

## SYNTHESIS AND BIOLOGICAL EVALUATION OF PYRIDO (2, 3-*d*)PYRIMIDINE-CARBOXYLATE DERIVATIVES

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### ABSTRACT

Pyrido(2,3-*d*)Pyrimidine-Carboxylate Derivatives were synthesized through nucleophilic substitution reactions with the use of amidines, followed by 4-haloanilines and malonic acid. Thus synthesized novel derivatives were confirmed by Elemental analysis, IR, <sup>1</sup>H NMR and MS. These novel derivatives have been screened for anticancer activity.

**Keywords:** Pyrido(2,3-*d*) pyrimidnes; Amidines; 4-Haloanilines; malonic acid and antitumar activity.

### INTRODUCTION

From the past few decades the research on Pyrido(2,3-*d*) pyrimidine derivatives revealed that derivatives had wide range of therapeutic applications such as antibacterial<sup>1-3</sup>, antifungal<sup>4-7</sup>, anti-inflammatory<sup>8</sup>, antiallergic<sup>9</sup>, antidiabetic<sup>10</sup>, antiviral<sup>11,12</sup> and antotumor<sup>13-16</sup>, antiherpes<sup>17</sup> and calcium channel blocking activity<sup>18,19</sup>. The versatile applications of Pyrido(2,3-*d*) pyrimidnes have given zeal to design and synthesize the novel derivatives with the aim to achieve anticancer activity.

In the present study Ethyl cyanoacetate **1** was converted to ethyl 3,3 bis (methyl thio)-2-cyano acrylate **3** with the help of carbon disulphide **2**. This was substituted with respective amidines to produce 2-methyl-4-(methylthio)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile**4**. Further condensation with aromatic 4-haloanilines to produce 4-(4-halo phenylamino)-2-methyl-6-oxo-1,6 dihydro pyrimidine-5-carbonitrile **5** And further treatment with malonic acid to give Ethyl-5-amino-8-(4-halophenyl)-2-methyl-4,7 dioxo-3,4,5,6,7,8 hexahydro pyrido(2,3-*d*)pyrimidine-6-carboxylate **6a-f** derivatives, respectively (**Scheme 1**). The synthesized compounds were purified by pre coated TLC Plates using solvent Methanol:Hexane (1:1 ratio). Thus synthesized novel derivatives were characterized in **Table 1**. The antitumor activity of all the synthesized compound wase investigated by using the MTT assay method. . In these all the synthesized compounds showed significant activity.

### EXPERIMENTAL

#### General:

Melting points were determined using an open-ended capillary method and are uncorrected. The reaction was monitored by TLC. FT-IR was recorded on a Jasco FT-IR spectrophotometer , <sup>1</sup>H NMR spectra were recorded at 300 MHZ on a Bruker FT-NMR spectrophotometer and mass spectra on a Varian atlas CH-7 mass spectrometer at 70 ev. The elemental analysis was obtained on a VARIO-EL instrument and all compounds showed satisfactory elemental analysis.

#### Synthesis of Ethyl 3, 3 bis (methyl thio)-2-cyano acrylate, (3):

To an ice cold solution of potassium hydroxide(0.2mol) in 10 ml of water and 30 ml of DMF was added, with cooling and stirring, followed by carbon di sulphide (0.1 mol). The mixture was added with ethyl cyanoacetate (0.1mol ) stirred for one hour at room temperature, cooled and added drop wise with DMS (0.2mol) maintaining temperature at 20 °C .The reaction mixture was allowed to stand at room

temperature for 12 hours and poured in to 500 ml of ice water mixture. The solid obtained was filtered, washed with cold water and dried. Recrystallization from N-hexane yields a crystalline product.

**Synthesis of 2-substituted- 4-(methyl thio)-6-oxo-1, 6-dihydro pyrimidine -5-carbo nitrile, (4):**

To an ice cold suspension of sodium hydride (0.02mol) in 20 ml of dimethyl formamide was added with stirring respective amidines [viz, acetamide (0.02mol)]. The mixture was stirred for 30 minutes and treated drop wise under cooling and stirring with solution of step 1 product (0.01mol) in 15ml of DMF. The reaction mixture stirred at 10 °C for four hours. After allowing standing for 24 hours, the reaction mixture was poured in to 800ml of ice water mixture. The solid obtained was filtered and dried. Recrystallization from hexane yielded a colorless crystalline compound.

