RESEARCH ARTICLE

Facile Synthesis of 5,6,7,8-tetrahydropyrimido[4,5-d] pyrimidine-2,4 (1H,3H) -dione Analogs via One Pot Multicomponent Reaction

Shumaila Shafi¹, Ammara Chand¹, Mehwish Nawaz¹, Rashida Parveen¹, Saba Munawar¹, Mateeba Ishfaq¹, Munazza Sheikh¹, Ayesha Maryam¹, Tahira Saleem¹,²*

¹Department of Chemistry, The Women University Multan, Multan, 60000, Pakistan
²Department of Chemistry, Institute of Chemical Sciences, Bahauddin Zakariya University, Multan, 60800, Pakistan

Abstract: Indeed, MCR technology is widely acknowledged now for its impact on drug discovery projects and is strongly supported by industry as well as academia. Uracil is an important one of the five nucleobases and significantly important because of their biological properties; of which 6-amino uracil is most important and can act as nucleophile and electrophile. 6-Aminouracils being rich are used as starting materials for the synthesis of heterocyclic compounds of biological significance such as pyrido-, pyrazolo, pyrimido and pyrimidines derivatives. 5,6,7,8-tetrahydropyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione have been synthesized by various conventional methods. However, these methods have drawbacks such as unsatisfactory yields and tedious work-up etc. In the present work, we would like to report a new route for the synthesis of 5,6,7,8-tetrahydropyrimido[4,5-d]pyrimidine-2,4 (1H,3H)-dione in MCRs.

Keywords: Multi Component Reaction (MCR), Green Synthesis, 6-Amino Uracil

INTRODUCTION

To construct various heterocyclic scaffolds, a number of advancements has introduced in implementation and design of multi component reactions (MCRs), as interest in preparation of diversity-oriented compounds has increased which cause an increase in consideration of this paradigm to synthesize a library of compounds [1].

MCRs that are easy to handle, can also be used to assemble the complex molecules in inert atmosphere without using dry conditions. In MCRs non-linear approach and convergent way is used and in one step all the moieties that determine the properties are introduced except sequentially. Thus, MCRs can be used to quickly generate the SARs (structure-activity relationships [2-8]. Any form of compounds can be synthesized with their high bond-forming efficacy and atom economy, unusual waste formation and the purification and separation of products are economical while using MCRs [9]. MCRs are the masterworks of reaction designs and synthetic efficiency [10, 11].

Many interesting pharmacological activities are exhibited by a variety of derivatives of pyrimidine [12-19]. A number of biological significant compounds were used to regulate the growth of plants in their protection area [20]. These studies revealed the introduction of expedient methods to synthesize pyrimido[4,5-d]pyrimidine-2,4-dione [21]. Now a days,
pyrimido-pyrimidines (annulated uracil) is a very interesting class of compounds as they display a large number of pharmacological activities with their effective inhibitory characteristics about tyrosine kinase area for the receptors of epidermal growth [22], dihydrofolate-reductase [23] and 5-phosphoribosyl-1-pyrophosphate synthetase [24]. A number of reports has given about their antiviral [25], antioxidant [26], antitumor [27], hepatoprotective and antifungal activities [28].

Uracil is an important constituent of nucleic acid and five nucleobases are a significant class of compound [29] due to their biological characteristics, from this class 6-amino uracil is an important compound that can act as an electrophile as well as nucleophile [30]. This compound being rich is used to synthesize biologically significant heterocyclic compounds as starting reagent such as pyrazolo, pyrimidine, pyrimido [31] and pyrido- derivatives [32]. 5,6,7,8-tetrahydropyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione have been synthesized by traditional methods. Thus there is a strong need to develop some better techniques to synthesize 5,6,7,8-tetrahydro pyrimido [4,5-d]pyrimidine-2,4(1H,3H)-dione that must be simple, economical, can give high yield in a short time. This method provides a new way to synthesize 5,6,7,8-tetrahydropyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione in MCRs.

Experimental

First of all, 0.39 mmol of 6-amino-uracil was taken, then dissolved it in H2O and THF (1:1) proceeded this reaction on ultrasonic bath. Then in a separate beaker took formaldehyde (29 µl) and aniline in 2:1 ratio. Added this prepared mixture into the vial drop by drop and sonicated it on ultrasonic bath for 3 hours by monitoring it with TLC. Then the reaction mixture was cooled at room temperature to obtain the final product in vial. To get the pure crystals of the product, washed it by using 50% ethanol.

