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Synthesis and evaluation of anti-bacterial activity of substituted benzimidazole derivatives containing heterocyclic compounds

Sushil Kumar [1], Maneesha D. Sati[2] and S. C. Sati[1]
[1]*Department of Chemistry, H. N. B. Garhwal University (A Central University)*
Srinagar Garhwal, Uttarakhand, India .246174
[2]*Department of Chemistry, Govt. PG College Augustyamuni, Rudraprayag. India*

Abstract
Benzimidazole is one of the heterocycles associated with diverse biological potential such as anthelmintic, antacid, antimicrobial, anticancer, and antiviral. Based on the importance of this moiety in medicinal drugs, both clinically approved and in clinical trials, many novel synthetic approaches have been reported for benzimidazoles. Nonetheless, there is a need for novel benzimidazoles to overcome drug resistance and side effects. The present paper covers the synthesis and evaluation anti-bacterial activity of substituted benzimidazole derivatives containing heterocyclic compounds. All the compounds were characterized by UV, IR, ^{1}H NMR, ^{13}C NMR, mass spectral data and CHN elemental analysis

Keywords: *Benzimidazole, 2-([1,1'-biphenyl]-4-yl)-6-methyl-1H-benzo[d]imidazole and 2-([1,1'-biphenyl]-4-yl)-6-chloro-1H-benzo[d]imidazole anti- bacterial activity, UV, IR, ^{1}H NMR, ^{13}C NMR, mass spectra, CHN analysis*

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I. Introduction

Heterocyclic compounds are of prime interest due to their role in the synthesis of various other organic compounds, especially pharmaceutical compounds, making them of immense significance for pharmacology and industrial purposes [1- 4]. Benzimidazole is extensively studied as heterocyclic scaffold. Due to the structural resemblance, benzimidazole and purines based nucleic acid are isosteric of each other. It has been projected as an important moiety in pharmaceutical study to show a wide range of pharmaceutical activities and hence to synthesize its derivatives became the part of interest for organic and medicinal chemists. Due to its presence in a variety of bioactive substances, including antihypertensives, anti-inflammatories, antivirals, analgesics, anticancer, proton pump inhibitors, anticonvulsants, antifungals, anticoagulants, antihistaminics, antiparasitics, and antiulcers, the development of the benzimidazole core has emerged over the recent years[5-12].

Experimental section

Melting point were determined in open capillary tubes and are uncorrected. The time required for the completion of the reaction was monitored by TLC using Silica gel G plates and spots were exposed in Iodine chamber.IR spectra were recorded on Perkin Elmer 1800(FTIR) spectrophotometer. IH NMR spectra (DMSO) were taken on a DRX – 300 spectrometer 300 MHz using TMS as internal standard and chemical shifts are expressed in ppm

II. Material And Methods

The U.S. based chemical company Sigma Aldrich was the procurer of all chemicals. Iodine spray (10% w/w I_2 in silica gel), UV light, and NMR methods were used to visualize the results of thin-layer chromatography conducted on preconditioned silica gel G/Ultraviolet-254 plates (Macherey-Nagel, 0.2 mm, Germany) using quantitative grade solvents.

Methodology of Synthesis:

Compound 1:

The 2-([1,1'-biphenyl]-4-yl)-6-methyl-1H-benzo[d]imidazole can be prepared by the condensation reaction of 4-methyl-1,2-phenylenediamine and 4-biphenylcarboxylic acid (or its derivative like 4-biphenylcarboxaldehyde) under strong acidic or specific catalytic conditions.

A common method for synthesising 2-substituted benzimidazole derivatives is the reaction between an *o*-phenylenediamine and a carboxylic acid (or aldehyde). For this specific compound, the key steps involve:

Reactants: 4-methyl-1,2-phenylenediamine (also known as 4-methyl-*o*-phenylenediamine) and 4-biphenylcarboxylic acid (or 4-biphenylcarboxaldehyde)

Conditions: The condensation is typically performed under strong acidic conditions, such as in polyphosphoric acid (PPA), or in the presence of a catalyst (e.g., sodium metabisulfite, $Na_2S_2O_5$) in a solvent (e.g., ethanol). High temperatures are often required.

