Base catalyzed microwave assisted synthesis, characterization of 6-bromo-pyrazolo-[1, 5-a]-pyrimidine-3-ethyl-carboxylate & its biological evaluation as CDKs inhibitor

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Abstract

Nano-catalysts have been playing a great role in the synthetic chemistry for their function in the reactions to give higher yields when compared to the micro-catalysts, as well as, moment end of the reaction. So we focused on the attention to use different nano-catalysts for the synthesis of 5-amino-pyrazolo-4-ethyl-carboxylate (3). In order to follow green synthesis, microwave assisted synthesis was maintained by different types of base hydrolysis to afford 6-Bromo-Pyrazolo- [1, 5-a]-Pyrimidine-3-Ethyl-Carboxylate (5). Instead of synthesizing different derivatives of compounds, different bases & nano-catalysts used to study the rate of the reaction & resulted successfully that it depends on the type of nano-catalysts & on the strength of the bases. We were characterized FT-IR, H1NMR & LC-MS. Later, the compound (5) was screened the biological activity & showed positive results by acting as inhibitor against Cyclin-dependent protein kinase (CDKs).

Keywords: 5-amino-pyrazolo-4-ethyl-carboxylate, green chemistry, nano-catalysts, microwave assisted synthesis, 6-bromo-pyrazolo-[1, 5-a]-pyrimidine-3-ethyl-carboxylate & kinase inhibitor

1. Introduction

Medicinal chemistry has been playing an essential role because without drugs no life is sustain. Various drugs possess different pharmacological activities depending upon their chemical composition. All Heterocyclic compounds have specific biological activity for having Hetero-atoms such as N, O & S. These atoms act as antagonists for abnormal cells which can cause impairments in the human body. Pyrazolo-Pyrimidines show a vast contribution towards the drug chemistry by acting as an anti to several diseases to man [1-4].

Our review has synthesized 6-Bromo-Pyrazolo-[1,5-a]-Pyrimidine-3-Ethyl-Carboxylate (5) by changing bases [5-9] for successive trials under microwave conditions [10-11] from 5-ammino-1H-pyrazolo-4-carboxylate, which was prepared by Ethyl-cyanoacetate & hydrazine hydrate [12-15] by using different metal-oxide nano-catalysts [16] under microwave heating conditions in absence of solvent [17,19]. It was reported that we used different kind of Nano-catalysts for (3) & different types of Bases for (5). This review helps to study the rate of reactions for the same scheme by using different Nano-catalysts & reagents. We found the reactions completed at different times based on the catalyst & strength of bases. Finally, the compound (5) was tested for pharmacological test shows a positive attitude against CDKs protein kinase with reference drug Roscovitine [20-23].

Fig 1: Binding Mechanism of (5) with CDKs cell
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Fig 1: Structure of Roscovitine & 6-Bromo-Pyrazole-[1, 5-a]-Pyrimidine-3-Ethyl-Carboxylate.

2. Instruments used
Melting points were determined in open capillary tubes in Buchi B-540 melting point apparatus. The reaction was monitored by thin layer chromatography using silica gel glass plates. The reaction was visualized by short Ultraviolet lamp & isolated in iodine chamber. NMR spectral data was determined on a BRUKER 400MHZ NMR spectrometer & chemical shifts were expressed in terms of parts per million (ppm) against TMS as internal reference. Mass spectra was recorded on ion trap mass spectrometer such as LC-MS, D-trap & XCT plus. Rota-vapors were handled for solvent extraction. FT-IR spectrometer (Bruker) was used.

3. Experimental section
3.1 General Procedure for the Synthesis (3) Using Zno Nano-Catalyst
To a solution of Ethyl cyanoacetate (1) (10.0g, 10.63ml, 0.0884mol) was added followed by drop-wise addition of Hydrazine hydrate (2) under microwave condition at 80-100°C (scheme -1). The solution turned brown in color which was heated under microwave at 100°C. The solid precipitate was quenched with water & extracted to ethyl acetate. The organic phase was dried over sodium sulphite, filtered & concentrated to afford 5-amino-1H-pyrazole-4-ethyl-carboxylate (3) as white solid compound. The compound was monitored by thin layer chromatography & Iodine chamber.

Synthesis

The scheme-1 was carried under the same conditions for MnO2, Fe2O3 & Fe3O4 as given in the table-4.1.

