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Synthesis, Anti-bacterial and Anti-fungal evaluation of 3-(Substitutedphenyl)-N-(4*H*-1,2,4-triazol-4 yl)acrylamide

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The Series of 3-(Substitutedphenyl)-N-(4H-1,2,4-triazol-4-yl)acrylamide compounds were prepared by reaction of 4-amino-1,2,4-Triazole with Acetyl Chloride followed by Claisen-Schmidt Condensation reaction with different aromatic aldehydes. The structures of new compounds were confirmed by IR, ^{1}H-NMR and ^{13}C-NMR spectral data. Anti-bacterial and Anti-fungal activities were evaluated and compared with the standard drugs, some compounds of the series exhibited promising anti-microbial and anti-fungal activity compared to standard drugs.

Keywords: Triazole; Chalcone; Antibacterial; Antifungal.

1. INTRODUCTION

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 [1-3] and antimycoticones such as fluconazole, intraconazole, voriconazole [4,5]. There are marketed drugs containing the 1,2,4-triazole group, e.g.: Triazolam [6], Alprazolam [7], Etizolam [8] and Furacylin [9]. In addition to these important biological applications, 1,2,4-triazoles are also of great utility in preparative organic chemistry.

Chalcone 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. Chalcone 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 [10], antifungal [11,12], antibacterial [13], antimalarial [14] and antitumor activities[15]. For these reasons, the synthesis of Chalcone and their functionalized derivatives is a primary objective.

Our literature survey revealed that Chalcone are yet to be explored with 1,2,4-triazole ring system. herein we report the activity of Chalcone 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 niger 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. Two compounds **(3b, 3c)** found most active in-vitro against eight strains of microorganisms. These compounds also tested for their antifungal profile in

which **3b** and **3c** found most active against mucor, aspergillus and pecillium fungi compared to standard drug.

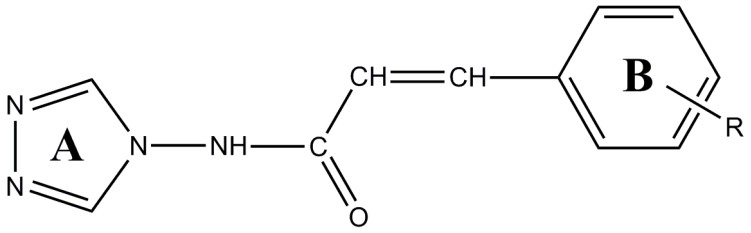


Fig. 1. Schematic diagram of Chalcone**.**

2. EXPERIMENTAL

2.1. Material and methods

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 $CDCl_3$ and DMSO. 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.

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

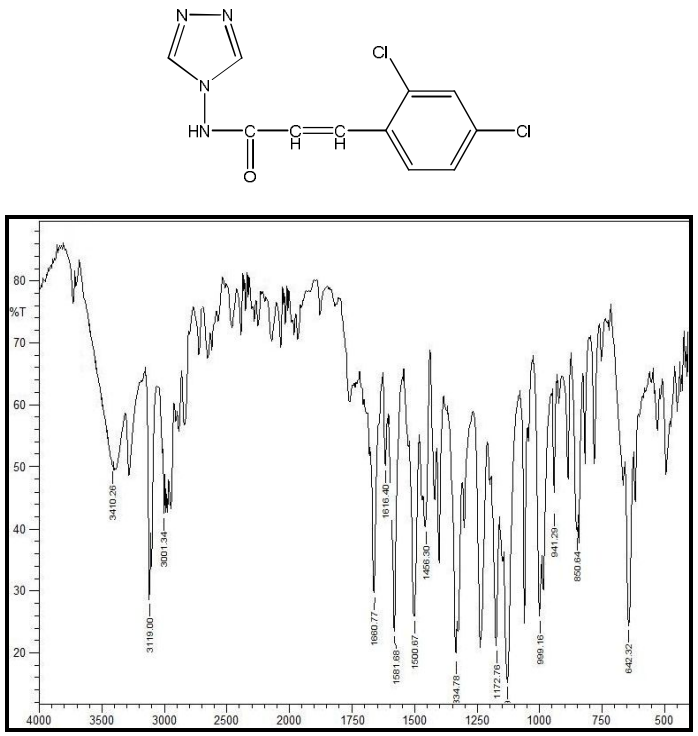
To a solution of 4-amino-4H-1,2,4-triazole **(1)** (0.01 mol) in dry benzene (50 mL), acetyl chloride (0.01 mol) was added drop by drop at 0–5 °C. The reaction mixture was stirred for 1 h and kept overnight. The reaction mixture was distilled off and then poured onto ice. The solid thus obtained was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

