MEDWIN PUBLISHERS
Committed to Create Value for Researchers

International Journal of Pharmacognosy & Chinese Medicine
ISSN: 2576-4772

Synthesis and Biological Activity of 6-Substituted Pyrimidine-2,4-Dionesderivatives

Kaur A[1], Kaur B[1], Gupta VK[2] and Gupta M[1]*

[1]Department of Pharmaceutical Chemistry, Amar Shaheed Baba Ajit Singh Jujhar Singh Memorial College of Pharmacy, India

[2]Dreamz College of Pharmacy, Sunder Nagar, India

***Corresponding author:** Monika Gupta, Department of Pharmaceutical Chemistry, Amar Shaheed Baba Ajit Singh Jujhar Singh Memorial College of Pharmacy, India, Email: monikaguptaa@gmail.com

Review Article

Volume 5 Issue 1

Received Date: February 07, 2021

Published Date: February 22, 2021

DOI: 10.23880/ipcm-16000211

Abstract

Cancer is a terrible disease and second leading cause of death, behind cardio-vascular disease in the world. At present, there are three main methods of cancer treatment: surgery, radiation therapy and chemotherapy. Pyrimidineis a six-membered heterocyclic aromatic organic compound containing two nitrogen atoms at positions 1 and 3. Pyrimidine derivatives occupy a distinct and unique place in chemotherapy. The chemotherapeutic efficacy of pyrimidine derivatives is related to their ability to inhibit vital enzymes responsible for DNA biosynthesis as dihydrofolatereductase (DHFR), thymidylatesynthetase (TSase), thymidine phosphorylase (TPase) and reverse transcriptase (RTase). In the present study involves synthesis of 6-Substituted pyrimidine 2,4-diones derivatives. The synthesized compounds were subjected to antimicrobial activity against Gram negative *E. coli* (MTCC 40) and *S. aureus* (MTCC 87). The synthesized compounds possessed good to moderate antibacterial activity. Compounds 1, 2, 3a possessed good antibacterial activity when compared with standard however the compounds 2a, 2b, 2d, 1b, 1d, 1e possessed moderate activity. 1c, 1d, 1a, 2e, 2c were observed to be totally inactive compounds. The derivatives with electron withdrawing substituent on the phenyl ring at para position had poor activity in comparison to derivatives possessing no or electron donating substituents. The structures of the synthesized compounds were established by IR and NMR spectral studies.

Keywords: Pyrimidine; Antimicrobial; Thymidylatesynthetase; Anticancer

Introduction

Cancer is a terrible disease and second leading cause of death, behind cardio-vascular disease, in the world. At present, there are three main methods of cancer treatment: surgery, radiation therapy and chemotherapy. Therefore, designing new anticancer drugs with high-efficiency and broad-spectrum activity is a significant study area today. Drug resistance, generally caused because of long term cancer treatment is rapidly becoming a major worldwide problem. Hence, the design of new compounds to deal with the resistance problem has become one of the most important goals of anticancer research today. Current studies on the properties of modified DNAs revealed that they led to many great discoveries in modern biological and medicinal field, such as new drug and biosensors discovery [1].

The present work targets the nitrogenous base moieties in this research for modification with designed functional groups. Modifying the nucleobases of 2-deoxynucleosides with heterocyclic molecules is incredibly important for biological and medicinal chemistry [2]. Pyrimidine derivatives occupy a distinct and unique place in chemotherapy. The chemotherapeutic efficacy of pyrimidine derivatives is related to their ability to inhibit vital enzymes

responsible for DNA biosynthesis as dihydrofolatereductase (DHFR), thymidylatesynthetase (TSase) and reverse transcriptase (RTase). Large array of pyrimidine derivatives possess a variety of pharmacological properties. These properties include anticancer, antimicrobial, antiprotozoal, antihypertensive and antihistaminic activities [3]. Pyrimidine (1,3-diazine) is a six-membered heterocyclic aromatic organic compound containing two nitrogen atoms at positions 1 and 3 [4]. Several hydro and oxo derivativesof the pyrimidine which are particularly important in biological systems are normally referred to by their nonsystematic names such as 2,4-(1*H*,3*H*)-pyrimidinedione (Uracil), 5-methyluracil(Thymine) and 4-aminopyrimidine-2-(1*H*)-one (Cytosine) [5,6].

The pyrimidine nucleus also constitutes the major part of vital molecules including vitamins such as thiamine and folic acid [7]. Uracil was used to synthesize antibacterial antitumor agent's anticancer antibacterial and antiviral drugs [8-12]. The drugs which is having pyrimidine nucleus have capacity mimic pyrimidine nucleotide to such an extent it interfere with vital cellular activity such as synthesis and functioning of nucleic acid. These inhibit purine nucleotide inter conversion by incorporation into DNA leading to strand breakage. These decrease intracellular level of guanine nucleotide and results in inhibition of glycoprotein synthesis [13]. 6-Amino-uracil derivatives represent very important classes of functionalized uracils. 6-Amino-uracils are key intermediates in the synthesis of purine, which constitute the basic nucleus of a number of drugs, for example- caffeine, penciclovir, theobromineand theophylline.

