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Synthesis of 6-oxopyrimidin-1(6H)-yl benzamide derivatives and evaluation of their antibacterial and cytotoxic activity

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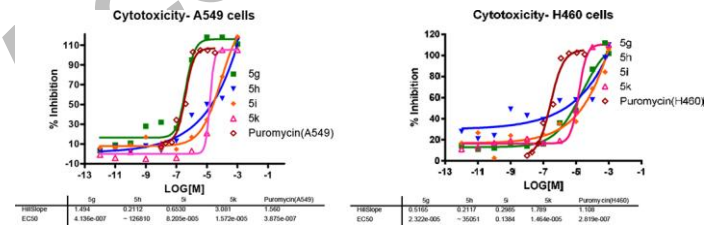
Abstract

A series of novel 2-alkylamino and 2, 4-dialkyl amino 6-oxopyrimidin-1(6H)-yl) benzamide derivatives were prepared in good yields from a base catalysed ring opening of oxadiazolo [3, 2-a] pyrimidin-5-one and evaluated for their antibacterial and cytotoxicity. Most of the compounds exhibited antibacterial activity. In particular, compounds **5b** and **5k** exhibited considerable antibiotic activity against *Klebsiella pneumonia* and *Bacillus cereus*. In addition, compounds **5g** and **5i** also inhibited the growth of two human tumor cell lines (A549 and H460) at micromolar concentrations.



KEYWORDS: Pyrimidones, oxadiazolo [3, 2-a] pyrimidin-5-ol, oxadiazolo[3,2-a]pyrimidin-5-one, Fused Oxadiazole, Cytotoxic agents, Cancer Cell Lines.

INTRODUCTION

Pyrimidine is a prominent pharmacophore prevailing in many heterocyclic natural products[1]. Pyrimidines and their derivatives have a long and distinguished history extending from the days of their discovery as important constituents of nucleic acids (like Uracil, Cytocine and Thymine... etc.) to their current use in the chemotherapy of AIDS. During the last two decades, several pyrimidine derivatives were found to have wide-spread clinical applications including chemotherapy. There are a large number of pyrimidine based antimetabolites which act as antagonists of endogenous substrates. The structural modification may be either on the pyrimidine ring or on the pendant sugar group. Lamivudine[2] is one of the examples which acts as an effective anti-AIDS drug when used in combination with Zidovudine. (Figure 1)

Likewise, there are several pyrimidone-based marketed drugs such as Uramustine,[3] Tegafur,[4] Fluorouracil[5] (Antineoplastic), Flucytosine[6] (Antifungal) and Idoxuridine[7] (Antiviral) (Figure 2). In addition, analgesic,[8] anticancer,[9] antioxidant,[10] antibacterial,[11] antiviral,[12] anti-inflammatory,[13] antiplatelet,[14] antiproliferative[15] and antitumor[16] activities were reported for pyrimidine-based derivatives.

In the course of our research devoted to the development of new class of pyrimidine and condensed pyrimidine moieties, we have focused on the synthesis and biological

evaluation of several 6-oxopyrimidin-1(6H)-yl benzamide derivatives, which may show their promising antibacterial and cytotoxic activities. Especially, condensed pyrimidine[17] derivatives possessing anti-inflammatory and analgesic activities are well documented in the literature.[18] This background of pyrimidine based derivatives motivated us to synthesize the titled derivatives for their biological evaluation.

There is an extensive work reported on nucleophilic displacement followed by rearrangement of electron-poor five-membered systems such as 1,3,4-oxadiazoles,[19] 1,3,4-thiadiazoles,[20] nitroimidazoles,[19, 21] bis(1,3,4-thiadiazol-2-yl)-1,3,5-triazinium halides,[22] isothiazole,[23] and isoxazoles.[24] Pace *et al.*,[25] studied the ANRORC rearrangements of 1,2,4-oxadiazoles as a valid approach for the obtaining heterocycles such as 1,2,4-triazoles,[25] 1,2,4-oxadiazoles,[26] 1,2,4-triazines,[25b,27] 1,2,4-oxadiazinones,[28] indazoles[29] and Amino-1,2,4-triazoles.[25] Taking advantage of the literature precedence, we intended to derive 2-alkylamino 6-oxopyrimidin-1(6H)-yl) benzamide derivatives (**6a-k**) by nucleophilic displacement on oxadiazolo[3,2-a]pyrimidin-5-one (**2**).

