CHAPTER 4

Experimental
General:

The melting points were taken in open capillaries and are uncorrected. The Infra-red spectra were recorded on Perkin Elmer RXI spectrometer using KBr. Ultra-voilet spectra in 95% methanol were measured on USB 2000 Ocean-Optics spectrophotometer and wave lengths, $\lambda_{\text{max}}$ were expressed in nm. The $^1$H NMR spectra were measured on Bruker DRX-300 MHz in deuteriochloroform or hexamethyldeuteriodi-methylsulfoxide with TMS as internal standard. The mass spectra were obtained in FAB mode on Jeol Sx 102 using Argon/Xenon as the FAB gas. m-Nitrobenzyl alcohol was used as the matrix. Peaks at m/z 136, 137, 154, 289, 307 which appears in the mass spectrum are due to matrix. The purity of the compounds were checked by TLC on glass plates coated with silica Gel G (Merk-Germany) using benzene and ethyl acetate as mobile phase and visualized by iodine vapour.

All the solvents and chemicals used were AR grade. 2-Hydroxyacetophenone, dehydroacetic acid, phosphorousoxychloride, 4-hydroxycoumarin, N,N-dimethyl formamide and hydroxyl ammonium sulphate were obtained from E. Merck (Germany).

3-Formylchromone$^8$, 4-amino-3alkyl-5-mercapto-1,2,4-triazoles$^{43}$, 2-ureidomethyl enecyclohexane-1,3-dione$^{46}$, 2-amino-3-formyl-
chromone$^1$ and triacetic acid lactone$^{13}$ were prepared according to published procedures.

**The reaction of 3-formylchromone with 4-amino-3-methyl-5-mercapto-1,2,4-triazole.**

**Formation of 3-(3-methyl-5-mercapto-1,2,4-triazolyliminomethyl) chromone (38)**

3-Formyl chromone 1 (1 gm, 5.75 mmole) was dissolved in dry benzene (10ml) and 4-amino-3-methyl-5-mercapto-1,2,4-triazole (0.747 gm, 5.75 mmole) added to it followed by p-toluene sulfonic acid (few crystals). The reaction mixture was refluxed on water bath for 16 hours, concentrated and allowed to stand at room temperature when light yellow solid precipitated out. It was filtered, washed with alcohol, dried and recrystallized from chloroform to give 3-(3-methyl-5-mercapto-1,2,4-triazolyliminomethyl) chromones, 38.

yield : 1.07gm (65%)

M.P. : 210-212°C

**Spectral Data:**

- $\text{UV(MeOH)} \lambda_{\text{max}}$: 247.25, 298.33, 338.14 nm.
- $\text{IR (KBr)} \lambda_{\text{max}}$: 3424, 3060, 2931, 2752, 2373, 1649, 1591, 1494, 1456, 1405, 1284, 1226, 1094, 981, 832, 752 cm$^{-1}$.

$^1$H NMR (300 MHz, DMSO-d$_{16}$):
δ 2.37 s, 3H (CH₃); 7.57-7.93 m, 3H (Ar-H);
8.17d, 1H, 9 Hz (C-5); 9.13 s, 1H (C-2); 10.32
s, 1H (-CH=N-).

MS (rel. int.): m/z 286 (M⁺ 84%), 172 (52), 167 (4), 165 (8),
139 (14), 138 (29), 137 (47), 136 (80), 120
(15), 114 (12).

The reaction of 3-formylchromone with 4-amino-3-ethyl-5-
mercapto-1,2,4-triazole

Formation of 3-(3-ethyl-5-mercapto-1,2,4-triazolyliminomethyl)
chromone (39)

A mixture of 3-formyl chromone (1) (1.0 gm, 5.75 mmole) and 4-
amino-3-ethyl-5-mercapto-1,2,4-triazole (0.827 gm, 5.74 mmole) was
dissolved in dry benzene (10 ml) and refluxed for 18 hour in the
presence of catalytic quantity of p-toluene sulfonic acid. The reaction
mixture was cooled at room temperature to afford light yellow solid. It
was filtered, washed with benzene and recrystallized from chloroform to
give 3-(3-ethyl-5-mercapto-1,2,4-triazolyliminomethyl) chromones 39.

