Synthesis of some new 1,4-dihydropyrimido[1,2-a]benzimidazoles and evaluation of their biological activity

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ABSTRACT

Synthesis of a series of pyrimido[1,2-a]benzimidazoles (4a-j) was achieved from acetoacetamides, name of aldehyde and benzimidazole using refluxing with DMF and isolated by methanol with high yield and purity. The pyrimido[1,2-a]benzimidazoles of the products were supported by FTIR, PMR and mass spectral data.

Keywords: pyrimidines, acetoacetamides, benzimidazole synthesis.

INTRODUCTION

An improved method for the synthesis of some new 1,4-dihydropyrimido[1,2-a]benz-imidazoles from aromatic aldehydes, Acetoacetamide compounds and 2-amino benzimidazole with significant enhancement in reaction rates, short reaction time (30 min h.), good to excellent yields (59-80%) and ambient temperature. Crystisation using ethanol. The biological evaluation revealed that the newly synthesized compounds (4a-j) and exhibited good antimicrobial activity and moderate antimycobacterial activity.

Polysubstituted pyrimido[1,2-a]benzimidazoles possess a wide spectrum of biological activities and they are structurally related to natural purine bases.

From the standpoint of biological activity, fused heteroaromatic systems are often of much greater interest than the constituent monocyclic compounds. Antimicrobial [1-4], antimalarial [5], antiproliferative [6], protein kinase inhibitor, [7], T cell activation, [8], angioprotein receptors and/or vascular endothelial growth factor receptor-2 (VEGFR-2) inhibitory activities. [9], hypotensive, spasmylytic, and antiaggregant activities [10], anesthetic activity [11] and diuretic [12], antiinflammatory [13, 14], etc. activities have been reported for certain pyrimido[1,2-a]benzimidazole derivatives.

To circumvent these problems, we have developed a new protocol for the synthesis of novel pyrimido[1,2-a]benzimidazoles (4a-j) with the advantage of short reaction time, high yield and environmentally friendliness (Scheme-a).

EXPERIMENTAL SECTION

Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was routinely checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine. IR spectra were recorded Shimadzu FT-IR-8400 instrument using potassium bromide (KBr) pellet method. Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using direct injection probe technique. \(^1\)H NMR was determined in DMSO-\(d_6\) solution on a bruker Ac 400 MHz spectrometer. Elemental analysis of the all the synthesized compounds
was carried out on elemental vario EL III carlo erba 1108 model and the results are in agreements with the structures assigned.

**Typical experimental procedure for the synthesis of 1,4-dihydropyrimido[1,2-a]benzimidazoles.**

A mixture of the 2-amino benzimidazole (0.01mol), N-(4-methylthiazol-2-yl)-3-oxobutanamide (0.01mol) and an appropriate aromatic aldehyde (0.01mol) was refluxed in 10 ml of DMF for 30 min. After cooling, methanol (~15 ml) was added. The reaction mixture was allowed to stand overnight and then filtered to give the solid 1,4-dihydropyrimido[1,2-a]benzimidazoles products 4a-j, which were crystallized from ethanol. Thin Layer Chromatography was performed on silica gel-G using hexane:ethylacetate solvent system.

**Reaction Scheme**

![Reaction Scheme](image)

**Scheme-a**

**4-(4-chlorophenyl)-2-methyl-N-(4-methyl-1,3-thiazol-2-yl)-1,4-dihydropyrimido[1,2-a]benzimidazole-3-carboxamide (4a)**

mp 198 °C; white crystals; 1H NMR (DMSO-d6) δ ppm: 1.25 (s, 3H, Hδ), 2.50 (s, 3H, Hα), 6.50 (s, 1H, Hη), 7.12-7.16 (m, 2H, Hk), 7.21-7.25 (m, 2H, Hj), 7.59-7.32 (d, 2H, Hm), 7.49-7.52 (d, 2H, Hn), 7.66 (s, 1H, Hj), 10.02 (s, 1H, Hk), 10.09 (s, 1H, Hm). FT IR (cm⁻¹): 3308 (N-H stretching of secondary amine), 3051 (C-H stretching of aromatic ring), 2976 (C-H asymmetrical stretching of CH₃ group), 2827 (C-H asymmetrical stretching of CH₃ group), 1670 (C=O stretching of amide), 1618 (N-H deformation of pyrimidine ring), 1583 and 1508 (C=C stretching of aromatic ring), 2976 (C-H asymmetrical stretching of CH₃ group), 1296 (C-N stretching), 1045 (C-H in plane deformation of aromatic ring), 1454 (C-H asymmetrical deformation of CH₃ group), 1386 (C-H symmetrical deformation of CH₃ group), 1296 (C-N stretching), 1045 (C-H in plane deformation of aromatic ring), 798 (C-H out of plane bending of 1,4-disubstitution), 736 (C-Cl stretching); MS: m/z: 435; Anal. Calcd. for C₂₂H₁₈ClN₄OS: C, 60.61; H, 4.16; N, 3.67; Found: C, 60.25; H, 4.02; N, 3.32 %; Yield: 68%.

