Evaluation of anticancer and antimicrobial activities of novel dihydropyrimidinone derivatives

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Abstract

Dihydropyrimidinone (DHPM) can be synthesized through a simple one-pot Biginelli reaction. Off-late medicinal properties of DHPM have been discovered. In our recent work, we have shown that the florescent property can be appended to this molecule while retaining its therapeutic attributes. DHPM derivatives have been substituted with different alkylating groups like ethyl-, mono-, di-, and tri- ethanol amines. This current paper discusses the multifunctionality of DHPM derivatives in form of anticancer and antimicrobial agent. DHPM derivatives exhibit improved inhibitory effects in *in vitro* anticancer assay. The synthesized DHPM derivatives are tested for their anti-cancer effects on A549 cell lines, as well as their anti-bacterial and anti-fungal effects on clinically significant pathogens like *Staphylococcus aureus*, Methicillin Resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, Colistin Resistant *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*. These discoveries reveal good opportunity to develop multifunctional anticancer medicine based on DHPM. The synthesis and the presence of radical moieties in the DHPM derivatives have been supported by chemical studies.

Keywords: Dihydropyrimidinone derivatives; anti-cancer; antibacterial; antifungal; IC50

Introduction:

Lung cancer is the leading cause of cancer related deaths with more than 1.38 million deaths worldwide.[1] Non-small-cell lung cancer (NSCLC) belongs to heterogeneous class of tumours and a frequently diagnosed cancer representing approximately 85% of all lung cancer diagnoses.[2, 3] Chemotherapy and radiotherapy are considered beneficial for patients with metastatic cancer and introduction of angiogenesis inhibitors, epidermal growth factor receptor inhibitors, and other new anticancer agents results in increased number of lung cancer survivors.[4] These are the traditional treatment process for most of the cancer ailments. Chemotherapy causes bacterial and fungal infections due to compromised immunity because of neutropenia.[5-7] Prophylaxis using common agents against bacteria and fungi often lacks effectivity due to emergence of resistant species.[8] Antimicrobial resistance arises as a global threat estimated to cause 10 million deaths in 2050.[9] Investigation and development of new drug molecules are in demand to counteract multi-drug resistant organisms. A drug with low toxicity and specific to cancerous cell could retain the immunity of the body at larger scale.

Dihydropyrimidinone (DHPM) derivatives discovered by Biginelli reaction is characterized by its multi-functionalized scaffold exhibiting various biological activities such as inhibition of calcium channels, anti-microbial, anti-viral, anti-oxidant and anti-cancer activity.[10] In our previous study, we synthesized dihydropyrimidinones (DHPMs) from benzyl alcohol by Biginelli reaction in a one-pot system which had shown robust anti-cancer activity.[11] Earlier reports showed that ethyl substituent groups are effectively increased the potency of the DHPM molecule by acting as calcium channel blocker.[4] Several withdrawal substituent groups (Cl^-, F^-, NO_2^- etc.) have been already used with Biginelli adducts like monastrol, piperastrol etc. However, studies related to the amine substituent of DHPM molecules amine are rare.[12] The amine analogs are utilized in