Yield: 1.07 gm (62%)

M.P.: 240°C

Spectral Data:

UV (MeOH) λ_max: 251.75, 297.22, 339.61 nm.

IR (KBr) ν_max: 3451, 3098, 2932, 2365, 1654, 1568, 1462, 1231,
1145, and 761 cm⁻¹.
$^1$H NMR (300 MHz, CDCl$_3$):

\[ \delta \ 1.33 \text{ t, 3H (CH$_3$); 2.82} \text{ q, 2H (CH$_2$); 7.48-7.78} \text{ m, 3H (Ar-H); 8.33} \text{ d, 1H, J=9Hz (C-5); 8.70} \text{ s, 1H (C-2); 10.37} \text{ s, 1H (-CH=N-); 10.46} \text{ br s, 1H (NH).} \]

MS (rel. int): m/z 300 (M$^+$ 100%), 172 (83), 165 (4), 138 (4), 123 (2), 120 (8), 114 (4).

The reaction of 3-formylchromone with 4-amino-5-mercapto-3-propyl-1,2,4-triazole:

**Formation of 3-(5-mercapto-3-propyl-1,2,4-triazolylinomethyl) chromone (40):**

3-Formylchromone (1.0 gm, 5.75 mmole) was dissolved in dry benzene (10 ml) and (0.908 gm, 5.74 mmol) 4-amino-5-mercapto-3-propyl-1,2,4-triazole, added to it. The reaction mixture was refluxed on water bath for 20 hours in the presence of catalytic amount of p-toluene sulfonic acid. It was concentrated, allowed to stand at room temperature when light yellow solid precipitated out. It was filtered, washed with a mixture of benzene and petrol, dried and recrystallized from chloroform to give 3-(5-mercapto-3-propyl-1,2,4-triazolylinomethyl) chromone 40.

Yield : 1.105 gm (61%)

M.P.: 225°C.

**Spectral Data:**

UV (meOH) $\lambda_{max}$: 251.38, 296.11, 350 nm.
IR(KBr) $\nu_{\text{max}}$: 3088, 2948, 2773, 1646, 1563, 1464, 1282, 1236 and 764 cm$^{-1}$.

$^1$H NMR (300 MHz, CDCl$_3$):

$\delta$ 1.041 t, 3H (CH$_3$); 1.82 m, 2H (CH$_2$); 2.78 t, 2H (CH$_2$); 7.48-7.79m, 3H (Ar-H); 8.30-8.33dd, 1H, J=7.8 Hz, 0.9Hz, (C-5); 8.71 s, 1H (C-2); 10.35 s, 1H (-CH=N-); 11.22 br s, 1H (NH).

MS (rel. int.): m/z 314 (M$^+$ 84%), 172 (100), 165 (12), 138 (18), 123 (9), 120 (12), 114 (12).

The reaction of 3-(3-alkyl-5-mercapto-1,2,4-triazolyliminomethyl) chromone 38-40

Formation of 2-(4-oxo-4H-[1] benzopyran-3-yl) [1] benzopyrano-[3,2-e] pyrimidin-5(5H)-one, (42) and 1-(4-oxo-4H-[1] benzopyran-3-yl) [1] benzopyrano-[3,2-d] pyridazin-5(5H)-one (43)

The compounds 38-40 (1.0 gm, 3.49 mmole) were taken in nitrobenzene (10 ml) and refluxed on an oil bath for 4 hours. The reaction mixture was adsorbed on a column of silica Gel and eluted with benzene. Appropriate fractions were combined, evaporated and crystallized from benzene petroleum ether to afford compound 2-(4-oxo-4H-[1] benzopyran-3-yl) [1] benzopyrano-[3,2-e] pyrimidin-5(5H)-one, 42 as a yellow crystals.

Yield : 0.72 gm (60%),

M.P. : 220-222°C
Spectral Data:

UV (MeOH) \( \lambda_{\text{max}} \): 244.25, 291.66, 402.10 nm.

IR (KBr) \( \nu_{\text{max}} \): 1652, 1602, 1531, 1402 cm\(^{-1}\).

