Novel synthesis of 3-(((E)-benzylidene)amino)-5-((Z)-3,4-dimethoxybenzylidene)-2-phenyl-3,5-dihydro-4H-imidazol-4-one derivatives as potent antimicrobial agents

Rohit B. Manawar, Mukesh B. Parmar, Indresh J. Nayaka, Brinda H. Pandit, Manish K. Shah*
Chemical Research Laboratory, Department of Chemistry, Saurashtra University, Rajkot - 360005, India
*E-mail address: drmksresearch2000@gmail.com

ABSTRACT

Novel synthesis of 4H-imidazol-4-one derivative by reaction of (Z)-3-amino-5-(3,4 dimethoxybenzylidene)-2-phenyl-3,5-dihydro-4H-imidazol-4-one with derivative of different aromatic aldehydes and Salicyldehyde. New synthesized compound are thermally stable and neither air-nor moisture sensitive at RT. Successive library of synthesized compound characterized using IR, NMR, Elemental, Mass, and its antimicrobial activity study.

Keywords: 4H-imidazol-4-one, Anti-microbial Agent, Anti-fungal, IR spectroscopy, NMR spectroscopy, Elemental analysis, Mass spectroscopy, Bioactivity, C. albicans, A. niger, S. pyogenes, S. aureus, P. aeruginosa, E. coli

1. INTRODUCTION

In today’s world Control of microbial population is key to prevent transmission and growth of disease, Infection, Decomposition, Contamination and spoilage caused by them. Human evolution and development is directly depends to a large extent on the control of
microbial population. Recently the application of nitrogen-based ligands species, such as Schiff bases [1-5] chalcones has also consider as a highly bioactive compound against microorganism [6-10]. In present studies have information regarding bio-activity of metal complex [11-15] and ligand which was synthesized using appropriate 1-4-dioxane as solvents for the reaction.

3,5-Dimethoxy benzaldehyde and Hippuric acid react together in presences of Acetic anhydride and Sodium acetate resulting production of Oxazolone derivative [16-29] which further derivatized to amino functional group [30-37] which further treated with various Salicylaldehyde derivative [32, 38-40]. For coupling reaction Piperidine used as basic catalyst and reflux on magnetic stirrer and oil bath leads to formation of 5-((Z)-3,4-dimethoxybenzylidene)-3-(((E)-2-hydroxybenzylidene)amino)-2-phenyl-3,5-dihydro-4H-imidazol-4-one and its analogue various derivative [41-53].

Compound bearing azo group exhibit various biological activity [54-56], though vast amount of work has been done on imidazolone, little attention has been created to synthesize imidazolone using azo salicyldehyde hydrazone and oxazolone. Literature survey reveals that imidazol-4-one exhibit promising biological and pharmacological activity. A novel method using microwave induced solvent free synthesis of 1-arlyl-2-(1E)-aryl-vinyl-4-arylmethylene-2-imidazoline-5-ones was reported [57]. Recently analysis of imidazolone derivative, its mode of action, their biodegradation and various applications have been studied [58]. Recently pharmaceutical study and particularly leishmanicidal activity of 5-imidazolone has been carried out [59-61]. Now a day an efficient method for the synthesis of long chain dialkyldiamo imidazolone by the reaction of diethylene triamine and several fatty acids under nonsolvent microwave irradiation using calcium oxide as support is used [62]. Interest in the chemistry of imidazolone continues unabated because of their usefulness as antibacterial and anti-inflammatory [63] agents.

2. RESULT AND DISCUSSION

2.1. Materials and methods

Required starting material and solvents were purchased from Sigma Aldrich, Merck and spectrochem, and checked by TLC. 1H NMR spectra were taken on Bruker NMR spectrometer [400 MHz], using TMS [used as internal standard], IR spectra were taken with FT-IR Spectrophotometer [Thermo Scientific]. Mass spectra were obtained from GCMS QP2010 mass spectrometer, moreover Elemental data was recorded by Carlo Erba EA 1108 elemental analyser.

