CHAPTER II
EXPERIMENTAL

1. CHEMICAL STUDIES

A. SYNTHESIS OF SUBSTITUTED O-NITROPHENOLS:

i) SYNTHESIS OF O-NITRO ACETOAMINOPHENI (VB1)

83.3g (45.3ml, 0.83 M) of concentrated H$_2$SO$_4$ was added continuously in a thin stream with stirring into 133.3ml of water kept in a litre three necked flask. 50g (0.583 M) of sodium nitrate was dissolved into this diluted acid. The mixture was allowed to cool in an ice bath.

50g (0.33 M) of paracetamol was mixed with 20ml of water and the slurry was added drop wise from a separating funnel to the vigorously stirred mixture in the reaction flask. The temperature was maintained at about 20°C. the stirring was continued for 2 hours after the addition of all the paracetamol slurry. 67ml of water was added in the flask, shaken well and the contents were allowed to settle. The wash liquor was poured off. The washing with water was repeated thrice to remove acid. The product was filtered and dried.

YIELD : 4.2g ; 25.42. %

m.p. : 155 - 159°C (uncorrected)

ii) SYNTHESIS OF 2-NITRORESORCINOL2 (VB2):

25ml concentrated H$_2$SO$_4$ (98 %) was added carefully to 5.5g (0.05 M) of resorcinol in a 150ml beaker with continuous stirring. Then the mixture was warmed to 60 - 65°C on a water bath and allowed to stand for 15 minutes. The slurry of 4,6-disulphonic acid was cooled at 0 - 10°C. A cold mixture of 4ml concentrated HNO$_3$ (72%) and 5.6ml of concentrated H$_2$SO$_4$ was added carefully into the cooled slurry of 4,6-disulphonic acid using well supported dropping funnel. The temperature of the reaction mixture was controlled below 20°C by using crushed ice bath. The mixture was allowed to stand for 15 minutes after the addition was complete. 15g of crushed ice was added into the reaction mixture to keep the temperature below 20°C. The resulting yellow - brown solution was transferred to a 250ml round bottom flask and steam distilled to collect about 250ml of distillate. The steam distillate was cooled and filtered to get yellow-orange precipitate of 2-nitroresorcinol.
iii) SYNTHESIS OF 2,4-DINITROPHENOL (VB3): A solution of 15.6g (0.15 M) of anhydrous sodium carbonate in 500ml water was placed in 1000ml round bottom flask fitted with reflux condenser. 12.5g (0.0625 M) of commercial 1 - chloro - 2, 4 - dinitrobenzene was added into it. The mixture was refluxed for 24 hours to pass the oil phase into the solution. The yellow solution was acidified with HCl. It was cooled and filtered. The crystalline dinitrophenol was separated. The product was dried and recrystallized from ethanol.

YIELD : 2.0g  
: 17.57 %  
m.p. : 113 - 115°C  
(uncorrected)

iv) SYNTHESIS OF O-NITRCINOL (VB4): 7.0g of orcinol (0.056 M) was added to a stirred mixture of concentrated HNO₃ (75ml) and water (113ml) at room temperature. The stirring was continued for 24 hours. The separated solid was filtered, washed with water and dried in air, recrystallized from ethanol.

YIELD : 2.2g  
: 23.06 %  
m.p. : 52-53°C  
(uncorrected)

v) SYNTHESIS OF 2-NITRO-4-CHLOROPHENOL (VB5): 7.0g 4-chlorophenol (0.0054 M) was added to a stirred mixture of concentrated HNO₃ (7.5ml) and water (113ml) at room temperature and stirring was continued for 24 hours. The separated solid was filtered, washed with water, dried in air and recrystallized from ethanol.

YIELD : 9.25g  
: 97.25 %  
m.p. : 80 - 82°C (uncorrected)
vi) SYNTHESIS OF 2-NITRO-4-CRESOL (VB6):
16.66g (9.06ml, 0.513 M) of concentrated H₂SO₄ was added continuously in a thin stream with stirring into 26.33ml of water kept in 1 litre three necked flask. 10g (1.17M) of sodium nitrate was dissolved into this diluted acid. The mixture was allowed to cool in an ice bath. 7.0g (0.67M) of p-cresol was dispersed in 4ml of water and drop wise added to the vigorously stirred mixture in the flask. The temperature was maintained at about 20°C. The stirring was continued for next 2 hours after all the cresol had been added. 13.4ml of water was added in the flask with shaking. The concentrate was allowed to settle down. The wash liquor was poured off. The washing with water was repeated thrice to remove acid. The liquor was kept in freeze for overnight, the oily product was separated.
YIELD : 5.0g :
: 50.45 %
: Oily liquid, m.p. cannot be reported.

vii) SYNTHESIS OF 2-NITRO-4-CHLORO-M-CRESOL (VB7):
16.66g (9.06ml, 0.17 M) of concentrated H₂SO₄ was added continuously in a thin stream into 1 litre water in 3-necked flask and then dissolves 10g NaNO₃ sodium nitrate in dilute acid. The solution was cooled in ice bath. 9.49ml (0.067 M) of chlorocresol was dispersed into 4ml of water and added drop wise to the vigorously stirred mixture in the flask. The temperature was maintained at about 20°C. The mixture was kept continuously stirring for next 2 hours after the addition was completed. 13.4ml of water was added in the flask with shaking. The concentrates were allowed to settle down. The washing with water was repeated thrice to remove acid. The product was frozen overnight. The separated solid filtered and dried.
YIELD :
: 7.0g
: 56.08 %
m.p. :
: 91-93°C
(uncorrected)

viii) SYNTHESIS OF 3-NITRO-4-HYDROXY BENZOIC ACID (VB8):
7.5175g of p-hydroxy benzoic acid (PHBA) (0.05447M) was added at room temperature to a stirred mixture of fuming HNO₃ (75ml) and concentrated H₂SO₄
The stirring was continued for 24 hours. Separated solid was filtered and washed with water, dried in air and recrystallized from ethanol.

