SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVAL IMIDAZOLE BASED COMPOUNDS

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ABSTRACT
Some new imidazole derivatives (3i-xii) were synthesized as per design synthetic protocol scheme. The structures of newly prepared compounds were confirmed by modern analytical technique and elemental analysis. All the synthetic compounds were screened for their antimicrobial activity against bacterial strains, with good to remarkable activity. The MIC (minimum inhibitory concentration) values were determined by comparison to ciprofloxacin (antibacterial) and fluconazole (antifungal) as standard drugs. Among them, compound 3w and 3x exhibited notable antimicrobial activity. These compounds may be used as new templates for the screening of potential antimicrobial agents.

KEYWORDS: Imidazole, Biphenyl ethanone, Antibacterial activity and Antifungal activity.

INTRODUCTION
Imidazole is an important group of compounds reported to have different biological activities and the present study was undertaken in order to synthesize some new derivatives of imidazole and related fused heterocyclic compounds and screen for their antimicrobial activity.1-3

Imidazoles are a class of five membered heterocyclic compounds having two nitrogen and three carbon atoms. Imidazole is an important group of compounds reported to have different biological activities and the present study was undertaken in order to synthesize some new derivatives of imidazole and related fused heterocyclic compounds and screen for their antimicrobial activity.1-3

Among the various classes of heterocyclic compounds, imidazole is an important component of pharmacologically active compounds and a part of various available marketed drugs like Azathioprine (Leukemia), Metronidazole (Protozoal and antimicrobial activity, trichomoniasis, amoebiasis and giardiasis), Dacarbazine (Hodgkin’s disease), Tinidazole (Metronidazole), Ornidazole (Antiprotozoal and antibacterial activity), Satranidazole (C 10213 Go), (Trichomoniasis and amoebiasis), Cimetidine (Duodenal and gastric ulcers), Carbimazole (Thyroid disorders), Tolazoline (Vasodilator action), Naphazoline (Vasconstrictor), Tetrahydrozoline (Vasoconstrictor).12-15 Recent studies have been revealed that the substituted imidazole derivatives attracted attention due to their broad spectrum of pharmacological activities such as anti-inflammatory, analgesic, antimicrobial, antiviral, antifungal, antibacterial, anti-tubercular, anti-cancer, anti-hypertensive, anti-obesity and anti-convulsant15,16

In the present studies it has been thought to synthesize newer imidazole derivatives and the structures of the synthesized compounds confirmed on the basis of their elemental analysis and modern analytical techniques such as IR, 1H-NMR and Mass spectral data results.

Experimental Protocol
General materials and instruments
All the chemicals and solvents procured from E. Merck and S. D. Fine chemicals (India). Melting points were determined by open tube capillary method and are uncorrected. Thin layer chromatography (TLC) plates prepared by silica gel G were used to monitor the reaction as well as to confirm the purity of the compound by using solvent systems as toluene: ethyl acetate: formic acid (5:4:1), benzene: acetone (9:1) were used to run the TLC. The spots were visualized under iodine...
vapours/UV light. IR spectra were obtained on a PerkinElmer 1720 FT-IR spectrometer using KBr Pellets. 1H-
NMR spectra were recorded on DPX-300 and BRUKER-
400 Ultra Shield™ NMR spectrometer, using TMS as
internal standard in CDCl3/DMSO-d6. Microanalysis of
the compounds was done on Perkin-Elmer model 240
analyzer and the values were found within ±0.4% of
the theoretical values. Mass spectrometry was recorded
on LC-MS/MS (WATERS, mass lynn version 4.1)
spectrometer.

Synthesis

The compounds of the synthetic protocol scheme were
obtained in the following steps.

Synthesis of 1-(Biphenyl-4-yl) ethanone (1): The
starting material biphenyl ethanone (1) was prepared by
heating biphenyl with anhydrous AlCl3 in presence of
CS2 and acetic anhydride. The usual work up of the
reaction mixture followed by recrystallized from ethanol
gave pure compound. The purity of the compound was
verified with the help of TLC (B: A, 9 : 1). Percentage
yield was found 85% and noted Mp.158-160 C. IR
spectra are very informative and provided evidence for
the formation of the expected structures.

