# CHAPTER - IV

## Experimental

A. Chemical Studies
B. Biological Studies

# EXPERIMENTAL WORK

## A. CHEMICAL STUDIES

All melting points reported were taken on Veego make Silicone oil bath-type melting point apparatus and are uncorrected. NMR spectra (on Varian 300 MHz) were recorded in $CDCl_3$ (unless specified) with TMS as internal reference (chemical shift in δ, ppm), IR spectra (Shimadzu FT-IR, 8300) in KBr (ν max in $cm^{-1}$) and UV spectra (Shimadzu, UV- 240) in methanol (λ max in nm; figure within parenthesis refer to log ε values). Mass spctra at 70 eV on a MASPEC msw 9629 instrument. Elemental analysis has been performed in Carlo-Erba elemental analyzer. Silica gel G (E. Merck) was used for TLC and iodine vapours used for exposure of TLC plates. Anhydrous sodium sulphate (S.D. fine chemicals) was used as drying agent.

The chemical studies of the present work are discussed under the following heads:

**Series I:** **1-Substituted-4-phenyl-*s*-triazolo[4,3-*a*]quinazolin-5(4*H*)-ones (1-5)**

**Series II:** **1-Substituted-4-(3-methylphenyl)-*s*-triazolo[4,3-a] quinazolin-5(4*H*)-ones (6-10)**

**Series III:** **1-Substituted-4-(4-methylphenyl)-*s*-triazolo[4,3-*a*] quinazolin-5(4*H*)-ones (11-15)**

**Series IV:** **1-Substituted-4-(4-methoxyphenyl)-*s*-triazolo[4,3-*a*] quinazolin-5(4*H*)-ones (16-20)**

**Series V:** **1-Substituted-4-(4-chlorophenyl)-*s*-triazolo[4,3-*a*] quinazolin-5(4*H*)-ones (21-25)**

**Series VI:** **1-Substituted-4-(2-pyridyl)-*s*-triazolo[4,3-*a*]quinazolin-5(4*H*)-ones (26-30)**

**Series I**

**1-Substituted-4-phenyl-*s*-triazolo[4,3-*a*]quinazolin-5(4*H*)-one (1-5)**

**General Procedure:**

**2-Thioxo-3-substituted quinazolin-4(3*H*)-one (III)**

A solution of the respective arylamine (0.02 moles) in dimethyl sulfoxide (10ml) was stirred vigorously. To this was added carbon disulphide (1.6ml) and aqueous sodium hydroxide (1.2ml, 20 molar) dropwise during 30 min. with stirring. Dimethyl sulphate (2.5gm, 0.02mole) was added gradually keeping the reaction mixture stirring in freezing mixture for 2 h. The reaction mixture was then poured into ice water. The solid obtained was filtered, washed with water, dried and recrystallized from ethanol.

Methyl anthranilate (1.5gm, 0.01mole) and the above prepared N-(aryl)-methyl dithiocarbamic acid (0.01 mole), were dissolved in ethanol (20ml). To this anhydrous potassium carbonate (100 mg) was added and refluxed for the specified time. The reaction mixture was cooled in ice and the solid separated was filtered and purified by dissolving in 10% alcoholic

sodium hydroxide solution and reprecipitated by treating with dilute hydrocholoric acid. The solid obtained was filtered, washed with water, dried and recrystallized.

**2-Thioxo-3-phenyl quinazolin-4(3*H*)-one (IIIa)**

Aniline was subjected to the above described procedure to yield a solid product (the reaction time was 18 h). It was recrystallized from ethanol to afford **(IIIa)** 85.3 % . It showed a m.p. of 305-306°C (Lit[233] >300°C)

Anal:

TLC : Rf 0.73 ($C_6H_6$ : MeOH::9:1)

IR (KBr) : 3222, 3134, 3028, 1622, 842 and 759 $cm^{-1}$

**2-Methylthio-3-substituted quinazolin-4(3*H*)-ones (IV)**

The 2-thioxo-3-substituted quinazolin-4(3*H*)-one (0.01 mole) was dissolved in 40 ml of 2% aicoholic sodium hydroxide solution. To this dimethyl sulphate (1.26 gm, 0.01 moles) was added dropwise with stirring and stirring was continued for 1 h after completion of addition. The reaction was then poured into ice water. The solid obtained was filtered, washed with water, dried and recrystallized.

**2-Methylthio-3-phenyl quinazolin-4(3*H*)-one (IVa)**

A solid was obtained when 2-thioxo-3-phenyl quinazolin-4(3*H*)-one **(IIIa)** was reacted with dimethyl sulphate by the above procedure. It was recrystallized from ethanol-chloroform (75:25) mixture to afford **(IVa)** in 88 % yield. It showed a m.p. of 124-126°C (Lit[234] 124°C).

Anal:

| | |
|---|---|
| TLC | : Rf 0.65 ($C_6H_6$: $CHCl_3$:: 9:1) |
| IR (KBr) | : 1689, 1604, 779 and 761 $cm^{-1}$ |

**2-Hydrazino-3-substituted quinazolin-4(3*H*)-one (V)**

The 2-methylthio-3-substituted quinazolin-4(3*H*)-one (0.01 mole) was dissolved in ethanol (25ml). To this hydrazine hydrate (99%) (5.0gm, 0.1 mole) and anhydrous potassium carbonate (100 mg) was added and refluxed for specified time. The reaction mixture was cooled and poured into ice-water. The solid so obtained was filtered, washed with water, dried and recrystallized to afford the product **(V).**

**2-Hydrazino-3-phenyl quinazolin-4(3*H*)-one (Va)**

2-Methylthio-3-phenyl quinazolin-4(3*H*)-one **(IVa)** when reacted by the above procedure yielded a solid (the reaction time was 22 h). The solid

was recrystallized from chloroform-benzene (25:75) mixture to afford **(Va)** 80.5 %. The solid showed a m.p. of 158-160°C (Lit[234] 142° C).

Anal:

| | |
|---|---|
| TLC | : Rf 0.58 ($C_6H_6$: MeOH:: 9:1) |
| IR (KBr) | : 3318, 3275, 1678 and 798 $cm^{-1}$ |

**1-substituted-4-phenyl-*s*-triazolo[4, 3-*a*]quinazolin-5(4*H*)-ones (1-5)**

The 2-hydrazino-3-substituted quinazolin-4(3*H*)-one **(V)** (0.01 mole) and formic acid (25ml) was taken in a round bottomed flask and refluxed for the specified time, cooled and poured into ice water. The solid obtained was filtered, washed with water, dried and recrystallized to yield **(1)**.

The above procedure was repeated with acetic acid, propionic acid, butyric acid and chloroacetyl chloride to yield the title compounds **2**, **3**, **4** and **5** respectively.

**4-phenyl-*s*-triazolo[4, 3-*a*]quinazolin-5(4*H*)-one (1)**

2-Hydrazino-3-phenyl quinazolin-4(3*H*)-one **(Va)** when reacted by the above procedure yielded a solid (the reaction time was 20 h) which was recrystallized from chloroform-ethanol (75:25) mixture to afford **(1)** 89.3 %. It showed a m.p. of 262-265°C (Lit[234] 197°C).

Anal:

| | | | | |
|---|---|---|---|---|
| TLC | : Rf 0.5 ($C_6H_6$ : MeOH:: 9:1) | | | |
| UV (MeOH) | : 232 nm (4.3) and 286 nm (4.0) | | | |
| IR (KBr) | : 3114, 3048, 1692, 1611, 1594 and 715 $cm^{-1}$ | | | |
| Elemental analyses | : | C | H | N |
| | % Calculated : | 68.69 | 03.84 | 21.36 |
| | % Found : | 69.65 | 03.85 | 21.83 |

**1-Methyl-4-phenyl-*s*-triazolo[4,3-*a*]quinazolin-5(4*H*)-one (2)**

2-Hydrazino-3-phenyl quinazolin-4(3*H*)-one **(Va)** when reacted by the above procedure yielded a solid (the reaction time was 29 h). It was recrystallized from chloroform-ethanol (75:25) mixture to afford (2) 86.5 %. The solid showed a m.p. of 276-279°C.

How can it methan if HCOOH is there I mixed have been CH3CN

Anal:

| | | | | |
|---|---|---|---|---|
| TLC | : Rf 0.43 ($C_6H_6$ : MeOH:: 9:1) | | | |
| UV (MeOH) | : 231 nm (4.4) and 283 nm (4.0) | | | |
| IR (KBr) | : 1699, 1626, 1608 and 763 $cm^{-1}$ | | | |
| NMR ($CDCl_3$) | : δ 2.4- 2.5 (s, 3*H*; $CH_3$) and 7.3-7.7 (m, 9*H*; ArH). | | | |
| Elemental analyses | : | C | H | N |
| | % Calculated : | 69.55 | 04.38 | 20.28 |
| | % Found : | 69.24 | 04.35 | 20.20 |

**1-Ethyl-4-phenyl-*s*-triazolo[4, 3-*a*] quinazolin-5(4*H*)-one (3)**

2-Hydrazino-3-phenyl quinazolin-4(3*H*)-one **(Va)** when reacted by the above procedure yielded a solid (the reaction time was 30 h). It was recrystallized from ethanol to afford **(3)** 80 %. The solid showed a m.p. of 190-193°C.

