



## Synthesis, Evaluation and Biological Potential of 1,2,4,5-Tetra substituted Imidazole Derivatives for Antibacterial Activity

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### ABSTRACT

Imidazole's have occupied a unique position in heterocyclic chemistry, and derivatives of imidazole have attracted considerable interests in recent years for their different pharmacological properties. Imidazole is nitrogen-containing heterocyclic ring which possesses biological and pharmaceutical importance. Thus, imidazole compounds have been an interesting source for researchers for more than a century. The imidazole ring is a constituent of many important natural products, including purine, histamine, histidine, and nucleic acid. Being a polar and ionisable aromatic compound, it improves pharmacokinetic characteristics of lead molecules and thus is used as a remedy to optimize solubility and bioavailability parameters of proposed poorly soluble lead molecules. There are several methods used for the synthesis of imidazole-containing compounds, and also their various structure reactions offer scope in the field of medicinal chemistry. The imidazole derivatives possess extensive spectrum of biological activities such as antibacterial, anticancer, antitubercular, antifungal, analgesic, and anti-HIV activities. This paper aims synthesis and antibacterial activity of novel imidazole derivatives.

**Keywords:** Imidazole; Heterocyclic; Aromatic; Anti-convulsant; Anti-ulcer; Anti-allergic; Anti-viral.

### 1. INTRODUCTION

#### 1.1 Background of Study

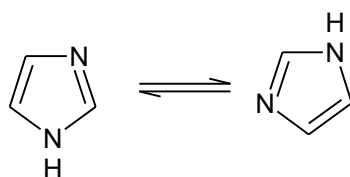
Medicinal chemistry is the discipline concerned with determining the effect of chemical structure on biological activity. Medicinal chemistry is related with the discovery, development, interpretation and identification of MOA of biologically active structures at the molecular level.<sup>1</sup>

Heterocyclic compounds represent an important function in medicinal chemistry and serves as a key template for the development of various chemotherapeutic agents. Most of the researcher has maintained their interest in nitrogen containing heterocyclic compounds through decades of historical development of medicinal synthesis. Heterocycles forms major class of medicinal and organic chemistry and are used industrially and biologically. There are wide range of therapeutic activity of synthetic heterocycles such as anticancer, antimicrobial, antidepressant, antimalarial, anticonvulsant, anthelmintic and insecticidal agents. The explorations for the new biologically active heterocyclic compounds continue to be an area of major research in medicinal synthetic chemistry.<sup>2</sup>

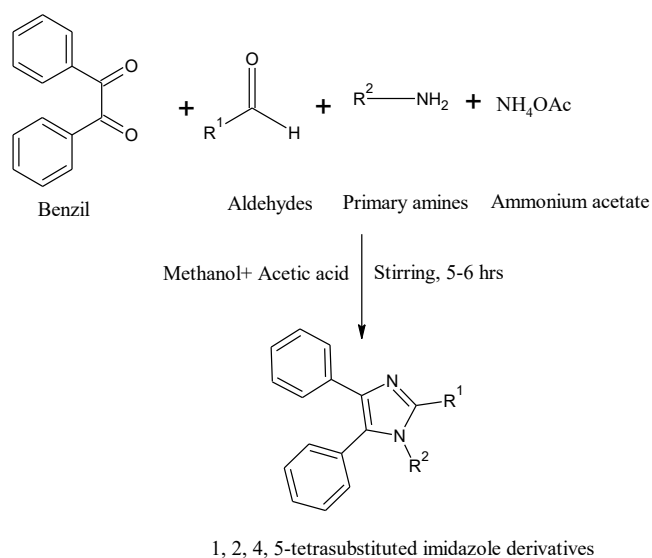
#### 1.2 Imidazole

##### 1.2.1 Introduction

Imidazole (1, 3-diaza-2, 4-cyclopentadiene) is a five member planar ring system with 3C and 2N in 1 and 3 positions having molecular formula  $C_3H_4N_2$ . IUPAC name for the imidazole is 1, 3-diazole, one of the annular N bear a H atom and can be regarded as pyrrole type N. Imidazole is basic aromatic in nature, less basic than ammonia and more basic than pyridine. It exhibits tautomerism because hydrogen atom that can be located on either of the two nitrogen atoms.<sup>3</sup>

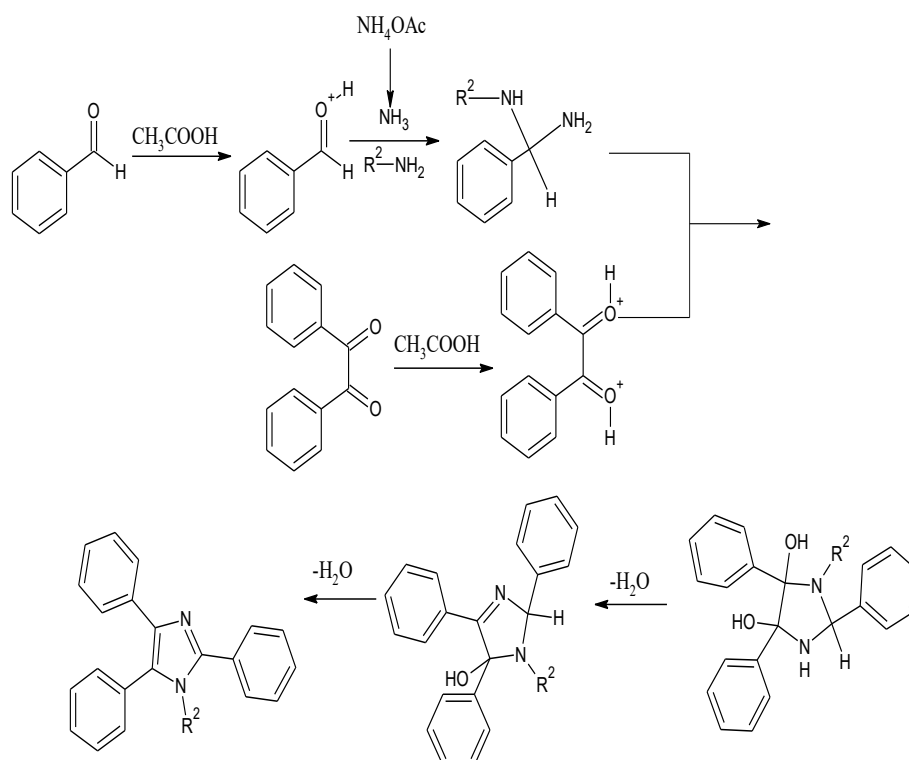


## 2. SCHEME FOR SYNTHESIS



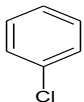
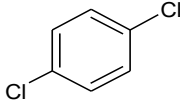
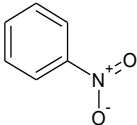
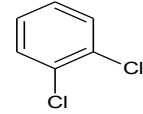
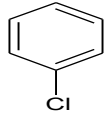
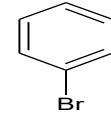
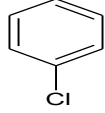
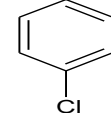
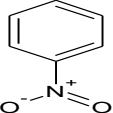
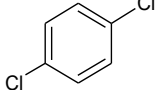
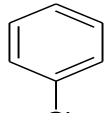
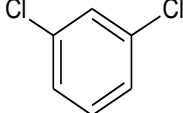
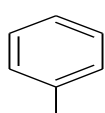
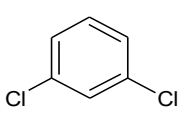
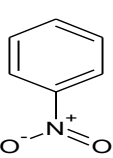
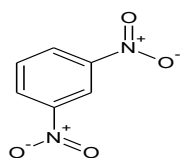
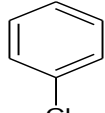
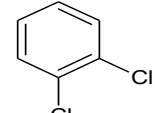
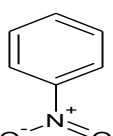
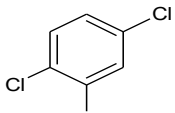
Where  $R^1$  – Substituted phenyl group,  $R^2$  – Substituted phenyl group

