

Efficient Synthesis, Spectral Characterization of novel Di substituted 1- ethyl – 7 –methylpyrido [2, 3-d] pyrimidine-2, 4, 5 (1H, 3H, 8H) - trione Derivatives and Anti-Microbial studies

Kubendiran Kumaran^{1*}, Kasi Raguraman Jaisankar¹,
S. Raja Mohamed Kamil¹, C. Baskaran², A. Jegatheesan³

¹Department of Chemistry, Islamiah College, Vaniyambadi, TN.,India.

²Department of Zoology, Presidency College, Chennai-5, TN., India.

³Department of Physics, Paavaai Group of Institution, Namakkal district, TN., India.

*Corres.author: kumaranchem9@gmail.com,
Mobile no.09003915019

Abstract: Several series of pyrido[2,3-d]pyrimidine derivatives were synthesized by reaction of aryl-methylene acetoacetates with different aminopyrimidines. A series of Diacyl substituted 1-ethyl – 7 – methylpyrido [2, 3-d] pyrimidine- 2, 4, 5 (1H, 3H, 8H) - trione at position 3, 8 with either acetyl or phenyl substituted derivatives are prepared. The ¹HNMR, ¹³CNMR, FTIR, Spectra were used to assign conformations to several of the disubstituted compounds.

Keywords: Disubstituted pyrido[2,3-d]pyrimidine, t-butyl acetoacetate, Anti-Microbial studies.

Introduction

Organic compounds containing pyridine scaffold as a core unit are known to exhibit various biological and pharmaceutical activities^{1a-c}. Recently reported a series of 5-substituted uracil/pyrimidine derivatives are anti-inflammatory agent possessing anti-oxidative activity also antiviral² chemotherapy. The development of the chemistry of pyrimidine derivatives during the last decade has been largely associated with the wide-scale employment of these compounds. The syntheses of pyrimidine/ uracil have been

developed during these years and existing methods have been significantly improved.

Several methods of synthesis of uracil are known: mainly two ways. i.e., annulations of pyrimidine ring over pyridine or vice versa³ and the interaction of urea derivatives with nitrile derivatives, the formation^{4-5a-b} of uracil/pyrimidine moiety (Substituted). Ring annulations of this type are considered to commence with Michael addition⁶ by C-5 of the 6-amino-1-ethylpyrimidine - 2, 4 (1H, 3H) – dione. Subsequent attack by the endo cyclic amino group on the carbonyl group of the initial Micheal adducts completes the cyclization.

Pyrimidine derivatives are well-known for their biological activity⁷. Numerous pyrimidine compounds have found application in medicine and therapeutic agents. Some are used in the chemotherapy of cancer and some are used against HIV and viral⁸ diseases. Usually functionalization of uracil at the C-5 and C-6 positions leads to biologically interesting molecules. Numerous reports delineate the antitumour⁹, antiviral, antioxidant, antibacteria¹⁰, antifungal and hepatoprotective activities of these compounds.

Results and Discussion

Synthesis and characterization

Disubstituted Pyrido [2, 3 - d] pyrimidines (6) were synthesized in good yield by thermal reaction of 1-ethyl-6-amino uracil (4) with the appropriate 1, 3-dicarbonyl compounds. The 1-ethyl-6-amino uracil was prepared from ethylurea (1), cyanoacetic acid (2) and acetic anhydride in a reaction vessel. The solution was stirred at 60^oC-70^oC for 3hrs. After cooling, the white crystal were filtered off and washed with ethanol to get 75% of

intermediate (3). This was stirred in hot water 10% of NaOH was added in portion so the solution was basic the whole solution. The reaction mixture was refluxed for 20min and then cooled to 0^oC and neutralized with 1.5N HCl, after cooling, white color precipitate was observed, the solid precipitate was filtered and dried to get 6-amino-1-ethylpyrimidine-2, 4 (1H, 3H)-dione^{11, 12} (4) (80%).

