International Journal of Behavioral Social and Movement Sciences
Volume 07, Issue 03, Jul-Sep 2016 | ISSN: 2277-7547 | www.ijobsms.org

Synthesis, Antimicrobial, and Anticancer Evaluation of N-(2-Phenyl-4-Oxothiazolidin-3-yl)-6-Methyl-4-Phenyl-2-Oxo-1,2,3,4-Tetrahydropyrimidine-5-Carboxamide Derivatives

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Received: 01 September, 2016; **Accepted:** 12 September, 2016; **Published:** 22 September, 2016

Abstract: In the current work, new pyrimidine derivativeswere made from 2-nitrobenzaldehyde, ethyl acetoacetate, and urea and tested for their in vitro antibacterial efficacy against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, two fungus strains, and one Gram-positive strain (*Candida albicans* and *Aspergillus niger*). A human breast cancer cell line was tested in vitro using these synthesized compounds (MCF-7). IR and 1H NMR spectrum studies were used to describe the newly synthesized compounds. All synthetic compounds have a significant biological potential against the investigated bacteria, according to the results.

Keywords: *Pyrimidine, Thiazolidinone, Anticancer Activity, Antimicrobial Activity*

Introduction

Cancer is defined as the abnormal development of aberrant cells that divide at an abnormally fast rate. Furthermore, cancer is the world's greatest cause of death, affecting nearly every household. As a result, there is an ongoing need to develop novel chemical compounds that may be useful in the treatment of cancer.[1] Infectious disease therapy continues to be a significant and difficult issue. Many drugs discovered to tackle infectious diseases have been limited in clinical usage due to their significant toxicity risk, pharmacokinetic problems, and bacterial resistance. The discovery of antimicrobials that are structurally unrelated to current molecules is a major research focus to combat this developing problem.[2] Pyrimidine is recognized as a significant heterocyclic moiety, because of its wide range of biological and pharmacological actions. 1,3-diazine ring, which possesses nitrogen at the first and third positions, is found in nucleotides, vitamins, nucleic acids, purines, coenzymes, uric acid, and

proteins. The inclusion of pyrimidine in the structure of DNA and RNA may account for its broad medicinal applications.[3] Pyrimidine and its analogs are known to have a broad variety of biological applications i.e. antimicrobial[4], antiviral[5], anticancer[6], anti-inflammatory[7], antioxidant[8], antimalarial[9], and analgesic[10], etc. We reported the preparation of certain novel substituted Pyrimidine analogs and assessed their biological properties as anticancer and antibacterial medicines in light of the aforementioned facts.

Experimental

Melting points are deliberately in capillary tubes with the help of Sonar melting point equipment are uncorrected. IR spectra were obtained using the FTIR Bruker ATR instrument (cm^{-1}). With the help of the Bruker DRX-300 FTNMR instrument, ^{1}H NMR spectrums in DMSO-d*6* were recorded to use a tetramethylsilane reference. TLC using silica gel G in the particular solvent-based ethyl acetate: benzene (6:4, v/v) with iodine vapors as a detecting agent was used to determine the purity of the produced compounds.

Chemistry

In ethanol, the reaction of 2-Nitro benzaldehyde, ethyl acetoacetate, and urea yielded the corresponding compound 1. In the next step compound (1) in ethanol and the required amount of hydrazine hydrate, was refluxed to afford compound 2. Further hydrazide derivatives were also reacted with substituted aldehydes to produce the Hydrazone of pyrimidine derivatives (3), and at last hydrazone of pyrimidine derivatives and a sufficient quantity of thioglycolic acid were reacted to yield the title derivatives (Scheme-1). The spectroscopic and analytical analyses were used to characterize pyrimidine derivatives.