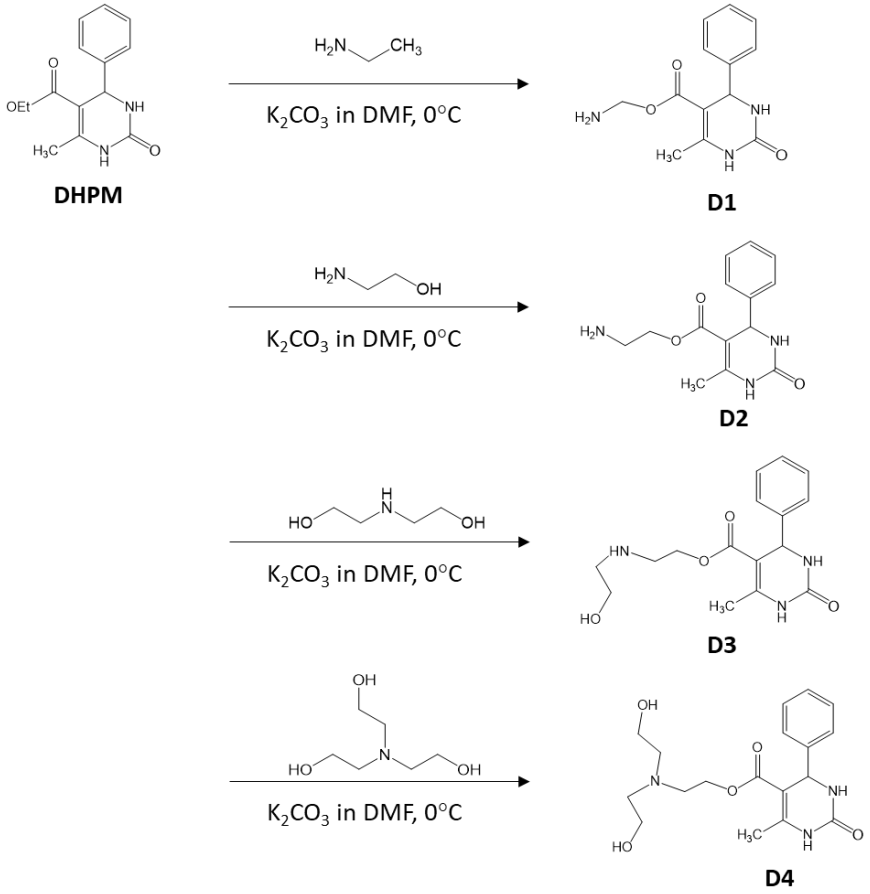
various applications as an alkylating agent in biological fields.[13] Amines are used as specific agents with their nucleophilic nature which are capable of interacting and forming covalent bonds with DNA molecules and proteins leading to the disruption of cellular functions.[14] Chemotherapy during treatment causes bacterial and fungal infections due to compromised immunity. Antimicrobial resistance emerges as a global threat estimated to cause 10 million deaths in 2050.[4] Investigation and development of new drug molecules are in demand to counteract multi-drug resistant organisms. A drug with low toxicity and specific to cancerous cell could retain the immunity of the body at larger scale.

In this study we have synthesized ethylamine, ethanolamine, diethanolamine, and triethanolamine substituent DHPM derivatives and explored their therapeutic potentials. To this end, we screened our DHPM derivatives to evaluate anti-cancer activities against A549 cell lines along with anti-bacterial and anti-fungal activities against clinically important pathogens such as *Staphylococcus aureus,* Methicillin Resistant *Staphylococcus aureus* (MRSA), *Escherichia coli,* Colistin Resistant *Escherichia coli* and *Pseudomonas aeruginosa, Candida albicans* and *Aspergillus niger*.

Methodology:

Preparation of Dihydropyrimidinone derivatives

Synthesis and optmization of DHPM through oe-pot Biginelli reaction have been discussed in our previous reports.[11, 15] Here, we prepared several amine derivatives of DHPM by substituting ethyl oxo- with ethylamine, ethanolamine, diethanolamine and triethanolamine. The synthesized pure DHPM compound has been substituted with various amine groups such as ethylamine(D1),



Scheme 1: Synthetic route of various amine derivatives from dihydropyrimidinone with chemical structures.

ethanolamine(D2), diethanolamine(D3) and triethanolamine(D4) used for the preparation of DHPM derivatives. In brief, 0.175 g of DHPM were dissolved in 5 ml of DMF, then 0.5 ml of the amine groups were added as ethylamine, ethanolamine, diethanolamine, triethanolamine and 700 mg of K_2CO_3 were added together. Then the mixture was poured into ice and same amount of water, referred in Scheme 1. Immediately a white precipitate forms but the stirring is continued

for 20 minutes to complete the reaction. Then the precipitate was filtered and washed in a little water, then dried well. The precipitate was recrystallized from ethanol solution.

Characterization tools

UV-Vis spectra are recorded with a Shimadzu UV-1800 spectrophotometer (scan range 200-800 nm) using ethanol as solvent. Fourier Transform Infra-Red (FTIR) spectra are recorded using Shimadzu IR affinity series 1S in the region of 4500-500 cm^{-1} in KBr pallet. Nuclear Magnetic Resonance (NMR) spectroscopy was carried out by a Bruker BioSpin GmbH model using deuterochloroform as solvent of molecules.