The 6-amino-1-ethyl pyrimidine-2, 4 (1H,3H)-dione (4) in t-butyl acetoacetate heated to 130^o for 3-4hrs (solvent free condition) , The reaction monitored by TLC, The whole organic media concentrated to residue and purified by column chromatography using pet ether and Ethyl acetate to get 50% of 1-ethyl-7-methylpyrido[2,3-d] pyrimidine - 2, 4, 5 (1H, 3H, 8H) - trione (5). The intermediate (5) was treated with acid chloride to afford the diacyl derivatives of 1-ethyl-7-methylpyrido [2, 3-d] pyrimidine-2, 4, 5 (1H, 3H, 8H) - trione 6(a-h). Also validated by different reaction conditions (Table 1). For all the basic conditions reaction work well, when the reaction was carried out in Triethylamine (TEA).

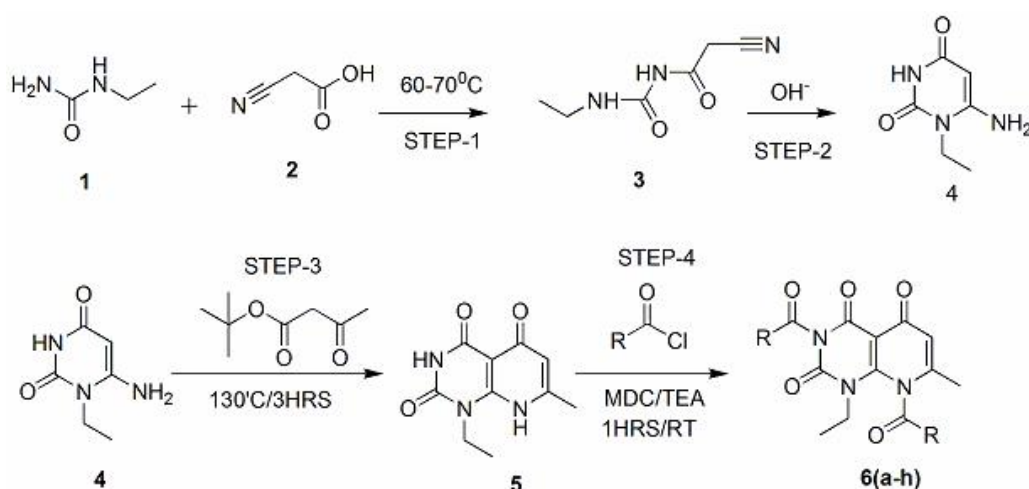


Table.1 Optimization of different condition

Entry	Catalyst	Yields(%)
1	Tri Ethyl Amine	>50%
2.	Diisopropyl ethyamine	>35%
3.	K ₂ CO ₃	<25%
4.	Na ₂ CO ₃	<25%
5.	Cs ₂ CO ₃	<30%

Experimental:

All starting materials were commercially available chemicals and were used without further purification. Flash column chromatography was carried out in silica gel 230-400 mesh. The NMR spectra were obtained with 400MHz spectrometers using DMSO as solvent and TMS as an internal reference. LCMS spectra were obtained using Agilent 1200 series LC and Micromass zQ spectrometer.

Characterization data of compounds:**6-amino-1-ethylpyrimidine-2, 4(1H, 3H)-dione (4)**

Brown Solid, Yield 90%, Mol. Formula: $C_6H_9N_3O_2$, Mol. Wt. 155.15, LCMS: 156.2 (M+1); IR (KBr $_{max} cm^{-1}$): 1665.74, 1611.64 (C=O); 3374.60 (NH₂); 3208.63 (NH); ¹HNMR (DMSO, ppm): 1.08 (t, 3H), 3.78 (q, 2H), 4.52 (s, 1H), 6.77 (s, 2H), 10.26 (s, 1H).