They find wide applications as starting materials for the synthesis of a number of fused uracils of biological significance, for example, pyrano-, pyrido-, pyrazolo-, pyrimido-, pyridazino-pyrimidines.There are a large number of pyrimidine-based antimetabolites. 5- Fluorouracil (5-FU) and 5-thiouracil were early recognized as effective therapies for cancer [14]. The 1-(2,3-dihydro-5*H*-1,4-benzodioxepin-3-yl)-5-fluorouracil and the 1- [*o*-(hydroxymethyl) phenoxyethyl-1-methoxy]-5-fluorouracil were proved to display potent activity against MCF-7 human breast cancer cell line. These derivatives act as 5-FU prodrugs. Uracil derivatives of the general structures were synthesized as potential anticancer agents [15]. The majority of uracil derivatives synthesized displayed good inhibitory activity against U937 cells through inhibition of histone deacetylases (HDACs) [16]. Capecitabine (Xeloda) is an orally-administered chemotherapeutic agent used in the treatment of metastatic breast and colorectal cancers [17]. Several pyrimidine derivatives were recognized as useful therapies against Human Immunodeficiency Viruses (HIV), Hepatitis B Viruses (HBV), Herpes Simplex Viruses (HSV) and Influenza Viruses [18]. Several pyrimidine derivatives have long been identified as potent bactericidal and fungicidal agents. 2,4-Diamino-5-(3,4,5-trimethoxybenzyl) pyrimidine (Trimethoprim) was discovered as a potent acteriostatic drug mainly in the prophylaxis and treatment of urinary tract infections. Trimethoprim belongs to a class of chemotherapeutic agents known as dihydrofolatereductase inhibitors (DHFRI) [19]. The enzyme is the target of several important anticancer and antibacterial drugs. Trimethoprim binds bacterial DHFRase 105 times tighter than it does to vertebrate DHFRase [20]. Pyrimethamine is one of the oldest drugs used for treatment and prophylaxis of malaria [21].

Experimental

All of the solvents used were LR grade and purified before use in different reactions. Chemicals used were of LR grade and obtained from Hi Media and Loba chem. Thin layer chromatography was carried on pre coated (Merck 60 F454) and self-prepared silica gel coated plates were monitor the reaction. Various solvents system used for developing the chromatograms were chloroform and methanol, toluene, ethyl acetate and formic acid, benzene and ethanol in ratio of 7:3. UV chambers were used for visualization of TLC Spots. The identification and characterization of the compound were carried out by determining by following melting point on a melting point apparatus by capillary method and were uncorrected. All the IR spectra of the synthesized compound were recorded on Bruker alpha-E FTIR-ATR. Proton magnetic resonance (^{1}HNMR) spectra were recorded on Bruker Advance II 400 (400 MHz) NMR spectrometer (chemical shift in *ppm*) in DMSO or $CDCl_3$ using Tetramethylsilane (TMS) as standard.Mass spectra were run on Micro spectra using TOF based detector at SAIF Punjab University, Chandigarh.

Synthesis of 6-Aminopyrimidine-2,4-Diones (1)

A mixture of 0.6 urea (1.0 mol) and 0.93gm (1.1 mol) cyanoacetic acid and 5ml acetic anhydride was heated at 100-120˚C for 3 hours. The excess of acetic anhydride and acetic acid formed during the reaction were removed under reduced pressure. Add 5% NaOH solution was added slowly to cooled residue the precipitate of 6-aminopyrimidine-2, 4-diones were formed.

Aminopyrimidine-2,4-Diones (1): State: white powder; Yield: 72.64%; Melting point: 220-280˚C; R_f 0.75 (Chloroform: Methanol: Ethylacetate 8:1:2); IR(ATR,cm^{-1}) 3310 (-NH stretch), 1690 (-C=O stretch),1477 (aromatic C=C stretch), 1579 (-NH stretch); ^{1}H NMR($CDCl_3$,400 MHz; δ*ppm*)δ3.39-3.815 (d, 2H, $-NH_2$)δ 7.26 (s, 1H, –NH), δ7.49 (s, 1H, -NH).

N-(2,6-Dioxo-1,2,3,6-Tetrahydropyrimidine-4yl) benzamide (1a): State: pale yellow powder; Yield: 65.49%; Melting point: 118˚C; R_f 0.58 (Chloroform: Methanol: Ethylacetate 8:1:2); IR(ATR,cm^{-1}) 3396 (-NH stretch), 1705

(acyclic-C=O stretch),1743 (cyclic C=O) 1467(aromatic C=C stretch); ^{1}H NMR($CDCl_3$,400 MHz; δ*ppm*) δ 7.51-7.55 (m,2H,-NH) , δ 7.58-7.61 (s,1H,C-H), δ 7.38-7.43 (m,5H,Ar-H), δ 7.45-7.47 (m,1H, -NH)

N-(2,6-Dioxo-1,2,3,6-Tetrahydropyrimidine-4yl)4-Nitrobenzamide (1b): State: brownish yellow powder; Yield: 52%; Melting point: 171-174˚C; R_f 0.61 (Chloroform: Methanol: Ethylacetate 8:1:2); IR (ATR,cm^{-1}) 3247 (-NH stretch), 1688 (-C=O stretch amide) , 1787 (cyclic C=O), 1419 (aromatic C=C stretch), 1345 (-NO_2 stretch); ^{1}H NMR(DMSO-d^6,400 MHz; δ*ppm*) δ8.33-8.42(m,4H,Benzene ring –CH), δ8.9(s, 1H,–NH) δ8.29(s,1H,C-H), δ 8.35-8.36(d, 2H,–NH in Pyrimidine ring).