General Procedure for Synthesis of Pyrimidine Derivatives Synthesis of Pyrimidine (Compound 2)

In the mixture of 2-nitrobenzaldehyde, ethyl acetoacetate, urea and ethanol add a few drops of concentrated hydrochloric and reflux for three hours. Progress of completion of the reaction was tracked with the help of TLC. Once the reaction was over, the solid precipitated was cooled and filtered. The crude was then rinsed with ethanol and cold water and the residual was recrystallized with ethyl acetate- hexane and ethanol (3:1) to produce the final compound.[11]

Synthesis of Hydrazide of Pyrimidine

Compound 2, (0.2M), ethanol (250 ml), required quantity of hydrazine hydrate (0.30 M), and

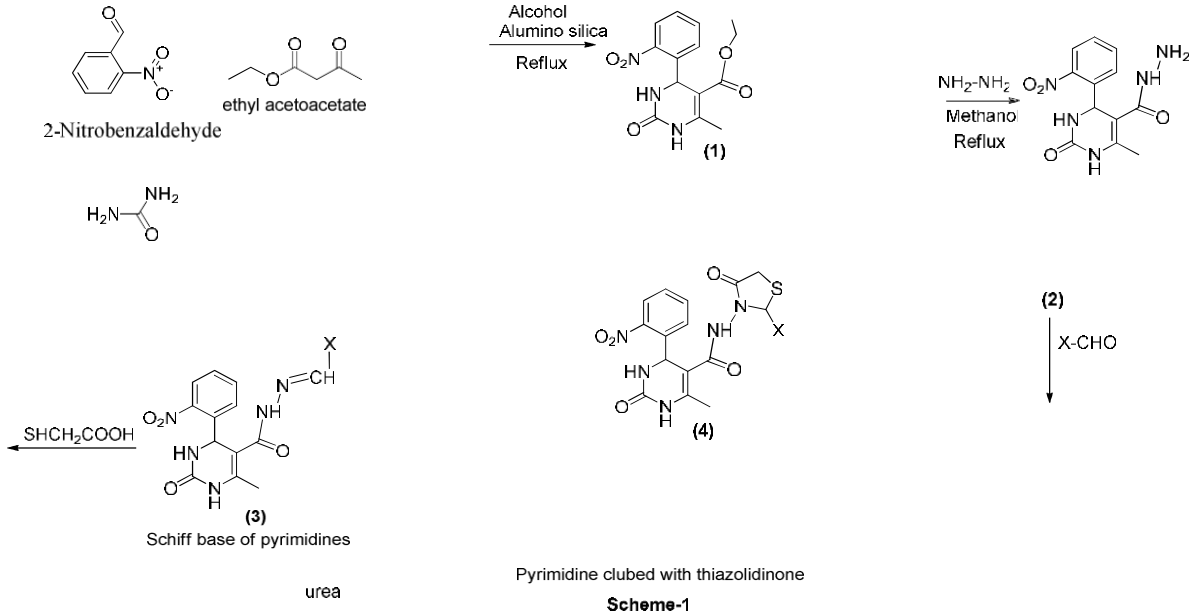
were refluxed for about 3 hours and then chilled. Filtering and recrystallization from ethanol yielded the titled compound.

Synthesis of Hydrazone of Pyrimidine Derivatives

A mixture of (0.025 M) compound 3 and the needed quantity of aldehydes (0.025 M), and methanol (100 ml) was taken RBF, glacial acetic acid was employed as a catalyst and refluxed for about 2 hours. After the finishing of the reaction, the mixture was transferred to crushed ice and the precipitate was recovered and purified by recrystallization from methanol to get the respective hydrazones.

Synthesis of Pyrimidine Derivatives Clubbed with Thiazolidinone

A combination of (0.015 M) pyrimidine derivative hydrazones and the needed quantity of thioglycolic acid (0.015 M) and Dimethylformamide (50 ml) was refluxed for 6 hours. Anhydrous $ZnCl_2$ was used as a catalyst. After the finishing of the reaction, the mixture was transferred on crushed ice and the precipitate was recovered and purified by recrystallization from methanol to get the respectively titled compounds.[12] Scheme-1 depicts the synthetic method for titled derivatives.



Scheme-1

Table-1: Physical Data of Pyrimidine Derivatives (T1-T20)

