CHAPTER – 2

Section-B

Aqua mediated and microwave assisted synthesis of Ethyl-2-Amino-7-hydroxy-4-(substituted phenyl)-4H-chromene-3-carboxylates
2.9 AIM OF THE CURRENT WORK DONE

The importance of the 2-Amino chromene as a significant chemical entity has already been aptly justified in the previous section of this chapter. Looking at the type of the reaction some new chemical entities could easily be generated by changing any of the reactants. Hence, it was decided to change the active methylene compound for this section. In Section A, Malanonitrile was used which afforded 2-Amino-3-cyano derivative. In current work cyano ethyl acetate, another active methylene compound, generated 2-Amino-3-carboxylate derivative. The literature survey revealed that these kinds of novelty compounds have hardly been reported and its method of synthesis is not very well cited.

Moreover, the literature survey revealed that the Structure activity relationship studies of ethyl 2-Amino-6-cyclopentyl-4-(1-cyano-2-ethoxy-2-oxo)-4H-chromene-3-carboxylate (HA 14-1; Fig. 1), an antagonist of the antiapoptotic Bcl-2 proteins, are reported. Bcl-2 and related proteins are key regulators of apoptosis or programmed cell death implicated in human disease including cancer. The cell-permeable Bcl-2 binding peptides could induce apoptosis of human myeloid leukemia in vitro and suppress its growth in severe combined immuno deficient mice. In vitro binding studies demonstrated the interaction of HA14-1 with this Bcl-2 surface pocket that is essential for Bcl-2 biological function. HA14-1 effectively induced apoptosis of human acute myeloid leukemia (HL-60) cells over expressing Bcl-2 protein that was associated with the decrease in mitochondrial membrane potential and activation of caspase-9 followed by caspase-3. Cytokine response modifier A, a potent inhibitor of Fas-mediated apoptosis, did not block apoptosis induced by HA14-1. Bcl-2 belongs to a growing family of proteins that regulate apoptosis or programmed cell death. The Bcl-2 family includes both death antagonists such as Bcl-2 and Bcl-xL and death agonists such as Bax, Bak, Bid, and Bad.

Fig. 1

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A series of analogues of Fig-1 with varied functional groups at the 6-position of the chromene ring were synthesized. These candidates were evaluated for their binding interactions with three antiapoptotic proteins: Bcl-2, Bcl-XL, and Bcl-w. They were also assayed for their in vitro cytotoxicities against a set of Jurkat cells with varied levels of Bcl-2 and Bcl-XL proteins and a non-small-cell lung carcinoma cell line (NCI-H460). It was found that the 6-bromo of Fig. 1 was not essential for its bioactivity and the 6-position can accommodate a variety of alkyl groups. Fig. 1 and its analogues bind to all of the three antiapoptotic Bcl-2 proteins tested. Positive correlations were observed between the binding affinities of these candidates to the antiapoptotic Bcl-2 proteins and their in vitro cytotoxicities, suggesting that the antiapoptotic Bcl-2 proteins are likely to be the cellular targets of Fig. 1 and its analogues. In this study, the binding interactions of the small molecules to antiapoptotic Bcl-2 proteins were studied by assaying their abilities to compete against a Bak peptide binding to the antiapoptotic Bcl-2 proteins. Inhibitory constants, instead of dissociation constants, were obtained in such assays. The most active compound had a >3-fold increase of binding affinity to the antiapoptotic Bcl-2 proteins and a >13-fold increase of in vitro cytotoxicity over Fig. 1. Though Jurkat cells with transgenic over expression of Bcl-2 or Bcl-XL protein can develop resistance to standard cancer therapies, such cells failed to develop resistance to Fig. 1.
based candidates. Fig. 1 also sensitizes Jurkat cells to cisplatin. These studies provide further support that Fig. 1 and its analogues function as antagonists for antiapoptotic Bcl-2 proteins and that they have the potential, either as a single agent or as a combination therapy with other anticancer agents, to treat cancers with the over expression of antiapoptotic Bcl-2 proteins.

The compounds synthesized in this section are structurally very similar to the compound shown in fig. 1. Moreover, the SAR study in the paper suggested that the substitution on position no. 6 is not an important aspect for the biological activity of this type of compounds.

Several researchers world wide have explored the chemistry as well as biology 2-Amino-4H-chromene derivatives in recent years and some fairly good reviews as well as publications are cited in the references 3-43.

The detailed literature survey on this class of compounds with 2-Amino-4-substitutedphenyl-3-carboxylate derivatives have not been explored and hence synthetic work on this chemical entity was initiated and evaluated for its biological activity. The route of synthesis is an environmentally friendly green chemistry approach, wherein the reaction is carried out using water as solvent and potassium carbonate as the base catalyst to prepare these compounds under microwave irradiations by which the reaction can be completed in few minutes.

Thus, the opportunity to synthesize some new chemical entities as well as to explore their biological activity was the main rational behind initializing the work included in this chapter.
2.10 REACTION SCHEME

Reagents & Conditions: a.) K$_2$CO$_3$, H$_2$O, MWI-320 watts, Open Vessel, 2 to 4 mins.

