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ANTIMICROBIAL AND ANTIFUNGAL EVALUATION OF NEWLY SYNTHESIZED OXADIAZOLE DERIVATIVES BEARING 2, 4, 5-TRIPHNENYL IMIDAZOLE MOIETY

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Keywords:

2, 4, 5-Triphenyl imidazole, oxadiazole, antimicrobial agent and antifungal agents

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ABSTRACT: A series of Oxadiazole derivatives (4a-d) have been synthesized from (2, 4, 5-triphenyl-imidazole-1-yl)-acetic acid hydrazide under various reaction conditions. Elemental analysis, IR, ^{1}H NMR and mass spectral data confirmed the structure of the newly synthesized compounds. All the synthesized Oxadiazole derivatives have been investigated for their anti-fungal and antimicrobial activity showing moderate to good activity. The anti-fungal activity of the newly synthesized compounds was compared with the standard drug Voriconazole. The oxadiazole derivatives showed moderate to good ant-fungal activity. The compounds having 4-methoxy group showed good activity (81%). The Oxadiazole derivative having 4-flourophenyl group showed highest activity (82.14%). The results of anti-microbial and anti-fungal activity clearly indicated that oxadiazole ring bearing 2, 4, 5-triphneylimidazole have moderate activity.

INTRODUCTION: Imidazoles are probably the most well known heterocycle which is common and important feature of a variety of natural products and medicinal agents. Derivatives of imidazole were reported for anti-inflammatory, analgesic, anti-convulsant, tuberculostatic, antimicrobial, antifungal and anticancer activities [1-6]. Furthermore, oxadiazole were also reported to exhibit anti-inflammatory and antimicrobial activity [7-12]. Now days, multi drug treatment of inflammatory conditions associated with microbial infections poses a unique problem especially for patients with impaired liver or kidney functions. Hence, mono therapy with a drug having both anti-inflammatory and antimicrobial activities is highly desirable, both from the pharmacoeconomic as well as patient compliance points of view [13-15]. Encouraged by these observations and in continuation of the research programme on the synthesis of five member heterocyclic compounds, it was decided to synthesize various 2, 4, 5 triphenyl-1-substituted imidazoles derivatives and to evaluate them for their pharmacological activities. Here we are presenting our findings in this paper.

