SHORT COMMUNICATION

Synthesis and characterization of novel 2-oxo-4-((4-(3-oxomorpholino)phenyl)amino)-2H-chromene-3-carbonitrile derivatives

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ABSTRACT

A highly functionalized heterocyclic library were synthesized, and characterized by common spectroscopic methods. This novel synthetic rout involves nucleophilic substitution reaction of 3-cyano-4chloro coumarin with 4-(4-aminophenyl)morpholin-3-one in the presence of base and methanol as a solvent in good yield and high purity. Key starting material 4-(4-aminophenyl)morpholin-3-one synthesized from aniline. The reaction of aniline with ethylene oxide gives INT-1 which on reaction with Chloroaacetyl chloride yield INT-b. Nitration of INT-b gives INT-c, which on reduction with Sn/HCl gives int-D. Further INT-3 was synthesized from 4-hydroxy coumarin. The Vilsmeier-haack reaction of 4-hydroxy coumarin gives INT-01 which on reaction with hydroxylamine hydrochloride and sodium acetate furnished INT-03. All the synthesized compound of libraries characterized using ¹H NMR, Mass, and IR spectroscopic technique.

Keywords: 4-(4-aminophenyl)morpholin-3-one, 3-cyano-4chlor coumarin, antimicrobial activity
1. INTRODUCTION

In recent decades, chromenone and its derivatives have attracted considerable attention from medicinal and synthetic organic chemists because of a wide range of biological activities displayed by this class of compounds which is described below. The corresponding 2-substituted-3-Cyanochromenones are molecules of current interest as they have potent biological activity. It is well recognized that incorporation of nitro group into the chromenone skeleton have significant biological activity\textsuperscript{1,2}. Vasselin A. D. et al.\textsuperscript{3} have synthesized a new series of fluoro, methoxy and amino substituted isoflavones and demonstrated as potent antitumor agents. The substituted isoflavones were synthesized using palladium catalyzed coupling methodologies to construct the central aryl carbon-carbon single bond. The new isoflavone derivatives were tested for \textit{in vitro} activity in human breast (MDA-MB-468 and MCF-7) and colon (HT29 and HCT-116) cancer cell lines. Low micromolar GI\textsubscript{50} values were obtained in a number of cases, with the MDA-MB-468 cell line being the most sensitive overall. This study is suggesting that isoflavone derivatives can act as substrates for CYP1A1 bioactivation. Chen S. F. et al.\textsuperscript{4} have developed a series of nitrocoumarin and nitrochromene derivatives and shown to inhibit the phosphatidylinositol-specific phospholipase C(PLC) (ICW C10 pg/mL) isolated from human melanoma.

The inhibition of PLC by nitrocoumarin was time-dependent and irreversible. The inhibition of PLC was shown to interfere with inositolide metabolism in whole cells in a manner consistent with their proposed mode of activity. Dauzonne D. et al\textsuperscript{5} have synthesized some novel flavone-8-acetic acid derivatives and evaluated for reversible inhibitors of aminopeptidaseN(APN/CD13) activity. The cell surface APN/CD13, overexpressed in tumor cells, plays a critical role in angiogenesis. In this context, they have tested a series of novel flavone-8-acetic acid derivatives and found that the 2', 3-dinitroflavone-8-acetic acid proved to be the most efficient and exhibited an IC\textsubscript{50} of 25 µM which is 2.5 times higher than that of bestatin, the natural known inhibitor of APN/CD13. The presence of other substituents such as OMe groups at the 3 or 4 position of the A phenyl group, or the existence of steric constraints, did not improve selectivity and potency. The results were indicated that derivatives, which bear a CH\textsubscript{2}COOH group in the 8-position and two NO\textsubscript{2} substituents in both 2' and 3 positions, inhibited efficiently APN activity and this to the same extent as bestatin. Deletion or replacement of the NO\textsubscript{2} group in the 2'-position gave compounds with a lesser degree of potency against APN activity whereas the presence of an electron-donating methoxy group in the ortho or para position of the nitro substituent led to slightly lowered inhibitory effects.

