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Synthesis and Biological Evaluation of Chalcone Derivatives Linked Triazoles

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ABSTRACT
In this work, an attempt was made to synthesize chalcone 3-(Substitutedphenyl)-N-(4*H*-1, 2, 4-triazol-4-yl)acrylamide by condensation of substitutedbenzaldehyde with N-(4*H*-1,2,4-triazol-4-yl)acetamide under basic conditions. A simple condensation reaction of substitutedbenzaldehyde and N-(4*H*-1, 2, 4-triazol-4-yl)acetamide using Sodium hydroxide as a base was carried out for the study. The synthesized chalcone derivative was characterized by FTIR, ^{1}H-NMR & ^{13}C-NMR and studied for their Antimicrobial and Antifungal activities and compared with the standard drugs, some compound of the series exhibited promising anti-microbial and anti-fungal activity compared to standard drugs.

Keywords: Triazole, Chalcone, Antibacterial, Antifungal.

INTRODUCTION

Chalcones come under an aromatic ketone that forms the central core for a variety of important biological compounds. Claisen–Schmidt condensation between acetophenone and benzaldehyde gives chalcone. This reaction is catalyzed by acids and bases under homogeneous or heterogeneous conditions. Chalcones derivatives have received a great deal of attention due to their relatively simple structures, and wide variety of pharmacological activities reported for these compounds include anti-inflammatory [1], antifungal [2-3] antibacterial[4], antimalarials [5] and antitumor activities. [6] For these reasons, the synthesis of chalcones and their functionalized derivatives is a primary objective.

In the last few decades, the chemistry of 1, 2, 4-triazoles has received considerable attention owing to their synthetic and effective biological importance. 1, 2, 4-Triazole moieties have been incorporated into a wide variety of therapeutically interesting drug candidates including anti-inflammatories, CNS stimulants, sedatives, antianxiety compounds, antimicrobial agents [7-9] and antimycoticones such as fluconazole, intraconazole, voriconazole. [10-11] There are marketed drugs containing the 1, 2, 4-triazole group, e.g.: Triazolam [12], Alprazolam [13], Etizolam [14] and Furacylin. [15]

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In addition to these important biological applications, 1, 2, 4-triazoles are also of great utility in preparative organic chemistry, for example in the presence of various reagents, undergo different types of reactions to yield other heterocyclic compounds.

Our literature survey revealed that Chalcones are yet to be explored with 1, 2, 4-triazole ring system. herein we report the activity of Chalcones derivatives by synthesizing a series of five molecules **(3a–e)** and evaluating their antibacterial activity against eight microorganism strains of Gm^{+ve} as well as Gm^{-ve} and antifungal profile against Mucor, Penicillium and Aspergillus fungi.

In this study, only the HY-ALI feature associated with 'B' ring of the Chalcone moiety was changed by keeping the basic skeleton intact. Three compounds (3b, 3d and 3e) found most active in-vitro against eight strains of microorganisms. These compounds also tested for their antifungal profile in which 3b and 3e found most active against mucor, aspergillus and pecillium fungi compared to standard drug.

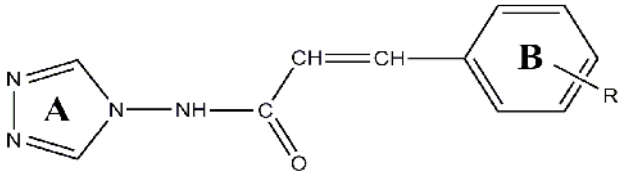


Figure I

MATERIALS AND METHOD

Melting points were determined in open capillary tubes and are uncorrected. All the chemicals and solvents used were of

Laboratory Grade and solvents were purified by suitable methods. IR (Infrared spectrum) (KBr, cm^{-1}) were recorded on a Shimadzu-8400 FT-IR spectrometer using KBr disc, ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Brucker Avance II 400 NMR spectrometer using TMS as an internal standard (chemical shift in δ,ppm) in $CDCl_3$. The homogeneity of the products was checked by TLC using Silica Gel GF_{254} (E.Merck) and the eluent system was a mixture of Acetone - Toluene in 2:8 proportions.

General procedure for the preparation of N-(4*H*-1, 2, 4-triazol-4-yl)acetamide (2)

To a solution of 4-amino-4*H*-1, 2, 4-triazole **(1)** (0.01 mol) in dry benzene (50 ml), acetylchloride (0.01 mol) was added drop by drop at 0–5°C. The reaction mixture was stirred for 1 h and kept overnight. The reaction mixture was distilled off and then poured onto ice. The solid thus obtained was recrystallised from suitable solvent. Physical and analytical data are given in Table I.

General procedure for the preparation of 3-(substitutedphenyl)-N-(4*H*-1, 2, 4-triazol-4-yl)acrylamide (3a–3e)

A solution of N-(4*H*-1, 2, 4-triazol-4-yl)acetamide (0.01 mol) in absolute ethanol (50 ml) is refluxed with various aromatic aldehydes in the presence of 2 % NaOH (5ml) for 10 h, concentrated, cooled and poured onto ice. The solids thus obtained were recrystallised from appropriate solvents. Physical, analytical and spectroscopic data of compounds are as follows, respectively.