A general procedure using polyphosphoric acid involves the following steps:

Mixing: Combine the 4-methyl-1,2-phenylenediamine and 4-biphenylcarboxylic acid in polyphosphoric acid. Reflux the mixture at a high temperature, typically between 180-200 °C, for 4-10 hours. Partially cool the reaction mixture to about 50 °C and pour it onto crushed ice. Neutralise the resulting suspension with an aqueous base, such as a 10% sodium hydroxide solution. Retrieve the pure product by collecting the precipitated crude product using vacuum filtration, washing it with water, drying it, and then recrystallizing it from a suitable solvent (e.g., acetone or ethanol).

It is a white crystalline solid that has a high amount of solubility in both H_2O and polar organic solvents.

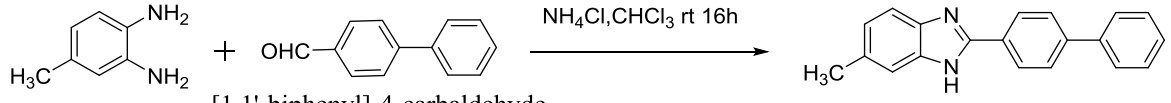
Physical Appearance	Crystalline
Chemical Formula	$C_{20}H_{16}N_2$
Molecular weight	284g
M.P.	225-227°C
Pka	5.6 (25°C)
Refractive Index	1.71
Density	1.20g/cm^3
Yield	52%
Mass Fragmentation	284$[M]^+$, 285 $[M+H]^+$, 286$[M+2H]^+$

FTIR: 3100-3400 N-H proton, 3000 C-H in the rings,1500-1600 fused aromatic systems like benzimidazole and biphenyl, 900 for mono-, di-, or poly-substituted rings,3000- 2800 C- H Stretching.

^{1}HNMR (400 MHz, DMSO, δ ppm):12.56(3H),7.51 (6H),7.57(7H),7.43(9H),2.80(1H),7.84 (H2a), 7.26(H3a), 7.28(H5a), 7.96(H6a),7.75(H2`a), 7.49(H3`a), 7.41(H4`a), 7.57 (H5`a), 7.73 (H6`a).

^{13}C NMR (100 MHz, DMSO, δppm):152.6(C-2),139.6(C-4),130.1(C-5),109.7(C-6),123.0(C-7),136.7(C-8),123.2(C-9),121.3(C-10),141.6(C-1a),127.9(C-2a),128.4(C-3a),136.0(C-4a),127.0(C5a),127.1(C-6a),139.9(C-1'a),127.2(C-2'a),129.7(C-3'a),127.4(C-4'a),129.5(C-5'a),129.5(C-6'a).

