



## Evaluation of Antioxidant and Antitumor Activities of Some Prepared Nitrogen Heterocycles

## KEYWORDS

1,2,4-triazine, Synthesis, Spectral Studies, Antioxidant activity, Antitumor activity

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**ABSTRACT** 4-Benzylidene-2-(p-chlorophenyl)-4H-oxazol-5-one (2) was prepared via cyclocondensation of N-(p-chlorobenzoyl) glycine with benzaldehyde in presence of fused sodium acetate and acetic anhydride. Condensation of 2 with semicarbazide hydrochloride in presence of fused sodium acetate afforded the corresponding 3,5-disubstituted-2-(aminocarbonyl)-1,2,4-triazine-6-one (3). Acetylation of 3 with acetic anhydride yielded the monoacetyl and diacetyl derivatives (4 and 5). Methyl  $\alpha$ -(p-chlorobenzoyl) amino- $\beta$ -phenyl acrylate (6) was synthesized via the reaction of 2 with triethylamine in methanol. 5,6-disubstituted-4-hydroxypyrimidine-2-thiole (7) was obtained via cyclocondensation of ester (6) with thiourea in presence of anhydrous potassium carbonate. Synthesized compounds were characterized by EI-MS and NMR spectroscopy. The biological activity studies of triazines and pyrimidine-2-thiole were carried out against antioxidant and antitumor activities.

### 1- Introduction

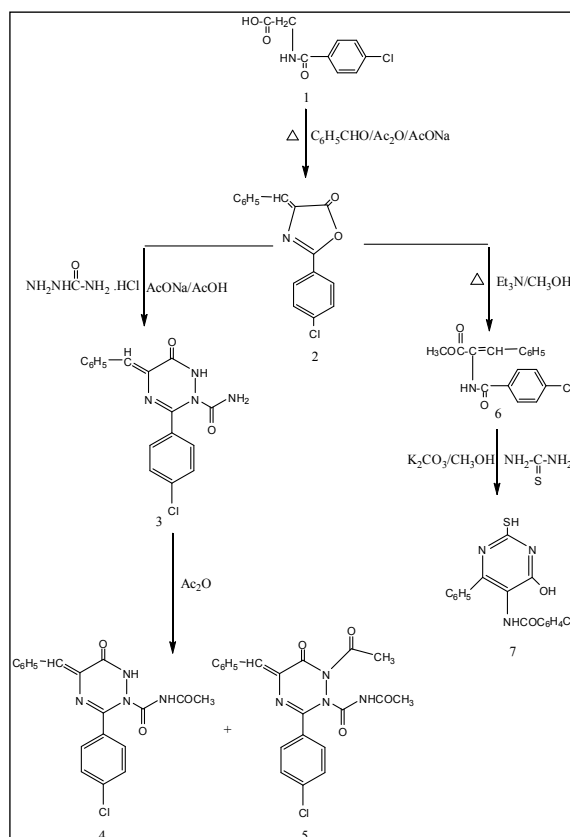
Triazine derivatives have occupied a unique position in medicinal chemistry. Triazine derivative have attracted considerable pharmaceutical interest due to their antitumor<sup>1-5</sup>, anticonvulsant<sup>6</sup> and antileukemic<sup>7,8</sup> activities and cytotoxic effects<sup>9</sup>. Triazine has been used to form many types of functional groups other than amines and heterocycles and used as protecting groups in natural product synthesis. Thus, they are reactive groups, which are adaptable to different synthetic transformations. Among the compound having good antimicrobial properties<sup>10</sup>, s-triazine derivatives constitute an important class of compounds possessing diverse pharmacological activities including broadly active triazine compounds. This paper reported the preparation of some nitrogen heterocycles using 4-benzylidene-2-(p-chlorophenyl)-4H-oxazol-5-one (2) as a key starting material which was obtainable in the reaction of N-(p-chlorophenyl)-glycine (1) with benzaldehyde in presence of acetic anhydride and fused sodium acetate.

### 2- Results and Discussion.

#### 2.1. Chemistry

4-Benzylidene-2-(p-chlorophenyl)-4H-oxazol-5-one (2) was prepared from N-(p-chlorobenzoyl)-glycine (1) and benzaldehyde in the presence of acetic anhydride and fused sodium acetate according to a published literature procedure<sup>11</sup>. The condensation of 4-benzylidene-2-(p-chlorophenyl)-4H-oxazol-5-one (2), semicarbazide, sodium acetate using glacial acetic acid as reaction solvent to afford the corresponding 5-benzylidene-3-(p-chlorophenyl)-2-(aminocarbonyl)-1,2,4-triazine-6-one (3) in good yield<sup>12</sup>. The formation of the 1,2,4-triazine (3) was proceeding through the attack of semicarbazide on the carbonyl carbon of 4-benzylidene-2-(p-chlorophenyl)-4H-oxazol-5-one (2), followed by elimination of water molecule.

