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SYNTHESIS AND ANTI-MICROBIAL SCREENING OF SOME NOVEL N-(5-BENZYL- 1, 3, 4-OXADIAZOL-2-YL)-2-CHLOROACETAMIDE AMINO DERIVATIVES

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ABSTRACT

Objective: The objective of this study is to synthesize some novel N-(5-Benzyl-1, 3, 4-oxadiazol-2-yl)-2-substituted chloroacetamide derivatives and to study their anti-microbial activity. **Materials and Methods:** Condensation of hydrazine carboxamide with phenyl acetic acid gave 2-benzyl-1,3,4-oxadiazole amine (I) which on treatment with chloroacetyl chloride at 80 °C yielded N-(5-benzyl-1,3,4-oxadiazol-2-yl)-2-chloroacetamide (II). Further, compound II reacted with various substituted amines in presence of glacial acetic acid to furnish N-(5-benzyl-1, 3, 4-oxadiazole-2-yl)-2-acetamide derivatives. The structures of the synthesized compounds have been established based on their analytical and spectral data. **Results:** The synthesized compounds were evaluated for their anti-microbial screening using *in vitro* free radical scavenging assay for their antibacterial activities. Compounds **III a** exhibited significant antibacterial activity. **Conclusion:** The obtained results clearly revealed that most of these derivatives showed promising broad spectrum antibacterial activity when compared to that of the standard drugs. We appreciate further detailed studies with these drugs as potential antimicrobial agents.

KEY WORDS: 1, 3, 4-oxadiazole, anti-microbial activity, *Escherichia coli*, *Streptococcus aureus*.

1.0 INTRODUCTION

Oxadiazoles are the heterocyclic compounds containing one oxygen and two nitrogen atoms in a five membered ring structure.[1] 1, 3, 4-oxadiaole derivatives show better metabolic stability, water solubility and lower lipophilicity.[2] There are numerous literature reports confirming the multidirectional effect of compounds containing the 1, 3, 4-oxadiazole ring in its structure. Derivatives of this type have antibacterial, antimalarial, anti-inflammatory, anti-depressive, anticancer, analgesic and antiviral effect.[3-4] The literature review reveals that 1, 3, 4-oxadiazoles and their amino derivatives are known have promising antibacterial activity.[5-6] 1, 3, 4-oxazdiazole have been known for more than eighty years, but it is only in the last decade that investigations in this molecule have been intensified. This is primarily due to the number of uses of 1, 3, 4-oxadiazoles in the most diverse areas. The compounds bearing 1, 3, 4-oxadiazoles currently used in clinical medicine such as an antiretroviral, anticancer agents. They are present in some antihypertensive drugs and antibiotics.[7]

There are various problems arising with the use of antimicrobials such as local tissue irritation, interference with wound healing process, hypersensitivity reactions, systemic toxicity, narrow antimicrobial spectrum, emergence of resistance. A wide variety of heterocyclic systems have been explored for developing pharmaceutically important molecules.[8] Among them the derivatives of oxadiazoles have been playing vital role in the synthetic and medicinal chemistry.[9] Oxadiazole moiety and its various derivatives have been explored in past years and is still be used for future development of new drugs against various pharmacological and pathological conditions.[10] Further 1, 3, 4-oxadiazole heterocycles can contribute substantially in increasing the pharmacological activity by participating in hydrogen bonding interactions with the receptors. Substituted 1, 3, 4-oxadiazoles are of considerable pharmaceutical and medical interest.[11-14] Resistance to number of antimicrobial agents among a variety of clinically significant species of bacteria is becoming an increasingly important global problem.[15] So, increasing the clinical importance of drug-resistant microbial pathogens has lent additional urgency in microbiological research. Oxadiazole derivatives have been found to possess broad spectrum antimicrobial activity and therefore are useful substructures for further molecular exploration.[16-17]

Oxadiazole has attracted wide attention of the researchers in search of new therapeutic molecules. Out of its four isomers, 1, 3, 4-oxadiazole moiety is widely exploited for various applications.[18] The structural modification leads to variation in antimicrobial activities of the

molecules.[19-22] This prompted us to extend the research to synthesize N-(5-Benzyl-1, 3, 4-oxadiazol-2-yl)-2-substituted chloroacetamide derivatives with an aim to screen their antibacterial activity. Also, literature survey revealed that various oxadiazole derivatives exhibited antimicrobial, anti-inflammatory, antifungal, antitubercular, anticonvulsant and anticancer activities. Development of new potent antimicrobial agents still a challenge and motivated by the mentioned data, the present study aims at the synthesis and antimicrobial activity investigations of some novel 1, 3, 4-oxadizoles derivatives.