**YIELD**

- 7.5g
- 75.30%

**m.p.**

178-180°C (uncorrected)

### B. SYNTHESIS OF SUBSTITUTED O-AMINOPHENOLS

#### i) SYNTHESIS OF 1-AMINOPHENOL (VB9):

1.5g freshly prepared Raney Nickel (2.5ml) was added in small portion into a warm clear solution of 2-nitrophenol (7g, 0.05 M) and hydrazine hydrate (100 %, 15ml). The mixture was refluxed on water bath for 1 hour. The mixture was filtered & evaporated to two-third of its volume to concentrate. It was poured in cold water (50ml). The separated solid was filtered and recrystallized using ethanol.

**YIELD**

- 2.8 g
- 51.09%

**m.p.**

163 - 167°C (uncorrected)

#### ii) SYNTHESIS OF O-AMINOPARACETAMOL (VB10):

2.4g freshly prepared Raney Nickel (4ml) was added in small portion into a warm clear solution of o-nitroacetaminophen (8g, 0.01 M) and hydrazine hydrate (100 %, 16ml). The mixture was refluxed on water bath for 1 hour. The mixture was filtered & evaporated to two-third of its volume to concentrate. It was poured in cold water (50ml). The separated solid was filtered and recrystallized using ethyl acetate.

**YIELD**

- 5.5g
- 71.00%

**m.p.**

160 - 163°C (uncorrected)

#### iii) SYNTHESIS OF O-AMINORESORCINOL (VB11):

3.0g freshly prepared Raney Nickel (5.0ml) was added in small portion into a warm clear solution of o-nitroresorcinol (1.786g, 0.0115 M) in absolute alcohol...
(30ml) and hydrazine hydrate (100 %, 3ml). The mixture was refluxed on water bath for 1 hour. The mixture was filtered & evaporated to two-third of its volume to concentrate. It was poured in cold water (250ml). The separated solid was filtered and recrystallized using methanol.

YIELD : 1.4g
   : 97.22 %
m.p. : above 240°C
   (uncorrected)

iv) SYNTHESIS OF 2,4-DIAMINOPHENOL (VB12):

Preparation of NaSH:
20g (0.0835 M) of crystallized sodium sulphide, Na₂S.9H₂O was dissolved in 75ml of water. 5g (0.155 M) of finely powdered sulphur was added into it and warmed until the solution was clear. The mixture of 13.8g (0.075 M) of 2,4-dinitrophenol and 100ml water was heated in 1000ml beaker to boil. The sodium polysulphide solution thus prepared was placed in a dropping funnel. The solution was added slowly into contents of the beaker over 30 · 35 minutes with vigorous stirring. The mixture was boiled and kept for 20 minutes.

The mixture was allowed to cool by using ice, filtered and washed with cold water. The mixture was transferred to 500ml beaker containing 75ml of water and 17.5ml of concentrated HCl. The mixture was boiled for 15 minutes. 2,4-dinitrophenol was dissolved leaving the sulfur and any unchanged 2,4-dinitrophenol.

The 2,4-diaminophenol was filtered and precipitated by addition of excess of concentrated aqueous ammonia solution. The precipitate was filtered off and recrystallised from boiling water.

YIELD : 1.2g
   : 89.55 %
m.p. : above 240°C
   (uncorrected)

v) SYNTHESIS OF O-AMINO ORCINOL (VB13):

Freshly prepared Raney Nickel was added (3g) in small portion to a warm clear solution of nitro-orcinol (2.1g, 0.115 M) in absolute alcohol (2.5ml). The mixture was refluxed on a water bath for 1 hours, filtered, concentrated and poured into
250ml cold water. The solid was separated. It was filtered off and recrystallized using methanol.

YIELD : 1.7g
: 98.83 %
m.p. : decomposed at 162°C
(uncorrected)

vi) SYNTHESIS OF 2-AMINO-4-CHLOROPHENOL (VB14):
1.5g freshly prepared Raney Nickel (2.5ml) was added in small portion into a warm clear solution of 2-nitro-4-chlorophenol (9.2g, 0.053M) in absolute alcohol (75ml) and hydrazine hydrate (100 %, 15ml). The mixture was refluxed on water bath for 1 hour. The mixture was filtered & evaporated to two-third of its volume to concentrate. It was poured in cold water (250ml). The separated solid was filtered and recrystallized using ethanol.

