EXECUTIVE SUMMARY OF MINOR RESEARCH PROJECT

1. Title of the Project	:	"Molecular Structure and Vibrational Spectroscopic Analysis of some Antimicrobial Activity Compounds – A Combined Experimental and Quantum Chemical Approach"
2. Name and Address of the Principal Investigator	:	Dr. F. Liakath Ali Khan Associate Professor of Physics & Controller of Examinations Islamiah College (Autonomous) Vaniyambadi- 635 752
3. Name and Address of the Institution	:	Islamiah College (Autonomous) Vaniyambadi – 635 752 Tirupattur District, Tamil Nadu.
4. UGC Approval Letter No. and Date	:	F. MRP- 6848 /16 (MRP/UGC-SERO), Dated 30.06.2017
5. Date of Implementation	:	15.08.2017
6. Tenure of the Project	:	2 Years
7. Total Grant Allocated	:	Rs. 307,550.00
8. Total Grant Received	:	Rs.2,94,050.00
9. Final Expenditure	:	Rs. 312,096.00
10. Title of the Project	:	"Molecular Structure and Vibrational Spectroscopic Analysis of some Antimicrobial Activity Compounds – A Combined Experimental and Quantum Chemical Approach"
11. ***Objective of the Projective***	:	

The objective of the Minor Research Project on **Molecular Structure and Vibrational Spectroscopic Analysis of some Antimicrobial Activity Compounds – A Combined Experimental and Quantum Chemical Approach** is Synthesis and Characterization of some novel halogenoderivatives of benzimidazole compound using Computational, Experimental FT-IR, UV-Visible, ^{1}NMR and Antimicrobial Activity Studies and to correlate the results obtained from Computational and Experimental studies of synthesized compounds with Antimicrobial activity and Molecular docking studies.

12. ***Whether objectives were achieved :***

Yes. The Principal Investigator extensively reviewed the literature related to Minor Research Project and came to conclusion to focus studies on ***Molecular Structure and Vibrational Spectroscopic Analysis of Some Antimicrobial Activity Compounds – A Combined Experimental and Quantum Chemical Approach.*** On the basis of this concept four novel halogenoderivatives of benzimidazole compounds such as 6-nitro-2-(4-nitrophenyl) -1H-benzimidazole, 6 amino-2-(4 nitrophenyl) -1H-benzimidazole, 6-chloro-2-(4 aminophenyl) - 1H-benzimidazole and 6 bromo -2-(4 chlorophenyl) -1 H benzimidazole were synthesized and characterized with the help of computational method and experimental studies such as UV-Visible spectroscopy, FT-IR spectra in the region of 4000-400 cm-1 1H NMR technique and antimicrobial activity disc diffusion method. The results obtained from computational method are compared with experimental, molecular docking and antimicrobial studies. Hence the study archived the concept and all the objective of the Minor Research Project to the greater extent.

13. ***Achievements from the Project:***

The Minor Research Project on "***Molecular Structure and Vibrational Spectroscopic Analysis of Some Antimicrobial Activity Compounds – A Combined Experimental and Quantum Chemical Approach"*** has enlightened the importance of antimicrobial activity of four synthesized novel halogenoderivatives of benzimidazole compounds. The synthesized compounds were evaluated for *in vitro* anti-bacterial activity against Gram-positive and Gram-negative bacteria. These are the agents commonly causes urinary tract infection, bronco pneumonia and bronchitis infections.

Spectral studies such as FT-IR in the region of 4000-400 cm^{-1}, UV-Visible and

^{1}H NMR experimental techniques and computational theoretical studies such as

molecular structure parameter, vibrational frequencies, electronic absorption spectra, HOMO-LUMO, molecular electrostatic potential (MEP), Natural bond orbital (NBO), of all halogenoderivatives of benzimidazole compound investigated using the HF and DFT (B3LYP, B3PW91) methods with 6-31+G (d, p) and 6-31++G (d, p) basis sets support with experimental data.