It was identified as **2-([1,1'-biphenyl]-4-yl)-6-methyl-1H-benzo[d]imidazole.**

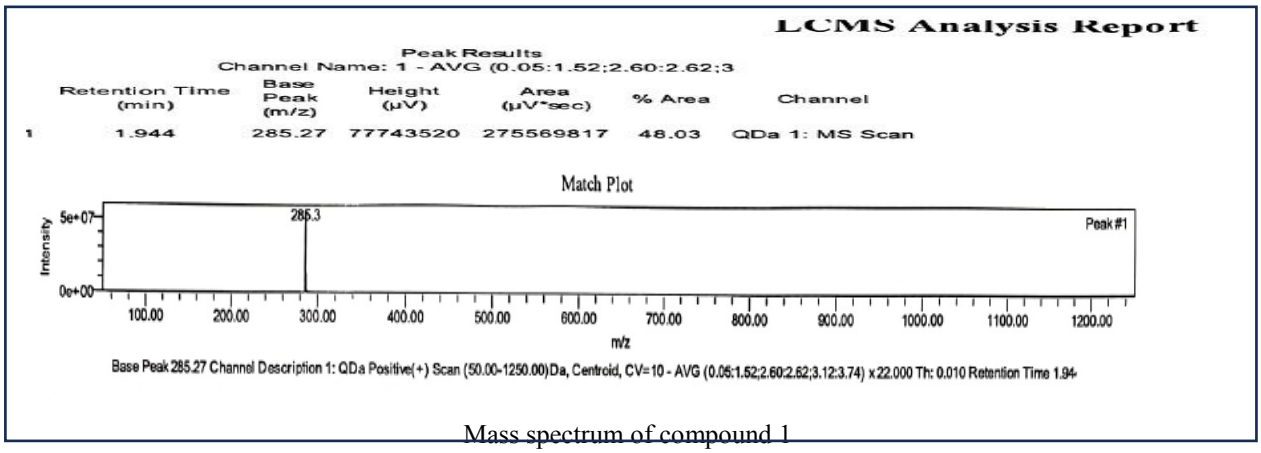


4-methylbenzene-1,2-diamine + [1,1'-biphenyl]-4-carbaldehyde

2-([1,1'-biphenyl]-4-yl)-6-methyl-1*H*-benzo[*d*]imidazole

Chemical Formula: $C_{20}H_{16}N_2$

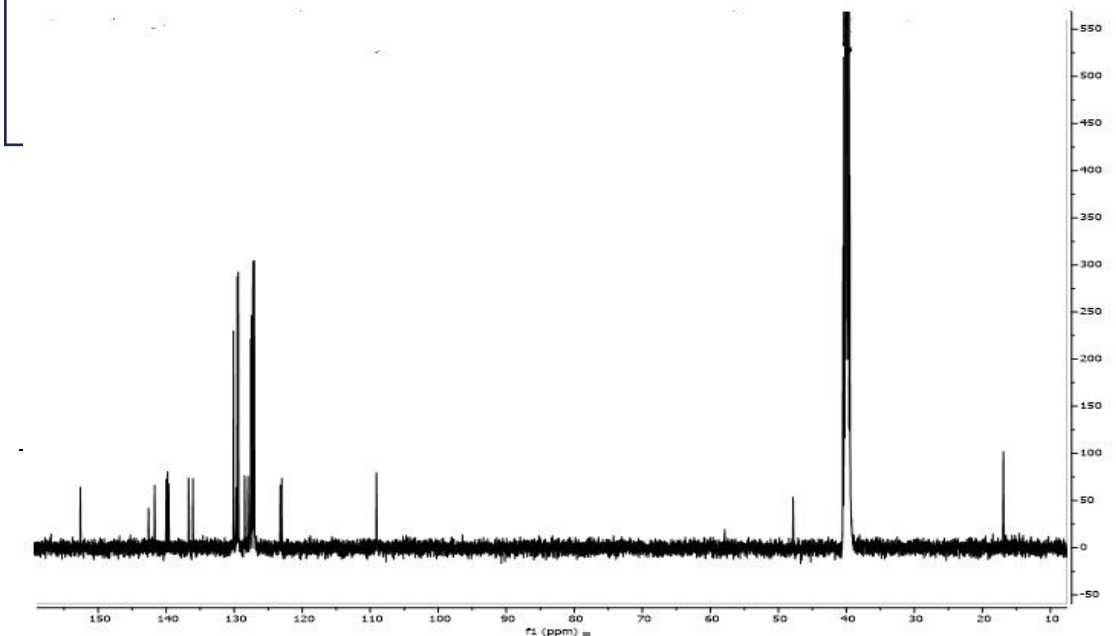
Molecular Weight: 284



Mass spectrum of compound 1

H

NMR Spectrum of Compound 1



^{13}C NMR

Spectrum of Compound 1

Compound 2

The 2-([1,1'-biphenyl]-4-yl)-6-methyl-1H-benzo[d]imidazole can be prepared by the condensation reaction of 4-methyl-1,2-phenylenediamine and 4-biphenylcarboxylic acid (or its derivative like 4-biphenylcarboxaldehyde) under strong acidic or specific catalytic conditions.