2.2. General procedure for the synthesis of (MK-201)

To the Hippuric acid (5 mmol) solid was added to sodium acetate (10 mmol) and Acetic anhydride (10 mmol) stir for half an hour. After homogenous solution appeared, substituted Benzaldehyde (5 mmol) was added and refluxed until the colour changed to dark orange and mixture is liquefied then reflux for 3 hours. The reaction was cooled at room temperature and methanol was added as solvent. The reaction mixture was kept overnight at freezing (14 ºC) temperature and Precipitate was filtered. Resulted Oxazolone is treated with hydrazine hydrate 80% with solvent THF in presence of catalytic amount of Gla. acetic acid. The reaction was monitored by TLC and after the completion of the reaction; Precipitate was filtered and washed with water several times and dried Derivative MK-201 is collected.
2. 3. Spectral data of (MK-201)

Mp: 150-152 °C. Elemental Analytical Calculation for C_{18}H_{17}N_{3}O_{3} (223.35 gm/mol) C, 66.86%; H, 5.30%; N, 13.00%; O, 14.84%; Found: C, 66.95%; H, 5.21%; N, 13.08%; O, 14.76%: MS (m/z) 323 (M), ¹H NMR (400 MHz, CDCl₃), δ 7.886, (d, 2H, -H); 7.692, (s, 2H, NH₂); 7.570,7.487 (t, 3H, CH(CH)₂); 7.170 (s, 1H, C-H); 7.040-7.019 (d, 1H, Ar-H); 6.968 (s, 1H, Ar-H), 6.844-6.823 (d, 1H, Ar-H); 3.877, 3.620 (s, 6H, -OCH₃).

2. 4. General method for synthesis of (MKL-201 to MKL-222)

((Z)-3-amino-5-(3,4-dimethoxybenzylidene)-2-phenyl-3,5-dihydro-4H-imidazol-4-one) MK-201 reacts to various aldehyde and salisyldehyde derivative in presence of Piperidine as catalyst and 1-4 Dioxane as solvent which is result in desired product MKL-201-222. The Crude product was recrystallized by ethanol.

2. 5. Physical property of (MKL-201 to MKL-222)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Compound Code</th>
<th>Mol. Weight (gm/mole)</th>
<th>Substitution</th>
<th>Yield (%)</th>
<th>Colour</th>
<th>Solubility</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MKL-201</td>
<td>427</td>
<td>OH H H H H</td>
<td>72</td>
<td>Light yellow</td>
<td>Chloroform</td>
</tr>
<tr>
<td>2</td>
<td>MKL-202</td>
<td>506</td>
<td>OH H H Br H</td>
<td>91</td>
<td>Clourless</td>
<td>DMF</td>
</tr>
<tr>
<td>3</td>
<td>MKL-203</td>
<td>461</td>
<td>OH H H Cl H</td>
<td>68</td>
<td>Cream Yellow</td>
<td>Chloroform</td>
</tr>
<tr>
<td>4</td>
<td>MKL-204</td>
<td>480</td>
<td>Cl H H H Cl</td>
<td>69</td>
<td>Light yellow</td>
<td>MeOH</td>
</tr>
<tr>
<td>5</td>
<td>MKL-205</td>
<td>498</td>
<td>OH H N(Et)₂ H H</td>
<td>65</td>
<td>Clourless</td>
<td>Chloroform</td>
</tr>
<tr>
<td>6</td>
<td>MKL-206</td>
<td>445</td>
<td>H H H Cl H</td>
<td>66</td>
<td>Yellow</td>
<td>DMF</td>
</tr>
<tr>
<td>7</td>
<td>MKL-207</td>
<td>471</td>
<td>H OMe OMe H H</td>
<td>62</td>
<td>Clourless</td>
<td>MeOH</td>
</tr>
<tr>
<td>8</td>
<td>MKL-208</td>
<td>456</td>
<td>H H NO₂ H H</td>
<td>70</td>
<td>Off white</td>
<td>DMF</td>
</tr>
<tr>
<td>9</td>
<td>MKL-209</td>
<td>429</td>
<td>H H F H H</td>
<td>72</td>
<td>Light yellow</td>
<td>DMF</td>
</tr>
<tr>
<td>10</td>
<td>MKL-210</td>
<td>427</td>
<td>H H OH H H</td>
<td>90</td>
<td>Clourless</td>
<td>Chloroform</td>
</tr>
<tr>
<td>11</td>
<td>MKL-211</td>
<td>461</td>
<td>OH H H Cl H</td>
<td>68</td>
<td>Light yellow</td>
<td>Chloroform</td>
</tr>
<tr>
<td>12</td>
<td>MKL-212</td>
<td>490</td>
<td>H Br H H H</td>
<td>54</td>
<td>Yellow</td>
<td>Chloroform</td>
</tr>
</tbody>
</table>
2. 6. Spectral data of (MKL-201)