Compound	Molecular Formula	X	Melting Point (^{o}C)	Molecular Weight	R Value* f	% Yield
1	$C_{21}H_{19}N_5O_5S$	Benzaldehyde	185-187	453.47	0.67	69
2	$C_{22}H_{21}N_5O_6S$	4-methoxy benzaldehyde	183-185	483.50	0.68	80
3	$C_{21}H_{19}N_5O_6S$	2-hydroxy benzaldehyde	180-182	469.47	0.59	82
4	$C_{21}H_{18}N_6O_7S$	3-nitro	168-170	498.47	0.72	74
		benzaldehyde				
5	$C_{21}H_{18}N_6O_7S$	2-nitro benzaldehyde	190-192	498.47	0.60	77
6	$C_{21}H_{19}N_5O_6S$	4-hydroxy benzaldehyde	224-226	469.47	0.75	76
7	$C_{22}H_{21}N_5O_6S$	3-methoxy benzaldehyde	157-159	483.50	0.68	79
8	$C_{22}H_{21}N_5O_6S$	2-methoxy benzaldehyde	163-165	483.50	0.72	72
9	$C_{21}H_{18}N_6O_7S$	4-nitro benzaldehyde	158-160	498.47	0.59	83
10	$C_{21}H_{18}ClN_5O_5S$	3-chloro benzaldehyde	180-182	487.92	0.71	78
11	$C_{21}H_{18}BrN_5O_5S$	4-bromo benzaldehyde	159-161	532.37	0.58	71
12	$C_{23}H_{24}N_6O_5S$	4-dimethyl amino benzaldehyde	171-173	496.54	0.55	76
13	$C_{22}H_{21}N_5O_7S$	4-hydroxy-3-methoxy benzaldehyde	208-210	499.5	0.50	83
14	$C_{25}H_{28}N_6O_5S$	4-diethylamino benzaldehyde	172-174	524.59	0.66	72
15	$C_{21}H_{18}ClN_5O_5S$	4-chloro benzaldehyde	167-169	487.92	0.59	83
16	$C_{21}H_{17}Cl_2N_5O_5S$	2,4-dichloro benzaldehyde	175-177	522.36	0.61	65
17	$C_{21}H_{18}ClN_5O_5S$	2-chloro benzaldehyde	189-191	487.92	0.59	63
18	$C_{21}H_{18}BrN_5O_5S$	3-bromo benzaldehyde	232-234	532.37	0.62	81
19	$C_{23}H_{23}N_5O_7S$	3,4-dimethoxy benzaldehyde	196-198	513.52	0.61	68
20	$C_{22}H_{21}N_5O_5S$	4-methyl benzaldehyde	201-203	467.50	0.72	75

Spectral Data

Spectral Characterization of Synthesized Compounds (T1–T20)

T1

6-Methyl-4-(2-nitrophenyl)-2-oxo-N-(4-oxo-2-phenylthiazolidin-3-yl)-1,2,3,4-tetrahydropyrimidine-5-carboxamide

IR (KBr, cm⁻¹): 3408 (NH), 2924 (Ar–C–H), 2851 (CH_3), 1656 (C=O), 1570 (Ar C=C), 1526 (NO_2), 1359 (C–N), 670 (C–S).

¹H NMR (DMSO-d_6, 400 MHz, δ ppm): 8.38–6.66 (m, 10H, Ar–H), 7.67 (s, 1H, NH), 6.63 (s, 2H, NH-pyrimidine), 6.60 (s, 1H, NCHS), 3.49 (s, 2H, CH_2-thiazolidinone), 1.23 (s, 3H, CH_3).

T2

N-(2-(4-Methoxyphenyl)-4-oxothiazolidin-3-yl)-6-methyl-4-(2-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide

IR (KBr, cm⁻¹): 3415 (NH), 2924 (Ar–C–H), 2855 (CH_3), 1648 (C=O), 1611 (Ar C=C), 1525 (NO_2), 1346 (C–N), 1166 (OCH_3), 752 (C–S).

¹H NMR (DMSO-d_6, 400 MHz, δ ppm): 9.64–7.69 (m, 9H, Ar–H), 7.53 (s, 1H, NH), 7.12 (s, 2H, NH-pyrimidine), 7.06 (s, 1H, NCHS), 3.86 (s, 3H, OCH_3), 3.28 (s, 2H, CH_2-thiazolidinone), 1.23 (s, 3H, CH_3).

T3

N-(2-(2-Hydroxyphenyl)-4-oxothiazolidin-3-yl)-6-methyl-4-(2-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide

IR (KBr, cm⁻¹): 3422 (NH), 2925 (Ar–C–H), 1616 (C=O), 1570 (Ar C=C), 1525 (NO_2), 1349 (C–N), 754 (C–S).