**4-(4-fluorophenyl)-2-methyl-N-(4-methyl-1,3-thiazol-2-yl)-1,4-dihydropyrimido[1,2-a]benzimidazole-3-carboxamide (4b)**

mp 185 °C; white crystals; ¹H NMR (DMSO-d6) δ ppm: 1.34 (s, 3H, Hδ), 2.24 (s, 3H, Hα), 5.29 (s, 1H, Hη), 6.58 (s, 1H, Hj), 6.92-7.93 (d, 2H, Hk), 7.05-7.09 (m, 2H, Hj), 7.18-7.24 (m, 2H, Hj), 7.39-7.41 (d, 2H, Hm), 9.35 (s, 1H, Hj), 9.56 (s, 1H, Hk). FT IR (cm⁻¹): 3274 (N-H stretching of secondary amine), 3057 (C-H stretching of aromatic ring), 2976 (C-H asymmetrical stretching of CH₃ group), 2845 (C-H asymmetrical stretching of CH₃ group), 1651 (C=O stretching of amide), 1608 (N-H deformation of pyrimidine ring), 1560 and 1504 (C=C stretching of aromatic ring), 1458 (C-H asymmetrical deformation of CH₃ group), 1386 (C-H symmetrical deformation of CH₃ group), 1280 and 1253 (C-N stretching), 1074 (C-H in plane deformation of aromatic ring), 798 (C-H out of plane bending of 1,4-disubstitution). MS: m/z: 419; Anal. Calcd. for C₂₂H₁₈FN₄OS: C, 62.99; H, 4.33; N, 16.70. Found: C, 62.23; H, 4.02; N, 16.28 %; Yield: 66%.

**4-(4-methylphenyl)-2-methyl-N-(4-methyl-1,3-thiazol-2-yl)-1,4-dihydropyr imido[1,2-a]benzimidazole-3-carboxamide (4c)**

mp 250 °C; ¹H NMR (DMSO-d6) δ ppm: 1.27 (s, 3H, Hδ), 2.25 (s, 3H, Hα), 2.72 (s, 3H, Hj), 6.47 (s, 1H, Hj), 7.05-7.10 (m, 4H, Hk′), 7.29-7.31 (d, 2H, Hj′), 7.53-7.55 (d, 2H, Hm), J=8.40 Hz), 7.64 (s, 1H, Hn), 9.96 (s, 1H, Hk), 10.10 (s, 1H, Hm). FT IR (cm⁻¹): 3288 (N-H stretching of secondary amine), 3057 (C-H stretching of aromatic ring), 2976 (C-H asymmetrical stretching of CH₃ group), 2888 (C-H symmetrical stretching of CH₃ group), 1651 (C=O stretching of amide), 1624 (N-H deformation of pyrimidine ring), 1562,1529 and 1502 (C=C stretching of aromatic ring), 1458 (C-H asymmetrical deformation of CH₃ group), 1394 (C-H symmetrical deformation of CH₃ group), 1257 and 1220 (C-N stretching), 1078 (C-H in plane deformation of aromatic ring), 817 (C-H out of plane bending of 1,4-disubstitution). MS: m/z: 416; Anal. Calcd. for C₂₂H₂₁N₄OS: C, 66.48; H, 5.09; N, 16.85. Found: C, 66.14; H, 4.91; N, 16.11 %; Yield: 76%.
4-(4-methoxyphenyl)-2-methyl-N-(4-methyl-1,3-thiazol-2-yl)-1,4-dihydro pyrimido[1,2-a]benzimidazole-3-carboxamide (4d)

mp 211°C; 1H NMR (DMSO-d6) δ ppm: 1.27 (s, 3H, H3), 3.25 (s, 3H, H6), 2.72 (s, 3H, H9), 6.47 (s, 1H, H2), 7.05-7.10 (m, 4H, H7a-e), 7.29-7.31 (d, 2H, H8), 7.53-7.55 (d, 2H, H10), 7.64 (s, 1H, H11), 9.96 (s, 1H, H12), 10.10 (s, 1H, H1). FT IR (cm⁻¹): 3288 (N-H stretching of secondary amine), 3057 (C-H stretching of aromatic ring), 2976 (C-H asymmetrical stretching of CH₂ group), 2888 (C-H asymmetrical stretching of CH₃ group), 1651 (C=O stretching of amide), 1562,1529 and 1502 (C=C stretching of aromatic ring), 1458 (C-H asymmetrical deformation of CH₃ group), 1394 (C=H symmetrical deformation of CH₃ group), 1257 and 1220 (C-N stretching), 1241 (C-O-C stretching), 1078 (C-H in plane deformation of aromatic ring), 817 (C-H out of plane bending of 1,4-disubstitution), MS: m/z 431; Anal. Calcd. for C₂₃H₂₈N₂O₃S: C, 64.02; H, 4.91; N, 16.23. Found: C, 63.85; H, 4.51; N, 16.02%. Yield: 71%.

