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# Synthesis and biological evaluation of pyrido (2, 3-*D*) pyrimidine-carboxylate derivatives

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## ABSTRACT

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 and Ethyl-5-amino-8-(4-halophenyl)-2-amino-4,7 dioxo-3,4,5,6,7,8 hexahydropyrido(2,3-*d*)pyrimidine-6-corboxylate 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, $^1$HNMR and MS. These novel derivatives have been screened for antibacterial, antifungal and antitumor activity.

**Keywords:** Pyrido (2,3*d*) pyrimidnes, $CS_2$, Amidines, Spectral data (Elemental, IR, NMR & MS), antibacterial activity, antifungal activity and antitumor activity.

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## INTRODUCTION

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

## MATERIALS AND METHODS

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 rescpective amidines to produce 2-methyl-4-(methylthio)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile 4 and 2-amino-4-(methyl thio)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile 5. Further condensation with aromatic 4-haloanilines to produce 4-(4-halo phenylamino)-2-methyl-6-oxo-1, 6 dihydro pyrimidine-5-carbonitrile 6a-6c and 4-(4-halo phenylamino)-2-amino-6-oxo-1, 6 dihydro pyrimidine-5-carbonitrile 7a-7c. 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 hexahydropyrido(2,3-d)pyrimidine-6-corboxylate 8a-8c and Ethyl-2,5 diamino-8-(4-halophenyl)-4,7 dioxo-3,4,5,6,7,8 hexahydropyrido(2,3-d)pyrimidine-6-corboxylate 9a-9c 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 by elemental analysis, IR, $^{1}$HNMR and MS. All these Elemental and $^{1}$HNMR data have been summarized in Table 1.

## EXPERIMENTAL

### IR data

Compounds 3, 4, 5, 6a-c and 7a-c showed sharp band between the region of 2250 to 2210 $cm^{-1}$ due to the presence of -C≡N group. The -C=O group shown strong absorption band in the region of 1680 to 1640 $cm^{-1}$ in the 3, 4, 5, 6a-c, 7a-c, 8a-c and 9a-c compounds. The -C≡N group absorption found in 3, 4, 5, 6a-c and 7a-c compounds was disappeared in 8a-c and 9a-c compounds which indicates the confirmation of final products. The compounds 5, 7a-c and 9a-c were shown the strong absorption in the region of 3450 to 3350 $cm^{-1}$ due to $–NH_2$group. The -C=C- and -C-H of aromatic shown the absorption in between 1600 to 1470 $cm^{-1}$ and 3050 to 3010 $cm^{-1}$ respectively by the 4, 5, 6a-c, 7a-c, 8a-c and 9a-c compounds. The aryl -C-F, -C-Cl and -C-Br groups shown strong absorption in the region of 1250 to 1180, 1150 to 1040 and 1080 to 1030 $cm^{-1}$ respectively by the compounds 8a & 9a, 8b & 9b and 8c & 9c.

