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SYNTHESIS, CHARACTERIZATION, ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF CERTAIN SUBSTITUTED IMIDAZOLONES

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ABSTRACT

A series of oxazolones (3) were prepared by cyclization reaction of hippuric acid (1) with different aromatic aldehydes (2) using sodium acetate and acetic anhydride as solvent. Two series of imidazolones (4&5) were synthesized from oxazolones (3) by condensation reaction. The 4-(substituted benzylidene)-2-phenyloxazol-5-ones (3) were refluxed with equimolecular quantity of aniline in dioxan in water bath for 6 hrs and urea in pyridine in an oil bath at 150^0c for 6 hrs to yield the 4-(substituted benzylidene)-1,2-diphenyl-1H-imidazol-5-ones (4) and 4-substituted benzylidene)-5-oxo-2-phenyl-4,5-dihydroimidazole-1-carboxamides (5) respectively. The yield of the synthesized imidazolones ranged from 60-72%. The structures of the synthesized compounds were characterized and confirmed by IR, ^{1}H-NMR,^{13}C-NMR, mass spectral data and physical analysis. The synthesized compounds were screened for their in vitro antimicrobial activity against standard pathological bacterial strains and fungal strains using disc diffusion and broth micro-dilution assays. All the compounds, exhibited a MIC values ranging between 1.6 to 204.8 µg and the MBC/MFC values ranging between 6.4 to >204.8µg for tested bacterial and fungal species. The compounds have better activity against the tested fungal species than bacteria species.

KEYWORDS:-Imidazolones, oxazolones, antibacterial & antifungal activities.

INTRODUCTION

The chemistry of nitrogen heterocyclic compounds especially imidazoles have attracted more attention during recent years due to their reactivity, pharmacological properties and are widely implicated in biochemical processes. Imidazole is a planer five-membered heterocyclic ring system with three carbon and two nitrogen atoms at 1st and 3rd positions (Shalini K *et al*., 2010). The imidazole ring is a constituent of several important naturally occurring organic molecules, including purine, histamine, histidine and nucleic acid (Priya V Frank *et al*., 2005). Many of the substituted imidazoles having herbicidal, antitubercular (Hadizadeh F *et al*., 2008) antiasthmatic, antibiotic, calcium antagonist antiarthritic, cardiotonic , antioxidant (Monica K *et al*., 2011; Hasan T *et al*., 2007) and antithrombotic activities, are present in these compounds. These types of compounds are also known as plant growth regulators and therapeutic agents (Satyanarayana VSV *et al*., 2010). Imidazole derivative compounds have been reported to possess antisenescence, anti-muscarinic, anthelminitic (Lakshmanan B *et al*., 2011; Das P *et al*., 2010) nonsedative anxiolytic, antiulcerative, antiparasitic (Abdul Jabar KA., 2009) inhibitors of Acyl CoA Cholesterol O-acyl transferase, HMG CoA reductase, and inhibitors of thromboxaneA_2 synthase, antifungal (Khabnadideh S *et al*., 2009; Thareja S *et al*., 2010) antiviral, antibacterial (Dahiya R *et al*., 2010; Rathod AK *et al*., 2011) antispasmodic and tranquillizers. The imidazolones and

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substituted imidazolones have been found to be associated with several pharmacological activities including anti-HIV (Ganguly S *et al*., 2011), antihistaminic, anticancer, antimalarial, antiparkinsonism, analgesic, anti-inflammatory (Jays J *et al*., 2011; Umarani N *et al*., 2011) anticonvulsant, monoamine oxidase inhibitory, sedative, hypnotics, CNS depressant, goitrogenic, local anesthetic, General anaesthetic. Angiotensin blocker (Block JH *et al*., 2004) and immunomodulatory activities, etc. During the past years a large number of imidazolone containing compounds have been in the market with diverse pharmacological properties e.g. clonidine, phentolamine for the treatment of hypertension, cimetidine as antiulcer, dacarbazine as anticancer, metronidazole as antiprotozoal drug, ketoconazole, econazole as antifungal agents (Shah RA *et al*., 2010) and two imidazolines priscol & privine are vasodilating and vasoconstricting drugs (Husain A *et al*., 2009). This observation prompted us to synthesize new imidazolones and evaluate their antimicrobial activity.

MATERIALS AND METHODS

Melting points were determined using a Thomas Hoover capillary melting point apparatus and were uncorrected. The purity of the synthesized imidazoles were checked by TLC using silicagel-G glass plate method using benzene: chloroform (6:4) with two drops of methanol as eluent and visualized in an iodine chamber (Table-1).IR spectra were recorded (in KBr) on FTIR 8300 Shimadzu spectrophotometer, ^{1}HNMR and ^{13}CNMR spectras were recorded on a Bruker AC 300 MHZ FTNMR spectrophotometer in $CDCl_3$ and chemical shift were recorded in parts per million downfield from tetramethylsilane. Mass spectra were recorded with a VG70-70H spectrophotometer.

Synthesis of oxazolones (3a-e)

The 4-(substituted benzylidene)-2-phenyloxazol-5(4H)-ones were prepared by refluxing with equimolecular quantity of hippuricacid (1) with five different aromatic aldehydes (2a-e) in the presence of acetic anhydride as a solvent with freshly fused sodium acetate for two hrs after cooling ethanol was added and kept overnight 5^0c a solid obtained was filtered, washed with alcohol dried in vaccum and recrystalised from benzene.