1H NMR (300 MHz, DMSO \( d_6 \)):

\( \delta 7.57-8.04 \text{ m, 6H (Ar-H); 8.24 d, 2H (H}_C; 9.14 \text{ s, 1H (H}_a; 9.58 \text{ s, 1H (H}_b. \)

MS (rel. int.): m/z 342 (M\(^+\) 90%), 315 (2), 284 (6), 267 (2), 223 (4), 190 (5), 172 (6), 144 (2), 120 (2), 107 (2).

Further elution of the column with benzene and ethylacetate (90:10, v/v) gave 1-(4-oxo-4H-[1] benzopyran-3-yl) [1] benzopyrano-[3,2-d] pyridazin-5(5H)-one 43. It was recrystallized from benzene petroleum mixture as yellow crystal.

Yield: 0.92 gm (40%),

M.P.: 240°C.

Spectral Data:

UV (MeOH) \( \lambda_{\text{max}} \): 241.25, 262.98, 288.69 nm.

IR (KBr) \( \nu_{\text{max}} \): 1672, 1616, 1579, 1454, 1392, 1332, 791 and 717 cm\(^{-1}\).

1H NMR (300 MHz, CDCl\(_3\)):

\( \delta 7.26-7.63 \text{ m, 6H (Ar-H); 7.65,dd, 1H(H}_C; 8.24 \text{ dd, 1H (H}_C; 9.80 \text{ s, 1H (H}_a; 10.30 \text{ s, (1H H}_b. \)

MS (rel. Int): m/z 342 (M\(^+\) 20%), 315 (6), 313 (6), 290 (9), 286 (6), 222 (6), 172 (3), 120 (17), 116 (3).
The reaction of 3-formylchromone with dimedone

Formation of 2-(4-oxo-4H-1-benzopyran-3-yl-methylidene)-5-, 5-
dimethyl cyclohexane-1, 3-dione (45):

3-Formylchromone (1 g, 5.75 mmol) was dissolved in absolute
alcohol (20 ml) and dimedone (0.805 g, 5.75 mmol), fused sodium
acetate (0.471 g, 5.75 mmol) were added to it. The reaction mixture
was refluxed on water bath for 5 hours, concentrated and allowed to
stand at room temperature. The white solid 2-(4-oxo-4H-1-benzopyran-
3-yl methylidene)-5, 5-dimethyl cyclohexane-1, 3-dione 45 crystallized
out from the reaction mixture, was filtered, washed with alcohol, dried
and recrystallized from chloroform.

M.P. = 220-222°C

Yield = 1.45 (85%)

Spectral Data:

UV (MeOH) \( \lambda_{\text{max}} \): 253.25, 292.03 nm

IR (KBr): 3421, 1630, 1569, 1467, 1408, 1349, 1323, 1148 and
764 cm\(^{-1}\)

\(^1\)H NMR (CDCl\(_3\)): \( \delta \) 0.978 6H, s, (CH\(_3\)), 2.23 2H, s (CH\(_2\)), 2.55 2H, s
(CH\(_2\)), 7.32-7.57 4H, m (ArH+CH), 7.65 1H, d,
J=7.8 Hz (H-6), 8.12 (1H, d, J=7.8 Hz, H-5), 8.32
1H, s (H-2).
MS (rel. Int): m/z 295 (M⁺-1, 25%), 294 (92), 278 (8), 258 (6), 232 (14), 229 (14), 176 (14), 165 (11), 154 (100), 121 (17), 107 (28) and 105 (11).

The reaction of 4-hydroxycoumarin with 2-ureidomethylene cyclohexane-1, 3-dione

Formation of 7-(4-hydroxycoumarin-3-yl) 10,10-dimethyl-8-oxo-8, 9,10,11-tetrahydro pyrano (3,2-c) coumarin (48).

To a solution of 4-hydroxycoumarin (0.764g, 4.71 mmol) and 2-ureidomethylene cyclohexane-1, 3-dione (1.0g, 4.71 mmol) in glacial acetic acid (30 ml), was added fused sodium acetate (1.0g). The reaction mixture was refluxed for six hours. It was then cooled at room temperature and added to water. The solid as obtained, was filtered, washed with water, dried and crystallized from ethanol to give 7-(4-hydroxycoumarin-3-yl) 10,10-dimethyl-8-oxo-8, 9,10,11-tetrahydro pyrano (3,2-c) coumarin 48 as white shining crystals.

M.P. 235-240

Yield 1.2g, 56%.

