International Journal of Engineering Research and Modern Education (IJERME)
ISSN (Online): 2455 - 4200
(www.rdmodernresearch.com) Volume I, Issue I, 2016

SYNTHESIS, CHARACTERIZATION BIOLOGICAL SCREENING OF SOME NOVEL TRIAZOLE DERIVATIVES

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Abstract:

An approach for the synthesis of new compound 1- benzotriazole -4-yl methyl-1H-benzotriazole were prepared from 1H benzotriazole was suspended in ethanol containing dimethyl formide, then formalin and benzotriazole were added to it with vigorous stirring. The structural elucidation of the synthesized compounds has been performed by FTIR, 1H NMR, 13CNMR and evaluated for antifungal and antibacterial activity against test organisms by Disc- method.

Key Words*:* Benztriazole, Dimethyl Formide, Formalin, Antifungal & Antibacteria

Introduction:

Triazoles have been reported to posse's antimicrobial activites[1-5]. Further, the benzotriazole derivatives constitute important classes of compounds possessing several pharmacological activities including antiviral[6], anti-inflammatory[7,8], and analgesic, activities. In view of this, we planned to synthesize some new one benzotriazole-4yl-methyl-1H-benzotriazole containing triazole varieties to get more potent compounds.

The reaction sequence for little compounds is outlined in scheme 1. One benzotriazole-4yl methyl-1H-1-benzotriazole was synthesized from a reaction of 1H-benzotriazole was suspended in ethanol containing dimethyl foramide. The suspension was warmed on a water bath and then Formalin and benzotriazole were added to it with vigorous stirring for two hour. The structures of new triazoles were established on the basis of their spectra data. These compounds showed uniform fragmentation pattern.

Materials and Methods:

Reagents and Solutions

Melting points were uncorrected and recorded on a REMI Series, Lab India Instrument. TLC analysis was done using pre-coated silica gel plates and visualization was done by using iodine. IR spectra were recorded in KBr on Schimadzu FT-IR Spectrometer. ^{1}H & ^{13}C-NMR spectra were recorded on a Bruker (AC 400MHz) using TMS as an internal standard. Elemental analysis was carried out on a Perkin-Elmer series –II CHNS/O Analyzer 2400. All the chemicals were obtained from Aldrich and all the solvents used were of commercial grade only.

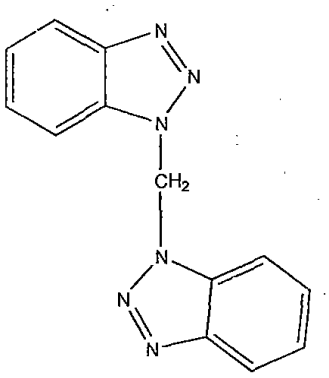
Chemistry

Synthesis of 1-benzotriazole-4-ylmethyl-1H- benzotiazole (I)

A mixture of 0.01 mole of 1H-benzotriazole was suspended in ethanol (20 mL) containing dimethyl formamide 6 mL. The suspension was warmed on a water bath and then formalin 0.01 mole and 0.01mole of benzotriazole were added to it with vigorous stirring for 2 hours. It was poured on crushed ice with constant stirring and kept for sometime filtered, dried and recrystallized from distilled water compound were synthesized.

The NMR spectra of the above compound shows three signals A multiplet at δ 7.89-

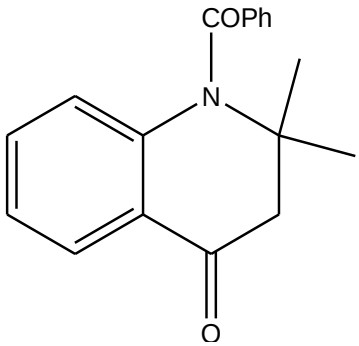
8.07, A multiplet at δ 7.40-7.61 singlet at δ 6.02-6.16. The multiplet at δ 7.89-8.07 is assigned due to the two aromatic protons 4_{Hb} of the benzotriazole. Another multiplet at δ 7.40-7.61 is assigned due to the two aromatic protons 4Ha of the benzotriazole. A singlet at δ 6.02-6.16 is due to the presence of methylene group of two protons (2_{Hc}) attached with two benzotriazole rings.