N-(2,6-Dioxo-1,2,3,6-Tetrahydropyrimidine-4yl)4-Hydroxybenzamide (1c): State: white powder; Yield: 58%; Melting point: 176˚C; R_f 0.57 (Chloroform: Methanol: Ethylacetate 8:1:2);IR(ATR,cm^{-1}): 3327 (-NH stretch), 1599 (-C=O stretch amide), 1789 (cyclic C=O), 1419 (aromatic C=C stretch), 1109 (C-F stretch); ^{1}H NMR(DMSO-d^{6}400 MHz; δ*ppm*) δ7.15-7.19(d,4H, Benzene ring), δ 7.32-7.54(d,2H,–NH Pyrimidine ring), δ 9.65 (s,1H,–NH) δ 3.46(s,1H,-CH).

N-(2,6-Dioxo-1,2,3,6-Tetrahydropyrimidine-4yl)3-Flurobenzamide(1d): State: pale yellow powder; Yield: 34%; Melting point: 184-187˚C; R_f 0.48 (Chloroform: Methanol: Ethylacetate 8:1:2); IR(ATR,cm^{-1}): 3224 (-NH stretch), 1599 (-C=O stretch amide), 1760 (cyclic C=O), 1410 (aromatic C=C stretch), 3398(-OH stretch); ^{1}H NMR(DMSO-d^{6}400 MHz; δ*ppm*) δ7.20-7.43 (m,4H, Benzene ring), δ7.79-7.83(m, 2H, –NH pyrimidine ring), δ 8.23(d,1H,Ar-OH), δ 6.92-6.94 (d,1H,-NH).

N-(2,6-Dioxo-1,2,3,6-Tetrahydropyrimidine-4yl)3-Nitrobenzamide (1e): State: brown powder; Yield: 46%; Melting point: 181-182˚C; R_f 0.63 (Chloroform: Methanol: Ethylacetate8:1:2); IR(ATR,cm^{-1}): 3271 (-NH stretch), 1532 (-C=O stretch amide), 1760 (cyclic C=O), 1410 (aromatic C=C stretch), 1345(-NO stretch); ^{1}H NMR(DMSO-d^{6}400 MHz; δ*ppm*) δ8.37-8.98(m,4H,Ar-H), δ8.94-8.98(d,2H, -NH Pyrimidine ring), δ 8.89 (s,1H,–NH).

Synthesis of 6-Amino-5-Nitrsopyrimidine-2,4-Diones (2)

0.5 gm Sodium nitrite (7.867 mmol) was dissolved in water and made a solution 6-aminopyrimidine-2,4-diones was acidified by drop wise addition of acetic acid over a period of 1 hour. The resulting solution was added to the sodium nitrite solution and heated the solution at 85˚C for 45 min. The mixture was thoroughly cooled precipitates were filtered, washed with cold water and recrystallized with 95% ethanol.

6-Amino-5-Nitrsopyrimidine-2,4-Diones(2): State: yellow powder; Yield: 76.43%; Melting point: 245-249˚C; R_f 0.69 (Chloroform: Methanol: Ethylacetate 7:1:2); IR (ATR,cm^{-1}) 3268 (-NH stretch), 1688 (-C=O stretch),1467(aromatic C=C stretch), 1578(-NO stretch).

1N-(5-Nitrso-2,6-Dioxo-1,2,3,6-Tetrahydropyrimidine-4-Yl)Benzamide (2a): State: off-white powder; Yield: 46%; Melting point: 152-156˚C; R_f 0.62 (Chloroform: Methanol: Ethylacetate 7.2:1:1.8); IR (ATR,cm^{-1}): 3268 (-NH stretch), 1622 (-C=O stretch amide),1467 (aromatic C=C stretch), 1760 (cyclic C=O), 1410 (aromatic C=C stretch), 1345(-NO stretch); ^{1}H NMR (DMSO-d^{6}400 MHz; δ*ppm*): δ7.4-7.8 (m, 5H, C-H benzene ring), δ 7.96-7.98 (m, 2H, pyrimidine ring –NH), δ8.12-8.14 (m,1H, –NH); Q-TOF-MS (m/z): 216 $[M+2]^+$.

N-(5-Nitrso-2,6-Dioxo-1,2,3,6-Tetrahydropyrimidine-4-Yl)-4-Nitrobenzamide (2b): State: Pale yellow powder; Yield: 52%; Melting point: 148-149˚C; R_f 0.61 (Chloroform: Methanol: Ethylacetate 7.2:1:1.8); IR(ATR,cm^{-1}): 3261 (-NH stretch), 1647 (-C=O stretch amide), 1787 (cyclic C=O), 1419 (aromatic C=C stretch), 1345 (-NO stretch); ^{1}H NMR (DMSO-d^{6}400 MHz; δ*ppm*) δ7.31(s,1H, –NH pyrimidine ring), δ 7.66(s,1H, –NH pyrimidine ring), δ8.31(s,1H, –NH), δ8.2-8.39(m,4H,benzene ring).