As per data obtained, it was confirmed that all the synthesized compounds possessed anti-bacterial activity against Gram-positive and Gram-negative organism. The selected microorganism for investigations were ***Staphylococcus aureus (MTCC-3160), Enterococcus faecalis (MTCC-3159) as Gram –positive bacteria and Pseudomonas aeruginosa (MTCC-4030), Escherichia coli (MTCC-1667) as Gram-negative bacteria and two fungal strains viz. Aspergillusniger (MTCC 282), Candida albicans (MTCC 227).*** The above microorganisms were chosen for present investigation, based on their clinical and pharmacological importance. In this present study all the synthesized compound shows their active against both types of the bacteria and fungi, which may indicate broad spectrum of properties this remarkable activity of synthesized compound may be arising from the benzimidazole ring, which plays an vital role in the anti-microbial activity and also due to nitro group attached to the benzimidazole ring hence it is noted that there is direct relationship between the biological activity and electron withdrawing group. The present study has revealed the antibacterial activity of all the synthesized compounds behave as potential source of useful drugs.

14. Summary of the Findings:

In resent year the main focus of medicinal chemistry has increases towards the synthesis and characterizing of benzimidazole derivatives are due to the remarkable medicinal and pharmacological properties of its derivatives. Benzimidazole occupies a central place in the class of heterocyclic compound used in pharmaceutical and medicinal chemistry. This compound is bicyclic in nature, which consists of the fusion of benzene and imidazole.

Nowadays infectious microbial diseases are causing many problems in world-

wide, because of resistances to many numbers of antimicrobial agents, despite of the availability of a huge numbers of antimicrobial compounds, the main matter of concern in the treatment of antimicrobial infections is the limited number of efficacious antimicrobial drug. Many of the currently available medicines are toxic, which enable recurrence because they are bacteriostic or fungistic and not bactericidal or fungicidal or lead to the development of resistance due to prolonged periods of administrations.

Apart from its applications in medicinal field, its application has been further expanded in the area of Nano material chemistry as optical sensors, NLO materials, with special application in environmental science, chemical technology and has obvious applications over other sensing devices such as easy of operations and low cost materials. Benzimidazole also proved to be an essential core for organic light emitting devices (OLEDs) with superior phosphorescence, thermal properties and morphological stabilities.

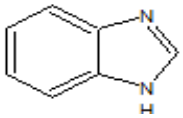
In order to expand the group of benzimidazole derivatives, we synthesized several new benzimidazole ring containing compounds. The O-phenylene diamine reacted with appropriate carboxylic acid under harsh dehydrating reaction condition to give the corresponding benzimidazole in good yield by Phillips reaction.

The synthesis, characterization and the study of antimicrobial activity of some novel halogenoderivatives of benzimidazole compounds remain a main focus of our project. Since this compounds exhibit a large number of biological activities towards antioxidant, Antimicrobial activity, antiflammatory – analgestic, anticancer, CNS depressant, androgen receptor antagonist, antitubercular, antihelmintic, diabetic drugs, anti-ulcer, anticonvulsant, antiviral-antifungal and antiprotozoal, It may be used in treatment of cardiovascular disease, neurology, and endocrinology. In addition the benzimidazole have played a very important role in the development of theory in heterocyclic chemistry and also extensively in organic synthesis. Benzimidazole nucleus is present in vitamin-B12.

In the present study, the antibacterial and antifungal activity four novel halogenoderivatives of benzimidazole compounds were synthesized, evaluated with the help of computational studies and tested against different pathogenic bacterial strains and fungal strains. The halogenoderivatives of benzimidazole compound in

6-position is scare in the literature. Introduction of halogenations substitution in 6-position of benzimidazole gives characterizing drug are used in clinical applications.