### Proposed mechanism for the synthesis of 1, 2, 4, 5-tetrasubstituted imidazole derivatives:

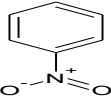
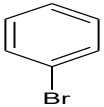
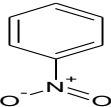
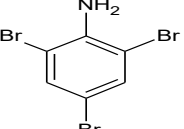
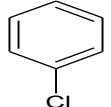
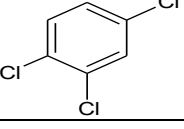
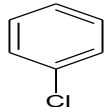
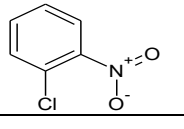
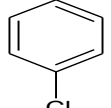
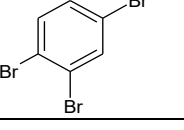
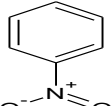
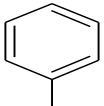
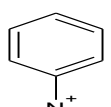
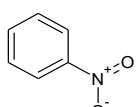
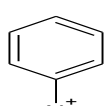
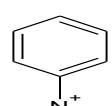
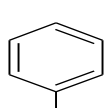
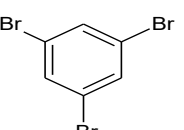
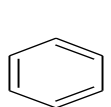
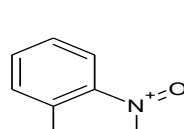




## 2.1 Structure of synthesized 1, 2, 4, 5-tetrasubstituted imidazole derivatives

Comp No.	R <sup>1</sup>	R <sup>2</sup>	IUPAC Name
1.			2-(4-chlorophenyl)-1-(2,5-dichlorophenyl)-4,5-diphenyl-1 <i>H</i> -imidazole
2.			2-(3-nitrophenyl)-1-(3,4-dichlorophenyl)-4,5-diphenyl-1 <i>H</i> -imidazole.
3.			2-(4-chlorophenyl)-1-(4-bromophenyl)-4,5-diphenyl-1 <i>H</i> -imidazole
4.			2-(4-chlorophenyl)-1-(4-chlorophenyl)-4,5-diphenyl-1 <i>H</i> -imidazole.
			2-(4-nitrophenyl)-1-(2,5-dichlorophenyl)-4,5-diphenyl-1 <i>H</i> -imidazole.
6.			2-(4-chlorophenyl)-1-(2,6-dichlorophenyl)-4,5-diphenyl-1 <i>H</i> -imidazole
7.			2-(4-chlorophenyl)-1-(3,5-dichlorophenyl)-4,5-diphenyl-1 <i>H</i> -imidazole
8.			2-(4-nitrophenyl)-1-(2,4-dinitrophenyl)-4,5-diphenyl-1 <i>H</i> -imidazole
9.			2-(4-chlorophenyl)-1-(3,4-dichlorophenyl)-4,5-diphenyl-1 <i>H</i> -imidazole
10.			2-(4-nitrophenyl)-4,5-diphenyl-1-(2,4,5-trichlorophenyl)-1 <i>H</i> -imidazole



11.			2-(4-nitrophenyl)-4,5-diphenyl-1-(4-bromophenyl)-1 <i>H</i> -imidazole
12.			2-(4-chlorophenyl)-4,5-diphenyl-1-(2,4,6-tribromophenyl)-1 <i>H</i> -imidazole.
13.			2-(4-chlorophenyl)-4,5-diphenyl-1-(2,4,5-trichlorophenyl)-1 <i>H</i> -imidazole.
14.			2-(4-chlorophenyl)-4,5-diphenyl-1-(3-nitro-4-chlorophenyl)-1 <i>H</i> -imidazole.
15.			2-(4-chlorophenyl)-4,5-diphenyl-1-(2,4,5-tribromophenyl)-1 <i>H</i> -imidazole
16.			2-(4-nitrophenyl)-4,5-diphenyl-1-(4-chlorophenyl)-1 <i>H</i> -imidazole
17.			2-(4-nitrophenyl)-4,5-diphenyl-1-(3-nitrophenyl)-1 <i>H</i> -imidazole
18.			2-(4-nitrophenyl)-4,5-diphenyl-1-(4-nitrophenyl)-1 <i>H</i> -imidazole
19.			2-(4-nitrophenyl)-4,5-diphenyl-1-(2,4,6-tribromophenyl)-1 <i>H</i> -imidazole
20.			2-(4-nitrophenyl)-4,5-diphenyl-1-(3-nitro-4-chlorophenyl)-1 <i>H</i> -imidazole



21.			2-(4-nitrophenyl)-4,5-diphenyl-1-(2,4,5-tribromophenyl)-1 <i>H</i> -imidazole
22.			2-(3-nitrophenyl)-4,5-diphenyl-1-(3,4-dichlorophenyl)-1 <i>H</i> -imidazole.
23.			2-(3-nitrophenyl)-4,5-diphenyl-1-(2,5-dichlorophenyl)-1 <i>H</i> -imidazole
24.			2-(3-nitrophenyl)-4,5-diphenyl-1-(4-chlorophenyl)-1 <i>H</i> -imidazole
25.			2-(3-nitrophenyl)-4,5-diphenyl-1-(3-nitrophenyl)-1 <i>H</i> -imidazole
26.			2-(3-nitrophenyl)-4,5-diphenyl-1-(3-nitro-4-chlorophenyl)-1 <i>H</i> -imidazole
27.			2-(3-nitrophenyl)-4,5-diphenyl-1-(2,4,5-tribromophenyl)-1 <i>H</i> -imidazole
28.			2-(3-nitrophenyl)-4,5-diphenyl-1-(4-bromophenyl)-1 <i>H</i> -imidazole
29.			2-(4-nitrophenyl)-4,5-diphenyl-1-(4-bromophenyl)-1 <i>H</i> -imidazole
30.			2-(4-nitrophenyl)-4,5-diphenyl-1-(2,4-dichlorophenyl)-1 <i>H</i> -imidazole



### 3. EXPERIMENTAL WORK:

The present chapter is divided into following sections

1. Materials and methods.
2. Characterization studies.
3. Synthesis and characterization of 1, 2, 4, 5-tetrasubstituted imidazole derivatives.
4. Result and discussion.

