World Inventia Publishers

Journal of Pharma Research

http://www.jprinfo.com/

Vol. 8, Issue 4, 2019 ISSN: 2319-5622 USA CODEN: JPROK3

Research Article

ANTIMICROBIAL SCREENING OF 1-METHYLSUBSTITUTED-2-SUBSTITUTED-4,5-DIPHENYL IMIDAZOLE DERIVAITVES

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Received on: 21-03-2019; Revised and Accepted on: 18-04-2019

ABSTRACT

R*ecently, there has been upward interest in findings of new antimicrobial agents from various sources to fight against infectious microbial pathogens. Consequently, a greater attention has been given to synthesize novel compounds. In this study, series of 1-methylsubstituted-2-substituted-4,5-diphenyl imidazole derivatives were designed and synthesized. The structures of synthesized compounds were recognized by IR, ^{1}H-NMR, Mass spectra and CHN analysis. The synthesized compounds were screened for their in-vitro antimicrobial activity against gram positive and gram negative microorganisms as bacterial strains and some fungal strains. Most of the compounds have shown significant antimicrobial activity when compared with standard drug.*

KEYWORDS: *Antimicrobial activity, CHN analysis, Imidazole derivatives, Spectral analysis.*

INTRODUCTION

During the past decades, the human population affected with life-threatening infectious diseases caused by multidrug-resistant gram positive and gram negative bacteria increased frightening level around the world. Due to this reason, new classes of antimicrobial agents are vital necessitate to fight against multidrug-resistant infections [1]. The synthetic study of analogues containing hetero atom nitrogen i.e. imidazole has been and continues to be one of the most vital and attractive areas of heterocyclic chemistry. Imidazole moiety forms the structures of some distinguished components of human organisms, i.e. the amino acid and component of DNA base structure. It is also present in the structure of many natural and synthetic drug molecules. The extensive biological importance of imidazoles has encouraged us to synthesize its derivatives. Imidazoles, being an important nucleus have a wide range of therapeutic activities like antimicrobial [2], analgesic [3], anti-inflammatory [4], anthelmintic [5], antileishmanial [6] and antiepileptic [7] activity. Some of the literature findings have promoted us to synthesize comparable compounds.

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DOI: https://doi.org/10.5281/zenodo.2647860

MATERIALS AND METHODS

All the chemicals and reagents used for reactions were of analytical grade. Progress of reactions and product formation was monitored by thin layer chromatographic method. Thin layer chromatography for compounds was performed using silica gel-G on glass plate in different solvents. Melting points were determined in open capillary method and are uncorrected. IR spectra were recorded on Thermo Nicolet IR 200 spectrophotometer using KBr disc method. ^{1}H NMR spectra were recorded on BRUKER AVANCE II 400 NMR Spectrometer, in DMSO-d_6 solvents using TMS as an internal standard. Mass spectra were recorded on Waters, Q-TOF Micromass. Elemental analysis was recorded on Perkin-Elmer Model 2400.

Biological Evaluation:

Antimicrobial Activity: [8, 9]

All the synthesized compounds were screened for their antimicrobial activity by cup plate diffusion method. The synthesized compounds were tested in-vitro for antibacterial activity against gram positive microorganism viz. *Staphylococcus aureus, Streptococcus pneumoniae* and gram negative microorganism *Escherichia coli*, *Salmonella Typhi* using nutrient agar medium. Similarly in-vitro antifungal activity was carried out against *Candida albicans* and *Aspergillus niger* using Sabouraud-Dextrose agar. The standard drug Norfloxacin and Griseofulvin were used for antibacterial and antifungal activity respectively.

Experimental:

General procedure for preparation of 2-substituted 4,5-diphenyl imidazoles: [10]

A mixture of benzil (25 mmole), substituted benzaldehyde (25 mmole) and ammonium acetate 10gm were

dissolved in glacial acetic acid and then refluxed for 3-5 hrs. After refluxing, the reaction mixture was kept overnight and filtered to remove any precipitate that may be present. Water was then added to filtrate & precipitate formed was collected. The filtrate was neutralized with ammonium hydroxide; the second crop of solid was collected. The two crops of precipitate was combined, dried and recrystallized from suitable solvents.