Preparation of imidazolones (4a-e & 5a-e)

The substituted oxazolones (3) were refluxed with equimolecular quantity of aniline in dioxan in waterbath for 6 hrs to yield the 4-(substituted benzylidene)-1,2-diphenyl-1H-imidazol-5(4H)-ones (4a-e) and urea in pyridine in an oil bath at 150^0c for 6 hrs to yield the 4-(substituted benzylidene)-5-oxo-2-phenyl-4,5-dihydroimidazole-1-carboxamides (5a-e).The mixture were cooled and poured in to crushed ice and filtered, dried in vaccum and recrystalised from ethanol.

SPECTRAL DATA

4-(4-methoxybenzylidene)-2-phenyloxazol-5(4H)-one.(compound 3a)

IR (ν in cm^{-1}): 1653 (C=N), 1600-1512 (C=C), 3103-3036 (=C-H), 1788-1770 (C=O, lactone), 1325-1311 (C-N), 2974-2843 (C-H,OCH_3), 1267-1163 (C-O);

^{1}HNMR (δ in ppm): 3.90 (S,3H,OCH_3), 6.98-7.03 (d,4H,P-subs phenyl), 7.49-7.61 (m,5H,Ar-H), 8.15 (s,1H,-CH=);

^{13}CNMR (δ in ppm): 167.96 (O-C=O), 162.39 (O-C=N), 162.11(Ar-OR), 131.87-132.93 (=C-N), 114.45-126.48 (R-C_6H_4-), 128.07-128.83 (C_6H_5-), 55.41 (CH_3).

4-benzylidene-2-phenyloxazol-5(4H)-one.(compound 3b).

IR (ν in cm^{-1}): 1653 (C=N), 1597-1489 (C=C), 3066-3041 (=C-H), 1793-1768 (C=O, lactone), 1327-1363 (C-N);

^{1}HNMR (δ in ppm): 6.98-7.36 (m,5H,Ar-H), 7.45-7.53 (m,5H,Ar-H), 8.16 (S,1H,-CH=);

^{13}CNMR (δ in ppm): 167.63 (O-C=O), 163.49 (O-C=N), 131.77-132.42 (=C-N), 128.33-128.89 (C_6H_5-).

4-(4-(dimethylamino)benzylidene)-2-phenyloxazol-5(4H)-one.(compound 3c)

IR (ν in cm^{-1}): 1649 (C=N), 1606-1529 (C=C), 3080-3045 (=C-H), 2899-2829 (C-H,CH_3), 1784-1763 (C=O, lactone), 1323-1294 (C-N);

^{1}HNMR (δ in ppm): 3.10 (S,6H,-N-$(CH_3)_2$), 6.73-7.26 (d,4H,P-subs phenyl), 7.50-7.55 (m,5H,Ar-H), 8.15 (S,1H,-CH=);

^{13}CNMR (δ in ppm): 167.51 (O-C=O), 160.29 (O-C=N), 147.0 (Ph-N), 131.90 (=C-N), 128.61-130.27 (C_6H_5-), 113.0-127.16 (R-C_6H_4-), 41.83 (CH_3).

4-(2-hydroxybenzylidene)-2-phenyloxazol-5(4H)-one.(compound 3d).

IR (ν in cm $^{-1}$): 1666-1626 (C=N), 1602-1533 (C=C), 3367 (=C-H), 1780-1708 (C=O, lactone), 3367 (OH), 1361 (C-N), 1255 (C-O);

^{1}HNMR (δ in ppm): 8.84 (S,1H,ortho substituted-OH), 7.27-7.35 (m,4H,Ar-H), 7.45-7.56 (m,5H,Ar-H), 7.93 (S,1H,-CH=);

^{13}CNMR (δ in ppm): 165.92 (O-C=O), 158.85 (O-C=N), 149.77 (Ar-OH), 132.43-133.33 (=C-N), 129.61-127.01 (C_6H_5-), 125.09-116.24 (R-C_6H_4-),

4-(4-hydroxy-3methoxybenzylidene)-2-phenyloxazol-5(4H)-one.(compound 3e)
IR (ν in cm $^{-1}$): 1653 (C=N), 1600-1508 (C=C), 3068-3037 (=C-H), 1797-1757 (C=O, lactone), 3414-3346 (OH), 1209 (C-O,OH), 2879-2841 (C-H,OCH_3), 1327-1288 (C-N), 1271 (C-O,OCH3);
^{1}HNMR (δ in ppm): 3.97 (S,3H,OCH_3), 9.82 (S,1H,ortho substituted-OH), 6.96-7.26 (m,3H,Ar-H), 7.51-7.62 (m,5H,Ar-H), 8.14 (S,1H,-CH=);
^{13}CNMR (δ in ppm): 166.58 (O-C=O), 161.48 (O-C=N), 140.08 (Ar-OH), 149.20 (Ar-O), 131.35-131.03 (=C-N), 130.29-126.91 (C_6H_5-), 126.16-113.26 (R-C_6H_4-), 53.81 (CH_3).