#### 3.1 Materials and Methods

1. All the chemicals used were purchased from commercial sources such as Himedia, SD fine Chemicals, Spectrochem and purified using standard procedure when required.
2. Melting points were recorded on open capillary tube on super fit melting point apparatus and are uncorrected.
3. The purity of all the final compounds was assessed by TLC.
4. Readymade TLC plates were procured from Merck (TLC Silica gel 60 on Aluminum sheets (20 x 20 cm) and cut to suitable sizes as required)
5. Completion of the reaction was monitored by TLC with n-Hexane, Ethyl Acetate, Pet Ether (in varying proportion) system.
6. TLC plates were visualized using Iodine Chamber & UV lamp.
7. IR spectra were recorded in KBr disk on "Shimadzu FTIR Affinity1" and are reported in centimetres ( $\text{cm}^{-1}$ ).
8.  $^1\text{H}$ NMR spectra were recorded using AVANCE II 400 NMR Spectrometer with tetramethylsilane (TMS) as the internal standard in DMSO.

Mass spectra were recorded using Mass Spectrometer; the spectra are recorded on WATERS Q-TOF MICROMASS (ESI-MS).

#### 3.2 Characterization Studies

All synthesized novel compounds are identified by means of physical and chemical parameters including melting point, boiling point, solubility, chemical tests, and elemental analysis. Analytical methods like TLC, IR, NMR and Mass spectroscopy are used for characterization of newly synthesized compounds, a brief outline of which is given below.

#### 3.3 Synthesis and characterization of novel 1, 2, 4, 5-tetrasubstituted imidazole derivatives

##### General procedure for the preparation of 1, 2, 4, 5-tetrasubstituted imidazole derivatives

In a representative experimental technique, a combination of Diketone Benzil (210 mg, 1 mmol) in 10 ml of Methanol was stirred and to this solution Substituted aldehydes (1.00 mmol), Substituted amines, (1 mmol), and Ammonium acetate (77 mg, 1.00 mmol) were added. Then the reaction mixture was stirred for a 4-5 hours as required to complete the reaction. Afterwards the completion of reaction monitored on TLC, the reaction mixture was transferred into crushed ice and agitated for five minutes. The solid detached was filtered under suction, washed away by means of ice-cold water (20 mL), and then recrystallized from hot ethanol to afford analytically pure product.

##### Compound 1: 2-(4-chlorophenyl)-1-(2, 5-dichlorophenyl)-4, 5-diphenyl-1H-imidazole

IR (KBr);  $\nu$   $\text{cm}^{-1}$ : 3064 (Ar-H stretching), 1674 (C=N stretching), 1593 (C-N stretching), 1450 (C=C stretching), 719 (C-Cl stretching). Mass spectrum showed the formation of molecular ion peak at  $m/z = 476.8$ . The reaction provided 65.15% yield. The TLC monitored by (Pet. Ether 1:9 Ethyl acetate) and  $R_f$  value was (0.42). Recrystallized from hot ethanol. M.P: 186 $^{\circ}$ C.



**Compound 2 : 2-(3-nitrophenyl)-1-(3, 4-dichlorophenyl)-4, 5-diphenyl-1H-imidazole**

IR (KBr);  $\nu$   $\text{cm}^{-1}$ : 3066 (Ar-H stretching), 1676 (C=N stretching), 1593 (C-N stretching), 1450 (C=C stretching), 1355 (C-NO<sub>2</sub> stretching), 719 (C-Cl stretching). Mass spectrum showed the formation of molecular ion peak at  $m/z = 485.7$ . The reaction provided 62.25% yield. The TLC monitored by (Pet. Ether 1:9 Ethyl acetate) and R<sub>f</sub> value was (0.51). Recrystallized from hot ethanol. M.P: 240 °C.

**Compound 3 : 2-(4-chlorophenyl)-1-(4-bromophenyl)-4, 5-diphenyl-1H-imidazole**

IR (KBr);  $\nu$   $\text{cm}^{-1}$ : 3062 (Ar-H stretching), 1678 (C=N stretching), 1593 (C-N stretching), 1450 (C=C stretching), 719 (C-Cl stretching), 642 (C-Br stretching). The <sup>1</sup>HNMR spectrum displayed  $\delta$ ppm at, 8.6370 (s, 1H, Ar-H), 8.2140 (s, 1H, Ar-H), 7.8476 (d, 2H, Ar-H, J=1.76), 7.8090 (s, 1H, Ar-H), 7.6399 (s, 1H, Ar-H), 7.4688 (d, 2H, Ar-H, J=1.76), 7.3508 (d, 2H, Ar-H, J=8.44), 7.2789 (s, 1H, Ar-H), 7.2217 (d, 2H, Ar-H, J=2.72), 7.0648 (s, 1H, Ar-H), 6.8765 (s, 1H, Ar-H), 6.5889 (d, 2H, Ar-H, J=1.68), 6.3765 (s, 1H, Ar-H), 6.1077 (s, 1H, Ar-H). Mass spectrum showed the formation of molecular ion peak at  $m/z = 485.8$ . The reaction provided 60.72% yield. The TLC monitored by (Pet. Ether 1:9 Ethyl acetate) and R<sub>f</sub> value was (0.57). Recrystallized from hot ethanol. M.P: 227 °C.

**Compound 4: 2-(4-chlorophenyl)-1-(4-chlorophenyl)-4, 5-diphenyl-1H-imidazole**

IR (KBr);  $\nu$   $\text{cm}^{-1}$ : 3064 (Ar-H stretching), 1674 (C=N stretching), 1593 (C-N stretching), 1450 (C=C stretching), 717 (C-Cl stretching). The reaction provided 55.55% yield. The TLC monitored by (Pet. Ether 1:9 Ethyl acetate) and R<sub>f</sub> value was (0.47). Recrystallized from hot ethanol. M.P: 177 °C.

**Compound 5 : 2-(4-nitrophenyl)-1-(2, 5-dichlorophenyl)-4, 5-diphenyl-1H-imidazole**

IR (KBr);  $\nu$   $\text{cm}^{-1}$ : 3064 (Ar-H stretching), 1676 (C=N stretching), 1593 (C-N stretching), 1450 (C=C stretching), 1325 (C-NO<sub>2</sub> stretching), 719 (C-Cl stretching). The <sup>1</sup>HNMR spectrum displayed  $\delta$ ppm at, 7.9898 (p, 5H, Ar-H, J=9.44 Hz), 7.8415 (s, H), 7.6835 (t, 3H, Ar-H, J=2.44 Hz), 7.5353 (p, 5H, Ar-H, J=7.8 Hz), 7.5000 (s, 1H, Ar-H), 7.4435 (s, 1H, Ar-H), 7.0943 (s, 1H, Ar-H). Mass spectrum showed the formation of molecular ion peak at  $m/z = 486.7$ . The reaction provided 64.76% yield. The TLC monitored by (Pet. Ether 1:9 Ethyl acetate) and R<sub>f</sub> value was (0.49). Recrystallized from hot ethanol. M.P: 281 °C.