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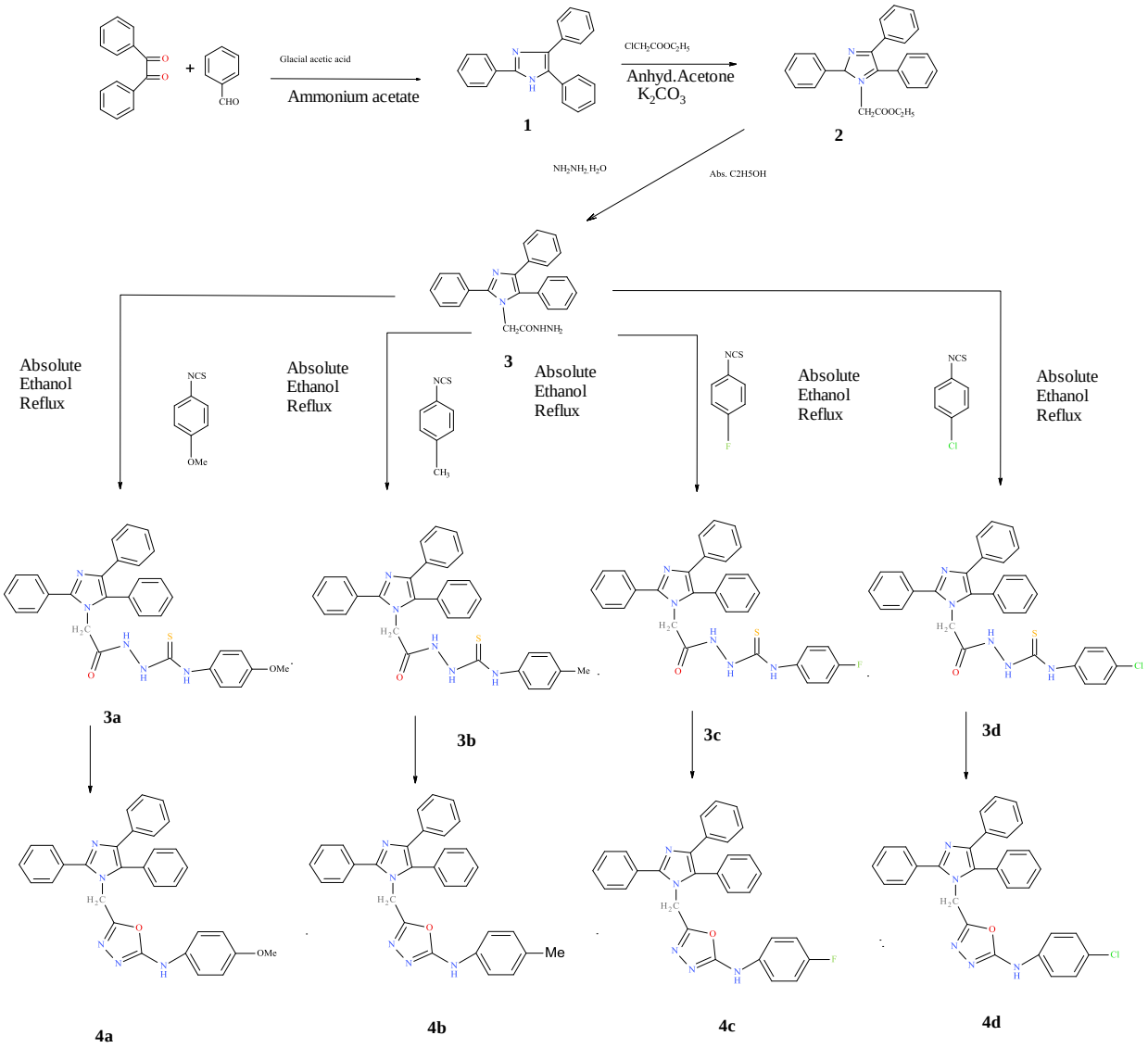
The reaction sequence leading to the formation of the desired heterocyclic compounds are outlined in **Scheme I**. (2,4,5-Triphenyl-imidazole-1-yl)-acetic acid hydrazide **3** was prepared by treating 2,4,5-triphenylimidazole with ethyl chloroacetate in the presence of anhydrous acetone and potassium carbonate followed by reaction with hydrazine hydrate in absolute ethanol. Hydrazide **3** on

treatment with various substituted phenylisothiocynate gave N^1-(2, 4, 5-triphenyl-imidazol-1-yl-ethanoyl) - N^4 - substitutedphenyl thiosemicarbazide 3**a-d**. The thiosemicarbazides were cyclised with 2% aqueous NaOH and Potassium Iodide to 1-{[2-(4-substituted phenyl amino)-(1, 3, 4-oxadiazol-5-yl)] methyl}-2, 4, 5-triphenylimidazole **4a-d.**



Scheme I

SCHEME: I

The IR spectrum of the compound **4a** showed absorption peaks at 1600-1695 cm^{-1} and 1600-1695 cm^{-1} due to C=C and C=N stretching vibration. The peak at 3310-3390 cm^{-1} appeared due to NH stretching vibration. The ^1H NMR spectra of compound 4**a** displayed a singlet at δ 3.73 showing the presence of a methoxy group. The NCH_2 proton appeared as a singlet at δ 4.45. A broad singlet at δ 12.69 indicated the presence of NH proton attached to oxadiazole ring. The IR spectrum of the compound 4**b** showed absorption peaks at 3310-3390 cm^{-1} (NH) stretching, and NH bending at (1530-1575) cm^{-1}, C-O-C group stretching at (3310-3395) cm^{-1}, C=C and C=N appears as a strong band at (1600-1695) cm^{-1} and (1600-1695) cm^{-1}. The ^1H NMR spectra of compound 4**b** displayed a singlet at δ 2.34 showing the presence of a methyl group. The NCH_2 proton appeared as a singlet at δ 4.45. A broad singlet at δ 12.13 indicated the presence of NH proton attached to oxadiazole ring.

The IR spectrum of the compound 4c showed absorption peaks at 3310-3389 cm^{-1} (NH)

stretching, and NH bending at (1530-1575) cm^{-1}, C-O-C group stretching at (3310-3390) cm^{-1}, C=C and C=N appears as a strong band at (1600-1695) cm^{-1} and (1600-1695) cm^{-1}. The ^1H NMR spectra of compound 4**b** displayed a singlet at δ 2.34 showing the presence of a methyl group. The NCH_2 proton appeared as a singlet at δ 4.45. A broad singlet at δ 12.57 indicated the presence of NH proton attached to oxadiazole ring.