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

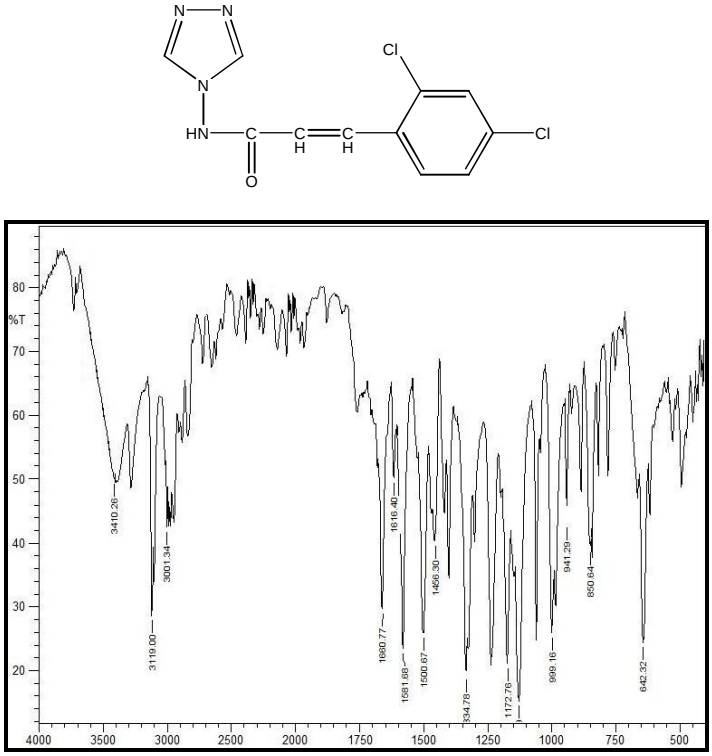
A solution of N-(4H-1,2,4-triazol-4-yl)acetamide (0.01 mol) in absolute ethanol (50 mL) is refluxed with various aromatic aldehydes in the presence of 2 % NaOH (5ml) for 10 h, concentrated, cooled and poured onto ice. The solids thus obtained were recrystallized from appropriate solvents. Physical, analytical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

2.3.1 3-(2,4-dichlorophenyl)-N-(4H-1,2,4-triazol-4-yl)acrylamide (3a)

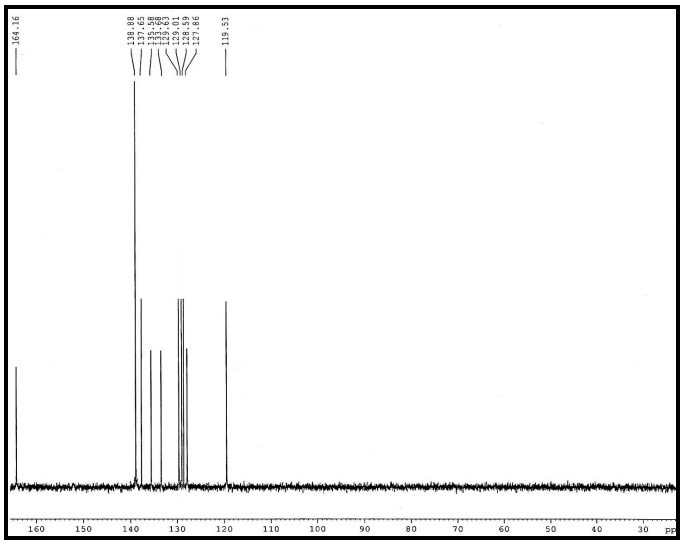
Grey powder, Yield 76%, m.p. 154^{o}C; TLC (Acetone : Toluene, 2:8). IR: (KBr, cm^{-1}) 3410.26 (N-H), 3119 (Ar C-H stretch), 3001 and 2915 (C-H stretch), 1660 (NH-C=O), 1616 (CH=CH of –Carbonyl-CH=CH-), 1581 (C=N in triazole ring), 1500 (C=C of aromatic ring); ^{1}HNMR (400 MHz, $CDCl_3$) δ/ppm: 8.1021 (s, 1H, N-H), 6.6938-6.6133 (d, 1H, -CO-CH=), 8.4906-8.4691 (d, 1H, =CH-Ar), 8.0904-8.0690 (d, 1H, Ar-H), 7.5188-7.4973 (d, 1H, Ar-H), 7.6521 (s, 1H, Ar-H), 9.1690 (s, 2H, -CH=N in triazole ring); ^{13}CNMR (400 MHz, DMSO) δ/ppm: δ 164.16 (-NH-C=0), δ 138.88 (triazole ring C), δ 137.65 (=C-Ar), δ 135.58; 133.68; 129.63; 129.01; 128.59; 127.86 (Aromatic C), 119.53 (Carbonyl-C=).