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| Table 1 – The elemental and $^1$HNMR spectral data for synthesized compounds | | | |
|---|---|---|---|
| **Compound** | **Molecular formulae/ Molecular weight** | **Elemental analysis Calculated ( Found )** | **Chemical shift in ppm (DMSO-$d_6$)** |
| **3** | $C_8H_{11}NO_2S_2$ 217.31 | C, 44.22(44.15); H, 5.10(5.19); N, 6.45(6.56) | 1.30 (T, 3H), 2.25 (S, 6H), 4.19 (M, 2H) |
| **4** | $C_7H_7N_3OS$ 181.21 | C, 46.40(46.15); H, 3.89(3.76); N, 23.19(23.31) | 0.9 (S, 3H), 2.25 (S, 3H), 8.0 (S, 1H) |
| **5** | $C_6H_6N_4OS$ 182.2 | C, 39.55(39.68); H, 3.32(3.19); N, 30.75(30.25) | 2.0 (S, 2H), 2.25 (S, 3H), 8.0 (S, 1H) |
| **6a** | $C_{12}H_9FN_4O$ 244.22 | C, 59.01(59.55); H, 3.71(3.92); N, 22.94(22.57( | 0.9 (S,3H), 4.0 (S, 1H), 6.44 (M, 2H), 6.72 (M, 2H), 8.0 (S, 1H) |
| **6b** | $C_{12}H_9ClN_4O$ 260.68 | C, 55.29(55.61); H, 3.48(3.96); N, 21.49(21.99) | 0.9 (S, 3H), 4.0 (S, 1H), 6.40 (D, 2H), 7.02 (D, 2H), 8.0 (S, 1H) |
| **6c** | $C_{12}H_9BrN_4O$ 305.13 | C, 47.24(47.01); H, 2.97(2.56) ; N, 18.36(18.90) | 0.9 (S, 3H), 4.0 (S, 1H), 6.35 (D, 2H), 7.18 (D, 2H), 8.0 (S, 1H) |
| **7a** | $C_{11}H_8FN_5O$ 245.21 | C, 53.88(53.11); H, 3.29(3.14) ; N, 28.56(28.19) | 2.0 (S, 2H), 4.0 (S, 1H), 6.44 (M, 2H), 6.72 (M, 2H), 8.0 (S, 1H) |
| **7b** | $C_{11}H_8ClN_5O$ 261.67 | C, 50.49(50.61); H, 3.08(3.66); N, 26.76(26.21) | 2.0 (S, 2H), 4.0 (S, 1H), 6.40 (D, 2H), 6.72 (M, 2H), 8.0 (S, 1H) |
| **7c** | $C_{11}H_8BrN_5O$ 306.12 | C, 43.16(43.98); H, 2.63(2.34); N, 22.88(22.67) | 2.0 (S, 2H), 4.0 (S, 1H), 6.35 (D, 2H), 7.18 (D, 2H), 8.0 (S, 1H) |
| **8a** | $C_{17}H_{17}N_4O_4F$ 360.34 | C, 56.66(56.45) ; H, 4.76(4.71) ; N, 15.55(15.49) | 0.9 (S, 3H), 1.30 (T, 3H), 2.0 (S, 2H), 3.53 (D, 1H ), 4.16 (M,1H), 6.95 (M, 1H), 7.62 (D, 1H ), 8.O (S, 1H ) |
| **8b** | $C_{17}H_{17}N_4O_4Cl$ 376.79 | C, 54.19(54.11 ; H, 4.55(4.45) ; N, 14.87(14.78) | 0.9 (S,3H), 1.30 (T, 3H), 2.0 (S, 2H), 3.53 (D, 1H ), 4.12(D, 2H ), 4.16 (D,1H), 7.25 (D, 1H ), 7.58(M, 1H), 8.0 (S, 1H ) |
| **8c** | $C_{17}H_{17}N_4O_4Br$ 421.25 | C, 48.47(48.39) ; H, 4.07(4.01) ; N, 13.30(13.17) | 0.9 (S,3H), 1.30 (T, 3H), 2.0 (S, 2H), 3.53 (D, 1H ), 4.12(D, 2H ), 4.16 (D,1H), 7.41 (D, 1H ), 7.53(M, 1H), 8.0 (S, 1H ) |
| **9a** | $C_{16}H_{16}N_5O_4F$ 361.12 | C, 53.18(53.22) ; H, 4.46(4.39) ; N, 19.38(19.28) | 1.30 (T, 3H ), 2.0 (S, 2H ), 3.53 (D, 1H), 4.12( D, 2H), 4.16 (M, 1H), 6.95( T, 1H ), 7.62 (T, 1H), 8.0 (S, 1H ) |
| **9b** | $C_{16}H_{16}N_5O_4Cl$ 377.78 | C, 50.87(50.76) ; H, 4.27(4.21) ; N, 18.54(18.45) | 1.30 (T, 3H), 2.0 (S, 2H), 3.53 (D, 1H), 4.12(D, 2H), 4.16 (M, 1H), 7.25 (T, 1H), 7.58 (T, 1H), 8.0(S, 1H). |
| **9c** | $C_{16}H_{16}N_5O_4Br$ 422.23 | C, 45.51(45.46) ; H, 3.82(3.71) ; N, 16.59(16.48) | 1.30 (T, 3H ), 2.0 (S, 2H ), 3.53 (D, 1H), 4.12( D, 2H), 4.16 (M, 1H), 7.41 (T, 1H), 7.53 (T, 1H), 8.0 (S , 1H ) |

## $^1$HNMR data

$^1$HNMR spectra of synthesized novel derivatives 8a-c and 9a-c showed the multiplet of aromatic protons in the region of 6.5 to 7.6 ppm. In all the derivatives a singlet is observed at 2 ppm which indicates the presence of -$NH_2$. And also a triplet at 1.30 ppm reveal

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that presence of $-CH_3$ attached to $-CH_2$ in ethoxy group. And in the compounds 8a-c the $-CH_3$ group showed the singlet at 0.9 ppm.