The IR spectrum of the compound 4d showed absorption peaks at 3308-3390 cm^{-1} (NH) stretching, and NH bending at (1529-1573) cm^{-1}, C-O-C group stretching at (3310-3390) cm^{-1}, C=C and C=N appears as a strong band at (1600-1695) cm^{-1} and (1600-1695) cm^{-1}. The ^1H NMR spectra of compound 4**b** displayed a singlet at δ 2.44 showing the presence of a methyl group. The NCH_2 proton appeared as a singlet at δ 4.45. A broad singlet at δ 12.57 indicated the presence of NH proton attached to oxadiazole ring.

Biological Studies:

Adult male Wister strain rats of either sex, weighing 180-200 g were used. The animals were allowed food and water *ad libitum*. They were housed in a room at 25 ± 2°C, and 50 ± 5% relative humidity with 12 h light/dark cycle. The animals were randomly allocated into groups at the beginning of all the experiment. All the test compounds and reference drug were administered orally, suspended in 0.5% carboxymethyl cellulose (CMC) solution.

Antibacterial and antifungal activities:

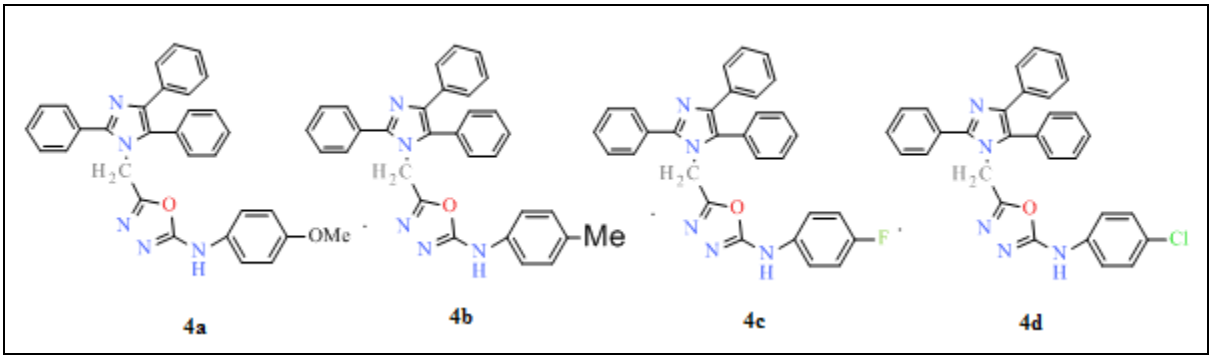
All the compounds have been screened for both antibacterial and antifungal activities using cup plate agar diffusion method [17] by measuring the inhibition zone in mm. Ofloxacin (100 µg/mL) was used as standard drug for antibacterial activity, and Vriconazole (100 µg/mL) as a standard drug for antifungal activity. The compounds were screened for antibacterial activity against *E. coli*, *B. subtalis* and *S. aureus* in nutrient agar medium, and for antifungal activity against *Candida albicans* in Sabouraud's dextrose agar medium. These sterilized agar media were poured into Petri-dishes and allowed to solidify. On the surface of the media microbial suspensions were spread with the help of sterilized triangular loop. A stainless steel cylinder of 8 mm diameter (pre-sterilized) was used to bore cavities. All the synthesized compounds (100µg/mL) were placed serially in the cavities with the help of micropipette and allowed to diffuse for 1 hr. DMF was used as solvent for all compounds and as control.

These plates were incubated at 37°C for 34 hr and 28°C for 48 hr, for antibacterial and antifungal activity respectively. The zone of inhibition observed around the cup after respective incubation was assured & percent inhibition of compounds was calculated. Results were presented in **Table 1.** The anti-microbial activity of the newly synthesized compounds 4**a-d** was compared with the standard drug ofloxacin. The oxadiazole derivatives 4**a-d** showed antimicrobial activity ranging from 48.38% to 82%. The compounds 4d having 4-chloro group showed moderate activity (82%) against *S. aureus* in comparison to standard drug ofloxacin. The compounds 4**c** having 4-fluoro group and 4d having 4-chloro group showed moderate activity (71%) against *E. coli* in comparison to standard drug ofloxacin.