4-(4-methoxybenzylidene)-1,2-diphenyl-1H-imidazol-5(4H)-one (compound 4a).
IR (ν in cm^{-1}): 1653 (C=N), 1600-1512 (C=C), 3066-3016 (=C-H), 1788-1770 (C=O, lactam), 1325-1311 (C-N), 2843-2810 (C-H,OCH_3),1267-1163 (C-O);.
^{1}HNMR (δ in ppm): 3.91 (S,3H,OCH_3), 6.90-7.01 (d,4H,P-subs phenyl), 7.21-7.29 (m,5H,Ar-H), 7.49-7.62 (m,5H,Ar-H), 8.17 (S,1H,-CH=);
^{13}CNMR (δ in ppm): 167.94 (N-C=O), 162.39 (N-C=N), 162.13(Ar-OR), 131.85-132.93 (=C-N), 128.08-128.83 (C_6H_5-), 114.46-126.50 (R-C_6H_4-), 55.41 (CH_3);
MS (m/z,%): 354.31(19), 339.32 (11), 326.15 (30), 247.08 (60), 235.18 (76), 222.10 (53), 194.18 (16), 160.15 (40), 145.01 (26), 119.22 (36), 103.24 (100).

4-(4-methoxybenzylidene)-5-oxo-2-phenyl-4,5-dihydroimidazole-1-carboxamide (compound 5a).
IR (ν in cm^{-1}): 3344-3259 (N-H), 1654 (C=N), 1602-1512 (C=C), 3018 (=C-H), 2845-2804 (C-H,OCH_3), 1788-1770 (C=O, lactam), 1627 (C=O, amide), 1431 (C-N,amide),1325 (C-N), 1267-1163 (C-O);
^{1}HNMR (δ in ppm): 3.85 (S,3H,OCH_3), 5.23 (S,2H,-CO-NH_2), 7.03-7.05 (d,4H,P-subs phenyl), 7.24-7.54 (m,5H,Ar-H), 8.26 (S,1H,-CH=);
^{13}CNMR (δ in ppm): 168.21 (N-C=O), 163.55 (N-C=N), 159.38 (Ar-OR), 154.07 (-CO-NH_2), 131.19 (=C-N), 130.05-128.65 (C_6H_5-), 116.10-127.37 (R-C_6H_4-), 56.37 (CH_3);
MS (m/z, %): 321.30 (14), 305.39 (32), 290.11 (21), 277.10 (81), 262.15 (69), 235.14 (40), 220.10 (16), 198.15 (54), 187.14 (11), 173.13 (24), 160.15 (19), 145.12 (44), 103.17 (100).

4-benzylidene-1,2-diphenyl-1H-imidazol-5(4H)-one (compound 4b).
IR (ν in cm^{-1}): 1653 (C=N), 1597-1554 (C=C), 3036 (=C-H), 1793-1768 (C=O, lactam), 1363-1327 (C-N);
^{1}HNMR (δ in ppm): 7.17-7.30 (m,5H,Ar-H), 7.46-7.54 (m,5H,Ar-H), 7.56-7.65 (m,5H,Ar-H), 8.18 (S,1H,-CH=);
^{13}CNMR (δ in ppm): 167.53 (N-C=O), 163.40 (N-C=N), 131.67-132.34 (=C-N), 128.25-128.81 (C_6H_5-);
MS (m/z,%): 324.36 (25), 296.35 (14), 247.04 (62), 222.19 (56), 205.26 (80), 194.14 (19), 130.20 (43), 119.11 (38), 103.17 (100).

4-benzylidene-5-oxo-2-phenyl-4,5-dihydroimidazole-1-carboxamide (compound 5b)
IR (ν in cm^{-1}): 3354-3257 (N-H), 1653 (C=N), 1597-1552 (C=C), 3068 (=C-H), 1793 -1768 (C=O, lactam), 1629 (C=O, amide), 1383 (C-N, amide), 1363-1327 (C-N);
^{1}HNMR (δ in ppm): 4.77 (S,2H,-CO-NH_2), 7.10-7.33 (m,5H,Ar-H), 7.43-7.88 (m,5H,Ar-H), 8.13 (S,1H,-CH=);
^{13}CNMR (δ in ppm): 167.81(N-C=O), 163.57 (N-C=N), 158.07 (-CO-NH_2), 131.13 (=C-N), 130.19-128.0 (C_6H_5-);
MS (m/z,%): 291.31 (19), 275.10 (60), 247.13 (76), 205.10 (28), 198.17 (33), 173.16 (40), 145.19 (20), 130.27 (43), 103.18 (100).

4-(4-(dimethylamino)benzylidene)-1,2-diphenyl-1H-imidazol-5(4H)-one (compound 4c).
IR (ν in cm^{-1}): 2916-2897 (C-H,CH_3), 1651-1643(C=N), 1604-1529 (C=C), 3103 (=C-H), 1763-1722 (C=O, lactam), 1323 (C-N);
^{1}HNMR (δ in ppm): 3.10 (S,6H,-N-$(CH_3)_2$), 6.72-6.76 (d,4H,P-subs phenyl), 7.13-7.34 (m,5H,Ar-H), 7.47-7.55 (m,5H,Ar-H), 8.15 (S,1H,-CH=);
^{13}CNMR (δ in ppm): 170.51 (N-C=O), 165.30 (N-C=N), 150.73 (Ar-N), 131.51 (=C-N), 130.11-128.07 (C_6H_5-), 116.56-127.08 (R-C_6H_4-), 41.31 (CH_3);
MS (m/z,%): 367.43 (14), 339.30 (22), 324.74 (11), 264.02 (68), 248.04 (62), 233.05 (16), 222.05 (34), 194.11 (19), 173.07 (10), 158.16 (42), 119.21 (39), 103.19 (100).