The main significance of the work is it will provide synthesized and more potent stable molecule for biological response as most of coumarin derivatives has significant biological activity. As we mentioned above, the significance and biological profile of this class of molecule so our continue efforts towards the synthesis of potential heterocyclic molecules

2. EXPERIMENTAL

All chemicals and solvents used to synthesised library were purchased from CDH chemical, Delhi of AR grade and were used without further purification. Melting points were taken in open capillary method and are uncorrected. IR spectra were recorded on FTIR-8400 spectrophotometer (Shimadzu, Kyoto, Japan), using DRS probe KBr pallet. $^1$H-NMR spectra
of the synthesized compounds were recorded on a Bruker-Avance-II (400 MHz) DMSO-d6 solvent. Chemical shifts are expressed in δ ppm downfield from TMS as an internal standard. Mass spectra were determined using direct inlet probe on a GCMS-QP 2010 mass spectrometer (Shimadzu, Kyoto, Japan). Physical constants of the synthesized compounds are shown in Table.

**Synthesis of INT-a**

In a Round bottom flask, aniline (0.01 mol) and solvent DMF was taken. To this resulting solution ethylene oxide gas passed under pressure. Reaction was continuously monitored on Thin Layer Chromatography. At the end, reaction mixture was cooled and quince in ice water to get int-a

**Synthesis of INT-b**

Chloroacetyl chloride was added in drop wise manner in a previously cooled mixture of INT-a (0.01) mol, DMF and K₂CO₃ (0.02 mol). Maintain the temperature further at 0 °C. After completion of the addition rise the temperature at 60 °C and maintain further 4 hr. progress of the reaction was monitored on TLC. After completion of the reaction, reaction mass was carefully poured into the crushed ice and filter the separated product.

**Synthesis of INT-C**

In a 250 ml RBF H₂SO₄ (0.03 mol) was taken and cooled to 0 °C. To this HNO₃ (0.03 mol) was added in a drop wise manner. To this nitrating mixture INT-b (0.01 mol) was added portion wise. After completion of the addition, reaction mixture was stir at 60 °C for 3 hr. Reaction mixture was cooled and Quince in ice water after the confirm on TLC that reaction was completed.

**Synthesis of INT-D**

In a 250 ml RBF Hydrochloric acid (3V) was taken and cools to 5 °C. To this Tin (Sn) metal was added. In this reaction mixture INT-b (0.01) was added and heat the reaction mixture at 70 °C for 5hr. Reaction mixture was cooled and Quince in ice water after the completion of the reaction. Neutralize the mixture with Sodium hydroxide solution until neutral pH. Extract the reaction mass with ethyl acetate and evaporate Ethyl Acetate to give INT-D

**Synthesis of 4-hydroxy coumarin (int-1)**

Take malanonitrile (0.1 mol) and Substituted phenols (0.1 mol) in a 250 ml RMF. To this add phosphorus oxychloride (40 ml) and previously dried anhydrous zinc chloride (30 gms). After the addition rise the temperature at 70 °C and maintain this temperature for 8-10 hr in a water bath. Reaction mixture was cooled and quince in crushed ice to afford INT-1 as a solid. Filter the solid and wash with water until free from acid. The separated solid product treated with 10% sodium bicarbonate solution and filter again to remove undisclosed residue. Finally the filtrate was cooled and acidifies with dilute hydrochloric acid. Cool and settle down the particle and Filter again to get pure INT-1
Synthesis of 4-chloro-3-formyl coumarin (int-2)

To a stirred mixture of 4-hydroxycoumarin (0.06 mol) in anhydrous DMF (0.6 mol) were added drop wise POCl₃ (0.18 mol) at −10 °C to −5 °C. The reaction mixture was then stirred for 1h at room temperature and further heated at 60 °C for 2 h. After the reaction completed, the mixture was poured onto crushed ice under vigorous stirring. Further allow to stir the mixture overnight at 0 °C. The pale yellow solid was collected by filtration and washed successively with Na₂CO₃ (5%) and water, and then was air–dried. Recrystallization from acetone gave 85% of 4-chloro-3-formyl coumarin as a pale yellow powder with m.p. 115–120 °C.