Acetylation of substituted -1,2,4-triazine (3) with acetic anhydride under reflux yielded the corresponding 5-benzylidene-3-(p-chlorophenyl)-2-(acetylamino)carbonyl-1,2-dihydro-1,2,4-triazine-6-one (4) and 5-benzylidene-3-(p-chlorophenyl)-2-(diacetylamino)carbonyl-1,2-dihydro-1,2,4-triazine-6-one (5) respectively.



Scheme 1: Synthesis of compounds 2, 3, 4, 5, 6, 7

Treatment<sup>13</sup> of 4-benzylidene-2-(p-chlorophenyl)-4H-oxazol-5-one (2) with triethylamine in methanol under reflux led to the formation of methyl  $\alpha$ -(p-chlorobenzoyl) amino- $\beta$ -phenyl acrylate (6). Cyclocondensation<sup>14</sup> of ester (6) with thiourea in the presence of anhydrous potassium carbonate in methanol yielded the corresponding 6-phenyl-5-(p-chlorophenyl)amino-4-hydroxy-pyrimidin-2-thiole (7).

## 2.2. Mass spectroscopy

The mass spectral decomposition modes of some nitrogen heterocycles have been suggested and investigated<sup>15,16</sup>.

1,2,4-Triazines derivatives 3, 4 and 5

The mass spectra of compounds 3, 4 and 5 show relatively weak and strong molecular ions and peaks typical of a cleavage and rearrangement processes type fragmentation. Thus compounds 3, 4 and 5 showed an intense molecular ion peak at 340, 382 and 424 corresponding to the molecular formulas  $C_{17}H_{13}N_4ClO_2$ ,  $C_{19}H_{15}N_4ClO_3$  and  $C_{21}H_{17}N_4ClO_4$ , respectively.

From the study of the mass spectra of compound 3 (Fig 1), it was found that the molecular ion for compound (3) fragmented further and involved two various possible pathways as illustrated by scheme 2. The molecular ion of compound 3 fragmented to produce ion at  $m/z$  339 after losing the hydrogen atom. The fragment ion at  $m/z$  339 fragmented via the suggested pathway A to a fragment ion at  $m/z$  297, corresponding to the molecular ion of 5-benzylidene-3-(*p*-chlorophenyl)-1,2-dihydro-1,2,4-triazine-6(5H)-one, by losing isocyanate group (NCO). The loss of phenyl acetylene ( $C_6H_5-C\equiv C-H$ ) from the ion of  $m/z$  297 gave peak at  $m/z$  195. The fragment ion of  $m/z$  195 was broken to give fragment ion at  $m/z$  153, which lost the isocyanate group (NCO). It underwent further loss of nitrogen atom (N) gave a stable peak at  $m/z$  139. The fragmentation led to ions at  $m/z$  111 and  $m/z$  75, respectively. The fragment ion at  $m/z$  339 was also found to undergo fragmentation via pathway B to produce the ion  $m/z$  296, which further fragmented and gave a fragment ion  $m/z$  282 by losing the nitrogen atom (N). The fragmentation led to ions at  $m/z$  152,  $m/z$  137 and  $m/z$  102, respectively.

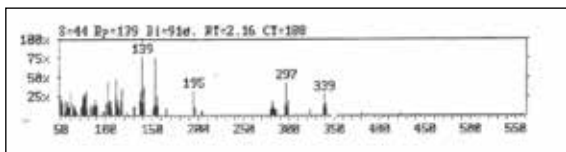


Figure 1: Mass spectrum of compound (3)

### Scheme 2: Fragmentation pathway of 1,2,4-triazine-6(5H)-one (3)

The loss of the hydrogen atom from the molecular ions of compounds 4 (Fig 2) and 5 (Fig 3) gave peaks at  $m/z$  381 and 423, respectively. The fragment ion of  $m/z$  381 was broken to give fragment ion of  $m/z$  339, which lost the ketene molecule.

The loss of the two ketene molecules from the fragment ion for compound 5 ( $m/z$  423) gave the fragment ions of  $m/z$  381 and  $m/z$  339, respectively.

The fragment ion of  $m/z$  339 for compounds 4 and 5 broke further via a pathway similar to compound 3 (Scheme 2). The EIMS of compounds 3 and 5 showed a base peak at  $m/z$  139, while the compound 4 showed a base peak at  $m/z$  297.