Anal:

| | |
|---|---|
| TLC | : Rf 0.45 ($C_6H_6$: MeOH:: 9:1) |
| UV (MeOH) | : 230 nm (4.3) and 283 nm (3.7) |
| IR (KBr) | : 1676, 1629, 770 and 750 $cm^{-1}$ |
| NMR ($CDCl_3$) | : $\delta$ 1.3-1.35 (t, 3*H*; $CH_2C\underline{H}_3$), 2.7-2.8 (q, 2*H*; $C\underline{H}_2CH_3$) and 7.2-8.5 (m, 9*H*; ArH) |

Elemental analyses :

| | | C | H | N |
|---|---|---|---|---|
| % Calculated | : | 70.33 | 04.86 | 19.29 |
| % Found | : | 70.60 | 04.59 | 19.72 |

**1-Propyl-4-phenyl-*s*-triazolo [4, 3-*a*] quinazolin-4(3*H*)-one (4)**

2-Hydrazino-3-phenyl quinazolin-4(3*H*)-one **(Va)** when reacted by the above procedure yielded a solid (the reaction time was 20 h). It was recrystallized from chloroform-ethanol (75:25) mixture to afford **(4)** in 76 % yield and m.p. of 190-194°C.

Anal:

TLC : Rf 0.46 ($C_6H_6$ : MeOH::9:1)

UV (MeOH) : 231 nm (4.4) and 284 nm (3.8)

IR (KBr) : 1692, 1631, 1608 and 767 $cm^{-1}$

NMR ($CDCl_3$) : δ 0.9-1.0 (t, 2*H*; $C\underline{H}_2CH_2CH_3$), 1.7-1.8 (sext, 2*H*; $-CH_2C\underline{H}_2CH_3$), 2.7-2.8 (t, 3*H*; $-CH_2CH_2C\underline{H}_3$) and 7.3-7.7 (m, 9*H*; ArH)

Mass (m/z) : 304 $M^+$, 290 (12%), 289 (49%), 276 (13%), 275 (19%), 221 (5%), 105 (13%) and 77 (25%).

**1-Chloromethyl-4-phenyl-*s*-triazolo[4,3-*a*]quinazolin-5(4*H*)-one (5)**

A solid was obtained by the above procedure when 2-hydrazino-3-phenyl quinazolin-4(3*H*)-one **(Va)** was taken as the starting (the reaction time was 12 h). It was recrystallized from chloroform-ethanol (75:25) mixture to afford **(5)** in 78.2 % yield. It showed a m.p. of 258-260°C.

Anal:

TLC : Rf 0.28 ($C_6H_6$ : MeOH::9:1)

UV (MeOH) : 232 nm (4.5) and 285 nm (4.1)

IR (KBr) : 1699, 1624, 869, 801, 783 and 753, $cm^{-1}$

Mass (m/z) : 310 $M^+$, 276 (94%), 275 (65%), 234 (4%), 208 (8%), 144 (3%), 130 (3%), 105 (28%) and 77 (100%, base peak).

**Series II**

**1-Substituted-4-(3-methylphenyl)-*s*-triazolo [4,3-*a*]quinazolin-5(4*H*)-one (6-10)**

**2-Thioxo-3-(3-methylphenyl) quinazolin-4(3*H*)-one (IIIb)**

3-methyl aniline as the aromatic amine yielded a solid by the above procedure (the reaction time was 21 h). It was recrystallized from ethanol to afford **(IIIb) in** 75 % yield with a m.p. of 286-289°C (Lit[233] 268-270°C).

Anal:

TLC : Rf 0.76 ($C_6H_6$ : MeOH::9:1)

UV (MeOH) : 218 nm (log ε 4.3) and 292 nm (4.2)

**2-Methylthio-3-(3-methylphenyl) quinazolin-4(3*H*)-one (IVb)**

2-Thioxo-3-(3-methylphenyl) quinazolin-4(3*H*)-one **(IIIb)** when reacted with dimethylsulphate by the above procedure yielded a solid which was recrystallized from ethanol-chloroform (75:25) mixture to afford **(IVb)** 81 % . The solid showed a m.p. of 148-150° C.

Anal:

TLC : Rf 0.67 ($C_6H_6$ : $CHCl_3$::9:1)

UV (MeOH) : 222 nm (4.5) and 276 nm (4.0)

IR (KBr) : 1678, 1605, 811 and 763 $cm^{-1}$

NMR ($CDCl_3$) : $\delta$2.4 (s, 3*H*; $CH_3$), 2.5 (s, 3*H*; $SCH_3$) and 7.1-8.2 (m, 8*H*; ArH).

**2-Hydrazino-3-(3-methylphenyl) quinazolin-4(3*H*)-one (Vb)**

2-Methylthio-3-(3-methylphenyl) quinazolin-4(3*H*)-one **(IVb)** on reaction with hydrazine hydrate as described above yielded a solid (the reaction time was 30 h). It was recrystallized from chloroform-benzene (25:75) mixture to afford **(Vb)** in 76 % yield with a m.p. of 195-197° C.

Anal:

TLC : Rf 0.61 ($C_6H_6$ : MeOH::9:1)

UV(MeOH) : 221 nm (log$\varepsilon$ 4.3) and 282 nm (log$\varepsilon$ 4.0)

IR (KBr) : 3320, 3205, 1674 and 762 $cm^{-1}$

NMR ($CDCl_3$) : $\delta$2.33 (s, 3*H*; $CH_3$), 4.93 (s, 2*H*; $NH_2$), 7.15-8.08 (m, 8*H*; ArH) and 8.63 (s, 1*H*; NH).

**4-(3-Methylphenyl)-*s*-triazolo[4,3-*a*]quinazolin-5(4*H*)-one (6)**

A solid was obtained by the above procedure when 2-hydrazino-3-(3-methylphenyl) quinazolin-4(3*H*)-one **(Vb)** was taken as the starting (the reaction time was 31 h). It was recrystallized from chloroform-ethanol (75:25) mixture to afford **(6)** 78 % with a m.p. of 256-258°C (Lit[235] 255-259°C).

Anal:

| | |
|---|---|
| TLC | : Rf 0.39 ($C_6H_6$ : MeOH::9:1) |
| UV (MeOH) | : 232 nm (4.5) and 286 nm (4.1) |
| IR (KBr) | : 3118, 3052, 1682, 1626, 812 and 765 $cm^{-1}$. |

**1-Methyl-4-(3-methylphenyl)-*s*-triazolo[4,3-*a*]quinazolin-5(4*H*)-one (7)**

A solid was obtained by the above procedure when 2-hydrazino-3-(3-methylphenyl) quinazolin-4(3*H*)-one **(Vb)** was taken as the starting (the reaction time was 31 h). It was recrystallized from chloroform-ethanol (75:25) mixture to afford (**7**) in 77 % yield. It showed a m.p. of 275-278°C (Lit[235] 274-277°C).

Anal:

| | | | | |
|---|---|---|---|---|
| TLC | : Rf 0.45 ($C_6H_6$ : MeOH::9:1) | | | |
| IR (KBr) | : 2330, 1683, 1627 and 766 $cm^{-1}$ | | | |
| NMR ($CDCl_3$) | : $\delta$ 2.43-2.45 (s, 3*H*; $CH_3$), 2.45-2.48 (s, 3*H*; $CH_3$) and 7.27-8.45 (m, 8*H*; ArH) | | | |
| Elemental analyses | : | C | H | N |
| | % Calculated | : 70.33 | 04.86 | 19.30 |
| | % Found | : 71.09 | 04.80 | 19.78 |

**1-Ethyl-4-(3-methylphenyl)-*s*-triazolo[4, 3-*a*]quinazolin-5(4*H*)-one ( 8)**

A solid was obtained by the above procedure from 2-hydrazino-3- (3-methylphenyl) quinazolin-4(3*H*)-one **(Vb)**. (The reaction time was 34 h). It

was recrystallized from ethanol to afford **(8)** in 77.4 % yield. It showed a m,p. of 258-262°C.

Anal:

| | |
|---|---|
| TLC | : Rf 0.47 ($C_6H_6$ : MeOH::9:1) |
| UV (MeOH) | : 231 nm (log ε 4.4) and 282 nm (log ε 4.0) |
| IR (KBr) | : 1680, 1602 and 829 $cm^{-1}$ |
| NMR ($CDCl_3$) | : δ 1.3-1.5 (t, 3*H*; $CH_2C\underline{H}_3$), 2.7-2.8 (q, 2*H*; $-C\underline{H}_2CH_3$), 2.5-2.6 (s, 3H, $CH_3$) and 7.2-8.5 (m, 8*H*; ArH) |
| Mass (m/z) | : 304 $M^+$, 290 (7%), 250 (2%), 238 (36 %), 220 (13%), 199 (100%, base peak), 198 (80%), 165 (44%) and 149 (11%). |

Elemental analyses :

| | C | H | N |
|---|---|---|---|
| % Calculated : | 71.04 | 05.30 | 18.41 |
| % Found : | 71.84 | 05.36 | 18.76 |

**1-Propyl-4-(3-methylphenyl)-*s*-triazolo[4, 3-*a*]quinazolin-5(4*H*)-one (9)**

A solid was obtained by the above procedure from 2-hydrazino-3-(3-methylphenyl) quinazolin-4(3*H*)-one **(Vb)** (the reaction time was 21 h). It was recrystallized from chloroform-ethanol (75:25) mixture to afford **(9)** in 70 % yield . It showed a m.p. of 260-265°C.