Synthesis of 4-chloro-3-cyano coumarin (int-3)

To a 20 mL solution of 4-chloro-3-formylcoumarin (0.01 mol) in glacial acetic acid was added sodium acetate (0.01 mol) and hydroxyl amine hydrochloride (0.01 mol) and the resultant solution was stir at 60 °C for 2 hr. completion of the reaction was checked by TLC. After completion of the reaction, Reaction mass was poured on to the cooled water and the solid was filtered and washed with water (25 ml). Crystallization of compound carried out from DMF:IPA (80:20) under 0-5 °C to Yields 45% of 4-chloro-3-cyano coumarin as a light green crystals m.p. 198–200 °C.

General synthesis for 2-oxo-4-((4-(3-oxomorpholino)phenyl)amino)-2H-chromene-3-carbonitrile derivatives

To a 100 ml flask K₂CO₃ (0.1 mol) was added to DMF and cool the resulting solution and stir well. To this cooled solution of 4-(4-aminophenyl)morpholin-3-one(0.1 mol) was added portion wise so that temperature do not rise above 25 °C. After addition is over, allow the reaction mass to stir further at Room temperature. The progress of reaction was monitored on Thin layer chromatography using Ethyl acetate and Hexane (8:20) solvent system. The reaction mass was Quenched on crushed ice after completion of reaction and filter the separated product.

3. REACTION SCHEME

Table 1. Physical constant of synthesized library.

<table>
<thead>
<tr>
<th>Code</th>
<th>Molecular formula</th>
<th>Substitution</th>
<th>Molecular Weight</th>
<th>M.P. °C</th>
<th>Percentage of Yield</th>
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<tbody>
<tr>
<td>V-2a</td>
<td>C₂₀H₁₅N₃O₄</td>
<td>H</td>
<td>361</td>
<td>162-164</td>
<td>80</td>
</tr>
<tr>
<td>V-2b</td>
<td>C₂₁H₁₇N₃O₄</td>
<td>2-Methyl</td>
<td>375</td>
<td>178-180</td>
<td>82</td>
</tr>
<tr>
<td>V-2c</td>
<td>C₂₁H₁₇N₃O₄</td>
<td>3-Methyl</td>
<td>375</td>
<td>154-156</td>
<td>78</td>
</tr>
<tr>
<td>V-2d</td>
<td>C_{21}H_{17}N_{3}O_{4}</td>
<td>4-Methyl</td>
<td>375</td>
<td>158-160</td>
<td>79</td>
</tr>
<tr>
<td>V-2e</td>
<td>C_{20}H_{14}N_{4}O_{6}</td>
<td>4-Nitro</td>
<td>406</td>
<td>176-178</td>
<td>72</td>
</tr>
<tr>
<td>V-2f</td>
<td>C_{20}H_{14}N_{4}O_{6}</td>
<td>2-Nitro</td>
<td>406</td>
<td>181-182</td>
<td>74</td>
</tr>
<tr>
<td>V-2g</td>
<td>C_{20}H_{14}ClN_{3}O_{4}</td>
<td>2-Chloro</td>
<td>395</td>
<td>148-150</td>
<td>70</td>
</tr>
<tr>
<td>V-2h</td>
<td>C_{20}H_{14}ClN_{3}O_{4}</td>
<td>4-Chloro</td>
<td>395</td>
<td>142-144</td>
<td>81</td>
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<tr>
<td>V-2i</td>
<td>C_{20}H_{14}BrN_{3}O_{4}</td>
<td>2-Bromo</td>
<td>440</td>
<td>182-184</td>
<td>80</td>
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<tr>
<td>V-2j</td>
<td>C_{20}H_{14}BrN_{3}O_{4}</td>
<td>4-Bromo</td>
<td>440</td>
<td>186-188</td>
<td>84</td>
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<tr>
<td>V-2k</td>
<td>C_{22}H_{19}N_{5}O_{4}</td>
<td>2,3-Dimethyl</td>
<td>389</td>
<td>172-174</td>
<td>78</td>
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<tr>
<td>V-2l</td>
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<td>2,5 Dimethyl</td>
<td>389</td>
<td>178-180</td>
<td>75</td>
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</tbody>
</table>