1-ethyl-7-methylpyrido [2, 3-d] pyrimidine-2, 4, 5(1H, 3H, 8H)-trione (5)

Off white Solid, Yield 85%, Mol. Formula: $C_{10}H_{11}N_3O_3$, Mol.Wt. 221.21, LCMS: 222.00 (M+1); IR(KBr $_{max} cm^{-1}$): 1761.65, 1705.21, 1666.76 (C=O); 3651.60, 3339.08 (NH); ¹HNMR (DMSO, ppm): 1.15 (t, 3H, CH₃), 2.50 (s, 3H, CH₃), 4.15 (q, 2H, CH₂), 6.31 (s, 1H, CH), 11.22 (s, 1H, NH), 11.77 (s, 1H, NH).

General procedure for preparation of disubstituted derivatives 6 (a-h)

To a thoroughly stirred solution of 1-ethyl-7-methylpyrido [2, 3-d] pyrimidine-2, 4, 5 (1H, 3H, 8H) – trione (5) (200mg, 10mmol) and substituted acid chloride (20mmol) in 5ml of DCM, 0.2ml of TEA was added. The reaction mixture was stirred for 2-3hrs and the reaction mixture was washed with water, 10% Sodium bicarbonate solution and brine solution. Then separated organic layer and purified by column chromatography. The pure compound isolated from 3 - 4 % of Methanol in dichloromethane. Table-2 showed all the derivatives physical and chemical properties.

Disubstituted pyrimidine (6a)

Off white Solid, Yield 75%, Mol. Formula: $C_{18}H_{19}N_3O_5$, Mol.Wt.357.36; LCMS: 358.0 (M+1); IR (KBr $_{max} cm^{-1}$): 1740.80, 1662.51, 1660.21, 1580.52, (C=O); ¹HNMR (DMSO, ppm): 1.14 (m, 2H), 1.230 (m, 4H), 1.29 (t, 3H), 1.44 (m, 2H), 1.88 (m, 1H), 2.197 (m, 1H), 2.80 (s, 3H), 4.33 (q, 2H), 6.76 (s, 1H); ¹³CNMR (DMSO): 9.59, 12.63, 12.73,

19.43, 21.59, 37.33, 38.86, 107.85, 113.43, 147.98, 151.61, 157.22, 159.03, 159.53, 171.89, 177.80.

Disubstituted pyrimidine (6b)

White Solid, Yield 80%, Mol. Formula: $C_{24}H_{31}N_3O_5$, Mol.Wt. 441.52, LCMS: 442.2 (M+1). IR (KBr $_{max} cm^{-1}$): 1762.56, 1669.21, 1674.25, 1598.21, (C=O); ¹HNMR (DMSO, ppm): 1.29 (t, 3H), 2.74 (s, 3H), 1.46 (m, 4H), 1.49 (m, 8H), 1.80 (m, 8H), 2.41 (m, 2H), 4.28 (q, 2H), 6.74 (s, 1H); ¹³CNMR (DMSO): 12.40, 19.64, 24.30, 24.68, 24.85, 24.92, 27.64, 27.68, 28.42, 28.64, 29.74, 37.24, 39.80, 41.21, 87.64, 103.22, 149.72, 158.22, 167.22, 167.44, 167.88, 179.32, 182.54.

Disubstituted pyrimidine (6c)

White Solid, Yield 85%, Mol. Formula: $C_{26}H_{23}N_3O_5$, Mol.Wt. 457.48, LCMS: 458.20 (M+1); IR (KBr $_{max} cm^{-1}$): 1745.80, 1669.21, 1667.51, 1582.52 (C=O); ¹HNMR (DMSO, ppm): 1.24 (t, 3H), 1.34 (s, 3H), 2.45 (t, 2H), 2.54 (s, 3H), 2.62 (s, 3H), 6.54 (s, 1H), 7.83 (m, 4H), 8.12 (d, 2H), 8.24 (d, 2H); ¹³CNMR(DMSO): 12.68, 20.56, 20.69, 21.64, 37.50, 38.87, 40.13, 108.24, 113.76, 127.26, 127.86, 129.20, 130.33, 130.74, 131.33, 135.35, 136.10, 138.76, 139.04, 148.54, 152.03, 157.46, 159.48, 160.36, 163.69, 169.31.