4-(4-(dimethylamino)benzylidene)-5-oxo-2-phenyl-4,5-dihydroimidazole-1-carboxamide (compound 5c).
IR (ν in cm^{-1}): 3342-3281 (N-H), 2922 (C-H,CH_3), 1641(C=N), 1595-1531 (C=C),3018 (=C-H), 1763-1741 (C=O, lactam), 1626.06 C=O(amide),1464 (C-N, amide), 1323-1294 (C-N);
^{1}HNMR (δ in ppm): 3.07 (S,6H,-N-$(CH_3)_2$), 5.01 (S,2H,-CO-NH_2), 6.54-6.75 (d,4H,P-subs phenyl), 7.18-7.34 (m,5H,Ar-H), 8.11 (S,1H,-CH=);
^{13}CNMR (δ in ppm): 173.78 (N-C=O), 168.80 (N-C=N), 156.15 (-CO-NH_2), 151.67 (Ar-N), 131.30-132.18 (=C-N), 126.85-130.12 (C_6H_5-), 111.69-126.85 (R-C_6H_4-), 39.98-39.90 (CH_3);
MS (m/z,%): 334.31 (18), 318.34 (10), 303.15 (15), 290.14 (73), 275.11 (67), 248.15 (25), 233.13 (11), 215.24

(12), 198.17 (33), 173.18 (49), 158.29 (20), 145.26 (35), 103.13 (100).

4-(2-hydroxybenzylidene)-1,2-diphenyl-1H-imidazol-5(4H)-one (compound 4d).
IR (ν in cm^{-1}): 3367-3279 (OH), 1666 (C=N), 1602-1535 (C=C), 3082 (=C-H), 1776-1710 (C=O, lactam), 1257 (C-O,OH), 1361 (C-N);
^{1}HNMR (δ in ppm): 8.85 (S,1H,ortho substituted-OH), 6.69-7.05 (m,4H,Ar-H), 7.26-7.35 (m,5H,Ar-H), 7.43-7.62 (m,5H,Ar-H), 7.93 (S,1H,-CH=);
^{13}CNMR (δ in ppm): 165.94 (N-C=O), 158.86 (N-C=N), 149.78 (Ar-OH), 132.43-133.35 (=C-N), 129.62-127.01 (C_6H_5-), 124.01-116.25 (R-C_6H_4-);
MS (m/z,%): 340.35 (15), 312.21(11), 247.07 (55), 237.22 (29), 221.15 (71), 194.06 (18), 146.10(39), 119.14 (33), 103.14 (100).

4-(2-hydroxybenzylidene)-5-oxo-2-phenyl-4,5-dihydroimidazole-1-carboxamide (compound 5d)
IR (ν in cm^{-1}): 3466 (N-H), 3367 (OH), 1666 (C=N), 1602-1533 (C=C), 3086 (=C-H), 1763-1707 (C=O, lactam), 1627.97 C=O(amide), 1257 (C-O,OH), 1363 (C-N);
^{1}HNMR (δ in ppm): 4.70 (S,2H,-CO-NH_2), 8.85 (S,1H,ortho substituted-OH), 7.26-7.35 (m,4H,Ar-H), 7.43-7.62 (m,5H,Ar-H), 7.93 (S,1H,-CH=);
^{13}CNMR (δ in ppm): 163.97 (N-C=N), 156.89 (-CO-NH_2), 147.81 (Ar-OH), 131.38 (=C-N), 130.46-126.84 (C_6H_5-), 125.80-114.28 (R-C_6H_4-);
MS (m/z,%): 307.31 (20), 291.34 (12), 263.15 (68), 221.28 (25), 198.12 (30), 118.30 (14), 173.24 (28), 146.16 (39), 103.15 (100).

4-(4-hydroxy-3-methoxybenzylidene)-1,2-diphenyl-1H-imidazol-5(4H)-one (compound 4e).
IR (ν in cm^{-1}): 3444-3273 (OH), 1651 (C=N), 1600-1508 (C=C), 3064-3014 (=C-H), 2881-2837 (C-H,OCH_3), 1795-1755 (C=O, lactam), 1209 (C-O,OH), 1271-1151 (C-O,Ar-O-CH_3), 1327 (C-N);
^{1}HNMR (δ in ppm): 3.94 (S,3H,OCH_3), 9.79 (S,1H,ortho substituted-OH), 6.70-6.93 (m,3H,Ar-H), 7.09-7.25 (m,5H,Ar-H), 7.48-7.77 (m,5H,Ar-H), 8.11 (S,1H,-CH=); **^{13}CNMR (δ in ppm):** 166.59 (N-C=O), 161.42 (N-C=N), 140.06 (Ar-OH), 149.18 (Ar-O), 131.33-130.98 (=C-N), 130.27-126.89 (C_6H_5-), 126.14-113.23(R_2-C_6H_4-), 53.78 (CH_3);
MS (m/z,%): 370.41 (20), 342.30 (24), 327.28 (12), 267.25 (37), 251.24 (75), 247.14 (60), 236.21 (17), 222.21 (53), 194.19 (27), 176.13 (31), 161.10 (14), 119.13 (36), 103.15 (100).