3-(4-chlorophenyl)-N-(4*H*-1, 2, 4-triazol-4-yl)acrylamide (3a)

White powder, Yield 74%, m.p. 159°C; TLC (Acetone: Toluene, 2:8). IR: (KBr, cm^{-1}) 3426 (N-H), 3123 (Ar C-H stretch), 2925 and 2852 (C-H stretch), 1691 (NH-C=O), 1653 (CH=CH of –Carbonyl-CH=CH-), 1595 (C=N in triazole ring), 1513 (C=C of aromatic ring), 762 (C-Cl). ^{1}HNMR (400 MHz, $CDCl_3$) δ/ppm: 8.31 (ss, 1H, N-H), 7.40-7.38 (d, 1H, -CO-CH=), 7.99-7.97 (d, 1H, =CH-Ar), 7.49-7.47 (d, 2H, Ar-H), 7.84-7.81 (d, 2H, Ar-H), 8.80 (ss, 2H, -CH=N in triazole ring). ^{13}CNMR (400 MHz, DMSO) δ/ppm: δ 166.13 (-NH-C=0), δ 156.03 (triazole ring C), δ 138.48 (=C-Ar), δ 130.73; 130.55; 129.56; 128.88 (Aromatic C), 128.13 (Carbonyl-C=).

3-(4-(dimethylamino) phenyl)-N-(4*H*-1, 2, 4-triazol-4-yl)acrylamide (3b)

Orange crystals, Yield 70%, m.p. 184°C; TLC (Acetone: Toluene, (2:8). IR: (KBr, cm^{-1}) 3416 (N-H), 3090 (Ar CH stretch), 2915 and 2830 (C-H stretch), 1643 (NH-C=O), 1595 (C=N in triazole ring), 1540 (C=C of aromatic ring), 1330 (C–N). ^{1}H NMR (400 MHz, $CDCl_3$) δ/ppm: 8.39 (ss, 1H, N-H), 6.71-6.70 (d, 1H, -CO-CH=), 7.68 (d, 1H, =CH-Ar), 6.72 (d, 2H, Ar-H), 7.67-7.66 (d, 2H, Ar-H), 8.53 (ss, 2H, -CH=N in triazole ring), 3.08 (ss, 6H, N-$(CH_3)_2$). ^{13}CNMR (400 MHz, DMSO) δ/ppm: δ 164.88 (-NH-C=0), δ 138.88 (triazole ring C), δ 135.58 (=C-Ar), δ 152.18; 129.63, 128.50; 127.86 (Aromatic C), 129.01 (Carbonyl-C=), δ 39.78; 39.57 (-N $(CH_3)_2$.

3-(naphthalen-1-yl)-N-(4*H*-1, 2, 4-triazol-4-yl)acrylamide (3c)

Yellow crystals, Yield 69%, m.p. 225°C; TLC (Acetone: Toluene, 2:8). IR: (KBr, cm^{-1}) 3458 (N-H), 3070 (Ar C-H stretch), 2930 and 2840 (C-H stretch), 1664 (NH-C=O), 1625 (CH=CH of –Carbonyl-CH=CH-), 1580 (C=N in triazole ring), 1535 (C=C of aromatic ring),. ^{1}HNMR (400 MHz, $CDCl_3$) δ/ppm: 8.21 (ss, 1H, N-H), 6.44-6.41 (d, 1H, -CO-CH=), 7.58-7.54 (d, 1H, =CH-Ar), 7.59-7.98 (m, 7H, Ar-H of Naphthalenyl), 8.90 (ss, 2H, -CH=N in triazole ring). ^{13}CNMR (400 MHz, DMSO) δ/ppm: δ 168.72 (-NH-C=0), δ 146.26 (triazole ring C), δ 143.96 (=C-Ar), δ 133.64; 132.00; 128.82; 128.34; 126.95; 126.32; 126.30; 124.22; 122.96 (Aromatic C), 120.01 (Carbonyl-C=).

3-(3-methoxyphenyl)-N-(4*H*-1,2,4-triazol-4-yl)acrylamide (3d)

Greenish crystals, Yield 72%, m.p. 126°C; TLC (Acetone : Toluene, 2:8). IR: (KBr, cm^{-1}) 3440 (N-H), 3110 (Ar C-H stretch), 2985 and 2838 (C-H stretch), 1671 (NH-C=O), 1635 (CH=CH of –Carbonyl-CH=CH-), 1587 (C=N in triazole ring), 1510 (C=C of aromatic ring). ^{1}HNMR (400 MHz, $CDCl_3$) δ/ppm: 8.12 (ss, 1H, N-H), 6.48-6.44 (d, 1H, -CO-CH=), 7.59-7.57 (d, 1H, =CH-Ar), 7.69-7.54 (m, 4H, Ar-H), 3.63 (ss, 3H, -O-CH_3), 8.48 (ss, 2H, -CH=N in triazole ring). ^{13}CNMR (400 MHz, DMSO) δ/ppm: δ 164.78 (-NH-C=0), δ 148.88 (triazole ring C), δ 142.48 (=C-Ar), δ 160.32; 134.22; 129.65, 120.82; 115.36; 113.24 (Aromatic C), 119.52 (Carbonyl-C=), δ 57.68 (-O-CH_3).

3-(2-hydroxyphenyl)-N-(4*H*-1,2,4-triazol-4-yl)acrylamide (3e)

Yellowish powder, Yield 65%, m.p.196°C; TLC (Acetone : Toluene, 2:8). IR: (KBr, cm^{-1}) 3435 (N-H), 3020 (Ar C-H stretch), 2925 and 2830 (C-H stretch), 1631 (NH-C=O), 1614 (CH=CH of –Carbonyl-CH=CH-), 1575 (C=N in triazole ring), 1535 (C=C of aromatic ring). ^{1}HNMR (400 MHz, $CDCl_3$) δ/ppm: 8.27 (ss, 1H, N-H), 6.40-6.38 (d, 1H, -CO-CH=), 7.79-7.77 (d, 1H, =CH-Ar), 6.44-7.76 (m, 4H, Ar-H), 8.70 (ss, 2H, -CH=N in triazole ring), 5.40 (ss, 1H, Aromatic C-OH). ^{13}CNMR (400 MHz, DMSO) δ/ppm: δ 162.52 (-NH-C=0), δ 148.61 (triazole ring C), δ 145.38 (=C-Ar), δ 157.27; 129.32; 128.96; 122.63, 121.20; 117.64 (Aromatic C), 116.18 (Carbonyl-C=).