**Compound 6 : 2-(4-chlorophenyl)-1-(2, 6-dichlorophenyl)-4, 5-diphenyl-1H-imidazole**

IR (KBr);  $\nu$   $\text{cm}^{-1}$ : 3062 (Ar-H stretching), 1674 (C=N stretching), 1593 (C-N stretching), 1450 (C=C stretching), 717 (C-Cl stretching). The <sup>1</sup>HNMR spectrum displayed  $\delta$ ppm at, 8.1278 (s, 1H, Ar-H), 7.9905 (p, 5H, Ar-H, J=9.36 Hz), 7.6495 (t, 3H, Ar-H, J=2.4 Hz), 7.5380 (p, 5H, Ar-H, J=7.88 Hz), 7.5029 (s, 1H, Ar-H), 7.3261 (s, 1H, Ar-H), 6.7863 (s, 1H, Ar-H). The reaction provided 67.25% yield. The TLC monitored by (Pet. Ether 1:9 Ethyl acetate) and R<sub>f</sub> value was (0.47). Recrystallized from hot ethanol. M.P: 185 °C.

**Compound 7 : 2-(4-chlorophenyl)-1-(3, 5-dichlorophenyl)-4, 5-diphenyl-1H-imidazole**

IR (KBr);  $\nu$   $\text{cm}^{-1}$ : 3062 (Ar-H stretching), 1681 (C=N stretching), 1593 (C-N stretching), 1450 (C=C stretching), 719 (C-Cl stretching). The <sup>1</sup>HNMR spectrum displayed  $\delta$ ppm at, 8.2641 (t, 3H, Ar-H, J=5.48), 8.01146 (s, 1H, Ar-H), 7.9371 (p, 5H, Ar-H, J=9.4), 7.7547(d, 2H, Ar-H, J=1.28), 7.5984 (p, 5H, Ar-H, J=15.6 Hz), 7.1611 (s, 1H, Ar-H). Mass spectrum showed the formation of molecular ion peak at  $m/z = 477.9$ . The reaction provided 60.44% yield. The TLC monitored by (Pet. Ether 1:9 Ethyl acetate) and R<sub>f</sub> value was (0.39). Recrystallized from hot ethanol. M.P: 162 °C.

**Compound 8: 2-(4-nitrophenyl)-1-(2, 4-dinitrophenyl)-4, 5-diphenyl-1H-imidazole**

IR (KBr);  $\nu$   $\text{cm}^{-1}$ : 3336 (Ar-H stretching), 1676 (C=N stretching), 1593 (C-N stretching), 1450 (C=C stretching), 1340 (C-NO<sub>2</sub> stretching). Mass spectrum showed the formation of molecular ion peak at  $m/z = 509.9$ . The reaction provided 62.25% yield. The TLC monitored by (Pet. Ether 1:9 Ethyl acetate) and R<sub>f</sub> value was (0.48). Recrystallized from hot ethanol. M.P: 212 °C.

**Compound 9: 2-(4-chlorophenyl)-1-(3, 4-dichlorophenyl)-4, 5-diphenyl-1H-imidazole**

IR (KBr);  $\nu$   $\text{cm}^{-1}$ : 3064 (Ar-H stretching), 1678 (C=N stretching), 1593 (C-N stretching), 1450 (C=C stretching), 719 (C-Cl stretching). The reaction provided 50.44% yield. Mass spectrum showed the formation of molecular ion peak at  $m/z = 476.9$ . The TLC monitored by (Pet. Ether 1:9 Ethyl acetate) and R<sub>f</sub> value was (0.43). Recrystallized from hot ethanol. M.P: 167 °C.



**Compound 10: 2-(4-nitrophenyl)-4, 5-diphenyl-1-(2, 4, 5 trichlorophenyl)-1H-imidazole**

IR (KBr);  $\nu$   $\text{cm}^{-1}$ : 3064 (Ar-H stretching), 1678 (C=N stretching), 1595 (C-N stretching), 1450 (C=C stretching), 1344 (C-NO<sub>2</sub> stretching), 719 (C-Cl stretching). The reaction provided 52.80% yield. The TLC monitored by (Pet. Ether 1:9 Ethyl acetate) and Rf value was (0.47). Recrystallized from hot ethanol. M.P: 182 °C.

**Compound 11: 2-(4-nitrophenyl)-4, 5-diphenyl-1-(4-bromophenyl)-1H-imidazole**

IR (KBr);  $\nu$   $\text{cm}^{-1}$ : 3062 (Ar-H stretching), 1660 (C=N stretching), 1593 (C-N stretching), 1454 (C=C stretching), 1382 (C-NO<sub>2</sub> stretching), 1315 (C-N stretching), 642 (C-Br stretching). The reaction provided 56.41% yield. The TLC monitored by (Pet. Ether 1:9 Ethyl acetate) and Rf value was (0.36). Recrystallized from hot ethanol. M.P: 185 °C.

**Compound 12: 2-(4-chlorophenyl)-4, 5-diphenyl-1-(2, 4, 6-tribromophenyl)-1H-imidazole**

IR (KBr);  $\nu$   $\text{cm}^{-1}$ : 3074 (Ar-H stretching), 1676 (C=N stretching), 1593 (C-N stretching), 1450 (C=C stretching), 719 (C-Cl stretching), 642 (C-Br stretching). The reaction provided 49.72% yield. The TLC monitored by (Pet. Ether 1:9 Ethyl acetate) and Rf value was (0.49). Recrystallized from hot ethanol. M.P: 277 °C.

**Compound 13: 2-(4-chlorophenyl)-4, 5-diphenyl-1-(2, 4, 5-trichlorophenyl)-1H-imidazole**

IR (KBr);  $\nu$   $\text{cm}^{-1}$ : 3064 (Ar-H stretching), 1676 (C=N stretching), 1593 (C-N stretching), 1450 (C=C stretching), 717 (C-Cl stretching). The reaction provided 50.93% yield. The TLC monitored by (Pet. Ether 1:9 Ethyl acetate) and Rf value was (0.53). Recrystallized from hot ethanol. M.P: 184 °C.

**Compound 14: 2-(4-chlorophenyl)-4, 5-diphenyl-1-(3-nitro-4-chlorophenyl)-1H-imidazole**

IR (KBr);  $\nu$   $\text{cm}^{-1}$ : 3064 (Ar-H stretching), 1674 (C=N stretching), 1593 (C-N stretching), 1336 (C-NO<sub>2</sub> stretching), 1450 (C=C stretching), 717 (C-Cl stretching). The reaction provided 54.48% yield. The TLC monitored by (Pet. Ether 1:9 Ethyl acetate) and Rf value was (0.47). Recrystallized from hot ethanol. M.P: 217 °C.

**Compound 15: 2-(4-chlorophenyl)-4, 5-diphenyl-1-(2, 4, 5-tribromophenyl)-1H-imidazole**

IR (KBr);  $\nu$   $\text{cm}^{-1}$ : 3064 (Ar-H stretching), 1674 (C=N stretching), 1593 (C-N stretching), 1450 (C=C stretching), 719 (C-Cl stretching), 642 (C-Br stretching). The reaction provided 50.51% yield. The TLC monitored by (Pet. Ether 1:9 Ethyl acetate) and Rf value was (0.48). Recrystallized from hot ethanol. M.P: 226 °C.