Disubstituted pyrimidine (6d)

Off white Solid, Yield 55%, Mol. Formula: $C_{22}H_{31}N_3O_5$, Mol.Wt. 417.50, LCMS: 418.20 (M+1); IR (KBr $_{max} cm^{-1}$): 1790.60, 1756.55, 1709.02, 1667.33, 1582.67 (C=O); ¹HNMR (DMSO, ppm): 1.15 (d, 18H), 1.28 (d, 3H), 2.47 (s, 2H), 2.81 (t, 5H), 4.26 (q, 2H), 6.70 (s, 1H); ¹³CNMR (DMSO): 12.50, 21.52, 28.69, 29.16, 29.92, 30.91, 37.29, 39.91, 28.87, 40.12, 46.71, 51.73, 107.85, 113.40, 148.08, 151.51, 157.35, 159.16, 159.70, 169.31, 174.76.

Disubstituted pyrimidine (6e)

Off white Solid, Yield 76%, Mol. Formula: $C_{24}H_{17}F_2N_3O_5$, Mol.Wt. 465.41, LCMS: 467.00 (M+1); IR (KBr $_{max} cm^{-1}$): 1755.68, 1697.54, 1633.92, 1579.83 (C=O); ¹HNMR (DMSO, ppm): 1.29 (t, 3H), 2.82 (s, 3H), 4.36 (q, 2H), 6.89 (d, 1H), 7.14 (m, 1H), 7.24 (m, 1H), 7.34 (m, 2H), 7.64 (m, 2H), 8.24 (m, 2H); ¹³CNMR (DMSO): 12.60, 21.64, 37.55, 108.07, 113.82, 116.30, 117.34, 117.56, 119.71, 125.58, 132.46, 136.88, 137.96, 148.36, 151.85, 159.18, 160.32, 162.91, 164.90.

Disubstituted pyrimidine (6f)

White Solid, Yield 80%, Mol. Formula: $C_{24}H_{17}F_2N_3O_5$, Mol.Wt. 465.41, LCMS: 467.00

(M+1); IR (KBr max cm^{-1}): 1758.68, 1696.54, 1632.92, 1580.23 (C=O); $^1\text{H NMR}$ (DMSO, ppm): 1.25 (t, 3H), 2.82 (s, 3H), 4.34 (d, 2H), 6.90 (s, 1H), 7.21(m, 4H) 8.02 (d,2H), 8.24 (d,2H); $^{13}\text{C NMR}$ (DMSO): 12.57, 20.64, 38.55, 107.07, 114.52, 115.90, 117.54, 117.86, 119.91, 125.88, 132.26, 136.18, 137.06, 148.86, 151.89, 159.78, 160.22, 162.51, 164.70.

Disubstituted pyrimidine (6g)

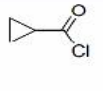
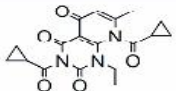
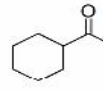
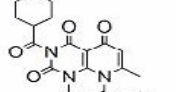
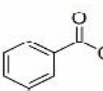
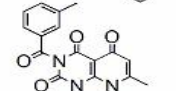
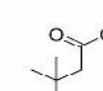
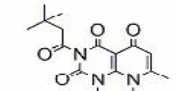
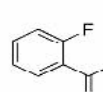
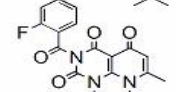
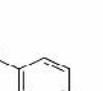
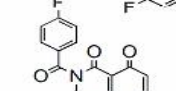
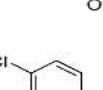
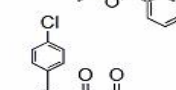

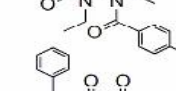
Off white Solid, Yield 75%, Mol. Formula: $\text{C}_{24}\text{H}_{17}\text{Cl}_2\text{N}_3\text{O}_5$, Mol.Wt. 498.31, LCMS: 499.2 (M+1); IR (KBr max cm^{-1}): 1794.60, 1754.55, 1710.02, 1666.23, 1580.67 (C=O); $^1\text{H NMR}$ (DMSO, ppm): 1.21 (t, 3H), 1.31 (s,3H), 2.45 (t,2H), 6.51 (s,1H),7.83(m, 4H), 8.01(d, 2H).8.21

(d, 2H); $^{13}\text{C NMR}$ (DMSO): 12.37, 21.64, 38.45, 107.17, 114.52, 115.70, 117.16, 118.86, 120.91, 125.78, 132.86, 136.48, 137.26, 148.76, 151.59, 158.98, 161.22, 162.31, 165.70.