Properties of the base compound

Base compound	:	**Benzimidazole**
Structure	:	
Formula	:	$C_7 H_6 N_2$
Molecular weight	:	118.17
Toxicity	:	Oral rat LD50:2910mg/Kg
Molar Refractivity	:	36.61±0.3 cm^3
Molar Volume	:	95.01 ± 3.0 cm^3
Index of Refraction	:	1.696 ± 0.02
Surface Tension	:	60.1 ± 3.0 dyne/cm^3
Density	:	1.242 ± 0.069 g/cm^3
Polarizibility	:	14.51 ± 0.5 cm^3
Synonyms	:	H-Benzimidazole; 1,3-benzodiazole;benzoglyoxaline
Melting point	:	176° C
Boiling point	:	360° C
Specific gravity	:	1
Solubility in water	:	Slightly soluble
Auto ignition	:	538° C
Stability	:	Stable under normal temperature and condition

Plan of the work:

1. To Synthesis and Characterize of 6-nitro-2-(4-nitrophenyl) -1H-benzimidazole

2. To Synthesis and Characterize of 6 amino-2-(4 nitrophenyl) -1H-benzimidazole

3. To Synthesis and Characterize of 6-chloro-2-(4 aminophenyl) - 1H-benzimidazole

4. To Synthesis and Characterize of 6 bromo -2-(4 chlorophenyl) -1 H benzimidazole

Studies under Investigation:

a) Experimental Studies using UV- visible spectroscopy, FT-IR spectra in the region of 4000-400 cm^{-1} and 1H NMR technique.

b) HOMO-LUMO energy band gap determination

c) Electrostatic Potential (MEP) Analysis

d) Natural Bond Orbital (NBO) Analysis

e) Computation methods - HF and DFT (B3LYP, B3PW91) methods with 6-31+G (d,p) and 6-31++G (d, p) basis sets.

f) Atomic Orbital (GIAO) analysis

g) Molecular Docking

h) Ramachandran Plot

i) Evaluation of anti-bacterial and anti-fungal activity

- The theoretical vibrational spectra of 6-nitro-2-(4-nitrophenyl)-1H-benzimidazole were calculated at the HF and DFT (B3LYP/B3PW91) methods with the 6-31+G(d,p) and 6-311++G(d,p) basis sets.

- FT-IR spectrum was recorded for both the experimental and theoretical methods. From FTIR spectra The C-H Symmetric stretching vibration of the title compound observed in and around the region of 3100 cm^{-1} for all the

DFT and HF basic sets and 3114 cm^{-1}experimentally.

- The asymmetric C-H was observed at 3061cm^{-1} experimentally whereas for DFT /B3LYP [6-311++G(d,p)/6-31+G(d,p)]we observed 3098cm^{-1} /3117cm^{-1} for DFT /B3PW91 [6-311++G(d,p)/6-31+G(d,p)] 3092 cm^{-1}/3112cm^{-1} and for HF [6-311++G(d,p)/6-31+G(d,p)] 3031cm^{-1}/3052cm^{-1}.

- The HOMO-LUMO energy gap is very important parameter for the stability of the structure [37] and also it reflect the biological activity of the compound. The energy value of the band gap is 3.4155 eV for HOMO to LUMO and 3.7232 for HOMO to LUMO +1.This ensure that the compound is stable.

- The global hardness is another good indicator of chemical stability. The global hardness of 6-nitro-2-(4-nitrophenyl)-1H-benzimidazole compound is 1.7075, which indicates the good chemical stability and the compound is stable, electronegativity (χ) is calculated as 5.5157, which measure the attraction of an atom to electron. The extremely low global softness of 0.2928 observed theoretical shows that the compound is nontoxic. The Electrophilicity index (ω) was found to be 8.9086 this ensures that there is strong energy transformation between HOMO and LUMO

- The molecular docking binding energy (kcal/mol) was also obtained to be -5.36 kcal/mol. for title compound 6-nitro-2-(4-nitrophenyl)-1H-benzimidazole. From this study, the obtained protein Id is 3EQA and corresponding bonded residues are SER 483, ARG 184 and THR 486 with bond distance are 2.5, 1.9, 3.3 and 2.4. The low value of binding energy (-5.36) shows the bio-active nature of the title compound. From Ramachandra plot, it is concluded that the selected protein has majority of the residues in allowed region thus indicating the 3EQA protein to be a stable protein.