**Compound 16: 2-(4-nitrophenyl)-4, 5-diphenyl-1-(4-chlorophenyl)-1H-imidazole**

IR (KBr);  $\nu$   $\text{cm}^{-1}$ : 3064 (Ar-H stretching), 1678 (C=N stretching), 1593 (C-N stretching), 1450 (C=C stretching), 1323 (C-NO<sub>2</sub> stretching), 719 (C-Cl stretching). The reaction provided 56.42% yield. The TLC monitored by (Pet. Ether 1:9 Ethyl acetate) and Rf value was (0.50). Recrystallized from hot ethanol. M.P: 163 °C.

**Compound 17: 2-(4-nitrophenyl)-4, 5-diphenyl-1-(3-nitrophenyl)-1H-imidazole**

IR (KBr);  $\nu$   $\text{cm}^{-1}$ : 3064 (Ar-H stretching), 1678 (C=N stretching), 1595 (C-N stretching), 1450 (C=C stretching), 1355 (C-NO<sub>2</sub> stretching). The reaction provided 60.54% yield. The TLC monitored by (Pet. Ether 1:9 Ethyl acetate) and Rf value was (0.44). Recrystallized from hot ethanol. M.P: 196 °C.

**Compound 18: 2-(4-nitrophenyl)-4, 5-diphenyl-1-(4-nitrophenyl)-1H-imidazole**

IR (KBr);  $\nu$   $\text{cm}^{-1}$ : 3064 (Ar-H stretching), 1678 (C=N stretching), 1593 (C-N stretching), 1450 (C=C stretching), 1325 (C-NO<sub>2</sub> stretching). The reaction provided 67.03% yield. The TLC monitored by (Pet. Ether 1:9 Ethyl acetate) and Rf value was (0.53). Recrystallized from hot ethanol. M.P: 277 °C.



**Compound 19: 2-(4-nitrophenyl)-4, 5-diphenyl-1-(2, 4, 6-tribromophenyl)-1H-imidazole**

IR (KBr);  $\nu$   $\text{cm}^{-1}$ : 3074 (Ar-H stretching), 1676 (C=N stretching), 1593 (C-N stretching), 1456 (C=C stretching), 1340 (C-NO<sub>2</sub> stretching), 642 (C-Br stretching). The reaction provided 59.62% yield. The TLC monitored by (Pet. Ether 1:9 Ethyl acetate) and Rf value was (0.49). Recrystallized from hot ethanol. M.P: 238 °C.

**Compound 20: 2-(4-nitrophenyl)-4, 5-diphenyl-1-(3-nitro-4-chlorophenyl)-1H-imidazole**

IR (KBr);  $\nu$   $\text{cm}^{-1}$ : 3064 (Ar-H stretching), 1678 (C=N stretching), 1593 (C-N stretching), 1450 (C=C stretching), 1344 (C-NO<sub>2</sub> stretching), 719 (C-Cl stretching). The reaction provided 49.30% yield. The TLC monitored by (Pet. Ether 1:9 Ethyl acetate) and Rf value was (0.47). Recrystallized from hot ethanol. M.P: 192 °C.

**Compound 21: 2-(4-nitrophenyl)-4, 5-diphenyl-1-(2, 4, 5-tribromophenyl)-1H-imidazole**

IR (KBr);  $\nu$   $\text{cm}^{-1}$ : 3074 (Ar-H stretching), 1674 (C=N stretching), 1593 (C-N stretching), 1456 (C=C stretching), 1346 (C-NO<sub>2</sub> stretching), 642 (C-Br stretching). The reaction provided 49.68% yield. The TLC monitored by (Pet. Ether 1:9 Ethyl acetate) and Rf value was (0.41). Recrystallized from hot ethanol. M.P: 217 °C.

**Compound 22: 2-(3-nitrophenyl)-4, 5-diphenyl-1-(3, 4-dichlorophenyl)-1H-imidazole**

IR (KBr);  $\nu$   $\text{cm}^{-1}$ : 3064 (Ar-H stretching), 1676 (C=N stretching), 1593 (C-N stretching), 1450 (C=C stretching), 1344 (C-NO<sub>2</sub> stretching), 719 (C-Cl stretching). The reaction provided 62.71% yield. The TLC monitored by (Pet. Ether 1:9 Ethyl acetate) and Rf value was (0.53). Recrystallized from hot ethanol. M.P: 182 °C.

**Compound 23: 2-(3-nitrophenyl)-4, 5-diphenyl-1-(2, 5-dichlorophenyl)-1H-imidazole**

IR (KBr);  $\nu$   $\text{cm}^{-1}$ : 3064 (Ar-H stretching), 1676 (C=N stretching), 1596 (C-N stretching), 1450 (C=C stretching), 1325 (C-NO<sub>2</sub> stretching), 719 (C-Cl stretching). The reaction provided 60.90% yield. The TLC monitored by (Pet. Ether 1:9 Ethyl acetate) and Rf value was (0.48). Recrystallized from hot ethanol. M.P: 177 °C.

**Compound 24: 2-(3-nitrophenyl)-4, 5-diphenyl-1-(4-chlorophenyl)-1H-imidazole**

IR (KBr);  $\nu$   $\text{cm}^{-1}$ : 3064 (Ar-H stretching), 1678 (C=N stretching), 1585 (C-N stretching), 1450 (C=C stretching), 1336 (C-NO<sub>2</sub> stretching), 719 (C-Cl stretching). The reaction provided 70.81% yield. The TLC monitored by (Pet. Ether 1:9 Ethyl acetate) and Rf value was (0.38). Recrystallized from hot ethanol. M.P: 196 °C.

**Compound 25: 2-(3-nitrophenyl)-4, 5-diphenyl-1-(3-nitrophenyl)-1H-imidazole**

IR (KBr);  $\nu$   $\text{cm}^{-1}$ : 3064 (Ar-H stretching), 1660 (C=N stretching), 1593 (C-N stretching), 1450 (C=C stretching), 1315 (C-NO<sub>2</sub> stretching). The reaction provided 58.37% yield. The TLC monitored by (Pet. Ether 1:9 Ethyl acetate) and Rf value was (0.43). Recrystallized from hot ethanol. M.P: 225 °C.

**Compound 26: 2-(3-nitrophenyl)-4, 5-diphenyl-1-(3-nitro-4-chlorophenyl)-1H-imidazole**

IR (KBr);  $\nu$   $\text{cm}^{-1}$ : 3064 (Ar-H stretching), 1676 (C=N stretching), 1593 (C-N stretching), 1450 (C=C stretching), 1340 (C-NO<sub>2</sub> stretching), 719 (C-Cl stretching). The reaction provided 55.34% yield. The TLC monitored by (Pet. Ether 1:9 Ethyl acetate) and Rf value was (0.41). Recrystallized from hot ethanol. M.P: 166 °C.

**Compound 27: 2-(3-nitrophenyl)-4, 5-diphenyl-1-(2, 4, 5-tribromophenyl)-1H-imidazole**

IR (KBr);  $\nu$   $\text{cm}^{-1}$ : 3074 (Ar-H stretching), 1676 (C=N stretching), 1593 (C-N stretching), 1450 (C=C stretching), 1350 (C-NO<sub>2</sub> stretching), 642 (C-Br stretching). The reaction provided 49.68% yield. The TLC monitored by (Pet. Ether 1:9 Ethyl acetate) and Rf value was (0.38). Recrystallized from hot ethanol. M.P: 287 °C.