In this paper, we report the synthesis of a new class of 2-alkylamino and 2, 4-dialkyl amino 6-oxopyrimidin-1(6H)-yl benzamide derivatives by using base mediated ring opening of oxadiazolo [3, 2-a] pyrimidin-5-one using DMF as solvent. The title compounds were subjected to *in vitro* antibacterial, and cytotoxicity studies to examine the relationship between structural modifications and biological activity. Compounds **5a-k** and **6a-k** showed significant anti-bacterial activity in comparison to streptomycin, a first line antibiotic drug. In addition, compounds **5g**, **5i** and **5k** showed significant

cytotoxicity activity in comparison to puromycin, an amino-nucleoside antibiotic derived from the *Streptomyces alboniger bacterium*.

RESULTS AND DISCUSSION

Chemistry

In this study previously reported oxadiazole,[30] was converted into new 2-alkylamino and 2, 4-dialkyl amino 6-oxopyrimidin-1(6H)-yl) benzamide derivatives (**5a-k** and **6a-k**). The synthesis of oxadiazole (**1**) was achieved by reaction of ethyl 4-bromobenzoate with hydrazine hydrate in ethanol to give hydrazide, which upon subsequent reaction with cyanogen bromide in ethanol furnished oxadiazole (**1**) in 72% yield (over two steps). For conversion of oxadiazole (**1**) into pyrimidin-5-one (**2**)**,** the required bis(1,3,5-trichloro phenyl) malonate reagent was prepared in 85% yield using a literature protocol by treating malonic acid with 2,4,6-trichlorophenol in phosphorous oxychloride. Oxadiazole (**1**) on reaction with bis (1,3,5-trichloro phenyl) malonate in chlorobenzene under reflux provided 2-(4-bromophenyl)-7-hydroxy-5H-[1,3,4]oxadiazolo[3,2-a]pyrimidin-5-one (**2**) in 92% yield.[31] The pyrimidin-5-one **2** was subjected to chlorination using phosphorous oxychloride to obtain chloro derivative **3** in 94% yield, which served as a synthetic precursor for the preparation of titled derivatives.

We wanted to evaluate the feasibility of ring opening of chloro derivative **3** by a reactive secondary amine like morpholine by screening different bases (triethyl amine, DIPEA, and DBU) in combination with different solvent systems at different temperature conditions. In ethanolic solvent system, room temperature reaction with TEA (Table 1,

entry 1) and reflux condition with DIPEA (entry 2) gave poor yields (entry 1-2). DCM solvent system with DBU provided moderate yield (entry 3), whereas in DMF solvent system, room temperature reaction with K_2CO_3 (entry 4) gave good yields in a relatively shorter time (2 h).

Based on optimized conditions (Table 1, entry 4) for ring opening reaction of chloro derivative (**3**) with morpholine (**4a**), similar reaction was attempted with different amines (**4b**-**k**) (Table 2).

In addition, ring opening of chloro derivative (**3**) was attempted with excess amines (**4a**-**k**) in DMSO at 90 ^{o}C for 16 h to get 2, 4-dialkyl amino 6-oxopyrimidin-1(6H)-yl) benzamide derivatives (**6a-k**) (Table 3).