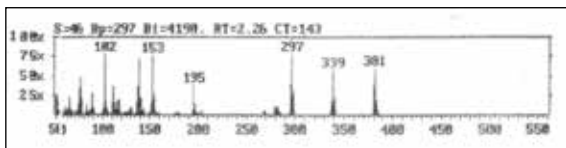


Figure 2: Mass spectrum of compound (4)

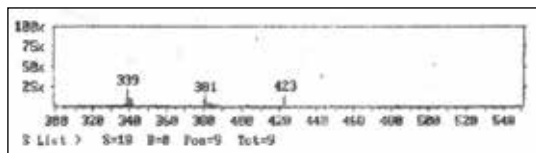
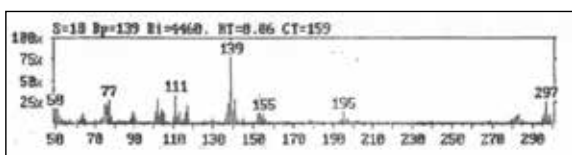


Figure 3: Mass spectrum of compound (5)

### Compound 6

The mass spectra of compound 6 (Fig 4) was fully consistent with the assigned structure thus, compound 6 showed intense molecular ion peak at  $m/z$  315, corresponding to the molecular formula  $C_{17}H_{14}NClO_3$ . The molecular ion of compound 6 fragmented via the suggested pathway A to fragment ion at  $m/z$  283, corresponding to the molecular ion of 4-benzylidene-2-(*p*-chlorophenyl)-4H-oxazol-5-one (compound 2). The loss of phenyl acetylene from a fragment ion at  $m/z$  283 gave peak at  $m/z$  182. The fragment ion of  $m/z$  182 was broken to give a stable ion at  $m/z$  139 which lost the iminocarbonyl group ( $NH=C=O$ ). The fragmentation led to ions of  $m/z$  110, 74 and  $m/z$  50, respectively (scheme 3). The molecular of compound 6 was also found to undergo fragmentation via pathway B to produce the stable ion at  $m/z$  139, which further fragmented and gave a fragment ion at  $m/z$  111. It underwent further loss of HCl and two carbon atom and gave peaks at  $m/z$  75 and  $m/z$  51, respectively.

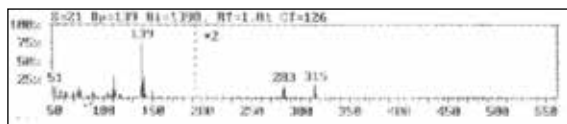
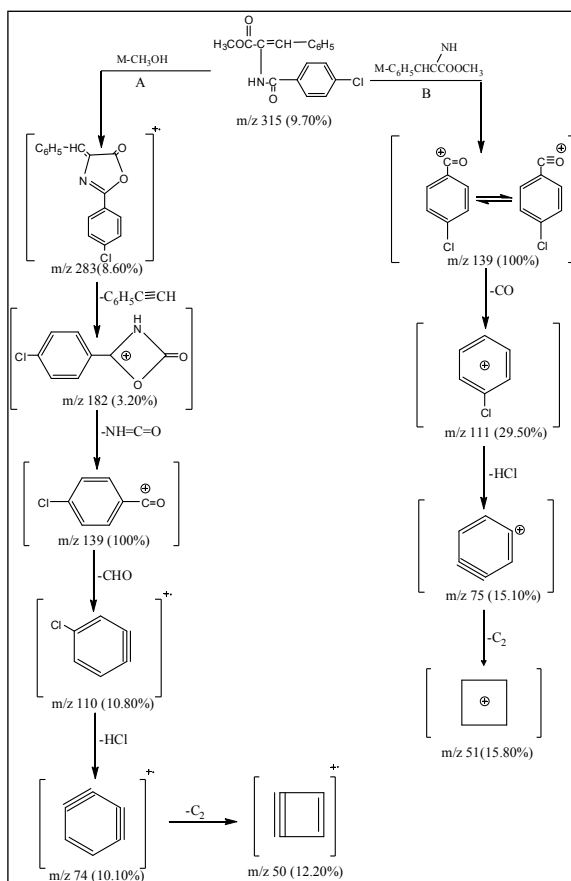


Figure 4: Mass spectrum of compound (6)



Scheme 3: Fragmentation pathway of compound (6)

3- Biological Assay

3.1. Antioxidant activity

The antioxidant of some prepared 1,2,4-triazine derivatives (3, 4 and 5) and pyrimidine derivative (7) were determined by the scavenging of synthetic radicals 2,2-diphenyl-1-picrylhydrazyl (DPPH) in polar organic solvent<sup>17</sup>. A methanol solution of the test compounds were prepared. Absorbance measurements were recorded immediately with a Milton Roy spectronic 201 UV-visible spectrophotometer. The decrease in absorbance at 515 nm was determined continuously, with data being recorded at 1 min intervals until the absorbance stabilized (16 min). Tocopherol was used as a reference standard at the same concentration of used tested compounds. The absorbance of the DPPH radical without antioxidant was also measured as control and 95% methanol was used as blank. All the determinations were performed in three replicates and averaged.