General procedure for preparation of mannich bases of 2-substitued 4,5-diphenyl imidazoles (A_1-A_{20}):

A mixture 0.001 mole of 2-substituted 4,5-diphenyl imidazoles & 0.001 mole of different aromatic amines and 0.001 mole of formaldehyde dissolved in 25 ml ethanol & refluxed for 3-5 hrs. The progress of the reaction was monitored by TLC using Benzene: Chloroform (1:4). The resultant mixture was poured on ice cold water and separated solid product **(A_1-A_{20})** was obtained, recrystallized from suitable solvents. Slight change in reaction time of each compound, completion of the reaction was confirmed by TLC.

1-((2,4,5-triphenyl-1H-imidazol-1-yl)methyl)piperidine (A_1):

M. P.: 252-254 ˚C, Yield: 76%, R_f: 0.53. IR (KBr) cm^{-1}: 3196.08 (C-H Ar. Str.), 2970.70 (aliphatic C-H), 1501.11 (C=C Ar. Str.), 1451.39 (C=N str.). ^{1}H-NMR δ ppm: 7.24-8.11 (15H, m, Ar-H), 3.65 (2H of CH_2, piperidine), 2.55 (2H of CH_2, methylene). Mass *M/Z*: M^+ 388, Anal. calcd. for $C_{27}H_{27}N_3$: C, 82.41; H, 6.92; N, 10.68. Found: C, 82.45; H, 6.95; N, 10.64.

4-((2,4,5-triphenyl-1H-imidazol-1-yl)methyl)morpholine (A_2):

M. P.: 263-265 ˚C, Yield: 65%, R_f: 0.63. IR (KBr) cm^{-1}: 3040.70 (C-H Ar. Str.), 2830.87 (aliphatic C-H), 1511.11 (C=C Ar, str.), 1471.04 (C=N str.) ^{1}H-NMR δ ppm: 7.26-8.11 (15H, m, Ar-H), 3.32 (2H of CH_2, Morpholine), 2.54-2.55 (2H of CH_2, methylene). Anal. calcd. for $C_{26}H_{25}N_3O$: C, 78.96; H, 6.37; N, 10.62. Found: C, 78.99; H, 6.41; N, 10.65.

1-methyl-4-((2,4,5-triphenyl-1H-imidazol-1-yl)methyl) piperazine (A_3):

M. P.: 274-276 ˚C, Yield: 70%, R_f: 0.39. IR (KBr) cm^{-1}: 3198.91 (C-H Ar. str.), 2975.11 (aliphatic C-H), 1500.66 (C=C Ar. Str.), 1452.12 (C=N str.). Mass M/Z: M^+ 403. Anal. calcd. for $C_{27}H_{28}N_4$: C, 79.38; H, 6.91; N, 13.71. Found: C, 79.41; H, 6.94; N, 13.74.

N'-((2,4,5-triphenyl-1H-imidazol-1-yl)methyl)isonicotino hydrazide (A_4):

M. P.: 216-218 ˚C, Yield: 72%, R_f: 0.46. IR (KBr) cm^{-1}: 3430.20 (N-H str.), 3041.60 (C-H Ar. Str.), 2966.6 (aliphatic C-H), 1742.9 (C=O str.), 1599.0 (C=C Ar. Str.), 1461.0 (C=N str.). ^{1}H-NMR δ ppm: 6.93-8.03 (18H, m, Ar-H), 8.05 (1H of NH), 2.54-2.55 (2H of CH_2, methylene). Anal. calcd. for $C_{28}H_{23}N_5O$: C, 75.49; H, 5.20; N, 15.72. Found: C, 75.52; H, 5.24; N, 15.75.

4-((2,4,5-triphenyl-1H-imidazol-1-yl)methylamino)benzene sulfonamide (A_5):

M. P.: 197-199 ˚C, Yield: 57%, R_f: 0.58. IR (KBr) cm^{-1}: 3426.4 (N-H str.), 3028.7 (C-H Ar. Str.), 2925.5 (aliphatic C-H), 1602.2 (C=C Ar. Str.), 1440.4 (C=N str.). Anal. calcd. for $C_{28}H_{24}N_4O_2S$: C, 69.98; H, 5.03; N, 11.66. Found: C, 69.94; H, 5.07; N, 11.70.