5-amino-1H-pyrazole-4-ethyl-carboxylate: White solid, m.p.122.5°C, (8.9g, % yield=89.00); IR(KBr): ν= 3339.72cm-1 (N-H stretch), 3230.43cm-1 (NH2 stretch), 2983.3 & 2908.67cm-1 (-CH stretch), 1717cm-1 (C=O ester); H'NMR: (300MHZ, DMSO) 1.266 (3H, t, CH3), 4.2 (2H, q, CH2), 5.99 (1H, s, CH), 11.844 (1H, s, -NH) & 7.4 (2H, s, -NH2). LC-MS: (M/Z) 273.0, 270.0; 225.9; 193.1, 192.1; 147.1. 

3.2 General Procedure for the Synthesis (5) Using Koh as A Base
To the compound (3), 2-Bromo-malonaldehyde (1.16g, 0.0077mol) was added in basic condition in Conc. hydrochloride under microwave conditions at 110°C to afford 6-Bromo-pyrazolo-[1,5-a]-pyrimidine-3-ethyl-carboxylate (scheme-2). The reaction process was monitored by thin layer chromatography. The solid crude was washed with ethanol & filtered to afford 6-bromo-pyrazolo-[1, 5-a]-pyrimidine-3-ethyl-carboxylate as yellow solid.

3.3 General procedures for (5) by Ethanol, H2O & Pyridine bases
The same procedures were followed for EtOH, H2O & Pyridine bases under the same conditions. The purification process for each reaction is depicted in the table-4.2.

6-Bromo-Pyrazolo-[1,5-a]-Pyrimidine-3-Ethyl-Carboxylate (by KOH base): Yellow solid, m. p. 278°C, (% yield 87.00) IR (KBr): ν= 3018cm-1(CH), 2976cm-1, 2918cm-1 & 2864cm-1 (-CH stretch), 1765cm-1 =O, ester), 683cm-1 (C-Br); HNMR (CDCl3, 300 MHz): δ= 1.410, 1.434 & 1.458 (m, 3H, CH3), 4.423-4.494 (m, 2H, CH2), 8.564 (s, 1H, C-C=H), 8.778, 8.786 (d, 2H, C=C=H), 8.917, 8.925 (d, 1H, C=C=H); LC-MS: m/z=273.0.

Synthesis

Fig 3

Fig 4
4. Results & Discussion

We reported the schemes planned under green chemistry by using microwave energy. The reaction was achieved at shorter reaction time is one of the good merit of our review. The yield of the product (3) was different for different nano-Catalysts; Also, the reaction time was varied as in the table-4.1. 5-amino-1H-pyrazole-4-Ethyl-carboxylate (3) was synthesized by one-pot step process by slow addition of Hydrazine hydrate to Ethyl-cyanoacetate using nano-catalysts under micro-oven solvent free conditions at 80-100°C. The obtained product was monitored by thin layer chromatography & iodine chamber. It was found that the yield of the product could improvise by using more oxygen coordinated metal Nano-catalysts. So that we came to know that more oxygen coordinated Nano-catalysts possess more active sites & results the higher yield. The compound (3) was treated with 2-Bromo-malonialdehyde in presence of different bases in Conc. hydrochloride under microwave heating to afford the compound (5). Moreover, the method for the synthesis of (5) under microwave conditions follows green chemistry. The product was monitored by thin layer chromatography as well as by Iodine chamber & confirmed by FT-IR, H¹ NMR & LC-MS. This experiment was revealed that the rate of the reaction depends on the strength of the bases, data of which, is shown in the table-4.2. The interesting experimental aspect was that the % yield of the compound (5) is specific for different kinds of bases, i.e., the Quantity of the (5) obtained, was increased proportional to the strength of the bases. The compound (3) was confirmed by 3339.72cm⁻¹ (N-H stretch), 3230.43cm⁻¹ (NH₂ stretch) & 1717cm⁻¹ (C=O ester). Likewise, the Compound (5) was confirmed by 1765cm⁻¹ (C=O, ester), 683cm⁻¹ (C-Br).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Reaction Condition</th>
<th>Nano-catalyst</th>
<th>Product</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl Cyanoacetate</td>
<td>MW, 2 Min</td>
<td>ZnO</td>
<td>(3)</td>
<td>84.00</td>
</tr>
<tr>
<td>Ethyl Cyanoacetate</td>
<td>MW, 1 Min</td>
<td>MnO₂</td>
<td>(3)</td>
<td>89.00</td>
</tr>
<tr>
<td>Ethyl Cyanoacetate</td>
<td>MW, 40 Sec</td>
<td>Fe₂O₃</td>
<td>(3)</td>
<td>92.00</td>
</tr>
<tr>
<td>Ethyl Cyanoacetate</td>
<td>MW, 30 Sec</td>
<td>Fe₃O₄</td>
<td>(3)</td>
<td>94.00</td>
</tr>
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</table>