4a) 6-phenyl-5,6,7,8-tetrahydropyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione
IR: 3348(N-H), 1713(C=O), 1628(C=O), 1593(C=C), 1396 (C-H), 1230(C-N), 753 (benzene).1H-NMR (δ in ppm): (300 MHz, DMSO-d6) δ: 10.13 (s, 1H, NH), 9.97 (s, 1H, NH), 7.37 (s, 1H, NH), 5.13 (s, 2H, CH2), 4.06 (s, 2H, CH2). 13C-NMR (δ in ppm): 163.7, 162.4, 154.8, 147.7, 129.7, 127.2, 115.7 ppm.

4b) 6-(4-chlorophenyl)-5,6,7,8-tetrahydropyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione
IR: 3320(N-H), 1710(C=O), 1628(C=O), 1591(C=C), 1388(C-H), 1020-1250 (C-N), 755 (benzene). 1H-NMR (δ in ppm): (300 MHz, DMSO-d6) δ: 10.27 (s, 1H, NH), 7.23 (d, 2H, ArH, J = 6.5 Hz), 7.11 (d, 2H, ArH, J = 6.5 Hz), 6.65 (s, 1H, NH), 5.17 (s, 2H, CH2), 4.12 (s, 2H, CH2). 13C-NMR (δ in ppm): 163.7, 162.4, 154.8, 147.7, 129.7, 127.2, 115.7, 83.3, 71.6, 52.1 Elemental Analysis: C12H11ClN2O2 C, 51.72; H, 3.98; Cl, 12.72; N, 20.10. Found: C, 51.91; H, 4.25; Cl, 12.86; N, 20.25

4c) 6-(3-chlorophenyl)-5,6,7,8-tetrahydropyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione
IR: 3328(N-H), 1720(C=O), 1637(C=O ), 1591(C=C), 1389(C-H), 1020(C-O), 1090, 1160(C-N), 755(benzene).1H-NMR (δ in ppm): (300 MHz, DMSO-d6) δ: 10.19 (s, 1H, NH), 10.11 (s, 1H, NH), 7.37 (s, 1H, ArH), 7.09 (m, 3H, ArH), 6.78 (s, 1H, NH), 5.23 (s, 2H, CH2), 4.35 (s, 2H, CH2). 13C-NMR (δ in ppm): 163.7, 162.4, 154.8, 151.0, 135.2, 131.0, 121.8, 114.7, 112.4, 83.3, 71.6, 52.1 Elemental Analysis: C12H11ClN2O2 C, 51.72; H, 3.98; Cl, 12.72;
N, 20.10. Found: C, 51.78; H, 3.99; Cl, 12.75; N, 20.18.

4d) 6-(2-chlorophenyl)-5,6,7,8-tetrahydropyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione
IR: 3350(N-H), 1712(C=O), 1630(C=O), 1590(C=C), 1388, 1450(N-O), 1299(C-H), 1018, 1112(C-N), 757(benzene). 1H-NMR (δ in ppm): (300 MHz, DMSO-d6) δ: 10.34 (s, 1H, NH), 10.09 (s, 1H, NH), 6.97-7.37 (m, 4H, ArH), 6.29 (s, 1H, NH), 5.33 (s, 2H, CH2), 4.46 (s, 2H, CH2) 13C-NMR (δ in ppm): 163.7, 162.4, 154.8, 150.8, 130.8, 129.0, 127.7, 124.9, 123.7, 83.3, 71.1, 51.6. Elemental Analysis: C17H16ClN2O2 C, 51.72; H, 3.98; Cl, 12.72; N, 20.10; Found: C, 51.85; H, 3.99; Cl, 12.84; N, 20.24.

4e) 6-(4-nitrophenyl)-5,6,7,8-tetrahydropyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione
IR: 3339(N-H), 1709(C=O), 1630(C=O), 1595(C=C), 1389, 1440(N-O), 1299(C-N), 1018-1115(C-N), 755 and 885(benzene). 1H-NMR (δ in ppm): (300 MHz, DMSO-d6) δ: 10.39 (s, 1H, NH), 10.18 (s, 1H, NH), 8.33 (d, 2H, ArH, J = 6.2 Hz), 8.14 (d, 2H, ArH, J = 6.2 Hz), 6.69 (s, 1H, NH), 5.21 (s, 2H, CH2), 4.19 (s, 2H, CH2) 13C-NMR (δ in ppm): 163.7, 162.4, 155.7, 154.8, 137.4, 124.8, 112.3, 83.3, 71.6. 52.1. Elemental Analysis: C17H16N3O2 C, 49.83; H, 3.83; N, 24.21; Found: C, 49.87; H, 3.91; N, 24.43.