Anal:

| | |
|---|---|
| TLC | : Rf 0.76 ($C_6H_6$ : MeOH::9:1) |
| UV (MeOH) | : 231 nm (log ε 4.6) and 283 nm (log ε 4.1) |

IR (KBr) : 1684, 1636, 1603, 838 and 770 $cm^{-1}$

NMR ($CDCl_3$) : $\delta$ 0.8-1.0 (t, 2*H*; $C\underline{H_2}CH_2CH_3$), 1.6-1.8 (sext, 2*H*; $CH_2C\underline{H_2}CH_3$), 2.6-2.8 (t, 3*H*; $CH_2CH_2C\underline{H_3}$), 2.4-2.5 (s, 3*H*; $CH_3$) and 7.2-8.5 (m, 8*H*; ArH)

Mass (m/z) : 318 $M^+$, 303 (18%), 290 (28%), 220 (21%), 205 (6%), 105 (12%), 91 (36%), 77 (6%) and 58 (100%, base peak).

**1-Chloromethyl-4-(3-methylphenyl)-*s*-triazolo[4,3-*a*]quinazolin-5(4*H*)-one (10)**

2-Hydrazino-3-(3-methylphenyl) quinazolin-4(3*H*)-one **(Vb)** when reacted by the above procedure yielded a solid (the reaction time was 13 h). It was recrystallized from dimethyl formamide to afford **(10)** in 75.7 % yield and a m.p. of 266-268°C.

Anal:

TLC : Rf 0.22 ($C_6H_6$ : MeOH::9:1)

UV(MeOH) : 233 nm (4.5) and 285 nm (4.0)

IR (KBr) : 1683, 1627, 1604 and 830 $cm^{-1}$

NMR ($CDCl_3$) : $\delta$ 2.40-2.45 (s, 3*H*; $CH_3$), 2.48-2.49 (s, 2*H*; $CH_2$) and 7.2-8.45 (m, 8*H*; ArH)

Elemental analyses :

| | C | H | N |
|---|---|---|---|
| % Calculated : | 62.87 | 04.03 | 17.25 |
| % Found : | 63.50 | 04.42 | 17.39 |

**Series III**

**1-Substituted-4-(4-methylphenyl)-*s*-triazolo[4,3-*a*]quinazolin-4(3*H*)-one (11-15)**

**2-Thioxo-3-(4-methylphenyl) quinazolin-4(3*H*)-one (IIIc)**

4-methyl aniline was subjected to the above described procedure to yield a solid (the reaction time was 21 h). It was recrystallized from ethanol to afford **(IIIc)** 75 %. It showed a m.p. of 302-305° C (Lit[233] 310°C).

Anal:

TLC : Rf 0.76 ($C_6H_6$ : MeOH::9:1)

UV(MeOH) : 218 nm (4.2) and 293 nm ( 4.2)

**2-Methylthio-3-(4-methylphenyl) quinazolin-4(3*H*)-one (IVc)**

2-Thioxo-3-(4-methylphenyl) quinazolin-4(3*H*)-one **(IIIc)** on reaction as described above yielded a solid. It was recrystallized from ethanol-chloroform (75:25) mixture to afford **(IVc)** 76 %. It showed a m.p. of 160-162° C.

Anal:

TLC : Rf 0.57 ($C_6H_6$ : $CHCl_3$::9:1)

UV(MeOH) : 224 nm (4.5) and 274 nm (4.2)

IR (KBr) : 1679, 1652, 1606, 812 and 764 $cm^{-1}$

NMR ($CDCl_3$) : $\delta$ 2.4 (s, 3*H*; $-CH_3$), 2.5 (s, 3*H*; $-SCH_3$) and 7.1-8.2 (m, 8*H*; -ArH).

**2-Hydrazino-3-(4-methylphenyl) quinazolin-4(3*H*)-one (Vc)**

2-methylthio-3-(4-methylphenyl) quinazolin-4(3*H*)-one **(IVc)** when reacted by the above procedure yielded a solid (the reaction time was 30 h) which was recrystallized from chloroform-benzene (25:75) mixture to afford **(Vc)** 72 %. It showed a m.p. of 170° C.

Anal:

TLC : Rf 0.64 ($C_6H_6$ : MeOH::9:1)

UV(MeOH) : 220 nm (4.7) and 281 nm (4.6)

IR (KBr) : 3334, 3314, 1674, 810 and 762 $cm^{-1}$

NMR ($CDCl_3$) : $\delta$ 2.33 (s, 3*H*; $-CH_3$), 5.1 (s, 2*H*; $-NH_2$), 7.12-8.07 (m, 8*H*;, -ArH) and 8.72 (s, 1*H*; -NH).

**4-(4-methylphenyl)-*s*-triazolo[4,3-*a*]quinazolin-5(4*H*)-one (11)**

A solid was obtained by the above procedure from 2-hydrazino-3-(4-methylphenyl) quinazolin-4(3*H*)-one **(Vc)** (the reaction time was 24 h). It was recrystallized from chloroform-ethanol (75:25) mixture to afford **(11)** 74 %. It showed a m.p. of 264-266°C.

Anal:

TLC : Rf 0.45 ($C_6H_6$ : MeOH::9:1)

UV(MeOH) : 231 nm (4.6) and 284 nm (4.2)

IR (KBr) : 3117, 3052, 1683, 1626 and 812 $cm^{-1}$

NMR ($CDCl_3$) : $\delta$ 2.48 (s, 3*H*; -$CH_3$), 7.4-7.7 (m, 4*H*; -ArH), 8.42-8.5 (m, 4*H*; -ArH) and 8.3 (s, 1*H*; -CH)

Elemental analyses :

| | C | H | N |
|---|---|---|---|
| % Calculated : | 69.55 | 04.34 | 20.28 |
| % Found : | 70.79 | 04.40 | 20.92 |

**1-Methyl-4-(4-methylphenyl)-*s*-triazolo[4,3-*a*]quinazolin-4(3*H*)-one (12)**

A solid was obtained by the above procedure from 2-hydrazino-3-(4-methylphenyl) quinazolin-4(3*H*)-one **(Vc)** (the reaction time was 31 h). It was recrystallized from chloroform-ethanol (75:25) mixture to afford **(12)** 76 %. The solid showed a m.p. of 263-265°C.

Anal:

TLC : Rf 0.46 ($C_6H_6$ : MeOH::9:1)

UV(MeOH) : 231 nm (4.6) and 284 nm (4.2)

IR (KBr) : 1682, 1627, 1604, 830 and 766 $cm^{-1}$

NMR ($CDCl_3$) : $\delta$ 2.42-2.43 (s, 3*H*; -$CH_3$), 2.45-2.48 (s, 3*H*;-$CH_3$) and 7.26-8.45 (m, 8*H*; -ArH)

Elemental analyses :

| | C | H | N |
|---|---|---|---|
| % Calculated : | 70.33 | 04.86 | 19.30 |
| % Found : | 70.04 | 05.07 | 19.55 |

**1-Ethyl-4-(4-methylphenyl)-*s*-triazolo[4,3-*a*]quinazolin-5(4*H*)-one (13)**

2-hydrazino-3-(4-methylphenyl) quinazolin-4(3*H*)-one **(Vc)** when reacted by the above procedure yielded a solid (The reaction time was 34 h). It was recrystallized from ethanol to afford **(13)** 80.8%. It showed a m.p. of 250-253°C.

Anal:

| | |
|---|---|
| TLC | : Rf 0.48 ($C_6H_6$ : MeOH::9:1) |
| UV(MeOH) | : 231 nm (4.6) and 283 nm (4.0) |
| IR (KBr) | : 1683, 1635, 1601, 829 and 770 $cm^{-1}$ |
| NMR ($CDCl_3$) | : $\delta$1.29-1.34 (t, 3*H*; -$CH_2\underline{CH_3}$), 2.49 (s, 3*H*; -$CH_3$), 2.71-2.76 (q, 2*H*; -$\underline{CH_2}CH_3$) and 7.3-8.3 (m, 8*H*; -ArH) |
| Mass (m/z) | : 304 $M^+$, 290 ( 9%), 105 (36%), 91 (26%), 77 (20%), 58 (100%, base peak) and 55 (6%). |

**1-Propyl-4-(4-methylphenyl)-*s*-triazolo[4,3-*a*]quinazolin-5(4*H*)-one (14)**

A solid was obtained by the above procedure when 2-hydrazino-3-(4-methylphenyl) quinazolin-4(3*H*)-one **(Vc)**was taken as the starting 2-hydrazino derivative (the reaction time was 21 h). It was recrystallized from chloroform-ethanol (75:25) mixture to afford **(14)** 70.0 %. It showed a m.p. of 228-230°C.