YIELD : 4.8g
: 62.90 %
m.p. : 140 - 142°C
(uncorrected)

vii) SYNTHESIS OF 2-AMINO-4-CRESOL (VB15):
3.0g freshly prepared Raney Nickel (5.0ml) was added in small portion into a warm clear solution of 2-nitro-4-cresol (5.0g, 0.033M) in absolute alcohol (75ml) and hydrazine hydrate (100 %, 7.5ml). The mixture was refluxed on water bath for 1 hour. The mixture was filtered & evaporated to two-third of its volume to concentrate. It was poured in cold water (250ml). The separated solid was filtered and recrystallized using ethanol.

YIELD : 3.9g
: 97.25 %
m.p. : 135 - 137°C
(uncorrected)

viii) SYNTHESIS OF 2-AMINO-4-CHLORO-M-CRESOL (VB16):
3g of freshly prepared Raney Nickel was added in small portions to the warm clear solution of 2-nitro-4-chloro-m-cresol (7.0g, 0.037M) in absolute alcohol (75ml) containing hydrazine hydrate (7.5ml, 100%). The mixture was refluxed on a water bath for 1 hour. The reaction mixture was filtered, concentrated and poured
into cold water (250ml). The separated solid was filtered and recrystallised using ethanol.
YIELD : 5.8g
: 98.63%
m.p. : 110-112°C
(uncorrected)

ix) SYNTHESIS OF 3-AMINO-4-HYDROXYBENZOIC ACID (VB17):
1.0g freshly prepared Raney Nickel (1.7ml) was added in small portion into a warm clear solution of 3-nitro-4-hydroxy benzoic acid (7.5g, 0.04M) absolute alcohol (50ml) and hydrazine hydrate (10ml 100%). The mixture was refluxed on water bath for 1 hour. The mixture was filtered & evaporated to two-third of its volume to concentrate. It was poured in cold water (500ml). The separated solid was filtered and recrystallized using ethanol.
YIELD : 6.2g
: 98.88%
m.p. : 125 - 128°C
(uncorrected)

x) SYNTHESIS OF 1-PHENYL AZO-2-NAPHTHOL7 (VB18):
20g (19.6ml, 0.125 M) of aniline was dissolved in a mixture of 55ml of concentrated HCl (1) and 55ml of water contained in a 500ml round bottom flask. Thermometer was placed in the solution and the flask was immersed in a bath of crushed ice (2). It was cooled until the temperature of the stirred solution dropped below 5°C. 16g sodium nitrate (3) was dissolved in 75ml of water and the solution was chilled by immersion in the ice bath. This solution of sodium nitrate was added in small volumes (2-3ml at a time) to the cold aniline HCl solution under continuous stirring. The temperature was controlled below 10°C by adding a few grams of ice to the reaction mix. (otherwise appreciable decomposition of the diazonium compound and or nitrous acid would occur). The last 5% the sodium nitrate solution was added more slowly. This was continued until a slight excess of nitrous acid was present.
Meanwhile, a solution of 7.8g of 2-naphthol (0.054 M) in 45ml of (10%) NaOH solution was prepared in an ice bath. The naphthol solution was stirred vigorously
and the diazonium salt solution was added very slowly. A red colour was developed, followed by separation of crystals of 1-phenylazo-2-naphthol. The mixture was allowed to stand for 30 minutes with occasional stirring. The solution was filtered through Buchner funnel with gentle wash of water. The crystals were drained thoroughly from water by pressing with glass stopper. Only one fourth of the crop was recrystallized from 30-35ml Glacial Acetic Acid (GAA). Remainder was retained for stannous chloride reduction. The recrystallized product was filtered with suction and washed with ethanol to eliminate acetic acid. The product was dried.

YIELD : 11.5g
: 74.77 %

m.p. : 128 - 129 °C
(uncorrected)

C. SYNTHESIS OF O-AMINOPHENOL HYDROCHLORIDES

i) SYNTHESIS OF 1-AMINOPHENOL HYDROCHLORIDE (VB19):

Dry HCl gas was passed through a warm clear solution of 2-aminophenol (2.8g, 0.002 M) in 200ml of ether till the precipitation was completed (2 hours). The separated solid was filtered and washed with ether. It was dried to give hydrochloride salt of 1-aminophenol.

YIELD : 2.4g
: 64.34 %

m.p. : 208 - 209 °C
(uncorrected)

ii) SYNTHESIS O-AMINOACETAMINOPHEN HYDROCHLORIDE (VB20):

Dry HCl gas was passed through a warm 200ml clear solution of dry acetone containing o-aminoparacetamol (2.5g, 0.015 M) till the precipitation was complete (2 hours). The product was filtered, washed with dry acetone and dried to give hydrochloride. This procedure was repeated twice.