2.0 MATERIALS AND METHODS

2.1 Synthetic Approach

STEP-1: Synthesis of 5-Benzyl-1, 3, 4-oxadiazol-2-amine (I)

In a 250 mL round bottomed flask, 5-benzyl-1, 3, 4-oxadiazole 2-amine (I) was synthesized by refluxing 1.87 g of hydrazine carboxamide in 6.5 mL phenyl acetic acid and few drops of H_2SO_4 for 12 h at 65-75 ^oC. The obtained precipitate was then cooled and filtered with distilled water and dried.

STEP-2: Synthesis of N-(5-Benzyl-1, 3, 4-oxadiazol-2-yl)-2-chloroacetamide (II)

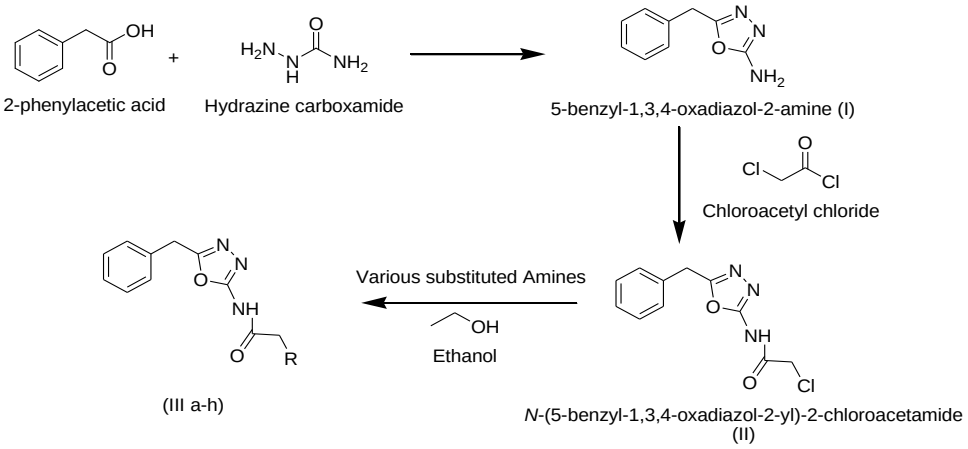
Compound-I in ethanol and substituted amine were added. Along with the mixture, few drops of glacial acetic acid was added and refluxed for nearly 5-6 h. The product formation was checked out through TLC. Then the reaction mixture was cooled and poured into ice cold water to get the solid product. The solid was filtered, washed with cold water, dried and recrystallized with ethanol.

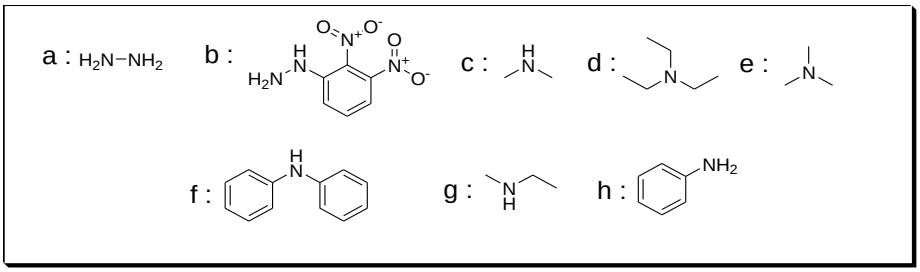
STEP-3: Synthesis of N-(5-Benzyl-1, 3, 4-oxadiazol-2-yl)-2-chloroacetamide amino derivatives (III a-e)

Compound-II in ethanol and substituted amine (a-e) were added. Along with the mixture, few drops of glacial acetic acid was added and refluxed for nearly 5-6 h. The product formation was checked out through TLC. Then the reaction mixture was cooled and poured into ice cold water to get the solid product. The solid was filtered, washed with cold water, dried and recrystallized with ethanol.