Anal:

| | |
|---|---|
| TLC | : Rf 0.60 ($C_6H_6$ : MeOH::9:1) |
| UV(MeOH) | : 231 nm (4.5) and 283 nm (4.0) |
| IR (KBr) | : 2965, 1684, 1635, 838 and 770 $cm^{-1}$ |
| NMR ($CDCl_3$) | : $\delta$ 0.93-0.98 (t, 3*H*; - $CH_2CH_2C\underline{H}_3$), 1.69-1.82 (sext, 2*H*; -$CH_2C\underline{H}_2CH_3$) , 2.48 (s, 3*H*; -$CH_3$), 2.6-2.7 (t, 2*H*; - $C\underline{H}_2CH_2CH_3$) and 7.2- 8.4 (m, 8*H*; -ArH) |

Elemental analyses :

| | C | H | N |
|---|---|---|---|
| % Calculated : | 71.68 | 05.70 | 17.59 |
| % Found : | 71.31 | 06.13 | 17.69 |

**1-Chloromethyl-4-(4-methylphenyl)-*s*-triazolo[4,3-*a*]quinazolin-5(4*H*)-one (15)**

A solid was obtained by the above procedure from 2-hydrazino-3-(4-methylphenyl) quinazolin-4(3*H*)-one **(Vc)** (the reaction time was 13 h). It was recrystallized from chloroform-ethanol (75:25) mixture to afford **(15)** 78.8 %. It showed a m.p. of 268-270°C.

Anal:

| | |
|---|---|
| TLC | : Rf 0.30 ($C_6H_6$ : MeOH::9:1) |
| UV(MeOH) | : 231 nm (4.6) and 283 nm (4.2) |
| IR (KBr) | : 1683, 1627, 1604, 830 and 766 $cm^{-1}$ |
| NMR ($CDCl_3$) | : $\delta$ 2.42-2.45 (s, 3*H*; -$CH_3$), 2.48-2.49 (s, 2*H*; -$CH_2$) and 7.2-8.4 (m, 8*H*; -ArH) |
| Mass (m/z) | : 324 $M^+$, 290 (100%, base peak), 289 (46%), 276 (8%), 235 (11%), 117 (9%), 105 (34%), 91 (55%) and 77 (45%). |

**Series IV**

**1-Substituted-4-(4-methoxyphenyl)-*s*-triazolo[4,3-*a*]quinazolin-5(4*H*)-one (16-20)**

**2-Thioxo-3-(4-methoxyphenyl) quinazolin-4(3*H*)-one (IIId)**

p-methoxy aniline on reaction as described above yielded a solid (the reaction time was 19 h). It was recrystallized from ethanol to afford **(IIId)** 78 %. The solid showed a m.p. of 296-300°C.

Anal:

| | |
|---|---|
| TLC | : Rf 0.68 ($C_6H_6$ : MeOH::9:1) |
| UV(MeOH) | : 220 nm (4.3) and 292 nm (4.3) |
| IR (KBr) | : 3218, 1725, 1680, 1593 and 759 $cm^{-1}$ |
| NMR ($CDCl_3$) | : $\delta$ 3.88 (s, 3*H*; -$OCH_3$), 7-8.1 (m, 8*H*; -ArH) and 10.36 (s, 1*H*; -NH). |

**2-Methylthio-3-(4-methoxyphenyl) quinazolin-4(3*H*)-one (IVd)**

2-Thioxo-3-(4-methoxyphenyl) quinazolin-4(3*H*)-one **(IIId)** when reacted with dimethylsulphate by the above procedure yielded a solid which was recrystallized from ethanol-chloroform (75:25) mixture to afford **(IVd)** 78 %. The product showed a m.p. of 142-145° C.

Anal:

| | |
|---|---|
| TLC | : Rf 0.35 ($C_6H_6$ : $CHCl_3$::9:1) |

UV(MeOH) : 225 nm (4.7) and 273 nm (4.3)

IR (KBr) : 2330, 1733, 1683 and 1254 $cm^{-1}$

NMR ($CDCl_3$) : $\delta$ 2.5 (s, 3*H*; $-SCH_3$), 3.87 (s, 3*H*; $-OCH_3$) and 7.0- 8.26 (m, 8*H*; ArH).

**2-Hydrazino-3-(4-methoxyphenyl) quinazolin-4(3*H*)-one (Vd)**

A solid was obtained by the above procedure when 2-methylthio-3-(4-methoxylphenyl) quinazolin-4(3*H*)-one **(IVd)** was taken as the starting 2-methylthio derivative (the reaction time was 30 h). It was recrystallized from ethanol to afford **(Vd)** 74 %. The solid showed a m.p. of 196-200° C.

Anal:

TLC : Rf 0.60 ($C_6H_6$ : MeOH::9:1)

UV(MeOH) : 222 nm (log 4.6) and 282 nm (log 4.5)

IR (KBr) : 3350, 3320, 1674, 1222, 835 and 767 $cm^{-1}$

NMR ($CDCl_3$) : $\delta$ 3.79 ( s, 3*H*; $-OCH_3$), 4.95 (s, 2*H*; $-NH_2$), 6.82-8.06 (m, 8*H*; -ArH) and 8.56 (s, 1*H*; -NH)

**4-(4-methoxyphenyl)-*s*-triazolo[4,3-*a*]quinazolin-5(4*H*)-one (16)**

2-Hydrazino-3-(4-methoxyphenyl) quinazolin-4(3*H*)-one **(Vd)** when reacted by the above procedure yielded a solid (the reaction time was 23 h). It was recrystaliized from chloroform-ethanol (75:25) mixture to afford **(16)** 72 %. The solid showed a m.p. of 248-250°C.

Anal:

| | | | | |
|---|---|---|---|---|
| TLC | : Rf 0.31 ($C_6H_6$ : MeOH::9:1) | | | |
| UV(MeOH) | : 231 nm (4.5) and 284 nm (4.2) | | | |
| IR (KBr) | : 3110, 3050, 1715, 1635, 1607and 828 $cm^{-1}$. | | | |
| Elemental analyses | : | C | H | N |
| | % Calculated : | 65.75 | 04.14 | 19.17 |
| | % Found : | 66.31 | 04.42 | 19.46 |

**1-Methyl-4-(4-methoxyphenyl)-*s*-triazolo[4,3-*a*]quinazolin-5(4*H*)-one (17)**

2-Hydrazino-3-(4-methoxyphenyl) quinazolin-4(3*H*)-one **(Vd)** on reaction with acetic acid as described above yielded a solid (the reaction time was 30 h). It was recrystallized from chloroform-ethanol (75:25) mixture to afford **(17)** 72 %. It showed a m.p. of 245-248°C.

Anal:

| | | | | |
|---|---|---|---|---|
| TLC | : Rf 0.33 ($C_6H_6$ : MeOH::9:1) | | | |
| UV(MeOH) | : 230 nm (4.8) and 284 nm (4.4) | | | |
| IR (KBr) | : 1707, 1627, 1605, 1279, 839 and 768 $cm^{-1}$ | | | |
| Elemental analyses | : | C | H | N |
| | % Calculated : | 66.66 | 04.61 | 18.29 |
| | % Found : | 66.23 | 04.62 | 18.10 |

**1-Ethyl-4-(4-methoxyphenyl)-*s*-triazolo[4,3-*a*]quinazolin-5(4*H*)-one (18)**

A solid was obtained by the above procedure when 2-hydrazino-3-(4-methoxyphenyl) quinazolin-4(3*H*)-one **(Vd)** was taken as the starting 2-hydrazino derivative (the reaction time was 32 h). It was recrystallized from ethanol to afford **(18)** 75.7 % . It showed a m.p. of 220-224°C.

Anal:

| | |
|---|---|
| TLC | : Rf 0.41 ($C_6H_6$ : MeOH::9:1) |
| UV(MeOH) | : 231 nm (4.6) and 284 nm (4.3) |
| IR (KBr) | : 1684, 1627, 1601, 1250 844 and 797 $m^{-1}$ |
| NMR ($CDCl_3$) | : $\delta$ 1.3-1.35 (t, 3*H*; $-CH_2\underline{CH_3}$), 2.69-2.76 (q, 2*H*; $-\underline{CH_2}CH_3$), 3.9 (s, 3*H*; $-OCH_3$) and 7.1-8.4 (m, 8*H*; -ArH) |
| Mass (m/z) | : 320 $M^+$, 304 (39%) , 290 (38%), 185 (20%), 223 (25%), 117 (28%) 105 (25%), 91 (69%). 77 (34%) and 57 (100%, base peak). |

**1-Propyl-4-(4-methoxyphenyl)-*s*-triazolo[4,3-*a*]quinazolin-5(4*H*)-one (19)**

2-hydrazino-3-(4-methoxyphenyl) quinazolin-4(3*H*)-one **(Vd)** on reaction with butyric acid as described above yielded a solid (the reaction time was 21 h). It was recrystallized from chloroform-ethanol (75:25)

mixture to afford **(19)** 72.2 % . It showed a m.p. of 226-228°C (Lit[235] 246-248°C).