Mp: 230-232 °C, Elemental Analytical Calculation for C_{25}H_{21}N_{3}O_{4} (427.46 gm/mole) C, 70.25%; H, 4.95%; N, 9.83%; O, 14.97%; Found: C, 70.40%; H, 4.98%; N, 9.68%; O, 14.94%: MS: m/z: 427 (M), 1H NMR (400 MHz, DMSO), δ 11.372 (s, 1H), 8.608 (s, 1H), 8.107-8.089 (d, 2H), 7.639-7.603 (d, 1H), 7.568-7.531 (t, 2H), 7.501-7.479 (d, 1H), 7.316-7.274 (t, 3H), 7.240-7.215 (d, 1H), 7.007-6.985 (d, 1H), 6.941-6.919 (d, 1H), 6.898 (s, 1H), 3.765 (s, 3H), 3.358 (s, 3H), IR, (cm⁻¹): ν (C-H) 3322.9; ν (OH) 3246.5; ν (C=N) 1690; ν (N-N) 948; ν (C-C) 1597.2; ν (C-N) 1354.9; ν (1,2, adj. H. Ar–C-H) 877; ν (-O-CH₃) 710.

2. 7. Spectral data of (MKL-202)

Mp: 158-160 °C, Elemental Analytical Calculation for C_{25}H_{20}BrN_{3}O_{3} (506.36 gm/mole) C, 59.30%; H, 3.98%; N, 8.30%; O, 12.64%; Br, 15.78%; Found: C, 58.30%; H, 3.78%; N, 8.30%; O, 12.84%; Br, 16.78%;: MS: m/z: 507 (M-1), 1H NMR (400 MHz, DMSO), δ 11.784 (s, 1H), 10.107 (s, 1H), 8.394 (s, 1H), 8.103-8.085 (d, 2H), 7.905 (s, 1H), 7.701-7.682 (d, 1H), 7.629-7.612 (d, 2H), 7.562-7.527 (t, 2H), 7.440-7.401 (t, 1H), 7.306 (s, 1H), 7.005-6.983 (d, 1H), 3.768 (s, 3H), 3.554 (s, 3H), IR, (cm⁻¹): ν (OH) 3214.8; ν (C=N) 1636.3; ν (C-C) 1597.2; ν (C-N) 1364.2; ν (1,2, adj. H. Ar–C-H) 890.8; ν (-O-CH₃) 795.

2. 8. Spectral data of (MKL-203)

Mp: 158-160 °C, Elemental Analytical Calculation for C_{25}H_{20}FNO_{3} (461.90 gm/mole) C, 65.01%; H, 4.36%; N, 9.10%; O, 13.85%; Cl, 7.67%; Found: C, 65.11%; H, 4.46%; N, 9.16%; O, 13.75%; Cl, 7.57%;: MS: m/z: 461 (M+1), 1H NMR (400 MHz, DMSO) δ 11.713 (s, 1H), 8.858 (dd, 1H), 8.135-8.116 (dd, 2H), 8.034-8.015 (dd, 2H), 7.937-9.19 (d, 1H), 7.681-7.540 (m, 3H), 7.327-7.246 (dd, 3H), 7.019-6.998 (d, 1H), 3.776 (s, 3H), 3.370 (s, 3H), IR, (cm⁻¹): ν (C-H) 3232.9; ν (OH) 3213.0; ν (C=N) 1634.4; ν (N-N) 950.5; ν (C-C) 1599.0; ν (C-N) 1397.8; ν (1,2, adj. H. Ar–C-H) 870.3; ν (-O-CH₃) 719.4.
2. 9. Spectral data of (MKL-204)