Aqua Mediated Synthesis of Ethyl-(2-Amino-7-hydroxy-4-substituted phenyl-4H-chromene-)3-carboxylate

2.10.1 PHYSICAL DATA TABLE

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<tr>
<th>Code</th>
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<th>M. F.</th>
<th>M. W.</th>
<th>M. P. °C</th>
<th>Time (min)</th>
<th>Yield %</th>
<th>$R_f$</th>
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<td>86</td>
<td>0.56</td>
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</tbody>
</table>

TLC solvent system for $R_f$ = Toluene:Ethyl acetate - 7:3. Microwave Irradiation: 320 Watts
2.11 PLAUSIBLE REACTION MECHANISM

2.11.1 Formation of Ethyl-(2-cyano-3-phenyl) acrylate Intermediate

As discussed in Section A the reaction mechanism proceeds first via the Knoevenagel Condensation route where in a nucleophilic addition of an active hydrogen compound takes place onto the carbonyl group which when followed by the dehydration reaction and subsequent elimination of water molecule (hence condensation) would afford us the condensate which is often an \( \alpha,\beta \)-conjugated enone. As shown above, first the base molecule, here potassium carbonate would first attack on the active hydrogen compound (Ethyl cyano acetate) by accepting a proton and thus forming a carbanion, shown herein as the resonating conjugate (2). This carbanion would then attack on the partially positively charged carbon of the carbonyl moiety, forming an unstable intermediate compound with negatively charged oxygen. This would then accept the hydrogen bonded to the \( \text{K}_2\text{CO}_3 \) and thus the base would then proceed through the anti elimination by cleaving a proton and thus a water molecule to afford the \( \alpha,\beta \)-unsaturated enone herein a Ethyl-(2-Cyano-3-phenyl) acrylate.
2.11.2 Formation of Ethyl-(2-amino-7-hydroxy-4-substitutedphenyl-4H-chromene)-3-carboxylate From Ethyl-(2-cyano-3-phenyl)-acrylate

The base would cleave the acidic proton from resorcinol thus forming an carbanion, this carbanion would then attack on Ethyl-(2-Cyano-3-phenyl)-acrylate to provide us with resonating conjugates. The negative charge on the Nitrogen would abstract the hydrogen out of base and satisfy its valency. The lone pair of that nitrogen would then accept a proton from water molecule and would become an ammonium ion. This is a very unstable moiety and hence the charge displaces on carbon forming a stabilized carbocation. The carbocation is quenched by the elimination of the water molecule by cleaving one hydrogen from the hydroxyl group forming a bond with the carbon containing the positive charge and thus giving Ethyl-(2-amino-7-hydroxy-4-substituted phenyl-4H-chromene)-3-carboxylate from Ethyl-(2-Cyano-3-phenyl)-acrylate.
2.12 EXPERIMENTAL

2.12.1 MATERIALS AND METHODS

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 and UV. All the reactions were carried out in Samsung MW83Y Microwave Oven which was locally modified for carrying out chemical reactions. IR spectra were recorded in Shimadzu FT-IR-8400 instrument using 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.

2.12.2 General Procedure: Ethyl-(2-amino-7-hydroxy-4 substituted phenyl-4H-chromene)-3-carboxylates

Equimolar amounts of neat reactants, substituted benzaldehydes, Ethyl cyanoacetate, and resorcinol were taken in an Erlenmeyer flask, and 10 ml saturated solution of K\(_2\)CO\(_3\) in demineralized water was added to it. The reaction mixture was subjected to MWI (Micro Wave Irradiations) for a specific time (see Physical data Table) at low power (320 W). The progress of the reaction was monitored by TLC examination at an interval of every 30 seconds. On completion of reaction, the reaction mixture was cooled and was triturated with 2–3 ml of ice cold water to get the solid product, leaving behind K\(_2\)CO\(_3\) dissolved in water. The product obtained was filtered, washed with cold water, dried, and recrystallized from ethanol.
2.13 ANALYTICAL DATA

2.13.1 Ethyl 2-amino-7-hydroxy-4-phenyl-4H-chromene-3-carboxylate (BN-1)

Yield: 90%; M.P.- 180-182 °C; IR (cm⁻¹): 3620 (O-H stretching of free primary alcohol), 3524-3489, (N-H stretching of free primary amine), 3146 (C-H stretching vibration of aromatic region), 1905-1066 (C-O stretching frequency of esters), 1752 (C=O stretching frequency for esters), 1604 (N-H deformation, in plane bending of N-H), 1558 (O-H in plane bending), 1361 (C-N stretching for carbon bonded to amino group), 952 (C-H in plane bending of phenyl ring), 696 (C-H out of plane bending for phenyl nucleus),

¹H NMR (DMSO-d₆) δ ppm: 6.46 (s, 2H, H₁), 4.79 (s, 1H, H₂), 7.14-7.18 (m, 6H, H₃, H₄, H₅, H₆, H₇, H₈, J=3.2 Hz), 7.05-7.06 (s, 1H, H₁₀, J=2.64 Hz), 6.86-6.83 (d, 1H, H₉, J=8.76 Hz), 9.25 (s, 1H, H₁₁), 3.95-4.00 (q, 2H, H₁₂), 1.08-1.12 (t, 3H, H₁₃), MS: m/z: 311.12; Anal. Calcd. for C₁₈H₁₇NO₄: C, 69.44; H, 5.50; N, 4.50; O, 20.56 Found: C, 69.39; H, 5.41; N, 4.40; O, 20.49.