YIELD : 6g
: 98.68 %

m.p. : 170 - 172 °C
(uncorrected)
iii) SYNTHESIS OF O-AMINO-RESORCINOL HYDROCHLORIDE (VB21):
Dry HCl gas was passed through a warm 200ml clear solution of dry ether containing o-aminoresorcinol (1.4g, 0.012 M) till the precipitation was complete (2 hours). The product was filtered, washed with dry acetone and dried to give hydrochloride. This procedure was repeated twice.
YIELD : 1.75g
: 97.22 %
m.p. : 143 - 145 °C
(uncorrected)

iv) SYNTHESIS OF 2,4-DIAMINOPHENOL HYDROCHLORIDE (VB22):
Dry HCl gas was passed through a warm 200ml clear solution of dry acetone containing 2,4-diaminophenol (1.24g, 0.01 M) till the precipitation was complete (2 hours). The product was filtered, washed with dry acetone and dried to give hydrochloride.
YIELD : 0.8g
: 50.00 %
m.p. : above 240°C
(uncorrected)

v) SYNTHESIS OF AMINO ORCINOL HYDROCHLORIDE (VB23):
Dry HCl gas was passed through a warm 200ml clear solution of benzene containing nitro-orcinol (1.7g, 0.012 M) till the precipitation was complete (2 hours). The product was filtered, washed with dry acetone and dried to give hydrochloride.
YIELD : 2.1g
: 98.13 %
m.p. : 93 - 95 °C
(uncorrected)

vi) SYNTHESIS OF 2-AMINO-4-CHLOROPHENOL HYDROCHLORIDE (VB24):
Dry HCl gas was passed through a warm 200ml clear solution of dry acetone containing 2-amino-4-chlorophenol (4.8g, 0.033 M) till the precipitation was complete (overnight). The mixture was concentrated and the separated solid was dried to give the hydrochloride.
vii) SYNTHESIS OF 2-AMINO-4-CRESOL HYDROCHLORIDE (VB 25):
Dry HCl gas was passed through a warm 200ml clear solution of dry acetone containing 2-amino-4-cresol (3.9g, 0.031 M) till the precipitation was complete (overnight). The mixture was concentrated and the separated solid was dried to give the hydrochloride.
YIELD: 4.5g
m.p. : above 240°C
(uncorrected)

viii) SYNTHESIS OF 2-AMINO-4-CHLORO-M-CRESOL HYDROCHLORIDE (VB26):
Dry HCl gas was passed through a warm 200ml clear solution of dry acetone containing 2-amino-4-chloro-m-cresol (5.3g, 0.033 M) till the precipitation was complete (overnight). The mixture was concentrated and the separated solid was dried to give the hydrochloride.
YIELD: 6.0g
m.p. : above 220°C
(uncorrected)

ix) SYNTHESIS OF 3-AMINO-4-HYDROXYBENZOIC ACID HYDROCHLORIDE (VB27):
Dry HCl gas was passed through a warm 200ml clear solution of dry ether containing 3-amino-4-hydroxybenzoic acid (6.2g, 0.041 M) till the precipitation was complete (2 hours). The oily phase was separated, product was filtered, washed with dry acetone and dried to give hydrochloride. This procedure was repeated twice.
YIELD: 7.6g
m.p. : above 240°C
(uncorrected)
x) SYNTHESIS OF 1-AMINO-2-NAPHTHOL HYDROCHLORIDE *(VB28)*:  
4g of the crude 1-phenylazo-2-naphthol (0.015 M) was placed in 500ml round bottom flask containing 100ml industrial spirit and fitted with reflux condenser. The mixture was boiled gently till the azo compound was dissolved. 20g of Tin (II) chloride was dissolved in 60ml of concentrated HCl by slight warming. This was added into the round bottom flask and boiled under reflux for 30 minutes. The azo compound was dissolved rapidly due to reduction by Tin (II) chloride. The solution turned to a very pale brown colour. The solution was decanted into a beaker containing ice to separate fine grayish white crystals of 1-amino-2-naphthol hydrochloride. The crystals were filtered and washed with 1:4 HCl. The crop was recrystallized from minimum volume of hot water containing a few drops of Tin (II) chloride solution in equal weight of hydrochloric acid. (this reduces atmospheric oxidation). The clear solution was cooled in ice bath and recrystallized product was collected again. The colourless crystals were dried in desiccator. The compound was protected from light during storage.

YIELD : 2g  
: 69.76 %  
m.p. : above 240°C  
(uncorrected)

xi) SYNTHESIS OF 2-AMINO-3-HYDROXY PYRIDINE HCl (VB29):  
Dry HCl gas was passed through a warm 200ml clear solution of dry ether containing 2-amino-3-hydroxy pyridine (5.0g, 0.047 M) till the precipitation was complete (2 hours). The separated product was filtered, washed with dry acetone and dried to give hydrochloride.

YIELD : 3.0g  
: 46.36 %  
m.p. : 178 - 180°C  
(uncorrected)

D : SYNTHESIS OF 5-BENZOAZOLINONES  
i) SYNTHESIS OF BENZOAZOLINONE (VB30):  
A mixture of o-aminophenol hydrochloride (2.4g, 0.0165 M) and urea (2.4g, 0.04 M) was fused at 140 - 150°C. The resulting solid was crystallized from benzene after decolourisation with activated charcoal.
ii) SYNTHESIS OF 5-CHLOROBENZOXAZOLINONE (VB31):
A mixture of 2-amino-4-chlorophenol hydrochloride (5.2g, 0.029 M) and urea (5.2g, 0.087 M) was mixed and fused at 140-150°C in a clean and dry porcelain dish. The resulting solid was recrystallised using methanol. The product came out as off-white crystalline compound.

YIELD : 2.2g
m.p. : 187-189°C
(uncorrected)

iii) SYNTHESIS OF 5-METHYLBENZOXAZOLINONE (VB32):
A mixture of 2-amino-4-cresol hydrochloride (4.5g, 0.0028M) and urea (4.5g, 0.075 M) was mixed and fused at 140-150°C in a clean and dry porcelain dish. The resulting solid was crystallized from water. The resulting solid was recrystallised using methanol. The product came out as off-white crystalline compound.