RESULT AND DISCUSSION

Chemistry

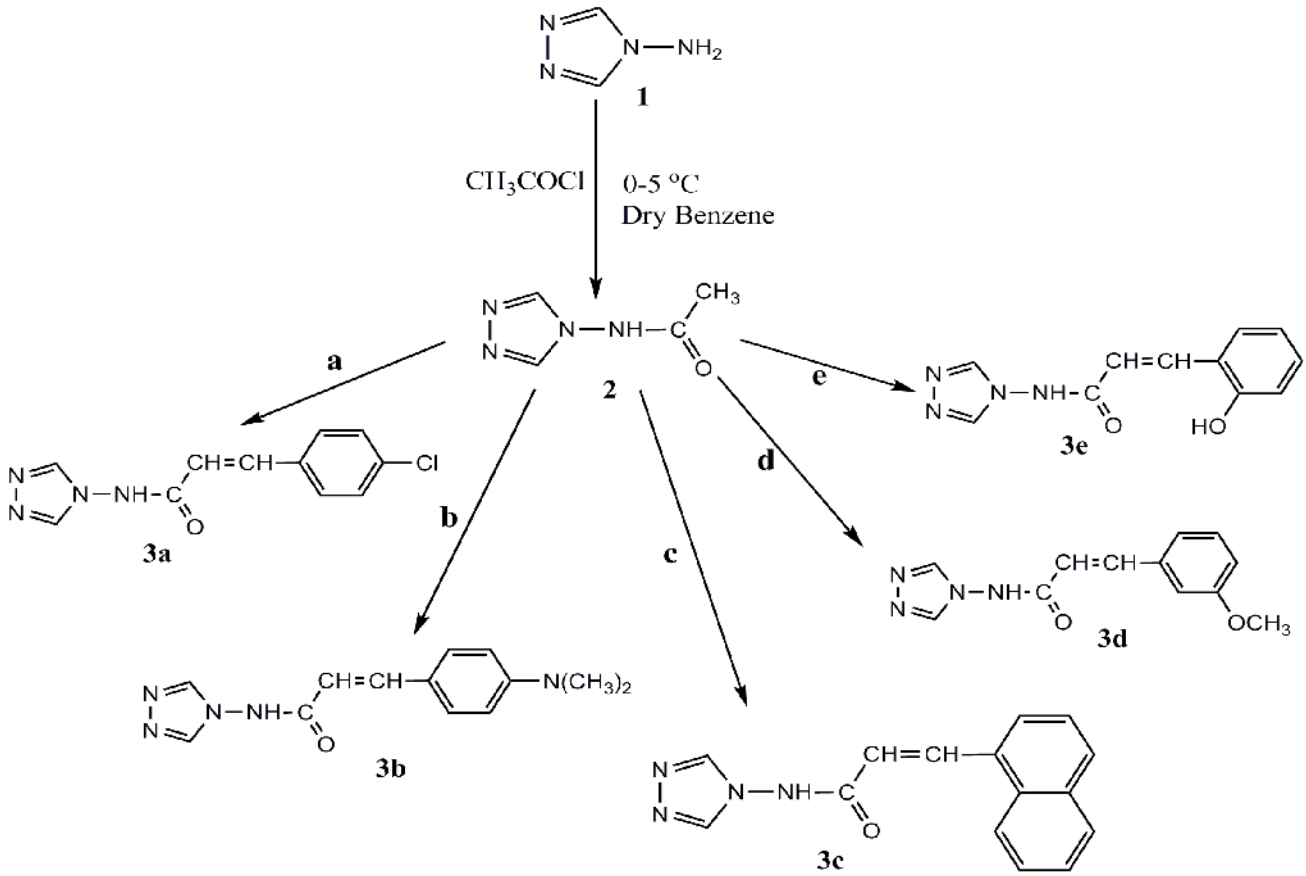
3-(Substitutedphenyl)-N-(4*H*-1,2,4-triazol-4-yl)acrylamide **(3a–e)** were synthesized according to the method shown in Scheme-1.

In the first step, synthesis of N-(4*H*-1,2,4-triazol-4-yl)acetamide were carried out by the acetylation of 4-amino-1,2,4-triazole by acetyl chloride and products were purified by recrystallization from suitable solvent (75–85% yield). Than in second step, synthesis of chalcone carried out by the reaction of N-(4*H*-1,2,4-triazol-4-yl)acetamide and different Aromatic aldehyde with 2%NaOH in absolute ethanol and the products were purified by recrystallization from suitable solvents.

The synthesized product has been fully characterized by IR, ^{1}H-NMR and ^{13}C-NMR spectroscopy data. The IR spectrum of the synthesized chalcone was recorded and it gives an absorption band near 1700-1650 cm^{-1} representing the presence of -C=O group. The absorption band at 1650-1580 cm^{-1} confirms the aromatic –C=C- group. The ^{1}H NMR and ^{13}C NMR of synthesized chalcone give ^{1}H NMR (400 MHz, $CDCl_3$) δ/ppm: 7.40-7.38 (d, 1H, -CO-CH=), 7.99-7.97 (d, 1H, =CH-Ar) and ^{13}C NMR (400 MHz, DMSO) δ/ppm: δ 166.13 (-NH-C=0), δ 138.48 (=C-Ar), 128.13 (Carbonyl-C=) confirms synthesis of chalcones.