Mp: 188-190 °C. Elemental Analytical Calculation for C\textsubscript{25}H\textsubscript{19}Cl\textsubscript{2}N\textsubscript{3}O\textsubscript{3} (480.35gm/mole) C, 62.51%; H, 3.99%; N, 8.75%; O, 9.99%; Cl, 14.76%; Found: C, 62.31%; H, 3.98%; N, 8.77%; O, 10.19%; Cl, 24.30%; MS: m/z: 479 (M+1), \textsuperscript{1}H NMR (400 MHz, DMSO), δ 8.627 (s, 1H), 8.102-8.085 (d, 2H), 7.632-7.615 (d, 2H), 7.597-7.528 (t, 3H), 7.462-7.422 (t, 1H), 7.276 (s, 1H), 7.245-7.224 (d, 2H), 3.765 (s, 3H), 3.568-3.358 (s, 30H), IR, (cm\textsuperscript{−1}): ν(C-H) 3067.6; ν(C=N) 1638.2; ν(C-N) 1375.4; ν(N-N) 944.9; 9; ν (1,2, adj. H. Ar–C-H) 872.2; ν (C-Cl), 841; ν (–O-CH\textsubscript{3}) 782.7.

3. BIOACTIVITY STUDY

Table 2. Bioactivity table.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Code No</th>
<th>E. coli</th>
<th>P. aeruginosa</th>
<th>S. aureus</th>
<th>S. pyogenes</th>
<th>C. albicans</th>
<th>A. niger</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MTCC 443</td>
<td>MTCC 3310</td>
<td>MTCC 96</td>
<td>MTCC 441</td>
<td>MTCC 227</td>
<td>MTCC 282</td>
</tr>
<tr>
<td>1</td>
<td>MKL 201</td>
<td>100</td>
<td>250</td>
<td>200</td>
<td>250</td>
<td>500</td>
<td>1000</td>
</tr>
<tr>
<td>2</td>
<td>MKL 202</td>
<td>125</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>3</td>
<td>MKL 203</td>
<td>100</td>
<td>125</td>
<td>200</td>
<td>200</td>
<td>500</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>4</td>
<td>MKL 204</td>
<td>100</td>
<td>200</td>
<td>62.5</td>
<td>100</td>
<td>1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>5</td>
<td>MKL 205</td>
<td>62.5</td>
<td>125</td>
<td>200</td>
<td>62.5</td>
<td>1000</td>
<td>500</td>
</tr>
<tr>
<td>6</td>
<td>MKL 206</td>
<td>125</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>1000</td>
<td>500</td>
</tr>
<tr>
<td>7</td>
<td>MKL 207</td>
<td>100</td>
<td>200</td>
<td>100</td>
<td>100</td>
<td>250</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>8</td>
<td>MKL 208</td>
<td>62.5</td>
<td>100</td>
<td>200</td>
<td>250</td>
<td>500</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>9</td>
<td>MKL 209</td>
<td>200</td>
<td>200</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>1000</td>
</tr>
<tr>
<td>10</td>
<td>MKL 210</td>
<td>62.5</td>
<td>125</td>
<td>62.5</td>
<td>100</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>11</td>
<td>MKL 211</td>
<td>200</td>
<td>250</td>
<td>100</td>
<td>100</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>12</td>
<td>MKL 212</td>
<td>62.5</td>
<td>125</td>
<td>200</td>
<td>100</td>
<td>500</td>
<td>1000</td>
</tr>
</tbody>
</table>
Antimicrobial activity of all synthesized compound 3-(((E)-benzylidene)amino)-5-((Z)-3,4-dimethoxybenzylidene)-2-phenyl-3,5-dihydro-4H-imidazol-4-one derivatives (MKL-201 to MKL-2014) were evaluated for their antibacterial and antifungal activity by measuring minimum inhibitory concentration (μg/mL). The results of antifungal activities (Table 2) indicated that most of the target compounds MKL-201-214 display very potent antifungal activity against *C. albicans* and *A. niger*, while Compound MKL-202, 205, 206, 207, 209, 213 show more potent activity better than Nystatin, Greseofulvin, Ciprofloxacin, Chloramphenicol.