**Synthesis of 4-(4-halo phenyl amino)-2-substituted-6-oxo-1, 6-dihydro pyrimidine-5-carbonitrile, (5):**

A mixture of above product (0.01mol) and freshly distilled aniline (0.01mol) in 30ml of ethanol was refluxed for 1 hour. After allowing to stand at room temperature for 24 hours. The reaction mixture was filtered, washed with cold ethanol and dried. Recrystallization from hexane yielded the product. In this work different aromatic amines like p-fluoro aniline, p-chloro aniline, p-bromo aniline were used.

**Synthesis of Ethyl-5-amino-8-(4-halo phenyl)-2-substituted-4,7-dioxo- 3,4,5,6,7,8-hexa hydro pyrido(2,3-d) pyrimidine -6-carboxylate, (6a-f):**

A mixture of above product (0.01mol for acetamide derivatives ) and malonic acid (0.02mol) reflux for one hour. The reaction mixture was stirred at 10 °C for four hours. After allowing standing for 24 hours, the reaction mixture was poured in to 400ml of ice water mixture. The solid obtained was filtered and dried. Recrystallization from hexane yielded a colorless crystalline compound.

**Ethyl-5-amino-8- (4-fluorophenyl) -2-acetyl-4,7-dioxo-3,4,5,6,7,8 hexahydro pyrido (2,3-d) pyrimidine-6-carboxylate (6a):**

IR (KBr):3132,2938,2598,2206, 1661,1621, 1550,1494, 1414, 1378,1310, 1258,1215,1132,1093,1027,900,818,782,670,634,612,574,518, 489. <sup>1</sup>HNMR(200MHz,DMSO-*d*<sub>6</sub>): 1.35 (T,3H) ,2.2 (S,1H) ,4.2 (M,1H) ,7.2 (T,3H) ,7.3 (M,1H) ,11.4 (S,1H). MASS:*m/e*(Abundance): 94.3405 (32.5%) , 109.4904 (32%) , 117.3907 (29%) ,141.6289 (27.02%) ,155.1755 (24.96%) , 167.3099 (26.72%) ,188.7449 (27.07%) ,201.6446 (28.53%) 215.3339 (60.73%) ,232.5310 (65.72%) ,249.8193 (100%) ,265.5188 (25.3%) 348.0324 (31.96%) ,360.5465 (24.15%) .

**Ethyl-5-amino-8- (4-chlorophenyl) -2- acetyl -4,7-dioxo-3,4,5,6,7,8 hexahydro pyrido (2,3-d) pyrimidine-6-carboxylate. (6b):**

IR (KBr): 2923,2591,2207,2096,1902, 1553,1490, 1413,1308,1256,1134, 1016,814,693,641,605,487. <sup>1</sup>HNMR(200MHz,DMSO-*d*<sub>6</sub>): 1.3 (M,1H) ,2.2 (S,1H) ,2.4 (S,1H) ,3.4 (S,2H) ,3.5 (S,3H) ,4.2 (M,3H) ,6.5 (M,2H) , 7.2 (M, 1H) , 7.3 (M, 1H) , 11.4 (S, 1H) .MASS:*m/e*(Abundance): 74.0212 (52.72%) ,93.9163 (78.19%) ,108.8854 (21.70%) ,120.8894 (17.86%) ,131.8193 (32.53%) ,146.9275 (12.62%) ,158.648 (83.61%) ,217.3430 (17.67%) ,249.9309 (21.17%) ,262.2604 (11.27%) .277.9503 (55.32%) ,301.6512 (6.92%) ,318.7645 (6.76%) ,341.6553 (7.02%) ,333.9842 (9.01%) ,378.6526 (5.027%) .