- The antimicrobial activities were carried for 6-nitro-2-(4-nitrophenyl)-1H-benzimidazole for four bacterial strains viz. ***Staphylococcus aureus (MTCC-3160), Enterococcus faecalis (MTCC-3159) as Gram –positive bacteria and***

Pseudomonas aeruginosa (MTCC-4030), Escherichia coli (MTCC-1667) as Gram-negative bacteria and two fungal strains viz. Aspergillusniger (MTCC 282),Candida albicans (MTCC 227).

- The compound 6-nitro-2-(4-nitrophenyl)-1H-benzimidazole showed the significant activity in following order: ***Staphylococcus aureus > Pseudomonas aeruginosa > Escherichia coli > Enterococcus faecalis and for fungal compound, Aspergillusniger > Candida albicans.***

- The spectral studies such as FT-IR, UV-visible and NMR for 6 amino-2-(4 nitrophenyl)-1H-benzimidazole was carried out. The υ(C-H) vibration of the 6 amino-2-(4 nitrophenyl)-1H-benzimidazole compound was observed at 3091-3097 cm^{-1} for DFT (B3LYP/B3PW91) calculations and 3062 cm^{-1} from experimental spectra and for HF the bands are observed in the region of 3030 cm^{-1}. This shows the strongly association of intermolecular hydrogen bonding of the benzimidazole compounds

- NBO analysis shows that the σ (N5-C13) participates as donor and the σ* (N4-C8) as acceptor and the intermolecular hyper conjugative interaction were observed with the stabilization energy of 173.23 Kcal/mol which reflects charge transfer takes place within the molecule.

- From HOMO and LUMO studies of 6 amino-2-(4 nitrophenyl)-1H-benzimidazole, the value of the energy separation between HOMO and LUMO is 2.8036 eV and 4.3793 eV for HOMO and LUMO+1. This low value shows that the title molecule is more reactive.

- The electrophilicity index (ω) is 6.7826, this high value shows that there is maximum flow between the donor and acceptor of the title compound. This ensures that, there is strong energy transformation between HOMO and LUMO.

- From the MEP studies of 6 amino-2-(4 nitrophenyl)-1H-benzimidazole, the negative region is localized on the oxygen atom of the nitrophenol ring N6, this is one of the possible sites for the electrophilic attack similarly the

positive region is localized around the H atoms of the benzimidazole ring indicates the possible site for nucleophilic attack.

- The molecular docking binding energy (kcal/mol) was also obtained to be -5.40 kcal/mol. The bonded residues corresponding to 3EQA protein is VAL 485, SER 484, ALA 110 and SER 107. This low value of binding energy shows the bio-active nature of the molecule.

- From Ramachandra Plot, the energetically allowed regions of the binding residue VAL 485,SER 484, ALA 110 and SER 107 has the torsion angle psi (ψ) against phi (ϕ) lying in the most allowed blue region as shown in the plot. From the plot, it is concluded that the selected protein has majority of the residues in allowed region thus indicating the 3EQA protein to be a stable protein.

- The antimicrobial activity for 6 amino-2-(4-nitrophenyl)-1H-benzimidazole compound was tested against ***Staphylococcus aureus (*****MTCC-3160),** ***Enterococcus faecalis*** **(MTCC-3159) as Gram –positive bacteria and** ***Pseudomonas aeruginosa*** **(MTCC-4030),** ***Escherichia coli*** **(MTCC-1667) as Gram-negative bacteria and** ***Aspergillusniger*** **(MTCC 282),*****Candida albicans*** **(MTCC 227)** as two fungal strains.