Table 4.2: Reactions brought by using different bases

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Reaction Condition</th>
<th>Bases</th>
<th>Time</th>
<th>Product</th>
<th>Purification</th>
<th>M.P &amp; color (°C)</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>(3)</td>
<td>MW</td>
<td>KOH</td>
<td>2 min</td>
<td>(5)</td>
<td>Washed by Ethanol &amp; filtered</td>
<td>278.1</td>
<td>Yellow</td>
</tr>
<tr>
<td>(3)</td>
<td>MW</td>
<td>EtOH</td>
<td>2.5 min</td>
<td>(5)</td>
<td>Washed by Hot Water &amp; Filtered</td>
<td>234.0</td>
<td>Yellow</td>
</tr>
<tr>
<td>(3)</td>
<td>MW</td>
<td>H₂O</td>
<td>4 min</td>
<td>(5)</td>
<td>Washed by Ethanol &amp; filtered</td>
<td>182-185</td>
<td>Yellow</td>
</tr>
<tr>
<td>(3)</td>
<td>MW</td>
<td>Pyridine</td>
<td>6 min</td>
<td>(5)</td>
<td>Extracted by Petroleum Ether &amp; Washed by ethanol &amp; Filtered</td>
<td>166-168</td>
<td>Brown</td>
</tr>
</tbody>
</table>

5. Analytical data

![Fig 5.1: FT-IR data for 5-ammino-1H-pyrazole-4-ethyl-carboxylate.](image-url)
Fig 5.2: $^1$HNMR data for 5-ammino-1H-pyrazole-4-ethyl-carboxylate.

Fig 5.3: FT-IR for 6-Bromo-Pyrazolo-[1, 5-a]-Pyrimidine-3-Ethyl-Carboxylate
Fig-5.4: HNMR data for 6-Bromo-Pyrazolo-[1, 5-a]-Pyrimidine-3-Ethyl-Carboxylate

Fig-5.5: LC-MS for 6-Bromo-Pyrazolo-[1,5-a]-Pyrimidine-3-Ethyl-Carboxylate
6. Biological activity

Anticancer activity:

6-Bromo-Pyrazolo-[1, 5-a]-Pyrimidine-3-Ethyl-Carboxylate (5) as Cyclin-dependent Kinases:

Cyclin-dependent Kinases are responsible to regulate the cell cycle (CDK4 & CDK6). CDK2 is regulatory key for DNA replication & mitosis of cells is controlled by CDK1 [24]. Other CDKs lead to the abnormalities in the mRNA transcription, motility, apoptosis & spermatogenesis. we have recently CDK5 as novel alternative and pharmacologically accessible target in the context of angiogenesis. The compound inhibited cell proliferation, cell migration & chemotaxis [25, 26]. The synthesized compound (5) showed good inhibitory activity against CDKs & proved to be an anticancer drug. This compound exhibited more potency at the N3 position attached, as given in the table- 6.1.

Table 6.1: Comparison of Inhibition activity (IC50) of Compound (5) with Roscovitine drug

<table>
<thead>
<tr>
<th>Concentration (μM)2</th>
<th>Compound (5) IC50 values</th>
<th>Roscovitine (μM)</th>
<th>Kinase</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>1.4</td>
<td>0.01</td>
<td>0.003</td>
</tr>
<tr>
<td>0.05</td>
<td>0.07</td>
<td>0.001</td>
<td>0.003</td>
</tr>
<tr>
<td>0.5</td>
<td>19.5</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>0.5</td>
<td>0.26</td>
<td>0.001</td>
<td>0.003</td>
</tr>
<tr>
<td>1</td>
<td>15.5</td>
<td>1</td>
<td>20.5</td>
</tr>
</tbody>
</table>

The mean IC50 values (μM) for Roscovitine and (5), obtained from this experiment are shown, together with the standard deviation (SD) from the mean.

7. Conclusion

This review reveals that, the importance of Pyrazolo-Pyrimidines, in therapy, herewith we are presumed to synthesize the same using simple & inexpensive starting materials by microwave assisted synthesis. Green chemistry has been done using Bases & Nano-catalysts is well. The use of different Nano-catalysts & Bases proves that the rate of reaction depends upon them & influenced quantitatively. The compound (5) exhibited the anticancer activity as competent for the reference drug Roscovitine. CDK4 inhibition was approached to the higher level for the Concentration of 1μM, likewise- CDK1, CDK2, CDK5 & CDK6 activation were controlled according to the concentrations of the Compound (5). So that the synthesized compound was a better drug for the treatment of Cancer.

8. Acknowledgements

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9. References

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