**Figure 1.** Reaction scheme.
3. 1. Structure of synthesized library

4. SPECTRAL DATA OF SYNTHESIZED COMPOUND

2-oxo-4-((4-(3-oxomorpholino)phenyl)amino)-2H-chromene-3-carbonitrile (V-2a)

Yellow solid, Rf Value 0.41 (Ethyl acetate:Hexane 8:2), M.P-162-164 °C, IR (KBR pallet) in CM, 3896, 3832, 3751, 3724, 3676, 3607, 3561, 3389, 3210, 2334, 1630, 1504, 1417, 1336, 1293, 1236, 1123, 1058, 1001, 960,850, 749, 650. ¹H NMR (CDCl₃) in δ PPM: 3.778 to 3.803 (2H, triplet, -CH₂-), 4.040 to 4.085 (2H, triplet, -CH₂-), 4.356 (2H, singlet, -CH₂-), 6.920 to 6.980 (1H, Multiplet, -ArH), 7.110 to 7.150 (1H, Multiplet, -ArH), 7.410 to 7.480 (1H, Multiplet, -ArH), 7.260 to 7.350 (4H, Multiplet, -ArH), 10.827 (1H, singlet, -NH-). MS (m/z): 361 (M⁺), Ana.calculated for Molecular formula C₂₀H₁₅N₃O₄is C: 66.48%, H: 4.18%, N: 11.63% found C: 67.45%, H: 4.15%, N: 11.60%.
8-methyl-2-oxo-4-((4-(3-oxomorpholino)phenyl)amino)-2H-chromene-3-carbonitrile

Yellow solid, Rf Value 0.40 (Ethyl Acetate:Hexane 8:2), M.P-178-180 °C, IR (KBR pallet) in CM, 3864, 3779, 3688, 2334, 1744, 1617, 1513, 1421, 1058, 829, 765, 651. \(^1\)H NMR (400 HZ, CDCl\(_3\)) in \(\delta\)PPM: 2.418 (3H, Singlet, -CH\(_3\)), 3.886 to 3.911 (2H, triplet, -CH\(_2\)-), 4.098 to 4.123 (2H, triplet, -CH\(_2\)-), 4.414 (2H, singlet, -CH\(_2\)-), 6.90 to 6.94 (1H, Multiplet, -ArH), 7.140 to 7.190 (1H, Multiplet, -ArH), 7.240 to 7.280 (1H, Multiplet, -ArH), 7.60 to 7.780 (4H, Multiplet, -ArH), 8.340 (1H, singlet, -NH-). MS (m/z): 375 (M\(^+\)), Ana.calculated for Molecular formula C\(_{21}\)H\(_{17}\)N\(_3\)O\(_4\) is C; 67.19%, H; 4.56%, N; 11.19% found C; 67.15%, H; 4.50%, N; 11.15%.

5. CONCLUSION

We have prepared a library of novel 4-(4-aminophenyl) morpholin-3-one containing different coumarin derivatives by chloramine coupling reaction using inorganic base and DMF as solvent at a low temperature which results in 2-oxo-4-((4-(3-oxomorpholino)phenyl)amino)-2H-chromene-3-carbonitrile derivatives.

The formation of 2-oxo-4-((4-(3-oxomorpholino)phenyl) amino)-2H-chromene-3-carbonitrile by this method was first developed by us. All synthesized compounds were obtained in good to moderate yield. All synthesized compounds were characterized by IR, NMR and Mass spectrometry.

References