4-(4,5-diphenyl-1-(piperine-1-ylmethyl)-1H-imidazol-2-yl) phenol (A_6):

M. P.: 201-203 ˚C, Yield: 62%, R_f: 0.71. IR (KBr) cm^{-1}: 3432.6 (O-H str.), 3062.0 (C-H Ar. str.), 2926.0 (aliphatic C-H), 1605.2 (C=C Ar. str.), 1485.4 (C=N str.). ^{1}H-NMR δ ppm: 9.51 (s, 1H, OH), 6.82-7.95 (14H, m, Ar-H), 3.35 (2H of CH_2, Piperidine), 2.55 (2H of CH_2, methylene). Anal. calcd. for $C_{27}H_{27}N_3O$: C, 79.19; H, 6.65; N, 10.26. Found: C, 79.22; H, 6.67; N, 10.30.

4-(-morpholinomethyl)-4,5-diphenyl-1H-imidazol-2-yl) phenol (A_7):

M. P.: 230-233 ˚C, Yield: 66%, R_f: 0.54. IR (KBr) cm^{-1}: 3429.4 (O-H str.), 3067.3 (C-H Ar. str.), 2960.6 (aliphatic C-H), 1606.4 (C=C Ar. str.), 1445.4 (C=N str.). Anal. calcd. for $C_{26}H_{25}N_3O_2$: C, 75.89; H, 6.12; N, 10.21. Found: C, 75.93; H, 6.16; N, 10.24.

4-(1-((4-methylpiperazin-1-yl)-4,5-diphenyl-1H-imidazol-2-yl)phenol (A_8):

M. P.: 242-245 ˚C, Yield: 68%, R_f: 0.62. IR (KBr) cm^{-1}: 3429.4 (O-H str.), 3067.3 (C-H Ar. str.), 2960.6 (aliphatic C-H), 1606.4 (C=C Ar. str.), 1445.4 (C=N str.). Anal. calcd. for $C_{27}H_{28}N_4O$: C, 76.39; H, 6.65; N, 13.20. Found: C, 76.42; H, 6.68; N, 13.23.

N'-((2-(4-hydroxyphenyl)-4,5-diphenyl-1H-imidazol-1-yl) methyl)isonicotinohydrazide (A_9):

M. P.: 231-234 ˚C, Yield: 64%, R_f: 0.48. IR (KBr) cm^{-1}: 3353.21 (O-H str.), 3208.33 (N-H str.), 3048.19 (C-H Ar. str.), 2921.63 (aliphatic C-H), 1687.91 (C=O str.), 1500.60 (C=C Ar. str.), 1452.12 (C=N str.). Anal. calcd. for $C_{28}H_{23}N_5O_2$: C, 72.87; H, 5.02; N, 15.17. Found: C, 72.91; H, 5.06; N, 15.21.

4-((2-(4-hydroxyphenyl)-4,5-diphenyl-1H-imidazol-1-yl) methyl)benzenesulfonamide (A_{10}):

M. P.: 257-259 ˚C, Yield: 53%, R_f: 0.35. IR (KBr) cm^{-1}: 3432.8 (O-H str.), 3175.8 (N-H str.), 3058.0 (C-H Ar. str.), 2928.3 (aliphatic C-H), 1610.3 (C=C Ar. str.), 1464.0 (C=N str.). Anal. calcd. for $C_{28}H_{24}N_4O_3S$: C, 67.72; H, 4.87; N, 11.28. Found: C, 67.75; H, 4.89; N, 11.32.

4-(4,5-diphenyl-1-(piperidin-1-ylmethyl)-1H-imidazol-2-yl) - 2-methoxyphenol (A_{11}):

M. P.: 243-245 ˚C, Yield: 74%, R_f: 0.72. IR (KBr) cm^{-1}: 3301.17 (O-H Str.), 3030.32 (Ar C-H Str), 2929.34 (aliphatic C-H), 1604.96 (Ar C=C Str.), 1456.25 (C=N Str.). Mass M/Z: M^+ 437. Anal. calcd. for $C_{28}H_{29}N_3O_2$: C, 76.51; H, 6.65; N, 9.56. Found: C, 76.54; H, 6.61; N, 9.53.