% scavenging of the DPPH free radical was measured using the following equation:-

% DPPH radical-scavenging = [(Absorbance of control - Absorbance of test sample) / (Absorbance of control)] × 100.

Tested samples had been submitted for qualitative evaluation of the antioxidant activity.

The provided samples had different antioxidant activity using DPPH radical scavenging method as shown by the following table 1.

Table 1: Antioxidant activity of some prepared compounds 3,4,5 and 7

Compounds No.	DPPH radical scavenging activity
3	+
4	-
5	+
7	-

(+) = weak      (++) = moderate      (+++) = Good  
 (++++) = strong      (-) = no activity

The results indicated that the compounds 3 and 5 were observed weak active, while the compounds 4 and 7 were found to be inactive.

3.2. Anticancer activity

Cytotoxic and antitumor activity of prepared compounds 3, 4, 5 and 7 were evaluated against cell lines MCF-7 and HePG-2 according to the method of Mosamann and Vijayan et al<sup>18,19</sup>. The drug vinblastine was used as standard. Inhibitory activity against breast carcinoma cells (MCF-7 cell line) and hepatocellular carcinoma cells (HePG-2 cell line) was tested by using different concentrations of the samples (50, 25, 12.5, 6.25, 3.125, and 1.56 µg), and cell viability (%) was determined by colorimetric method. The 50% inhibitory concentration (IC<sub>50</sub>) of the MCF-7 cell line was calculated from Table 2 and Fig 5.

Table 2: Evaluation of cytotoxicity of prepared compounds against cell line MCF-7

Sample conc. (µg)	Viability%				
	3	4	5	7	Vinblastine standard
50	8.77	9.92	8.86	32.21	7.82
25	17.12	20.34	13.24	51.82	15.18
12.5	23.94	25.65	29.48	64.58	29.60
6.25	42.67	46.26	42.66	84.95	48.75
3.125	79.74	76.14	53.85	93.14	60.35
1.56	87.28	90.43	79.12	98.04	76.24
0	100.00	100.00	100.00	100.00	100.00

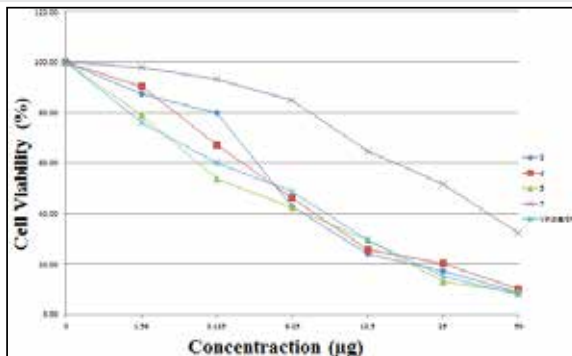


Figure 5:- Evaluation of cytotoxicity of prepared compounds against MCF-7 cell line.

The 50% inhibitory concentration (IC<sub>50</sub>) of the HePG-2 cell line was calculated from table 3 and figure 6.

Table 3: Evaluation of cytotoxicity of prepared compounds against cell line HePG-2

Sample conc.(µg)	Viability%				
	3	4	5	7	Vinblastine standard
50	7.14	13.76	8.94	36.89	14.38
25	12.79	22.53	15.91	65.71	16.13
12.5	23.25	37.28	24.82	76.43	24.25
6.25	47.46	54.61	39.73	88.65	45.13
3.125	73.28	76.22	68.05	94.72	55.00
1.56	86.71	89.74	81.56	98.54	72.13
0	100.00	100.00	100.00	100.00	100.00

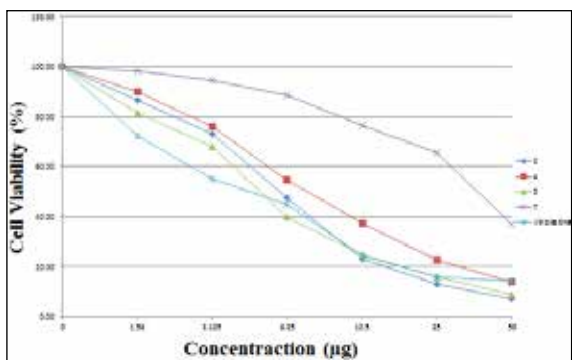


Figure 6:- Evaluation of cytotoxicity of prepared compounds against HePG-2 cell line.