The synthesized molecules were consistent with their assigned spectra such as IR, NMR spectral data conformed their formation. The completion of the reaction was checked by using TLC. Finally the synthesized compounds were evaluated for their antibacterial activity.

2.2 SCHEME





2.3 ANTI-BACTERIAL EVALUATION

Microorganism used : *Escherichia coli* (G^{-ve})

Streptococcus aureus (G^{+ve})

Standard used : Amoxicillin (against *E. coli*)

Clindamycin (against *S. aureus*)

Assay : Cup Plate Method

Sample : III (a-e)

Requirements

- Nutrient Agar
- Petri dishes
- Test organism
- Sterile Pipettes
- Test sample
- Standard drug

Determination of antimicrobial activity-In brief

- Each bacterial strain was suspended in nutrient broth and incubated for 12 h at 37 ^{o}C. Nutrient agar (NA) was used for testing the antibacterial activity.

- Nutrient Agar (NA) plates were seeded with 12 h broth culture (young culture) of different bacteria (0.1mL).
- In each of these plates, 2 wells (10 mm) were cut out using sterile cork borer.
- Using sterilized dropping pipettes, 0.1mL of test sample was carefully added into the wells and allowed to diffuse at 4 ^{o}C for 2 h.
- The plates were then incubated at 37 ^{o}C for 18-24 h for checking the inhibitory/resistance activity was evaluated by measuring the diameter of inhibition zone.
- The experiment was carried out in triplicate and the mean of the diameter of the inhibition zones was calculated.

3.0 RESULTS AND DISCUSSION

Analytical data of synthesized compounds

All the synthesized compounds were first analyzed by performing thin layer chromatography until single spot is obtained. The synthesized compounds were completely characterized by IR, ^{1}H NMR data. The IR spectra of compounds showed broad bands in the region 3100-3400cm^{-1} due to NH stretching. The ^{1}H NMR spectra also support the structure of the synthesized compounds. All the compounds showed the peaks for aromatic hydrogens in ^{1}H NMR spectroscopy between 6-8 ppm. All the analytical data showed satisfactory results were shown in Table 1 & 2.

Table 1: IR data of some synthesized compounds.

COMPOUNDS	IR Data
I	**IR (Cm-^{1}):** 3195(N-H), 3056(Alkane st C-H), 1655(st C-O-C), 861(Ar bd C-H), 1587(st N-H).
III (a)	**IR (Cm^{-1}):** 3322(N-H), 3087(N-H 2^{o}Amine), 1629(Ar st N-H), 1570(st Amine C-N), 1490(st N-O), 1409(C-H), 827(Ar C-H).
III (b)	**IR (Cm^{-1}):** 1074(N=N)
III (c)	**IR (Cm^{-1}):** 3198(N-H), 1654(C-O-C), 1580(C=N), 1453(R_3N), 1328(C-H), 864(Ar ring).
III (d)	**IR (Cm^{-1}):** 3200(N-H), 1654(C-O-C), 1580(C=N), 1432(R_3N), 1327(C-H), 884(Ar ring), 847(CCl^{-}).
III (e)	**IR (Cm^{-1}):** 3200(N-H), 1654(C-O-C), 1579(C=N), 1426(R_3N), 1318(C-H), 864(Ar ring), 847.90(CCl^{-}).

Table 2: NMR data of some synthesized compounds.

COMPOUNDS	NMR data
III (a)	**NMR (ppm):** NH (12.449), Aromatic ring (7-8), Hydrazine NH (4.013), Benzyl CH_2 (2.543), -CH_2 (2.500), NH_2 (1.960).
III (e)	**NMR (ppm):** NH (12.196), Aromatic ring (6-9), Benzyl CH_2 (2.509), -CH_2 (4.0), 3 CH_3 groups (2.0).