2.13.2 Ethyl-2-amino-7-hydroxy-4-(4-methoxyphenyl)-4H-chromene-3-carboxylate (BN-2)

Yield: 85%; M.P.- 166-168 °C; IR (cm⁻¹): 3614 (O-H stretching of free primary alcohol), 3551-3406 (N-H stretching of free primary amine), 3186 (C-H stretching vibration of aromatic region), 1750 (C=O stretching frequency of esters), 1606 (N-H deformation, in plane bending of N-H), 1514 (O-H in plane bending), 1301 (C-O stretching frequency for aromatic ether group), 1089 (C-H in plane bending of phenyl ring), 692 (C-H out of plane bending for phenyl nucleus),

¹H NMR (DMSO-d₆) δ ppm: 6.45 (s, 2H, H₁), 4.74 (s, 1H, H₂), 7.14 (s, 2H, H₃ & H₆), 7.04 (d, 2H, H₄ & H₅), 6.68-6.70 (d, 2H, H₇ & H₈, J₇₈=8 Hz, J₆₇=8 Hz), 9.22 (s, 1H, H₉), 6.28-6.83 (d, 1H, H₁₀), 3.95-4.00 (q, 2H, H₁₂), 1.08-1.12 (t, 3H, H₁₃), MS: m/z: 341.13; Anal. Calcd. for C₁₉H₁₉NO₅: C, 66.85; H, 5.61; N, 4.10; O, 23.43 Found: C, 66.79; H, 5.56; N, 4.01; O, 23.36.
2.13.3 Ethyl 2-amino-4-(3-bromophenyl)-7-hydroxy-4H-chromene-3-carboxylate (BN-3)

Yield: 88%; M.P. 174-176 °C; IR (cm⁻¹): 3610 (O-H stretching of free primary alcohol), 3520-3410, (N-H stretching of free primary amine), 3012 (C-H stretching vibration of aromatic region), 1266 (C-O stretching frequency of esters), 1725 (C=O stretching frequency for esters), 1610 (N-H deformation, in plane bending of N-H), 1553 (O-H in plane bending), 1341 (C-N stretching for carbon bonded to amino group), 1005 (C-H in plane bending of phenyl ring), 650 (C-H out of plane bending for phenyl nucleus), MS: m/z: 389.03; Anal. Calcd. for C₁₈H₁₆BrNO₄: C, 55.40; H, 4.13; Br, 20.48; N, 3.59; O, 16.40 Found: C, 55.29; H, 4.04; Br, 20.39 N, 3.50; O, 16.32.

2.13.4 Ethyl 2-amino-4-(3-chlorophenyl)-7-hydroxy-4H-chromene-3-carboxylate (BN-4)

Yield: 80%; M.P. 120-122 °C; IR (cm⁻¹): 3632 (O-H stretching of free primary alcohol), 3504-3469, (N-H stretching of free primary amine), 3123 (C-H stretching vibration of aromatic region), 1254 (C-O stretching frequency of esters), 1720 (C=O stretching frequency for esters), 1615 (N-H deformation, in plane bending of N-H), 1523 (O-H in plane bending), 1345 (C-N stretching for carbon bonded to amino group), 1010 (C-H in plane bending of phenyl ring), 697 (C-H out of plane bending for phenyl nucleus), MS: m/z: 345.08; Anal. Calcd. for C₁₈H₁₆ClNO₄: C, 62.52; H, 4.66; Cl, 10.25; N, 4.05; O, 18.51 Found: C, 62.46; H, 4.52; Cl, 10.17 N, 4.00; O, 18.49.
2.13.5 Ethyl 2-amino-7-hydroxy-4-(3-nitrophenyl)-4H-chromene-3-carboxylate (BN-5)

Yield: 92%; M.P.- 142-144 °C; IR (cm⁻¹): 3642 (O-H stretching of free primary alcohol), 3503-3473, (N-H stretching of free primary amine), 3121 (C-H stretching vibration of aromatic region), 1262 (C-O stretching frequency of esters), 1732 (C=O stretching frequency for esters), 1624 (N-H deformation, in plane bending of N-H), 1528 (O-H in plane bending), 1332 (C-N stretching for carbon bonded to amino group), 978 (C-H in plane bending of phenyl ring), 690 (C-H out of plane bending for phenyl nucleus), MS: m/z: 356.10; Anal. Calcd. for C₁₈H₁₆N₂O₆: C, 60.67; H, 4.53; N, 7.86; O, 26.94 Found: C, 60.63; H, 4.49; N, 7.83; O, 26.88.

