7. Abstract of the invention:-

The present invention relates to the synthesis of molecules showing cytostatic/cytotoxic potential against leukemia. Study relates to a process for the preparation of 1, 4-dihydropyridine derivatives and their anti leukemic activity.

5. CLAIMS:-

We Claim:-

1) Synthesis of Diethyl 4-(4-chlorophenyl)-2,6-diphenyl-1,4-dihydropyridine-3,5-dicarboxylate **(3a)** as described in **Example-1** and its its antileukemic activity against K-562 cell line as given in **Table.**

2) Synthesis of Diethyl 4-(4-bromophenyl)-2,6-diphenyl-1,4-dihydropyridine-3,5-dicarboxylate **(3b)** as described in **Example-2** and its antileukemic activity against K-562 cell line as given in **Table.**

3) Synthesis of Diethyl 4-(4-cyanophenyl)-2,6-diphenyl-1,4-dihydropyridine-3,5-dicarboxylate **(3c)** as described in **Example-3** and its antileukemic activity against K-562 cell line as given in **Table.**

4) Synthesis of Diethyl 4-(3-fluorophenyl)-2,6-diphenyl-1,4-dihydropyridine-3,5-dicarboxylate **(3d)** as described in **Example-4** and its antileukemic activity against K-562 cell line as given in **Table.**

5) Synthesis of Diethyl 4-(3-chlorophenyl)-2,6-diphenyl-1,4-dihydropyridine-3,5-dicarboxylate **(3e)** as described in **Example-5** and its antileukemic activity against K-562 cell line as given in **Table.**

6) Synthesis of Diethyl 4-(2,5-dimethoxyphenyl)-2,6-diphenyl-1,4-dihydropyridine-3,5-dicarboxylate **(3f)** as described in **Example-6** and its antileukemic activity against K-562 cell line as given in **Table.**

7) Synthesis of Diethyl 4-(naphthalen-2-yl)-2,6-diphenyl-1,4-dihydropyridine-3,5-dicarboxylate **(3g)** as described in **Example-7** and its antileukemic activity against K-562 cell line as given in **Table.**

8) *Synthesis of Diethyl 2,6-diphenyl-1,4-dihydro-4,4'-bipyridine-3,5-dicarboxylate* ***(3h)*** *as* described in **Example-8** and its antileukemic activity against K-562 cell line as given in **Table**.

9) Synthesis of Diethyl 4-(furan-2-yl)-2,6-diphenyl-1,4-dihydropyridine-3,5-dicarboxylate **(3i)** as described in **Example-9** and its antileukemic activity against K-562 cell line as given in **Table**.

10) Synthesis of Diethyl 4-(5-bromothiophen-2-yl)-2,6-diphenyl-1,4-dihydropyridine-3,5-dicarboxylate **(3j)** as described in **Example-10** and its antileukemic activity against K-562 cell line as given in **Table**.

11) *These compounds could be considered new lead compounds in the treatment of leukemia.*

6. DATE:- 21st February 2014

SIGNATURES:-

Dr. M. M. V. Ramana

Dr. R. S. Lokhande

Ms. Ankita L. Mehta

21 FEB 2014

4. DESCRIPTION:-

Title: -

A process for the Synthesis of 1, 4-dihydropyridine derivatives and their antileukemic activity.

Abstract of the invention:-

The present invention relates to the synthesis of molecules showing cytostatic/cytotoxic potential against leukemia. Study relates to a process for the preparation of 1, 4-dihydropyridine derivatives and their anti leukemic activity.

Background of invention and Prior art:-

1,4-Dihydropyridines belong to the class of nitrogen-containing heterocycles having a 6-membered ring. Among the different dihydropyridine isomers, 1, 4-dihydropyridines merit special attention, not only because of the general interest in their chemistry, but particularly because of their increasing pharmaceutical importance.

1,4-Dihydropyridines, a class of calcium channel blockers are used as therapeutic agents to treat angina and hypertension. Several of them are also reported to exhibit a variety of biological and pharmacological activities, viz., bronchodilatory, vasodilatory, hepatoprotective, neuroprotectant, platelet aggregation inhibitory and cerebral anti-ischemic. They are also reported to possess potential anticancer properties. Hence, this field has attracted the attention of several medicinal chemists, pharmacologists and oncologists to investigate further in view of the growing resistance to chemotherapy by cancer cells.