Infrared Spectra for 3-(2,4-dichlorophenyl)-N-(4H-1,2,4-triazol-4-yl)acrylamide (3a)



Infrared Spectra for 3-(2,4-dichlorophenyl)-N-(4H-1,2,4-triazol-4-yl)acrylamide (3a)



^{13}C NMR Spectra for 3-(2,4-dichlorophenyl)-N-(4H-1,2,4-triazol-4-yl)acrylamide (3a)

2.3.2 N-(4H-1,2,4-triazol-4-yl)-3-(3,4,5-trimethoxyphenyl)acrylamide (3b)

White powder, Yield 69%, m.p. 162^{o}C; TLC (Acetone : Toluene, 2:8). IR: (KBr, cm^{-1}) 3415 (N-H), 3026 (Ar C-H stretch), 2928 and 2830 (C-H stretch), 1684 (NH-C=O), 1615 (CH=CH of –Carbonyl-CH=CH-), 1525 (C=N in triazole ring), 1513 (C=C of aromatic ring); ^{1}HNMR (400 MHz, $CDCl_3$) δ/ppm: 8.1811 (s, 1H, N-H), 6.4951-6.4532 (d, 1H, -CO-CH=), 7.4806-7.4610 (d, 1H, =CH-Ar), 6.1904-6.1690 (d, 2H, Ar-H), 3.3415 (s, 9H, -O-CH_3), 8.2645 (s, 2H, -CH=N in triazole ring); ^{13}CNMR (400 MHz, DMSO) δ/ppm: δ 168.26 (-NH-C=0), δ 148.08 (triazole ring C), δ 141.65 (=C-Ar), δ 153.85; 138.23; 130.36; 103.81 (Aromatic C), 118.35 (Carbonyl-C=), 56.32 (-OCH_3).

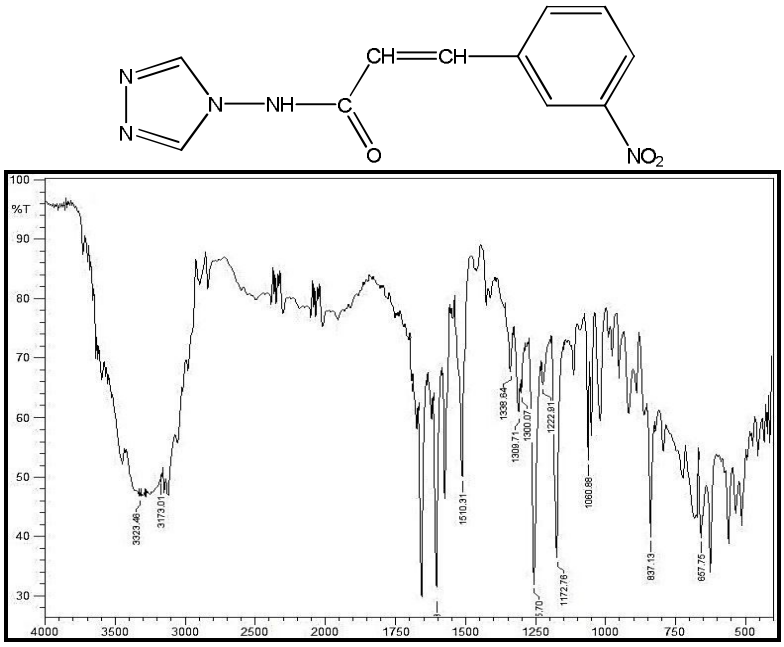
2.3.3 3-(4-methoxyphenyl)-N-(4H-1,2,4-triazol-4-yl)acrylamide (3c)