Spectral Data:

UV (MeOH) λ_max: 261.11, 309.43.

IR (KBr) ν_max: 3432, 1722, 1617, 1374, 1326, 1193, 1034, 755 cm⁻¹

¹H NMR (DMSO): δ 0.98 s, 3H (CH₃), 1.10 s, 3H (CH₃) 2.16 d, 1H, J_ab=15.9 Hz (H₃), 2.39 d, 1H, J_ba = 15.9 Hz (H₃), 2.64 d, 1H, J_cd=17.7 Hz (H₃), 2.79 d, 1H. J_dc=17.7
Hz (H₂), 5.26 s, 1H, 7.27-7.73 m, 6H (Ar-H), 7.93 d, 1H, J=7.8 (H-1), 8.01 d, 1H, J=8.1 Hz (H-5).

MS (rel int.): m/z 456 (M⁺,30), 335 (15), 307 (20), 295 (100), 279 (6), 239 (20), 121 (8) and 107 (12).

The reaction of 2-amino-3-formylchromone (50) with triacetic acid lactone (61)


2-Amino-3-formylchromone (1.0 g, 5.29 mmol) was dissolved in pyridine (30 ml) containing piperidine (0.89 ml, 10.5 mmol) and triacetic acid lactone (0.999 g, 7.936 mmol) added to it. The reaction mixture was kept at room temperature for 7 days. The product 3-acetoacetyl-5-oxo-5H-[1]benzopyrano[3, 2-e]pyridin-2-one 62 crystallised out as yellow solid. It was filtered, washed with cold water and dried. The mother liquor was poured in to ice-cold water and acidified with HCl. The solid, which precipitated out was filtered, washed with water, dried and recrystallised from chloroform-methanol to afford more 62.

M.P. 180°C.

Yield 1.3g, 82%;

Spectral Data:

UV (MeOH) λ<sub>max</sub>: 263.76, 299.08, 396.33 nm.

IR (KBr)ν<sub>max</sub>: 1652, 1647, 1642, cm⁻¹;

¹H NMR (300MHz, DMSO):
The reaction of 2-amino-3-formylchromone (50) with triacetic acid lactone (61)

Formation of 6-methyl-2-(4-oxo-4H-1-benzopyran-3-yl)-3-(2'-hydroxybenzoyl)-4-pyridone (68).

1.0 g (5.29 mmol) of 2-amino-3-formylchromone was dissolved in 20 ml of alcohol and potassium acetate 0.519 g (5.29 mmol) and triacetic acid lactone 0.99 g (7.93 mmol) added to it. The resultant mixture was refluxed on water bath for 12 hours. It was concentrated and allowed to stand at room temperature when light yellow solid crystallized out from the reaction mixture. It was filtered, washed with alcohol and dried. The compound was recrystallized from chloroform and methanol to afford 6-methyl-2-(4-oxo-4H-1-benzopyran-3-yl)-3-(2'-hydroxybenzoyl)-4-pyridone 68 as yellow shining crystals.

M.P. : 215-220°C
Yield : 1.38 g

Spectral Data:

UV(MeOH)\(\lambda_{\text{max}}\): 242.37, 261.11, 296.48, 370.62 nm.
IR: 3398, 3249, 1650, 1616, 1620, 1508, 1463, 1432, 1331 and 760 cm\(^{-1}\).

\(^1\)H NMR (DMSO \(d_6\)):

\(\delta\) 213 s, 3H (CH\(_3\)), 7.07 s, 1H (H-5), 7.31-7.70 m, 6H (Ar-H), 8.02 d, 1H, J=9Hz (H-5'), 8.61 s, 1H (H-2'), 9.58 br s, 1H (NH) and 16.68 br s, 1H (OH).

MS (rel. Int.): m/z 374 (M\(^+\)+1, 48), 345 (39), 336 (83), 298(36), 289(22), 283 (14), 256 (8), 254 (3), 240 (17), 228 (17), 222 (6), 211 (6), 192 (100), 190 (20), 166 (3), 154 (61), 120 (6), 121 (4), 107 (11), 91 (8), 89 (11) and 76 (8).