Therefore, in view of growing incidents of different cancer cells resistance to various potent anticancer agents and dihydropyridines exhibiting an ability to chemosensitize such cells it has been thought worthwhile to synthesize some new dihydropyridine derivatives.

Research on anticancer activity of 1,4-dihydropyridine are well known in the prior arts including U.S. Pat. No. **US 20130005773 A1; WO 2002012235 A1.**

1,4-Dihydropyridine derivatives have been synthesized by a modified Hantzsch one-pot synthesis involving the three-component condensation reaction of ethyl benzoyl acetate with appropriate aldehyde and ammonium acetate by conventional methods.

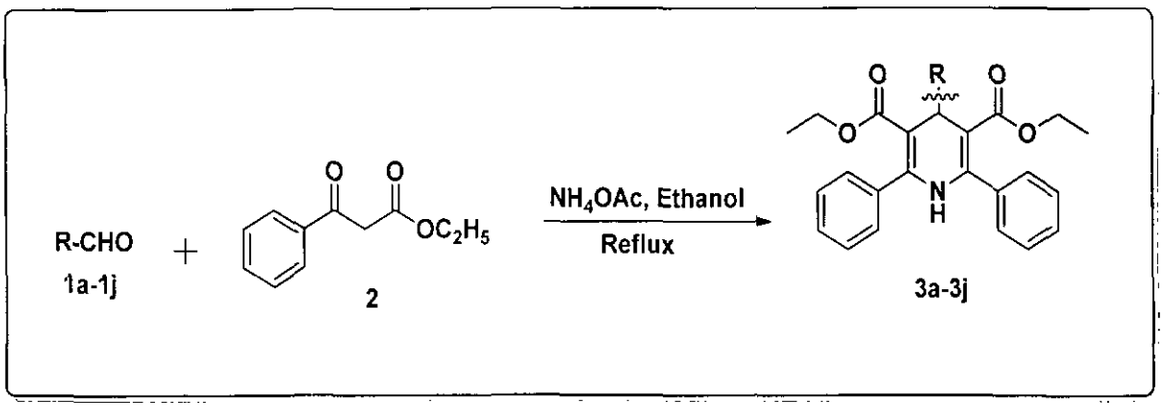
The present invention describes the synthesis of 1,4-dihydropridine derivatives **3a-3j (Scheme)** and their cytotoxicity evaluation (**Table**) against leukemia cell line K562.

Description of the invention:-

The present invention is successful in attaining all the above objectives. According to the invention there is provided a process for the preparation of 1,4-dihydropyridine derivatives (**Scheme**).

Table shows the results of antileukemic activity of the synthesized compounds against **K-562** cell line. These results are expressed in terms of percent growth in the presence of the test compounds. The GI_{50} values are shown in **Table.**

SCHEME



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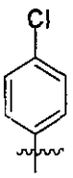
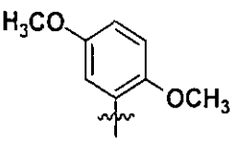
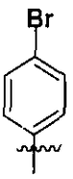
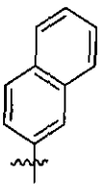
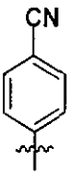
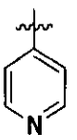
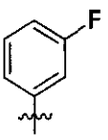
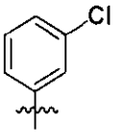
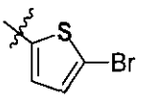
1a		1f	
1b		1g	
1c		1h	
1d		1i	
1e		1j	

Table

Tumor cell cytotoxicity of compounds 3a-3j on human cancer cell line K-562

Sr.No	Compounds	GI_{50} (μM)
1	3a	22.7
2	3b	25.0
3	3c	48.2
4	3d	47.4
5	3e	93.8
6	3f	30.1
7	3g	43.6
8	3h	70.5
9	3i	47.0
10	3j	52.6

In conclusion, compounds 3a, 3b, 3f show promising antileukemic activity against **K562** cell line.

Examples:-

Compounds **1a-1l** and **2** are commercially available.

Example-1

Preparation of Diethyl 4-(4-chlorophenyl)-2,6-diphenyl-1,4-dihydropyridine-3,5-dicarboxylate (3a).