Greenish powder, Yield 77%, m.p. 116^{o}C; TLC (Acetone : Toluene, 2:8). IR: (KBr, cm^{-1}) 3429 (N-H), 3086 (Ar C-H stretch), 2938 and 2856 (C-H stretch), 1678 (NH-C=O), 1612 (CH=CH of –Carbonyl-CH=CH-), 1518 (C=N in triazole ring), 1505 (C=C of aromatic ring); ^{1}HNMR (400 MHz, $CDCl_3$) δ/ppm: 8.1239 (s, 1H, N-H), 6.4329-6.4117 (d, 1H, -CO-CH=), 7.5960-7.5621 (d, 1H, =CH-Ar), 6.9604-6.9490 (d, 2H, Ar-H), 7.6440-7.6209 (d, 2H, Ar-H), 3.8415 (s, 3H, -O-CH_3), 8.4645 (s, 2H, -CH=N in triazole ring); ^{13}CNMR (400 MHz, DMSO) δ/ppm: δ 166.62 (-NH-C=0), δ 146.80 (triazole ring C), δ 140.52 (=C-Ar), δ 159.51; 130.36; 122.69; 113.18 (Aromatic C), 119.95 (Carbonyl-C=), 56.43 (-OCH_3).

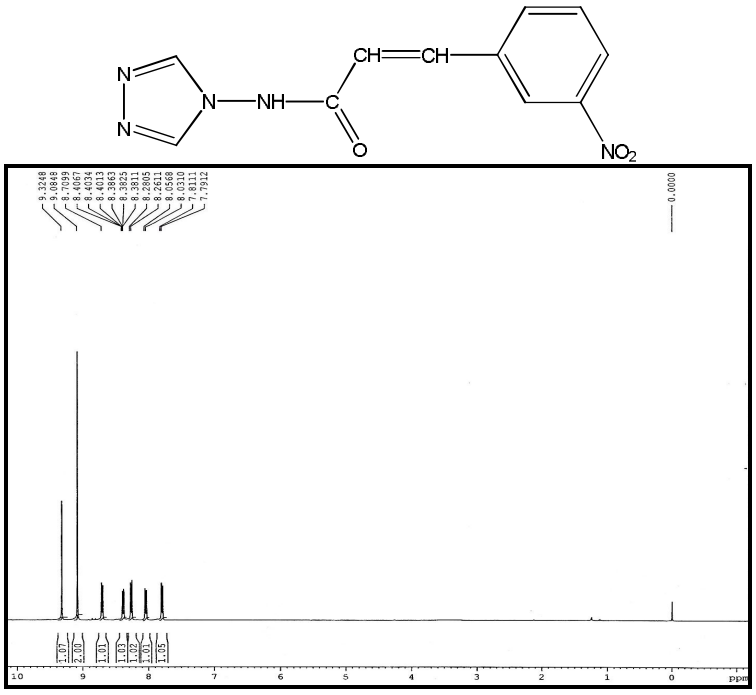
2.3.4 3-(3-nitrophenyl)-N-(4H-1,2,4-triazol-4-yl)acrylamide (3d)

White powder, Yield 68%, m.p. 151^{o}C; TLC (Acetone : Toluene, 2:8). IR: (KBr, cm^{-1}) 3323 (N-H), 3173 (Ar C-H stretch), 3010 and 2978 (C-H stretch), 1655 (NH-C=O), 1605 (CH=CH of –Carbonyl-CH=CH-), 1563 (C=N in triazole ring), 1510 (C=C of aromatic ring); ^{1}HNMR (400 MHz,