Anal:

| | |
|---|---|
| TLC | : Rf 0.54 ($C_6H_6$ : MeOH::9:1) |
| UV(MeOH) | : 231 nm (4.6) and 283 nm (3.3) |
| IR (KBr) | : 1683, 1626, 1602, 1254, 1223 and 769 $cm^{-1}$ |
| Mass (m/z) | : 334 $M^+$, 319 (46%), 306 (12%), 305 (10%), 135 (37%), 77 (9%) and 90 (9%). |

**1-Chloromethyl-4-(4-methoxyphenyl)-*s*-triazolo[4,3-*a*]quinazolin-5(4*H*)-one (20)**

2-Hydrazino-3-(4-methoxyphenyl) quinazolin-4(3*H*)-one **(Vd)** on reaction with chloroacetyl chloride as described above yielded a solid (the reaction time was 13 h). It was recrystallized from chloroform-ethanol (75:25) mixture to afford **(20)** 80.4 %, which showed a m.p. of 254-255°C.

Anal:

| | |
|---|---|
| TLC | : Rf 0.29 ($C_6H_6$ : MeOH::9:1) |
| UV(MeOH) | : 231 nm (4.6) and 283 nm (4.1) |
| IR (KBr) | : 1709, 1627, 1605, 1322 and 1256 $cm^{-1}$ |

| Elemental analyses | : | | C | H | N |
|---|---|---|---|---|---|
| | | % Calculated | : 59.92 | 03.84 | 16.44 |
| | | % Found | : 60.66 | 04.61 | 16.84 |

**Series V**

**1-Substituted-4-(4-chlorophenyl)-*s*-triazolo[4,3-*a*]quinazolin-5(4*H*)-one (21-25)**

**2-Thioxo-3-(4-chlorophenyl) quinazolin-4(3*H*)-one (IIIe)**

p-chloro aniline as the amine yielded a solid by the above procedure (the reaction time was 20 h). It was recrystallized from ethanol to afford **(IIIe)** 73.4 %. It showed a m.p. of 319-321°C (Lit[234] 307°C).

Anal:

TLC : Rf 0.66 ($C_6H_6$ : MeOH::9:1),

UV(MeOH) : 221 nm (4.5) and 293 nm (4.4)

**2-Methylthio-3-(4-chlorophenyl) quinazolin-4(3*H*)-one (IVe)**

A solid was obtained by the above procedure when 2-thioxo-3-(4-chlorophenyl) quinazolin-4(3*H*)-one **(IIIe)** was taken as the starting 2-thioxo derivative. It was recrystallized from ethanol-chloroform (75:25)

mixture to afford **(IVe)** 79.6 % . It showed a m.p. of 136-139°C (Lit[234] 137°C).

Anal:

| | |
|---|---|
| TLC | : Rf 0.62 ($C_6H_6$ : $CHCl_3$::9:1) |
| UV(MeOH) | : 227 nm (4.5) and 274 nm (4.1) |
| IR (KBr) | : 1682, 1652, 811and 763 $cm^{-1}$ |

**2-Hydrazino-3-(4-chlorophenyl) quinazolin-4(3*H*)-one (Ve)**

2-methylthio-3-(4-chlorophenyl) quinazolin-4(3*H*)-one **(IVe)** when reacted by the above procedure yielded a solid (the reaction time was 31 h) which was recrystallized from chloroform-benzene (25:75) mixture to afford **(Ve)** 82.7 %. It showed a m.p. of 162-165°C (Lit[234] 152° C).

Anal:

| | |
|---|---|
| TLC | : Rf 0.61 ($C_6H_6$ : MeOH::9:1) |

**4-(4-chlorophenyl)-*s*-triazolo[4,3-*a*]quinazolin-5(4*H*)-one (21)**

2-Hydrazino-3-(4-chlorophenyl) quinazolin-4(3*H*)-one **(Ve)** on reaction with formic acid as described above yielded a solid (the reaction time was 21 h). It was recrystallized from chloroform-ethanol (75:25) mixture to afford **(21)** 89.9 %. It showed a m.p. of 228-231°C (Lit[234] 205°C).

Anal:

| | |
|---|---|
| TLC | : Rf 0.40 ($C_6H_6$ : MeOH::9:1) |
| UV(MeOH) | : 217nm (4.9) and 284 nm (4.0) |
| IR (KBr) | : 2330, 1717, 1652 and 1489 $cm^{-1}$. |
| NMR ($CDCl_3$) | : $\delta$ 7.2-8.0 (m, 8*H*; -ArH) and 8.8-8.9 (s, 1*H*; -ArH). |
| Mass (m/z) | : 296 $M^+$, 146 (83%), 119 (100%, base peak), 92 (42%) and 90 (28%). |

**1-Methyl-4-(4-chlorophenyl)-*s*-triazolo[4,3-*a*]quinazolin-5(4*H*)-one (22)**

2-Hydrazino-3-(4-chlorophenyl) quinazolin-4(3*H*)-one (**Ve**) when reacted by the above procedure yielded a solid (The reaction time was 30 h). It was recrystallized from dimethylformamide to afford (**22**) 89.3 %. It showed a m.p. of 316-319°C.

Anal:

| | |
|---|---|
| TLC | : Rf 0.40 ($C_6H_6$ : MeOH::9:1) |
| UV(MeOH) | : 231 nm (5.1) and 272 nm (3.9) |
| IR (KBr) | : 1688, 1627, 1609 and 844 $cm^{-1}$ |
| NMR ($CDCl_3$) | : $\zeta$ 3.0-3.1 (s, 3*H*; $-CH_3$), 7.3-7.4 (m, 4*H*; -ArH) and 7.5-7.8 (m, 4*H*; -ArH) |
| Mass (m/z) | : 310 $M^+$, 309 ($[M-1]^+$ 12 %),275 ( 6%), 247 (10%), 205 (7%), 91 (10%) and 77 ( 8%). |

**1-Ethyl-4-(4-chlorophenyl)-*s*-triazolo [4,3-*a*]quinazolin-5(4*H*)-one (23)**

2-Hydrazino-3-(4-chlorophenyl) quinazolin-4(3*H*)-one (**Ve**) on reaction with as described above yielded a solid (the reaction time was 32 h). It was recrystallized from ethanol to afford **(23)** 77.1 %. The solid showed a m.p. of 267-270°C.

Anal:

| | |
|---|---|
| TLC | : Rf 0.41 ($C_6H_6$ : MeOH::9:1) |
| UV(MeOH) | : 230 nm (4.5) and 283 nm (3.9) |
| IR (KBr) | : 1688, 1631, 840 and 792 $cm^{-1}$ |
| NMR ($CDCl_3$) | : $\delta$1.3-1.38 (t, 3H, -$CH_2\underline{CH_3}$), 2.72-2.79 (q, 2H, -$\underline{CH_2}CH_3$), and 7.27-8.45 (m, 8H, -ArH) |
| Mass (m/z) | : 324 $M^+$, 323 ( 6%), 199 ( 38%), 171 (15%), 130 ( 21%), 119(18%), 111 (100%, base peak), 103 ( 23 %), 102 ( 82%), 90 (76%), 76 ( 23%) and 57 ( 35%). |

**1-Propyl-4-(4-chlorophenyl)-*s*-triazolo[4,3-*a*]quinazolin-5(4*H*)-one (24)**

2-Hydrazino-3-(4-chlorophenyl) quinazolin-4(3*H*)-one (**Ve**) was subjected to the above described procedure to yield a solid (the reaction time was 22 h). It was recrystallized from chloroform-ethanol (75:25)

mixture to afford **(24)** 82.4 %. It showed a m.p. of 210-215°C (Lit[234] 233-234°C).

Anal:

| | |
|---|---|
| TLC | : Rf 0.55 ($C_6H_6$ : MeOH::9:1) |
| UV(MeOH) | : 230 nm (4.6) and 283 nm (4.0) |
| IR (KBr) | : 1709, 1689, 1606, 961 and 840 $cm^{-1}$ |
| Mass (m/z) | : 339 $M^+$, 310 (16%), 325 (6%), 200 (18%), 102 (18%), and 76 (9%). |

**1-Chloromethyl-4-(4-chlorophenyl)-*s*-triazolo[4,3-*a*]quinazolin-5(4*H*)-one (25)**

2-Hydrazino-3-(4-chlorophenyl) quinazolin-4-(3*H*)-one **(Ve)** was subjected to the above described procedure to yield a solid (the reaction time was 12 h). It was recrystallized from dimethyl formamide to afford **(25)** 80.1 % . It showed a m.p. of 244-247°C (Lit[235] 246-252°C).