TABLE 1: ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF COMPOUNDS 4a-d

	Antibacterial activity						**Antifungal activity**	
	E. coli		***B. subtalis***		***S. aureus***		***C. albicans***	
Compd	**Zone of inhibition (mm)**	**% inhibition**	**Zone of inhibition (mm)**	**% inhibition**	**Zone of inhibition (mm)**	**% inhibition**	**Zone of inhibition (mm)**	**% inhibition**
4a	22	70.96	19	65.51	21	75.00	21	81.48
4b	15	48.38	17	58.62	19	67.85	16	59.25
4c	22	70.96	15	51.72	23	82.14	21	77.77
4d	22	70.96	19	65.51	21	67.85	19	70.37
Ofloxacin	31	100	29	100	28	100	---	---
Vriconazole	---	---	---	---	---	---	27	100

The anti-fungal activity of the newly synthesized compounds 4**a-d** was compared with the standard drug Voriconazole. The oxadiazole derivatives 4**a-d** showed ant-fungal activity ranging from 59% to 81%. The compounds 4a having 4-methoxy group showed moderate activity (81%) against *C. albicans* in comparison to standard drug voriconazole. The compounds 4c having 4-fluoro group showed moderate activity (78%) against *C. albicans* in comparison to standard drug voriconazole. The Oxadiazole derivative 4**c** having 4-flourophenyl group showed highest activity (82.14%) against *S. aureous*, whereas compounds 4c and 4**d** showed high activity (70.96%) against *E. coli* when compared with standard drug Ofloxacin. The results of anti-microbial and anti-fungal activity clearly indicated that oxadiazole ring bearing 2, 4, 5-triphneylimidazole have moderate activity.



GRAPHICAL ABSTRACT

ANTIMICROBIAL AND ANTIFUNGAL EVALUATION OF NEWLY SYNTHESIZED OXADIAZOLE DERIVATIVES BEARING 2, 4, 5-TRIPHNENYL IMIDAZOLE MOIETY

A series of azole derivatives 4**a-d** have been synthesized from (2, 4, 5-triphenyl-imidazole-1-yl)-acetic acid hydrazide under various reaction conditions. Elemental analysis, IR, ^{1}H NMR and mass spectral data confirmed the structure of the newly synthesized compounds. All the synthesized oxadiazole derivatives have been investigated for their anti-inflammatory showing moderate to good activity.

Experimental Section:

All the chemicals used in the synthesis were supplied by E. Merck and S. D. Fine Chemicals. Melting point was determined by open capillary tube method and is uncorrected. Homogeneity of the compounds were checked on thin layer chromatography using iodine vapors as visualizing agent. IR spectra were obtained on a Perkin-Elmer 1720 FT-IR spectrometer (KBr pellets). ^{1}H NMR spectra were obtained on a Bruker DRX-300 (300 MHz FT NMR) spectrometer in $CDCl_3$ using tetramethysilane (TMS) as the internal reference (chemical shifts in δ, ppm). Mass spectra were recorded on a Jeol SX-102 spectrometer. Synthesis of 2,4,5-triphenyl-1*H*-imidazole **1** was carried out by the method reported in literature [18].

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Synthesis of (2, 4,5-triphenyl-imidazole-1-yl)-acetic acid ethyl ester 2. A mixture of 2,4,5-triphenylimidazole (0.01 mole) and ethyl chloroacetate (0.01 mole) in dry acetone (40 mL) was refluxed on a heating mantle for 30 hr. The reaction mixture was cooled to RT. The crystals thus obtained was filtered, washed with water, dried and purified by recrystallization from ethanol to yield colourless crystalline compound **2**. Yield 65%; m.p. 260^{o}C; IR (KBr): 1668 (C=O), 1595 cm^{-1} (C=N); ^{1}H NMR ($CDCl_3$): δ 1.31-1.37 (t, 3H, CH_3), 4.08-4.16 (q, 2H, CH_2), 4.48 (s, 2H, NCH_2), 7.20-7.45 (m, 9H, ArH), 7.54, 7.66 & 8.10 (6H, 3d, ArH); MS: *m/z* 382 (M^+). Anal. Calcd for $C_{25}H_{22}N_2O_2$: C, 78.51; H, 5.8; N, 7.32. Found: C, 78.31; H, 5.69; N, 7.16%.