Pharmacological results

Antibacterial activity



Scheme I: Synthesis of 3-(Substitutedphenyl)-N-(4*H*-1,2,4-triazol-4-yl)acrylamide 3a-e. Reagents: (a) 4-chlorobenzaldehyde, NaOH/EtOH; (b) 4-dimethylaminobenzaldehyde, NaOH/EtOH; (c) 1-naphthaldehyde, NaOH/EtOH; (d) 3-methoxybenzaldehyde, NaOH/EtOH; (e) 2-hydroxybenzaldehyde, NaOH/EtOH.

Table I: Physical data of synthesized compounds 2, 3a-e and 4a-e.

Compound	R	m.p. (°C)	Yield (%)	Mol. Formula	Mol. Weight	Recrystallization solvent*
2	-	155	84	$C_4H_6N_4O$	126.12	1
3a	4-Cl	159	74	$C_{11}H_9ClN_4O$	248.67	1
3b	4-N(CH_3)	186	70	$C_{13}H_{15}N_5O$	257.29	2
3c	Naphthalene	225	69	$C_{15}H_{12}N_4O$	264.28	3
3d	3-OCH_3	126	72	$C_{12}H_{12}N_4O_2$	244.25	1
3e	2-OH	196	65	$C_{11}H_{10}N_4O_2$	230.22	2

*1. Methanol, 2. Ethanol, 3. Acetone

Table II: Antibacterial activity of Chalcones (3a-e).

Compounds	Antimicrobial Activity of Synthetic Compound (In mm)							
	Bacillus megaterium	*Bacillus subtilus*	*Micrococcusluteus*	*Staphylococcus aureus*	*Escherichia coli*	*Enterobacter*	*Proteus vulgaris*	*Pseudomonas aeruginosa*
Chloramphenicol	+++	+++	+++	+++	+++	++	++	++
3a	-	-	-	-	-	-	-	-
3b	++	-	++	-	-	-	-	-
3c	-	-	-	-	-	-	-	-
3d	++	-	-	-	-	-	-	-
3e	-	-	++	-	-	++	-	-

+ : <6 mm (poor), ++ : <12 mm (good), +++ : <18 mm (Excellent), - : Without activity

Table III: Antifungal activity of Chalcones derivatives (3a-e).

Compounds	Antifungal Activity of Synthetic compound (In mm)		
	Mucor	*Penicillium*	*Aspergillus Niger*
Fluconazole	++	+++	++
3a	+	++	+
3b	+	++	++
3c	+	++	+
3d	+	++	+
3e	++	++	+

+ : <25 mm (poor), ++ : <50 mm (good), +++ : <75 mm (Excellent)

All the synthesized compounds were screened for their in vitro antibacterial activity. *Bacillus megaterium, Bacillus subtilus, Micrococcus luteus, Staphylococcus aureus, Escherichia coli, Enterobacter, Proteus vulgaris and Pseudomonas aeruginosa* strains were used to determine antibacterial activity in which first four are gram positive bacteria while later four are gram negative bacteria. Antibacterial activities of all samples were screened by the agar well diffusion method. [16-17] Compounds 3b, 3d and 3e were most potent and comparable to activities of standard antibiotic chloramphenicol against *Bacillus megaterium, Micrococcus luteus and Enterobacter*. Weak activity was obseved with the other compound 3a and 3c.

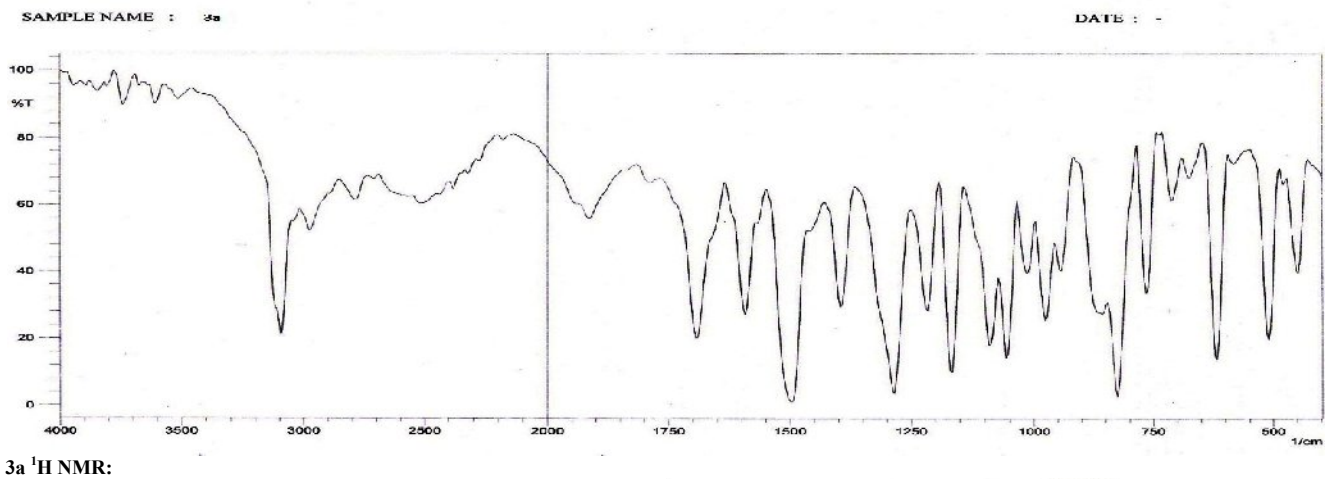
Table IV: Minimum inhibitory concentration (MIC) values (MIC50 & MIC90) of Most Potent compounds measured on standard bacterial strains.