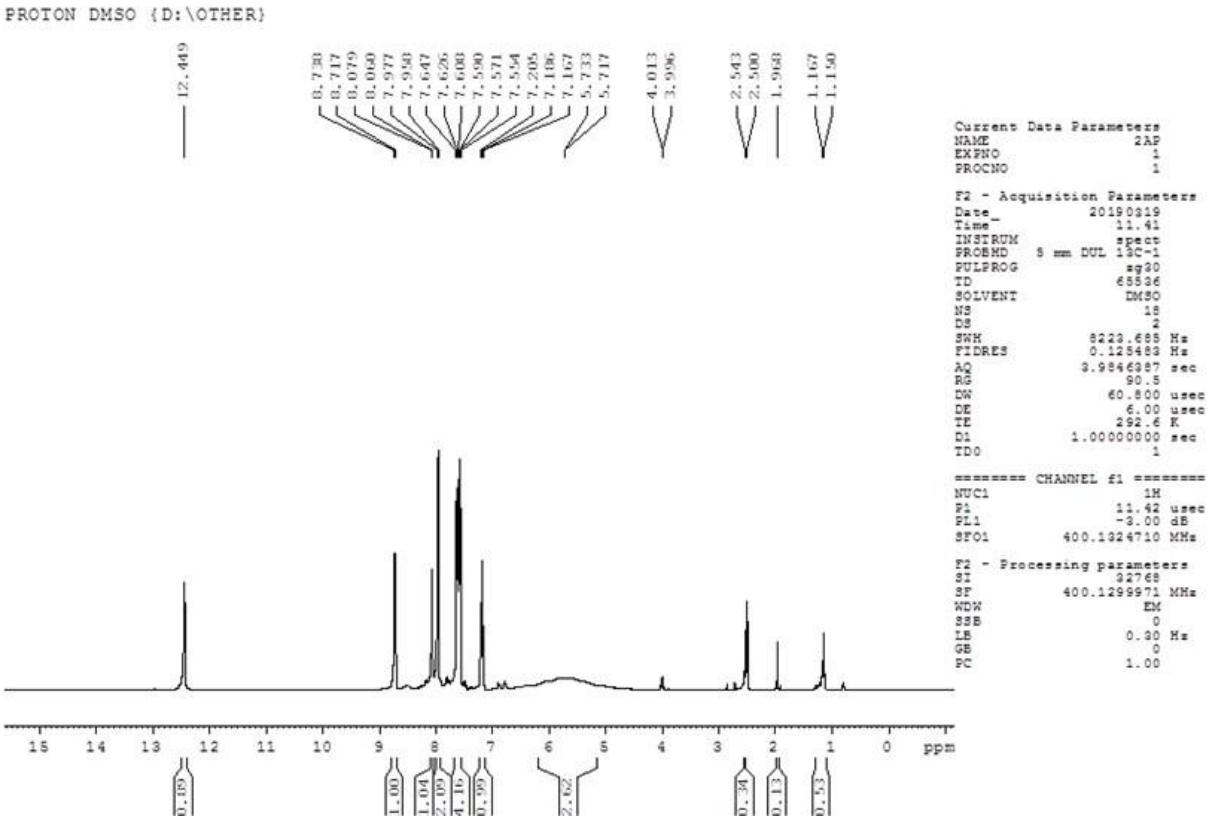


Figure 1: NMR Spectra for the compound III (a).