2.13.6 Ethyl-2-amino-7-hydroxy-4-(3-methoxyphenyl)-4H-chromene-3-carboxylate (BN-6)

Yield: 87%; M.P.- 158-160 °C; IR (cm⁻¹): 3614 (O-H stretching of free primary alcohol), 3512-3475, (N-H stretching of free primary amine), 3143 (C-H stretching vibration of aromatic region), 1247 (C-O stretching frequency of esters), 1730 (C=O stretching frequency for esters), 1612 (N-H deformation, in plane bending of N-H), 1562 (O-H in plane bending), 1345 (C-N stretching for carbon bonded to amino group), 964 (C-H in plane bending of phenyl ring), 682 (C-H out of plane bending for phenyl nucleus), MS: m/z: 341.13; Anal. Calcd. for C₁₉H₁₉NO₅: C, 66.85; H, 5.61; N, 4.10; O, 23.43 Found: C, 66.81; H, 5.57; N, 4.05; O, 23.40.
2.13.7 Ethyl 2-amino-4-(3-ethoxy-4-hydroxyphenyl)-7-hydroxy-4H-chromene-3 carboxylate (BN-7)

Yield: 85%; M.P.- 126-128 ºC; IR (cm\(^{-1}\)): 3623 (O-H stretching of free primary alcohol), 3527-3464, (N-H stretching of free primary amine), 3139 (C-H stretching vibration of aromatic region), 1256 (C-O stretching frequency of esters), 1742 (C=O stretching frequency for esters), 1611 (N-H deformation, in plane bending of N-H), 1548 (O-H in plane bending), 1371 (C-N stretching for carbon bonded to amino group), 958 (C-H in plane bending of phenyl ring), 694 (C-H out of plane bending for phenyl nucleus), MS: m/z: 371.14; Anal. Calcd. for C\(_{20}\)H\(_{21}\)NO\(_6\): C, 64.68; H, 5.70; N, 3.77; O, 25.85 Found: C, 64.64; H, 5.66; N, 3.71; O, 25.78.

2.13.8 Ethyl 2-amino-4-(4-(dimethylamino)phenyl)-7-hydroxy-4H-chromene-3 carboxylate (BN-8)

Yield: 82%; M.P.- 132-134 ºC; IR (cm\(^{-1}\)): 3615 (O-H stretching of free primary alcohol), 3517-3472 (N-H stretching of free primary amine), 3162 (C-H stretching vibration of aromatic region), 1242 (C-O stretching frequency of esters), 1752 (C=O stretching frequency for esters), 1632 (N-H deformation, in plane bending of N-H), 1498 (O-H in plane bending), 1326 (C-N stretching frequency for aryl tertiary amine), 1351 (C-N stretching for carbon bonded to amino group), 1023 (C-H in plane bending of phenyl ring), 836 (C-H out of plane bending for phenyl nucleus), MS: m/z: 354.16; Anal. Calcd. for C\(_{20}\)H\(_{22}\)N\(_2\)O\(_4\): C, 67.78; H, 6.25; N, 7.90; O, 18.06, Found: C, 67.73; H, 6.21; N, 7.87; O, 18.02.
2.13.9 Ethyl 2-amino-7-hydroxy-4-(4-nitrophenyl)-4H-chromene-3-carboxylate (BN-9)

Yield: 90%; M.P.- 148-150 ºC; IR (cm\(^{-1}\)): 3632 (O-H stretching of free primary alcohol), 3517-3484, (N-H stretching of free primary amine), 3092 (C-H stretching vibration of aromatic region), 1278 (C-O stretching frequency of esters), 1757 (C=O stretching frequency for esters), 1623 (N-H deformation, in plane bending of N-H), 1498 (O-H in plane bending), 1353 (C-N stretching for carbon bonded to amino group), 977 (C-H in plane bending of phenyl ring), 716 (C-H out of plane bending for phenyl nucleus), MS: \textit{m/z}: 356.10; Anal. Calcd. for C\(_{18}\)H\(_{16}\)N\(_2\)O\(_6\): C, 60.67; H, 4.53; N, 7.86; O, 26.94 Found: C, 60.63; H, 4.47; N, 7.82; O, 26.89.

2.13.10 Ethyl-2-amino-4-(3,4-dimethoxyphenyl)-7-hydroxy-4H-chromene 3-carboxylate (BN-10)

Yield: 95%; M.P.- 174-176 ºC; IR (cm\(^{-1}\)): 3624 (O-H stretching of free primary alcohol), 3516-3491 (N-H stretching of free primary amine), 3138 (C-H stretching vibration of aromatic region), 1243 (C-O stretching frequency of esters), 1753 (C=O stretching frequency for esters), 1603 (N-H deformation, in plane bending of N-H), 1573 (O-H in plane bending), 1374 (C-N stretching for carbon bonded to amino group), 974 (C-H in plane bending of phenyl ring), 759 (C-H out of plane bending for phenyl nucleus), MS: \textit{m/z}: 371.14; Anal. Calcd. for C\(_{20}\)H\(_{21}\)NO\(_6\): C, 64.68; H, 5.70; N, 3.77; O, 25.85 Found: C, 64.63; H, 7.65; N, 3.72; O, 25.80.
Chapter 2: Aqua mediated and Microwave Assisted synthesis of Ethyl-2-amino-7-