The results of 50% inhibitory concentration (IC<sub>50</sub>) data are summarized in table 4.

Table 4: IC<sub>50</sub> (µg) values of prepared compounds after 72 h continuous exposure of tumor cell lines

Compound	Tumor type/cell line	
	MCF-7	HePG-2
3	5.6	5.9
4	5.9	7.9
5	4.2	5.1
7	27.3	38.60
Vinblastine standard	6.10	4.60

The IC<sub>50</sub> Values is the concentration that induces 50% growth inhibition compared with untreated control cells.

MCF-7: Human breast carcinoma cell line.

HePG-2: Human hepatocellular carcinoma cell line.

Compounds 3, 4 and 5 were found to be more active than

standard antitumor drug vinblastine against MCF-7 cell lines. Compound 7 was observed to be weakly active than standard antitumor drug against MCF-7 cell line.

In comparison with standard antitumor drug vinblastine, compounds 3,4 and 5 were found to be more active against the HePG-2 cell line, while compound 7 was observed to be more weakly active against HePG-2.

### Conclusions

In conclusion we have described the preparation and biological activities of a new nitrogen heterocyclic compounds. 1,2,4-triazine derivatives and pyrimidine derivative showed inhibitor activity against MCF-7 cell line and HePG-2 cell line. The antioxidant activity of some prepared compounds were also evaluated. The biological data revealed that with slight modifications in the structure one can plan for the drug design.

### Experimental

Melting points were determined on MEL-TEMP II melting point apparatus and uncorrected. Infra-red spectra were recorded on a Perkin-Elmer 1420 spectrometer and a Bio-rad FTS7 (KBr). NMR spectra were recorded on a General Electric QE 300 instrument and chemical shifts were given with respect TMS. Mass spectra were recorded on GC/MS with CI (chemical ionization) and a Hewlett-Packard MS Engine ThermoSpray and ionization by electron impact to 70 ev. The accelerating voltage was 6 KV, the temperature of the ion source was = 200°C and the emission current = 100mA. Microanalyses were conducted using an elemental analyzer 1106.

#### 4-Benzylidene-2-(p-chlorophenyl)-4H-oxazol-5-one (2)

A mixture of N-(p-chlorobenzoyl) glycine (0.01 mole), benzaldehyde (0.01 mole), fused sodium acetate (0.03 mole) and acetic anhydride (0.01 mole) was fused on a hot plate for 3-5 min. The reaction mixture was heated on a water bath for 2 hr, then cooled and poured on to water. The solid obtained was filtered off, washed with hot water, dried and purified by benzene to give 2 as yellow crystals, yield 79%, m.p. 140°C. IR(KBr): 1768 (C=O), 1632 (C=N), 1615, 1605, 1583 (C=C), 1215, 1098 (C-O) cm<sup>-1</sup>. <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>): δ, 6.91-7.83 (m, 10H, Ar-H and H-olefinic) ppm. MS: m/z (%): 285 (M++2, 4.20), 284 (M++1, 6.50), 283 (M+, 8.60), 282(5.80), 149(10.20), 143(5.30), 142(6.70), 141(29.50), 140(23.10), 139(100), 138(13.20), 137(3.60), 118(3.80), 117(3.40), 113(12.50), 112(6.30), 111(28.90), 110(11.20), 109(3.20), 108(4.20), 105(8.30), 104(7.80), 91(5.80), 90(6.30), 89(8.30), 77(10.20), 76(7.40), 75(15.20), 74(10.30), 63(6.70), 61(8.60), 57(9.30), 55(11.20), 51(15.80), 50(12.30). Anal. Calcd. for C<sub>16</sub>H<sub>10</sub>NClO<sub>2</sub>: C, 67.84; H, 3.53; N, 4.95. Found: C, 67.68; H, 3.35; N, 4.71.