4f) 6-(3-nitrophenyl)-5,6,7,8-tetrahydropyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione
IR: 3335(N-H), 1715(C=O), 1621(C=O), 1594(C=C), 1389(N-O), 1019 and 1093(C-N), 755(benzene). 1H-NMR (δ in ppm): (300 MHz, DMSO-d6) δ: 10.25 (s, 1H, NH), 10.16 (s, 1H, NH), 8.46 (s, 1H, ArH), 8.17-8.03 (m, 3H, ArH), 6.83 (s, 1H, NH), 5.31 (s, 2H, CH2), 4.37 (s, 2H, CH2) 13C-NMR (δ in ppm): 163.7, 162.4, 154.8, 150.5, 148.8, 130.5, 120.4, 113.4, 107.2, 83.8, 71.6. 52.1. Elemental Analysis: C17H16N3O2 C, 49.83; H, 3.83; N, 24.21; Found: C, 49.85; H, 3.85; N, 24.31.

4g) 6-(2-nitrophenyl)-5,6,7,8-tetrahydropyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione
IR: 3335(N-H), 1715(C=O), 1621(C=O), 1594(C=C), 1389(N-O), 1019 and 1093(C-N), 755(benzene). 1H-NMR (δ in ppm): (300 MHz, DMSO-d6) δ: 10.47 (s, 1H, NH), 10.13 (s, 1H, NH), 8.05-7.37 (m, 4H, ArH), 6.23 (s, 1H, NH), 5.41 (s, 2H, CH2), 5.16 (s, 2H, CH2). 13C-NMR (δ in ppm): 163.7, 162.4, 154.8, 143.3, 140.0, 135.1, 126.0, 119.0, 110.7, 83.3, 70.6, 51.1. Elemental Analysis: C17H16N3O4 C, 49.83; H, 3.83; N, 24.21; Found: C, 49.91; H, 3.92; N, 24.35.

4h) 6-(2-methyl-5-nitrophenyl)-5,6,7,8-tetrahydropyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione
IR: 3530(N-H), 1712(C=O), 1630(C=O), 1590(C=C), 1388, 1450(N-O), 1299(C-H), 1018, 1112(C-N), 757(benzene). 1H-NMR (δ in ppm): (300 MHz, DMSO-d6) δ: 10.29 (s, 1H, NH), 10.17 (s, 1H, NH), 8.42 (s, 1H, ArH), 8.25 (d, 1H, ArH, J = 6.3 Hz), 7.55 (d, 1H, ArH, J = 6.3 Hz), 6.65 (s, 1H, NH), 5.29 (s, 2H, CH2), 4.43 (s, 2H, CH2), 2.21 (s, 3H, CH3). 13C-NMR (δ in ppm): 163.7, 162.4, 154.8, 148.2, 145.8, 138.9, 132.6, 124.7, 107.1, 83.3, 71.9, 52.4, 17.9. Elemental Analysis: C17H16N3O4 C, 51.48; H, 4.32; N, 23.09; C, 51.52; H, 4.42; N, 23.15.

4i) 6-(2,4-dichlorophenyl)-5,6,7,8-tetrahydropyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione
IR: 3340(N-H), 1713(C=O), 1620(C=O), 1592(C=C), 1388(C-H), 1018 and 1092(C-N), 755(benzene). 1H-NMR (δ in ppm): (300 MHz, DMSO-d6) δ: 10.55 (s, 1H, NH), 10.29 (s, 1H, NH), 7.98 (s, 1H, ArH), 7.65 (d, 1H, ArH, J = 6.5 Hz), 7.52 (d, 1H, ArH, J = 6.5 Hz), 6.37 (s, 1H, NH), 5.34 (s, 2H, CH2), 4.76 (s, 2H, CH2) 13C-NMR (δ in ppm): 163.7, 162.4, 154.8, 148.9, 131.3, 127.8, 125.2, 124.6, 117.1, 83.3, 71.1, 51.6. Elemental Analysis: C18H10Cl2N2O2 C, 46.03; H,
3.22; Cl, 22.64; N, 17.89; Found: C, 46.14; H, 3.32; Cl, 22.71; N, 17.92.