A common method for synthesising 2-substituted benzimidazole derivatives is the reaction between an *o*-phenylenediamine and a carboxylic acid (or aldehyde). For this specific compound, the key steps involve:

Reactants: 4-methyl-1,2-phenylenediamine (also known as 4-methyl-*o*-phenylenediamine) and 4-biphenylcarboxylic acid (or 4-biphenylcarboxaldehyde)

Conditions: The condensation is typically performed under strong acidic conditions, such as in polyphosphoric acid (PPA), or in the presence of a catalyst (e.g., sodium metabisulfite, $Na_2S_2O_5$) in a solvent (e.g., ethanol). High temperatures are often required.

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It is an off-white solid that has a high amount of solubility in both H_2O and polar organic solvents.

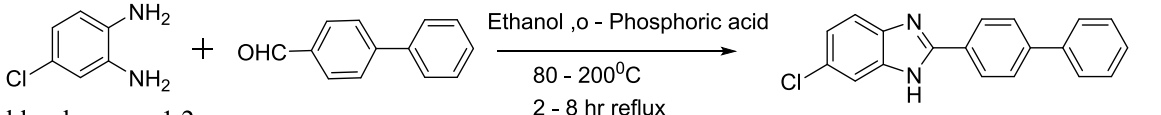
. Physical Appearance	Crystalline
Chemical Formula	$C_{19}H_{13}ClN_2$
Molecular weight	305g
M.P.	200-205°C
Refractive Index	1.70
Density	1.20g/cm^3
Yield	50%
Mass Fragmentation	305$[M]^+$, 306 $[M+H]^+$, 307$[M+2H]^+$

FTIR: 3100-3400 N-H proton, 3000 C-H in the rings,1500-1600 fused aromatic systems like benzimidazole and biphenyl, 900 for mono-, di-, or poly-substituted rings,3000- 2800 C- H Stretching**.**

^{1}HNMR (400 MHz, DMSO, δ ppm): 12.56 (3H),7.14(6H),7.51 (7H),7.42 (9H),2.44(10H),7.88 (H2a), 7.16(H3a), 7.38 (H5a), 7.86(H6a),7.74 (H2`a), 7.49(H3`a), 7.40 (H4`a), 7.47 (H5`a), 7.78 (H6`a).

^{13}C NMR (100 MHz, DMSO, δ ppm): 157.1(C-2),132.9(C-4),129.3(C-5),116.1 (C-6),126.9(C-7),129.1(C-8),114.9(C-9)**,**140.2(C-1a), 127.4(C-2a), 128.9(C-3a), 135.2(C-4a), 127.9(C-5a),127.7(C-6a)**,**143.4(C-1'a),127.1(C-2'a),117.9(C-3'a), 118.2(C-4'a),129.2(C-5'a),135.5(C-6'a).