- The Gram-positive bacteria ***Staphylococcus aureus, Enterococcus faecalisca*** uses boils, skin infection. The Gram-negative bacteria ***Pseudomonas aeruginosa, Escherichia coli*** commonly causes urinary tract infection, respiratory infection. The fungus ***Aspergillusniger, Candida albicans*** causes bronchopulmons, urinary tract infection.

- 6 amino-2-(4-nitrophenyl)-1H-benzimidazole compound shows, it response to Gram positive bacteria more than the Gram negative bacteria. It shows strong antibacterial activity against the ***Staphylococcus aureus*** and ***Enterococcus faecalis*** this result is better than the reference compound, moderate activity is shown again the Gram-negative bacteria and fungi. This clearly indicates the title compound has the capacity of inhibiting the metabolic growth of the

investigating bacteria and fungi to some extent.

- The 6 amino-2-(4-nitrophenyl)-1H-benzimidazole compound showed the significant activity in the following order: ***Staphylococcus aureus*** > ***Enterococcus faecalis*** > ***Pseudomonas aeruginosa*** > ***Escherichia coli. For fungal activity, Aspergillusniger*** > **Candida *albicans.***

- The vibrational assignments for 6-chloro-2-(4-aminophenyl)-1H-benzimidazole were performed on the theoretically and predicted wave numbers by HF and DFT (B3LYP/B3PW91) methods with 6-311 G (d, p) and 6-311 ++ G (d, p) basis sets.

- For 6-chloro-2-(4-aminophenyl)-1H-benzimidazole, we observed this stretching of C-H vibration in the 3025-3040 cm^{-1} for all the DFT basis sets methods whereas for HF it is the region of 2960-2980 cm^{-1}. and for NBO analysis, the highest stabilization of the energy is from π*(N3-C5) to π*(C7-C11) with a value of 262.01 Kcal/mol.

- From HOMO LUMO studies, the highest occupied molecular orbital energy level is -5.681 eV and the lowest unoccupied molecular energy level is -2.9589 eV and the energy gap between them is 4.2472 eV. The other parameters such as electron affinity, electronegativity, global hardness and softness, and electrophilicity index can be determined using the HOMO-LUMO values. This shows the compound is stable and charge transfer take place within a molecule.

- The antimicrobial activity of title compound 6-chloro-2-(4-aminophenyl)-1H-benzimidazole shows an good anti-fungal against the ***Aspergillus niger*** the zone of inhibition is 18±0.6 to 31±0.4 for different concentrations . As the results are compared with reference compound Ciprofloxacin, the benzimidazole derivative shows a better activity than the reference drug this activity is due to presence of the Chloro group in the benzimidazole moiety and the ortho and para position of the halogen compound in the benzimidazole moiety will improve the antimicrobial inhibition activity against all tested organism.

- For compound 6-chloro-2-(4-aminophenyl)-1H-benzimidazole, the significant activity observed in the following order ***Staphylococcus aureus* > *Escherichia coli* > *Pseudomonas aeruginosa* > *Enterococcus faecalis and fungal activity Aspergillusniger* > *Candida albicans***
- From Molecular docking studies, the binding energy (kcal/mol) was also obtained to be -6.50 kcal/mol. The bonded residues corresponding to 3EQA protein is GLY 254 and ASP 369. This low value of binding energy shows the title compound is bio-active nature of the molecule. From Ramachandra Plot it is also concluded that the selected protein has majority of the residues in allowed region thus indicating the 3EQA protein to be a stable protein.
- The optimized molecular structure, vibrational frequencies, corresponding vibrational assignment of 6 bromo-2-(4-chlorophenyl)-1H-benzimidazole have been investigated. The spectrum of the title compound shows υ (N-H) strong bond in the region of 3511 cm^{-1} experimentally while the theoretical υ (N-H) bond for HF/DFT is observed in the region of 3490-3528 cm^{-1} this shows polymeric association of intermolecular hydrogen bonding.
- From NBO analysis, the hyperconjugative interaction was observed between the donor σ (C15-Cl25) to the acceptor σ* (C14-H23) with stabilization energy 13.42 kcal/mol. The interaction between the donor π (C10-C13) distributes the energy to anti bonding acceptor π* (C6-C11) with stabilization energy 19.43 kcal/mol. These are the some important transition for the stability of the title compound 6 bromo-2-(4-chlorophenyl)-1H-benzimidazole. From the studies of HOMO & LUMO the HOMO energy value is -6.2241 eV, LUMO energy value is -2.0035 eV, LUMO +1 energy value is -1.17444 eV and the energy value between the HOMO-LUMO is 4.2405 eV using DFT/B3LYP method.
- By using Auto dock software, 6-bromo-2-(4-Chlorophenyl)-1H-benzimidazole molecules are docked with 3EQA protein. The ligand of 6-amino-2-(4-nitrophenyl)-1H-benzimidazole molecules binds at the active site of the substrate. The molecular docking binding energy (kcal/mol) was also