YIELD : 3.2g
m.p. : 198-202°C
(uncorrected)

iv) SYNTHESIS OF 5-AMINOBENZOXAZOLINONE (VB33):
A mixture of diaminophenol hydrochloride (0.8g, 0.005 M) and urea (4.5g, 0.075 M) was mixed and fused at 140-150°C in a clean and dry porcelain dish. The resulting solid was crystallized from water. The resulting solid was recrystallised using methanol. The product came out as off-white crystalline compound.

YIELD : 0.5g
m.p. : 130-135°C
(uncorrected)
v) SYNTHESIS OF 5-HYDROXYBENZOXAZOLINONE (VB34):
A mixture of o-amino resorcinol hydrochloride (oily liquid) (1.75g, 0.01 M) and urea (4.5g, 0.075 M) was mixed and fused at 140-150°C in a clean and dry porcelain dish. The resulting solid was recrystallised using ethanol. The product came out as off-white crystalline compound.

YIELD : 1.6g
m.p. : decomposed at 68°C
(uncorrected)

vi) SYNTHESIS OF 5-CARBOXYBENZOXAZOLINONE (VB35):
A mixture of 3-amino-4-hydroxybenzoic acid hydrochloride (7.2g, 0.038 M) and urea (2.7g, 0.045M) was mixed and fused at 140-150°C in a clean and dry porcelain dish. The resulting solid was crystallized from water. The product was recrystallised using ethanol resulting into off-white crystalline compound.

YIELD : 6.91g
m.p. : 171-173°C
(uncorrected)

vii) SYNTHESIS OF 5-ACETAMIDOBENZOXAZOLINONE (VB36):
A mixture of o-aminoacetaminophen hydrochloride (3g, 0.015 M) and urea (3g, 0.05 M) was mixed and fused at 140-150°C in a clean and dry porcelain dish. An oily dark brown coloured compound was obtained. It was kept in freeze for 15 days. Benzene extract of it was also found to be oily. Aqueous layer was again extracted with solvent ether. This gave a light yellow product. The product was recrystallised using ethanol.

YIELD : 5.6g
m.p. : above 240°C
(uncorrected)

viii) SYNTHESIS OF 4-HYDROXY-5-METHYLBENZOXAZOLINONE (VB37):
A mixture of amino-orcinol hydrochloride (2.1g, 0.011M) and urea (2.1g, 0.036 M) was mixed and fused at 140-150°C in a clean and dry porcelain dish. The
resulting solid was crystallized from water. The product was recrystallised using 70% ethanol resulting into off-white crystalline compound. The product was recrystallised using ethanol.

YIELD : 1.9g
m.p. : 185 - 188°C
(uncorrected)

ix) SYNTHESIS OF 4-METHYL-5-CHLOROBENZOXAZOLINONE (VB38):
A mixture of 2-amino-4-chloro-m-cresol hydrochloride (5.0g, 0.03M) and urea (3.0g, 0.05M) was mixed and fused at 140-150°C in a clean and dry porcelain dish. The resulting solid was crystallized from water. The product was recrystallised using 70% ethanol resulting into off-white crystalline compound.

YIELD : 0.5g
m.p. : 162 - 165°C
(uncorrected)

x) SYNTHESIS OF 5,6-(BUT-2,4-DIENE)-BENZOXAZOLINONE (VB39):
A mixture of 1-amino-2-naphthol hydrochloride (2.4g, 0.012M) and urea (2.4g, 0.04M) was mixed and fused at 140-150°C in a clean and dry porcelain dish. The resulting solid was recrystallised using 70% ethanol resulting into off-white crystalline compound.

YIELD : 1.7g
m.p. : 190 - 195°C
(uncorrected)

xi) SYNTHESIS OF (VB40):
A mixture of 2-amino-3-hydroxy pyridine hydrochloride (3.0g, 0.021M) and urea (3.0g, 0.05M) was fused at 140 - 150°C in a clean and dry porcelain dish. The resulting black solid was crystallized from benzene, after decolourisation with activated charcoal to give product. The product was recrystallised using ethanol.

YIELD : 0.9g
m.p. : 133 - 138°C (uncorrected)
E. SYNTHESIS OF 5-BENZOXAZOLINONES ADDUCTS WITH GABA:

i) CONDENSATION OF GABA WITH VB30 (VB41):

VB10 compound (1.5g, 0.011 M) was dissolved in 20ml methanol in clean dry round bottom flask. GABA (1.27g, 0.011M) was dissolved into 10ml absolute alcohol. GABA solution was added into the solution of the compound in small portions. The mixture was refluxed for few minutes on water bath.

In a clean dry beaker, weighed DCC (2.28g, 0.011 M), and triethylamine (3.05ml, 0.011 M). 30ml THF was added into it to make the solution clear. This clear solution was added drop wise through the condenser into reaction mixture kept in round bottom flask with continuous shaking over a period of 15 minutes. After completion of addition, the reaction was continued to reflux for 2 hours in water bath. The round bottom flask with reaction mixture was cooled to room temperature. The reaction mixture was filtered and filtrate was concentrated on water bath. The solid was precipitated out by addition of water and the product was crystallized using methanol.