¹H NMR (DMSO-d_6, 400 MHz, δ ppm): 9.94–7.39 (m, 9H, Ar–H), 7.35 (s, 1H, NH), 6.63 (s, 2H, NH-pyrimidine), 6.61 (s, 1H, NCHS), 3.99 (s, 1H, OH), 3.24 (s, 2H, CH_2-thiazolidinone), 1.23 (s, 3H, CH_3).

T4

6-Methyl-4-(2-nitrophenyl)-N-(2-(3-nitrophenyl)-4-oxothiazolidin-3-yl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide

IR (KBr, cm⁻¹): 3419 (NH), 1615 (C=O), 1573 (Ar C=C), 1527 (NO_2), 1349 (C–N), 672 (C–S).

¹H NMR (DMSO-d_6, 400 MHz, δ ppm): 9.78–7.52 (m, 9H, Ar–H), 7.39 (s, 1H, NH), 7.18 (s, 2H, NH-pyrimidine), 6.63 (s, 1H, NCHS), 3.21 (s, 2H, CH_2-thiazolidinone), 1.23 (s, 3H, CH_3).

T7

N-(2-(3-Methoxyphenyl)-4-oxothiazolidin-3-yl)-6-methyl-4-(2-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide

IR (KBr, cm^{-1}): 3372 (NH), 2925 (Ar–C–H), 2856 (CH_3), 1658 (C=O), 1607 (Ar C=C), 1570 (NO_2), 1360 (C–N), 1098 (OCH_3), 671 (C–S).

^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 9.81–7.36 (m, 9H, Ar–H), 7.39 (s, 1H, NH), 6.76 (s, 2H, NH-pyrimidine), 6.63 (s, 1H, NCHS), 4.02 (s, 3H, OCH_3), 3.88 (s, 2H, CH_2-thiazolidinone), 1.23 (s, 3H, CH_3).

T8

N-(2-(2-Methoxyphenyl)-4-oxothiazolidin-3-yl)-6-methyl-4-(2-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide

IR (KBr, cm^{-1}): 3414 (NH), 2923 (Ar–C–H), 1654 (C=O), 1610 (Ar C=C), 1568 (NO_2), 1363 (C–N), 1032 (OCH_3), 669 (C–S).

^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 8.14–7.69 (m, 9H, Ar–H), 7.64 (s, 1H, NH), 7.40 (s, 2H, NH-pyrimidine), 7.20 (s, 1H, NCHS), 3.24 (s, 2H, CH_2-thiazolidinone), 1.24 (s, 3H, CH_3).

T9

6-Methyl-4-(2-nitrophenyl)-N-(2-(4-nitrophenyl)-4-oxothiazolidin-3-yl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide

IR (KBr, cm^{-1}): 3414 (NH), 2924 (Ar–C–H), 1654 (C=O), 1601 (Ar C=C), 1518 (NO_2), 1488 (C–N), 752 (C–S).

^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 9.79–7.79 (m, 9H, Ar–H), 7.37 (s, 1H, NH), 6.77 (s, 2H, NH-pyrimidine), 6.63 (s, 1H, NCHS), 3.22 (s, 2H, CH_2-thiazolidinone), 1.23 (s, 3H, CH_3).

The remaining compounds (T10, T11, T12, T15, T16, T17, T18, and T20) should be formatted in the same manner for consistency throughout the manuscript.

Antimicrobial Assay

The antimicrobial assay for the synthesized compounds was performed on *Bacillus subtilis* and *Staphylococcus aureus,* (Gram +Ve Bacteria), *Escherichia coli* (Gram –Ve bacteria) and *Aspergillus niger* and *Candida albicans* (fungal strain). The samples for testing and standard

were ready in Sabouraud dextrose broth I.P. (fungi) or double strength nutrition broth I.P. (bacteria). The samples were cultivated for respective conditions at 24˚C for 24 hours (bacteria), 37°C (*C. Albicans*) for 48 hours, and 25°C (*A. Niger*) for 7 days. The results are given in terms of MIC.[13-14]