Cell culture

A549 cells were grown in complete DMEM with 10% foetal bovine serum (FBS), 1% L-Glutamine, 1% Antibiotic-antimycotic solution. The cells were grown aseptically in tissue culture flasks (T 25 cm^2) at 37℃ with 5% CO_2. PBMCs from healthy donors were isolated by density gradient centrifugation using Ficoll-Paque overlay method, and grown using medium comprised of RPMI-1640, 10% heat inactivated filtered foetal bovine serum (FBS), 1% Streptomycin and Penicillin solution and incubated in humidified 5% CO_2 incubator at 37 ℃.

Cytotoxicity assay

The *in vitro* cytotoxicity of DHPM derivatives using MTT assay was carried out in 96-well, flat bottomed microtiter plates. A volume of 200µL of 5×10^3cells were seeded in wells and incubated for 24 hrs. On the following day cells were treated with drug concentration ranging from 100µM to 0.78 µM for 24hrs. After incubation, 10-15µL of 5mg/mL 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenylterazolium bromide (MTT) was added and then the plates were further incubated for 3 hours at 37 ℃in the dark. Media was carefully aspirated and100 µL of DMSO was added to each well and absorbance was read at 590 nm by using Varioskan Flash multimode microtiter plate

reader. IC50 values were determined by non-linear regression analysis. Experiments were performed in triplicates and Standard Deviation was calculated.

The *in vitro* cytotoxicity of DHPM derivatives on freshly isolated PBMCs from healthy blood donors, was determined by seeding 1×10^5 cells per well and treating with different concentrations (in µM) (25, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500 and 550) of the derivative compound at 37 ℃ with 5% CO_2 for 72 hrs to determine the maximum cytotoxic effect. After incubation, MTT assay was performed and absorbance was read at 590nm. The mean percentage of post treatment viable cells was calculated in MS Excel.

***In vitro* antibacterial screening**

Antibacterial activity of DHPM derivatives were tested against two gram-positive bacteria: *Staphylococcus aureus* and Methicillin Resistant *Staphylococcus aureus* (MRSA) and three gram-negative bacteria: *Escherichia coli,* Colistin-resistant *Escherichia coli* (a patient isolate found to be colistin resistant with MIC value of 8 µg/ml) and *Pseudomonas aeruginosa.* The Minimum Inhibitory Concentrations (MIC) of the compounds were tested against organisms using micro broth dilution method.[16] Initially, the isolates were grown overnight at 37 ℃ in nutrient agar plates. The bacterial suspension was prepared from overnight culture and adjusted to a turbidity of 0.5 McFarland value. The final desired inoculate size was 5×10^5 CFU/mL. The test compounds were two-fold serially diluted from highest to lowest concentration (1000µg/mL to 7.8 µg/mL for the derivatives). In a 96-well plate, the drugs were added in an order of lowest to highest concentrations with the final inoculum size (5×10^5 CFU/mL diluted in Muller Hinton broth) and the plates were incubated at 37 ℃ for 24 hrs. Gentamycin was used as a standard antibacterial drug (positive control) and the drug solvent was used as the negative control. Uninoculated broth

was used as sterility control and the inoculated broth without antibiotic was used as growth control for the quality assessment.

***In vitro* antifungal screening**

The DHPM derivatives were screened for their antifungal activity against *Candida albicans* and *Aspergillus niger* respectively. The antifungal activity was tested using well diffusion method in 2% Muller Hinton agar plates. The fungal cultures were grown and maintained in sterile sabouraud dextrose agar medium. The turbidity of fungal suspension was adjusted to 2.2 McFarland standards and uniformly swabbed in the agar petridishes, to which 25µL of the synthesized compounds were added to each well at different concentrations (250µg/mL, 500µg/mL and 1000µg/mL). The solvent used in the preparation of the drug was used as the negative control and itraconazole (10µg/mL) was used as the positive control. The plates were then incubated at 26 ℃ for 72 hrs and the zone of inhibition was measured.