4-(4-hydroxy-3-methoxybenzylidene)-5-oxo-2-phenyl-4,5-dihydroimidazole-1-carboxamide (compound 5e).
IR (ν in cm^{-1}): 3437-3261 (OH), 3340-3146 (N-H), 3146 (=C-H), 2808 (C-H,OCH_3), 1797-1739 (C=O, lactam), 1674-1658 (C=N), 1600-1512 (C=C), 1624 (C=O amide), 1327 (C-N), 1271-1153 (C-O,Ar-O-CH_3), 1209 (C-O,OH);
^{1}HNMR (δ in ppm): 3.97 (S,3H,OCH_3), 4.64 (S,2H,-CO-NH_2), 9.83 (S,1H,ortho substituted-OH), 6.89-7.18 (m,3H,Ar-H), 7.46-7.83 (m,5H,Ar-H), 8.06 (S,1H,-CH=);
^{13}CNMR (δ in ppm): 167.81 (N-C=O), 164.55 (N-C=N), 159.24(-CO-NH_2), 141.90 (Ar-OH), 149.27 (Ar-O), 131.21 (=C-N), 130.19-128.07 (C_6H_5-), 122.72-112.92 (R_2-C_6H_4-), 56.72 (CH_3);
MS (m/z,%): 337.30 M^+ (15), 321.14 (12), 306.22 (19), 293.13 (75), 278.11 (62), 251.18 (13), 236.23 (10), 218.19 (27), 198.27(46), 173.12 (38), 161.27 (22), 145.11 (51), 103.18 (100).

ANTIMICROBIAL ACTIVITY

Micro-organisms

Two strains of bacteria used were Bacillus cereus (MTCC-430), and Escherichia coli (MTCC-1302). These standard strains were obtained from Microbial Type Culture Collection and gene bank (MTCC); Institute of Microbial Technology, Chandigarh, India. Two Fungal species isolates from the same source were used, these being Aspergillus niger (MTCC-2425), Aspergillus niger (MTCC-1344) and the Candida albicans (MTCC-183) *Candida norvegensis* (MTCC-227).

All bacterial strains were cultivated in Mueller Hinton Agar (MHA) media, and fungi were cultivated in Sabouraud Dextrose Agar (SDA) media. The stock culture was maintained on agar slant at 4 °C. These strains were subculture on a fresh appropriate agar plate 24 h prior to any antimicrobial test.

Agar diffusion assay

The antimicrobial activity was undertaken agar disc diffusion test according to the National Committee for Clinical Laboratory Standards (NCCLS, 2001) using 100 µl of suspension of the tested microorganisms; containing 2.0×10^6 CFU/ml for bacteria and2.0×10^5 CFU /ml spore for fungal strains. The suspension was standardized by adjusting the optical density to 0.1 at 600 nm. This was used to inoculate by flooding the surface of MHA and SDA plates for bacteria and fungi respectively. Then sterilized discs were prepared at 50µg/disc for synthesized compounds and 10µg/disc for standard antibiotics (ciprofloxacin and clotrimazole) separately. A disc prepared with only the corresponding volume of DMSO was used as negative control. The petri plates were then

incubated and antimicrobial activity was evaluated by measuring the diameter of the zones of inhibition around the disc.

Broth micro-dilution assay

The minimum inhibitory concentration (MIC) and minimum bactericidal or fungicidal concentration (MBC/MFC) were determined by twofold serial micro broth dilution method (NCCLS, 1999) in Mueller Hinton or Sabouraud broth. The synthesized compounds and standard antibiotics (ciprofloxacin and clotrimazole) were dissolved in 1% DMSO aqueous solution at concentration of 10 mg/ml. These solutions were used in the determination of the antimicrobial activity against the reference strains. The compounds were diluted two fold concentration from 0.2 to 204 µg/ml and the starting inoculums of 2.0 $\times 10^7$ CFU/ ml was used. Solvent (DMSO) blanks were included. The test tubes were incubated at 37°C for bacteria and 28°C for fungi. The lowest concentration showing no visible growth was considered as the MIC. For the determination of MBC/MFC, a portion of liquid (5 µl) from each well that showed no change in colour was plated on MHA and incubated at 37 °C for 24 h. The lowest concentration that yielded no growth after this sub-culturing was taken as the MBC/MFC.

RESULT AND DISCUSSION

The objective of the present work was to synthesize, purify, characterize and evaluate the antimicrobial activity of synthesized imidazolones..The benzoyl glycine (1) reacts with P-OCH_3/ H/P-$N(CH_3)_2$/o-OH/P-OH & m-OCH_3 substituted benzaldehyde (2a-e) to give respective substituted oxazolone derivatives (3a-e). The IR spectra of all the derivatives (3a-e) were showed absorption band at 1797-1708 cm^{-1} due to carbonyl stretching of lactone, 1666-1626 cm^{-1} due to –C=N stretching, 1606-1508 cm^{-1} due to –C=C- aromatic stretching, 1363-1288 cm^{-1} due to –C-N stretching. The ^{1}HNMR (δ) spectra of all the derivatives were showed peak at 7.93-8.16 ppm due to –CH= , 7.45-7.62 ppm due to Phenyl group, 6.73-7.35 ppm due to substituted Phenyl group (except 3_b). The ^{13}CNMR (δ) spectra of all the derivatives were showed peak at 167.96-165.92 ppm due to carbonyl group of oxazolone, 158.85-163.49 ppm due to – C=N, 131.03-133.33 ppm due to =C-N, 126.91-130.29 ppm due to Phenyl group, 113.0-127.16 ppm due to substituted Phenyl group (except 3b).