Synthesis of 2-(Biphenyl-4-yl)-2-oxoacetaldehyde (2): -
Compound 2-(Biphenyl-4-yl)-2-oxoacetaldehyde (2) was
synthesized from biphenyl ethanone (1) in presence of
selenium dioxide, usual work up of the reaction mixture
gave a yellow liquid which was found pure on TLC
examination (TEF 5: 4 : 1). The structure of compound
was confirmed on the basis of spectral studies.

General procedure for synthesis of 4-(Biphenyl-4-yl)-
2-(substituted phenyl)-1H-imidazole (3i-xii): -
Biphenyl-2-oxoacetaldehyde (2) was refluxed with
different aromatic aldehyde in presence of ammonium
acetate and glacial acetic acid. The usual work up of the
reaction mixture followed by recrystallized from acetone
to get the desired products (3i-xii). The structures of
compounds were confirmed on the basis of their IR and
1H-NMR spectral studies. The compound was found pure
on TLC examination (BA 9: 1) and (TEF 5: 4 : 1) and its
spectral data was found satisfactory for the proposed
structures.

Synthesis of 4-(biphenyl-4-yl)-2-phenyl-1H-imidazole
(3i): Yield: 70%, Mp: 134-137°C, Rf = 0.53. IR (KBt,
cm−1): 3450(C-H, N-H), 3041(C-H, Ar-H), 2871(C-H,
CH3), 1595(C=N), 1564 (C=C). 1H-NMR (DMSO-d6, δ,
ppm): 10.93 (H, s, N-H), 6.53-8.79 (H, m, Ar-H), 7.92
(1H, s, CH, imidazole). ESI-MS (m/z): 296 (M+).
Anal.cald. for C21H19N2O: C, 65.11; H, 5.44; N, 9.45.
Found: C, 75.11; H, 4.44; N, 9.51.

Synthesis of 4-(4-(biphenyl-4-yl)-1H-imidazol-2-yl-phenol (3ii): Yield: 75%, Mp: 151-154°C, Rf = 0.54. IR
(KBr, cm−1): 3397(C-H, N-H), 3213(OH), 3035(C-H,
Ar-H), 2931(C-H, CH3), 1675(C=N), 1556 (C=C). 1H-
NMR (DMSO-d6, δ, ppm): 10.51 (H, s, N-H), 8.01-6.79
(H, m, Ar-H), 9.01 (1H, s, CH, imidazole), 9.46 (H, s,
OH). ESI-MS (m/z): 312 (M+). Anal.cald. for C21H19N2O:
C, 80.75; H, 5.16; N, 8.97. Found: C, 80.79; H, 4.11; N, 8.95.

Synthesis of 4-(biphenyl-4-yl)-2-(3-chlorophenyl)-1H-
imidazole (3iii): Yield: 77%, Mp: 148-151°C, Rf = 0.47.
IR (KBr, cm−1): 3405(C-H, N-H), 3021(C-H, Ar-H),
2947(C-H, CH3), 1623(C=N), 1564 (C=C), 733(C-Cl).
1H-NMR (DMSO-d6, δ, ppm): 9.97 (H, s, N-H), 7.85-
6.59 (H, m, Ar-H), 8.93 (1H, s, CH, imidazole). ESI-MS
(m/z): 330 (M+). Anal.cald. for C21H19ClN2O: C, 76.24;
H, 4.57; N, 8.47. Found: C, 76.27; H, 4.54; N, 8.37.

Synthesis of 3-(4-(biphenyl-4-yl))-1H-imidazol-2-
ylphenol (3iv): Yield: 64%. Mp: 132-133°C, Rf = 0.53.
IR (KBt, cm−1): 3451(C-H, N-H), 3267(OH), 3058(C-H,
Ar-H), 2879(C-H, CH3), 1578(C=N), 1551 (C=C). 1H-
NMR (DMSO-d6, δ, ppm): 10.23 (H, s, N-H), 7.95-6.73
(H, m, Ar-H), 8.31 (1H, s, CH, imidazole), 9.37 (H, s,
OH). ESI-MS (m/z): 312 (M+). Anal.cald. for C21H19N2O:
C, 64.75; H, 5.16; N, 8.97. Found: C, 60.79; H, 5.21;
N, 8.95.