Ethyl benzoyl acetate **(2)** (2 mmol) and 4-chlorobenzaldehyde **(1a)** (1 mmol) were taken into a RB flask and dissolved in ethanol by shaking. Ammonium acetate (1.3 mmol) was added, stirred and the reaction mixture was heated under reflux for 3-4 h, on a heating mantle. After completion of the reaction (monitored by TLC), ethanol was removed by distillation and the residue was cooled, triturated with crushed ice. The product was filtered, washed with small portions of cold water and dried. The isolated product was purified by recrystallization from aqueous ethanol, where by Diethyl 4-(4-chlorophenyl)-2,6-diphenyl-1,4-dihydropyridine-3,5-dicarboxylate **(3a)** was obtained as solid (82% Yield).

m.p.: 182-184 ^{0}C

IR (cm^{-1}): 3271, 3077, 2977, 1688, 1596, 784.

^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.503 (d, 2H, *J*=4.8 Hz), 7.405 (m, 10H), 7.298 (d, 2H, *J*=4.8 Hz), 5.981 (s, 1H, NH), 5.190 (s, 1H), 3.919 (m, 4H), 0.906 (t, 6H, *J*=6.9 Hz)

^{13}C NMR (75 MHz, $CDCl_3$) δ: 166.67, 145.97, 145.72, 136.54, 132.16, 129.34, 129.25, 128.40, 128.11, 119.24, 104.08, 59.87, 39.73, 13.66.

HRMS: *m/z* cal. mass for $C_{29}H_{27}ClNO_4$ $[M+H]^+$ = 488.97, obs. mass $[M+H]^+$ = 488.19.

Example-2:

Preparation of Diethyl 4-(4-bromophenyl)-2,6-diphenyl-1,4-dihydropyridine-3,5-dicarboxylate (3b).

It was prepared as described in **Example-1** by using 4-bromobenzaldehyde **(1b)** (1 mmol) in place of 4-chlorobenzaldehyde **(1a)** and using ethyl benzoyl acetate **(2)** (2 mmol), whereby

Diethyl 4-(4-bromophenyl)-2,6-diphenyl-1,4-dihydropyridine-3,5-dicarboxylate **(3b)** was obtained as solid (80 % Yield).

m.p.: 201-203 ^{0}C

IR (cm^{-1}): 3267, 3062, 2976, 1687, 1595, 590.

^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.632 (s, 1H, NH), 7.753 (d, 2H, *J*=6 Hz), 7.542 (d, 2H, *J*=6 Hz), 7.41 (m, 10H), 4.95 (s, 1H), 3.804 (q, 4H, *J*=7.2 Hz), 0.773 (t, 6H, *J*=7.2 Hz)

^{13}C NMR (75 MHz, $CDCl_3$) δ: 166.55, 147.51, 146.31, 135.67, 131.62, 129.73, 129.13, 128.99, 128.87, 119.24, 100.82, 59.10, 39.75, 13.40.

HRMS: *m/z* cal. mass for $C_{29}H_{27}BrNO_4$ $[M+H]^+$ = 533.43, obs. mass $[M+H]^+$ = 533.71.

Example-3

Preparation of Diethyl 4-(4-cyanophenyl)-2,6-diphenyl-1,4-dihydropyridine-3,5-dicarboxylate (3c). It was prepared as described in **Example-1** by using 4-cyanobenzaldehyde **(1c)** (1 mmol) in place of 4-chlorobenzaldehyde **(1a)** and using ethyl benzoyl acetate **(2)** (2 mmol), whereby Diethyl 4-(4-cyanophenyl)-2,6-diphenyl-1,4-dihydropyridine-3,5-dicarboxylate **(3c)** was obtained as solid (80 % Yield).

m.p.: 196-198 ^{0}C

IR (cm^{-1}): 3435, 3055, 2987, 2228, 1688, 1600.

^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.668 (d, 2H, *J*=8.4 Hz), 7.633 (d, 2H, *J*=8.4 Hz), 7.420 (m, 10H), 6.040 (s, 1H, NH), 5.269 (s, 1H), 3.929 (m, 4H), 0.890 (t, 6H, *J*=7.2 Hz)

^{13}C NMR (75 MHz, $CDCl_3$) δ: 166.48, 152.55, 146.23, 136.24, 132.28, 129.54, 128.66, 128.48, 128.07, 119.23, 110.29, 103.41, 60.01, 40.65, 13.62.

HRMS: *m/z* cal. mass for $C_{30}H_{27}N_2O_4$ $[M+H]^+$ = 479.54, obs. mass $[M+H]^+$ = 479.63.