#### 5-Benzylidene-3-(p-chlorophenyl)-2-(aminocarbonyl)-1,2-dihydro-1,2,4-triazine-6(5H)-one (3)

A mixture of oxazolinone (2, 0.01 mole), semicarbazide hydrochloride (0.01 mole) and fused sodium acetate (0.03 mole) in acetic acid (30 ml) was heated under reflux for 3 hr, then cooled and poured into water. The resulting solid was filtered off, washed with hot water, dried and purified by ethanol to give 3 as yellow crystals, yield 76%, m.p. 220°C. IR(KBr): 3415, 3205 (NH<sub>2</sub>), 3285 (NH), 1695-1683 (C=O), 1633 (C=N), 1605, 1587 (C=C), cm<sup>-1</sup>. <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>): δ, 6.21 (s, 2H, NH<sub>2</sub>), 7.13-8.10 (m, 10H, Ar-H and H-olefinic), 10.63 (s, 1H, NH) ppm. MS: m/z (%): 342 (M++2, 8.80), 341 (M++1, 15.40), 340 (M+, 12.10), 339 (M+-1, 27.50), 338(12.10), 323(6.60), 299(17.60), 298(9.90), 297(41.80), 296(14.30), 286(6.60), 285(6.60), 284(7.70), 283(12.10), 282(18.70), 281(9.90), 204(5.50), 203(4.40), 197(9.90), 195(31.90), 165(8.80), 155(25.30), 154(13.00), 153(74.70), 152(13.10), 141(36.30), 140(15.40), 139(100), 138(29.70), 137(18.70), 118(6.60), 117(35.20), 116(17.60), 113(20.90), 112(14.30), 111(48.40), 110(22.00), 106(8.80), 105(18.70), 104(18.70), 103(14.30), 102(42.90), 101(15.40), 91(12.10), 90(12.10),

89(19.80), 85(14.30), 77(30.80), 76(23.10), 75(26.40), 74(26.40), 65(13.90), 64(8.80), 63(14.30), 60(29.70), 57(16.50), 56(13.20), 55(19.80), 51(19.80), 50(25.30). Anal. Calcd. for C<sub>17</sub>H<sub>13</sub>N<sub>4</sub>ClO<sub>2</sub>: C, 60.00; H, 3.82; N, 16.47. Found: C, 59.83; H, 3.78; N, 16.44.

#### 5-Benzylidene-3-(p-chlorophenyl)-2-(acetylaminocarbonyl)-1,2-dihydro-1,2,4-triazine-6(5H)-one (4) and

#### 5-Benzylidene-3-(p-chlorophenyl)-2-(acetylaminocarbonyl)-1-acetyl-1,2-dihydro-1,2,4-triazine-6(5H)-one (5).

A solution of 3 (0.01 mole) in acetic anhydride (20 ml) was heated under reflux for 1 hr, then cooled and poured into ice-water. The resulting product was filtered off, washed with water, dried and purified by recrystallization from benzene to give 4 as pale yellow crystals, yield 43%, m.p. 146°C. IR(KBr): 3225 (NH), 1701-1687 (C=O), 1632 (C=N), 1605, 1589 (C=C) cm<sup>-1</sup>. <sup>1</sup>H-NMR (CDCl<sub>3</sub>): δ, 2.35 (s, 3H, CH<sub>3</sub>), 7.12-7.91 (m, 10H, Ar-H and H-olefinic), 9.83(br-s, 1H, NH), 10.73(s, 1H, NH) ppm. MS: m/z (%): 384 (M++2, 6.20), 383 (M++1, 19.30), 382 (M+, 13.80), 381 (M+-1, 58.20), 380(36.50), 342(5.70), 341(22.00), 340(18.40), 339(56.10), 338(16.50), 299(31.30), 298(24.10), 297(100), 296(38.70), 283(8.40), 282(10.70), 281(6.90), 280(10.50), 269(4.50), 268(4.50), 204(5.30), 203(3.60), 198(2.60), 197(14.30), 196(7.40), 195(36.80), 155(26.70), 154(19.10), 153(75.90), 152(14.30), 141(20.90), 140(20.80), 139(70.20), 138(36.50), 117(19.30), 116(18.10), 113(15.30), 112(6.90), 111(35.60), 110(14.80), 105(5.70), 104(8.40), 103(16.50), 102(85.90), 101(9.50), 91(6.00), 90(10.00), 89(27.70), 88(8.40), 83(12.60), 77(20.80), 76(47.20), 75(33.90), 74(13.40), 64(7.90), 63(22.20), 62(10.00), 51(26.30), 50(24.10).

Anal. Calcd. for C<sub>19</sub>H<sub>15</sub>N<sub>4</sub>ClO<sub>3</sub>: C, 59.68; H, 3.93; N, 14.66. Found: C, 59.59; H, 3.88; N, 14.44.