Synthesis of 4-(biphenyl-4-yl)-2-(4-chlorophenyl)-1H-
imidazole (3vii): Yield: 72%. Mp: 185-187°C, Rf = 0.47.
IR (KBt, cm−1): 3379(C-H, N-H), 3086(C-H, Ar-H),
2951(C-H, CH3), 1663 (C=N), 1491(C=C), 719(C=C).
1H-NMR (DMSO-d6, δ, ppm): 9.73 (H, s, N-H), 7.89-
6.57 (H, m, Ar-H), 9.11 (1H, s, CH, imidazole). ESI-MS
(m/z): 330 (M+). Anal.cald. for C21H19ClN2O: C, 55.30;
H, 4.61; N, 15.71. Found: C, 55.41; H, 4.75; N, 15.92.

Synthesis of 4-(biphenyl-4-yl)-2-(4-bromophenyl)-1H-
imidazole (3viii): Yield: 81%. Mp: 142-145°C, Rf = 0.57.
IR (KBt, cm−1): 3447(C-H, N-H), 3171 (C-H, Ar-H),
2817 (C-H, CH3), 1652 (C=N), 1539 (C=C), 803 (C-Br).
1H-NMR (DMSO-d6, δ, ppm): 10.01 (H, s, N-H), 7.91-
6.85 (H, m, Ar-H), 8.71 (1H, s, CH, imidazole). ESI-MS
(m/z): 375 (M+). Anal.cald. for C21H19BrN2O: C, 57.21;
H, 4.03; N, 7.47. Found: C, 56.79; H, 4.07; N, 7.51.

Synthesis of 4-(biphenyl-4-yl)-2-(4-methoxyphenyl)-
1H-imidazole (3x): Yield: 71%. Mp: 160-163°C, Rf =
0.63. IR (KBt, cm−1): 3031(C-H, Ar-H), 2905(C-H, CH3),
1662(C=N), 1571 (C=C). 1H-NMR (DMSO-d6, δ, ppm):
9.97 (H, s, N-H), 7.85-6.21 (H, m, Ar-H), 9.03 (1H, s,
CH, imidazole), 3.85 (H, s, OCH3). ESI-MS (m/z): 326
(M+). Anal.cald. for C21H19O2N: C, 68.91; H, 5.56; N,
8.51. Found: C, 68.79; H, 5.51; N, 8.58.

Synthesis of 3-(4-(biphenyl-4-yl))-1H-imidazol-2-yl-
phenol (3xi): Yield: 78%. Mp: 140-143°C, Rf = 0.55. IR
(KBr, cm−1): 3421(C-H, N-H), 3307(OH), 3041(C-H,
Ar-H), 2867(C-H, CH3), 1571(C=N), 1543 (C=C).
1H-NMR (DMSO-d6, δ, ppm): 10.11 (H, s, N-H), 7.89-6.68
(H, m, Ar-H), 8.73 (1H, s, CH, imidazole), 9.31 (H, s,
OH). ESI-MS (m/z): 312 (M+). Anal.cald. for C21H19N2O:
C,
55.45; H, 5.19; N, 9.13. Found: C, 55.51; H, 5.21; N, 9.21.

Synthesis of 4-(biphenyl-4-yl)-2-(4-fluorophenyl)-1H-imidazole (3xii): Yield: 67%, Mp: 171-175 °C. IR (KBr, cm⁻¹): 3127(C-H, Ar-H), 2945(CH₃), 1635(C=N), 1589(C=C), 861 (C-CH₃), 1H-NMR (DMSO-d₆, δ, ppm): 11.01 (H, s, N-H), 8.11-7.23 (H, m, Ar-H), 9.25 (1H, s, CH, imidazole). ESI-MS (m/z): 314 (M⁺). Anal.calcd. for C₂₇H₁₅FN₅: C, 63.27; H, 2.87; N, 16.10. Found: C, 63.28; H, 2.90; N, 16.07.