**Ethyl-5-amino-8- (4-bromophenyl) -2- acetyl -4,7-dioxo-3,4,5,6,7,8 hexahydro pyrido (2,3-d) pyrimidine-6-carboxylate (6c):**

IR (KBr): 3170,3071,2992,2942,2203,1657,1604, 1558,1509, 1471,1418, 1397,1378,1314, 1263,1221,1158, 1112,1095,1026,956,870,846, 780,719,660,623, 541,521,505. <sup>1</sup>HNMR(200MHz,DMSO-*d*<sub>6</sub>): 1.3 (T,1H) ,2.2 (S,1H) ,4.2 (M,3H) ,7.1 (M,1H) ,7.2 (M,1H) ,11.4 (S,1H) .MASS:*m/e*(Abundance): 77.7532 (37.82%) ,89.3361 (43.96%) ,108.3128 (32.01%) ,124.0297 (35.82%) ,146.7202 (36.57%) ,183.5436 (38.52%) ,200.8523 (43.72%) , 217.1962 (48.01%) ,249.7169 (100%) ,264.1582 (36.19%) ,355.1588 (30.52%) ,383.5034 (26.76%) ,421.3979 (31.76%) .

**Ethyl-2,5-diamino-8- (4-fluorophenyl) 2-benzyl- 4,7-dioxo-3,4,5,6,7,8 hexahydro pyrido (2,3-d) pyrimidine-6-carboxylate (6d):**

IR (KBr): 3434,2083,1656,1561,1123,619 <sup>1</sup>HNMR(200MHz,DMSO-*d*<sub>6</sub>): 0.07 (S,1H) ,1.2 (S,2H) ,2.08 (S,1H) ,2.9 (S,2H) ,3.4 (S,1H) ,3.7 (S,1H) ,6.6 (S,1H) , 7.0 (T, 3H) , 8.02 (S, 1H) , 8.6 (S,1H) ..  
MASS:*m/e*(Abundance): 68.1382 (6.17%) ,82.0256 (6.80%) ,103.9129 (7.17%) ,113.8159 (5.72%) ,137.8441 (5.57%) , 150.6908 (7.18%) , 176.4536 (21.72%) ,192.4484 (6.92%) ,205.3677 (100%) , 217.5851 (6.57%) , 247.9336 (6.42%) , 262.1373 (65.32%) ,288.5457 (7.18%) , 301.8948 (6.60%) , 324.2994 (7.10%) , 339.0495 (7.21%) ,362.4446 (7.32%) , 377.9562 (7.54%) , 398.8542 (7.11%) , 422.2755 (4.92%).

**Ethyl-2,5-diamino-8- (4-chlorophenyl) 2-benzyl -4,7-dioxo-3,4,5,6,7,8 hexahydro pyrido (2,3-d) pyrimidine-6-carboxylate (4e):**

IR (KBr): 3785,3412,3129,2956,2590, 2011,1905,1685, 1618,1561,1490, 1390, 1311,1264,1176,1128,1071,1016,899,813,785,685,670 , 643, 632,604, 573,484.  
<sup>1</sup>HNMR(200MHz,DMSO-*d*<sub>6</sub>): 0.1 (M,1H) ,1.3 (M,3H) ,2.9 (S,1H) ,4.2 (M,1H) ,4.3 (M,3H) ,6.5 (D,1H) 6.8 (S,1H) , 7.2 (M, 3H) ,7.4 (S,1H) ,10.8 (S,1H). MASS:*m/e*(Abundance): 74.1739 (52.16%) , 94.5471 (50.16%) , 104.1117 (57.18%) , 121.8711 (58.32%) ,135.6927 (57.52%) , 165.0146 (59.17%) , 249.6610 (100%) ,.67%) ,302.1690 (45.19%) , 321.5307 (45.53%) , 332.8958 (40.19%) ,353.8196 (45.17%) , 393.0101 (46.19%) , 405.8843 (37.93%) , 433.3561 (42.72%) , 438.2912 (37.96%).