YIELD : 1.6g

: 76.92%

m.p. : 63 - 67 °C

(uncorrected)

ii) CONDENSATION OF GABA WITH VB31 (VB42):

VB31 compound (2.2g, 0.014 M) was dissolved in 20ml methanol in clean dry round bottom flask. GABA (1.5g, 0.014 M) was dissolved into 10ml absolute alcohol. GABA solution was added into the solution of the compound in small portions. The mixture was refluxed for few minutes on water bath. In a clean dry beaker, weighed DCC (2.67g, 0.014 M) and triethylamine (3.56ml, 0.014 M). 30ml THF was added into it to make the solution clear. This clear solution was added drop wise through the condenser into the reaction mixture kept in the round bottom flask with continuous shaking over a period of 15 minutes. After completion of addition, the reaction was continued to reflux for 2 hours in water bath. The round bottom flask with reaction mixture was cooled to room temperature. The reaction mixture was filtered and filtrate as concentrated on
water bath. The solid was precipitated out by addition of water and the product was crystallized using methanol.

YIELD : 2.5g

m.p. : 218 - 221°C

(uncorrected)

iii) CONDENSATION OF GABA WITH VB32 (VB43):

VB32 compound was dissolved (3.3g, 0.02 M) in 20ml absolute alcohol in dry round bottom flask. GABA (2.3g, 0.02 M) was dissolved into 10ml absolute alcohol. GABA solution was added into the solution of the compound in small portions. The mixture was refluxed for few minutes.

In a clean dry beaker, weighed DCC (4.12g, 0.02 M) and 5.5ml (4.034g, 0.04 M). 30ml THF was added into it to make the solution clear. This clear solution was added drop wise through the condenser into the reaction mixture kept in the round bottom flask with continuous shaking over a period of 15 minutes. After completion of addition, the reaction was continued to reflux for 2 hours in water bath. The round bottom flask with reaction mixture was cooled to room temperature. The reaction mixture was filtered and filtrate was concentrated on water bath. The solid was precipitated out by addition of water and the product was crystallized using methanol.

YIELD : 3.5g

m.p. : 113 - 117°C

(uncorrected)

iv) CONDENSATION OF GABA WITH VB33 (VB44):

VB33 compound (0.5g, 0.033M) was dissolved in 10ml methanol in clean dry round bottom Flask. GABA (0.38g, 0.033M) dissolved in 10ml absolute alcohol in a dry test tube. This GABA solution was added into the solution of the compound in small portions. The mixture was refluxed for few minutes on water bath.

In a clean dry beaker, weighed DCC (0.68, 0.033 M) and triethylamine (0.91ml, 0.033 M) . 20ml THF was added into it to make the solution clear. This clear solution was added drop wise through the condenser into the reaction mixture kept in the round bottom flask with continuous shaking over a period of 15 minutes. After completion of addition, the reaction was continued to reflux for 2 hours in
water bath. The round bottom flask with reaction mixture was cooled to room temperature. The reaction mixture was filtered and filtrate was concentrated on water bath. The solid was precipitated out by addition of water and the product was crystallized using methanol.

**YIELD**: 0.5g  
**m.p.**: 76-79°C  
(uncorrected)

v) CONDENSATION OF GABA WITH VB34 (VB45):

VB34 compound (2.0g, 0.0132 M) was dissolved in 20ml methanol in clean dry round bottom Flask. GABA (1.437g, 0.0132M) was dissolved in 10ml absolute alcohol. This GABA solution was added into it in small portions. The mixture was refluxed for few minutes on water bath.

In a clean dry beaker, weighed DCC (2.57g, 0.0132 M) and triethylamine (3.43ml, 0.0132 M). 30ml THF was added into it to make the solution clear. This clear solution was added drop wise through the condenser into the reaction mixture kept in the round bottom flask with continuous shaking over a period of 15 minutes. After completion of addition, the reaction was continued to reflux for 2 hours in water bath. The round bottom flask with reaction mixture was cooled to room temperature. The reaction mixture was filtered and filtrate was concentrated on water bath. The solid was precipitated out by addition of water and the product was crystallized using methanol.

**YIELD**: 2.4g  
**m.p.**: 168-171°C  
(uncorrected)

vi) CONDENSATION OF GABA WITH VB35 (VB46):

VB35 compound (3.2g, 0.018 M) was dissolved in 20ml absolute alcohol (methanol) in clean dry round bottom Flask. GABA (2.047g, 0.018 M) was added in 10ml absolute alcohol. This GABA solution was added into it in small portions. The mixture was refluxed for few minutes on water bath.

In a clean dry beaker, weighed DCC (3.672g, 0.0132 M) and triethylamine (4.88ml, 0.0357 M). 20ml methanol was added into it to make the solution clear. This clear solution was added drop wise through the condenser into the reaction mixture kept
in the round bottom flask with continuous shaking over a period of 15 minutes. After completion of addition, the reaction was continued to reflux for 2 hours in water bath. The round bottom flask with reaction mixture was cooled to room temperature. The reaction mixture was filtered and filtrate was concentrated on water bath. The solid was precipitated out by addition of water and the product was crystallized using methanol.