Antimicrobial Evaluation
The inhibition of microbial growth under standardized condition may be utilized for demonstrating the therapeutic efficacy of any subtle change in the antibiotic molecule. Which may not be detected by chemical method will be revealed by a reduction in the anti-microbial activity and hence microbiological assays are very useful for resolving doubts regarding possible loss of potency of antibiotics and their preparations of the antibiotic having a known activity. The in-vitro antibacterial and antifungal activities of the synthesized compounds were carried out by microdilution susceptibility test using cup-plate technique. Antibacterial activity of newly synthesized compounds (3i-xii) was screened against bacterial strains viz. Escherichia coli (E. coli, MTCC 2961), Staphylococcus aureus (S. aureus, MTCC 3160), Bacillus subtilis (B. subtilis, MTCC 121), Klebsiella pneumoniae (K. pneumoniae, MTCC 3040) and Micrococcus luteus (M. luteus, MTCC 7527). The anti-fungal activity was screened against fungal strains viz. Candida albicans (C. albicans, MTCC 227), Aspergillus niger (A. niger, MTCC 277) and Aspergillus flavus (A. flavus, MTCC 418). The MIC (minimum inhibitory concentration) values were determined in compare to standard drug Ciprofloxacin (anti-bacterial) and Fluconazole (anti-fungal). The MIC is considered to be the lowest drug concentration for which there is no microbial growth.

Antibacterial Activity
Experimental Procedure
In-vitro antibacterial activity of the synthesized compounds was tested by disc diffusion method under standard condition using Muller Hinton Agar medium. The test organisms were first cultured in Nutrient broth and incubated for 24 hrs at 37°C and then freshly prepared bacterial cells were spread onto the Muller Hinton agar plates in a laminar flow cabinet. The test compounds which were previously dissolved in DMSO were then soaked onto sterile discs of Whatman filter paper no. 1 (6 mm diameter). The discs were then placed onto the surface of the previously prepared inoculated plates and incubated. After 24 hrs of incubation at 37°C, the diameter of zone of inhibition was measured for each compound in mm. The activity was compared with standard antibiotic ciprofloxacin (positive control) and a disc impregnated with dimethylsulfoxide (DMSO) was used as a negative control. All the tests were performed in triplicate and the average was taken as final reading. Compounds which have shown good zone of inhibition were selected for minimum inhibitory concentration (MIC) determination.

Antifungal activity
Experimental Procedure
In-vitro antifungal activity of the synthesized compounds was tested by disc diffusion method under standard conditions using Potato dextrose agar medium. Sterile discs of Whatman filter paper no.1 (6 mm diameter) containing specific amounts of an antifungal agent fluconazole (300 mg for the synthesized compounds) were placed on the surface of an agar plate inoculated with a standardized suspension of the microorganisms tested. The plates were incubated at 28±2°C for 72 hrs for evaluating antifungal activity. A paper disc impregnated with dimethylsulfoxide (DMSO) was utilized as negative control.

The nutrient agar medium was prepared and autoclaved at 15 lbs pressure for 20 minutes and this media was poured into petri plates and was allowed to solidify. On the surface of media microbial suspension was spread with the help of sterilized cotton swab. Cups were made by boring into agar surface with a previously sterilized cork borer and scooping out the punched part of agar. Four cavities or cups were made in the medium and different concentrations of the test compounds and standard drug Fluconazole were poured in these cavities. The plates were kept at room temperature for 1 hr and then incubated at 37±0.5°C for 24 hrs. The diameter of the zone of inhibition formed around the cavities (cups) after 24 hrs incubation was measured and percentage inhibition of the compound were evaluated. A solvent control was also run to know the activity of the blank.

Determination of MIC
MIC of the compound was determined by agar streak dilution method. A stock solution of the synthesized compounds (100 µg/mL) in DMSO was prepared and graded quantities of the test compounds were incorporated in specified quantity of molten sterile agar (Muller Hinton agar). A specified quantity of the medium (40-50°C) containing the compound was poured into a Petri dish to give a depth of 3-4 mm and allowed to solidify. Suspension of the micro-organism was prepared to contain approximately 10⁵ cfu/mL and applied to plates with serially diluted compounds in DMSO to be tested and incubated at 37°C. At the end of the incubation period, the MIC values were determined. All determinations were done in triplicates and the average was taken as final reading. The standard antibiotic, ciprofloxacin (100 µg/mL) used as positive control and 100 mL of DMSO used as a negative control. The MIC was considered to be the lowest concentration of the test substance exhibiting no visible growth of bacteria or fungi on the plate.
RESULT AND DISCUSSION

Chemistry

The title compounds (3i-xii) were synthesized as per synthetic scheme outline. In this scheme biphenyl ethanone (1; starting material) was treated with selenium dioxide to get 2-(biphenyl-4-yl)-2-oxoacetaldehyde (2). Compound 2 was refluxed with different aromatic aldehydes in presence of ammonium acetate and glacial acetic acid, followed by treatment with chlorobenzene in THF to get twelve new imidazole derivatives (3i-xii). The structures of newly prepared compounds were established on the basis of modern analytical techniques (FT-IR, $^1$H-NMR and mass spectral data) and elemental analysis. The final compounds were purified by recrystalization with suitable solvent and found pure on TLC examination.