**Ethyl-2,5-diamino-8- (4-bromophenyl) 2-benzyl -4,7-dioxo-3,4,5,6,7,8 hexahydro pyrido (2,3-d) pyrimidine-6-carboxylate (4f) :**

IR (KBr): 3322,2085,1193,1122,754,657,618. <sup>1</sup>HNMR(200MHz,DMSO-*d*<sub>6</sub>): 0.1 (D,1H) ,1.2 (S,1H) ,2.1 (D,1H) ,2.9 (D,2H) ,3.8 (S,1H) ,6.6 (M,1H) ,7.2 (M,3H) , 7.4 (S,1H) .MASS:*m/e*(Abundance): 163.5082 (72.50%) , 90.7657 (87.90%) , 135.6515 (65.76%) , 154.0540 (42.76%) ,185.1653 (43.96%) , 250.0333 (100%) , 308.8417 (41.02%) , 361.1454 (46.92%) ,398.1652 (38.62%) , 423.9146 (37.17%) , 43.5131 (38.15%) , 469.7823 (42.92%) ,483.9555 (36.17%).

**Antitumor Activity:**

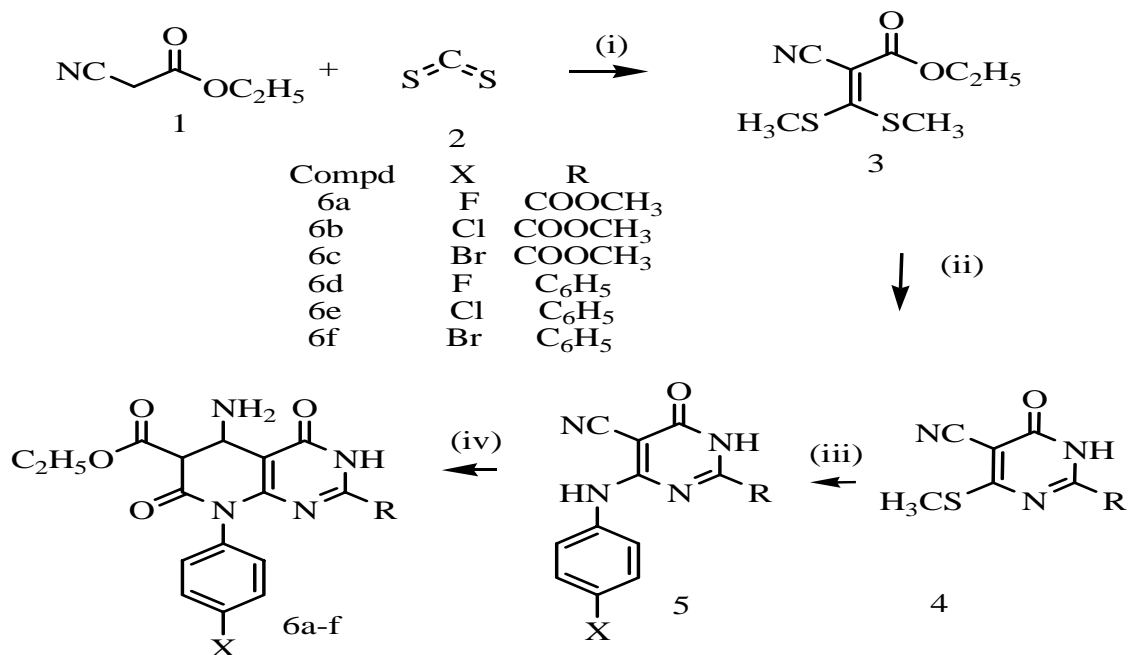
In this present study the cytotoxic activity of synthesized pyrimidine derivatives using three human cancer cell lines [i.e.colon cancer (HT29), liver cancer (HepG2), cervical cancer (Hela)] were evaluated with MTT assay. In these all the synthesized compounds showed significant activity.

The LC<sub>50</sub> of the synthesized pyrimidine derivatives was found to be >100 µg/ml for all these cell lines. Based on cytotoxicity results the synthesized pyrimidine derivatives posses cytotoxic effect on these three human cancer cell lines. The antitumor activity data on cell line was summarized in **Table 2**.