Example-4

Preparation of Diethyl 4-(3-fluorophenyl)-2,6-diphenyl-1,4-dihydropyridine-3,5-dicarboxylate (3d).

It was prepared as described in **Example-1** by using 3-fluorobenzaldehyde **(1d)** (1 mmol) in place of 4-chlorobenzaldehyde **(1a)** and using ethyl benzoyl acetate **(2)** (2 mmol), whereby Diethyl 4-(3-fluorophenyl)-2,6-diphenyl-1,4-dihydropyridine-3,5-dicarboxylate **(3d)** was obtained as solid (78 % Yield).

m.p.: 123-125 ^{0}C

IR (cm^{-1}): 3250, 3061, 2986, 1677, 1596, 1210, 1189.

^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.409 (s, 1H), 7.396 (m, 10H), 6.948 (m, 3H), 6.028 (s, 1H, NH), 5.240 (s, 1H), 3.939 (m, 4H), 0.925 (t, 6H, *J*=7.2 Hz)

^{13}C NMR (75 MHz, $CDCl_3$) δ: 166.62, 161.43, 149.27, 136.48, 134.03, 129.53, 129.37, 128.42, 128.15, 128.08, 126.68, 126.12, 103.84, 59.92, 40.03, 13.68.

HRMS: *m/z* cal. mass for $C_{29}H_{27}FNO_4$ $[M+H]^+$ = 472.32, obs. mass $[M+H]^+$ = 472.19.

Example-5

Preparation of Diethyl 4-(3-chlorophenyl)-2,6-diphenyl-1,4-dihydropyridine-3,5-dicarboxylate (3e).

It was prepared as described in **Example-1** by using 3-chlorobenzaldehyde **(1e)** (1 mmol) in place of 4-chlorobenzaldehyde **(1a)** and using ethyl benzoyl acetate **(2)** (2 mmol), whereby Diethyl 4-(3-chlorophenyl)-2,6-diphenyl-1,4-dihydropyridine-3,5-dicarboxylate **(3e)** was obtained as solid (78 % Yield).

m.p.: 148-150 ^{0}C

IR (cm^{-1}): 3283, 3064, 2981, 1683, 1600, 696, 760.

^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.535 (s, 1H), 7.466 (m, 3H), 7.389 (m, 10H), 6.026 (s, 1H, NH), 5.211 (s, 1H), 3.952 (m, 4H), 0.93 (t, 6H, *J*=6.9 Hz)

^{13}C NMR (75 MHz, $CDCl_3$) δ: 166.61, 149.269, 145.934, 136.486, 134.03, 129.53, 129.36, 128.41, 128.15, 128.07, 126.69, 126.11, 103.84, 59.91, 40.02, 13.67

HRMS: *m/z* cal. mass for $C_{29}H_{27}ClNO_4$ $[M+H]^+$ = 488.04, obs. mass $[M+H]^+$ = 488.16.

Example-6

Preparation of Diethyl 4-(2,5-dimethoxyphenyl)-2,6-diphenyl-1,4-dihydropyridine-3,5-dicarboxylate (3f).

It was prepared as described in **Example-1** by using 2,5-dimethoxybenzaldehyde **(1f)** (1 mmol) in place of 4-chlorobenzaldehyde **(1a)** and using ethyl benzoyl acetate **(2)** (2 mmol), whereby Diethyl 4-(2,5-dimethoxyphenyl)-2,6-diphenyl-1,4-dihydropyridine-3,5-dicarboxylate **(3f)** was obtained as solid (84 % Yield).

m.p.: 170-172 ^{0}C

IR (cm^{-1}): 3290, 3058, 2992, 1705, 1598, 1053.

^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.431 (m, 10H), 7.101 (d, 1H, *J*=3 Hz), 6.861 (d, 1H, *J*=9 Hz), 6.762 (dd, 1H, *J*=3, 9 Hz), 5.903 (s, 1H, NH), 5.447 (s, 1H), 3.899 (q, 4H, *J*=7.2Hz), 3.774 (s, 6H), 0.931 (t, 6H, *J*=7.2 Hz)

^{13}C NMR (75 MHz, $CDCl_3$) δ: 167.09, 153.38, 152.47, 145.55, 137.22, 135.82, 128.96, 128.30, 128.09, 116.79, 112.31, 111.81, 103.18, 59.55, 55.99, 55.67, 37.37, 13.75.

