B. cereus* compared to the reference and this is a lower activity.

The antifungal activity studies shows that compound **5b** shows the maximum percentage activity against *F. oxysporium*, whereas compound **5b** and compound **5c** show approximately equal activities against *M. phaeolina*, which is also the greatest activity among four compounds compared to the reference. Compound **5d** shows least activity in case of both the fungi compared to the reference drug activity.

CONCLUSIONS

Based on comparison of the antibacterial activity of various compounds, the following conclusions could be drawn.

1. No zone of inhibition is observed with the pure solvent (DMF).

2. Decreasing order of the antimicrobial activity is as follows:

against *E. coli:* **5d > 5b > 5a > 5c**; against *B. cereus:* **5d > 5b > 5c > 5a**;

against *F. oxysporium* : **5b > 5c > 5a > 5d**; and against *M. phaeolina*: **5b** > **5c > 5a > 5d**.

3. The structure – activity relationship in these compounds (see Scheme 2) indicates that bromo and nitro substitution increases the activity against bacteria, while chloro substitution have highest activity against fungi. Similarly chloro, methoxy and bromo substitutions have least activity against *E. coli, B. cerus* and fungal species, respectively.

It also appears that substitutions with highest activity against bacteria have lowest activity against fungi.

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