The reaction of 2-amino-3-formylchromone (50) with 5, 5-dimethyl-cyclohexane-1,3-dione:

Formation of 3,3-dimethyl-5-oxo-cyclohexa[2,3-b] azaxanthone (78):

2-Amino-3-formylchromone 1 g (5.29 mmol) was dissolved in 40 ml of pyridine containing 0.899 ml (10.5 mmol) of piperidine and added 5, 5-dimethylcyclohexane-1, 3-dione, 0.740 gm (5.29 mmol) to it. The reaction mixture was allowed to stand at room temperature for 5 days. The solid as obtained, was filtered, washed with water, dried and recrystallised from chloroform to afford 3,3-dimethyl-5-oxo-cyclohexa[2,3-b] azaxanthone 78 as yellow shining crystals.

M.P. 250\(^0\)C

Yield 1.4gm, 90%
Spectral Data:

UV (MeOH) \( \lambda_{\text{max}} \): 261.11, 331.90 nm.

IR (KBr) \( \nu_{\text{max}} \): 3429, 1685, 1651, 1596, 1472, 1403, 1317 and 768 cm\(^{-1}\).

\(^1\)H NMR (CDCl\(_3\)): \( \delta \) 1.17 s, 6H (2 x CH\(_3\)), 2.64 s, 2H (CH\(_2\)), 3.14 s, 2H (COCH\(_2\)), 7.47-7.83 m, 3H (Ar-H), 8.33 1H, d, J=7.8 Hz, (H8), 9.27 s. 1H.

MS (rel. int.): m/z 294 (M\(^+\)+1, 100), 278 (17), 276 (6), 262 (6), 238 (6), 211 (6), 202 (3), 180 (3), 120 (3) and 115 (4).

The reaction of 2-amino-3-formylchromone 50 with 3-methyl-1-phenyl-5-pyrazolone.

Formation of methylidene-bis-4,4-(3-methyl-5-oxo-1-phenylpyrazole) (81):

2-Amino-3-formylchromone 1.0g (5.29 mmol) was dissolved in 20 ml of dry methanol, added 5 drops of piperidine and 0.922g (5.29 mmol) of 3-methyl-1-phenyl-5-pyrazolone to it. The resultant mixture was refluxed on water bath for 2 hours. It was allowed to cool at room temperature. The reaction mixture was poured into ice-cold water and acidified with HCl. The solid methylidene-bis-4,4-(3-methyl-5-oxo-1-phenylpyrazole) 81 which precipitated out was filtered, washed with water, dried and recrystallized from chloroform-methanol as yellow crystals. The filtrate was extracted with chloroform, washed with water, dried over anhydrous sodium sulfate and evaporated. The solid as obtained was recrystallized from chloroform-methanol to get more methylidene-bis-4, 4-(3-methyl-5-oxo-1-phenylpyrazole) 81.
M.P. – 150-160°C
Yield – 1.52 gm

Spectral Data:

UV (MeOH)\(\lambda_{\text{max}}\): 240.38, 406 nm.

IR (KBr): 3350, 1627, 1592, 1550, 1498 and 1328 cm\(^{-1}\);

\(^1\)HNMR (CDCl\(_3\)): \(\delta\) 2.32 s, 6H (CH\(_3\)), 7.26-7.92 m, 11H (Ar-H + methylene proton).

MS (rel. int.): m/z 358 (M\(^+\), 100), 357 (50), 340 (5), 281(6), 117 (4), 104 (4), 90 (20) and 77(35).
**Piper cubeba**

**Extraction and isolation**

The seed of the plant were collected from Mysore, India. The plant material was further identified in the Department of Botany, AMU, Aligarh (India). The seeds of *Piper cubeba* (5 Kg) were air dried under shade and powdered. The powdered material was exhaustively extracted with petrol and then with benzene at room temperature for 15 days.

Both petrol and benzene extracts were concentrated under reduced pressure to afford gummy residues (30g). The TLC of both the extracts displayed same type of spots. The two extracts were, therefore, combined and subjected to column chromatography, using benzene ethyl acetate as eluant. The compound isolated was labeled as KCB.

**KCB:**

It was obtained using benzene-ethyl acetate (75.25, v/v) as eluant and crystallized from benzene to yield white crystalline solid. It was identified as cubebin through comparison with authentic sample (m.p., IR, NMR and mass).