BIOLOGICAL ACTIVITY

Antibacterial Activity

The antibacterial activity of compounds **5a-i** and **6a-i** were assessed and compared against *Streptomycin* using agar well diffusion method.[32] The antibacterial activity was assayed by measuring the diameter of the inhibition zone. About 1 mg ml^{-1} of the test compounds (**5a-k & 6a-k**) and 1 mg ml^{-1} of *Streptomycin* (control) were dispensed into the wells. The plates were incubated at 37 ℃ for 24 h. The sensitivity of the test organisms to the compounds (**5a-k** & **6a-k**) was determined by measuring the diameters of the zone of inhibition surrounding the wells. The diameters of the zones of inhibition were measured with a ruler and indicated in Table – 4.

Cytotoxicity Assay

The synthesized compounds were tested for cytotoxicity by measuring their effect on the percentage viability of two different cell lines, adenocarcinomic human alveolar basal epithelial cells (A549) and human lung cancer cells (H460) by applying the XTT reagent.[33] Compounds were tested over a range of concentrations from 1mM to 0.051 μM, and the calculated IC_{50} values i.e. the concentration (μM) of compounds able to cause 50% of cell death with respect to the control culture, are reported in **Table 5**. Result shows that few compounds inhibited the growth of human cancer cell lines with IC_{50} values in the micromolar range. Compound **5g** with longer and bulky side chain showed significant activity (IC_{50} 0.4µM) against A549 cell line, lipophilic group containing **5i** and **5k** showed moderate inhibition against A549 cell line, while **5k**, **5g** & **5i** showed moderate activity in H460 cell line compared to puromycin. Compounds with apolar and shorter side chains were less active (IC_{50} about 10 fold higher).

From the Cytotoxic Activity results of 2-alkylamino 6-oxopyrimidin-1(6H)-yl-benzamide derivatives (**5**) on Human Lung carcinoma Cell Sublines, it was observed that the better results were shown by **5g** and **5i**. Thus the compounds **5g** and **5i** were assayed for cytotoxic activity against non tumoral mammalian cell lines MDCK and Vero cells. Compound **5g** has shown IC_{50} value of 29.4 µM on MDCK and 38.3 µM on Vero cells when compared with standard Puromycin having IC_{50} value of 0.29 µM for MDCK and 0.57 µM for Vero cells, whereas **5i** did not show any cytotoxicity on both the tested cell lines. The results were analyzed using the Graph Pad Prism4 program (Figure 1).

The A549 and H460 cell lines were maintained in Ham's F12k and RPMI Medium supplemented with 10% FBS and 1% antibiotics (100 U/mL penicillin, 100 µg/mL streptomycin) respectively. The cells were grown at 37 ℃ in a humidified incubator with 5% CO_2. Cells were sub-cultured by treating with cell dissociation buffer. Both cell lines were seeded in a 96-well microtitre plate at a concentration of 7500 cells per well. Cells were allowed to attach for 24 h at 37 ℃, 5% CO_2. The cells were exposed to the test compounds and positive drug control, Puromycin (SIGMA). The microtitre plate was incubated for further 72 h and thereafter cytotoxicity was measured using the XTT (Invitrogen) reagent (1mg/ml XTT and 7.7µg/ml Phenazine methosulfate in PBS). The plates were incubated for 2 to 4 h and absorbance was read at 450 nm using a Spectra Max Multi-Mode Microplate Reader (Molecular Devices). The results were analysed using the Graph Pad Prism4 program.

Experimental Section:

General experimental details: Analytical grade solvents and commercially available reagents were used without further purification. The column chromatography was carried over silica gel (60-120 mesh), purchased from Sisco Research Laboratories Pvt Ltd. Melting points were determined in open capillaries in electrical melting point apparatus and are uncorrected. ^{1}H NMR and ^{13}CNMR spectra were recorded on 400 MHz Varian spectrometer in DMSO-d_6 or $CDCl_3$ using tetramethylsilane (TMS) as an internal standard. Chemical shifts are given in δ relative to TMS, the coupling constants are given in Hz. IR spectra in KBr disk were recorded from 4000 to 400 cm^{-1} on Avatar 330 FT-IR spectrometer equipped with DTGS detector. Mass spectra were recorded using Agilent

1100 MSD spectrometer in electro spray mode. The starting compound **1** was prepared by previously reported direct cyclization of 4-bromobenzohydrazide and Cyanogen bromide