**Compound 28: 2-(3-nitrophenyl)-4, 5-diphenyl-1-(4-bromophenyl)-1H-imidazole**

IR (KBr);  $\nu$   $\text{cm}^{-1}$ : 3064 (Ar-H stretching), 1668 (C=N stretching), 1595 (C-N stretching), 1450 (C=C stretching), 1325 (C-NO<sub>2</sub> stretching), 682 (C-Br stretching). The reaction provided 61.44% yield. The TLC monitored by (Pet. Ether 1:9 Ethyl acetate) and Rf value was (0.41). Recrystallized from hot ethanol. M.P: 235 °C.

**Compound 29: 2-(4-nitrophenyl)-4, 5-diphenyl-1-(4-bromophenyl)-1H-imidazole**

IR (KBr);  $\nu$   $\text{cm}^{-1}$ : 3062 (Ar-H stretching), 1660 (C=N stretching), 1593 (C-N stretching), 1450 (C=C stretching), 1325 (C-NO<sub>2</sub> stretching), 642 (C-Br stretching). The reaction provided 63.91% yield. The TLC monitored by (Pet. Ether 1:9 Ethyl acetate) and Rf value was (0.44). Recrystallized from hot ethanol. M.P: 170 °C.

**Compound 30: 2-(3-nitrophenyl)-4, 5-diphenyl-1-(4-nitrophenyl)-1H-imidazole**

IR (KBr);  $\nu$   $\text{cm}^{-1}$ : 3064 (Ar-H stretching), 1681 (C=N stretching), 1593 (C-N stretching), 1450 (C=C stretching), 1346 (C-NO<sub>2</sub> stretching). The reaction provided 63.79% yield. The TLC monitored by (Pet. Ether 1:9 Ethyl acetate) and Rf value was (0.47). Recrystallized from hot ethanol. M.P: 197 °C.

**3.4 Physiochemical data of 1, 2, 4, 5-tetrasubstituted imidazole derivatives****Table :Physiochemical data of 1, 2, 4, 5-tetrasubstituted imidazole derivatives.**

Comp. No.	Nature	Colour	Solubility	Molecular Formula	Molecular Weight	R <sub>f</sub> value	M.P. (°C)	% Yield
1	Solid	Orange	CDCl <sub>3</sub>	C <sub>27</sub> H <sub>17</sub> Cl <sub>3</sub> N <sub>2</sub>	475.79	0.42	186	65.15%
2	Solid	Yellowish	CDCl <sub>3</sub>	C <sub>27</sub> H <sub>17</sub> Cl <sub>2</sub> N <sub>3</sub> O <sub>2</sub>	486.34	0.51	240	62.25%
3	Solid	Dull Brown	CDCl <sub>3</sub>	C <sub>27</sub> H <sub>18</sub> BrClN <sub>2</sub>	485.80	0.57	227	60.72%
4	Solid	Yellowish	DMSO	C <sub>27</sub> H <sub>18</sub> Cl <sub>2</sub> N <sub>2</sub>	441.35	0.47	177	55.55%
5	Solid	Brown	CDCl <sub>3</sub>	C <sub>27</sub> H <sub>17</sub> Cl <sub>2</sub> N <sub>3</sub> O <sub>2</sub>	486.34	0.49	281	64.76%
6	Solid	White	CDCl <sub>3</sub>	C <sub>27</sub> H <sub>17</sub> Cl <sub>3</sub> N <sub>2</sub>	475.79	0.47	185	67.25%
7	Solid	White	DMSO	C <sub>27</sub> H <sub>17</sub> Cl <sub>3</sub> N <sub>2</sub>	475.79	0.39	162	60.44%
8	Solid	Dull Brown	CDCl <sub>3</sub>	C <sub>27</sub> H <sub>17</sub> N <sub>5</sub> O <sub>6</sub>	507.45	0.48	212	62.25%
9	Solid	Yellowish	CDCl <sub>3</sub>	C <sub>27</sub> H <sub>17</sub> Cl <sub>3</sub> N <sub>2</sub>	475.79	0.43	167	50.44%
10	Solid	Pale Yellow	DMSO	C <sub>27</sub> H <sub>16</sub> Cl <sub>3</sub> N <sub>3</sub> O <sub>2</sub>	520.79	0.47	182	52.80%
11	Solid	Dark Brown	DMSO	C <sub>27</sub> H <sub>18</sub> BrN <sub>3</sub> O <sub>2</sub>	496.35	0.36	185	56.41%
12	Solid	Dull White	DMSO	C <sub>27</sub> H <sub>16</sub> Br <sub>3</sub> ClN <sub>2</sub>	643.59	0.49	277	49.72%
13	Solid	Orange	DMSO	C <sub>27</sub> H <sub>16</sub> Cl <sub>4</sub> N <sub>2</sub>	510.24	0.53	184	50.93%
14	Solid	Yellowish	DMSO	C <sub>27</sub> H <sub>17</sub> Cl <sub>2</sub> N <sub>3</sub> O <sub>2</sub>	486.34	0.47	217	54.48%
15	Solid	Dark Brown	DMSO	C <sub>27</sub> H <sub>16</sub> Br <sub>3</sub> ClN <sub>2</sub>	643.59	0.48	226	50.51%
16	Solid	Light Orange	DMSO	C <sub>27</sub> H <sub>17</sub> ClN <sub>3</sub> O <sub>2</sub>	451.90	0.50	163	56.42%
17	Solid	Light Green	DMSO	C <sub>27</sub> H <sub>18</sub> N <sub>4</sub> O <sub>4</sub>	462.45	0.44	196	60.54%
18	Solid	Brown	DMSO	C <sub>27</sub> H <sub>18</sub> N <sub>4</sub> O <sub>4</sub>	462.45	0.53	277	67.03%
19	Solid	Dull White	DMSO	C <sub>27</sub> H <sub>16</sub> Br <sub>3</sub> N <sub>3</sub> O <sub>2</sub>	654.14	0.49	238	59.62%
20	Solid	Light Green	DMSO	C <sub>27</sub> H <sub>17</sub> ClN <sub>4</sub> O <sub>4</sub>	496.90	0.47	192	49.30%
21	Solid	Brown	DMSO	C <sub>27</sub> H <sub>16</sub> Br <sub>3</sub> N <sub>3</sub> O <sub>2</sub>	654.14	0.41	217	49.68%
22	Solid	Light Yellow	DMSO	C <sub>27</sub> H <sub>17</sub> Cl <sub>2</sub> N <sub>3</sub> O <sub>2</sub>	486.34	0.53	182	62.71%
23	Solid	Light Brown	DMSO	C <sub>27</sub> H <sub>17</sub> Cl <sub>2</sub> N <sub>3</sub> O <sub>2</sub>	486.34	0.48	177	60.90%
24	Solid	Light Yellow	DMSO	C <sub>27</sub> H <sub>17</sub> ClN <sub>3</sub> O <sub>2</sub>	451.90	0.38	196	70.81%
25	Solid	Cream	DMSO	C <sub>27</sub> H <sub>18</sub> N <sub>4</sub> O <sub>4</sub>	462.45	0.43	225	58.37%
26	Solid	Shiny White	DMSO	C <sub>27</sub> H <sub>17</sub> ClN <sub>4</sub> O <sub>4</sub>	496.90	0.41	166	55.34%
27	Solid	Shiny cream	DMSO	C <sub>27</sub> H <sub>16</sub> Br <sub>3</sub> N <sub>3</sub> O <sub>2</sub>	654.10	0.38	287	49.68%
28	Solid	Dull Brown	DMSO	C <sub>27</sub> H <sub>18</sub> BrN <sub>3</sub> O <sub>2</sub>	496.35	0.41	235	61.49%
29	Solid	Light Orange	DMSO	C <sub>27</sub> H <sub>18</sub> BrN <sub>3</sub> O <sub>2</sub>	496.35	0.44	170	63.91%
30	Solid	Orange	DMSO	C <sub>27</sub> H <sub>17</sub> Cl <sub>2</sub> N <sub>3</sub> O <sub>2</sub>	462.45	0.47	197	63.79%