The filtrate was concentrated, then cooled. The solid formed was filtered off, dried and purified by recrystallization with benzene to give 5 as pale yellow crystals, yield 33%, m.p. 123°C. IR(KBr): 3210 (NH), 1703-1683 (br.C=O), 1629 (C=N), 1606, 1590 (C=C) cm<sup>-1</sup>. <sup>1</sup>H-NMR(CDCl<sub>3</sub>): δ, 2.35-2.43 (br.s, 6H, 2×COCH<sub>3</sub>), 7.13-7.83 (m, 10H, Ar-H and H-olefinic), 9.98 (s, 1H, NH) ppm. Ms: m/z (%): 424 (M+, 1.60), 423(1.80), 422(1.30), 385(4.30), 384(3.10), 383(4.70), 382(4.70), 381(13.20), 380(8.50), 342(2.20), 341(8.10), 340(9.20), 339(21.30), 338(10.10), 299(9.80), 298(7.80), 297(24.90), 296(9.60), 283(9.60), 282(8.70), 281(5.20), 280(5.80), 203(3.40), 202(2.50), 197(5.80), 196(3.40), 195(12.10), 194(4.90), 155(10.50), 154(6.50), 153(31.60), 152(23.80), 141(27.40), 140(14.80), 139(100), 138(24.00), 137(12.30), 117(20.20), 116(12.60), 115(4.30), 113(12.80), 112(8.50), 111(32.30), 110(7.60), 105(11.20), 104(15.20), 103(9.90), 102(27.60), 101(13.00), 91(6.10), 90(9.20), 89(12.10), 77(26.00), 76(19.30), 75(21.70), 64(4.70), 63(11.20), 51(16.60), 50(16.80). Anal. Calcd. for C<sub>21</sub>H<sub>17</sub>N<sub>4</sub>ClO<sub>4</sub>: C, 59.43; H, 4.01; N, 13.21. Found: C, 59.34; H, 3.99; N, 13.17.

#### Mehtly α-(p-chlorobenzoyl) amino-β-phenyl acrylate (6).

A mixture of oxazolinone (2, 0.01 mole) and triethylamine (0.03 mole) in methanol (30 ml) was heated under reflux 2 hr. the reaction mixture was cooled and poured into dilute hydrochloric acid (1%). The solid formed was filtered off, washed with water, dried and purified by recrystallization with ethanol to give 6 as colorless crystals, yield 56%, m.p. 156°C. IR(KBr): 3225 (NH), 1758 (C=O of ester), 1689 (C=O of amide), 1610, 1589 (C=C), 1205, 1085 (C-O) cm<sup>-1</sup>. <sup>1</sup>H-NMR(CDCl<sub>3</sub>): δ, 3.98 (s, 3H, OCH<sub>3</sub>), 6.89-7.81 (m, 10H, Ar-H and H-olefinic), 9.85 (br-s, 1H, NH) ppm. MS: m/z (%): 317 (M++2, 2.40), 316 (M++1, 1.60), 315 (M+, 9.40), 314 (M+-1, 7.20), 284(6.50), 283(8.60), 282(5.80), 149(9.40), 142(5.00), 141(29.50), 140(20.10), 139(100), 138(14.40), 118(4.30), 117(4.30), 113(11.50), 112(5.00), 111(29.50), 110(10.80), 108(3.60), 105(7.20), 104(7.50), 91(5.80), 90(6.50), 89(8.60), 77(10.10), 76(7.20), 75(15.10), 74(10.10), 63(6.50), 61(6.50), 55(10.10), 51(15.80), 50(12.20). Anal. Calcd. for C<sub>17</sub>H<sub>14</sub>N-

C103: C, 64.76; H, 4.44; N, 4.44. Found: C, 64.67; H, 4.23; N, 4.31.

#### 6-phenyl- 5- (p-chlorophenyl) amino-4-hydroxyl-pyrimidine-2-thiole (7).

A mixture of ester (6, 0.01 mole), thiourea (0.01 mole) and anhydrous potassium carbonate (0.03 mole) in methanol (30 ml) was heated under reflux for 3 hr. The reaction mixture was cooled and acidified with dilute hydrochloric acid (1%). The resulting solid was filtered off, washed with water, dried and purified by recrystallization with ethanol to give 7 as pale yellow crystals, yield 71%, m.p. 223°C. IR(KBr): 3310-2951 (br. OH), 3236 (NH), 1705 (C=O), 1631 (C=N), 1605, 1581 (C=C), 1174, 1084 (C-O) cm<sup>-1</sup>. <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>): δ, 7.21-7.81(m, 9H, Ar-H), 9.21(s, 1H, SH), 9.89 (s, 1H, NH), 10.37 (br.s, 1H, OH) ppm. MS: m/z (%): 359 (M<sup>++2</sup>, 5.10), 358 (M<sup>++1</sup>, 9.20), 357 (M<sup>+</sup>, 16.90), 313(1.50), 312(12.30), 304(7.70), 302(12.30), 301(9.10), 285(15.40), 283(18.50), 282(9.20), 281(7.70), 252(6.20), 251(6.20), 227(9.20), 226(13.80), 213(10.80), 207(7.70), 194(10.80), 191(9.20), 169(16.90), 167(4.60),