Disubstituted pyrimidine (6h)

Off white Solid, Yield 80%, Mol. Formula: $\text{C}_{24}\text{H}_{19}\text{N}_3\text{O}_5$, Mol.Wt.429.42, LCMS: 430.44 (M+1); IR (KBr max cm^{-1}): 1755.68, 1697.54, 1633.92, 1579.83 (C=O); $^1\text{H NMR}$ (DMSO, ppm): 1.28 (t, 3H), 1.34 (s, 3H), 2.55 (t, 2H), 6.71 (s, 1H), 7.44 (m, 4H), 8.11 (d, 2H), 8.41 (d, 2H); $^{13}\text{C NMR}$ (DMSO): 12.01, 19.6, 37.21, 87.5, 103.8, 127.2, 127.6, 127.9, 128.5, 128.6, 128.9, 132.5, 132.8, 134.5, 134.8, 149.6, 161.5, 168.2, 169.2, 182.4.

Table-2 Synthesis of disubstituted derivatives 6 (a-h)

Final	Entry	Product	M.Pt $^{\circ}\text{C}$	%Yield ^a
6a			150-152	75
6b			144-146	80
6c			110-112	85
6d			112-114	55
6e			148-150	76
6f			152-154	80
6g			146-148	75
6h			140-142	80

^a Yields refer to the isolated pure compound by column chromatography

Biological Activity:

Anti microbial Activity

The prepared pyrimidine/uracil derivatives are screened for their antibacterial activity¹³⁻¹⁶ in comparison with standard antibiotic Ciprofloxacin (100µg/mL) in-vitro by well diffusion (perez, 1999; Bagambouta et al., 2004). Lawn culture was prepared using the test organism on Muller Hinton Agar (MHA), The inoculated plates were kept aside for a few minutes. Using well cutter, four wells were made in those plates at required distance. In each step of well cutting, the well cutter was thoroughly wiped with alcohol. Using sterilized micropipettes 30µl of different solvents with selected derivatives was added in to the well. The plates were incubated at 37°C for overnight. The activity of the derivatives was determined by measuring the diameters of zone of inhibition. For each bacterial strain, controls were maintained where pure solvents (DMSO).

Antibacterial activity of all the synthesized derivatives was evaluated against pathogenic bacterial strains viz., E.Coli, Staphylococcus aureus, Pseudomonas aeruginosa, also Anti fungal activity of these derivatives are evaluated against fungal strains viz., Aspergillus flavus, Cryptococcus neoformans, Candida albicans using

well diffusion methods. The zones of inhibition of the compounds against above bacterial and fungal strains are summarized in Table 3. The results obtained showed that most of the compounds possess high activity of Cryptococcus neoformans.

Conclusion

This review has presented a brief account of several attempts to develop the process and improved the percentage of yield. This is first time to publish Diacyl derivative of Substituted pyrimidine derivatives. It's give only Cryptococcus neoformans (Fungal organism) gave very good result comparing to other fungal and bacterial in studies.

Acknowledgement

The authors would like to express thanks to the Department of chemistry, Islamiah College, Vaniyambadi for support to this work. We also special thanks to Pon Saravanakumar, A. Prabhu, and friends are behind this work and encouragement. Also thankful to CECRI, KARIKUDI and NMR Research Centre, Bangalore for providing Analytical data.