SRB Assay

The SRBs assay was performed for anticancer activity of the active compound in RPMI 1640 (2 mM L- glutamine and 10% fetal bovine serum) medium. The suitable amount (90µL) of the medium was transferred into 96 microwell plates and about 5000 cells were inoculated after that this was incubated at 37 ˚C, 95% air, 5% CO_2, and 100% relative humidity. To create a stock of 10^{-2} concentrations experimental medicines were solubilized in a suitable solvent. At the time of the experiment, a full medium was used to make four 10-fold serial dilutions. Aliquots of 10µL were added to the respective microwell and incubated for 48 hours. After that 50µL of cold TCA (30% w/v) was added and incubated for 60 minutes for completion of the reaction. In the next step, the supernatant was removed and plates were washed with tap water followed by air drying. Each well was treated with a 50 µl solution of sulforhodamine B (SRB) in 1 percent acetic acid at 0.4 percent (w/v) and incubated at room temperature for 20 minutes. After that, this dye was removed by rinsing the well plates 4 times with a 1% acetic acid solution. The plates were dried in air at room temperature and treated with a 10mM Trizma base to solubilize the protein-bound dye. The absorbance of the test (n=6) and the control samples were measured using an Eliza plate reader at 690nm. The dose-response parameter for each compound was calculated using 4 different concentration levels of active compound, each concentration was tested using test and control growth well plates.[15-16]

Table-2: *In Vitro* Antimicrobial Activity of the Title Compounds (T1-T20)

Compound	Minimum inhibitory concentration (µg ml^{-1})				
	Bacterial Strains			Fungal Strains	
	E. coli	***S. aureus***	***B. subtilis***	***C. albicans***	**A. *Niger***
T1	12.5	12.5	25	6.25	6.25
T2	12.5	25	50	12.5	12.5
T3	12.5	12.5	6.25	12.5	25
T4	12.5	25	12.5	6.25	6.25
T5	50	25	12.5	25	25
T6	25	12.5	25	50	25
T7	25	25	3.12	50	25

T8	12.5	12.5	6.25	25	25
T9	50	25	3.12	**3.12**	**1.56**
T10	6.25	25	12.5	25	25
T11	25	12.5	25	25	12.5
T12	6.25	25	12.5	25	25
T13	25	12.5	25	25	25
T14	25	12.5	25	25	12.5
T15	**1.56**	**3.12**	**1.56**	12.5	25
T16	25	25	12.5	6.25	25
T17	12.5	25	12.5	12.5	25
T18	25	12.5	25	12.5	**1.56**
T19	25	25	12.5	6.25	25
T20	12.5	25	12.5	12.5	25
Ciprofloxacin (standard drug)	0.01	0.15	0.12	---	--
Clotrimazole (standard drug)	--	--	--	0.10	0.30

Table-3: Anticancer activity of titled compounds (T1-T20)

Compound No	Cancer cell line (Human Breast Cancer Cell Line MCF-7) **LC50 (**µMolar)
T1	>100
T2	>100
T3	>100
T4	>100
T5	>100
T6	>100
T7	0.038
T8	0.082
T9	0.201
T10	>100
T11	0.109
T12	0.018
T13	>100
T14	>100
T15	>100
T16	>100
T17	>100
T18	>100
T19	>100
T20	>100
Adriamycin (Doxorubicin).	**0.005**

One of the purposes of this study was to develop novel pyrimidine analogs that could have biological applications. The antibacterial, antifungal, and anticancer properties of the produced analogs were tested. *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* (Bacterial strains), and fungal strains *Candida albicans* and *Aspergillus Niger* were utilized in antimicrobial research (Table-2). The antimicrobial potential was measured using the tube dilution method. Compound 15 showed high antibacterial activity against *Escherichia coli* MTCC 443(1.56 *µg/ml*), *Staphylococcus aureus* MTCC 3160 (3.12 *µg/ml*), Bacillus subtilis MTCC 441 (1.56 *µg/ml*) and compound 9 showed high antifungal activity against Candida albicans MTCC 227 (3.12 *µg/ml*), and *Aspergillus Niger* MTCC 281(1.56 *µg/ml*). Anticancer potential of produced analogs was performed against Human Breast Cancer Cell Line MCF-7. Compounds 7, 8, 9, 11, and 12 suppressed the growth of the human breast cancer cell line MCF-7 significantly as compared to the standard drug Adriamycin (Doxorubicin).

Conclusion

From 2-nitrobenzaldehyde, and ethyl acetoacetate, a series of new pyrimidine derivatives (T1–T20) were prepared. Antimicrobial and anticancer research was carried out. Some of the synthesized substances have been shown to have potent actions.

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