4j) *Ethyl 4-(5,7-dioxo-1,4,5,6,7,8-hexahydropyrimido[4,5-d]pyrimidin-3(2H)-yl)benzoate*

IR: 3348 (N-H), 1713(C=O), 1613(C=O), 1594(C=C), 1389(C-H), 1018 and 1093(C=N), 755(benzene). 1H-NMR (δ in ppm): (300 MHz, DMSO-d6) δ: 10.46 (s, 1H, NH), 10.19 (s, 1H, NH), 8.2 (d, 2H, Ar-H, J = 6.4 Hz), 7.47 (d, 2H, Ar-H, J = 6.4 Hz), 6.71 (s, 1H, NH), 5.53 (s, 2H, CH2), 4.18 (s, 2H, CH2), 4.07 (q, 2H, CH2), 2.12 (t, 3H, CH3) 13C-NMR (δ in ppm): 165.9, 163.7, 162.4, 154.8, 153.9, 130.8, 119.6, 111.7, 83.3, 71.6, 60.9, 52.1, 14.1 Elemental Analysis: C18H18N4O4 C, 56.96; H, 5.10; N, 17.71; Found: C, 56.99; H, 5.17; N, 17.74.

4k) *3-(5,7-dioxo-1,4,5,6,7,8-hexahydropyrimido[4,5-d]pyrimidin-3(2H)-yl)benzoic acid*

IR: 3345(N-H), 1720(C=O), 1610(C=O), 1596(C=C), 1389(C-H), 1019 and 1100(C-N), 755(benzene). 1H-NMR (δ in ppm): (300 MHz, DMSO-d6) δ: 11.35 (s, 1H, NH), 10.27 (s, 1H, NH), 10.05 (s, 1H, NH), 7.40 (s, 1H, Ar-H), 7.17 (m, 3H, Ar-H), 6.73 (s, 1H, NH), 5.34 (s, 2H, CH2), 4.37 (s, 2H, CH2) 13C-NMR (δ in ppm): 166.3, 163.7, 162.4, 154.8, 149.5, 129.0, 119.8, 119.5, 112.6, 83.3, 71.6, 52.1 Elemental Analysis: C13H12NaO4 C, 54.17; H, 4.20; N, 19.44; Found: C, 54.19; H, 4.28; N, 19.47.

**Result and Discussion**

By using this method a great variety of 6-aminouracil derivatives were obtained via following reactions. This one pot, catalyst free reaction of formaldehyde with a variety of anilines yield 6-aminouracil derivatives containing different substituents with altered positions is generally given bellow. The product obtained then react with 6-aminouracil giving the derivatives of 6-aminouracil (4a-k).

The compounds synthesized indicate N-H stretching of secondary amine in region 3350-3320cm⁻¹ and two C=O stretching in region 1600-1750cm⁻¹ as each compound contains two carbonyl functional groups.

**Table 1: Physical Parameters of Synthesized Compounds**

| Serial No. | Compound Code | Structure of Compound | % age yield | Melting point (°C) |
|-----------|---------------|-----------------------|-------------|-------------------|
| 1         | 4a            | ![Structure of Compound](image1) | 78%         | 240°C             |
| 2         | 4b            | ![Structure of Compound](image2) | 75%         | 250°C color change |
| 3         | 4c            | ![Structure of Compound](image3) | 74%         | >265°C            |
| 4         | 4d            | ![Structure of Compound](image4) | 79%         | >255°C slight change in color |
| 5         | 4e            | ![Structure of Compound](image5) | 74%         | >270°C            |
| 6         | 4f            | ![Structure of Compound](image6) | 75%         | >265°C            |
| 7         | 4g            | ![Structure of Compound](image7) | 73%         | >250°C            |

These compounds also indicate the presence of aromatic ring stretching of C=C in region 1500-
1650 cm\(^{-1}\), the stretching in the region of 1000-1250 cm\(^{-1}\) is due to C-N in amine and the stretching in the region of 1300-1400 cm\(^{-1}\) is due to C-H groups. The stretching in the region of 700-900 cm\(^{-1}\) is due to aromatic ring system. Few compounds that are synthesized showed strong bands in the region of 1330-1365 cm\(^{-1}\) due to the presence of NO\(_2\) group. According to proton NMR spectrum (4a) CH group showed one singlet peak at \(\delta 4.06\) ppm, NH groups showed three singlet peaks at \(\delta 10.13, 9.97\) and 6.26 respectively and a multiplet due to benzene ring. 10 separate resonances further confirmed this structure by \(^1\)H-decoupled \(^{13}\)C NMR spectrum (4a).