General procedure for the preparation of 5- substituted 2-(4-bromophenyl)-7-chloro-5H-[1,3,4]oxadiazolo[3,2-a] pyrimidin-5-ol (5a–i). The mixture of 2-(4-bromophenyl)-7-chloro-5H-[1,3,4]oxadiazolo[3,2-a]pyrimidin-5-one **3** (0.5 g, 1.53 mmol), potassium carbonate (0.63 g, 4.59 mmol) and the appropriate amine **4** (1.53 mmol) in dimethylformamide (5 mL) was stirred at room temperature for 2-16 h. The completion of the reaction was indicated by TLC and the solvent was evaporated and residue was poured into water, precipitate formed was filtered off, washed with water and then purified by flash column chromatography using hexane and ethyl acetate to give the corresponding oxadiazolo[3,2-a]pyrimidin-5-ol derivative (**5**).

General procedure for the preparation of 5,7-disubstituted 2-(4-bromophenyl)-5H-[1,3,4]oxadiazolo[3,2-a]pyrimidin-5-ol (6a–k). The mixture of 2-(4-bromophenyl)-7-chloro-5H-[1,3,4]oxadiazolo[3,2-a]pyrimidin-5-one **3** (0.5 g, 1.53 mmol) and the appropriate Amine (**4)** (7.65 mmol) in dimethylsulfoxide (5 mL) was stirred at 90 °C for 16 h. The completion of the reaction was indicated by TLC and the reaction mixture was poured into water, precipitate formed was filtered off, washed with water and then purified by flash column chromatography using Hexane and Ethyl acetate to give the corresponding oxadiazolo[3,2-a]pyrimidin-5-ol derivative (**6)**.

CONCLUSION

In conclusion, we have synthesized the 2-alkylamino 6-oxopyrimidin-1(6H)-yl) benzamide (**5a-k**) and 2, 4-dialkyl amino 6-oxopyrimidin-1(6H)-yl) benzamide derivatives (**6a-k**) from key intermediate **3**. The compounds **5a-k** and **6a-k** exhibited very good anti-bacterial activity against both Gram positive and Gram negative bacteria in comparison to Streptomycin, a first line antibiotic drug (Table 4). Compounds **5g**, **5i** and **5k** showed potent cytotoxicity activity. The structure and biological activity relationship of title compounds showed that the presence of Pyrimidone nuclei and amino guanidine skeleton, as well as biologically active Aminoethyl cyclohexane, morpholine, piperidine, 3-aminopyrrolidine, Dimethyl amine, 2,2,2-trifluoroethylamine, groups attached to the pyrimidone ring are responsible for good antibacterial activity and cytotoxic activity. Based on these results, new compounds are being synthesized by keeping pyridone nuclei with suitable substituents for their cytotoxicity activity which will be reported in due course.

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34. For **5K** and **6K**: A solution of Boc compound in 1,4-dioxane (10 vol) was added 4N HCl in dioxane (5 vol) at room temperature under N_2 atmosphere and the reaction mixture was stirred at room temperature for 16 h. Solvent was evaporated under reduced pressure and triturated with diethyl ether to afford corresponding amines in quantitative yields.