Results:

UV-Visible and FTIR spectral analysis

The absorption peak at 284 nm for 3, 4-dihydropyrimidinones (DHPM) were observed in the UV-Visible spectroscopy. The spectra (**Figure 1a**) of the products in ethanol showed characteristic absorption band λ_{max} at 284 nm due to n→π* transition of C=O group of such compounds.[11] The various amine group substituted DHPMs showing the absorbance peak ataround 286 nm. Due to covalent attachment of nucleophile amine groups to DHPM side-chain consists of C=O, the molecular energy of the derivative molecules decreses. This has been displayed by slight red-shift of the absorption peak. When the amine group chains are longer, the corresponding absorption peaks are sharper than their shorter amine group counterparts. This indicates the heavier molecules have lesser molecular energies. Less molecular enrgy makes molecules more stable. Though the

role of molecular energy towards the medicinal effect of a molecule has not been totally identified but it is likely that a molecule with lower energy can be less interactive.

In the FTIR spectra of these molecules show that the peaks corresponding to amine groups (1700, 3200 cm^{-1} etc.) strengthen than in DHPM due to presence of more amine groups in the derivative molecules (**Figure 1b**). The FTIR spectra of pure DHPM compound and various amine groups substituted DHPM displayed two absorption bands for -NH at first and third position in the range 3270-2970 cm^{-1}. The ester carbonyl stretching frequency was observed in the range of 1600-1750 cm^{-1}. Absorption band for -C=O was observed in the range of 1420-1600 cm^{-1}. The C-N bond in dihydropyrimidinone ring showed absorption band in the range of 1150-1350 cm^{-1}. Compounds were showed absorption band for C-O-C stretch at 1296 cm^{-1} respectively. These results demonstrate amine group attachment to the DHPM molecule.

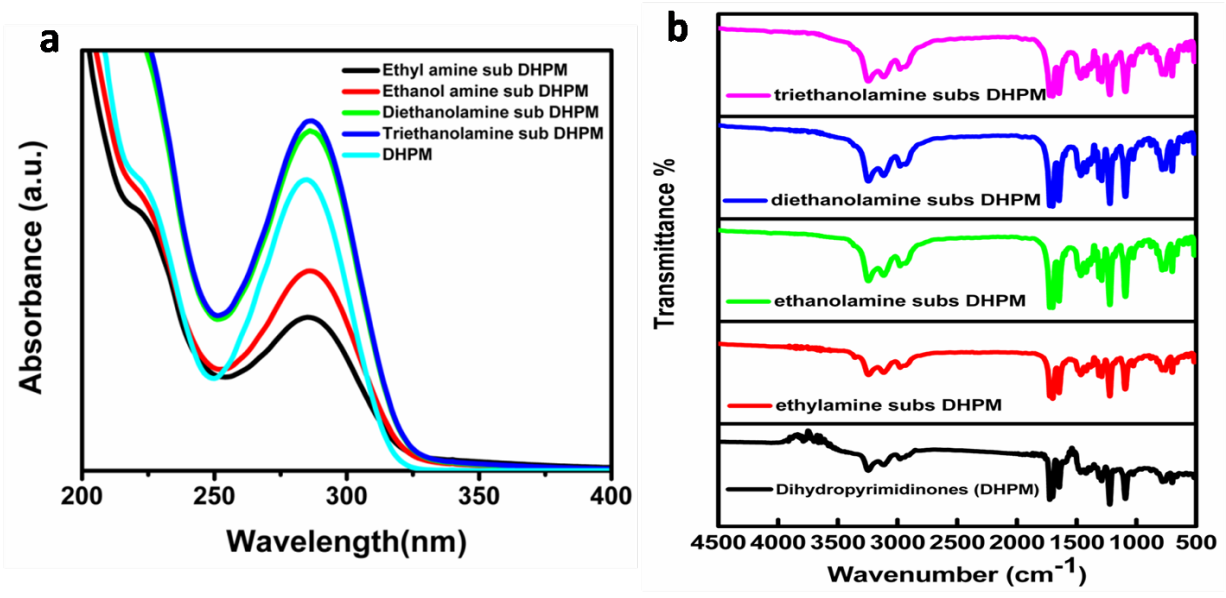


Figure 1: a) UV-Visible spectroscopic analysis of various amine groups substituted DHPM derivatives b) FT-IR spectroscopic analysis for DHPM and various amine groups substituted DHPM derivatives.