165(15.90), 154(20.00), 152(13.80), 140(81.50), 138(78.50), 116(6.20), 115(9.20), 108(10.80), 107(18.50), 106(16.90), 105(100), 102(10.80), 100(15.40), 89(9.20), 77(47.70), 72(18.50), 50(15.40). Anal. Calcd. for C<sub>17</sub>H<sub>12</sub>N<sub>3</sub>ClO<sub>2</sub>S: C, 57.14; H, 3.36; N, 11.76. Found: C, 57.03; H, 3.21; N, 11.57.

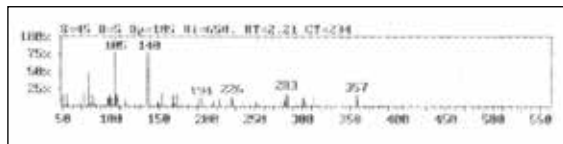


Figure 7: Mass spectrum of compound (7)

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#### REFERENCE

- | 1- Gibson, N.W.; Erickson, L.C.; Hickman, J.A.; Cancer. Res., 1984, 44, 1767. | 2- Pilch, D.S.; Kirolos, M.A.; Liu, X.; Plum, G.E.; Breslauer, K.J.; Berenil Biochem., 1995, 34, 9962. | 3- Jean-Claude, B.J.; Mustafa, A.; Damian, Z.; Marté, Ji.; Vasilescu, D.E.; Yen, R.; Chan, T.H.; Leyland. Jones, B.; Biochem. Pharmacol., 1999, 57, 753. | 4- Smith, R.H. Jr.; Scudiero, D. A.; Michejda, C.J.; J. Med. Chem., 1990, 33, 2579. | 5- Unsalan, S.; Rollas, S.; Indian J. Chem. Sec. B. Org. Cdem. Inc. Med. Chem., 2007, 46B, 185. | 6- Kumar, A.; Mukerjee, S.K.; Bhattacharya, S. K.; Pharmazie., 1983, 38, 66. | 7- Katsoulas, A.; Rachid, Z.; Brahimi, F.; McNamee, J.; Jean-Claude, B.J.; Leukemia Res., 2005, 29, 693. | 8- Seiter, K.; Liu, D.; Loughran, T.; Siddiqui, A., Baskind, P.; Ahmed, T.; J. Clin. Oncol, 2002, 20(15), 3249. | 9- Manolov, I.; Machulla, H.J.; Momekov, G.; Pharmazie, 2006, 61(6), 511. | 10- Bhaskar, S. D.; Shuddhodan, N.K.; Baseer, M. S.; Der Pharmacia lett. , 2010, 2(4), 126. | 11- El-Deen, I.M.; Chinese J. Chem., 1998, 16(6), 533. | 12- Kamble, V.T.; Dawane, B.S.; Chavan, S.A.; Bhosale, R.B.; Aust. J. Chem.; 2007, 60, 302. | 13- El-Deen, I.M.; Chinese J. Chem., 1999, 17(4), 391. | 14- Hasanen, J.A.; El-Deen, I.M.; El-Desoky, R.M.; Abdalla, A.M.; Res. Chem. Intermed; Published online, 16 April 2013. | 15- Ibrahim, H.K.; El-Tamani, S.H.; El-Shaarawy, R.F.; El-Deen, I.M.; Maced. J. Chem. Eng., 2008, 27(1), 65. | 16- El-Deen, I.M.; Ibrahim, H.K.; Chem. Paper, 2004, 58(3), 200. | 17- Benzie, F.F.; Strain, J.J.; Ferric reducing Antioxidant power assay; Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration methods in enzymology, 1999, 299; 15-23. | 18- Mosmann, T.; J. Immunol. Methods, 1983, 65, 55. | 19- Vijayan, P., Raghu, C.; Ashok, G.; Dhanaraj, S.A.; Suresh, B.; Indian J. Med. Res., 2004, 120, 29. |