- **MP:** 120-22°C
- **Yield:** 3.5g

**Spectral Data:**

- **UV (MeOH)λ_max:** 239.74, 287.20 nm.
- **IR (KBr)ν_max:** 3418, 1629, 1491 and 926 cm⁻¹.
**Zanthoxylum simularis**

**Extraction and Isolation:**

The plant was collected from Nepal Border and a sample of it was identified by the Department of Botany, Tribhuvan University of Nepal. The stems wood (15 kg) was chapped into small piece and shade dried. It was defatted with petroleum ether and extracted with chloroform in cold for 15 days. The solvent was removed under reduced pressure to furnish a brown residue (30 g). It was adsorbed on silica gel (60-120 mesh) and subjected to column chromatography using chloroform–petrol (60-80) as eluant. The compounds isolated were labelled as ZS-1 and ZS-2.
Elution of the column with chroform-methanol petrol (50:50, v/v) gave a fraction which on evaporation afforded a yellowish mass (5 g). The yellowish residue was further chromatographed over silica gel using chloroform-petrol (70:30, v/v) as eluent. The appropriate fractions were combined and evaporated to give a solid which on crystallization from chloroform yielded ZS-1. It was characterized as limonin through direct comparison with (IR, \(^1\)H NMR, \(^{13}\)C NMR and mass) with an authentic sample.

M.P.: 275°C
Yield: 100 mg

**Spectral Data:**

IR (Nujol) \(\nu_{\text{max}}\): 1755, 1710, 1460, 1380, 1160, 920, 880 and 875 cm\(^{-1}\).

\(^1\)H NMR (300 MHz, DMSO):

\[\delta 1.007 (\text{C-8 Me}), 1.03 (\text{C-18Me}), 1.11, 1.19 (\text{C-4-Methyls}), 1.23-1.88 (4\text{H, m, C-10, C-11 CH}_2), 2.25-2.3 (1\text{H, dd, H-9, } J=14.76, 3.2 \text{ Hz}), 2.58-2.66 (1\text{H, dd, H-2 } J=16.59, 3.9 \text{ Hz}), 3.07-3.17 (1\text{H, t, H-1}), 3.31 (1\text{H, m, H-5}), 4.11 (1\text{H, s, H-15}), 4.50 (1\text{H, d, H-19, } J=13.02 \text{ Hz}), 4.94 1\text{H, d, H-19 } J=13.05 \text{ Hz}), 5.47 (1\text{H, s, H-17}), 6.15 (1\text{H, m, H-21}), 7.66 (1\text{H, m, H-20}), 7.72, (1\text{H, m, H-23}).

\(^{13}\)C NMR (300 MHz):

\[\delta 78.2 (\text{C-1}), 35.39 (\text{C-2}), 169.67 (\text{C-3}), 79.24 (\text{C-4}), 57.9 (\text{C-5}), 35.9(\text{C-6}), 207.58(\text{C-7}), 50.15 (\text{C-8}), 46.3(\text{C-9}), 50.15(\text{C-10}) 17.36(\text{C-11}), 28.9(\text{C-12}),\]
37.5(C-13), 66.49(C-14), 53.59(C-15), 166.6(C-16),
77.24 (C-17), 0.0 (C-18), 64.6(C-19), 120.04 (C-20),
141.4 (C-21), 109.9(C-22), 143.04 (C23).

MS (rel. int.): m/z 413 (7), 348 (23), 347 (100), 329 (15), 287 (8),
241 (5), 227 (5), 145 (10), 136 (13), 135 (30), 121
(18), 108 (28), 95 (50), 91 (25) and 69 (27).

Further elution of the extract with chloroform afforded 4',5-
dihydroxy-6", 6"'-dimethylpyrano (2", 3", 7, 6) flavone 106.

M.P.: 295-98°C
Yield: 60 mg

IR (Nujol): 3400, 1650, 1600, 1450, 1350, 1240, 1180, 1130 and
840 cm⁻¹

¹H NMR (60 MHz, CDCl₃ + DMSO d₆):

δ 1.5 (6 H, s, CH₃), 5.6 (1H, d, J=~10 Hz), 6.2 (1H, s, H-8), 6.5 (1H, s, H-3), 6.70-7.55 (3H, m, Olefinic

MS (rel. int.): m/z 336 (M⁺, 52), 322 (40), 321 (100), 203 (60), 159
(10), 118 (5).