It was confirmed as **2-([1,1'-biphenyl]-4-yl)-6-chloro-1H-benzo[d]imidazole.**



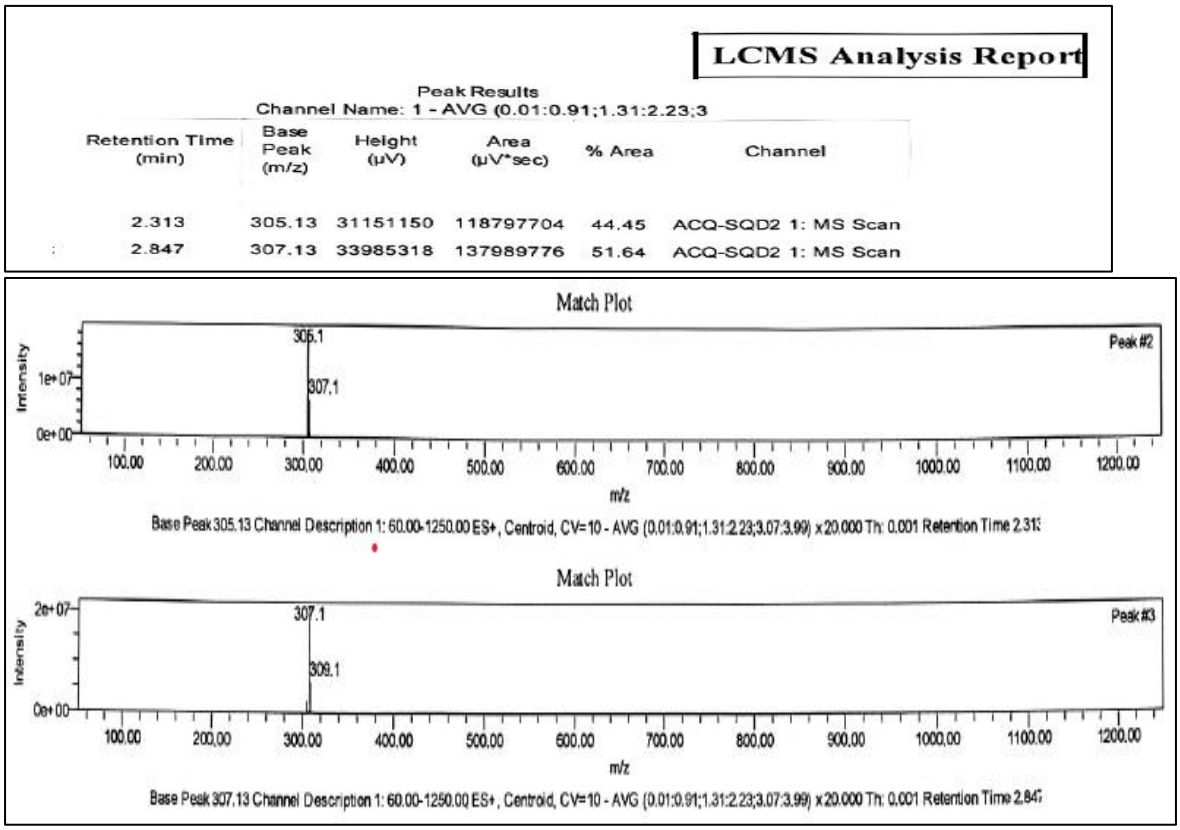
4-chlorobenzene-1,2-diamine

[1,1'-biphenyl]-4-carbaldehyde

2-([1,1'-biphenyl]-4-yl)-6-chloro-1*H*-benzo[*d*]imidazole