Synthesis of 3-methyl-1-morpholin-4-ylmethy1-2, 6-diphenylpiperidin-4-one (II)

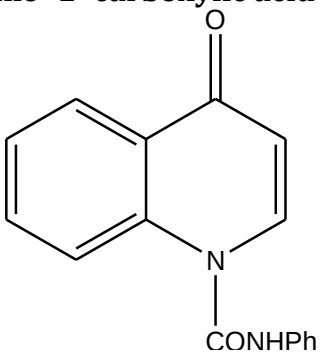
The NMR spectra of the above compound shows nine signals multiplet at δ 7.20 -7.37, multiplet at δ 7.38-7.47, doublet at δ 7.1-7.2, singlet at δ 4.2-4.3, singlet at δ 3.24-3.4, triplet at δ 3.5-3.6, triplet at δ 2.8-2.9, singlet at δ 2.5 and singlet at δ 1.2. The multiplet at δ 7.20 -7.37 is assigned to the four aromatic protons for both phenyl groups attached in piperidone ring. A multiplet at δ 7.38-7.47 is assigned to the four aromatic protons for both phenyl groups attached in piperidone ring. The doublet at δ 7.1-7.2 is assigned due to the H_c of the aromatic protons. The singlet at δ 4.24.3 is assigned due to the presence of the H_e protons of piperidone ring. The singlet singlet at δ 3.24-3.4 is assigned due to H_i and H_j of the piperidone and methylene protons. The triplet at δ 3.5-3.6 is assigned due to the $4H_g$ protons of the piperidone ring. The triplet at δ 2.8-2.9 is assigned due to the H_f methylene protobs of the morpholine. The singlet at δ 2.5 is assigned due to the H_i protons of piperidone. The singlet at δ 1.2 is assigned to the $3H_d$ protons of the piperidone.

Synthesis of 1-Benzoyl- 2,2- dimethyl-2,3-dihydro-1H-quinolin-4-one (III)



^{1}HNMR Spectral Data (DMSO/TMS), δ values in ppm
δ 7.9 (d, 1H), 7.8 (s, 1H,), 7.7 (s, 1H), 7.4 (d, 2H), 7.5 (s), 2.8 (s, 3H), 1.3 (d).
^{13}CNMR Spectral Data (DMSO/TMS), δ values in ppm
δ198 (carbonyl, C-2), 163 (1- amide), 132 (C-8a), 138 (Cl,C-Ar), 131 (C-6), 133 (Cl,C-Ar), 128.1 (C-2'), 124 (C-8), 129 (C-4a), 123, 54 (aliphatic- C), 25.8 (aliphatic -C).

Synthesis of 4-Oxo- 4H-quinoline -1- carboxylic acid phenylamide (IV)



^{1}HNMR Spectral Data (DMSO/TMS), δ values in ppm
δ 7.8 (s, 1H,), 7.7 (s, 1H), 7.6 (d, 2H), 7.3 (d), 7.5 (s), 6 (s, NH)
^{13}CNMR Spectral Data (DMSO/TMS), δ values in ppm
δ 187 (carbonyl, C-2), 147 (N- urea), 132 (C-8a), 139 (C-Ar), 138 (C-6), 134 (C-Ar), 130, 128.6 (C-2'), 113.7(1-ethylene), 13.8 (aliphatic -C).

Antimicrobial Screening:

The screening of newly synthesized antimicrobial chemotherapeutic compounds provides effective measures for dreadful disease. The biochemical studies will help to test the efficacy and existing of the compounds that are antimicrobial in nature.

In this work the compounds I and II were tested against gram positive bacteria and gram positive bacteria. This test is just finding out whether both gram positive and gram negative organisms are equally susceptible to these compounds.

Media for the inoculation of bacteria:

The following medium was used for bacterial culture,

Peptone	5.0 g
Beef extract	3.0 g
Sodium chloride	3.0 g
Bactosugar	20.0 g
Distilled water	1000 ml

The above constituents were dissolved by heating the medicine at 80°C and the pH of the medium was adjusted to 7.2 this medium was sterilized 15/lb pressure for 20 minutes. The cultures were maintained on slants by periodic sub culturing at a ten days interval.

Preparation of Assay Medium:

The following medium which meets the growth requirement of both gram positive and gram negative bacteria was used to prepare the slants and plates.

Dipotassium hydrogen phosphate	-	7.0 g
Potassium dihydrogen phosphate	-	3.0g
Sodium citrate	-	0.5g
Magnesium sulphate	-	0.1 g
Ammonium chloride	-	2.0 g
Peptone	-	2.0 g
Glucose	-	4.0 g
Bactoagar	-	20.0g
Distilled water	-	1000ml

The medium was dispensed into 5.0 ml test tubes and covered with cotton. The test tubes sterilized at 15/lb pressure for 20 minutes and slanted. Bacteria were inoculated into slants under sterile conditions and were incubated at 37°C. They were sub cultured in fresh slants for every ten days.

The above autoclaved medium was poured that in sterilized Petri dishes with an even depth throughout for the preparation of plates.