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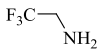
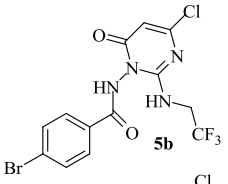
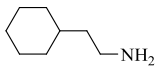
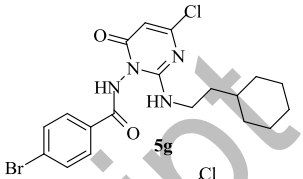
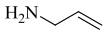
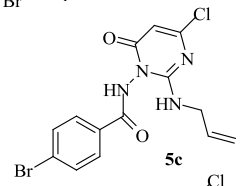
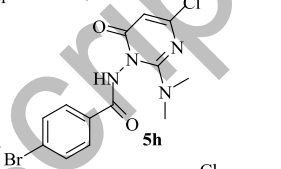
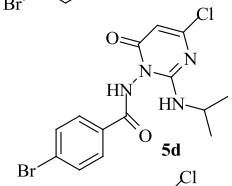
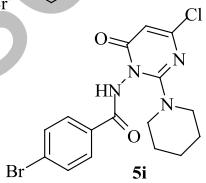
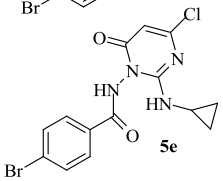
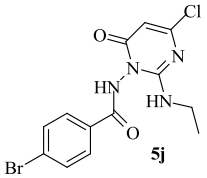
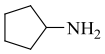
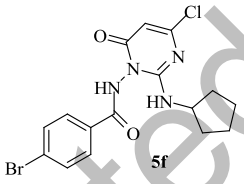
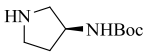
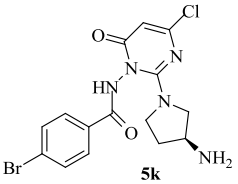
Table 1. Optimization of ring opening[a] reaction

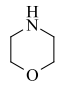
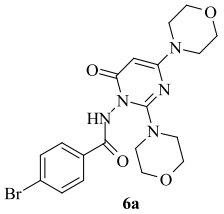
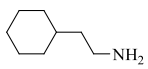
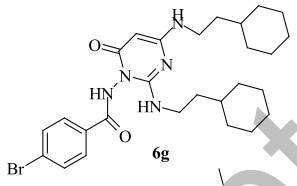
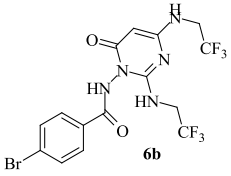
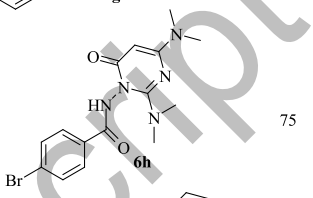
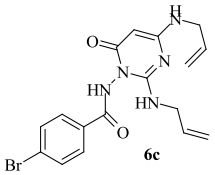
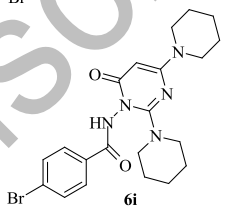
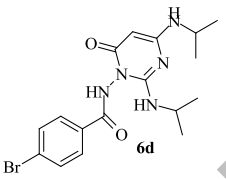
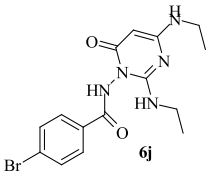
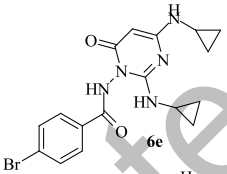
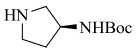
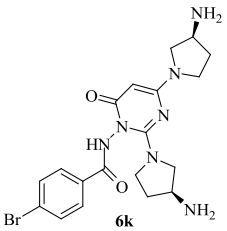
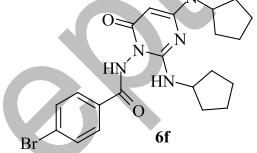
Entry	Base	Solvent	Time	Temp (^{o}C)	Yields[b] (%)
1	TEA	EtOH	20 h, 16 h[c]	RT	10
2	DIEA	EtOH	16 h	Reflux	22
3	DBU	DCM	16 h	RT	47.2
4	K_2CO_3	DMF	2 h	RT	78

Table 2. Synthesis of 2-alkylamino 6-oxopyrimidin-1(6H)-yl-benzamide derivatives using Scheme-2.