Chemical Formula: $C_{19}H_{13}ClN_2$

Molecular Weight: 305

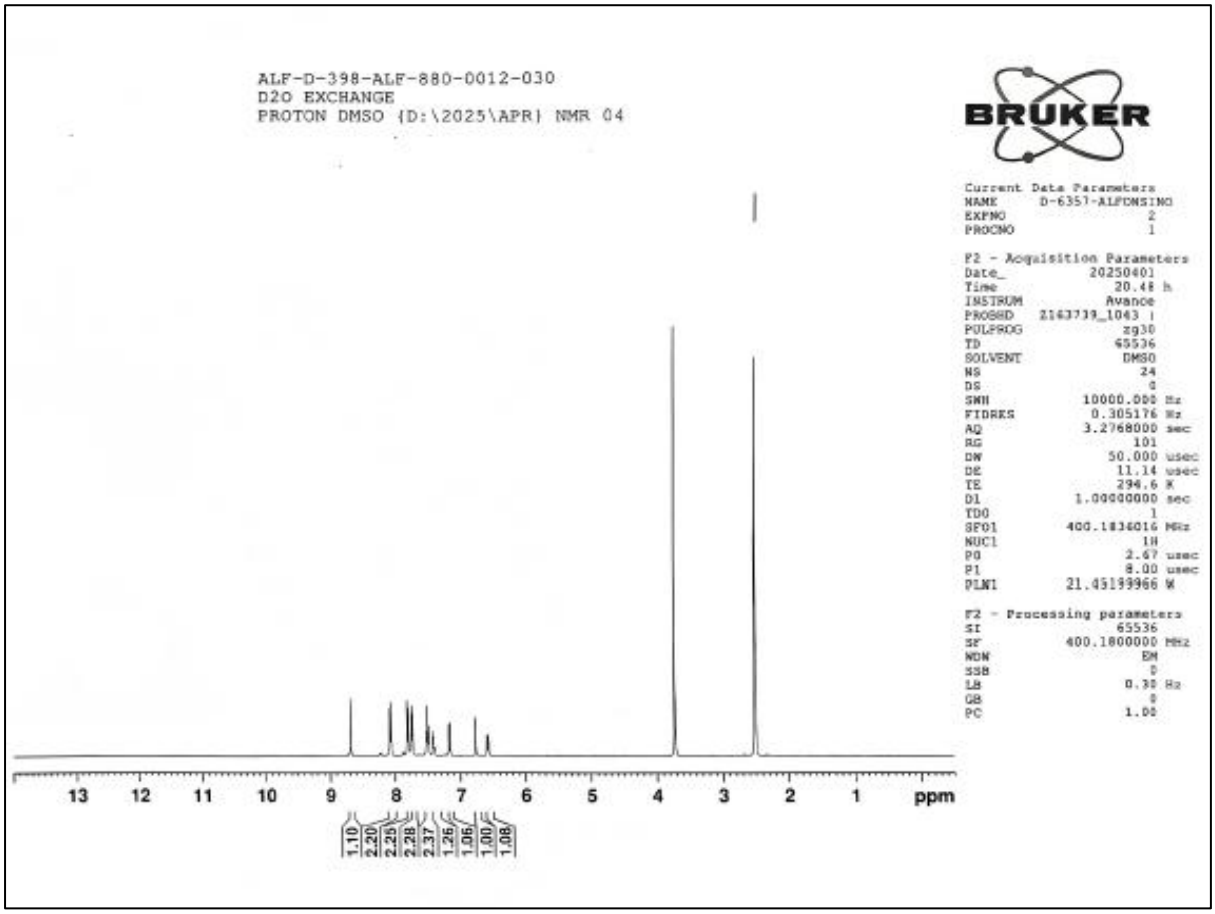


LCMS Analysis Report

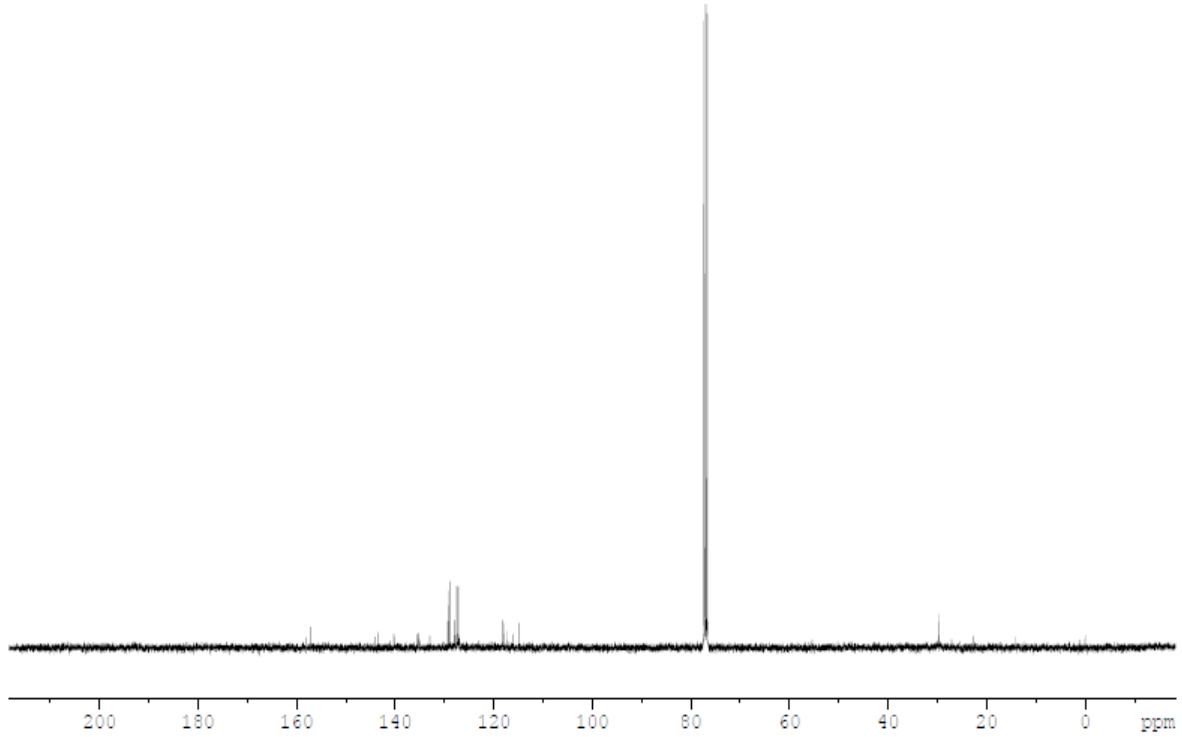
Peak Results
Channel Name: 1 - AVG (0.01:0.91;1.31:2.23;3