4-(3-bromophenyl)-2-methyl-N-(4-methyl-1,3-thiazol-2-yl)-1,4-dihydro pyrimido[1,2-a]benzimidazole-3-carboxamide (4e)

mp 203°C; 1H NMR (DMSO-d6) δ ppm: 1.34 (s, 3H, H3), 2.24 (s, 3H, H6), 5.29 (s, 1H, H2), 6.58 (s, 1H, H9), 6.92-7.93 (d, 2H, H7a-e), 7.05-7.09 (m, 2H, H8), 7.18-7.24 (m, 2H, H10), 7.39-7.41 (d, 2H, H11), 9.35 (s, 1H, H12), 9.56 (s, 1H, H1). FT IR (cm⁻¹): 3274 (N-H stretching of secondary amine), 3053 (C-H stretching of aromatic ring), 2976 (C-H asymmetrical stretching of CH₃ group), 2845 (C-H asymmetrical stretching of CH₃ group), 1651 (C=O stretching of amide), 1608 (N-H deformation of pyrimidine ring), 1560,1529 and 1504 (C=C stretching of aromatic ring), 1458 (C-H asymmetrical deformation of CH₃ group), 1392 (C=H symmetrical deformation of CH₃ group), 1280 and 1253 (C-N stretching), 1074 (C-H in plane deformation of aromatic ring), 1080 (C-Br stretching), 819 (C-H out of plane bending of 1,3-disubstitution), MS: m/z 479; Anal. Calcd. for C₂₂H₁₉BrN₂O₃S: C, 55.01; H, 3.78; N, 14.58. Found: C, 54.79; H, 3.45; N, 14.19%. Yield: 80%.

2-methyl-N-(4-methyl-1,3-thiazol-2-yl)-4-(4-nitrophenyl)-1,4-dihydro pyrimido[1,2-a]benzimidazole-3-carboxamide (4g)

mp 203°C; 1H NMR (DMSO-d6) δ ppm: 1.34 (s, 3H, H3), 2.24 (s, 3H, H6), 5.29 (s, 1H, H2), 6.58 (s, 1H, H9), 6.92-7.93 (d, 2H, H7a-e), 7.05-7.09 (m, 2H, H8), 7.18-7.24 (m, 2H, H10), 7.39-7.41 (d, 2H, H11), 9.35 (s, 1H, H12), 9.56 (s, 1H, H1). FT IR (cm⁻¹): 3274 (N-H stretching of secondary amine), 3053 (C-H stretching of aromatic ring), 2976 (C-H asymmetrical stretching of CH₃ group), 2845 (C-H asymmetrical stretching of CH₃ group), 1651 (C=O stretching of amide), 1608 (N-H deformation of pyrimidine ring), 1560,1529 and 1504 (C=C stretching of aromatic ring), 1458 (C-H asymmetrical deformation of CH₃ group), 1392 (C=H symmetrical deformation of CH₃ group), 1280 and 1253 (C-N stretching), 1074 (C-H in plane deformation of aromatic ring), 1074 (C-Cl stretching), 805 (C-H out of plane bending of 1,3-disubstitution), MS: m/z 435; Anal. Calcd. for C₂₂H₁₈ClN₂O₅S: C, 60.61; H, 4.16; N, 16.07. Found: C, 60.22; H, 4.01; N, 15.77%. Yield: 77%.
2-methyl-N-(4-methyl-1,3-thiazol-2-yl)-4-(2-nitrophenyl)-1,4-dihydro pyrimido[1,2-a]benzimidazole-3-carboxamide (4i)