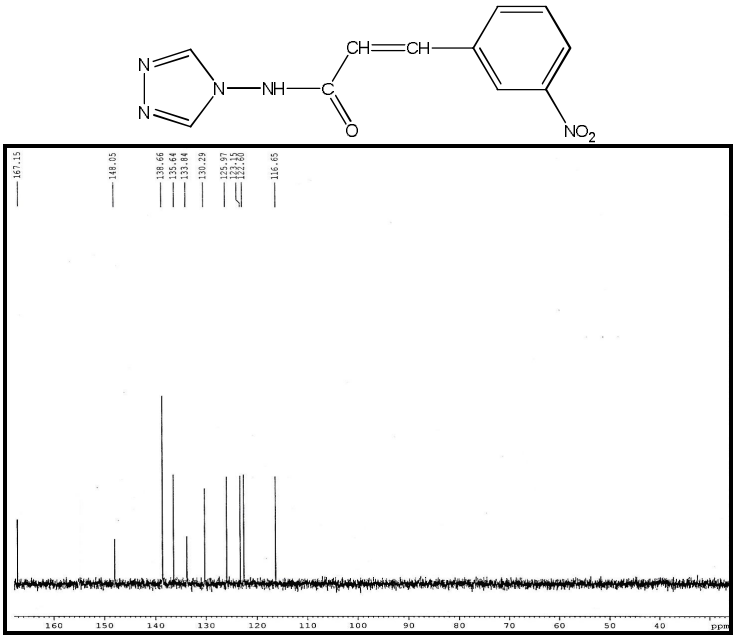
$CDCl_3$) δ/ppm: 8.1149 (s, 1H, N-H), 7.8111-7.7912 (d, 1H, -CO-CH=), 8.0568-8.0310 (d, 1H, =CH-Ar), 8.2805-8.2611 (d, 1H, Ar-H [-C-CH-CH-]), 8.4067-8.3811 (Multiplet, 1H, Ar-H [-CH-CH-CH-]), 8.7099 (d, 1H, Ar-H [-CH-CH-C-NO_2]), 9.0848 (s, 2H, -CH=N in triazole ring), 9.3248 (s, 1H, Ar-H [-C-CH-C-]); ^{13}CNMR (400 MHz, DMSO) δ/ppm: δ 167.15 (-NH-C=0), δ 138.66 (triazole ring C), δ 135.64 (=C-Ar), δ 148.05; 133.84; 130.29; 125.97; 123.15; 122.60 (Aromatic C), 116.65 (Carbonyl-C=).



Infrared Spectra for 3-(3-nitrophenyl)-N-(4*H*-1,2,4-triazol-4-yl)acrylamide (3d)



^{1}H NMR Spectra for 3-(3-nitrophenyl)-N-(4*H*-1,2,4-triazol-4-yl)acrylamide (3d)



^{13}C NMR Spectra for 3-(3-nitrophenyl)-N-(4*H*-1,2,4-triazol-4-yl)acrylamide (3d)

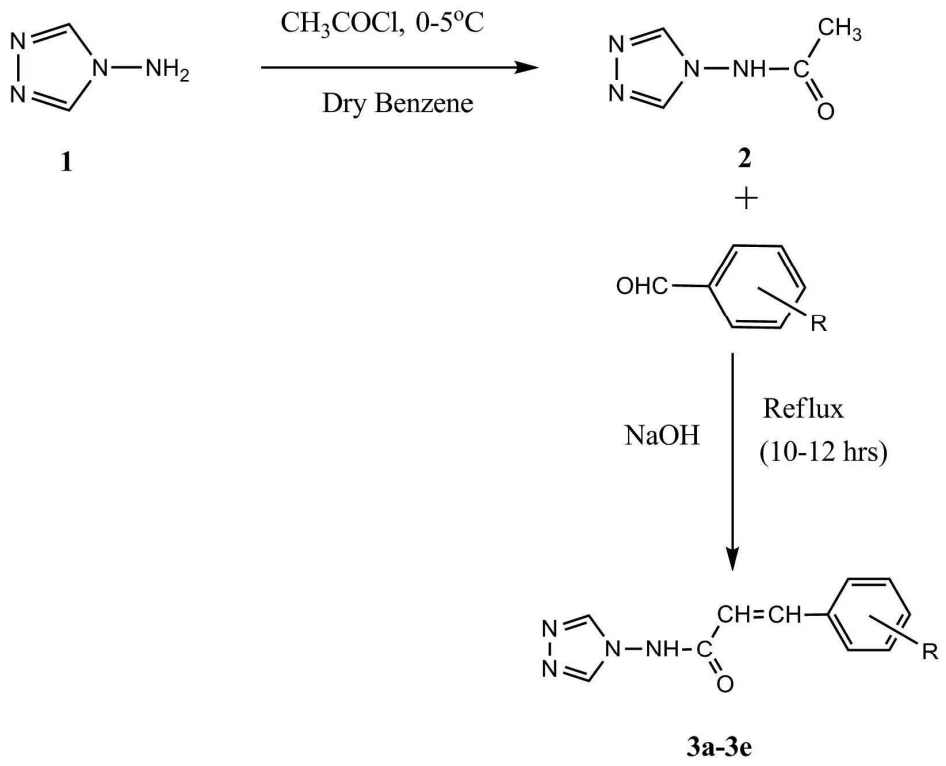
2.3.5 3-(4-bromophenyl)-N-(4H-1,2,4-triazol-4-yl)acrylamide (3e)