YIELD : 3.45g  
: 75.77%

m.p. : 121 - 125°C  
(uncorrected)

vii) CONDENSATION OF GABA WITH VB36 (VB47):

VB36 compound was dissolved (3.89g, 0.02 M) in 25ml absolute alcohol in dry round bottom Flask. GABA (2.3g, 0.02M) was dissolved into 10ml alcohol. This GABA solution was added into it in small portions. The mixture was refluxed for few minutes.

In a clear dry beaker, weighed DCC (412g, 0.02 M) and triethylamine 5.52ml (4.034g, 0.040 M). 30ml THF was added into it to make the clear solution. This clear solution was added drop wise through the condenser into the reaction mixture kept in the round bottom flask with continuous shaking over a period of 15 minutes. After completion of addition, the reaction was continued to reflux for 2 hours in water bath. The round bottom flask with reaction mixture was cooled to room temperature. The solids were filtered out and filtrate was concentrated on water bath. The solid was precipitated out by addition of water and the product was crystallized using methanol.

YIELD : 3.0g  
: 43.13%

m.p. : 85-88°C  
(uncorrected)

viii) CONDENSATION OF GABA WITH VB37 (VB48):

VB37 compound was dissolved (3.3g, 0.02M) in 20ml absolute alcohol in dry round bottom flask. GABA (2.3g, 0.02 M) was dissolved into in 10ml absolute alcohol. This GABA solution was added into it in small portions. The mixture was refluxed for few minutes.
In a clean dry beaker, weighed DCC (412g, 0.02M) and triethylamine 5.5ml (4.034g, 0.0402M). 30ml THF was added into it to make the solution clear. This clear solution was added drop wise through the condenser into the reaction mixture kept in the round bottom flask with continuous shaking over a period of 15 minutes. After completion of addition, the reaction was continued to reflux for 2 hours in water bath. The round bottom flask with reaction mixture was cooled to room temperature. The solids were filtered out and filtrate was concentrated on water bath. The solid was precipitated out by addition of water and the product was crystallized using methanol.

YIELD : 3.8g
: 79.83%
m.p. : 188 - 191 °C
(uncorrected)

ix) CONDENSATION OF GABA WITH VB38 (VB49):

VB38 compound was dissolved (3.95g, 0.02 M) in 20ml absolute alcohol in dry round bottom Flask. GABA (2.3g, 0.02M) was dissolved into 10ml absolute alcohol. This solution was added into it in small portions. The mixture was refluxed for few minutes.

In a clean dry beaker, weighed DCC (412g, 0.02 M) and triethylamine 5.52ml (4.034g, 0.04 M). 30ml THF was added into it to make the solution clear. This clear solution was added drop wise through the condenser into the reaction mixture kept in the round bottom flask with continuous shaking over a period of 15 minutes. After completion of addition, the reaction was continued to reflux for 2 hours in water bath. The round bottom flask with reaction mixture was cooled to room temperature. The solids were filtered out and filtrate was concentrated on water bath. The solid was precipitated out by addition of water and the product was crystallized using methanol.

YIELD : 0.8g
: 95.80%
m.p. : 152 - 155 °C
(uncorrected)

x) CONDENSATION OF GABA WITH VB39 (VB50):

VB39 compound (1.7g, 0.009 M) was dissolved in 20ml methanol in dry round bottom Flask. GABA (0.05g, 0.009 M) was dissolved into 5ml absolute alcohol.
This GABA solution was added into it in small portions. The mixture was refluxed for few minutes.

In a clean dry beaker, weighed DCC (1.89g, 0.009 M) and triethylamine (2.52ml, 0.0091M). 30ml THF was added into it to make the solution clear. This clear solution was added drop wise through the condenser into the reaction mixture kept in the round bottom flask with continuous shaking over a period of 15 minutes. After completion of addition, the reaction was continued to reflux for 2 hours in water bath. The round bottom flask with reaction mixture was cooled to room temperature. The reaction mixture was filtered and filtrate was concentrated on water bath. The solid was precipitated out by addition of water and the product was crystallized using methanol.

YIELD : 1.8g
: 77.75%
m.p. : 207 - 210 °C
(uncorrected)

xi) CONDENSATION OF GABA WITH VB40 (VB51):

VB40 compound was dissolved (0.8g, 0.0058 M) in 10ml methanol in dry round bottom Flask. GABA (0.67g, 0.0058 M) was dissolved into 5ml absolute alcohol. This GABA solution was added into it in small portions. The mixture was refluxed for few minutes.

In a clean dry beaker, weighed DCC (1.121g, 0.0058 M) and triethylamine (1.61ml, 0.0058M). 15ml THF was added into it to make the solution clear. This clear solution was added drop wise through the condenser into the reaction mixture kept in the round bottom flask with continuous shaking over a period of 15 minutes. After completion of addition, the reaction was continued to reflux for 2 hours in water bath. The round bottom flask with reaction mixture was cooled to room temperature. The reaction mixture was filtered and filtrate was concentrated on water bath. The solid was precipitated out by addition of water and the product was crystallized using methanol.