#### 4. BIOLOGICAL EVALUATION:

Biological activities of synthesized novel 1, 2, 4, 5-tetrasubstituted imidazole derivatives were screened for antimicrobial and antifungal activity. Total thirty derivatives were subjected for biological evaluation. The following section discussed, in brief the antibacterial and antifungal screening strategies, and the screening of synthesized compounds for their antibacterial activity.

##### 4.1 ANTIBACTERIAL ACTIVITY:

The antibacterial activity of synthesized compounds were determined by screening them against two gram positive viz. *Staphylococcus aureus*, *Bacillus subtilis* and two gram negative culture viz. *Escherichia coli* and *Salmonella typhi* using agar cup method. The basic principle of antimicrobial activity lies in the comparison of inhibition of growth of microorganism produced by the known concentration of antimicrobial agents to be tested with that produced by known concentration of standard antimicrobial agent having known activity. The following section discusses, in brief the antibacterial activity and screening strategies.

##### 4.1.1 Method

Method	Agar cup method
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##### 4.1.2 Preparation of nutrient broth:

###### Composition

- Peptone (Bacteriological) : 20 g
- Beef extract (Bacteriological) : 5 g
- Sodium chloride : 5g
- Distilled water up to : 1000 ml

Nutrient Broth was prepared by dissolving all these and steamed for about 2 hr. Adjust the reaction mixture pH to about 7.2 and autoclaved at 15 lbs. pressure for 20 minute. One day prior to the testing, the microorganisms obtained commencing the laboratory stock were subcultured into sterilized nutrient broth and incubated at 37 °C for 18-24 hrs. The culture growth thus obtained was used as inoculant for the antimicrobial testing.

##### 4.1.3 Preparation of nutrient agar medium:

The nutrient agar medium was prepared by using the following ingredients,

- Peptone (Bacteriological) : 20 g
- Beef extract (Bacteriological) : 5 g
- Sodium chloride : 5g
- Agar (Bacteriological) : 20 g
- Distilled water up to : 1000 ml

Weigh up the required amounts of peptone, beef extract were dissolved in distilled water by gentle warming, and then the specified quantity of agar was dissolved by warming on boiling water bath. Then the pH of the above solution was adjusted by addition of sodium chloride and the volume of ultimate solution is made up to 1000 ml with distilled water. Then the above organized nutrient agar media was sterilized by means of autoclave at 121 °C for 15 minute at 15 Psi pressure.



#### 4.1.4 Preparation of test solution:

0.1g of the test compound was dissolved in 10 ml of DMSO. Now the concentration of solution was 1%. These sample solutions were made in suitably labelled sterilized test tubes.

#### 4.1.5 Preparation of standard solution:

Standard drug used in this testing is Penicillin. It is water soluble; the concentration of this drug is adjusted so as to contain 10 units of penicillin.

#### 4.1.6 Antibacterial Activity of Synthesized 1, 2, 4, 5-tetrasubstituted imidazole Derivatives:

Table : Antibacterial Activity of Synthesized 1, 2, 4, 5-Tetra Substituted Imidazole Derivatives

Compound Code	Zone of inhibition in millimetre (mm)			
	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>	<i>Salmonella typhi</i>
1	22	14	0	12
2	24	11	0	12
3	20	14	0	0
4	19	12	0	12
5	16	14	12	11
6	15	14	12	0
7	16	11	12	12
8	16	0	0	0
9	13	0	0	0
10	16	0	12	0
11	0	14	0	0
12	16	15	0	0
13	14	14	0	0
14	20	15	14	13
15	23	12	0	0
16	19	15	0	0
17	25	13	13	13
18	23	18	0	0
19	21	21	15	16
20	20	16	0	0
21	16	15	0	0
22	18	17	0	0
23	20	26	15	0
24	16	15	0	0
25	17	14	0	0
26	16	14	0	0
27	0	16	0	0
28	0	16	0	0
29	16	15	13	14
30	20	13	15	20
DMSO(Controlled)	-ve	-ve	-ve	-ve
Penicillin (Standard)	28	24	11	16

4.1.7 Graphical representation of antibacterial activity (Graph 1 to 3)

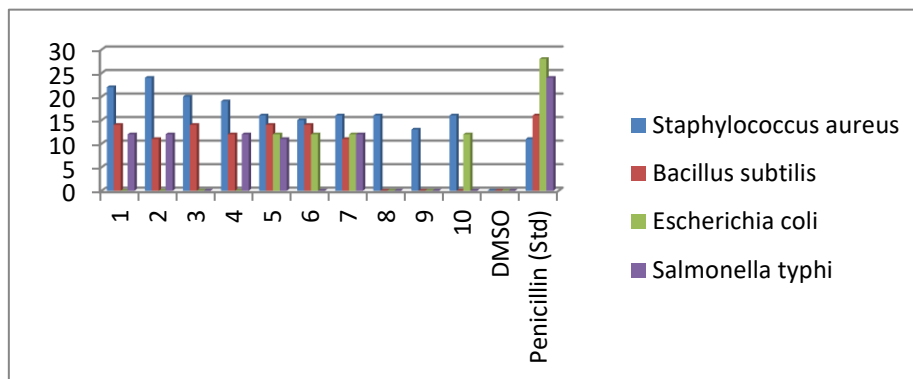


Fig: 4.1.8.1 Comparison of antibacterial activity of 1, 2, 4, 5-Tetra Substituted Imidazole Derivatives (Compound 1 to 10)