Table3. Antimicrobial potentialities of the tested compounds expressed as size (mm/mg sample) of inhibition zone

Microorganism	compound								Cip	Amp
	6a	6b	6c	6d	6e	6f	6g	6h		
E.Coli	12	10	11	10	11	14	11	9	22	-
Pseudomons aeuginosa	11	12	10	14	10	12	10	15	22	-
Staphylococcus aureus	10	11	14	10	12	10	16	14	27	-
Aspergillus Flavus(F)	11	10	10	11	6	10	15	9	-	18
Cryptococcus neoformans(F)	12	12	12	13	11	21	18	16	-	16
Candida albicans (F)	<10	<10	<10	<10	<10	<10	<10	<10	-	14

Cip. :Ciprofloxin, Amp. :Amphotericin B, (F) : Fungus microorganism

References:

- 1 (a) Kappe, C.O. *Tetrahedron* 1993, 49, 6937 and references cited therein; (b) Sasaki, S.; Cho, N et al., *J. Med.Chem.* 2003, 46, 113; (c) Ibrahim, D.A. El_Metwally, A.M. *Eur. J. Med. Chem.* 2010, 45, 1158;
- 2 Jones, A. S.; Sayers, J.R.; Walker, R.T.; Clercq, E.D.J. *Med. Chem.* 1988, 31, 268.
- 3 *Comprehensive Heterocyclic Chemistry - II*; Kataritzky, A.R., Rees, C.W., Scriven, E.F.V., Eds.: 1996; Vol, p-591.
- 4 Mark C. Bagley, David D. Hughes, Roger Lloyd and Vicki E. C. Powes *Tetrahedron Letters* 42 (2001) 6585-6588.
- 5 (a) Srivastava, P.; Saxena, A.S.; Ram, V.J *Synthesis* 2000, 541 and references cited therein. (b) Gordeev, M.F.; Patel, D.V.; Wu, J.; Gordon, E.M. *Tetrahedron Lett.* 1996, 37, 4643.
- 6 Edward C. Taylor, James E. Dowling, Thomas Schrader and Beena Bhatia, *Tetrahedron* 54(1998)9507-9518.
- 7 (a) Quiroga, J.; Mejia, et al., *Tetrahedron* 2001, 57, 6947-6953., (b) Quiroga, J.; Cisneros, Letter 2001, 42, 5625-5627. (c) Quiroga, J.; Insuasty, B et al., *J. Heterocycl. Chem.* 2001, 38, 339-341.
- 8 Magnus, M.A.; Confalone, F.N.; Storace, L. *Tetrahedron Lett.* 2000, 41, 3015-3019
- 9 Piper, J.R.; Montgomery, J. A. ; Sirotnak, F.M. *Chem. Abstr.* 1991, 115, 92204. (b) Gangjee, A.; Vasudevan, A.; Queener, S.; Kisliuk, R. J. *Med. Chem.* 1995, 38, 1778-1785.
- 10 Suzuki, N. *Chem. Pharm. Bull.* 1980, 28, 761-768.
- 11 *Monatshefte für Chemie* 127, 917-925 (1996).
- 12 Per G. Kjellin, Lund; Carl G.A. Persson, Loberod; Us patent No. 4,546,182.
- 13 Perez, C., Pauli, M., Bazerque, P. 1990. An antibiotic assay by the agar-well diffusion method. *Acta Biol. Med. Exp.*, 15: 13- 115.
- 14 Bagamboula, C.F., Uyttendaele, M., Debevere, J. 2004. Inhibitory effect of thyme and basil essential oils, carvacrol, thymol, estragol, linalool and p-cymene towards *Shigella sonnei* and *S. flexneri*. *Food Microbiol.* 21: 33-42.
- 15 Erdemoglu, N., Kuş Peli, E., Yes, E., Ilada, R. 2003. Anti-inflammatory and antinociceptive activity assessment of plants used as remedy in Turkish folk medicine. *J. Ethnopharmacol.*, 89: 123-1.
- 16 Perez, C., Pauli, M., Bazerque, P. 1990. An antibiotic assay by the agar-well diffusion method. *Acta Biol. Med. Exp.*, 15: 13- 115.