mp 189 °C; MS: ¹H NMR (DMSO-d₆) δ ppm: 1.34 (s, 3H, Hₐ), 2.24 (s, 3H, H₉), 5.29 (s, 1H, Hₜ), 6.58 (s, 1H, Hₐ), 6.92-7.09 (m, 2H, H₈, H₆), 7.18-7.24 (m, 2H, H₆), 7.39-7.41 (d, 2H, Hₐ), 9.35 (s, 1H, H₉), 9.56 (s, 1H, Hₚ). FT IR (cm⁻¹): 3274 (N-H stretching of secondary amine), 3053 (C-H stretching of aromatic ring), 2976 (C-H asymmetrical stretching of CH₃ group), 2845 (C-H asymmetrical stretching of CH₃ group), 1651 (C=O stretching of amide), 1608 (N-H deformation of pyrimidine ring), 1560, 1529 and 1504 (C=C stretching of aromatic ring), 1458 (C-H asymmetrical deformation of CH₃ group), 1392 (C-H symmetrical deformation of CH₃ group), 1280 and 1253 (C-N stretching), 1074 (C=O stretching of pyrimidine ring), 1500, 1542 and 1500 (C-C stretching of aromatic ring), 1316 (Nitro N-O), 1280 and 1253 (C-N stretching), 1078 (C-H in plane deformation of aromatic ring), 645 (C-H out of plane bending of 1,2-disubstitution). MS: m/z 446; Anal. Calcd. for C₂₂H₁₈N₆O₃S: C, 59.18; H, 4.06; N, 18.82. Found: C, 59.02; H, 3.82; N, 18.55%; Yield: 72%.

4-(4-hydroxyphenyl)-2-methyl-N-(4-methyl-1,3-thiazol-2-yl)-1,4-dihydro pyrimido[1,2-a]benzimidazole-3-carboxamide (4j)

mp 186 °C; ¹H NMR (DMSO-d₆) δ ppm: 1.27 (s, 3H, Hₐ), 10.25 (s, 1H, Hₚ), 2.72 (s, 3H, H₉), 6.47 (s, 1H, H₌), 7.05-7.10 (m, 4H, Hₐ-h), 7.29-7.31 (d, 2H, Hₐ-h), 2.72-2.75 (d, 2H, Hₐ-h), 7.64 (s, 1H, Hₚ), 9.10 (s, 1H, H₉). FT IR (cm⁻¹): 3599 (Free -OH), 3288 (N-H stretching of secondary amine), 3057 (C-H stretching of aromatic ring), 2976 (C-H asymmetrical stretching of CH₃ group), 2888 (C-H asymmetrical stretching of CH₃ group), 1651 (C=O stretching of amide), 1624 (N-H deformation of pyrimidine ring), 1562, 1529 and 1502 (C=C stretching of aromatic ring), 1458 (C-H asymmetrical deformation of CH₃ group), 1394 (C=O stretching of pyrimidine ring), 1257 and 1220 (C-N stretching), 1078 (C=O stretching of aromatic ring), 817 (C-H out of plane bending of 1,4-disubstitution). MS: m/z 417; Anal. Calcd. for C₂₂H₁₉N₅O₂S: C, 63.29; H, 4.59; N, 16.78. Found: C, 63.06; H, 4.12; N, 16.47%: Yield: 70%.

RESULTS AND DISCUSSION

The synthesis of 1,4-dihydropyrimido[1,2-a]benzimidazoles is based on the Biginelli like cyclocondensation of aromatic aldehydes and acetoacetic acid derivatives with 2-amino benzimidazole containing a guanidine fragment. Synthesis of N-(4-methylthiazol-2-yl)-3-oxobutanamide was achieved using previously published method [15].

There are literary data about the synthesis of 1,4-dihydropyrimido[1,2-a]benzimidazoles by treatment of 2-amino benzimidazole with aldehydes and ethyl acetoacetate. The cyclocondensations were achieved by heating of the starting materials in ethanol with catalytic amounts of hydrochloric acid under reflux conditions. The use of acetoacetamides in these or similar reactions has not been described [16-21].

Recognizing these facts, we have synthesised new series of 1,4-dihydropyrimido[1,2-a]benzimidazoles (4a-j) containing an acetoacetamide fragment. The structures of all the newly synthesized compounds were elucidated by FT-IR, mass spectra, ¹H NMR and elemental analyses. The newly synthesized compounds were subjected to antimicrobial activity.

Biological evaluation

Table-1: - In vitro Antimicrobial Screening Results for 4a-j

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<th>Code</th>
<th>Minimal inhibition concentration (µg mL⁻¹)</th>
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<td>Gram-positive</td>
</tr>
<tr>
<td>4a</td>
<td>200</td>
</tr>
<tr>
<td>4b</td>
<td>250</td>
</tr>
<tr>
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Ampicillin 250 100 100 100 - - -
Chloramphenicol 50 50 50 50 - - -
Iprofloxacin 50 50 25 25 - - -
Norfloxacin 10 10 10 10 - - -
Nystatin - - - - 100 100 100
Gresefulvin - - - - 500 100 100

3560
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