NMR analysis

However, neither UV-Vis absorption spectra nor the FTIR spectra can clarify the chemical structure of a unknown compound. To establish the chemical structure of the resultant molecules NMR study has been carried out. The NMR spectra of DHPM derivatives have been mentioned in **Figure 2(a-d)**. Detailed NMR analysis of DHPM derivatives has been given in **Table SI 1-SI 4**. Around 4.08 ppm and 1.17 ppm, respectively, the triple of three protons and the quartet of two protons are observed. Both of them possessed an ethyl group presence as indicated by a coupling constant (J) of 7.2 Hz. The isolated ethyl group is detected by the singlet of three hydrogen atoms at a value of 2.20 ppm. Multiplets with two protons are indicative of an aromatic system and may represent the pyrimidine aldehyde at 8.00 ppm and 7.19 ppm. Around 7.25 ppm, a multiplet of three protons is seen, once more indicating aromaticity. The benzylic H peak and the two amide hydrogen peaks combine to form the peak for 7 H atoms at 7.85 ppm. At 3.97 ppm, a singlet with six protons indicated the existence of the two CH_3 groups that make up the diethylamino moiety, while a singlet with three protons at 2.2 ppm indicated the presence of an isolated ethyl group. Each peak of the 7.25 ppm aromatic protons contains two hydrogen atoms. It should be noticed that there are minor peaks between only 8 ppm and 7.92 ppm, where there should be likely evidence of singlets composed of just one H-proton. For structural elucidation, NMR spectroscopy is used to examine the products in **Figure 2(a-d)**. Chemical structures of D1, D2, D3 and D4 have been confirmed with these NMR spectral analysis.