2-methoxy-4-(1-(morpholinemethyl)-4,5-diphenyl-1H-imidazol-2-yl)phenol (A_{12}):

M. P.: 224-226 ˚C, Yield: 58%, R_f: 0.67. IR (KBr) cm^{-1}: 3490. 36 (O-H Str.), 3062.01 (Ar C-H Str), 2931.31 (aliphatic C-H), 1610.87 (Ar C=C Str.), 1449.68 (C=N Str.). Anal. calcd. for $C_{27}H_{27}N_3O_3$: C, 73.45; H, 6.16; N, 9.52. Found: C, 73.41; H, 6.20; N, 9.47.

2-methoxy-4-(1((4-methylpiperazin-1-yl)methyl)-4,5-diphenyl-1H-imidazol-2-yl)phenol (A_{13}):

M. P.: 262-264 ˚C, Yield: 67%, R_f: 0.61. IR (KBr) cm^{-1}: 3377.69 (O-H Str.), 3060.04 (Ar C-H Str), 2824.24 (aliphatic C-H), 1600.36 (Ar C=C Str.), 1447.85 (C=N Str.). Anal. calcd. for $C_{28}H_{30}N_4O_2$: C, 73.98; H, 6.65; N, 12.33. Found: C, 73.95; H, 6.70; N, 12.36.

N' ((2-(4-hydroxy-3-methoxyphenyl)-4,5-diphenyl-1H-imidazol-1-yl)methyl)isonicotino hydrazide (A_{14}):

M. P.: 238-241 ˚C, Yield: 52%, R_f: 0.58. IR (KBr) cm^{-1}: 3510.59 (O-H Str.), 3378.21 (N-H Str.), 3032.84 (Ar C-H Str), 2917.08 (aliphatic C-H), 1604.88 (C=O str.), 1499.60 (Ar C=C Str.), 1451.32 (C=N Str.). ^{1}H-NMR δ ppm: 8.99 (s, 1H, OH), 6.86-

7.67 (17H, m, Ar-H), 7.92 (1H of NH), 3.92 (3H of CH_3), 2.55 (2H of CH_2, methylene). Anal. calcd. for $C_{29}H_{25}N_5O_3$: C, 70.86; H, 5.13; N, 14.25. Found: C, 70.89; H, 5.17; N, 14.21.

4-((2-(4-hydroxy-3-methoxyphenyl)-4,5-diphenyl-1H-imidazol-1-yl)methylamino) benzene sulfonamide (A_{15}):

M. P.: 218-221 ˚C, Yield: 63%, R_f: 0.35. IR (KBr) cm^{-1}: 3511.5 (O-H Str.), 2925.3 (Ar C-H Str), 2854.8 (aliphatic C-H), 1607.3 (C=O str.), 1498.5 (Ar C=C Str.), 1452.3 (C=N Str.). ^{1}H-NMR δ ppm: 9.06 (s, 1H, OH), 6.88-8.01 (17H, m, Ar-H), 7.55 (1H of NH), 3.91 (3H of CH_3), 2.53 (2H of CH_2, methylene). Anal. calcd. for $C_{29}H_{26}N_4O_4S$: C, 66.14; H, 4.98; N, 10.64. Found: C, 66.17; H, 4.99; N, 10.67.

1-((2-(2-chlorophenyl)-4,5-diphenyl-1H-imidazol-1-yl) methyl)piperidine (A_{16}):

M. P.: 230-233 ˚C, Yield: 60%, R_f: 0.45. IR (KBr) cm^{-1}: 3062.1 (C-H Ar. Str.), 2925.2 (aliphatic C-H), 1505.8 (Ar.C=C Str.), 1450.9 (C=N str.), 773.0 (C-Cl). ^{1}H-NMR δ ppm: 6.82-7.92 (14H, m, Ar-H), 3.54 (2H of CH_2, piperidine), 2.54-2.55 (2H of CH_2, methylene). Anal. calcd. for $C_{27}H_{26}ClN_3$: C, 75.77; H, 6.12; N, 9.82. Found: C, 75.75; H, 6.16; N, 9.84.