Followed by oxazolone derivatives (3a-e) were condensed with aniline yielded corresponding substituted imidazolone derivatives (4a-e). The IR spectra of all the derivatives were showed absorption band at 1795-1710 cm^{-1} due to carbonyl stretching of lactam, 1666.0-1643cm^{-1} due to –C=N stretching, 1604-1508 cm^{-1} due to –C=C- aromatic stretching, 1363-1311cm^{-1} due to –C-N stretching. The ^{1}HNMR (δ) spectra of all the derivatives were showed peak at 7.93-8.18 ppm due to –CH= , 7.09-7.35 ppm due to 1-Phenyl group, 7.43-7.77 ppm due to 2-Phenyl group, 6.69-7.05 ppm due to substituted Phenyl group (except 4b). The ^{13}CNMR (δ) spectra of all the derivatives were showed peak at 170.51-165.94 ppm due to carbonyl group of imidazolone, 158.86-165.3 ppm due to – C=N, 130.98-133.35 ppm due to =C-N, 126.89-130.27 ppm due to Phenyl group, 113.23-127.08 ppm due to substituted Phenyl group (except 4b).

Followed by oxazolone derivatives (3a-e) were condensed with urea yielded corresponding substituted imidazolone carboxamide derivatives (5a-e). The IR spectra of all the derivatives were showed absorption band at 3466-3257 NH stretching, 1797-1707 cm^{-1} due to carbonyl stretching of lactam, 1674.0-1641cm^{-1} due to –C=N stretching, 1604-1512 cm^{-1} due to –C=C- aromatic stretching, 1629-1624 cm^{-1} due to carbonyl stretching of amide, 1363-1323 cm^{-1} due to –C-N stretching. The ^{1}HNMR (δ) spectra of all the derivatives were showed peak at 7.93-8.26 ppm due to –CH= , 7.18-7.83 ppm due to Phenyl group, 6.54-7.35 ppm due to substituted Phenyl group (except 5b). The ^{13}CNMR (δ) spectra of all the derivatives were showed peak at 173.78-167.81 ppm due to carbonyl group of imidazolone, 163.55-168.8 ppm due to – C=N, 159.24-154.07 ppm due to amide group, 131.13-132.18 ppm due to =C-N, 126.84-130.46 ppm due to Phenyl group, 111.69-127.37 ppm due to substituted Phenyl group (except 5b).The mass spectra of the compounds (4a-e &5a-e) were showed molecular ion peak corresponding to their molecular formula. The spectral data of synthesized compounds were in conformity with previously were characterized by spectral data (Silverstein GH *et al*., 1998).

The invitro antimicrobial activities of compounds 4a-e&5a-e against the microorganisms tested and their antimicrobial efficacy were qualitatively and quantitatively examined by the presence or absence of inhibition zone diameters and MIC values respectively.

The results of antimicrobial activity of compounds and reference antibiotics by agar disc diffusion assay are summarized in Table-2. The data obtained from the disc diffusion method indicated that the compounds exhibited microbial growth inhibition of all the microorganisms at the tested concentration

and displayed a variable degree of antimicrobial activity on the bacterial and fungal species. It has been observed that some of the compounds revealed moderate to good antimicrobial activity. The compounds 4_{a-e} & 5_{a-e} were showed zone of inhibition diameters ranging from 4.6 to 7.8 mm against *B. cereus* and 3.8 to 5.4 mm against *E. coli* at 50µg/disc concentration. In comparison with clotrimazole, compounds were showed zone of inhibition diameters ranging from 8.0 to 13.8 mm & 8.3 to 15.9 mm against *A. niger* MTCC-2425 and *A. niger* MTCC-1344 respectively and 9.3 to 15.6 mm & 10.6 to 16.2 mm against *C. albicans* and *C. norvegensis* respectively at 50µg/disc concentration.

The results obtained suggest marked antibacterial and antifungal activity of the imidazolones at the MIC levels examined. Compounds showed antimicrobial activities on fungi species, Gram positive and negative bacteria. Within the tested interval (0.1 to >204.8 µg) compounds showed evident MIC values on the entire set of the tested microbial organism. The results of the MIC of compounds and reference antibiotics are recorded in Table-3. It shows that the MIC values are ranging from 25.6µg to102.4µg for *B. cereus*, 102.4µg to 204.8µg for *E. coli*, 3.2µg to 12.8µg for *A. niger* MTCC-2425, 1.6µg to 12.8µg for *A. niger* MTCC-1344, 1.6µg to 6.4µg for *C. albicans* and 1.6µg to 6.4µg for *C. norvegensis*. The results from the minimum bactericidal or fungicidal concentration (MBC/MFC) determination are shown in Table-4. Compounds displayed the MBC and MFC ranged between 51.2 µg and 204.8 µg for *B. cereus*, >204.8 µg for *E. coli* , 6.4µg and 25.6µg for *A. niger* MTCC-2425, 6.4µg and 51.2µg for *A. niger* MTCC-1344, 6.4µg and 25.6 for *C. albicans* and *C. norvegensis* respectively. These results were coincided with the MIC values of reported article (Mamolo MG et al., 2004).

When structure activity relationships are concerned, the antimicrobial activity might be increased by the presence of methoxy group as substituents at R_2 or R_3 position on the benzene ring. Furthermore, compounds bearing dimethylamino or hydroxyl group as substituents at R_3 or R_1 position on the benzene ring, both enhanced the antifungal activity. These substituents had no effect on the *E. coli*.