2.13.11 Ethyl-2-amino-7-hydroxy-4-p-tolyl-4H-chromene-3-carboxylate (BN-11)

```
Yield: 85%; M.P.- 192-194 ºC; IR (cm⁻¹): 3612 (O-H stretching of free primary alcohol), 3533-3467, (N-H stretching of free primary amine), 3174 (C-H stretching vibration of aromatic region), 1270 (C-O stretching frequency of esters), 1744 (C=O stretching frequency for esters), 1585 (N-H deformation, in plane bending of N-H), 1542 (O-H in plane bending), 1373 (C-N stretching for carbon bonded to amino group), 964 (C-H in plane bending of phenyl ring), 746 (C-H out of plane bending for phenyl nucleus), MS: m/z: 325.13; Anal. Calcd. for C₁9H₁₉NO₄: C, 70.14; H, 5.89; N, 4.31; O, 19.67 Found: C, 70.10; H, 5.73; N, 4.25; O, 19.58.
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2.13.12 Ethyl-2-amino-7-hydroxy-4-(2-hydroxyphenyl)-4H-chromene-3-carboxylate (BN-12)

```
Yield: 80%; M.P.- 188-190 ºC; IR (cm⁻¹): 3617 (O-H stretching of free primary alcohol), 3517-3473, (N-H stretching of free primary amine), 3158 (C-H stretching vibration of aromatic region), 1277 (C-O stretching frequency of esters), 1752 (C=O stretching frequency for esters), 1574 (N-H deformation, in plane bending of N-H), 1568 (O-H in plane bending), 1338 (C-N stretching for carbon bonded to amino group), 1002 (C-H in plane bending of phenyl ring), 710 (C-H out of plane bending for phenyl nucleus), MS: m/z: 327.11; Anal. Calcd. for C₁₈H₁₇NO₅: C, 66.05; H, 5.23; N, 4.28; O, 24.44 Found: C, 65.98; H, 5.19; N, 4.21; O, 24.34.
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2.13.13 Ethyl-2-amino-4-(furan-2-yl)-7-hydroxy-4H-chromene-3-carboxylate (BN-13)

Yield: 86%; M.P.- 176-178 °C; IR (cm⁻¹): 3616 (O-H stretching of free primary alcohol), 3513-3477, (N-H stretching of free primary amine), 3137 (C-H stretching vibration of furan ring system), 1252 (C-O stretching frequency of esters), 1725 (C=O stretching frequency for esters), 1597 (N-H deformation, in plane bending of N-H), 1498 (O-H in plane bending), 1357 (C-N stretching for carbon bonded to amino group), 973 (C-H in plane bending of phenyl ring), 796 (C-H out of plane bending for phenyl nucleus), MS: m/z: 301.10; Anal. Calcd. for C₁₆H₁₅NO₅: C, 63.78; H, 5.02; N, 4.65; O, 26.55 Found: C, 63.69; H, 4.94; N, 4.52; O, 26.48.

2.13.14 Ethyl-2-amino-4-(2-chlorophenyl)-7-hydroxy-4H-chromene-3-carboxylate (BN-14)

Yield: 90%; M.P.- 152-154 °C; IR (cm⁻¹): 3622 (O-H stretching of free primary alcohol), 3534-3486, (N-H stretching of free primary amine), 3145 (C-H stretching vibration of aromatic region), 1246 (C-O stretching frequency of esters), 1723 (C=O stretching frequency for esters), 1584 (N-H deformation, in plane bending of N-H), 1548 (O-H in plane bending), 1371 (C-N stretching for carbon bonded to amino group), 1052 (C-H in plane bending of phenyl ring), 754 (C-H out of plane bending for phenyl nucleus), MS: m/z: 345.08; Anal. Calcd. for C₁₈H₁₆ClNO₄: C, 62.52; H, 4.66; Cl, 10.25; N, 4.05; O, 18.51 Found: C, 62.44; H, 4.56; Cl, 10.17; N, 4.01; O, 18.47.
2.13.15 Ethyl-2-amino-4-(4-chlorophenyl)-7-hydroxy-4H-chromene-3-carboxylate (BN-15)

Yield: 85%; M.P.- 176-178 ºC; IR (cm\(^{-1}\)): 3625 (O-H stretching of free primary alcohol), 3534-3478, (N-H stretching of free primary amine), 3276 (C-H stretching vibration of aromatic region), 1266 (C-O stretching frequency of esters), 1757 (C=O stretching frequency for esters), 1580 (N-H deformation, in plane bending of N-H), 1508 (O-H in plane bending), 1381 (C-N stretching for carbon bonded to amino group), 987 (C-H in plane bending of phenyl ring), 742 (C-H out of plane bending for phenyl nucleus), MS: m/z: 345.08; Anal. Calcd. for C\(_{18}\)H\(_{16}\)ClNO\(_4\): C, 62.52; H, 4.66; Cl, 10.25; N, 4.05; O, 18.51 Found: C, 62.45; H, 4.54; Cl, 10.19; N, 4.00; O, 18.45.