Antibacterial activity: Based on the antimicrobial results for all the synthesized 3-(((E)-benzylidene)amino)-5-((Z)-3,4-dimethoxybenzylidene)-2-phenyl-3,5-dihydro-4H-imidazol-4-one derivatives MKL-204, 205, 206, 208, 210, 212 exhibited good antibacterial activity (Table 3) against the various gram positive (*S. aureus, S. pyogenes*) and gram negative (*E. coli, P. aeruginosa*) bacterial strains and MIC values were comparable to those observed against the standard drugs Nystatin, Greseofulvin, Ciprofloxacin, Chloramphenicol. The MIC of the control organism is calculated to check the accuracy of the drug concentrations. The lowest concentration inhibition is called MIC. Several methods used for primary and secondary screening every synthesized drug was diluted microgram/ml. Primary screen and Secondary screen are in order. The first screening 1000 micro/ml, 500 micro/ml, and 250 micro/ml concentration. The active drugs found in primary screening were further diluted to 200 micro/ml, 100 micro/ml, 50 micro/ml, 25 micro/ml, 12.5 micro/ml, and 6.250 micro/ml concentrations. Highest dilution showing at least 99% inhibition zone is considered as MIC.

4. CONCLUSIONS

All the final MKL-201 to MKL-222 compounds were successfully synthesized and Purified by Column chromatography and characterized by various spectroscopic techniques like $^1$H NMR, $^{13}$C NMR and Mass analysis. Shortlisted fourteen compounds were carried out for their antibacterial and anti-fungal activity using 2 gram positive and 2 gram negative
bacteria as well as two fungal stain. From this whole research study we came to conclude that all the compounds found active and among these some are emerged out as potent antibacterial and anti-fungal agents are MKL-205, 206, 208, 209, 213.

ACKNOWLEDGMENT

We are thankful to Department of Chemistry, Saurashtra University Rajkot Gujarat India. We are also thankful to NFDD (National Facility for Drug Discovery) Rajkot and Department of Pharmacy Sau. Uni. Rajkot for analytical support and antimicrobial activity during research work.

References


Sosale Chandrasekhar and Malempati Srimannarayana, The Erlenmeyer synthesis with a thioazlactone, *Arkivoc* 2009 (xii) 290-295


[38] Vikas Sharma, Prabodh C. Sharma, Vipin Kumar, A mini review on pyridoacridines: Prospective lead compounds in medicinal chemistry, Journal of Advanced Research (2015) 6, 63-71


[47] M. B. Madhusudana Reddy and M. A. Pasha, Molecular iodine–catalyzed, mild, effective, ecofriendly, microwave-assisted, one-pot synthesis of 5-arylmethylidene-2-phenyloxazol-4-ones (azlactones) under solvent-free conditions, Synthetic Communications 1, 40: 1895-1898, 2010


I. Ostromistensky ‘Note on Bacteriostatic Azo Compounds. J. Am. Chem. Soc. 1934, 56, 1713-1714

D. E. Fink and D. L. Vivian; *J. Econ. ‘Entomol.Scintific Notes’* 1936, 29, 804.


Figure 1. Reaction Scheme of Amine derivative (MK-201)
Figure 2. Mass spectrum of (MK-201)
Figure 3. IR spectrum of (MK-201)
Figure 4. $^1$HNMR spectrum of (MK-201)
Figure 5. $^{13}$C NMR spectrum of (MK-201)
**Figure 6.** Reaction Scheme of (MKL-201 to MKL-222)
Figure 7. Mass spectrum of (MKL-201)
Figure 8. IR spectrum of (MKL-201)
Figure 9. $^1$HNMR spectrum of (MKL-201
Figure 10. Mass spectrum of (MKL-202)
Figure 11. IR spectrum of (MKL-202)
Figure 12. $^1$HNMR spectrum of (MKL-202)
Figure 13. Mass spectrum of (MKL-203)
Figure 14. IR spectrum of (MKL-203)
Figure 15. $^1$HNMR spectrum of (MKL-203)
Figure 16. Mass spectrum of (MKL-204)
Figure 17. IR spectrum of (MKL-204)
Figure 18. $^1$HNMR spectrum of (MKL-204)