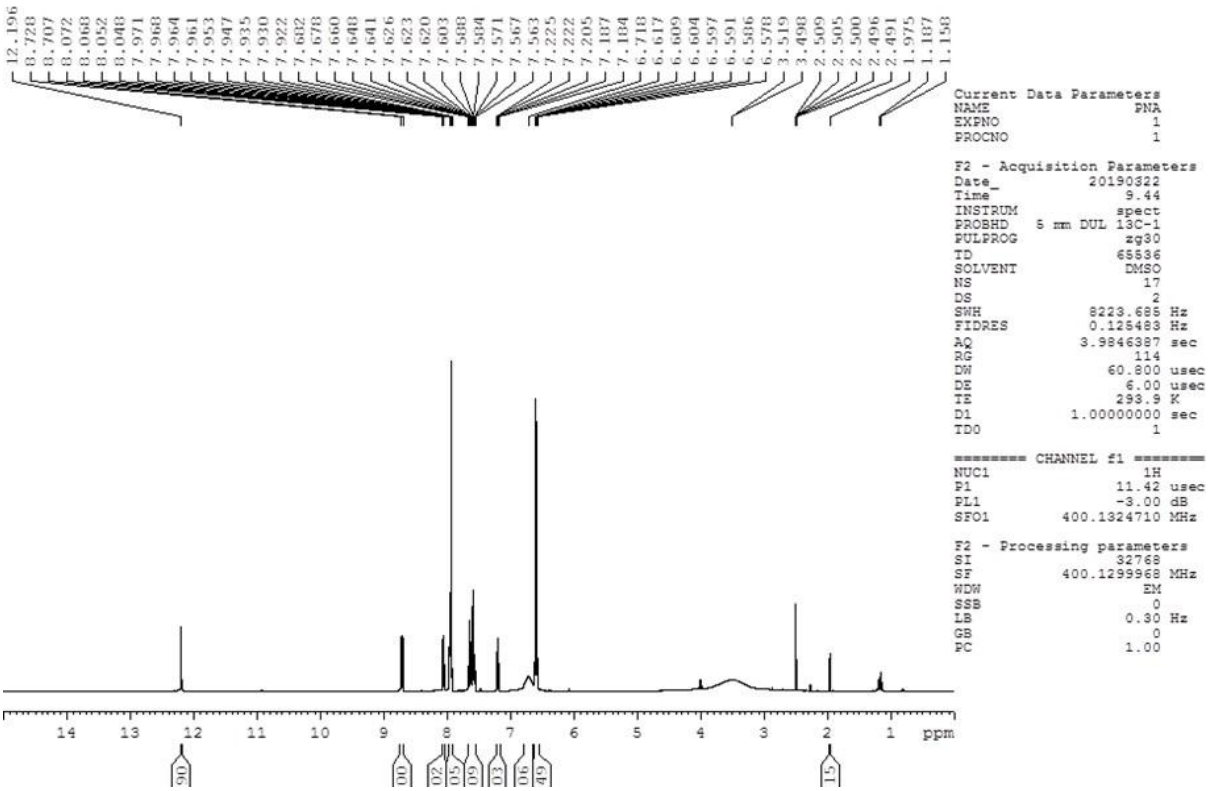


Figure 2: NMR Spectra for the compound III (e).

3.1 Anti-bacterial screening

All newly synthesized compounds were tested for their *in vitro* antibacterial activity against Gram-positive bacterium such as *Streptococcus aureus* as well as Gram-negative bacterium such as *Escherichia coli* by cup and plate method. The standard used for the comparison is amoxicillin (against *E.coli*) and clindamycin (against *S. aureus*) and used at a concentration of 100 µmol/mL. DMSO was used as solvent. The activity towards the bacterium was calculated from the zone of inhibition.

Analysis of the results revealed that the compound **III a** showed higher activity compared to that of standard amoxicillin and clindamycin against *E.coli* and *S. aureus* respectively. The following compounds showed significant activity against gram positive and gram negative bacteria compared to that of standard drug.

Comparison between synthesized compound against Gram positive and Gram negative bacteria

Comparing the gram positive and gram negative bacteria of all the synthesized compounds, the synthesized compound **III a** shows highest activity against gram negative bacteria than gram positive bacteria. Table 3 summarizes the results of antibacterial activity of the compounds against various bacteria.

Table 3: Preliminary Screening for antimicrobial activity of the synthesized compounds against standard organism.

Sl. No	Micro Organism	Sample code	Compound No.	Mean Diameter Inhibition Zone (mm)
1.	**Escherichia coli**	M1	III a	**32**
		M2	III b	25
		M3	III c	16
		M4	III d	20
		M5	III e	22
2.	**Streptococcus aureus**	M1	III a	26
		M2	III b	22
		M3	III c	14
		M4	III d	18
		M5	III e	22
3.	**Amoxicillin (Against *E.coli*)**	-	-	32
4.	**Clindamycin (Against *S. aureus*)**	-	-	30

CONCLUSION

The novelty in our study was that we have investigated the antimicrobial property of some new N-(5-Benzyl-1, 3, 4-oxadiazol-2-yl)-2-chloroacetamide derivatives. The obtained results clearly revealed that most of these derivatives showed promising broad spectrum antibacterial activity when compared to amoxicillin and clindamycin respectively.

Conflict of Interest

The authors have no conflict of interest in the subject matter or materials discussed in this article.

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