Anal:

| | |
|---|---|
| TLC | : Rf 0.40 ($C_6H_6$ : MeOH::9:1) |
| UV(MeOH) | : 232 nm (4.4) and 285 nm (3.8) |
| IR (KBr) | : 1690, 1627, 1609 843 and 771 $cm^{-1}$ |
| NMR ($CDCl_3$) | : $\delta$ 4.6-4.7 (s, 2H, $-CH_2$), 7.3-7.5 (m, 4H, -ArH) and 7.6-7.7 (m, 4H, -ArH) |

**Series VI**

**1-Substituted-4-(2-pyridyl)-*s*-triazolo[4,3-*a*]quinazolin-5(4*H*)-one (26-30)**

**2-Thioxo-3-(2-pyridyl) quinazolin-4(3*H*)-one ( IIIf )**

A solid was obtained by the above procedure from 2-Amino pyridine (the reaction time was 19 h). It was recrystallized from ethanol to afford ( **IIIf** ) 83 % which showed a m.p. of 260-262°C.

Anal:

| | |
|---|---|
| TLC | : Rf 0.67 ($C_6H_6$ : MeOH::9:1) |
| UV(MeOH) | : 227 nm (4.1) and 295 nm (4.2) |
| IR (KBr) | : 3220, 1675, 1618, 850, 795 and 743 $cm^{-1}$ |
| NMR ($CDCl_3$) | : $\delta$ 7.15-8.74 (m, 8H, -ArH) and 10.71 (s, 1H, -NH). |
| Mass (m/z) | : 255 $M^+$, 254 (18%), 226 (9%), 197 ( 22%), 194 (19%), 168 (9%), 119 (11%), 102 ( 11%), 90 (9%) and 78 (40%). |

**2-Methylthio-3-(2-pyridyl) quinazolin-4(3*H*)-one ( IVf )**

2-Thioxo-3-(2-pyridyl) quinazolin-4(3*H*)-one **(IIIf)** when reacted with dimethyl sulphate by the above procedure yielded a solid

which was recrystallized from ethanol-chloroform (75:25) mixture to afford **(IVf)** 88 % with a m.p. of 145-146° C.

Anal:

| | |
|---|---|
| TLC | : Rf 0.61 ($C_6H_6$ : $CHCl_3$::9:1) |
| UV(MeOH) | : 211 nm (4.5) and 268 nm (3.9) |
| IR (KBr) | : 1676, 1654, 765, and 744 $cm^{-1}$ |
| NMR ($CDCl_3$) | : $\delta$ 2.5 (s, 3*H*; -$SCH_3$), and 7.2- 8.6 (m, 8*H*; ArH). |

**2-Hydrazino-3-(2-pyridyl) quinazolin-4(3*H*)-one ( Vf )**

A solid was obtained by the above procedure from 2-methylthio-3-(2-pyridyl) quinazolin-4(3*H*)-one **( IVf )** (the reaction time was 24 h). It was recrystallized from chloroform-acetone (50:50) mixture to afford **(Vf)** 83 % with a m.p. of 235-236° C.

Anal:

| | |
|---|---|
| TLC | : Rf 0.57 ($C_6H_6$ : MeOH::9:1) |
| UV(MeOH) | : 219 nm (4.2) and 292 nm (4.1) |
| IR (KBr) | : 3300, 3280, 1683, 1566 and 759 $cm^{-1}$ |
| NMR ($CDCl_3$) | : $\delta$ 5.1-5.3 (s, 2H, -$NH_2$), 7.0-8.8 (m, 8H, -ArH) and 9.4-9.5 (s, 1H, -NH) |

**4-(2-pyridyl)-*s*-triazolo[4,3-*a*]quinazolin-5(4*H*)-one (26)**

2-Hydrazino-3-(2-pyridyl) quinazolin-4(3*H*)-one **(Vf)** was subjected to the above described procedure to yield a solid (the reaction time was 22 h). It was recrystallized from chloroform-ethanol (75:25) mixture to afford **(26)** 88 % with a m.p. of 222-225°C

Anal:

| | | | | |
|---|---|---|---|---|
| TLC | : Rf 0.39 ($C_6H_6$ : MeOH::9:1) | | | |
| UV(MeOH) | : 235 nm (4.4) and 281 nm (4.2) | | | |
| IR (KBr) | : 3110, 3050, 1710, 1683, 784 and 764 $cm^{-1}$ | | | |
| NMR ($CDCl_3$) | : $\delta$ 7.26-8.6 (m, 8*H*; ArH) and 9.23 (s,1*H*; -CH) | | | |
| Elemental analyses | : | C | H | N |
| | % Calculated : | 63.87 | 03.45 | 26.61 |
| | % Found : | 64.32 | 03.22 | 26.91 |

**1-Methyl-4-(2-pyridyl)-*s*-triazolo[4,3-*a*]quinazolin-5(4*H*)-one (27)**

2-Hydrazino-3-(2-pyridyl) quinazolin-4(3*H*)-one **(Vf)** was subjected to the above described procedure to yield a solid (The reaction time was 32 h). It was recrystallized from chloroform-ethanol (75:25) mixture to afford **(27)** 76 % with a m.p. of 276-279°C.

Anal:

| | |
|---|---|
| TLC | : Rf 0.41 ($C_6H_6$ : MeOH::9:1) |
| UV(MeOH) | : 224 nm (4.4) and 277 nm (4.3) |

IR (KBr) : 1683, 1648 and 1605 $cm^{-1}$

Elemental analyses :

| | | C | H | N |
|---|---|---|---|---|
| % Calculated | : | 64.97 | 03.99 | 25.25 |
| % Found | : | 65.95 | 03.46 | 24.38 |

**1-Ethyl-4-(2-pyridyl)-*s*-triazolo[4,3-*a*]quinazolin-5(4*H*)-one (28)**

2-Hydrazino-3-(2-pyridyl) quinazolin-4(3*H*)-one **(Vf)** was subjected to the above described procedure to yield a solid (the reaction time was 33 h). It was recrystallized from ethanol to afford **(28)** 73.1%. It showed a m.p. of 346-350°C.

Anal:

TLC : Rf 0.42 ($C_6H_6$ : MeOH::9:1)

UV(MeOH) : 223 nm (4.7) and 276 nm (4.6)

IR (KBr) : 1680, 1648, 1627, 932 and758 $cm^{-1}$

NMR ($CDCl_3$) : δ 2.4-2.6 (q, 2*H*; -$C\underline{H}_2CH_3$), 3.25-3.5 (t, 3*H*; -$CH_2C\underline{H}_3$), and 6.95-8.5 (m, 8*H*; -ArH)

Elemental analyses :

| | | C | H | N |
|---|---|---|---|---|
| % Calculated | : | 65.97 | 04.50 | 24.04 |
| % Found | : | 66.73 | 03.87 | 23.96 |

**1-Propyl-4-(2-pyridyl)-*s*-triazolo[4,3-*a*]quinazolin-5(4*H*)-one (29)**

2-Hydrazino-3-(2-pyridyl) quinazolin-4(3*H*)-one **(Vf)** when reacted by the above procedure yielded a solid (the reaction time was

23 h). It was recrystallized from chloroform-ethanol (75:25) mixture to afford **(29)** 76 %. It showed a m.p. of 330-334°C.

Anal:

| | |
|---|---|
| TLC | : Rf 0.50 ($C_6H_6$ : MeOH::9:1) |
| UV(MeOH) | : 223 nm (4.6) and 276 nm (4.5) |
| IR (KBr) | : 1686, 1683, 1648, 1627, 931 and 759 $cm^{-1}$ |
| Mass (m/z) | : 305 $M^+$, 290 (15%), 275 (23%), 222 (17%) 149 (11%), 105 (19%) 91 (61%), 77 (100%, base peak) and 55 (19%). |

**1-Chloromethyl-4-(2-pyridyl)-*s*-triazolo[4,3-*a*]quinazolin-5(4*H*)-one (30)**

2-Hydrazino-3-(2-pyridyl) quinazolin-4(3*H*)-one **(Vf)** when reacted by the above procedure yielded a solid (the reaction time was 13 h). It was recrystallized from chloroform-ethanol (75:25) mixture to afford **(30)** 69.0 %. It showed a m.p. of 340-343°C.

Anal:

| | |
|---|---|
| TLC | : Rf 0.29 ($C_6H_6$ : MeOH::9:1) |
| UV(MeOH) | : 232 nm (4.3) and 283 nm (4.0) |
| IR (KBr) | : 1713, 858 and 807 $cm^{-1}$ |
| NMR ($CDCl_3$) | : $\delta$ 1.265 (s, 2*H*; $-CH_2$) and 7.27-8.66 (m, 8*H*; -ArH) |
| Mass (m/z) | : 310 $[M-1]^+$, 276 (24%), 145 (10 %), 102 (30%), 78 (100%, base peak ) and 77 (12 %). |

## B. BIOLOGICAL STUDIES

All the animal experiments have been carried out according to the internationally valid guidelines and they were approved by the "Institutional Animal Ethical Committee". Animals were maintained in wire-mesh cages in a restricted-access room with constant conditions (23 ± 2°C, 12 h light) for one week before the experiments. The animals were fed standard lab pellets and purified water ad libitum. Prior to the experiments animals were fasted for 12 h. The following animals were used in the biological studies.