Compounds	MIC50 of Antibacterial Agent (mg/ml)				MIC90 of Antibacterial Agent (mg/ml)			
	Bacillus megaterium	*Micrococc usluteus*	*Staphylococcus aureus*	*Enterobacter*	*Bacillus megaterium*	*Micrococcuslut eus*	*Staphylococcus aureus*	*Enterobacter*
3b	755.86	1060.45	-	-	1360.54	1908.8	-	-
3d	853.24	-	-	-	1535.84	-	-	-
3e	-	1184.83	-	1492.54	-	2132.7	-	2686.57
Chloramphenicol	340.14	327.65	339.9	481	612.24	589.78	611.83	865.8

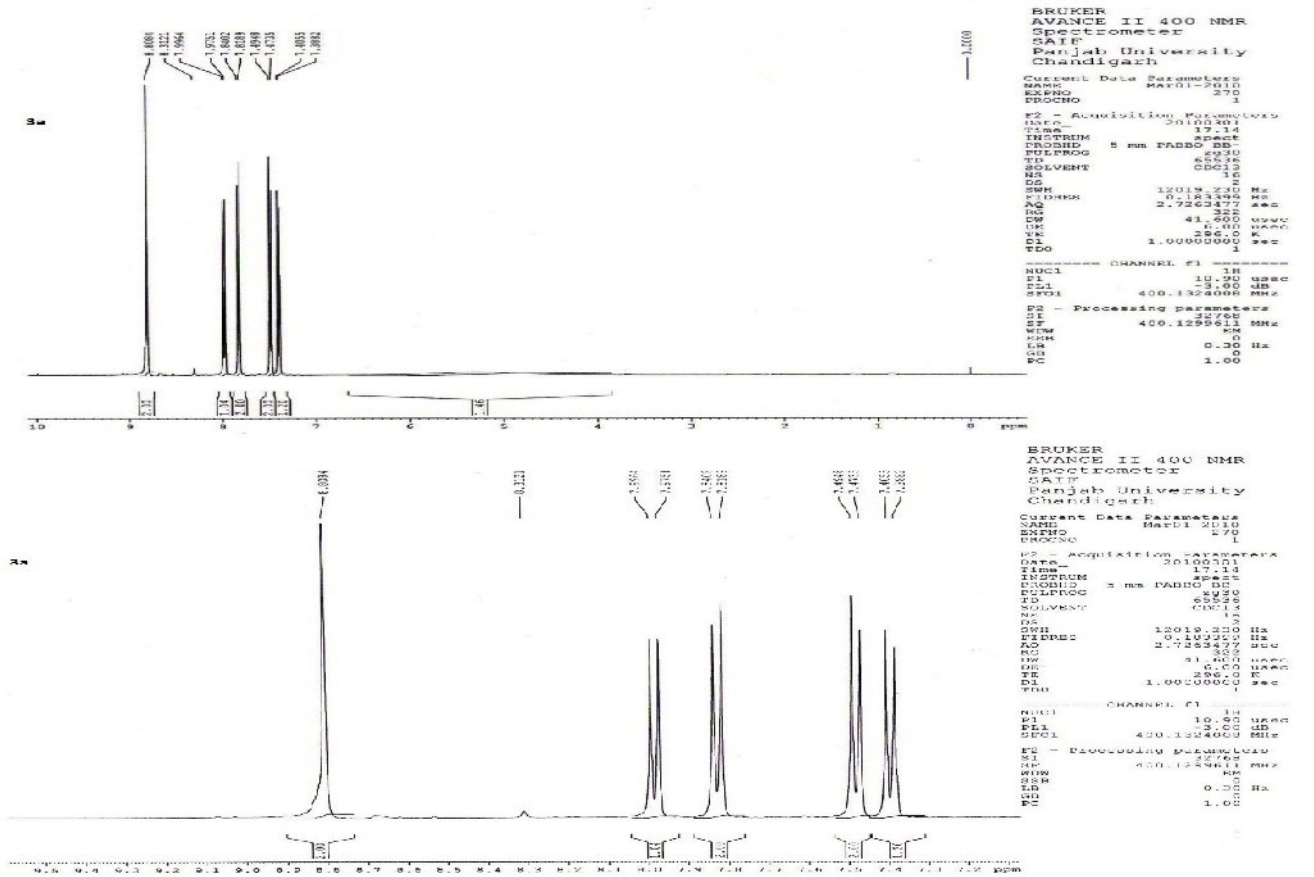
Table V: Minimum inhibitory concentration (MIC) values (MIC50 & MIC90) of Most Potent compounds measured against three fungi strains.