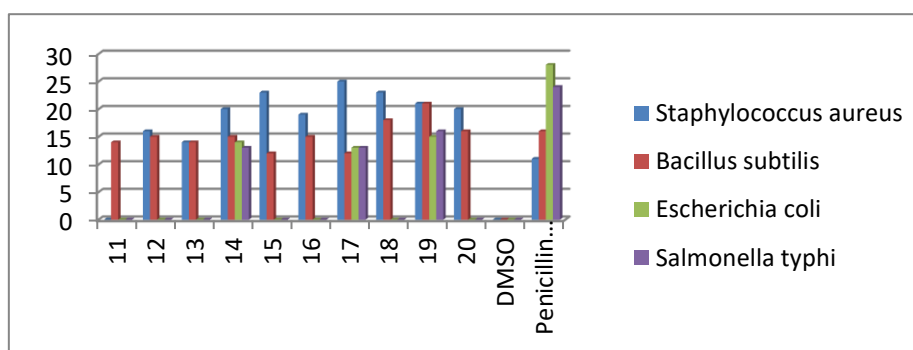


Fig: 4.1.8.2 Comparison of antibacterial activity of 1, 2, 4, 5-Tetra Substituted Imidazole Derivatives (Compound 11 to 20)

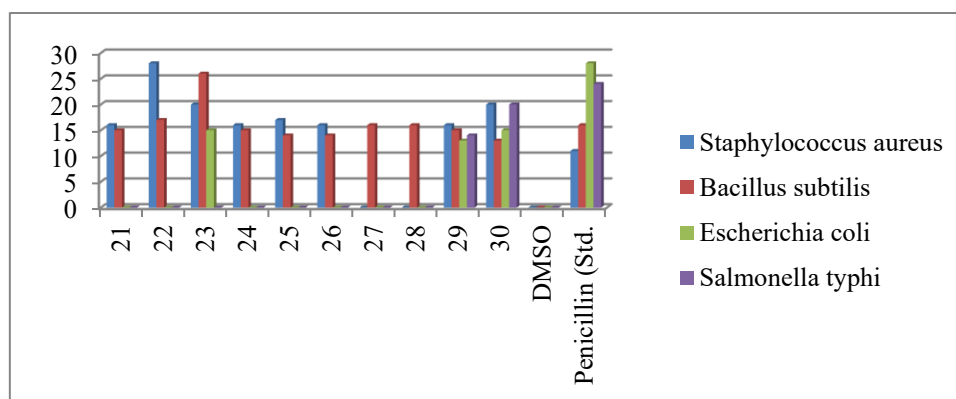


Fig: 4.1.8.3 Comparison of antibacterial activity of 1, 2, 4, 5-Tetra Substituted Imidazole Derivatives (Compound 21 to 30)

5 RESULT AND DISCUSSION

The antibacterial activity of synthesized compounds were evaluated against *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Bacillus subtilis* using agar cup method and results are shown in Table no.9- I as zone of inhibition (in mm). *Penicillin* was used as standard for synthesized compound. Antibacterial screening against *Escherichia coli* amongst which compound number 5 (12 mm), 6 (12mm), 7 (12 mm), 10 (12 mm), 14 (14 mm), 17 (13 mm), 19 (15 mm), 23 (15 mm), 29 (13 mm), 30 (15 mm) showed more potent antibacterial activity as compared standard *Penicillin* (11 mm), Whereas, compound number 1, 2, 3, 4, 8, 9, 11, 12, 13, 15, 16, 18, 20, 21, 22, 24, 25, 26, 27, and 28 showed no antibacterial activity against *Escherichia coli* as compared standard *Penicillin*.



The antibacterial screening against *Salmonella typhi* revealed that compound 30 (20 mm) showed more potent activity as compared standard Penicillin (16 mm), compound number 19 (16 mm) showed equipotent activity as compared standard Penicillin, while compound number 1 (12 mm), 2 (12 mm), 4 (12 mm), 5 (11 mm), 7 (12 mm), 14 (13 mm), 17 (13 mm), 29 (14 mm) showed moderate activity as compared standard Penicillin, Whereas, compound 3, 6, 8, 9, 10, 11, 12, 13, 15, 16, 18, 20, 21, 22, 23, 24, 25, 26, 27, and 28 showed no activity against *Salmonella typhi* as compared standard Penicillin.

The antibacterial screening against *Staphylococcus aureus* revealed that compound number 1 (22 mm), 2 (24 mm), 3 (20 mm), 4 (19 mm), 14 (20 mm), 15 (23 mm), 16 (19 mm), 17 (25 mm), 18 (23 mm), 19 (21 mm), 20 (20 mm), 23 (20 mm), 30 (20 mm) showed moderate activity as compared standard Penicillin, whereas, compound number 5 (16 mm), 6 (15 mm), 7 (16 mm), 8 (16 mm), 9 (13 mm), 10 (16 mm), 12 (16 mm), 13 (14 mm), 21 (16 mm), 12 (18 mm), 24 (16 mm), 25 (17 mm), 26 (16 mm), and 29 (16 mm) showed weaker activity as compared standard Penicillin (28 mm), Whereas, compound 11, 17, and 28 showed no activity against *Staphylococcus aureus* as compared standard Penicillin.

The antibacterial screening against *Bacillus subtilis* revealed that compound number 23 (26 mm) showed more potent activity as compared standard Penicillin (24 mm), while compound number 18 (18 mm), 19 (21 mm) showed moderate activity as compared standard Penicillin, whereas, compound number 1 (14 mm), 2 (11 mm), 3 (14 mm), 4 (12 mm), 5 (14 mm), 6 (14 mm), 7 (11 mm), 11 (14 mm), 12 (15 mm), 13 (14 mm), 14 (15 mm), 15 (12 mm), 16 (15 mm), 17 (13 mm), 20 (16 mm), 21 (15 mm), 22 (17 mm), 24 (15 mm), 25 (14 mm), 26 (14 mm), 27 (16 mm), 28 (16 mm), 29 (15 mm), and 30 (13 mm) showed weaker activity, Whereas, compound 8, 9, and 10 showed no activity against *Bacillus subtilis* as compared standard Penicillin. One can be concluded from the above antibacterial screening of 1, 2, 4, 5-Tetra Substituted Imidazole derivatives that, compound number 5, 7, 14, 17, 19, 29 and 30 showed significant antimicrobial activity against all microbial species.

## 6. CONCLUSION

The present dissertation work is aimed to one pot multicomponent synthesis of some 1, 2, 4, 5-tetrasubstituted imidazole derivatives and screen them for antibacterial and antifungal activities. New compounds had been synthesized with an effort to potentiate them by means of fulfilling structural requirements for desired biological activity. 1, 2, 4, 5-tetrasubstituted imidazole derivatives can be synthesized with good yield by the assumed scheme. Total thirty analogues have been synthesized by using Benzil, substituted amines, substituted aldehydes and ammonium acetate, as substituted compounds and were tested for their identification and purity. The purity of all compounds was achieved by determining melting point, and Rf value. All the compounds were achieved in an agreeable yield and confirmed by IR spectra, NMR spectra, and Mass spectra. The yields of all synthesized compounds were found to be in the range of 40-70% and the characterization was done through melting point and TLC. The synthesized analogues were evaluated for their antibacterial and antifungal activity.

The antibacterial activity of synthesized compounds were assessed against to *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, and *Bacillus subtilis* using agar plate method and results are displayed in table 9-I and zone of inhibition in mm. Penicillin was used as a reference standard for synthesized compounds. We conclude from the antibacterial screening of 1, 2, 4, 5-substituted imidazole derivatives that compound number 5, 7, 14, 17, 19, 29, and 30 showed significant activity against all microbial species used for screening.

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