N-(5-Nitrso-2,6-Dioxo-1,2,3,6-Tetrahydropyrimidine-4-Yl)-3-Nitro Benzamide (2c):State: Yellow powder; Yield: 63%; Melting point: 181-184˚C; R_f 0.43 (Chloroform: Methanol: Ethylacetate 7.2:1:1.8); IR(ATR,cm^{-1}) 3252 (-NH stretch), 1548 (-C=O stretch amide), 1736 (cyclic C=O), 1410 (aromatic C=C stretch), 1352(-NO stretch); ^{1}H NMR (DMSO-d^{6}400 MHz; δ*ppm*) δ8.27-8.37(m, 4H, Benzene ring), δ 7.31(s,1H,–NH, pyrimidine ring), δ 7.56 (s,1H, –NH Pyrimidine ring), δ 9.58 (s, 1H,–NH).

N-(5-Nitrso-2,6-Dioxo-1,2,3,6-Tetrahydropyrimidine-4-Yl)-4-Hydroxybenzamide (2d): State: white powder; Yield: 58%; Melting point: 171-176˚C; R_f 0.54 (Chloroform: Methanol: Ethylacetate 7.2:1:1.8); IR(ATR,cm^{-1}) 3224 (-NH stretch), 1588 (-C=O stretch amide), 1762 (cyclic C=O), 1433 (aromatic C=C stretch), 3398(-OH stretch), 1360 (-NO); ^{1}H NMR(DMSO-d^{6}400 MHz; δ*ppm*) δ8.21-8.23(d,1H,Ar-OH), δ 7.91-8.21(m,2H,-NH Pyrimidine ring), δ 7.25-7.54(m,4H,C-H Benzene ring), δ 7.79-7.82(m,1H,-NH).

N-(5-Nitrso-2,6-Dioxo-1,2,3,6-Tetrahydropyrimidine-4-Yl)-3-Fluorobenzamide (2e): State: brown powder; Yield: 64%; Melting point: 182-184˚C; R_f 0.59 (Chloroform: Methanol: Ethylacetate 7.2:1:1.8); IR(ATR,cm^{-1}) 3319 (-NH stretch), 1599 (-C=O stretch amide), 1692 (cyclic C=O), 1419 (aromatic C=C stretch), 1162 (C-F stretch); ^{1}H NMR(DMSO-d^6,400 MHz; δ*ppm*) δ7.11-7.16 (m,4H,Ar-H), δ8.00-8.04 (m,2H, –NH of Pyrimidine ring), δ7.59(s,1H,–NH).

3-Chloro-N-(2,6-Dioxo-1,2,3,6-Tetrahydropyrimidine-4-Yl)-5-Nitrobenzamide (3a): State: brown-powder; Yield: 64%; Melting point: 182-184˚C; R_f 0.59 (Chloroform: Methanol: Ethylacetate 7.2:1:1.8); ^{1}H NMR($CDCl_3$,400 MHz; δ*ppm*) δ 2.82-3.20(m,6H, –CH_3), δ 8.29(s, 1H, -NH), δ 8.34(s,1H, –NH), δ 8.28 (s,1H,C-H), δ 7.73-7.82 (m,3H,Ar-H).

Synthesis of *N*-(2,6-Dioxo-1,2,3,6-Tetrahydropyrimidine-4-Yl) Benzamide

The benzoic acid (3.93 mmol), N-methyl morpholine (3.93mmol) and isobutylchloroformate (3.93mmol) in THF were stirred in flat bottom flask for 10 minat 0-5˚C. After 10 min, 6-aminopyrimidine-2,4-dione was added and stirred for further 30 min. After completion of the reaction the THF is evaporated. The precipitates obtained were dissolved in specific solvent and washed successively with citric acid, sodium bicarbonate and finally with brine solution. The organic layer was dried under vacuumand solid residue was obtained.

Synthesis of *N*-(5-Nitrso-2,6-Dioxo-1,2,3,6-Tetrahydropyrimidine-4-Yl) Benzamide

The benzoic acid (3.93 mmol), N-methyl morpholine (3.93mmol) and isobutylchloroformate (3.93mmol) in THF were stirred in flat bottom flask for 10 min at 0-5˚C. After 10 min,6-amino-5-nitrso pyrimidine-2,4-dione was added and stirred for further 30 min. After completion the reaction the THF was evaporated. The precipitates obtained were dissolved in specific solvent and washed successively with citric acid, sodium bicarbonate and finally with brine solution. The organic layer was dried under vacuum and solid residue was obtained.

Antimicrobial Evaluation

Study of Antibacterial Activity by Cup-Plate Agar Diffusion Method: In this present study, the antibacterial activity was carried out by cup-plate agar diffusion method. Here response of organism towards the synthesized compounds was measured and compared with the standard reference drug.

Materials and Method: Antimicrobial activity of new synthesized compounds was carried out by the cup-plate agar diffusion method against Gram +ve bacteria like *Staphylococcus aureus* (MTCC 87), and Gram –ve bacterial strains like *Escherichia coli* (MTCC 40).

Microbial Cultures: Two strains of bacteria were used as test microorganism. The bacterial strains used in the study were Gram +ve bacteria *Staphylococcus aureus* (MTCC 87) and Gram –ve bacterial strains like *Escherichia coli*(MTCC 40). All microorganisms were obtained from Institution of Microbial Technology (IMTECH) Chandigarh.

Inoculum Preparations: Nutrient agar media (HIMEDIA) was applied for growing and diluting the microorganism suspensions. Bacterial strains were grown to exponential phase in nutrient agar at 37^0 C for 18h.