2.13.16 Ethyl-2-amino-4-(4-fluorophenyl)-7-hydroxy-4H-chromene-3-carboxylate (BN-16)

Yield: 87%; M.P.- 200-202 ºC; IR (cm\(^{-1}\)): 3627 (O-H stretching of free primary alcohol), 3514-3499, (N-H stretching of free primary amine), 3141 (C-H stretching vibration of aromatic region), 1256 (C-O stretching frequency of esters), 1722 (C=O stretching frequency for esters), 1595 (N-H deformation, in plane bending of N-H), 1568 (O-H in plane bending), 1351 (C-N stretching for carbon bonded to amino group), 962 (C-H in plane bending of phenyl ring), 796 (C-H out of plane bending for phenyl nucleus), MS: m/z: 329.11; Anal. Calcd. for C\(_{19}\)H\(_{16}\)FNO\(_4\): C, 65.65; H, 4.90; F, 5.77; N, 4.25; O, 19.43 Found: C, 65.61; H, 4.83; F, 5.59; N, 4.12; O, 19.32.
2.14  SPECTRAL DISCUSSION

2.14.1  IR Spectral study

IR spectra were recorded on Shimadzu FT-IR-8400 model using KBr pellet method. Various functional groups present in molecule were identified by characteristic frequency obtained for them. The characteristic bands of Hydroxyl groups were obtained for stretching at 3400-3650 cm$^{-1}$, and those for bending were obtained at 1050-1250 cm$^{-1}$. The characteristic bands of amino group were obtained for stretching at 3500-3400 cm$^{-1}$ with a deformation due to in plane bending at 1650-1580 cm$^{-1}$. The characteristic bands of the Ester group were seen for the Carbonyl function of the group at 2250-2100 cm$^{-1}$ while the C-O stretching frequency was seen at 1095-1066 cm$^{-1}$, also the C=O stretching frequency was observed around 1710 to 1760 cm$^{-1}$. The general aromatic C-C stretching bands were observed at 1460-1408 cm$^{-1}$ while the out of plane bending frequency of C-H was seen between 952-696 cm$^{-1}$. The characteristic bands for halogen groups like chlorine and bromine were found at 740-700 cm$^{-1}$ & 600-500 cm$^{-1}$. Also characteristic stretching frequencies of 1,3-Disubstituted and 1,4-Disubstituted phenyl ring were found at 671 cm$^{-1}$ and 823 cm$^{-1}$ respectively suggesting the correct formation of the desired products (BN-1 to BN-16).

2.14.2  MASS SPECTRAL STUDY

Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using Direct Injection Probe technique. Systematic fragmentation pattern was observed in mass spectral analysis. Molecular ion peak was observed in agreement with molecular weight of respective compound. The probable Mass fragmentation pattern for the representative compound of each series is discussed below.
2.14.2.1 PLAUSIBLE MASS FRAGMENTATION PATTERN OF BN-01

Ethyl 2-amino-7-hydroxy-4-phenyl-4H-chromene-3-carboxylate (BN-1)

1. The target compound showed the characteristic molecular ion peak 311 \textit{m/z}.
2. The bond cleavage between C\textsubscript{4}-C\textsubscript{5} generated a molecular ion which corresponds to a characteristic peak at 234 \textit{m/z} (A).
3. A bond cleavage between C\textsubscript{3}-C\textsubscript{18} generated a molecular ion which corresponds to a characteristic peak at 239 \textit{m/z} (B).
4. Bond cleavages between C\textsubscript{18}-O\textsubscript{20} generated a molecular ion which corresponds to a characteristic peak at 267 \textit{m/z} (C).
5. Bond cleavages between C\textsubscript{4}-C\textsubscript{11} and O\textsubscript{1}-C\textsubscript{2} generated a molecular ion which corresponds to a characteristic peak at 206 \textit{m/z} (D).
6. Bond cleavages between C\textsubscript{4}-C\textsubscript{5} and O\textsubscript{20}-C\textsubscript{21} generated a molecular ion which corresponds to a characteristic peak at 206 \textit{m/z} (E).
7. Bond cleavages between C₂-N₁₇, O₁-C₂ and C₄-C₁₁ generated a molecular ion which corresponds to a characteristic peak at 188 m/z (F).
8. Bond cleavages between C₃-C₁₈, and C₁₄-O₂₃ generated a molecular ion which corresponds to a characteristic peak at 220 m/z (G).
9. Bond cleavages between C₃-C₄ and O₁-C₁₆ generated a molecular ion which corresponds to a characteristic peak at 181 m/z (H).
10. Bond cleavages between C₃-C₄, O₁-C₁₆, C₄-C₅ & C₄-C₁₁ generated a molecular ion which corresponds to a characteristic peak at 132 m/z (I).
11. Bond cleavages between C₄-C₅, C₃-C₄, & O₁-C₁₆ generated a molecular ion which corresponds to a characteristic peak at 105 m/z (J).
12. Bond cleavages between C₁₁-C₁₂, C₁₅-C₁₆, generated a molecular ion which corresponds to a characteristic peak at 69 m/z (K).
13. Bond cleavage between O₂₀-C₂₁ generated a molecular ion which corresponds to a characteristic peak at 282 m/z (L).
14. The other fragment caused due to bond cleavage between C₄-C₅, generated a molecular ion which corresponds to a characteristic peak at 77 m/z (M).
15. Bond cleavages between C₃-C₄, & C₄-C₁₁ generated a molecular ion which corresponds to a characteristic peak at 92 m/z (N).
Ethyl-2-amino-7-hydroxy-4-(4-methoxyphenyl)-4H-chromene-3-carboxylate (BN-02).