**Mass Spectral data**

In Mass Spectra all final Synthesized compounds 8a, 8b, 8c, 9a, 9b & 9c showed expected molecular ion ($m^+$) peaks , those values 360.02, 376.17, 421.11, 361.02, 377.59 & 421.30 respectively. Compounds 8b, 9b, & 8c, 9c showed M+2 Peaks in the ratio of 3:1 & 1:1 respectively.

**ANTIMICROBIAL ACTIVITY**

All the synthesized six pyrido pyrimidine carboxylate derivatives were subjected to antibacterial activity by disc diffusion method, against gram positive bacteria *Staphylococcus aureus, Bacillus cereus,* gram negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa* and fungal organisms *Aspergillus niger, Candida albicans*. In these all the compounds show significant antibacterial and antifungal activity against all the tested respective microorganisms. The synthesized compounds were used at the concentration of 50µg/ml and DMSO as a solvent. The standard drug used for antibacterial activity, Ciprofloxacin HCl, 5 µg/disc) .The standard drug used for antifungul activity, ketoconazole, 50 µg/disc.

In these study compound 9c (Ethyl-2,5-diamino-8-(4-bromophenyl)-4,7-dioxo-3,4,5,6,7,8 hexahydro pyrido(2,3-d)pyrimidine-6-corboxylate ) showed, maximum activity against Staphylococcus aureus, compound 9b (Ethyl-2,5-diamino-8-(4-chlorophenyl)-4,7-dioxo-3,4,5,6,7,8 hexahydro pyrido(2,3-d)pyrimidine-6-corboxylate ) showed, maximum activity against Bacillus cereus, compound 9a (Ethyl-2,5-diamino-8-(4-fluorophenyl)-4,7-dioxo-3,4,5,6,7,8 hexahydro pyrido(2,3-d)pyrimidine-6-carboxylate) showed maximum activity against gram negative organisms viz, *Escherichia coli and Pseudomonas aeruginosa.*

Compound 9b (Ethyl-2,5-diamino-8-(4-chlorophenyl)-4,7-dioxo-3,4,5,6,7,8 hexahydro pyrido(2,3-d) pyrimidine-6-corboxylate ) showed maximum activity against Aspergillus niger and compound 8a (Ethyl-5-amino-8-(4-flourophenyl)-2-methyl-4,7-dioxo-3,4,5,6,7,8 hexahydro pyrido(2,3-d)pyrimidine-6-corboxylate ) showed maximum activity against *Candida albican*.

**Table 2 –** Antimicrobial activity of synthesized compounds

| Compound | ZONE OF INHIBITION (mm). | | | | | |
|---|---|---|---|---|---|---|
| | ***Staphylococcus aureus*** | ***Bacillus cereus*** | ***E.coli*** | ***Pseudomonas aeruginosa*** | ***Asprgillus niger*** | ***Candida albicans*** |
| **8a** | 24 | 21 | 23 | 21 | 25 | 27 |
| **8b** | 20 | 22 | 18 | 20 | 23 | 19 |
| **8c** | 19 | 18 | 16 | 15 | 22 | 19 |
| **9a** | 24 | 23 | 26 | 25 | 29 | 24 |
| **9b** | 25 | 25 | 18 | 19 | 30 | 20 |
| **9c** | 26 | 23 | 17 | 18 | 24 | 24 |
| **standard** | 28 | 29 | 28 | 28 | 31 | 29 |

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It shows that fluorine substituted compounds has more antibacterial activity against all the tested microorganisms. The data was showed in the table 2.

## 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), and cervical cancer (Hela)] were evaluated with MTT assay. In these all the synthesized compounds showed significant activity.