Structural investigations

The starting material 1-(biphenyl-4-yl) ethanone (biphenyl ethanone, 1) was prepared by heating biphenyl with anhydrous AlCl$_3$ in the presence of CS$_2$ and acetic anhydride. The usual work up of the reaction mixture followed by recrystallized from ethanol gave pure compound. The purity of the compound was verified with the help of TLC (B: A, 9:1), % age of yield was found 83% and melting point noted 151-152 °C. IR spectra are very informative and provided evidence for the formation of expected structures. In general, IR spectra of acetophenone showed a strong band at 1673 cm$^{-1}$ for the confirmation of C=O. Whereas $^1$H-NMR spectra left no doubt with a singlet of aldehydic proton at 9.810 ppm.

Compound 2 [2-(biphenyl-4-yl)-2-oxoacetaldehyde] was synthesized from 1-(biphenyl-4-yl) ethanone (1) in the presence of selenium dioxide and after work out of reaction gave a yellow liquid which was found pure on TLC examination (TEF 5: 4: 1). The structure of compound was confirmed on the basis of spectral studies. In IR spectra showed a band at 2851 cm$^{-1}$ for the confirmation of aldehydic C-H stretching was very clear. The $^1$H-NMR spectra left no doubt with a singlet of aldehydic proton at 9.810 ppm. Compound 2 [2-(biphenyl-4-yl)-2-oxoacetaldehyde] was refluxed with different aromatic aldehyde in the presence of ammonium acetate and glacial acetic acid. The usual work up of the reaction mixture followed by recrystallized from acetone to get the desired products (3i-xii). The compound was found pure on TLC examination (TEF 5: 4: 1) and its spectral data was found satisfactory for the proposed structures. The structure of this compound was confirmed on the basis of their IR and $^1$H-NMR spectral studies. In IR spectral studies, the compounds showed intense bands in the region 1535-1633 cm$^{-1}$ of C=N stretching due to the ring closure. In addition, the absorption bands at 1351-1367 cm$^{-1}$ are attributed to the C-N stretching vibrations, which also confirm the formation of desired imidazole ring in the compounds. Whereas $^1$H-NMR spectra further confirm the structure due to disappearance of the peak of aldehydic proton and appearance of a single peak of NH (imidazole ring) at 11.23 ppm due to ring closure.

![Scheme: Synthetic protocol.](image-url)
Evaluation of Anti-microbial Screening

Anti-bacterial activity

The results of anti-bacterial screening of all the newly synthesized compounds are showed in Table 2 and 3. Compound 3iv [4-(biphenyl-4-yl)-2-(3-chlorophenyl)-1H-imidazole] and compound 3x [4-(biphenyl-4-yl)-2-(4-methoxyphenyl)-1H-imidazole] showed notable activity against E. coli, B. subtilis and K. pneumoniae. Some of them showed moderate activity and others rest good activity. Compounds (3ii, 3viii & 3ix) were shown moderate activity against E. coli, S. aureus, M. luteus and K. pneumonia, whereas compounds (3iii, 3vii, 3xi & 3xii) showed mild activity against few bacterial strainsµg/mL.

Table 1: Antibacterial activity measure by zone of inhibition of title compounds (3i-xii).