Synthesis of (2,4,5-triphenyl-imidazole-1-yl)-acetic acid hydrazide 3:

A mixture of (2,4,5-triphenyl-imidazol-1-yl)-acetic acid ethyl ester (0.01 mole) and hydrazine hydrate (0.05 mole) in ethanol (50 mL) was refluxed on water bath for 12 hr. The reaction-mixture was cooled to RT. The crystals thus obtained were filtered, washed with water, dried and purified by recrystallization from ethanol to yield colourless crystalline compound **3**. Yield 66%; m.p. 240^{o}C; IR

(KBr): 3038 (NH), 1694 (C=O), 1597 cm^{-1} (C=N); ^1H NMR ($CDCl_3$): δ 4.35 (s, 2H, NCH_2), 4.71 (d, 2H, NH_2), 7.15-8.12 (m, 15H, ArH), 12.78 (bs, 1H, s, NH); MS: *m/z* 368 (M^+). Anal. Calcd for $C_{23}H_{20}N_4O$: C, 74.98; H, 5.47; N, 15.21. Found: C, 74.73; H, 5.29; N, 15.28%.

General for synthesis of N^1-(2,4,5-triphenyl-imidazol-1-yl-ethanoyl) - N^4 - substitutedphenyl thiosemicarbazide 3a-d:

A mixture of acid hydrazide (0.01 mole) and substitutedphenyl isothiocynate (0.01 mole) in absolute ethanol (50 mL) was refluxed on a water bath for 6 hr. The reaction-mixture was concentrated and allowed to stand at RT overnight. The needle shaped crystals of thiosemicarbazide thus obtained were filtered, washed with petroleum ether and purified by recrystallization from ethanol.

N^1-(2,4,5-triphenyl-imidazol-1-yl-ethanoyl) - N^4 - 4-methoxyphenyl thiosemicarbazide, 3a:

Yield 69%; m.p. 230°C; IR (KBr): 3238 (NH), 1683 (C=O), 1597 (C=N), 1099 cm^{-1} (C=S); ^1H NMR ($CDCl_3$): δ 3.74 (s, 3H, OCH_3), 4.48 (s, 2H, NCH_2), 7.2-7.5 (m, 19H, ArH), 8.12 (bs, 1H, NH) 10.91 (bs, 1H, CSNH), 12.70 (bs, 1H, CONH); MS: *m/z* 533 (M^+). Anal. Calcd for $C_{31}H_{27}N_5O_2S$: C, 69.77; H, 5.10; N, 13.12. Found: C, 69.81; H, 5.11; N, 12.92%.

N^1-(2,4,5-triphenyl-imidazol-1-yl-ethanoyl)-N^4-4-methylyphenylthiosemicarbazide, 3b:

Yield 70%; m.p. 238°C; IR (KBr): 3238 (NH), 1668 (C=O), 1595 (C=N), 1091 cm^{-1} (C=S); ^1H NMR ($CDCl_3$): δ 2.28 (s, 3H, CH_3), 4.53 (s, 2H, NCH_2), 7.12-7.54 (m, 19H, ArH), 8.08 (bs, 1H, NH), 12.41 (bs, 1H, CSNH), 12.59 (bs, 1H, CONH); MS: *m/z* 517 (M^+). Anal. Calcd for $C_{31}H_{27}N_5OS$: C, 71.93; H, 5.28; N, 13.53. Found: C, 71.83; H, 5.12; N, 13.29%.

N^1-(2,4,5-triphenyl-imidazol-1-yl-ethanoyl) - N^4 - 4-flourophenyl thiosemicarbazide, 3c:

Yield 67%; m.p. 251°C; IR (KBr): 3196 (NH), 1651 (C=O), 1606 (C=N), 1096 cm^{-1} (C=S); ^1H NMR ($CDCl_3$): δ 4.52 (s, 2H, NCH_2), 7.21-7.51 (m, 19H, ArH), 8.14 (bs, 1H, NH), 11.08 (bs, 1H, CSNH), 12.80 (s, 1H, CONH); MS: *m/z* 521 (M^+); Anal. Calcd for $C_{30}H_{24}FN_5OS$: C, 69.97; H, 4.64; N, 13.43. Found: C, 69.78; H, 4.57; N, 13.26%.