Compounds	MIC 50 of Antifungal Activity (mg/ml)			MIC 90 of Antifungal Activity (mg/ml)		
	Mucor	*Penicillium*	*Aspergillus Niger*	*Mucor*	*Penicillium*	*Aspergillus Niger*
Fluconazole	206.27	196.62	241.20	371.28	353.91	434.15
3a	1650.00	248.23	878.57	2970.00	446.81	1581.43
3b	611.11	296.61	323.68	1100.00	533.90	582.63
3c	423.08	321.10	455.56	761.54	577.98	820.00
3d	785.71	307.02	1537.50	1414.29	552.63	2767.50
3e	206.25	241.38	512.50	371.25	434.48	922.50

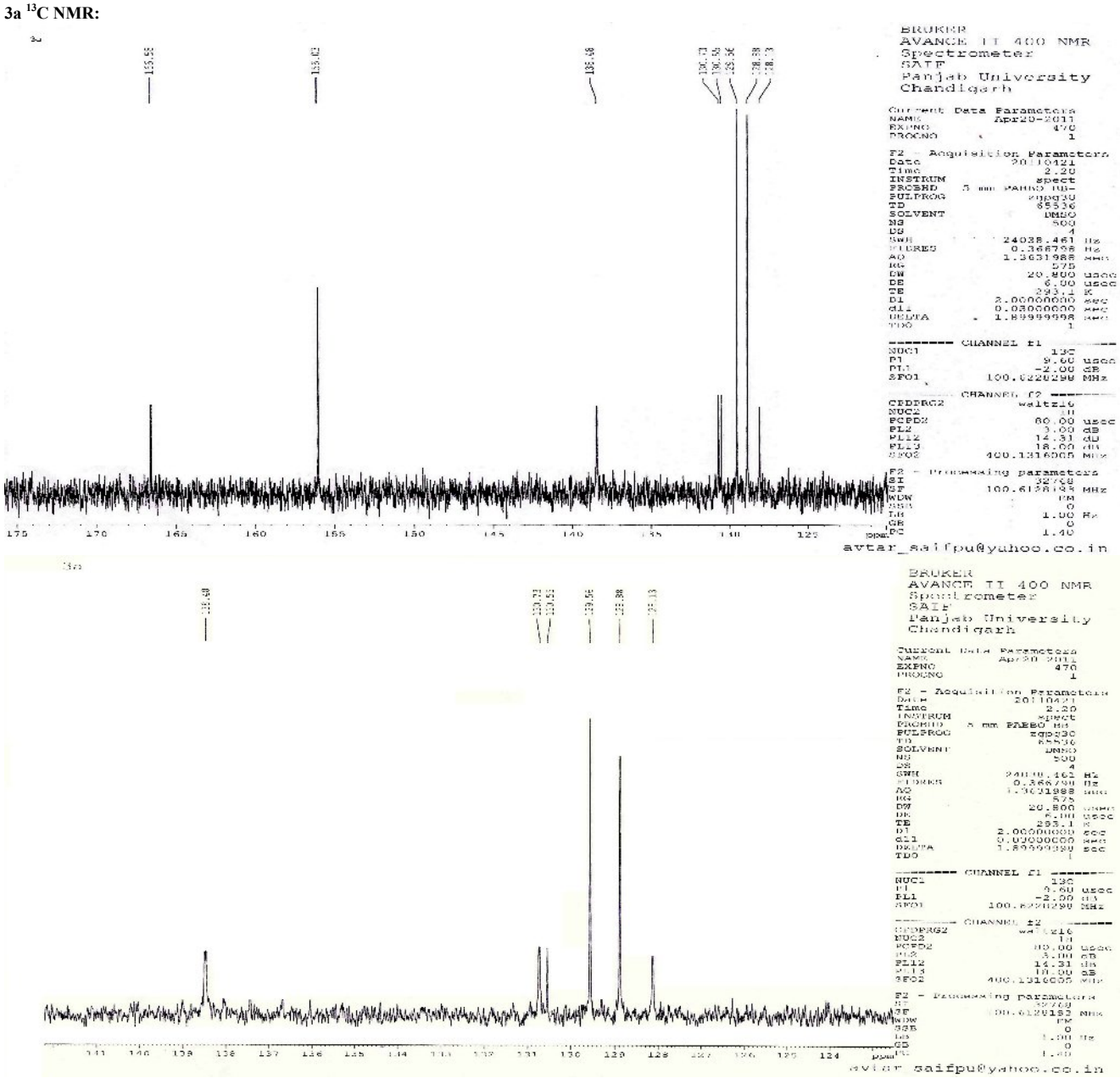
3a IR:



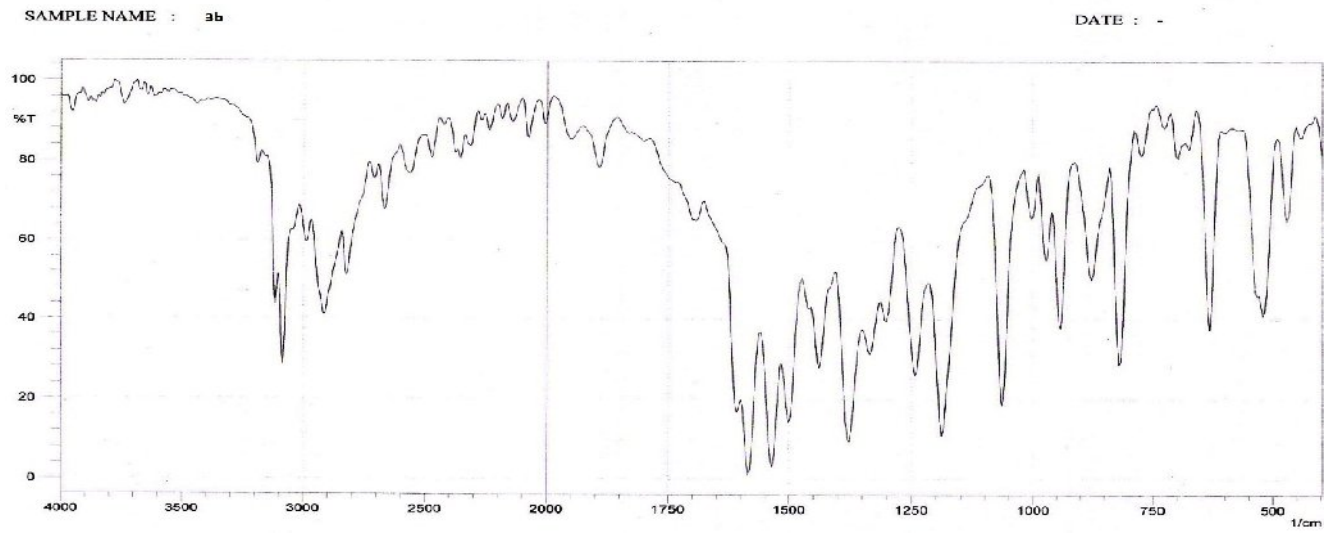
3a ^{1}H NMR:



3a ^{13}C NMR:



3b IR:



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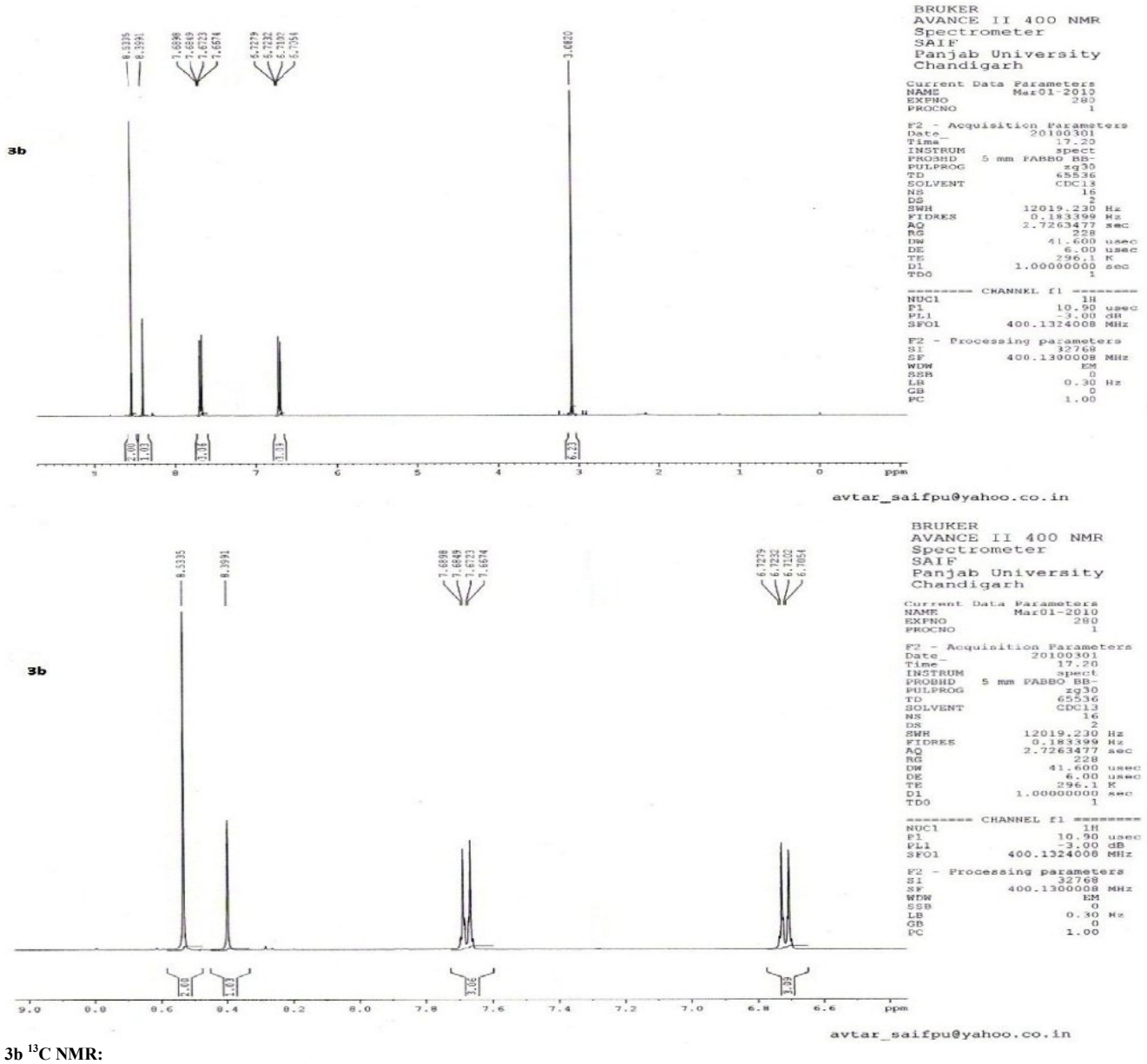
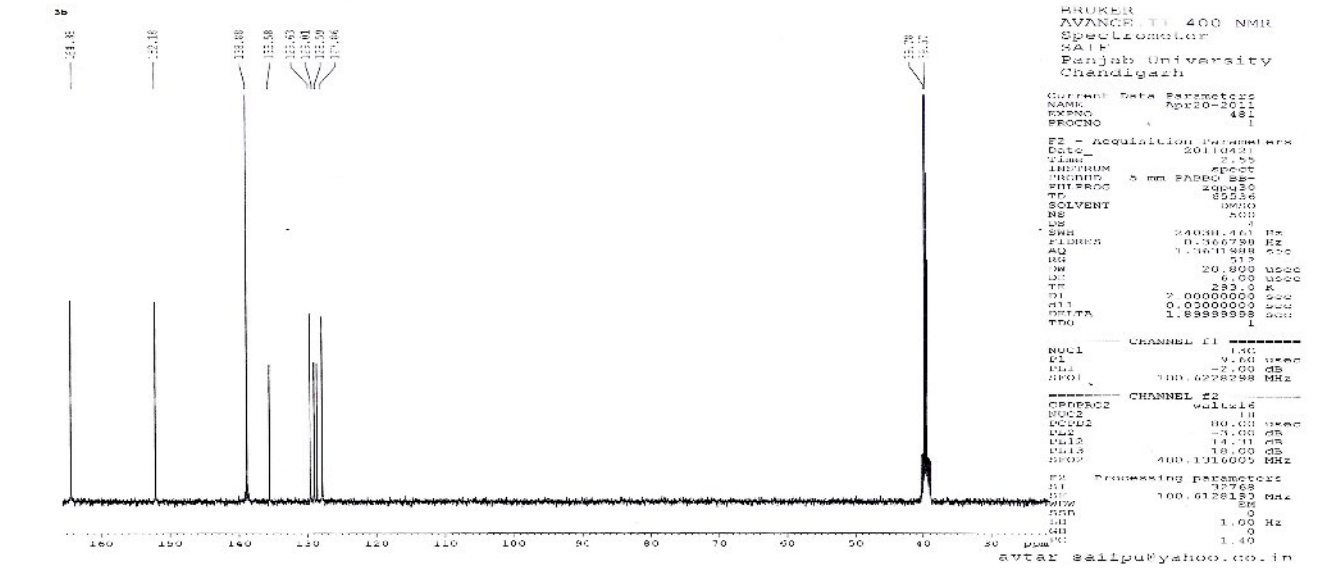
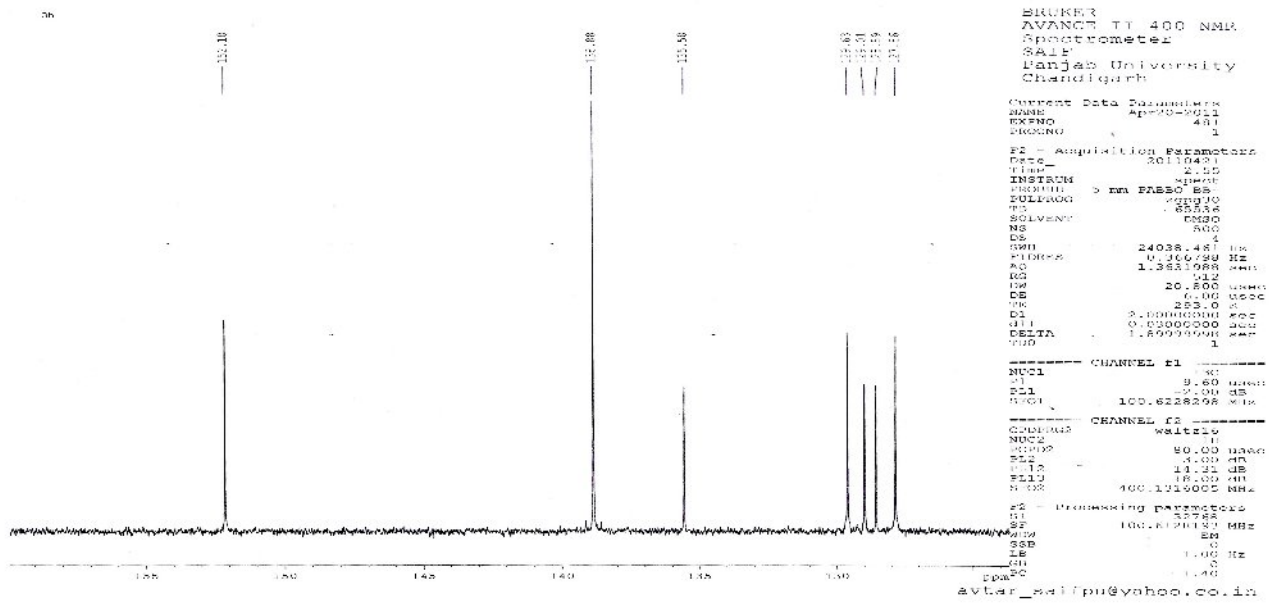