Retention Time (min)	Base Peak (m/z)	Height (µV)	Area (µV*sec)	% Area	Channel
2.313	305.13	31151150	118797704	44.45	ACQ-SQD2 1: MS Scan
2.847	307.13	33985318	137989776	51.64	ACQ-SQD2 1: MS Scan

Mass spectrum of compound 2



^{1}H NMR Spectrum of Compound 2



^{13}C NMR Spectrum of Compound 2

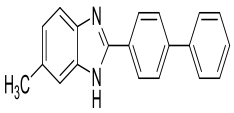
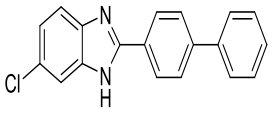
Antibacterial activity

The antibacterial activity of the synthesised substituted benzimidazole derivatives were screened using the agar well diffusion method against two bacterial strains. We observed that benzimidazole was inhibitory for the growth of almost all the test organisms used in this study, and the derivatives that we synthesised in the laboratory were more effective than the parent compound. The synthesised molecules carried two types of substituents as side chains, such as methyl and chloro groups, which led to the altered and enhanced activity of the compound [13-14]. Different molecules exhibited differential behaviour on Gram-positive and Grave bacteria. A landmark achievement of the study is that the synthesised compounds were highly active against phytopathogenic fungal strains.

The synthesised substituted benzimidazole derivatives D_1 and D_2 inhibited the growth of *Staphylococcus aureus* and *Klebsiella pneumoniae* in the range of 11.33± 0.58 mm to 18.00±1.00 in 5 mg and 10 mg for both gram - positive and gram-negative strains, using chloramphenicol (10 mcg) and Ampicillin (25 mcg) as standard reference drug.

These results shows that the synthesized substituted benzimidazole derivatives 2-([1,1'-biphenyl]-4-yl)-6-methyl-1H-benzo[d]imidazole and 2-([1,1'-biphenyl]-4-yl)-6-chloro-1H-benzo[d]imidazole showed moderate anti-bacterial activity against the tested fungal organisms.

Table - Antibacterial activity of synthesised substituted benzimidazole Compounds 1 and 2

Compound codes	**Name of Substituted Benzimidazole**	**Structures**	**Gram positive bacteria**		**Gram negative bacteria**	
			Staphylococcus aureus (mm)		*Klebsiella pneumoniae* (mm)	
			5 mg	10 mg	5 mg	10 mg
CD1	2-([1,1'-biphenyl] -4-yl)-6-methyl-1H -benzo[d]imidazole		11.33±0.58	14.00±1.00	12.33±0.58	15.33±0.58
CD2	2-([1,1'-biphenyl] -4-yl)-6-chloro-1H -benzo[d]imidazole		13.33±0.58	17.00±1.00	14.33±0.58	18.00±1.00
Reference drugs	Chloramphenicol (10mcg)		16.33±0.58		21.33±1.15	
	Ampicillin (25mcg)		13.67±0.58		12.00±1.00	
Abs. DMSO			0.00±0.00		0.00±0.00	

Reference drugs = Chloramphenicol (10mcg)
Ampicillin (25mcg)

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