Brown powder, Yield 74%, m.p. 218^{o}C; TLC (Acetone : Toluene, 2:8). IR: (KBr, cm^{-1}) 3426 (N-H), 3095 (Ar C-H stretch), 2938 and 2813 (C-H stretch), 1695 (NH-C=O), 1619 (CH=CH of –Carbonyl-CH=CH-), 1523 (C=N in triazole ring), 1502 (C=C of aromatic ring); ^{1}HNMR (400 MHz, $CDCl_3$) δ/ppm: 8.0213 (s, 1H, N-H), 6.4834-6.4612 (d, 1H, -CO-CH=), 7.4562-7.4323 (d, 1H, =CH-Ar), 7.6305-7.6198 (d, 2H, Ar-H), 7.5531-7.5374 (d, 2H, Ar-H), 8.2884 (s, 2H, -CH=N in triazole ring); ^{13}CNMR (400 MHz, DMSO) δ/ppm: δ 162.28 (-NH-C=0), δ 144.63 (triazole ring C), δ 142.57 (=C-Ar), δ 134.87; 131.43; 128.98; 122.74 (Aromatic C), 120.98 (Carbonyl-C=).

3. RESULTS AND DISCUSSION

3.1 Chemistry

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



3	R
a	-2-Cl,4-Cl
b	-3,4,5-OCH_3
c	-4-OCH_3
d	-3-NO_2
e	-4-Br

Scheme 1. Synthesis of 3-(Substitutedphenyl)-N-(4H-1,2,4-triazol-4-yl)acrylamide 3a-e

In the first step, synthesis of N-(4H-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 Chalcones carried out by the reaction of N-(4H-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.

Table 1. Physical data of synthesized compounds 2, 3a-e.

Compound	R	M.P. (°C)	Yield (%)	Mol. Formula	Mol. Weight	Recrystallization solvent*
2	-	155	84	$C_4H_6N_4O$	126.12	1
3a	-2-Cl,4-Cl	154	76	$C_{11}H_8Cl_2N_4O$	283.11	1
3b	-3,4,5-OCH_3	162	69	$C_{14}H_{16}N_4O_4$	304.12	2
3c	-4-OCH_3	116	77	$C_{12}H_{12}N_4O_2$	244.10	1
3d	-3-NO_2	151	68	$C_{11}H_9N_5O_3$	259.22	3
3e	-4-Br	218	74	$C_{11}H_9BrN_4O$	293.12	2

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

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 of synthesized chalcones give peaks at 6.69-6.61 δ/ppm for α carbon and 8.49-8.46 δ/ppm for β carbon while ^{13}C NMR shows peaks at δ 164.16 for carbonyl group, δ 137.65 for β carbon and δ 119.53 for α carbon confirms synthesis of chalcones.

3.2 Pharmacological results

3.2.1 Antibacterial activity

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 and 3c were most potent and comparable to activities of standard antibiotic chloramphenicol against Bacillus megaterium, Micrococcus luteus and Enterobacter. Weak activity was observed with the other compound 3a, 3d and 3e.

Table 2. Antibacterial activity of Chalcones derivatives **(3a-e).**

Antimicrobial Activity of Synthetic Compound (In mm)								
	Organisms							
Compounds	A	B	C	D	E	F	G	H
Chloramphenicol	+++	+++	+++	+++	+++	++	++	++
3a	-	-	-	++	-	-	-	-
3b	++	-	-	++	++	-	-	++
3c	++	-	-	++	-	-	-	++
3d	-	++	-	-	-	-	-	-
3e	-	-	-	-	-	-	-	++
A: *Bacillus megaterium*, B: *Bacillus subtilus*, C: *Micrococcusluteus*, D: *Staphylococcus aureus*, E: *Eschericha coli*, F: *Enterobacter*, G: *Proteus vulgaris*, H: *Pseudomonas aeruginosa*								
+ : <6 mm (poor), ++ : <12 mm (good), +++ : <18 mm (Excellent), - : Without activity								

3.2.2 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 3c provided the best antifungal activity and compared well with the activity of fluconazole. The compounds 3a, 3d and 3e also possess promising antifungal activity.

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

Antifungal Activity of Synthetic compound(In mm)			
Compounds	Organisms		
	A	B	C
Fluconazole	++	+++	++
3a	+	++	+
3b	++	+	++
3c	++	++	+
3d	++	+	+
3e	+	++	+
A: Mucor, B: Penicillium, C: Aspergillus Niger			
+ : <25 mm (poor), ++ : <50 mm (good) +++ : <75 mm (Excellent)			

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