1. The target compound shows the desired characteristic molecular ion peak of 341 m/z.
2. The bond cleavage between C₄-C₅ generated a molecular ion which corresponds to a characteristic peak at 234 m/z (A).
3. The bond cleavage between C₃-C₁₈ generated another molecular ion which corresponds to a characteristic peak at 268 m/z (B).
4. The bond cleavage between C₁₈-O₂₀ generated a molecular ion which corresponds to a characteristic peak at 294 m/z (C).
5. The bond cleavages between C₄-C₅ & C₃-C₁₈ generated a molecular ion which corresponds to a characteristic peak at 161 m/z (D).
6. The bond cleavages between C₄-C₅ & O₂₀-C₂₁ generated a molecular ion which corresponds to a characteristic peak at 206 m/z (E).
7. The bond cleavages between C₃-C₄ & C₄-C₁₁ generated a molecular ion which corresponds to a characteristic peak at 224 m/z (F).
8. The bond cleavages between C₃-C₁₈ & C₁₄-O₂₃ generated a molecular ion which corresponds to a characteristic peak at 251 m/z (G).
9. The bond cleavages between O₁-C₂, O₁-C₁₆, C₁₈-O₂₀, & C₄-C₁₁ generated a molecular ion which corresponds to a characteristic peak at 188 m/z (H).
10. The bond cleavage between C₃-C₄, C₄-C₅, O₁-C₁₆ & C₄-C₁₁ generated a molecular ion which corresponds to a characteristic peak at 132 m/z (I).
11. Bond cleavages between C₄-C₅, C₃-C₄, & O₁-C₁₆ generated a molecular ion which corresponds to a characteristic peak at 105 m/z (J).
12. Bond cleavages between C₁₁-C₁₂, C₁₅-C₁₆, generated a molecular ion which corresponds to a characteristic peak at 69 m/z (K).
13. Bond cleavage between O₂₀-C₂₁ generated a molecular ion which corresponds to a characteristic peak at 312 m/z (L).
14. The other fragment caused due to bond cleavage between C₄-C₅, generated a molecular ion which corresponds to a characteristic peak at 77 m/z (M).
2.14.3 \(^1\)H-NMR SPECTRAL STUDY

\(^1\)H-NMR spectra of the synthesized compounds were recorded on Bruker Avance II 400 spectrometer. Sample solutions were made in DMSO solvent using tetramethylsilane (TMS) as the internal standard unless otherwise mentioned. Numbers of protons identified from H-NMR spectrum and their chemical shift (\(\delta\) ppm) were in the agreement of the structure of the molecule. \(J\) values were calculated to identify o, m and p coupling. In some cases, aromatic protons were obtained as multiplet. The spectral interpretation can be discussed as under.

**Ethyl 2-amino-7-hydroxy-4-phenyl-4H-chromene-3-carboxylate (BN-01)**

1. The two protons (proton no. 1) of the amino group gave a characteristic singlet at 6.46 \(\delta\) ppm.

2. The methine proton (proton no. 2) on C-4 gave a characteristic singlet 4.79 \(\delta\) ppm. The assignment of this proton is the most important for the structure elucidation and as it is evident here the successful assignment of this singlet has confirmed our structure. The proton no. 2 is a bit downfield as compared to a proton singlet in isolation because of the strong electron withdrawing group like –COOC\(_2\)H\(_5\) present on the adjacent carbon which deshields the proton forcing it to go down field.

3. Now the aromatic protons no. 3, 4, 5, 6, 7, and 8 are all in the aromatic region of the NMR spectrum and gave a characteristic multiplet accounting for six protons between 7.14-7.18 \(\delta\) ppm.

4. The proton no. 9 gave a characteristic doublet for a single proton between 6.86-6.83 \(\delta\) ppm with a \(J\) value of 8.76 Hz suggesting that it is ortho coupled to another proton in its vicinity.

5. The proton no. 10 gave a characteristic singlet for a single proton in the region of 7.05 \(\delta\) ppm with the \(J\) value of 2.64 Hz which clearly indicates that it is meta coupled with the proton no. 9.

6. The proton of the hydroxyl group gave a characteristic singlet at 9.25 \(\delta\) ppm. As the proton is bonded directly to Oxygen, an electronegative entity, the
proton gets completely deshielded and the signal shifts to such a downfield region as expected.

7. The two protons of the ethyl group i.e. proton no. 12 is on the carbon directly bonded to the oxygen of the ester moiety thus they would be deshielded due to the electronegativity of the oxygen atom and hence are seen as the quartet between 3.95-4.00 δ ppm with a very high J value suggesting that it is ortho coupled to the other protons.

8. Proton no 13 of the ethyl group gave a characteristic triplet between 1.08-1.12 δ ppm as expected for a methyl group.

Thus, by observing and assigning the peaks in the NMR spectrum and by the calculation of the J values for each of the above proton it can be clearly suggested that the proposed structure for compound no. BN-01 has been confirmed.

**Ethyl-2-amino-7-hydroxy-4-(4-methoxyphenyl)-4H-chromene-3-carboxylate (BN-02)**

1. The two protons (proton no. 1) of the amino group were distinctively observed as a broad singlet at 6.45 δ ppm. As the protons are bonded to the electronegative Nitrogen atom they are de-shielded and found at such a downfield region of 6.45 δ ppm.