- Wistar rats of either sex weighing
    - i) 250-300 gm for alpha adrenergic blocking acitivity
    - ii) 175-200 gm for antiinflammatory activity.
- Hartley guinea pigs of either sex (7-8 weeks old, 250-300 gm)
- Albino mice of either sex (20 and 25 gm).

The standard bacterial strains for antibacterial studies were procured from the American Type Culture Collection (ATCC), Rockville, USA.

Reference compounds and chemicals used for the biological studies are as follows.

Urethane (Sigma Chemicals, USA)

Noradrenaline (Sigma Chemicals, USA)

Adrenaline (Sigma Chemicals, USA)

Histamine (Sigma Chemicals, USA)

Phenylephrine (Sigma Chemicals, USA)

Angiotensin-II (Sigma chemicals, USA)

Aminophylline (Unichem, Mumbai)

Chlorpheniramine maleate (Hoechst, Mumbai)

Cetirizine (Sun Pharma, Mumbai)

Ciprofloxacin (Sun Pharma, Mumbai)

Carregeenan (Irish moss) (Otto kemi, Mumbai)

Pentazocine (Ranbaxy, NewDelhi)

Diclofenac sodium (Ranbaxy, NewDelhi)

**Statistical Evaluation**

Statistical evaluation was done by "student's t-test" and probability value (p) less than 0.05 was considered significant.

All values were presented in terms of Mean ± S.E.M.
Potency of compounds was compared using data of standard drugs.

**1. Alpha adrenergic blocking acitivity**

The experiments were conducted on Wistar rats of either sex weighing 200-300 g. All animals were maintained on standard rat pellets and tap water ad libitum and housed in cages in groups of five animals, at 26° C. All experiments were done by acute administration of test compounds by i.v., i.p. and oral route. Potency of test compounds was compared using prazosin (selective $\alpha_1$-adrenergic blocker) as standard.

Two types of studies were carried out:

i) *In vivo* Studies

For *in vivo* studies, intraperitoneal route has been chosen for initial screening of $\alpha_1$-adrenergic blocking activity of test compounds at the dose of 5 mg/kg in the form of suspension using 0.5 % Sod. CMC as Suspending agent.

Oral route has been chosen for promising compounds for detail evaluation. Blood pressure was measured after 3 h of oral treatment of test compounds as well as in vehicle control rats.

ii) *In vitro* studies

The *in vitro* studies was carried out using rat thoracic aortic strip. Contractile response to phenylephrine was recorded in a cumulative manner for treated and control groups by mounting rat aortic strip.

**1.1 α-adrenergic blocking activity[236] of test compounds after intravenous administration in anesthetized rats *(in vivo* study).**

Albino rats either of sex weighing 250-300gm were used. Rats were anesthetized with urethane (120 mg/kg i.p.*)* and tracheotomy was performed. The blood pressure was measured directly through the left common carotid artery by Statham pressure transducer and recorded

on calibrated polygraph. The femoral vein was cannulated with a needle (24 no styles removed) connected to a fine polyethylene catheter for the injection of drugs. Heparin (500 iu/ml) solution was filled on the dome of the transducer and also in the fine polyethylene catheter cannulated to the carotid artery to prevent clotting. Following a 20-30 minutes stabilization period noradrenaline (1mcg/kg) adrenaline (1mcg/kg) angiotensin–II (100 ng/kg) and histamine (1 mcg/kg) were administered intravenously before and after administration of test compound ( –24) . The experiments with the test compound were carried out different doses in the range of 2, 6, 10 and 20 mg/kg on different animals. All agonists were dissolved in saline. Each dose of agonists was administered at an interval of 10 - 15 minutes. The volume of administration was less than 0.3 ml. The test compounds was dissolved in DMSO (Dimethylsulfoxide) as a vehicle. The effect of DMSO was studied on the separate groups of animals.

## 1.2 α-adrenergic blocking activity[236] of test compounds after intraperitoneal and oral administration in anesthetized rats *(in vivo* study).

In anaesthetized rats, femoral vein was cannulated for administration of various agonists like noradrenaline, adrenaline, histamine, and phenylephrine and left common carotid artery was cannulated for measurement of blood pressure and heart rate. Following 20-30 minutes of stabilization period, agonists like noradrenaline, adrenaline, histamine, and phenylephrine were administered through

femoral vein and mean change in arterial blood pressure and heart rate were recorded after 30 min of intraperitoneal administration of test compounds. In the same rat, test compounds in the form of suspension were given by i.p. route at the dose of 5 mg/kg and change in blood pressure and heart rates were recorded. Again similar amount and dose of agonists were administered and change in blood pressure and heart rates were recorded. Changes were compared before and after treatment of test compounds. Reduction in blood pressure response to the agonists like noradrenaline, adrenaline, and phenylephrine was indicative of $\alpha_1$-adrenergic blocking activity of tested compounds.

Compounds were further tested for their $\alpha_1$-adrenergic blocking activity by oral route at the dose of 10 mg/kg in the form of suspension. Acute oral dose of test compounds were administered and animals were kept in cages for 3 h at standard laboratory condition. After 3 h of treatment, animals were anaesthetized and cannulation was performed. Changes in blood pressure responses and heart rate was recorded after administration of agonists intravenously. Results were compared with vehicle control rats.

### 1.3.1 Measurement of Blood Pressure And Heart Rate *(in vivo* study)

Male Wistar rats anaesthetized with Urethane (120 mg / 100 g, i.p.). Polyethylene catheter for measuring arterial blood pressure and heart rate was cannulated into the left common carotid artery, and Femoral vein was cannulated for intravenous administration of agonists[237] like noradrenaline, adrenaline, histamine, and phenylephrine. Arterial blood pressure and heart rates were measured directly through carotid artery by pressure transducer (Model SS13L, Biopac Systems, Inc., CA, USA), which was connected to the cannulated catheter. The dome of transducer and catheter cannulated to carotid artery was filled with heparinized saline (100 IU/ml) to prevent clotting. Blood Pressure was measured through left common carotid artery by pressure transducer and recorded on computer. The magnitudes of effects elicited after injections were evaluated by measuring the changes in arterial blood pressure and heart rate and comparing them with basal values. Potency of compounds was compared using data of prazosin as standard $\alpha_1$-adrenoceptor blocker.

### 1.3.2 $\alpha_1$-Adrenoceptor Antagonism *in vitro*

Isolated rat thoracic aortic strip was used to evaluate the $\alpha_1$-adrenoceptor antagonisms. [238, 239]

Rats (200-300 g) were sacrificed by a blow on the head and bled to death by cutting the neck vessels. Helically cut aortic strip was prepared from thoracic aorta and mounted in an organ bath of 40 ml capacity as described by Furchgott and Bhadrakom (1953). Aorta preparation was suspended in physiological solution (in mM; NaCl 118, KCl 4.8,$CaCl_2$ 2.5, $MgSO_4$ 1.2; $KH_2PO_4$ 1.2, NaHCO3 24, and glucose 11) at 37°±0.5° C and (PH) 7.4) gassed with carbogen and under a 1 g tension.

In the *in vitro* study for $\alpha_1$-blocking action, after 3 h of oral administration of test compounds, aorta was mounted and stabilized for 2 h with washing after each 10 minutes interval. Phenylephrine was cumulatively added to the bath to induce contraction and contractile effect was recorded on 2-channel recorder using isotonic force transducer (UGO BASILE 7070). Results were compared with the vehicle control rat thoracic aortic strip.

## 2. Antihistaminic Activity

### Protection against histamine-induced bronchospasm on conscious guinea pigs (*in vivo* model)

A modification of the technique of Van Arman[240] was adopted to determine the antihistaminic potential of the synthesized compounds.

Guinea pigs of either sex (250-300 g) were fasted for 12 h. Six animals were taken in each group. The test compound, was administered orally at a dose of 10 mg/kg in 1% CMC and challenged with histamine aerosol (0.2% aqueous solution of histamine acid chloride 3 ml) in a vaponephrin pocket nebulizer sprayed into a closed transparent cage. The respiratory status reflecting the increasing degree of bronchoconstriction was recorded. The time for onset of convulsions (preconvulsion) was recorded. Animals remaining stable for more than 6 min. were considered protected against histamine induced bronchospasm. An intraperitoneal injection of chlorpheniramine maleate (Avil ; Hoechst, Mumbai) at a dose of 25 mg/kg was given for the recovery of the test animals. The mean preconvulsion time of test compounds treated animals was compared with control and is expressed in terms of percentage protection (Table 1).

$$\text{Percent protection} = ( 1- (T_1 / T_2) ( 100$$

$T_2$ . preconvulsive time of test compound ; $T_1$ - preconvulsive time of control

## 3. Sedative-hypnotic activity

Sedative-hypnotic activity was determined by measuring the reduction in motor activity, using actophotometer [241, 242].

The albino mice of either sex weighing between 20 and 25 gm, were divided into groups, each containing five animals. The animals were fasted for 24 h. All the compounds were suspended in 1% Na CMC and administered orally at a dose of 10 mg/kg. The animals in the control group were fed with same volume of Na CMC suspension. The chlorpheniramine maleate and cetirizine 5 mg/kg were used as standard drug for the comparison. Scores were recorded at 0.5, 1, 2 and 3 h after the drug administration (Table 2). The percent reduction in motor activity was calculated by the following formula and shown in Table 3.

$$\% \text{ Reduction in motor activity} = [(A-B)/A] \times 100$$

where A is basal score and B is score after drug treatment.