4-((2-(2-chlorophenyl)-4,5-diphenyl-1H-imidazol-1-yl) methyl)morpholine (A_{17}):

M. P.: 247-249 ˚C, Yield: 55%, R_f: 0.52. IR (KBr) cm^{-1}: 3044.04 (C-H Ar. str.), 2925.53 (aliphatic C-H), 1592.98 (C=C Ar. str.), 1452.75 (C=N str.), 768.31 (C-Cl). Anal. calcd. for $C_{26}H_{24}ClN_3O$: C, 72.63; H, 5.63; N, 9.77. Found: C, 72.66; H, 5.67; N, 9.80.

1-((2-(2-chlorophenyl)-4,5-diphenyl-1H-imidazol-1-yl) methyl)-4-methyl piperazine (A_{18}):

M. P.: 247-249 ˚C, Yield: 55%, R_f: 0.52. IR (KBr) cm^{-1}: 3032.84 (C-H Ar. str.), 2943.79 (aliphatic C-H), 1604.88 (C=C Ar. str.), 1451.32 (C=N str.), 770.87 (C-Cl). ^{1}H-NMR δ ppm: 7.28-8.11 (14H, m, Ar-H), 3.32 (2H of CH_2 Piperazine), 2.55 (2H of CH_2, methylene). Anal. calcd. for $C_{27}H_{27}ClN_4$: C, 73.21; H, 6.14; N, 12.65. Found: C, 73.25; H, 6.17; N, 12.68.

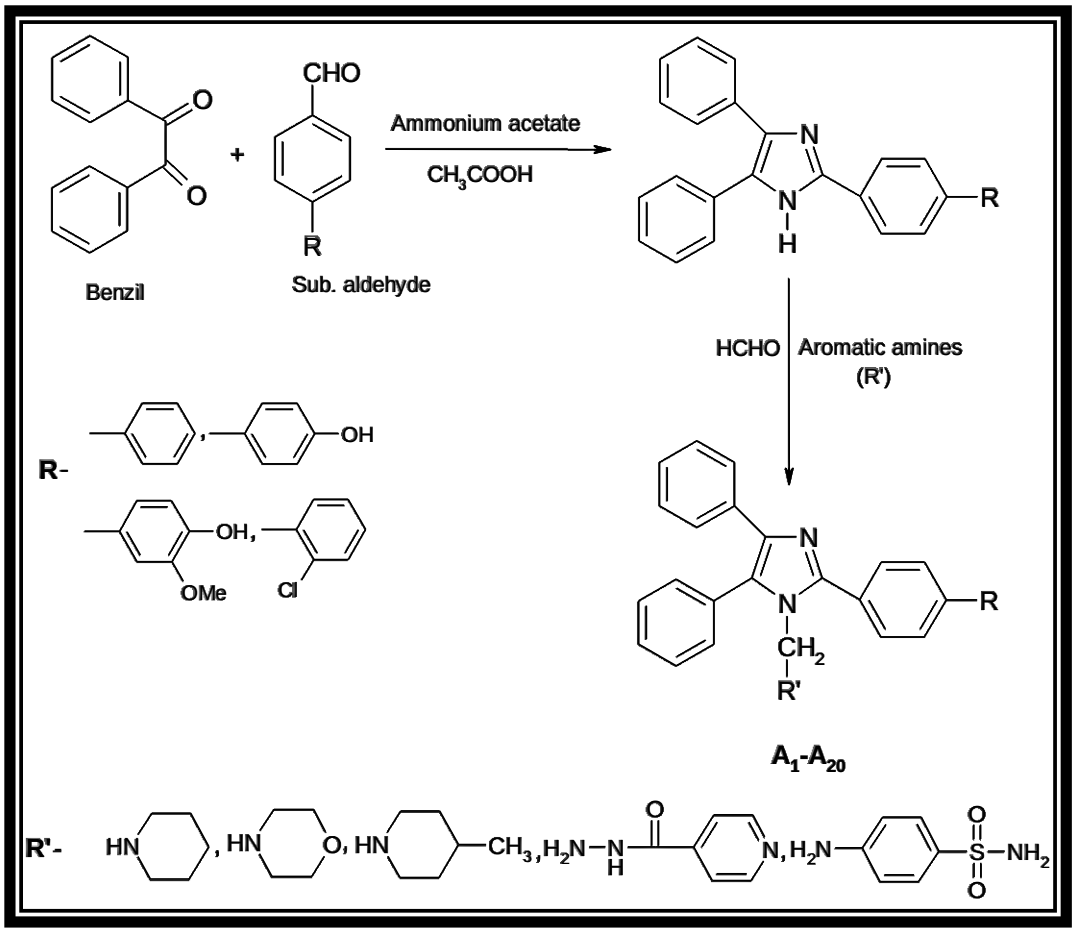
N'-((2-(2-chlorophenyl)-4,5,-diphenyl-1H-imidazol-1-yl) methyl)isonicotinohydrazide (A_{19}):