**Mechanism of reaction**

Mechanism of this reaction is given below which explain every step of this reaction and how the product is generated by this reaction.

![Scheme 1: Proposed mechanism for synthesis of amino-uracil derivatives](image)

**Conclusions**

In this method we overcome the drawbacks of poor yields and reaction time. This method provides a new way to synthesize 5,6,7,8-tetrahydropyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione in MCRs which is simple, economical and give high yield in a short time. In future the synthesized compounds will be used in pharmacological and pharmaceutical applications.

**Acknowledgement:**

We are grateful for the Department of Chemistry, The Women University Multan for their Lab facilities to conduct this study.

**References**

[1] Sunderhaus, J.D. and S.F. Martin, Applications of multicomponent reactions to the synthesis of diverse heterocyclic scaffolds. Chemistry–A European Journal, 2009. 15(6): p. 1300-1308.

[2] Ramachary, D.B. and C.F. Barbas III, Towards Organo-Click Chemistry: Development of Organocatalytic Multicomponent Reactions Through Combinations of Aldol, Wittig,
Knoevenagel, Michael, Diels–Alder and Huisgen Cycloaddition Reactions. Chemistry–A European Journal, 2004. 10(21): p. 5323-5331.

[3] Ramachary, D., N.S. Chowdari, and C.F. Barbas III, Organocatalytic Asymmetric Domino Knoevenagel/Diels–Alder Reactions: A Bioorganic Approach to the Diastereospecific and Enantioselective Construction of Highly Substituted Spiro [5, 5] undecane-1, 5, 9-triones. Angewandte Chemie International Edition, 2003. 42(35): p. 4233-4237.

[4] Guillena, G., D.J. Ramon, and M. Yus, Organocatalytic enantioselective multicomponent reactions (OEMCRs). Tetrahedron: Asymmetry, 2007. 18(6): p. 693-700.

[5] Yu, J., F. Shi, and L.-Z. Gong, Brønsted-acid-catalyzed asymmetric multicomponent reactions for the facile synthesis of highly enantioenriched structurally diverse nitrogenous heterocycles. Accounts of chemical research, 2011. 44(11): p. 1156-1171.

[6] de Graeff, C., E. Ruijter, and R.V. Ortu, Recent developments in asymmetric multicomponent reactions. Chemical Society Reviews, 2012. 41(10): p. 3969-4009.

[7] Marson, C.M., Multicomponent and sequential organocatalytic reactions: diversity with atom-economy and enantiocontrol. Chemical Society Reviews, 2012. 41(23): p. 7712-7722.

[8] Heravi, M.M., S. Asadi, and B.M. Lashkariani, Recent progress in asymmetric Biginelli reaction. Molecular diversity, 2013. 17(2): p. 389-407.

[9] Cimarelli, C., Multicomponent Reactions. 2019, Multidisciplinary Digital Publishing Institute.

[10] Dömling, A., Recent developments in isocyanide based multicomponent reactions in applied chemistry. Chemical reviews, 2006. 106(1): p. 17-89.

[11] Ram, V.J., et al., A convenient synthesis and hepatoprotective activity of imidazo [1, 2-c] pyrimido [5, 4-e] pyrimidine, tetraazaacenaphthene and tetraazaphenalene from cyclic ketene aminals through tandem addition-cyclization reactions. Bioorganic & medicinal chemistry, 2002. 10(5): p. 1275-1280.

[12] Aly, A., Synthesis and pharmacological activity of annelated pyrimidine derivatives. Phosphorus, Sulfur, and Silicon and the Related Elements, 2006. 181(6): p. 1285-1298.

[13] Pinna, C., et al., Purine-and pyrimidine-induced responses and P2Y receptor characterization in the hamster proximal urethra. British journal of pharmacology, 2005. 144(4): p. 510.

[14] Reznik, V., et al., Synthesis and pharmacological activity of ω-(4-phenylpiperazin-1-yl) alkylthiopyrimidines. Pharmaceutical Chemistry Journal, 2004. 38(12): p. 654-658.