3b ^{13}C NMR:





Antifungal activity

All the synthesized compounds were also screened for their in vitro antifungal activity against *Mucor, Aspergillus Niger* and *Penicillium* strains. The zone of inhibition was measured in millimeters. Antifungal activities of all compounds were screened by the turbidometry method. [18] Activity of extract was compared with standard antibiotics fluconazole fungi. DMSO was used as solvent. All compounds are active against *Mucor, A. Niger* and *penicillium*. Compounds 3b and 3e provided the best antifungal activity and compared well with the activity of fluconazole. The compounds 3a, 3c and 3d also possess promising antifungal activity.

Minimum inhibition concentration (MIC)

The minimal inhibitory concentrations (MIC 50 & 90) of the strongly active compounds were also measured. The MICs of the extracts were determined by broth dilution method with a little modification. [19] The extracts were serially diluted with normal saline (0.9%) to 5-50 mg/ml preparation dispensed (1.0 ml) into test tubes containing 1.0 ml of nutrient or potato dextrose both. Each sensitive bacterial or fungal isolate (100 µl) was inoculated into the test tubes. The tubes were mixed, covered with cotton wool and incubated at 37^{o}C for bacteria and 25^{o}C for fungi. Thereafter, the tubes were then examined for microbial growth. The MIC (MIC50 & MIC90) was defined as the minimum concentration of the extract that did not allow to growth (50% & 90%) of microorganism.

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