obtained to be - 6.73 kcal/mol. The bonded residues corresponding to 3EQA protein is ASP 354. This low value of binding energy shows the title compound is bio-active nature of the molecule.

- The data obtained from all the synthesized compounds, shows all the compounds are stable and excellent antimicrobial activity against microorganisms such as ***Staphylococcus aureus (MTCC-3160), Enterococcus faecalis (MTCC-3159) as Gram –positive bacteria and Pseudomonas aeruginosa (MTCC-4030), Escherichia coli (MTCC-1667) as Gram-negative bacteria and Aspergillusniger (MTCC 282),Candida albicans (MTCC 227) as two fungal strains.***

15. Contribution to the Society:

Computer-aided drug design uses computational approaches to discover, new drugs. The ligand-based computer-aided drug discovery approach involves the analysis of ligands known to interact with a target of interest. Today's medicinal chemist is part of a team that handles essentially all the components of the drug discovery process. They need excellent communication and interpersonal skills in order to be able to function effectively as a part of a multi-functional and multi-dimensional project team that consists of biologists, computational chemists, structural biologists, high throughput screeners, , information management technologists, toxicologists, etc.

Nowadays infectious microbial diseases are causing many problems in world-wide, because of resistances to many numbers of antimicrobial agents, despite of the availability of a huge numbers of antimicrobial compounds, the main matter of concern in the treatment of antimicrobial infections is the limited number of efficacious antimicrobial drug. Many of the currently available medicines are toxic,

which enable recurrence because they are bacteriostic or fungistic and not bactericidal or fungicidal or lead to the development of resistance due to prolonged periods of administrations.

The study of Minor Research Project on ***"Molecular Structure and Vibrational Spectroscopic Analysis of some Antimicrobial Activity Compounds – A Combined Experimental and Quantum Chemical Approach"*** focus on the role & response of synthesized novel halogenoderivatives of benzimidazole compounds against the antimicrobial activity against microorganisms such as ***Pseudomonas aeruginosa (MTCC-4030), Escherichia coli (MTCC-1667), Staphylococcus aureus (MTCC-3160), Enterococcus faecalis(MTCC-3159) and fungal compounds Aspergillusniger(MTCC 282) & Candida albicans(MTCC 227).***

The study highlights the activity of synthesized pharmaceutical compound towards antioxidant, antimicrobial activity, antiflammatory analgestic, anticancer, CNS depressant, androgen receptor antagonist, antitubercular, antihelmintic, diabetic drugs, anti-ulcer, anticonvulsant, antiviral-antifungal and treatment of cardiovascular disease, neurology, and endocrinology. The study also highlight introduction of halogen derivatives at 6th position of benzimidazole compound enhance bioactive stimulation to higher levels. The computational & molecular docking studies for all the synthesized compounds to a great extent will help the chemist for new drug design uses computational approaches to discover, develop, and analyze drugs and similar biologically active molecules.

Principal Investigator **Principal**