[15] Sondhi, S.M., et al., Synthesis, anti-inflammatory and analgesic activities evaluation of some mono, bi and tricyclic pyrimidine derivatives. Bioorganic & medicinal chemistry, 2005. 13(22): p. 6158-6166.
[16] Bruno, O., et al., Synthesis and pharmacological evaluation of 2, 5-cycloamino-5H-[1] benzopyrano [4, 3-d] pyrimidines endowed with in vitro antiplatelet activity. Bioorganic & medicinal chemistry letters, 2001. 11(11): p. 1397-1400.

[17] Gangjee, A., et al., Synthesis, antifolate, and antitumor activities of classical and nonclassical 2-amino-4-oxo-5-substituted-pyrrolo [2, 3-d] pyrimidines. Journal of medicinal chemistry, 2001. 44(12): p. 1993-2003.

[18] Banjanac, M., et al., Pyrimido-Pyrimidines: A Novel Class of Dihydrofolate Reductase Inhibitors. Food Technology & Biotechnology, 2009. 47(3).

[19] Mangalagiu, G., et al. New pyrrolo-pyrimidine derivatives with antifungal or antibacterial properties in vitro. in Annales pharmaceutiques françaises. 2001.

[20] Hamama, W.S., et al., Facile construction of substituted pyrimido [4, 5-d] pyrimidiones by transformation of enaminouracil. Journal of advanced research, 2013. 4(2): p. 115-121.

[21] Hamama, W., Enaminouraciles as Precursors for Synthesis of Pyrimido [4, 5-d] pyrimidine2, 4-diones. ZEITSCHRIFT FUR NATURFORSCHUNG B, 2000. 55(5): p. 443-447.

[22] Rewcastle, G.W., et al., Tyrosine kinase inhibitors. 12. Synthesis and structure− activity relationships for 6-substituted 4-(phenylamino) pyrimido [5, 4-d] pyrimidines designed as inhibitors of the epidermal growth factor receptor. Journal of medicinal chemistry, 1997. 40(12): p. 1820-1826.

[23] Gebauer, M.G., C. McKinlay, and J.E. Gready, Synthesis of quaternised 2-aminopyrimido [4, 5-d] pyrimidin-4 (3H)-ones and their biological activity with dihydrofolate reductase. European journal of medicinal chemistry, 2003. 38(7-8): p. 719-728.

[24] Fry, D.W., M.A. Becker, and R.L. Switzer, Inhibition of human 5-phosphoribosyl-1-pyrophosphate synthetase by 4-amino-8-(beta-D-ribofuranosylamino)-pyrimido [5, 4-d] pyrimidine-5'-monophosphate: evidence for interaction at the ADP allosteric site. Molecular pharmacology, 1995. 47(4): p. 810-815.

[25] Tenser, R.B., A. Gaydos, and K.A. Hay, Inhibition of herpes simplex virus reactivation by dipyridamole. Antimicrobial agents and chemotherapy, 2001. 45(12): p. 3657-3659.

[26] De la Cruz, J., et al., Inhibition of ferrous-induced lipid peroxidation by pyrimido-pyrimidine derivatives in human liver membranes. Lipids, 1992. 27(3): p. 192-194.

[27] Sanghvi, Y.S., et al., Antitumor and antiviral activity of synthetic. alpha.-and. beta.-ribonucleosides of certain substituted pyrimido [5, 4-d] pyrimidines: a new synthetic strategy for exocyclic aminonucleosides. Journal of medicinal chemistry, 1989. 32(3): p. 629-637.

[28] Singh, G., et al., Synthesis and antimicrobial evaluation of some new pyrido [2, 3-d] pyrimidines and their ribofuranosides. 2002.
[29] Patrick, G.L., An introduction to medicinal chemistry. 2013: Oxford university press.

[30] Parker, W.B., Enzymology of purine and pyrimidine antimetabolites used in the treatment of cancer. Chemical reviews, 2009. 109(7): p. 2880-2893.

[31] Edupuganti, R., et al., Synthesis and biological evaluation of pyrido [2, 3-d] pyrimidine-2, 4-dione derivatives as eEF-2K inhibitors. Bioorganic & medicinal chemistry, 2014. 22(17): p. 4910-4916.

[32] Tolstoluzhsky, N., et al., Efficient Synthesis of Uracil-Derived Hexa-and Tetrahydropyrido [2, 3-d] pyrimidines. European Journal of Organic Chemistry, 2013. 2013(24): p. 5364-5369.