HRMS: *m/z* cal. mass for $C_{31}H_{32}NO_6$ $[M+H]^+$ = 514.58, obs. mass $[M+H]^+$ = 514.67.

Example-7

Preparation of Diethyl 4-(naphthalen-2-yl)-2,6-diphenyl-1,4-dihydropyridine-3,5-dicarboxylate (3g).

It was prepared as described in **Example-1** by using 2-naphthaldehyde **(1g)** (1 mmol) in place of 4-chlorobenzaldehyde **(1a)** and using ethyl benzoyl acetate **(2)** (2 mmol), whereby Diethyl 4-(naphthalen-2-yl)-2,6-diphenyl-1,4-dihydropyridine-3,5-dicarboxylate **(3g)** was obtained as solid (80 % Yield).

m.p.: 187.189 ^{0}C

IR (cm^{-1}): 3408, 3062, 2977, 1695, 1598.

^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.950 (m, 17H), 6.046 (s, 1H, NH), 5.422 (s, 1H), 3.924 (m, 4H), 0.912 (t, 6H, *J*=7.2 Hz)

^{13}C NMR (75 MHz, $CDCl_3$) δ: 166.83, 145.70, 144.74,136.70, 133.60, 132.57, 129.26, 128.38, 128.21, 128.11, 127.99, 127.53, 126.76, 126.23, 125.64, 125.29, 104.28, 59.82, 40.32, 13.68.

HRMS: *m/z* cal. mass for $C_{33}H_{30}NO_4$ $[M+H]^+$ = 504.59, obs. mass $[M+H]^+$ = 504.24.

Example-8

Preparation of Diethyl 2,6-diphenyl-1,4-dihydro-4,4'-bipyridine-3,5-dicarboxylate (3h).

It was prepared as described in **Example-1** by using 4-pyridinecarboxaldehyde **(1h)** (1 mmol) in place of 4-chlorobenzaldehyde **(1a)** and using ethyl benzoyl acetate **(2)** (2 mmol), whereby Diethyl 2,6-diphenyl-1,4-dihydro-4,4'-bipyridine-3,5-dicarboxylate **(3h)** was obtained as solid (78 % Yield).

m.p.: 206-208 ^{0}C

IR (cm^{-1}): 3060, 2980, 2876, 1703, 1599.

^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.939 (d, 2H, *J*=9 Hz), 7.473 (m, 12H), 6.035 (s, 1H, NH), 5.410 (s, 1H), 3.914 (m, 4H), 0.910 (t, 6H, *J*=7.2 Hz)

^{13}C NMR (75 MHz, $CDCl_3$) δ: 164.749, 148.847, 148.18, 134.86, 134.57, 130.12, 128.08, 127.83, 123.70, 104.08, 59.81, 13.42.

HRMS: *m/z* cal. mass for $C_{28}H_{27}N_2O_4$ $[M+H]^+$ = 455.74, obs. mass $[M+H]^+$ = 455.59.

Example-9

Preparation of Diethyl 4-(furan-2-yl)-2,6-diphenyl-1,4-dihydropyridine-3,5-dicarboxylate (3i).

It was prepared as described in **Example-1** by using 2-furaldehyde **(1i)** (1 mmol) in place of 4-chlorobenzaldehyde **(1a)** and using ethyl benzoyl acetate **(2)** (2 mmol), whereby Diethyl 4-(furan-2-yl)-2,6-diphenyl-1,4-dihydropyridine-3,5-dicarboxylate **(3i)** was obtained as solid (83 % Yield).

m.p.: 172-174 ^{0}C

IR (cm^{-1}): 3259, 3054, 2987, 1674, 1596, 1211.

^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.404 (m, 10H), 7.346 (d, 1H, *J*=1.5 Hz), 6.327 (q, 1H, J=1.8, 1.5 Hz), 6.209 (d, 1H, *J*=3 Hz), 6.120 (s, 1H, NH), 5.421 (s, 1H), 3.992 (m, 4H), 0.961 (t, 6H, *J*=6.9 Hz)

^{13}C NMR (75 MHz, $CDCl_3$) δ: 166.58, 158.24, 146.60, 141.36, 136.50, 129.31, 128.30, 110.23, 104.88, 100.88, 59.86, 34.02, 13.74.

HRMS: *m/z* cal. mass for $C_{27}H_{26}NO_5$ $[M+H]^+$ = 444.71, obs. mass $[M+H]^+$ = 444.87.