Entry	Amine	Product	Yield (%)[a]	Entry	Amine	Product	Yield (%)[a]
1	 **4b**	 **5b**	76	6	 **4g**	 **5g**	81
2	 **4c**	 **5c**	71	7	 **4h**	 **5h**	76
3	 **4d**	 **5d**	72	8	 **4i**	 **5i**	78
4	 **4e**	 **5e**	82	9	 **4j**	 **5j**	79
5	 **4f**	 **5f**	74	10	 **4k**	 **5k**	85[b]

a. Yields are calculated after purification by column chromatography. b. see ref 34

Table 3. Synthesis of 2, 4-dialkyl amino 6-oxopyrimidin-1(6H)-yl) benzamide derivatives (6)

Entry	Amine	Product	Yield (%)[a]	Entry	Amine	Product	Yield (%)[a]
1	 **4a**	 **6a**	77	7	 **4g**	 **6g**	62
2	 **4b**	 **6b**	76	8	 **4h**	 **6h**	75
3	 **4c**	 **6c**	70	9	 **4i**	 **6i**	73
4	 **4d**	 **6d**	69	10	 **4j**	 **6j**	73
5	 **4e**	 **6e**	82	11	 **4k**	 **6k**	83[b]
6	 **4f**	 **6f**	79				

a. Yields are calculated after purification by column chromatography. b. see ref 34

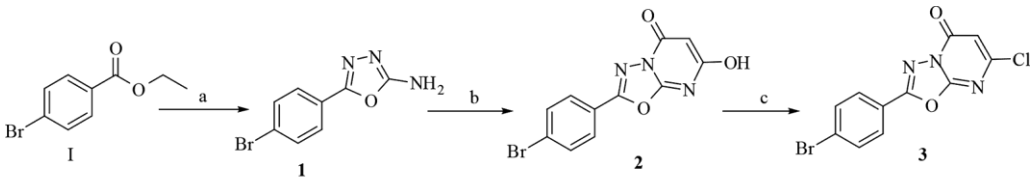
Table 4. Antimicrobial activity of the synthesized compounds 5a-i & 6a-i.c

	Diameter of inhibition (mm)					
Compound	Escherichia coli	Proteus vulgaris	Salmonella paratyphi	Klebsiella pneumonia	Staphylococcus aureus	Bacillus cereus
5a	7	2	5	4	3	6
5b	3	8	7	14	8	14
5c	2	4	6	11	6	12
5d	2	5	5	8	4	10
5e	2	3	3	2	6	8
5f	2	4	4	2	6	8
5g	2	3	2	2	4	2
5h	3	2	2	10	3	5
5i	5	7	4	6	2	6
5j	1	2	2	12	1	10
5k	7	2	15	13	7	10
6a	3	7	9	10	7	10
6b	2	2	1	4	3	5
6c	1	4	3	5	3	5
6d	1	2	2	4	4	8
6e	5	4	3	10	4	8
6f	4	7	4	6	3	10
6g	2	5	4	7	4	10
6h	5	3	2	12	3	9
6i	2	6	4	10	4	11
6j	2	5	2	9	2	7
6k	8	2	5	10	5	6
Streptomycin (1 mg /1	6	8	10	20	10	22

Table 5. Cytotoxic Activity of 5a-i & 6a-i Against Human Lung carcinoma Cell Sublines.

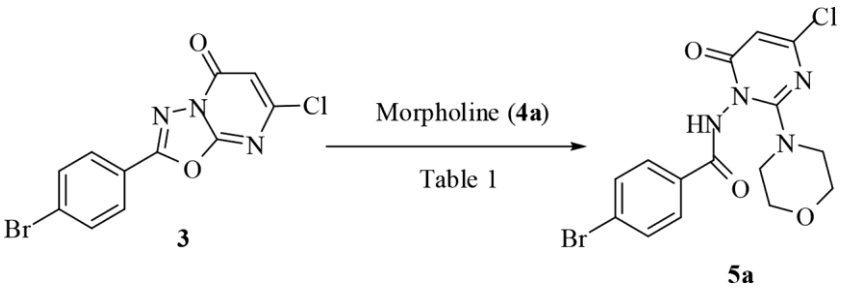
Compound	**A549**	**H460**	**Compound**	**A549**	**H460**
5a	>100	91	**6a**	>100	94
5b	88	93	**6b**	95	>100
5c	>100	>100	**6c**	>100	>100
5d	>100	>100	**6d**	>100	>100
5e	84	91	**6e**	88	92
5f	>100	94	**6f**	>100	95
5g	0.4	23	**6g**	84	88
5h	>100	>100	**6h**	>100	>100
5i	82	>100	**6i**	92	>100
5j	>100	>100	**6j**	>100	>100
5k	14	15	**6k**	86	94
Puromycin	0.387	0.281			