Preparation of Standard Drug Solutions: Amoxicillin was dissolved in DMSO to get a concentration of 100 and 200µg/ml for testing antibacterial activity.

Preparation of Test Solutions: Each test compound was dissolved in DMSO to get a concentration of 100 and 200µg/ml for testing antibacterial activity.

Procedure: The nutrient agar medium media was taken in 100 ml beaker and made up the volume up to 100 ml with water. Then the media was sterilized by autoclaving at 121˚C for 15 min at 15 *psi*. Afterwards the mixture was cooled to 45˚C, and then inoculums were added to the above cooled media, mixed properly and poured into the sterile petrifies for solidifying. Bores were made on the medium with sterile bore 0.1 ml of test solution and standard solution at a concentration 100µg/ml and 200µg/ml was taken. Then the petrifies were incubated at 37˚C for 24 h and zone of inhibition were observed and measured in mm.

Anticancer Evaluation

In-Vitro Anticancer Screening: The anticancer screening of all the synthesized compounds was conducted against K562 cell lines (Myelogenous leukemia cell lines) to determine the growth inhibitory effects of the compounds. Source of cell line was NCI, USA. Vehicle used for testing was Dimethylsulfoxide (DMSO). *In vitro* testing would be carried out using SRB assay protocols, each derivative would be tested at 4 dose levels (1×10^{-7} M, 1×10^{-6} M, 1×10^{-5} M, 1×10^{-4} M). The compound showing activity would further be subjected for calculation of **GI_{50}, TGI and LC_{50}** values.

Results and Discussion

As per the proposed protocol (Figure 1) the synthesis of varied 6-amino-5-nitrosopyrimidine-2,4-diones and 6-amino-5-pyrimidine-dione derivatives were carried out. The yield (%) of the said derivatives was found to be in range of 54-76. The melting points of compounds ranged from 113-260˚C and are uncorrected. The R_f were observed in ranges of 0.45-0.7 using different solvents and detecting system (Table 1). The IR spectra of the final derivatives exhibited the absorption band at 3250-3000 cm^{-1} and 1610-1540 cm^{-1}which confirmed the presence of –NH groups, 3030 cm^{-1}confirmed the presence of aromatic –C-H stretch, 1725-1700 cm^{-1} confirmed the presence of keto functional group.^{1}H NMR spectra had multiple in region δ 6.55-7.25 ppm indicated the presence of aromatic protons and multiple at δ 8.67-9.22 ppm confirms the presence of -NH_2 and the singlet in region of δ 6.67-9.05 ppm confirms the presence

of -CONH protons. The singlet at δ 9.21-10.51 ppm confirms the presence of –NH.

Reagents and Conditions

(a) Cyanoacetic acid, Acetic anhydride, reflux, 2 hrs; (b) $NaNO_2$, 50 %Glacial Acetic acid, stirring,45 min 85˚C; (c) Benzoic acid, N-Methyl morpholine, isochloroformate, THF, stirring, 30-35 min, 0-5˚C; (d) Benzoic acid, N-Methyl morpholine ,isochloroformate stirring for 10 min in THF, stirring for 30-35 min 40-5˚C

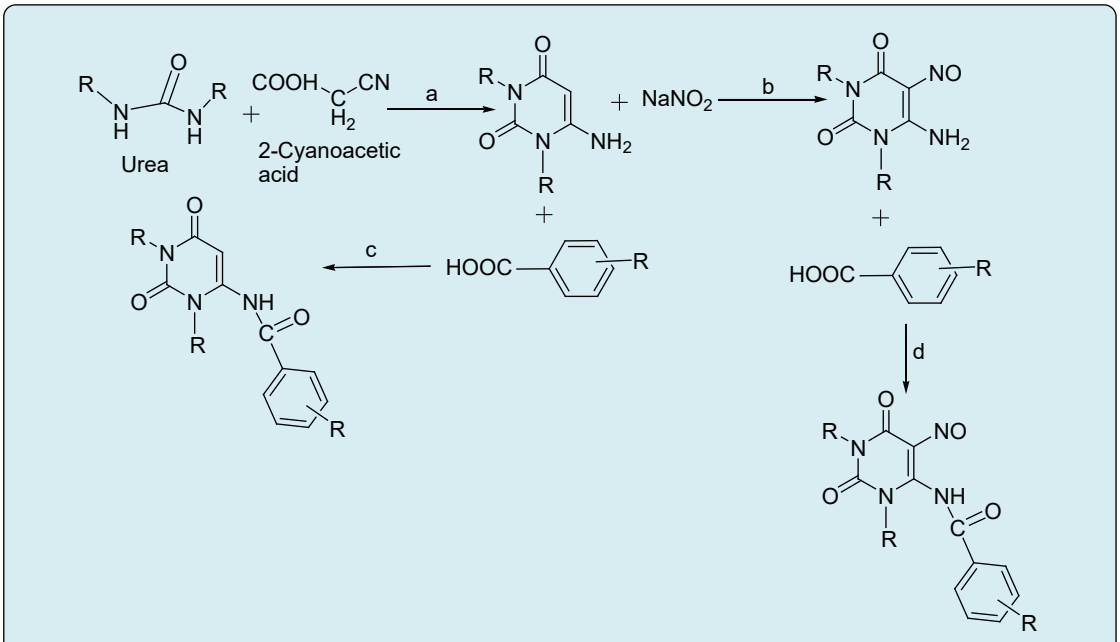

Figure 1: Reaction scheme for the synthesis of 6-substituted Pyrimidine 2,4 –dione derivatives.