| Compd. | E. coli (MTCC-1687) | S. aureus (MTCC-2940) | B. subtilis (MTCC-441) | M. luteus (MTCC 7527) | K. pneumonia (MTCC 3040) |
|--------|---------------------|-----------------------|------------------------|------------------------|--------------------------|
|        | 50 µg/mL ± SD | 100 µg/mL ± SD | 50 µg/mL ± SD | 100 µg/mL ± SD | 50 µg/mL ± SD | 100 µg/mL ± SD | 50 µg/mL ± SD | 100 µg/mL ± SD |
| 3i     | 8.11 ± 2.35 | 14.01 ± 2.01 | nt | nt | 8.32 ± 0.58 | 9.20 ± 1.32 | 10.31 ± 1.58 | 9.31 ± 1.11 |
| 3ii    | 14.13 ± 1.71 | 16.78 ± 1.21 | 16.51 ± 1.51 | 17.21 ± 2.51 | 13.21 ± 1.31 | 14.21 ± 1.32 | 14.47 ± 1.32 | 17.17 ± 1.44 |
| 3iii   | 12.17 ± 3.26 | 13.58 ± 1.27 | nt | nt | 13.51 ± 1.37 | 15.72 ± 2.32 | nt | nt |
| 3iv    | 20.31 ± 1.51 | 23.53 ± 1.23 | 14.18 ± 1.32 | 16.87 ± 1.17 | 19.17 ± 1.16 | 21.97 ± 0.67 | 15.21 ± 1.23 | 16.54 ± 1.18 |
| 3v     | 15.13 ± 1.33 | 17.37 ± 1.04 | 13.17 ± 1.12 | 14.65 ± 1.71 | 14.64 ± 1.52 | 15.61 ± 1.12 | 13.61 ± 1.53 | 15.67 ± 0.58 |
| 3vi    | 14.17 ± 1.12 | 16.31 ± 1.51 | 14.61 ± 1.45 | 15.23 ± 2.53 | 16.6 ± 1.73 | 15.21 ± 1.25 | 11.67 ± 1.53 | 18.31 ± 1.12 |
| 3vii   | 16.23 ± 2.35 | 15.31 ± 1.53 | nt | nt | 15.47 ± 1.23 | 17.68 ± 1.51 | 17.21 ± 1.00 | 18.21 ± 1.01 |
| 3viii  | 16.15 ± 1.13 | 17.36 ± 2.31 | 15.61 ± 1.45 | 16.23 ± 2.51 | 14.61 ± 1.53 | 15.31 ± 1.11 | 15.17 ± 1.39 | 17.61 ± 0.21 |
| 3ix    | 14.15 ± 1.11 | 16.36 ± 1.33 | 15.17 ± 1.21 | 16.33 ± 1.11 | 13.23 ± 1.11 | 14.83 ± 1.53 | 15.93 ± 1.15 | 17.31 ± 1.27 |
| 3x     | 18.15 ± 1.41 | 21.61 ± 1.03 | 15.11 ± 0.5 | 17.21 ± 1.23 | 17.01 ± 1.31 | 19.61 ± 1.41 | 15.16 ± 1.51 | 14.61 ± 2.13 |
| 3xi    | nt | nt | 13.61 ± 1.05 | 15.61 ± 1.33 | 11.51 ± 1.72 | 13.12 ± 1.51 | 9.61 ± 1.15 | 11.67 ± 2.36 |
| 3xii   | 11.68 ± 1.15 | 15.51 ± 1.67 | 11.23 ± 2.07 | 14.61 ± 1.57 | 12.33 ± 1.32 | 14.61 ± 1.51 | nt | nt |
| Cipro. | 27.51 ± 1.21 | 29.11 ± 1.17 | 29.31 ± 1.41 | 30.37 ± 1.71 | 28.45 ± 1.51 | 29.81 ± 1.61 | 28.33 ± 1.53 | 30.17 ± 1.11 |

Measure zone of inhibition in millimeter, SD; Standard Deviation, Compd.; Compounds, Cipro; Ciprofloxacin, nt; means not tested compounds.

Anti-fungal activity

The results of anti-fungal screening of all the newly synthesized compounds are presented in Table 4 and 5. Compound 3iv [4-(biphenyl-4-yl)-2-(3-chlorophenyl)-1H-imidazole] and compound 3x [4-(biphenyl-4-yl)-2-(4-methoxyphenyl)-1H-imidazole] were shown notable activity against E. coli, B. subtilis and K. pneumoniae. Some of them showed moderate activity and others rest good activity. Compounds (3ii, 3viii & 3ix) showed moderate activity against E. coli, S. aureus, M. luteus and K. pneumonia, whereas compounds (3iii, 3vii, 3xi & 3xii) showed mild activity against few bacterial strains.