Scheme 1. Synthesis of key intermediate **3**



Reagents and conditions: (a) (i) $NH_2NH_2.H_2O$, Ethanol; (ii) Cyanogen bromide Ethanol 72% (over two steps); (b) Bis(1,3,5-trichlorophenyl)malonate, Chlorobenzene, 140 °C, 2 h, 92%; (c) $POCl_3$, reflux, 5 h, 94%.

Scheme-2. Synthesis of 2-alkylamino 6-oxopyrimidin-1(6H)-yl) benzamide derivative (**5a**)



Scheme 3: Synthesis of 2, 4-dialkyl amino 6-oxopyrimidin-1(6H)-yl) benzamide derivatives (**6a-k**).

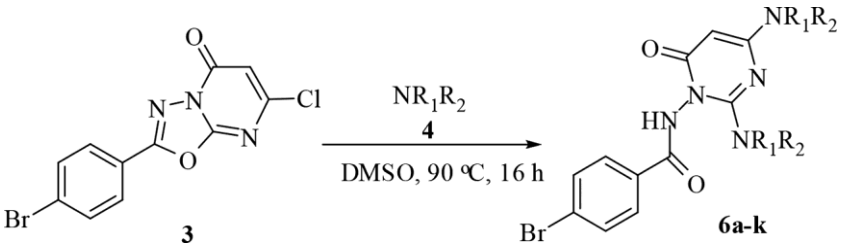
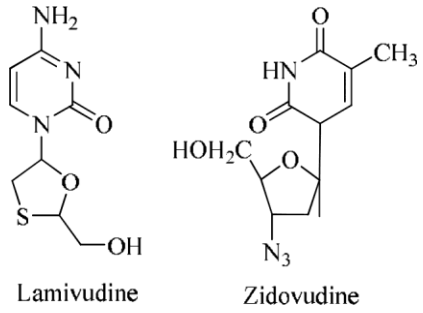


Figure 1. Structures of Lamivudine and Zidovudine



Lamivudine Zidovudine

Figure 2. Biologically active pyrimidone based drugs

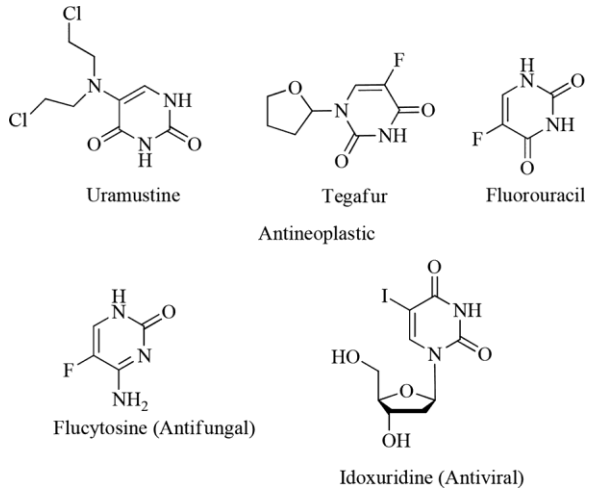


Figure 3: A & **B:** Inhibition of Compounds **5g**, **5h**, **5i** and **5k** against adenocarcinomin human alveolar basal epithelial cells (A549 and H460 cells) at 37 °C and 5 % CO_2. **C** & **D:** Inhibition of Compounds **5g** and **5i** against non tumoral mammalian cell lines (MDCK and Vero cells) at 37 °C and 5 % CO_2.