M. P.: 226-228 ˚C, Yield: 65%, R_f: 0.42. IR (KBr) cm^{-1}: 3419.9 (N-H str.), 3058.0 (C-H Ar. str.), 2927.4 (aliphatic C-H), 1603.52 (C=C Ar. str.), 1729.8 (C=O str.), 1450.2 (C=N str.), 767.9 (C-Cl). Anal. calcd. for $C_{28}H_{22}ClN_5O$: C, 70.07; H, 4.62; N, 14.59. Found: C, 70.11; H, 4.65; N, 14.63.

4-((2-(2-chlorophenyl)-4,5-diphenyl-1H-imidazol-1-yl) methylamino)benzene sulfonamide (A_{20}):

M. P.: 259-261 ˚C, Yield: 57%, R_f: 0.49. IR (KBr) cm^{-1}: 3431.9 (N-H str.), 3033.7 (C-H Ar. str.), 2921.2 (aliphatic C-H), 1601.1 (C=C Ar. str.), 1445.4 (C=N str.), 767.5 (C-Cl). ^{1}H-NMR δ ppm: 6.60- 8.04 (18H, m, Ar-H), 7.59 (s, 1H of NH), 3.83 (2H of CH_2, methylene). Anal. calcd. for $C_{28}H_{23}ClN_4O_2S$: C, 65.30; H, 4.50; N, 10.88. Found: C, 65.34; H, 4.48; N, 10.91.

SCHEME



RESULTS AND DISCUSSION

2-substituted 4,5-diphenyl imidazoles (A_1-A_{20}) were synthesized by reacting equimolar quantity of benzil, substituted benzaldehyde and ammonium acetate, which were then reacted with different aromatic amines to yield 1-methylsubstituted-2-substituted-4,5-diphenyl imidazoles. The structure of all synthesized compounds was recognized from their physical, analytical and spectral data.

All the synthesized compounds have been screened for their antimicrobial activity by Cup Plate diffusion method. The synthesized compounds were screened for their antibacterial activity against gram positive microorganism *Staphylococcus aureus, Streptococcus pneumoniae* and gram negative microorganism *Escherichia coli*, *Salmonella Typhi* at concentration of 100µg/ml by using nutrient agar. Norfloxacin was used as standard drug and Dimethylformamide as solvent control. The majority of compounds have exhibited moderate to equipotent activity against the pathogenic organisms used for the study. The compounds A_1, A_5, A_8, A_{11}, A_{17} and A_{19} exhibited equipotent activity. The remaining synthesized compounds have shown moderate antibacterial activity. The results and graphical representation of antibacterial activity were showed in table no. 1 and figure 1 resp.

The synthesized compounds were also screened for the antifungal activity against *Aspergillus niger* and *Candida albicans* at concentration of 100µg/ml using Sabouraud-Dextrose agar. Griseofulvin used as standard drug. Most of the compounds have shown promising antifungal activity against the pathogenic organisms used for the study. Amongst them compounds A_3, A_5, A_8, A_{12}, A_{14}, A_{16} and A_{17} have shown the superior activity as comparable to standard drug and remaining compounds were shown moderate to good antifungal activity. The results and graphical representation of antifungal activity were showed in table no. 2 and figure 2 resp.