Testing of Antimicrobacterial Activity:

The antimicrobacterial activity of the 4-benzotriazol-1-ylbutan-2-one and 1-morpholin-4-ylmethy1-1H-benzotriazole were studied by Disc- method of Norris and Ribbons[17] with necessary modifications, it is well known that various factors like media composition pH, diffusion property of the compounds and inoculums' concentration will influence. The size of zone of inhibition, hence it's necessary to maintain sloringent standardization for the reproducible results.

Disc Method:

The 4-benzotriazol-1-yibutan-2-one and 1-morpholin-4-ylmethy1-1H-benzotriazole were dissolved in DMSO and filter paper disc of 5mm diameter (whatman No.1) containing, (25 lag and 12.5 lag/disc) were prepared the discs were allowed to dry in an hot air oven at 80°C for the solvent evaporation. Disc socked in DMSO alone were similarly dried and used as control.

Procedure:

The plates containing nutrient agar were seeded with different organisms at a concentration of 2-3x10 colony forming unit (CFU) using sterile swab. The filter paper disc containing the synthesized compounds were placed on the surface of the bacterial seeded agar plates and incubated at 37°C for 24 hours.

The diameter of the inhibition zones were measured in four directions and averages were taken into account and compared with control disc from the diameter of inhibition zones the radius of the inhibition zones were calculated. The agar medium itself has the dispersion property; it takes up the compounds from the disc. Antimicrobial activity of benzotriazole and quinoline derivatives expressed as the inhibition produced by the disc diffusion method.

The antimicrobial activities for the given samples (III, IV and V) were carried out by Disc Diffusion Technique (Indian Phannacopoeia 1996, Vol 11 A-105). The test microorganisms of Gram positive *Staphylococcus aureus,* and Gram negative *Escherichia coli,* and Fungi *Canchda albicans, Aspergillus niger* were obtained from National Chemical Laboratory (NCL) Pune and maintained by periodical sub culturing on Nutrient agar and Sabouraud dextrose medium for bacteria and fungi respectively. The effect produced by the sample was compared with the effect produced by the positive control (Reference standard Ciprofloxacin 2µg/disc for bacteria and Fluconazole 10µg/disc for fungi).

Discussion:

Both the compound (I) [1-benzotriazol-4-ylmethy1-1H-benzotriazole] and compound (II) [3-methyl-l-morpholin-4-ylmethy1-2,6-diphenylpiperidin-4-one] showed significant antimicrobial activities against gram positive *(staphylococcus aerues* and *bacillus subtills)* and gram negative bacteria *(Escherichia coli* and *Pseudomonas aureginoso).* From the table, it clearly indicates that both compound (I) showed more or less equal activity against *S.aureus, B.subtills* and *E.coli and P.aiureginaosa* with the standard drug. It also indicates that the synthesized mannich bases showed higher inhibition zone than that of parent compound 1 H-Benzotriazole. The results are summarized in table:

Compound	Zone of inhibition (mm)			
	S.aureus (Gram +ve)	*B.subtills* (Gram +ve)	*E.coli* (Gram -ve)	*P.aureginosa* (Gram –ve)
Standard (ciprofloxacin)	**16**	**17**	**15**	**14**
Compound (I)	12	13	15	15
DMSO	4	4	4	4

The compound II, compound III and compound IV showed significant antimicrobial activities against gram positive (Staphylococcus aureus) and gram negative (*Escherichia coli)* Form the table; it clearly indicates that compound II, III and IV with standard drug The obtained results are tabulated as follows.

S. No	Samples	Diameter of zone of inhibition in mm			
		Staphylococcus aureus	***Escherichia coli***	***Canada albicans***	***Aspergillous niger***
1	Compound II	9	9	9	11
2	Compound III	NI	9	9	9
3	Compound IV	NI	8	9	9
	Std	**17**	**15**	**10**	**12**

NI — No inhibitory effect

Standard - Ciprofloxacin 2µg/disc for bacteria, Nystatin l0µg/disc for fungi. Solvent- DMS0 (Showed nil effect against the microorganisms under test)

Conclusion:

The present work describes the synthesis of Mannich bases of 1 Hbenzotriazole and quinoline and their pharmacological screening.

When 1H-benzotriazole is condensed with formaldehyde in DMF and ethanol it yielded 1-benzotriazol-4-ylmethyl-1H-benzotriazole.

Similar procedure was followed to prepare the Mannich base 3-methyl-1-morpholin-4-ylmethyl-2,6-diphenylpiperidin-4-one.

The compounds III and IV were simply characterized and the structure were confirmed through IR and NMR spectra

The NMR spectra of the synthesized compounds showed the expected absorption frequency range and signals.

The compounds were subjected to antimicrobial activity by paper disc method / disc diffusion technique. They showed good inhibition power.

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