Fig. No. 1 Synthesis of imidazolones

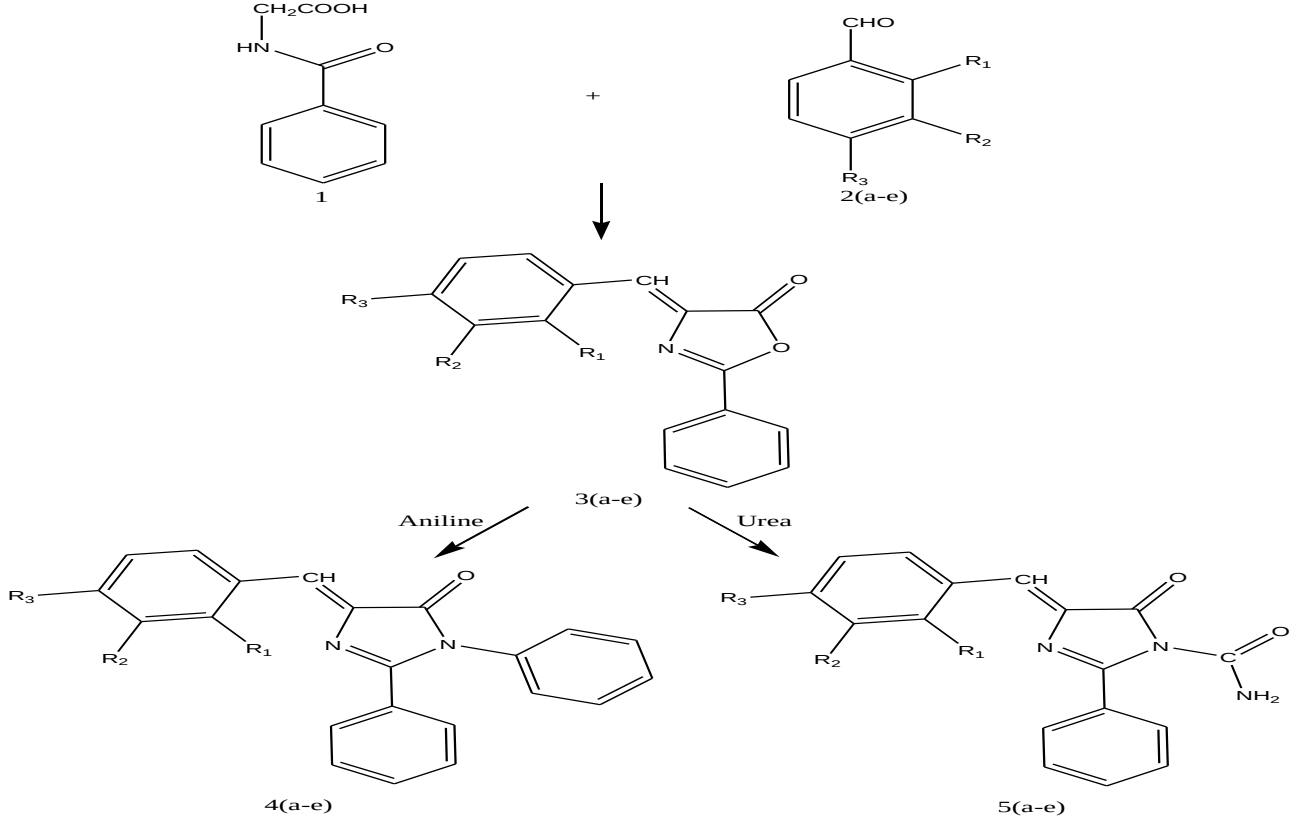

Table 1. Physical constant of synthesised imidazolones

Compounds	Molecular formula	R_1	R_2	R_3	Melting Point ^{O}C	R_f value	Yield %
4_a	$C_{23}H_{18}N_2O_2$	H	H	OCH_3	210-213	0.42	65
5_a	$C_{18}H_{15}N_3O_3$	H	H	OCH_3	192-193	0.45	68
4_b	$C_{22}H_{16}N_2O$	H	H	H	158-161	0.36	60
5_b	$C_{17}H_{13}N_3O_2$	H	H	H	146-148	0.39	62
4_c	$C_{24}H_{21}N_3O$	H	H	$N(CH_3)_2$	202-203	0.65	72
5_c	$C_{19}H_{18}N_4O_2$	H	H	$N(CH_3)_2$	164-165	0.69	61
4_d	$C_{22}H_{16}N_2O_2$	OH	H	H	225-227	0.8	66
5_d	$C_{17}H_{13}N_3O_3$	OH	H	H	149-150	0.84	63
4_e	$C_{23}H_{18}N_2O_3$	H	OCH_3	OH	207-209	0.61	61
5_e	$C_{18}H_{15}N_3O_4$	H	OCH_3	OH	179-181	0.66	65

Table 2. Antimicrobial activity (mm)* of the synthesized compounds series 4_{a-e}, 5_{a-e} and reference antibiotics determined by the agar disc diffusion assay