Compound	Molecular formula	Melting point (˚C)	yield%	Rf*
1	C4N3O2H5	220-228	72.64	0.75
2	C4N4O3H4	245-249˚C	76.43	0.69
2a	C10H8O3N3	152-156	46	0.62
2b	C10H7O5N4	148-149	52	0.61
2c	C10H7O5N4	181-184	63	0.43
2d	C10H8O4N3	171-176	58	0.54
2e	C10H7O5N3F	182-184	64	0.59
3a	C10 H11O5N4	164-168	68	0.53
1a	C10H9O3N3	118	65.49	0.58
1b	C10H8O5N4	171-174	52	0.61
1c	C10H8O3N3F	176	58	0.57
1d	C10H9O4N3	184-187	34	0.48
1e	C10H8O4N4	181-182	46	0.63

*chloroform and methanol, benzene and ethanol and toluene, ethyl acetate and formic acid

Table 1: Physical Characteristic of Synthesized Compounds.

Drug Concentrations (µg/ml)																
Experiment 1				Experiment 2				Experiment 3				Average Values				
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
1	79	78.9	85.2	97.6	78.9	74.3	83.6	87.4	83.2	78	77.9	86.2	80.3	77.1	82.2	90.4
2	82.2	81.7	75.3	89	83.1	72.7	82.7	85.2	82	82.7	76.1	74.5	82.4	79.1	78	82.9
3	74.1	71.2	55.1	71.9	73.1	60.8	64.4	63.1	77.4	85	63.4	61.8	74.9	72.3	61	65.6
1b	83.7	79.3	82.1	83.1	80.7	72.4	76.3	83.3	88.9	85.1	82.6	82.7	84.4	79	80.3	83
1c	82.8	77.4	57.7	62.7	76.6	67.5	64.1	67.1	77.7	81	78.7	78.5	79.1	75.3	66.8	69.4
1d	82.8	68.7	42.4	43.1	89.1	64.5	60.2	42.6	87.4	79.6	78.1	55	86.4	70.9	60.2	46.9
1e	96.9	84.8	63.8	67.8	83.1	80	75.7	74	95.6	91.3	88.7	88.4	91.9	85.4	76.1	76.8
2a	73.1	66.7	64.5	67.6	84.2	81.6	77.6	67.5	82.6	77.5	80.8	59.9	80	75.3	74.3	65
2c	73	65.8	63.6	73.9	86.9	83.8	80.6	88.9	86.3	81.4	86.3	44.5	82.1	77	76.8	69.1
2d	80.4	57.1	54.2	66.8	82.4	83.5	87.4	88.5	97.5	91.1	87	58.3	86.8	77.3	76.2	71.2
ADR	-3.9	-17.8	-34.3	-27.2	20.3	18.3	8	5.5	25.8	22.6	14.7	6.7	14.1	7.7	-3.9	-5

Table 2: *In vitro* percentage control growth of Human Leukemia cell line K-562 at different molar drug concentrations.

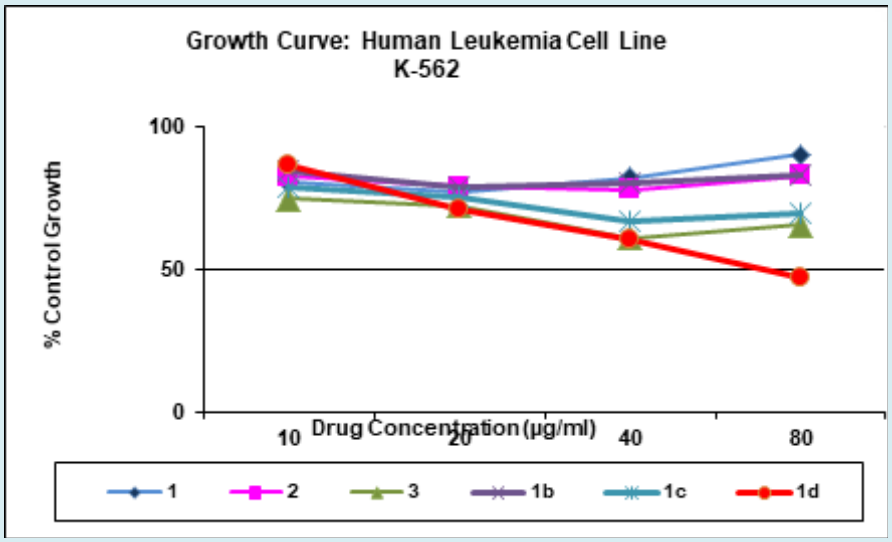

Figure 2: Graph showing % control growth against the drug concentrations and standard Andriyamycin.

Antimicrobial Activity

All the synthesized compounds had been screened for antimicrobial activity against *E.coli* and *S.aureus*. The synthesized compounds possessed good to moderate antibacterial activity. Compounds 1, 2, 3a possessed good antibacterial activity when compared with standard however the compounds 2a, 2b, 2d, 1b, 1d, 1e possessed moderate activity. 1c, 1d, 1a, 2e, 2c were observed to be totally inactive compounds. The derivatives with electron withdrawing substituents on the phenyl ring at *para* position had poor activity in comparison to derivatives possessing no or electron donating substituents. It may be concluded that the synthesized derivatives have the potential to act as antibacterial agents and the activity of the compounds varied according to the position and nature of substituents attached. The potency of the compounds can be further improved by studying their structure activity relationship.