Table No. 1: Antibacterial activity of synthesized compounds (A_1-A_{20})

Comp.	Zone of inhibition at 100 µg/ml (in mm)			
	S. aureus	S. pneumoniae	E. coli	S. Typhi
A_1	21	20	22	20
A_2	16	13	15	15
A_3	16	15	16	14
A_4	15	12	13	11
A_5	21	19	20	18
A_6	16	16	17	13
A_7	13	11	14	10
A_8	22	21	22	20
A_9	16	17	15	15
A_{10}	18	19	18	16
A_{11}	23	21	21	20
A_{12}	16	15	17	14
A_{13}	14	11	12	10
A_{14}	20	18	19	19
A_{15}	15	14	18	16
A_{16}	18	15	14	15
A_{17}	23	21	22	21
A_{18}	14	12	15	11
A_{19}	23	22	21	19
A_{20}	18	15	16	15
Standard	**24**	**24**	**23**	**23**

Table No. 2: Antifungal activity of synthesized compounds (A_1-A_{20})

Comp.	Zone of inhibition at 100 µg/ml (in mm)	
	A. niger	*C.* albicans
A_1	16	18
A_2	17	18
A_3	24	23
A_4	17	19
A_5	22	20
A_6	14	15
A_7	16	15
A_8	20	19
A_9	17	16
A_{10}	14	13
A_{11}	12	11
A_{12}	23	25

A_{13}	17	20
A_{14}	24	23
A_{15}	14	13
A_{16}	24	24
A_{17}	23	24
A_{18}	20	20
A_{19}	14	15
A_{20}	12	14
Standard	**25**	**26**

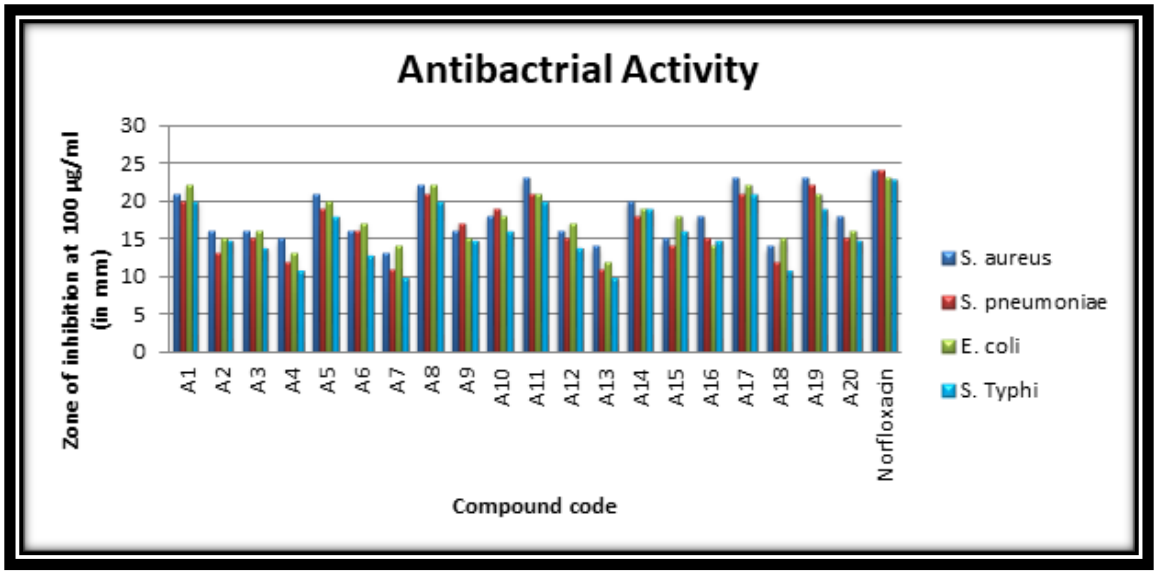


Fig. 1: Graphical Representation of Antibacterial activity (A_1-A_{20})