YIELD : 0.9g
: 75.37 %
m.p. : 133 - 146 °C
(uncorrected)
PHYSICOCHEMICAL STUDIES:

1] THIN LAYER CHROMATOGRAPHY

In order to ascertain the purity and homogeneity of synthesized compounds, primarily, thin layer chromatography was carried out. The solvent systems used for different series of compounds, are shown below:

Step A : VB1 to VB8
Benzene : Ethyl acetate : Methanol (70:10:20)

Step B : VB9 to VB18
Benzene : Ethyl acetate : Methanol (75:15:10)

Step C : VB19 to VB29
Benzene : Ethyl acetate : Methanol (65:15:20)

Step D : VB30 to VB40
Benzene : Ethyl acetate : Methanol (85:05:10)

Step E : VB41 to VB51
Benzene : Ethyl acetate : Methanol (60:30:10)

Silicagel-G was used as an adsorbent. The spots were located by using iodine vapour chamber. Rf value was calculated for each compound by using following formula.

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R_f = \frac{\text{Distance traveled by the compound}}{\text{Distance traveled by the solvent}}
\]

2] ROTATIONAL AND VIBRATIONAL ABSORPTION STUDIES

Infrared absorption spectra of all synthesized compounds in Nujol were obtained by using Infrarad spectrophotometer (Perkin-Elmer 883 model). The scanning time was 5.8 minutes.

3] NMR SPECTRAL STUDIES

The synthesized compounds were dissolved in deuterated chloroform (CDCl₃) and subjected to NMR spectral studies on Brucker 200 Mega Spectrometer by standard method.
4] ELEMENTAL ANALYSIS
All the compounds were subjected to elemental analysis and the results were tabulated.

PRELIMINARY PHARMACOLOGICAL SCREENING
1] NEUROPHARMACOLOGICAL SCREENING
The neuropharmacological screening procedure proposed by Irwin\textsuperscript{19}, is an excellent method to obtain clues of activity on central nervous system and other fields of activity of a test compound. Before undertaking a detailed pharmacological testing, it becomes imperative to understand whether a group of compounds under test is worthy for further attention and if it is, which among them have the most interesting pharmacological properties.

Method
Albino rats weighing between 100-120g were divided into the groups having two animals each. They were given the intraperitoneal injection of test compounds. The compounds were given in two doses, i.e. 100 mg / kg and 200 mg / kg. Injections were given at constant volume of 1.0ml/100gm of body weight. A group of control rats was given equal volume of 0.9% sterile saline. Observations were made after 15 minutes and effects of test compounds on the animals were scored with nine degrees from 0 to 8. The base score below 4 was considered as subnormal response and those above 4 were considered as supernormal. The base score for abnormal sign was 0 and the maximal score was 8.

2] EFFECTS ON CENTRAL NERVOUS SYSTEM\textsuperscript{14}
2.1 Motor Activity
2.1.1 Using Actophotometer :
The effect of test compounds on the spontaneous locomotor activity of mice was measured by employing Actophotometer. (Centronix, Consolidate Electronics, Mumbai). A group of four mice was used for each compound. A dose of 100 mg / kg in the fine suspension form in water with the help of Tween-80 was injected intraperitoneally.
Mice were housed in Actophotometer and control readings were recorded for every 5 minutes. The same group after receiving test compounds, was housed in
Actophotometer and locomotor activity was recorded for every 5 minutes. Marketed CNS depressant drug, Calm pose® injection (Ranbaxy Laboratory Limited, H.P.) containing 10 mg Diazepam in 2.0ml was given to one group at a dose of 20 mg/kg intraperitoneally.

2.1.2 Using Rotarod
Mice were placed on a rod of 2.0 cm in diameter turning at the rate of 10 rotation per minute. The control mouse was able to remain on the rod for about thirty minutes. The mice were treated with synthesized compounds at the dose of 50 mg/kg in fine suspension form in water with the help of Tween 80. Injection was given intraperitoneally. The treated mice were placed on the rod at the intervals and the time of the fall from the rod was noted. A group of two mice was used for one compound and average readings were taken. Marketed CNS depressant drug, Calm pose® injection (Ranbaxy Laboratory Limited, H.P.) containing 10 mg Diazepam in 2.0ml was given to one group at a dose of 20 mg/kg intraperitoneally.

2.2] Anticonvulsant Activity
Albino rats of either sex, weighing between 100-200g were used for testing. Each group contained two animals. Minimum shock to induce seizures was found to be 0.96 mA. Tween 80 and propylene glycol were tested before experiment for anticonvulsant action and found to be neutral. Marketed for anticonvulsant drug, Mazetol- (S.G. Chemicals, Baroda), containing Carbamazepine 200 mg was given to one group, 20 mg / kg intraperitoneal in propylene glycol. Test compounds were made into fine suspension in water, with the help of Tween 80 and given at the doses of 50 mg / kg intraperitoneally. All the test compounds and carbamazepine were given at a constant volume of 1ml / 100g of body weight. In totally ineffective test compound, the next higher dose 100 mg/kg intraperitoneally was tried to get result. The shock was delivered after each 30 minutes for two hours and degree of protection against convulsions was noted.

Approximate LD$_{50}$ value for intraperitoneal route for only most effective test compounds from each series was determined on albino rats weighing between 100-200g. The animals were observed for 24 hours.

Four groups each containing six animals were made and LD$_{50}$ value for test compound was calculated using graphical method of Miller and Tainter.
REFERENCES :-