K-562	LC50	TGI	GI50*
1	NE	NE	>80
2	NE	NE	>80
3	NE	NE	>80
1b	NE	NE	>80
1c	NE	NE	>80
1d	NE	NE	68.9
1e	NE	NE	>80
2a	NE	NE	>80
2c	NE	NE	>80
2d	NE	NE	>80
ADR	NE	50	<10

Table 3: TGI, LC_{50} and GI_{50} of the synthesized compounds against Human Leukemia cell line K-562.

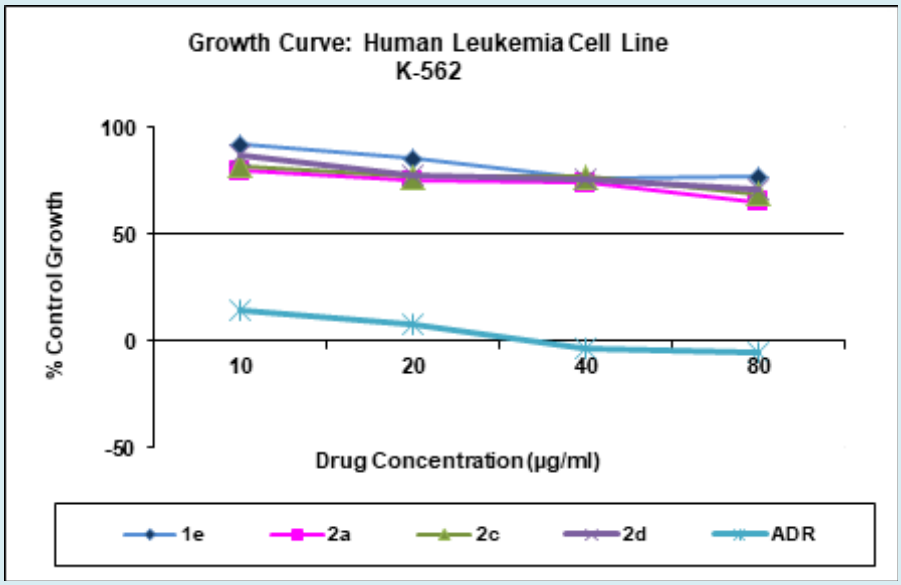

Figure 3: Graph showing % control growth against the drug concentrations and standard Andriyamycin.

Compound No.	Conc.(µg/Ml)	Zone of Inhibition(Mm)	
		Gram Positive Bacteria (MTCC 40)	Gram Negative Bacteria (MTCC 87)
1	100	16±0.5	-
	200	22±0.4	-
2	100	18±0.6	6±0.5
	200	20±0.4	9±0.4
2a	100	7±0.46	-
	200	9±0.5	12±0.3
2b	100	11±0.4	8±0.5
	200	11±0.3	11±0.6

2c	100	-	13±0.5
	200	-	16±0.3
2d	100	11±05	-
	200	12±05	-
2e	100	-	12±0.4
	200	-	13±.3
3a	100	13±0.5	14±0.3
	200	22±0.4	16±0.4
1a	100	-	-
	200	11±0.3	-
1b	100	8± 0.4	6±0.3
	200	13±0.3	8±0.4
1c	100	-	14±0.5
	200	-	16±0.2
1d	100	-	9±0.4
	200	9±0.5	11±0.4
1e.	100	8±0.3	-
	200	12±0.4	-
Amoxicillin	100	34±0.2	28±0.3
	200	38±0.2	30±0.4

- no activity

Table 4: *In vitro* antimicrobial activity of synthesized Compounds.

Anticancer Drug Screening

All the synthesized compounds were screened against K562 cell lines (Myelogenous leukemia cell lines) to determine the growth inhibitory effects of the compounds. *In vitro* testing was done using SRB assay protocol; each derivative was tested at 4 dose levels (10 µg/ml, 20µg/ml, 40µg/ml, 80µg/ml).

Conclusion

In conclusion, a total of ten 6-substituted Pyrimidine 2,4 –dione derivatives, have been synthesized and evaluated for their anti-cancer and antimicrobial activities. The synthesized compounds possessed good to moderate antibacterial activity. Compounds 1, 2, 3a possessed good antibacterial activity when compared with standard however the compounds 2a, 2b, 2d, 1b, 1d, 1e possessed moderate activity. 1c, 1d, 1a, 2e, 2c were observed to be totally inactive compounds. The derivative possessing electron withdrawing substituents on the phenyl ring at *para* position had poor activity in comparison to derivatives possessing no or electron donating substituents.

Acknowledgement

The authors are thankful to SAIF, Punjab University, Chandigarh, for providing spectral data of synthesized compounds. We are also grateful to Management, ASBASJSM College of Pharmacy for providing the necessary facilities.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Gazzar BAEl, Hafez HN (2009) Synthesis of 4-substituted pyrido [2,3-*d*] pyrimidin-4(1*H*)-one as analgesic and anti-inflammatory agents. Bioorg Med Chem 19(13): 3392-3397.