N^1-(2,4,5-triphenyl-imidazol-1-yl-ethanoyl) - N^4-4-chlorophenyl thiosemicarbazide, 3d:

Yield 65%; m.p. 223°C; IR (KBr): 3216 (NH), 1664 (C=O), 1591 (C=N), 1101 cm^{-1} (C=S); ^1H NMR ($CDCl_3$): δ 4.49 (s, 2H, NCH_2), 7.22-7.67 (m, 19H, ArH), 11.16 (bs, 1H, NH), 12.69 (bs,1H, CSNH), 12.83 (bs, 1H, CONH); MS: *m/z* 538 (M^+), 540 (M^++2). Anal. Calcd for $C_{30}H_{24}Cl\ N_5OS$: C, 66.97; H, 4.50; N, 13.02. Found: C, 66.81; H, 4.39; N, 12.86%.

General method for synthesis of 1-{[2-(4-substitiutedphenyl amino)-(1, 3, 4-oxadiazol-5-yl)] methyl}-2, 4, 5-triphenylimidazole, 4a-d:

In a 100 ml round bottom flask, thiosemicarbazide (0.02 mole) and ethanol (25 ml) was taken. To it, sodium hydroxide solution (5N, 2 ml) was added and mixture was cooled with continuous stirring for half an hour. To this mixture iodine in potassium iodide was added drop wise till the colour of iodine persisted at room temperature. The reaction mixture was refluxed for one hour on a water bath. When reaction was completed, reaction mass was poured over crushed ice in a beaker. The precipitated solid thus obtained was washed with sodium thiosulphate solution and recrystallized from absolute ethanol.

1-{[2-(4-methoxyphenyl amino)-(1,3,4-oxadiazol-5-yl)]methyl}-2,4,5-triphenylimidazole, 4a:

Yield 65%; m.p. 190°C; IR (KBr): 3310-3390(NH) stretching, and N-H bending at (1530-1575) cm^{-1}, C-O-C group stretching at (3310-3390) cm^{-1}, C=C and C=N appears as a strong band at (1600-1695) cm^{-1} and (1600-1695) cm^{-1} ; ^1H NMR ($CDCl_3$): δ 3.73 (s, 3H, OCH_3), 4.45 (s, 2H, N-CH_2), 6.90-7.11 (m, 4H, ArH), 7.22-7.53 (m, 15H, ArH), 12.69 (s, 1H, NH); MS: *m/z* 515 (M^+).

1-(4-methylphenyl amino)-(1,3,4-oxadiazol-5-yl)] methyl}-2,4,5-triphenylimidazole, 5b:

Yield 64%; m.p. 1910°C; IR (KBr): 3310-3390(NH) stretching, and N-H bending at (1530-1575) cm^{-1}, C-O-C group stretching at (3310-3390) cm^{-1}, C=C and C=N appears as a strong band at (1600-1695) cm^{-1} and (1600-1695) cm^{-1} ; ^1H NMR ($CDCl_3$): δ 2.34 (s, 3H, CH_3), 4.45 (s, 2H, N-CH_2), 6.90-7.11 (m, 4H, ArH), 7.22-7.53 (m, 15H, ArH), 12.69 (s, 1H, NH); MS: *m/z* 514 (M^+).

1-{[2-(4-fluorophenyl amino)-(1,3,4-oxadiazol-5-yl)] methyl}-2,4,5-triphenylimidazole, 4c:
Yield 68%; m.p. 193^{o}C; IR (KBr): 3310-3390(NH) stretching, and N-H bending at (1530-1575) cm^{-1}, C-O-C group stretching at (3310-3390) cm^{-1}, C=C and C=N appears as a strong band at (1600-1695) cm^{-1} and (1600-1695) cm^{-1}; ^{1}H NMR ($CDCl_3$): 4.45 (s, 2H, N-CH_2), 6.90-7.11 (m, 4H, ArH), 7.22-7.53 (m, 15H, ArH), 12.69 (s, 1H, NH); MS: *m/z* 514 (M^+).

1-{[2-(4-chlorophenyl amino)-(1, 3, 4-oxadiazol-5-yl)] methyl}-2,4,5-triphenylimidazole,4d:
Yield 68%; m.p. 193^{o}C; IR (KBr): 3310-3390(NH) stretching, and NH bending at (1530-1575) cm^{-1}, C-O-C group stretching at (3310-3390) cm^{-1}, C=C and C=N appears as a strong band at (1600-1695) cm^{-1} and (1600-1695) cm^{-1}; ^{1}H NMR ($CDCl_3$): 4.54 (s, 2H, N-CH_2), 6.90-7.11 (m, 4H, ArH), 7.22-7.53 (m, 15H, ArH), 12.69 (s, 1H, NH); MS: *m/z* 520 (M^+), 522 (M^++2).

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