Compound	Inhibition zone diameters of the test substances (mm)					
	Bacillus cereus MTCC-430	***Escherichia coli MTCC-1302***	***Aspergillusniger* MTCC-2425**	***Aspergillus niger* MTCC- 1344**	***Candida albicans MTCC-183***	***Candida norvegensis MTCC-227***
4_a	7.2 ± 0.1	4.5 ± 0.1	12.6 ± 0.3	15.7 ± 0.2	15.5 ± 0.3	16.0 ± 0.3
4_b	6.4 ± 0.2	4.8 ± 0.3	10.2 ± 0.2	13.9 ± 0.4	11.7 ± 0.2	13.0 ± 0.3
4_c	6.7 ± 0.2	4.0 ± 0.2	13.8 ± 0.3	14.5 ± 0.2	12.4 ± 0.3	13.5 ± 0.2
4_d	6.3 ± 0.1	4.6 ± 0.2	12.5 ± 0.2	13.7 ± 0.3	15.6 ± 0.4	16.2 ± 0.4
4_e	7.8 ± 0.1	5.4 ± 0.3	13.7 ± 0.3	15.9 ± 0.4	12.3 ± 0.2	15.8 ± 0.3
5_a	5.9 ± 0.1	4.1 ± 0.2	9.4 ± 0.2	8.6 ± 0.2	12.9 ± 0.3	13.1 ± 0.3
5_b	4.8 ± 0.1	4.0 ± 0.1	8.0 ± 0.3	8.3 ± 0.1	9.3 ± 0.1	9.6 ± 0.2
5_c	5.0 ± 0.1	4.3 ± 0.1	10.8 ± 0.2	9.5 ± 0.1	10.5 ± 0.2	10.3 ± 0.2
5_d	4.6 ± 0.1	3.8 ± 0.1	9.1 ± 0.1	10.4 ± 0.2	11.2 ± 0.3	12.7 ± 03
5_e	5.7 ± 0.1	4.3 ± 0.1	9.9 ± 0.2	9.2± 0.1	9.7 ± 0.2	10.2 ± 0.2
Ciprofloxacin	15.9 ± 0.3	16.7 ± 0.4	-	-	-	-
Clotrimazole	-	-	15.3 ± 0.3	16.2 ± 0.4	16.5 ± 0.3	17.3 ± 0.4

Values are given as mean ± SD (*n = 3*). (–): Not tested

*Anti-microbial activity synthesized compounds (4_{a-e}-5_{a-e}) (50 µg/disc) and standard antibiotics (10 µg/disc).

Table 3. Minimum inhibition concentration (µg/ml) of synthesized compounds 4_{a-e}, 5_{a-e} and reference antibiotics determined by the broth microdilution method

Compound	Minimum inhibition concentration (MIC) in µg/ml					
	Bacillus cereus MTCC-430	***Escherichia coli MTCC-1302***	***Aspergillus niger* MTCC-2425**	***Aspergillus niger* MTCC- 1344**	***Candida albicans MTCC-183***	***Candida norvegensis MTCC-227***
4_a	25.6	102.4	3.2	1.6	1.6	1.6
4_b	51.2	102.4	6.4	3.2	6.4	3.2
4_c	51.2	204.8	3.2	3.2	6.4	6.4
4_d	51.2	102.4	3.2	3.2	1.6	1.6
4_e	25.6	102.4	3.2	3.2	6.4	1.6
5_a	102.4	204.8	6.4	12.8	6.4	6.4
5_b	102.4	204.8	12.8	12.8	6.4	6.4
5_c	102.4	204.8	3.2	6.4	6.4	3.2
5_d	102.4	204.8	6.4	6.4	3.2	3.2
5_e	102.4	204.8	6.4	6.4	6.4	3.2
Ciprofloxacin	0.2	0.2	-	-	-	-
Clotrimazole	-	-	0.4	0.4	0.2	0.2

(–): Not tested

Table 4. Minimum bactericidal or fungicidal concentration (MBC/MFC) (µg/ml) of the synthesized compounds 4_{a-e}, 5_{a-e} and reference antibiotics determined by the broth microdilution method

Compound	MBC/MFC concentration in µg/ml					
	Bacillus cereus MTCC-430	***Escherichia coli MTCC-1302***	***Aspergillusniger* MTCC-2425**	***Aspergillus niger* MTCC- 1344**	***Candida albicans MTCC-183***	***Candida norvegensis MTCC-227***
4_a	51.2	204.8	6.4	6.4	6.4	6.4
4_b	102.4	204.8	25.6	12.8	25.6	6.4
4_c	102.4	>204.8	6.4	6.4	25.6	25.6
4_d	102.4	204.8	12.8	6.4	6.4	6.4
4_e	51.2	204.8	6.4	6.4	25.6	6.4
5_a	204.8	>204.8	25.6	51.2	25.6	25.6
5_b	204.8	>204.8	25.6	25.6	25.6	25.6
5_c	204.8	>204.8	25.6	25.6	25.6	6.4
5_d	204.8	>204.8	25.6	25.6	6.4	6.4
5_e	204.8	>204.8	25.6	25.6	25.6	6.4
Ciprofloxacin	0.8	0.8	-	-	-	-
Clotrimazole	-	-	1.6	0.8	0.8	0.8

(–): Not tested

CONCLUSION

In this study, two series substituted imidazolones were prepared and evaluated for their antimicrobial activities using disc diffusion and micro broth dilution assays. Compounds 4_{a-e}, 5_a and 5_e were showed relatively effective zone of inhibition against *B. cereus*. All compounds were showed less effective zone of inhibition against *E. coli*. It was observed that compounds 4_{a-e} had better activity against *Aspergillus* and *Candida species* respectively than the compounds $5_{a-e.}$ Only compound 5_a was showed comparatively strong activity than 5_{b-e} against *Candida species*. Considering the results obtained from antibacterial and antifungal tests together, it is possible to say that all the tested compounds showed good zone of inhibition against fungi than bacteria and also all compounds had better activity against *Candida* strains than *Aspergillus* strains.

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