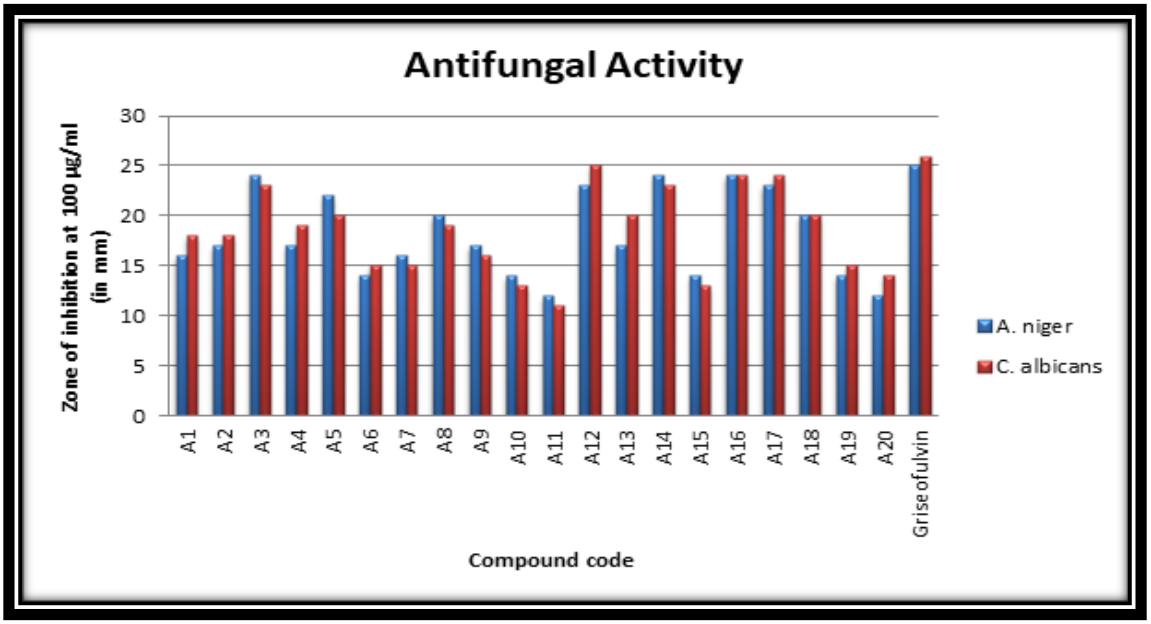


Fig. 2: Graphical representation of Antifungal activity (A_1-A_{20})

CONCLUSION

The objective of present research work is to synthesize novel 1-methylsubstituted-2-substitued-4,5-diphenyl imidazole derivatives and screened for their antimicrobial profile. The antimicrobial activity results revealed that some of the synthesized compounds were showed promising antibacterial and antifungal activities. The results of antibacterial activity revealed that the compounds A_1, A_5, A_8, A_{11}, A_{17} and A_{19} have exhibited equipotent activity against gram positive and gram negative microorganisms. Similarly, the results of antifungal activity revealed that the compounds A_3, A_5,

A_8, A_{12}, A_{14}, A_{16} and A_{17} have shown promising activity. From overall result, concluded that the incorporation of piperidine, morpholine, 1-methyl piperazine and sulphonamide on imidazole ring may contribute for the strengthening of antimicrobial activity.

ACKNOWLEDGEMENT

The authors are grateful to Management of Shri Gajanan Maharaj Shikshan Prasarak Mandal, Otur for constant encouragement and support. The authors are also gratefully acknowledged Dr. Ganesh Y. Dama, Principal, SGMSPM's Sharadchandra Pawar College of Pharmacy, Otur for providing necessary facility to carry out the research work and SAIF, Panjab University, Chandigarh for providing spectral data.

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How to cite this article:

VS. Wakale, DH. Nandal. ANTIMICROBIAL SCREENING OF 1-METHYLSUBSTITUTED-2-SUBSTITUTED-4,5-DIPHENYL IMIDAZOLE DERIVAITVES. J Pharm Res 2019;8(4):141-146. **DOI: https://doi.org/10.5281/zenodo.2647860**

Conflict of interest: The authors have declared that no conflict of interest exists.

Source of support: Nil