Example-10

Preparation of Diethyl 4-(5-bromothiophen-2-yl)-2,6-diphenyl-1,4-dihydropyridine-3,5-dicarboxylate (3j).

It was prepared as described in **Example-1** by using 5-bromo-2-thiophenecarboxaldehyde **(1j)** (1 mmol) in place of 4-chlorobenzaldehyde **(1a)** and using ethyl benzoyl acetate **(2)** (2 mmol),

whereby Diethyl 4-(5-bromothiophen-2-yl)-2,6-diphenyl-1,4-dihydropyridine-3,5-dicarboxylate **(3j)** was obtained as solid (79 % Yield).

m.p.: 185-187 ^{0}C

IR (cm^{-1}): 3203, 3063, 2977, 1678, 1596, 590.

^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.426 (m, 10H), 6.899 (d, 1H, *J*=3.6 Hz), 6.843 (d, 1H, *J*=3.6 Hz), 6.188 (s, 1H, NH), 5.471 (s, 1H), 3.998 (q, 4H, *J*=7.2 Hz), 0.964 (t, 6H, *J*=7.2 Hz)

^{13}C NMR (75 MHz, $CDCl_3$) δ: 166.35, 152.80, 146.11, 136.16, 129.54, 129.52, 128.41, 128.26, 123.79, 109.91, 103.14, 60.06, 35.68, 13.74.

HRMS: *m/z* cal. mass for $C_{27}H_{25}BrNO_4S$ $[M+H]^+$ = 539.61, obs. mass $[M+H]^+$ = 539.43.

5. CLAIMS:-

We Claim:-

1) Synthesis of Diethyl 4-(4-chlorophenyl)-2,6-diphenyl-1,4-dihydropyridine-3,5-dicarboxylate **(3a)** as described in **Example-1** and its its antileukemic activity against K-562 cell line as given in **Table.**

2) Synthesis of Diethyl 4-(4-bromophenyl)-2,6-diphenyl-1,4-dihydropyridine-3,5-dicarboxylate **(3b)** as described in **Example-2** and its antileukemic activity against K-562 cell line as given in **Table.**

3) Synthesis of Diethyl 4-(4-cyanophenyl)-2,6-diphenyl-1,4-dihydropyridine-3,5-dicarboxylate **(3c)** as described in **Example-3** and its antileukemic activity against K-562 cell line as given in **Table.**

4) Synthesis of Diethyl 4-(3-fluorophenyl)-2,6-diphenyl-1,4-dihydropyridine-3,5-dicarboxylate **(3d)** as described in **Example-4** and its antileukemic activity against K-562 cell line as given in **Table.**

5) Synthesis of Diethyl 4-(3-chlorophenyl)-2,6-diphenyl-1,4-dihydropyridine-3,5-dicarboxylate **(3e)** as described in **Example-5** and its antileukemic activity against K-562 cell line as given in **Table.**

6) Synthesis of Diethyl 4-(2,5-dimethoxyphenyl)-2,6-diphenyl-1,4-dihydropyridine-3,5-dicarboxylate **(3f)** as described in **Example-6** and its antileukemic activity against K-562 cell line as given in **Table.**

7) Synthesis of Diethyl 4-(naphthalen-2-yl)-2,6-diphenyl-1,4-dihydropyridine-3,5-dicarboxylate **(3g)** as described in **Example-7** and its antileukemic activity against K-562 cell line as given in **Table.**

8) *Synthesis of Diethyl 2,6-diphenyl-1,4-dihydro-4,4'-bipyridine-3,5-dicarboxylate* ***(3h)*** *as* described in **Example-8** and its antileukemic activity against K-562 cell line as given in **Table**.

9) Synthesis of Diethyl 4-(furan-2-yl)-2,6-diphenyl-1,4-dihydropyridine-3,5-dicarboxylate **(3i)** as described in **Example-9** and its antileukemic activity against K-562 cell line as given in **Table**.

10) Synthesis of Diethyl 4-(5-bromothiophen-2-yl)-2,6-diphenyl-1,4-dihydropyridine-3,5-dicarboxylate **(3j)** as described in **Example-10** and its antileukemic activity against K-562 cell line as given in **Table**.

11) *These compounds could be considered new lead compounds in the treatment of leukemia.*

6. DATE:- 21st February 2014

SIGNATURES:-

Dr. M. M. V. Ramana

Dr. R. S. Lokhande

Ms. Ankita L. Mehta

21 FEB 2014