**RESULTS AND DISCUSSION**

The GI<sub>50</sub> of the **compd 6d** (Ethyl-2,5-diamino-8- (4-Flurophenyl) -2-benzyl-4,7-dioxo-3,4,5,6,7,8 hexahydro pyrido (2,3-d) pyrimidine-6-carboxylate ) was found at 21 & 19 µg/ml on HT29 and HepG2 cell lines respectively. The GI<sub>50</sub> of the **compd 6b** (Ethyl-5-amino-8- (4-Chlorophenyl) -2-acetyl-4,7-dioxo-3,4,5,6,7,8 hexahydro pyrido (2,3-d) pyrimidine-6-carboxylate) was found at 24µg/ml on Hela cell lines.

The total growth inhibition (TGI) of the **compd 6e** (Ethyl-2,5-diamino-8- (4-chlorophenyl) -2-benzyl -4,7-dioxo-3,4,5,6,7,8 hexahydro pyrido (2,3-d) pyrimidine-6-carboxylate) was found at 39 & 40 µg/ml on HT29 and HepG2 cell lines respectively. The TGI of the **compd 6a** (Ethyl-5-amino-8- (4-fluorophenyl) -2-acetyl-4,7-dioxo-3,4,5,6,7,8 hexahydro pyrido (2,3-d) pyrimidine-6-carboxylate ) was found at 47 µg/ml on Hela cell lines.



(i) KOH, DMF, DMS at 20°C. (ii) Sodium hydride, Acetamidine & Ethanol.  
(iii) 4-Halo anilines & Ethanol. (iv) Malonic acid & Ethanol.

**Scheme-1**

**Table-1:** Characterization data for the synthesized compounds

S.No	Compd.	Molecular Formulae and Molecular Wt.	R <sub>f</sub> Value	Elemental Analysis Calculated( Experimental)
1	6a	C <sub>17</sub> H <sub>17</sub> N <sub>4</sub> O <sub>4</sub> F 360.34	0.68	C, 56.66 (56.45); H, 4.76 (4.71); F, 5.27 (5.23); N, 15.55 (15.49); O, 17.76 (17.68).
2	6b	C <sub>17</sub> H <sub>17</sub> N <sub>4</sub> O <sub>4</sub> Cl 376.79	0.88	C, 54.19 (54.11); H, 4.55 (4.45); Cl, 9.41 (9.37); N, 14.87 (14.78); O, 16.98 (16.89).
3	6c	C <sub>17</sub> H <sub>17</sub> N <sub>4</sub> O <sub>4</sub> Br 421.25	0.71	C, 48.47 (48.39); H, 4.07 (4.01); Br, 8.97 (8.88); N, 13.30 (13.17); O, 5.19 (5.11).
4	6d	C <sub>22</sub> H <sub>19</sub> N <sub>4</sub> O <sub>4</sub> F 422.40	0.76	C, 53.18 (53.22); H, 4.46 (4.39); F, 5.26 (5.16); N, 19.38 (19.28); O, 17.71 (17.59).
5	6e	C <sub>22</sub> H <sub>19</sub> N <sub>4</sub> O <sub>4</sub> Cl 438.863	0.63	C, 50.87 (50.76); H, 4.27 (4.21); Cl, 9.38 (9.28); N, 18.54 (18.45); O, 16.94 (16.86).
6	6f	C <sub>22</sub> H <sub>19</sub> N <sub>4</sub> O <sub>4</sub> Br 483.31	0.57	C, 45.51 (45.46); H, 3.82 (3.71); Br, 18.92 (18.88); N, 16.59 (16.48); O, 5.16 (5.08)

**Table-2:** Antitumor activity data for the synthesized compounds

Compound	GI <sub>50</sub> (µg/ml)			TGI(µg/ml)			LC <sub>50</sub> (µg/ml)		
	HT29	HepG2	Hela	HT29	HepG2	Hela	HT29	Hep2	Hela
<b>6a</b>	24	22	24	59	47	47	>100	>100	>100
<b>6b</b>	28	25	25	44	51	70	>100	>100	>100
<b>6c</b>	23	29	32	56	54	65	>100	>100	>100
<b>6d</b>	27	34	24	47	74	57	>100	>100	>100
<b>6e</b>	21	19	29	39	40	60	>100	>100	>100
<b>6f</b>	29	30	30	59	60	62	>100	>100	>100

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