2. El Taweel FMA, Sofan MA, Mashaly MA, Hanna MA, Elagamey AA (1990) Synthesis of some new pyranoquinoline, pyridine and pyrone derivatives. PharmazieJ of Bioorg Chem 45(9): 671-673.

3. Brown DJ, Evans RF, Cowden WB, Fenn MD (1994) Chemistryof Heterocyclic Compounds: The Pyrimidines. 52: 1.

4. Brown DJ (1984) Comprehensive Heterocyclic Chemistry Pergamon, Oxford 3: 57.

5. Bettendorff L, Wirtzfeld B, Makarchikov AF, Mazzucchelli G, Frederich M, et al. (2007) Discovery of a natural thiamine adenine nucleotide. Nature Chem Biomol 3(4): 211-212.

6. Agrody MEl, Latif MS, Hady NAEl, Fakery AH, Bedair AH (2001) Heteroaromatization with 4-hydroxycoumarin Part II: Synthesis of some new pyrano[2,3-d]-pyrimidines, [1,2,4]triazolo[1,5-c]pyrimidines and pyrimido[1,6-*b*] [1,2,4]triazine derivatives. Molecule 6(6): 519-527.

7. Bedair H, El-Haddy NA, Abd El Latif MS, Fakery AH, El-Agrody AM (2000) 4- Hydroxycoumarin in heterocyclic synthesis. Part III: Synthesis of some new pyrano[2,3-*d*] [1,2,4]triazolo[1,5- c]pyrimidine and pyrimido[1,6-*b*] [1,2,4]triazine derivatives. Farmaco 55(11-12): 708-714.

8. Eiden F, Denk F (1991) Synthesis and CNS activity of pyrane derivatives: 6,8-dioxabicyclo(3,2,1) octanes. Arch Pharm 324(6): 353-354.

9. Abdelrazek FM, Metz P, Metwally NH, El-Mahrouky SF (2006) Synthesis and molluscicidal activity of new cinnoline and pyrano[2,3-*c*]pyrazole derivatives. Arch Pharm Chem Life Sci 339(8): 456-460.

10. Bianchi G, Tava A (1987) Synthesis of (2*R*)(+)-2,3-dihydro-2,6-dimethyl-*4H*-pyran-4-one, a homologue of pheromones of a species in the hepialidae family. Agri Biomol Chem 51(7): 2001-2002.

11. Noda K, Nakagawa A, Nakajima Y, Ide H, Pyrido [2,3-d]-s-triazolo [4,3-c] pyrimidine derivatives. Agri Biomol Chem 88 (1978) P50908.

12. Bailey SW, Aylin JE (2009) The extremely slow and variable activity of dihydrofolatereductase in human liver and its implications for high folic acid intake. Nat Acad Sci Proc USA 106(36): 15424-15429.

13. Ghoshal K, Jacob ST (1997) An alternative molecular mechanism of action of 5-fluorouracil, a potent anticancer drug. Biochem Pharmacol 53(11): 1569-1575.

14. Callery P, Gannett P (2002) Cancer and cancer chemotherapy In: Foye'sPrinci. of Med Chem. Williams DA, Lemke TL, et al. (Eds.), Lippincott Williams and Wilkins, Philadelphia, pp: 934-935.

15. Al Safarjalani ON, Zhou X, Rais RH, Shi J, Schinazi RF, et al. (2005) 5-(Phenylthio)acyclouridine: a powerful enhancer of oral uridine bioavailability relevance to chemotherapy with 5-fluorouracil and other uridine rescue regimens Cancer Chemo. Pharmacol 55(6): 541-551.

16. Boisdron Celle M, Remaud G, Traore S, Poirier AL, Gamelin L, et al. (2007) 5-Fluorouracil-related severe toxicity: A comparison of different methods for the pretherapeutic detection of dihydropyrimidine dehydrogenase deficiency. Cancer Lett 249(2): 271-282.

17. Mai A, Massa S, Rotili D, Pezzi R, Bottoni P, et al. (2005) Exploring the connection unit in the HDAC inhibitor pharmacophore model: novel uracil-based hydroxamates. Bioorg Med Chem Lett 15(21): 4656-4661.

18. Spacilova L, Dżubak P, Hajduch M, Kŕupkova S, Hradila P, et al. (2007) Synthesis and cytotoxic activity of various 5-[alkoxy-(4-nitro-phenyl)-methyl]-uracils in their racemic Form. Bioorg Med Chem Lett 17(23): 6647-6650.

19. Singh P, Paul K (2006) Studies of interactions between uracil-based hybrid molecules and P-glycoprotein-Search for multidrug resistance modulators. Bioorg Med Chem 14(21): 7183-7186.

20. Saniger E, Campos JM, Entrena A, Marchal JA, Boulaiz H, et al. (2003) Neighbouring-group participation as the key step in the reactivity of acyclic and cyclic salicyl-derived *O*,*O*-acetals with 5-fluorouracil. Antiproliferative activity, cell cycle dysregulation and apoptotic induction of new *O*,*N*-acetals against breast cancer cells. Tetrahedron 59(40): 8017-8026.

21. Focher F, Spadari S (2001) Thymidine phosphorylase, a two-face Janus in anticancer chemotherapy. Curr Cancer Drug Targets 1(2): 141-153.