The compounds of electron releasing imidazole derivatives (3i, 3iv, 3vii, 3ix, 3x & xi) presented comparatively better anti-fungal activity than the compounds of electron withdrawing imidazole derivatives (3iii, 3vii & 3xii). Regarding the overall anti-fungal activity was found to be the most potent compound having meta substituted chloro group attached to the aromatic ring and methoxy group on para position.
Table 2: Antifungal activity as zone of inhibition of title compounds (3i-xii).

| Compd. | C. albicans (MTCC-3617) | A. niger (MTCC-281) | A. flavus (MTCC 418) |
|--------|-------------------------|----------------------|----------------------|
|        | 50 µg/mL ± SD | 100 µg/mL ± SD | 50 µg/mL ± SD | 100 µg/mL ± SD | 50 µg/mL ± SD | 100 µg/mL ± SD |
| 3i     | 15.67 ± 1.53 | 18.31 ± 1.54 | 15.67 ± 1.52 | 19.23 ± 1.08 | 16.54 ± 1.23 | 19.13 ± 2.01 |
| 3ii    | 17.12 ± 2.41 | 19.63 ± 1.21 | 19.71 ± 1.56 | 20.19 ± 1.61 | 19.67 ± 1.53 | 21.81 ± 1.64 |
| 3iii   | 11.61 ± 1.52 | 19.17 ± 2.26 | 17.67 ± 1.52 | 16.33 ± 1.01 | nt            | nt            |
| 3iv    | 16.21 ± 1.63 | 18.73 ± 1.08 | 19.50 ± 1.12 | 22.67 ± 1.57 | 21.50 ± 1.32 | 23.61 ± 1.53 |
| 3v     | nt           | nt            | 16.00 ± 1.00 | 14.31 ± 1.54 | 15.03 ± 1.42 | 16.32 ± 1.53 |
| 3vi    | 20.61 ± 1.04 | 24.21 ± 1.14 | 21.67 ± 1.08 | 21.31 ± 1.31 | 23.67 ± 2.08 | 13.33 ± 2.53 |
| 3vii   | 14.67 ± 1.63 | 22.04 ± 1.55 | 13.33 ± 1.53 | 16.01 ± 1.06 | 11.33 ± 1.33 | 18.56 ± 1.21 |
| 3viii  | 17.31 ± 1.34 | 19.09 ± 4.65 | 14.61 ± 1.53 | 18.83 ± 1.23 | 21.67 ± 1.51 | 22.39 ± 1.04 |
| 3ix    | 16.67 ± 2.51 | 15.31 ± 2.53 | 16.60 ± 1.53 | 15.31 ± 2.08 | nt            | nt            |
| 3x     | 17.13 ± 1.04 | 19.31 ± 1.53 | 16.01 ± 1.07 | 19.36 ± 1.53 | 18.21 ± 1.22 | 20.31 ± 1.53 |
| 3xi    | 19.63 ± 3.53 | 22.20 ± 1.21 | 13.33 ± 2.51 | 16.43 ± 1.12 | 11.31 ± 1.54 | 17.01 ± 1.32 |
| 3xii   | nt           | nt            | 16.61 ± 1.15 | 15.81 ± 1.04 | 15.61 ± 1.47 | 16.81 ± 1.01 |
| Fluco  | 31.23 ± 1.14 | 31.81 ± 1.72 | 30.17 ± 1.32 | 31.23 ± 1.21 | 28.56 ± 2.51 | 31.15 ± 2.13 |

CONCLUSION
A number of compounds 3i-xii [4-(biphenyl-4-yl)-2-(substituted phenyl)-IH-imidazole] have been successfully synthesized. The pharmacological study was performed to evaluate the effects of substituent on the antibacterial and antifungal activities. The biological activity result revealed that all the newly synthetic compounds 3i-xii [4-(biphenyl-4-yl)-2-(substituted phenyl)-IH-imidazole] exhibited better antibacterial activity as compared to antifungal activity in compare to reference drug. The results of anti-bacterial screening further revealed that among all the compounds, the compound (3iv) and (3x) were observed significant anti-bacterial activity against E. coli, B. subtilis and K. pneumoniae while compounds (3ii), (3viii) and (3ix) as well as compounds (3xi) and (3vii) showed moderate anti-bacterial activity in compare to standard drug ciprofloxacin. The results of anti-fungal screening showed that the compound (3ii) and (3viii) showed good anti-fungal activity against A. niger and A. flavus and compound (3xi) showed notable activity against C. albicans. The compound (3vii) and (3ix) were shown moderate activity against C. albicans and A. niger.

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