## 4. Anticancer activity

The test compounds were evaluated for anticancer activity in drug-screening programme at the National cancer Institute [NCI,USA].

In the 3-cell line, primary anticancer assay protocol [243], each cell line is innoculated and preincubated on a microtiter plate. Test agents are

then added at a single concentration and the culture incubated for 48 hours. End point determinations are made with sulforhodamine B, a protein binding dye. Results for each test agent are reported as the percentage of growth of the treated cells when compared to untreated controlled cells (Table 4). Compounds which reduce the growth of any one of the cell lines to 32% or less are passed on for evaluation in the full panel of 60 cell lines derived from nine different tissues and the compounds were tested at a minimum of five concentrations at 10 fold dilutions. A 48 h continuous drug exposure protocol was used and a SRB protein assay was used to estimate cell viability or growth. The $GI_{50}$ (Concentration causing 50% growth inhibition) was determined (Table 5), which corresponds to the $IC_{50}$ Value defined elsewhere [234].

## 5. Anti HIV Activity

The compounds were tested for their anti HIV activity. The MT-4 cells were grown in RPMI-1640 DM (Dutch modification) medium (Flow Laboratories, Irvine, Scotland), supplemented with 10 % (v/v) heat inactivated fetal calf serum (FCS) and 20 μg/ml gentamicin (E. Merck, Darmstadt, Germany). The cells were maintained at 37°C in a humidified atmosphere of 5% $CO_2$ in air. Every 3-4 days, cells were spun down and seeded at 3 x $10^5$ cells/ml in new cell culture flasks. At regular time intervals, the MT-4 cells were analyzed for the presence of mycoplasma and consistently found to be mycoplasma-free. HIV-1 ( strain HTLV-$III_B$LAI )[245] and HIV-2 (strain LAV-2ROD)[246] were obtained from the culture supernatant of HIV-1 or HIV-2 infected MT-4 cell lines[247]. The

virus titer of the supernatant was determined in MT- 4 cells. The virus stocks were stored at -70° until used.

Flat bottomed 96-well plastic microtiter plates (Falcon, Becton Dickinson, Mountain view, CA) were filled with 100 µl of complete medium using a Titertek Multidrop dispenser (Flow Laboratories). This eight-channel dispenser could fill a microtiter tray in less than 10sec. Subsequently, stock solutions (10 x final test concentration) of compounds were added in 25 µl volumes to two series of triplicate wells so as to allow simultaneous evaluation of their effects on HIV-and mock-infected cells. Serial five-fold dilutions were made directly in the microtiter trays using a Biomek 1000 robot (Beckman). Untreated control HIV- and mock-infected cell samples were included for each compound.

Fifty microlitres of HIV at 100 $CCID_{50}$ medium was added to either infected of mock-infected part of a microtiter tray. Exponentially growing MT-4 cells were centrifuged for 5 min at 140 x g and the supernatants were discarded. The MT-4 cells were resuspended at 6 x $10^5$ cells/ml in a flask which was connected with an autoclavable dispensing cassette of a Titertek Multidrop dispenser. Under slight magnetic stirring 50 µl volumes were then transferred to the microtiter tray wells. The outer row wells were filled with 200 µl of medium. The cell cultures were incubated at 37°C in a humidified atmosphere of 5% $CO_2$ in air. The cells remained in contact with the test compounds during the whole incubation period. Five days after infection the viability of mock and HIV-infected cells were examined spectrophotometrically by the MTT method.

**MTT assay**

The MTT assay is based on mitochondrial reduction of the yellow coloured 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) ondrial (Sigma Chemical Co., St. Lous, MO) by mitochondrial dehydrogenase of metabolically active cells to a blue formazan which can be measured spectrophotometrically. Therefore, to each well of the microiitre plates 20 µl of a solution of MTT (7.5 mg/ml) in phosphate-buffered saline was added using the Titertek Multidrop. The trays were further incubated at 37°C in a $CO_2$ incubator for 1 h. A fixed volume of medium (150 µl) was then removed from each cup using M96 washer (ICN flow) without disturbing the MT-4 cell cluster containing the formazan crystals.

Solubilization of the formazan crystals was achieved by adding 100 µl 10% (v/v) Triton X-100 in acidified isopropanol (2 ml concentrated HCl per 500 ml solvent) using the M96 washer. Complete dissolution of the formazan crystals could be obtained after the trays had been placed on a plate shaker for 10 min. Finally, the absorbances were read in a eight-channel computer-controlled photometer (Multiskan MCC, ICN Flow) at two wavelengths (540 and 690 nm). The absorbance measured at 690 nm was automatically subtracted from the absorbance at 540 nm, so as to eliminate the effects of non-specific absorption. Blanking was carried out directly on the microtiter plates with the first column wells which contained all reagents except the MT-4 cells. All data represent the average values for a minimum of three wells. The 50% cytotoxic

concentration ($CC_{50}$) was defined as the concentration of compound that reduced the absorbance ($OD_{540}$) of the Mock-infected control sample by 50%. The percent protection achieved by the compound in HIV-infected cells was calculated by the following formula:

$$\left(\frac{(OD_T)HIV}{(OD_C)mock}\right) - \left(\frac{(OD_C)HIV}{(OD_C)mock}\right)$$

Where ($OD_T$) HIV is the optical density measured with a given concentration of the test compound in HIV infected calls; ($OD_C$) mock is the optical density measured for the control untreated mock infected cells; all OD values determined at 540 nm· The dose achieving 50% protection according to the above formula was defined as the 50% effective concentration ($EC_{50}$). The results are presented in Table 6.

## 6. Antibacterial activity

Few of the title compounds were screened for their antibacterial activity by agar cup-plate method[248]. At a concentration of 200 µg/ml using DMF: water as solvent, against the following organisms *Salmonella typhi, Escheriachia coli, Vibreo cholerae, Staphylococcus epidermitis, Klebsiella pneumoniae, Pseudomonas aeruginosa and Shigella flexnari.* All Bacteria were grown on a Muller–Hinton Agar [Hi-media] plates (37°C, 24 h). The zone of inhibition of each strain was recorded. The

activity has been compared with the standard drug ciprofloxacin 10 μg/ml (Table 7).

## 7. Antitubercular activity

The synthesized compounds were screened for their *in Vitro* antimycobacterial activity against *Mycobacterium tuberculosis* at the Tuberculosis Antimicrobial Acquisition & Coordinating Facility (TAACF, USA).

Primary antimycobacterial ativity screening is conducted at 6.25 μg/ml against *Mycobacterium tuberculosis* $H_{37}RV$ (ATCC 27294) in BACTEC 12B medium using a broth microdilution assay, the Microplate Alamar Blue Assay (MABA)[249]. Compounds exhibiting fluorescence are tested in the BACTEC 460 radiometric system[249]. Compounds effecting <90% inhibition in the primary screen (*i.e.*, MIC >6.25 μg/ml) are not generally evaluated further. The results are expressed in terms of Minimum Inhibitory Concentration (MIC) and percentage inhibition of growth (Table 8).

## 8. Analgesic Activity

The analgesic activity of selected title compounds was studied in albino mice by Tail-flick Technique [250, 251].

The albino mice of either sex weighing between 20 and 25 gm, were divided into groups, each containing five animals. The animals were fasted for 12 h. The initial screening was done to select the mouse which showed a reaction time of 5 seconds or less. All the compounds were suspended in 1% Na CMC and administered orally at a dose of 10 mg/kg. The animals in the control group were fed with same volume of Na CMC suspension. The pentazocine 10 mg/kg was used as standard drug for the comparison.

Test for analgesia was carried out at 30 min, 1 h, 2 h and 3 h after administering the test compounds by immersing the tail of the animal for about 2-2.5 cm in water maintained at 55.5 ± 0.5° C. The time taken by the mouse to show the discomfort i.e., flicking of tail was taken as response time for analgesic activity. The results are recorded in Table 9.

## 9. Antiinflammatory Activity

The antiinflammatory activity of selected title compounds was studied by carrageenan induced paw oedema test in albino rats[252, 253] using plethysmometer.

The albino rats of either sex weighing between 175 and 200 gm, were divided into groups, each containing five animals. The animals were fasted for 12 h. The hind paw was marked just beyond tibio-tarsal junction, so that every time the paw is dipped in the mercury column upto the fixed mark to ensure constant paw volume. The initial paw volume was noted by mercury displacement technique. All the compounds were

suspended in 1% Na CMC and administered orally at a dose of 10 mg/kg. The animals in the control group were fed with same volume of Na CMC suspension. The diclofenac sodium 10 mg/kg was used as standard drug for the comparison. After 30 min 0.1 ml of 1% (w/v) carrageenan was injected in planter region of the paw and the paw volume was noted at 30 min, 1h ,2h, 3h, 4h and 5h after carrageenan administration. The results are recorded in Table 10.