The $GI_{50}$ of the compound 9b (Ethyl-2,5-diamino-8-(4-chlorophenyl)-4,7-dioxo-3,4,5,6,7,8 hexahydro pyrido(2,3-d)pyrimidine-6-carboxylate ) was found at 18 & 17 µg/ml on HT29 and HepG2 cell lines respectively. The $GI_{50}$ of the compound 8a (Ethyl-5-amino-8-(4-flourophenyl)-2-methyl-4,7-dioxo-3,4,5,6,7,8 hexahydropyrido(2,3-d) pyrimidine-6-carboxylate ) was found at 20µg/ml on Hela cell lines.

The total growth inhibition (TGI) of the compound 9b (Ethyl-2,5-diamino-8-(4-chlorophenyl)-4,7-dioxo-3,4,5,6,7,8 hexahydro pyrido(2,3-d)pyrimidine-6-carboxylate) was found at 35 & 41 µg/ml on HT29 and HepG2 cell lines respectively. The TGI of the compound 8a (Ethyl-5-amino-8-(4-fluorophenyl)-2-methyl-4,7-dioxo-3,4,5,6,7,8 hexahydropyrido(2,3-d)pyrimidine-6-carboxylate ) was found at 49 µg/ml on Hela cell lines.

**Table 3 –** Antitumor activity data for the synthesized compounds

| Compound | $GI_{50}$(µg/ml) | | | TGI(µg/ml) | | | $LC_{50}$(µg/ml) | | |
|---|---|---|---|---|---|---|---|---|---|
| | HT29 | HepG2 | Hela | HT29 | HepG2 | Hela | HT29 | Hep2 | Hela |
| **8a** | 22 | 19 | 20 | 56 | 44 | 49 | >100 | >100 | >100 |
| **8b** | 25 | 23 | 21 | 41 | 49 | 73 | >100 | >100 | >100 |
| **8c** | 22 | 27 | 29 | 52 | 56 | 68 | >100 | >100 | >100 |
| **9a** | 23 | 35 | 22 | 44 | 72 | 59 | >100 | >100 | >100 |
| **9b** | 18 | 17 | 26 | 35 | 41 | 62 | >100 | >100 | >100 |
| **9c** | 26 | 28 | 27 | 55 | 63 | 67 | >100 | >100 | >100 |

The $LC_{50}$ 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 3.

## EXPERIMENTAL SECTION

All melting points were determined in open capillary tubes and uncorrected. IR Spectra were recorded on ABB BOTTEM FT-IR Spectrophotometer using KBr disc and $^1$HNMR Spectra on 400MHZ- Joel DPX, DMSO-$d_6$ as solvent and using TMS as an internal standard. The homogeneity of the synthesized compounds was checked by TLC using silica gel as adsorbent.

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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 $^{o}$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 & 5**

To an ice cold suspension of sodium hydride (0.02mol) in 20 ml of dimethyl formamide was added with stirring respective amidines [viz, acetamidine (0.02mol), guanidine (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 $^{o}$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, 6a-6c, 7a-7c**

A mixture of above product (0.01mol) and freshly distilled aniline (0.01mol) in 30ml of ethanol was refluxed for 1 hour. After allowing standing 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, and 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-corboxylate, 8a-8c, 9a-9c**

A mixture of above product (0.01mol for acetamidine related derivatives and 0.01mol for guanidine related derivatives) and malonic acid (0.02mol) reflux for one hour. The reaction mixture was stirred at 10 $^{o}$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.

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## CONCLUSION

The compounds of pyrido (2, 3-d) pyrimidine -6- carboxylate derivatives was synthesized by Nucleophilic substitution reactions. All the compounds were characterized by Elemental analysis, IR, NMR and Mass Spectroscopy. All the final six compounds were tested against gram positive bacteria Staphylococcus aureus, Bacillus cereus, gram negative bacteria Escherichia coli, Pseudomonas aeruginosa and fungal organisms Aspergillus niger, Candida albicans. In this halogen substituted compounds, especially fluoro substituted compound showed more activity than other synthesized compounds. All the synthesized compounds showed cytotoxic activity on cell lines HT29, HepG2 and Hela.

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