2. The proton no. 2 or the methine proton which is very important for our structure elucidation is evidently observed as a sharp singlet at 4.74 δ ppm. The reason for it being downfield as compared to other isolated proton normally found at an upfield frequency of around 2 δ ppm is due to the fact that an electronegative ester group is attached to its neighboring carbon atom moreover it is surrounded by phenyl ring which acts as an electron sink thus making the proton significantly de-shielded and hence it is found at 4.74 δ ppm.
3. Now just by looking at the structure it is evident that the chemical environment of proton no. 3 and proton no. 6 as well as that of proton no. 4 and 5 are identical, hence their signals should also be the same. The broad singlet assigned for two protons in the aromatic region at 7.14 δ ppm are responses for proton no. 3 and proton no. 6.

4. As discussed above, the chemical environment for proton no. 4 as well as proton no. 5 is also similar hence their signals would also similar. This was evidently observed as a doublet between 7.04 δ ppm to 7.05 δ ppm which accounted for two protons.

5. The proton no. 7 and proton no. 8 could be assigned to the doublet shown in the ¹H-NMR spectrum between 6.68 δ ppm and 6.70 δ ppm. The J value for this proton was calculated to be 8 Hz suggesting that it is ortho coupled to another proton.

6. The proton no. 9 here is the hydroxyl proton bonded to the electronegative oxygen atom; hence as it is completely deshielded we observe a sharp singlet at 9.22 δ ppm.

7. The proton no 10 is observed as a doublet between 6.83 δ ppm to 6.82 δ ppm. The splitting occurs due to its proximity to the hydroxyl proton.

8. The two protons assigned as no. 11 here are observed as a singlet at 3.98-3.99 δ ppm and are comparatively downfield due to the oxygen atom directly bonded to it.

9. The proton no. 12 is observed as a characteristic triplet for 3 protons at 1.12 δ ppm to 1.13 δ ppm.

10. The methoxy protons are observed at 3.69 δ ppm as a singlet and are in the downfield region due to their proximity to the oxygen atom.

11. The methine proton, the Methoxy protons as well as all the protons of the aromatic region seen very clearly in the spectra confirms the proposed structure.

Thus, by observing and assigning the peaks in the NMR spectrum and by the calculation of the J values for each of the above proton it can clearly be suggested that the proposed structure for compound no. BN-02 has been confirmed.
2.15 SPECTRAL REPRESENTATIONS COMPOUNDS

2.15.1 IR SPECTRUM OF BN-01

![IR Spectrum of BN-01](image)

2.15.2 MASS SPECTRUM OF BN-01

![Mass Spectrum of BN-01](image)
2.15.3 $^1$H-NMR SPECTRUM OF BN-01

2.15.3.1 EXPANDED $^1$H-NMR SPECTRUM OF BN-01
2.15.3.2  \textit{EXPANDED} $^1\text{H}$-\textit{NMR SPECTRUM OF BN-01}

\begin{center}
\includegraphics[width=\textwidth]{figure1.png}
\end{center}

2.15.4  \textit{IR SPECTRUM OF BN-02}

\begin{center}
\includegraphics[width=\textwidth]{figure2.png}
\end{center}
Chapter 2: Aqua mediated and Microwave Assisted synthesis of Ethyl-2-amino-7-

2.15.5 MASS SPECTRUM OF BN-02

![Mass Spectrum of BN-02](image)

2.15.6 $^1$H-NMR SPECTRUM OF BN-02

![$^1$H-NMR Spectrum of BN-02](image)
2.15.6.1 \textit{EXPANDED} $^1$H-NMR SPECTRUM OF BN-02

![Graph of Expanded $^1$H-NMR Spectrum of BN-02]

2.15.6.2 \textit{EXPANDED} $^1$H-NMR SPECTRUM OF BN-02

![Graph of Expanded $^1$H-NMR Spectrum of BN-02]
2.16 RESULTS AND DISCUSSIONS

This chapter deals with the 2-Amino-3-cyano / ethyl carboxylate-4-substituted phenyl-benzopyran core structure. The importance of chromene / Benzopyran as a privileged structure was discussed at length in the introduction of this chapter which served as the rational for the synthesis of these kinds of compounds. Moreover, this chapter has quite a lot of interesting features in terms of synthesis methodology as well as biological activity study. First of all the reaction employed in this chapter is a 3 component reaction and as we all know multicomponent reactions have quite a lot of advantages over the normal conventional methodologies. Also, the medium by which the energy was supplied was Microwave irradiation which made this a Microwave assisted Organic Synthesis which again has its own advantage of using optimum time and resources. And last but not the least, none of the hazardous organic volatile solvents were employed in the reaction; instead we did this reaction using the universal solvent i.e. water which made our process appreciably green. The bioactivity of these compounds is all together another interesting aspect of the novel compounds enlisted in this chapter.

2.17 CONCLUSION

This chapter involves some interesting chemical aspects as far as organic chemical synthesis is concerned. The New chemical entities synthesized in this chapter were screened for Anti HIV study. The results of which are discussed separately.
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