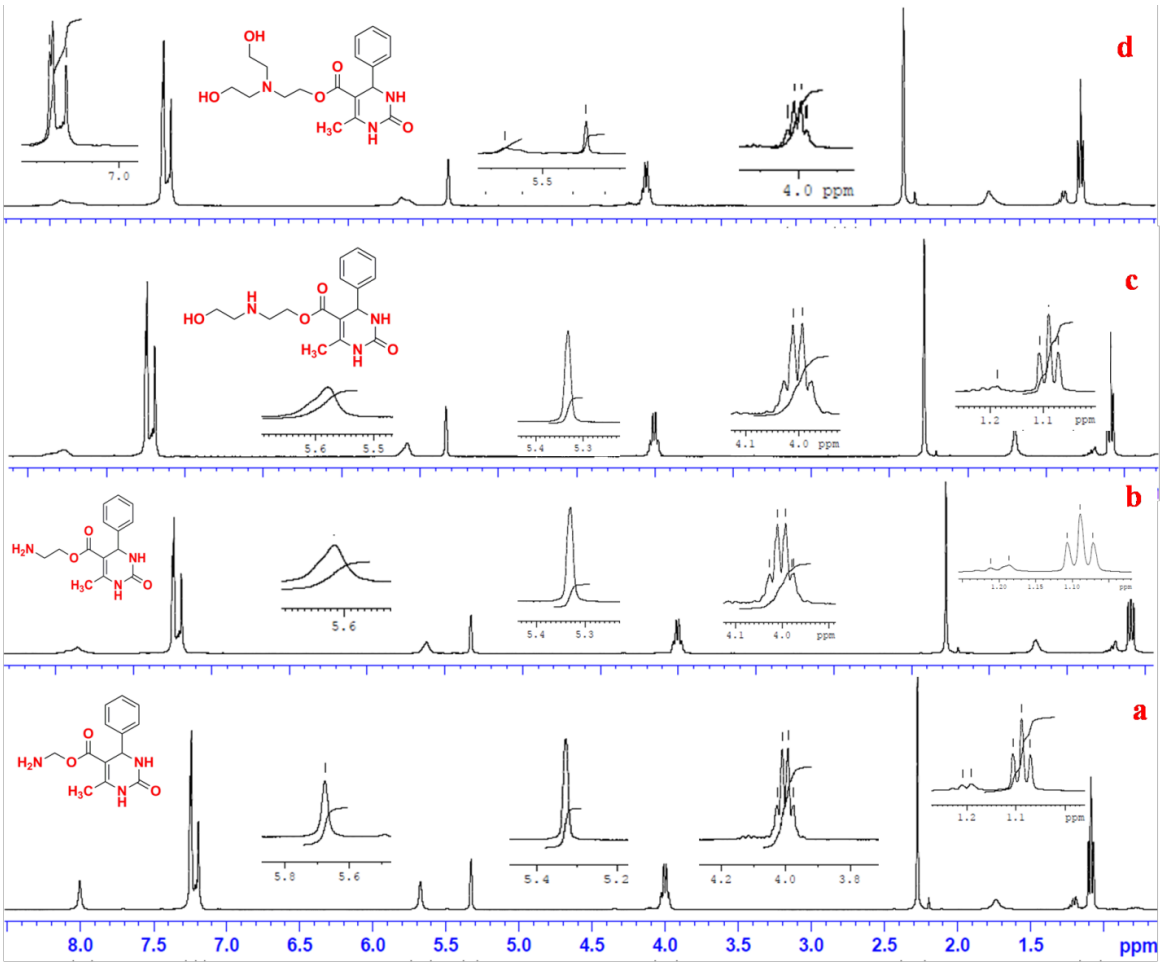


Figure 2: a) ^{1}H NMR analysis of ethylamine substituted dihydropyrimidinones b) ethanolamine substituted dihydropyrimidinones c) diethanolamine substituted dihydropyrimidinones d) triethanolamine substituted dihydropyrimidinones.

Cytotoxic activity:

Four different DHPM derivatives were tested ranging from highest (100µM) to lowest (0.78µM) concentrations in two-fold serial dilutions to assess cytotoxic activity. The compounds exhibited a dose dependent cytotoxicity against A549 cell-line. The DHPM derivatives synthesized along with the four different subgroups exhibited higher cytotoxic effect with notably less IC50 concentrations (almost 40-fold lesser than pure DHPM), the lowest IC50 being for D2 at 3.60 µM.

To establish the optimal treatment concentration for the drug, its IC50 values were calculated and compared (**Table 1**).

Table 1: Levels of cytotoxicity exhibited by the DHPM compounds against A549 cell line. IC50 is the concentration required to inhibit 50% of the cell growth.

S.No.	DHPM Compounds	Cytotoxicity (IC50 in µM)
1	D1 (ethylamine)	5.39
2	D2 (ethanolamine)	3.60
3	D3 (diethanolamine)	5.22
4	D4 (triethanolamine)	6.26
5	DHPM	202.60

Test compounds activity were dose dependent. PBMCs viability was found to remain more than 70% even after 72 hrs of drug treatment with highest drug concentrations (in µM) (25, 50, 100, 200, 300, 400, 500 and 550) as shown in **Figure 3**. The percentage of growth inhibition of the drugs against both the A549 cell line and PBMCs were compared at 100µM concentration and represented in **Table 2**. These results show the specificity of DHPM compounds with cancer cells.

Table 2: Comparison of percentage of growth inhibition of DHPM compounds against A549 and PBMCs at 100µM concentration. A549 -Non-Small Cell Lung Carcinoma (NSCLC), PBMCs-Peripheral Blood Mononuclear Cells.

S. No	DHPM Compounds	Percentage of growth inhibition (%)	
		A549	PBMCs
1	D1 (ethylamine)	95.63	35.23
2	D2 (ethanolamine)	99.54	42.98
3	D3 (diethanolamine)	95.65	36.68
4	D4 (triethanolamine)	96.71	33.37
5	DHPM	42.6	26.42

A lower IC50 value indicates a lesser amount of drug is required to be administered to exterminate poisonous cells whereas a higher viability percentage over a longer period means that the drug has diminutive detrimental effect towards healthy cells. Earlier DHPM derivatives exhibited IC50 values against MRC-5 cells as 44.16 and 32.04µM.[17] Venugopala *et al*., study reported DHPM

derivatives exhibited up to 20% growth inhibition at 50µg/mL.[18] In previously published paper, at 10µM, most of the DHPM derivatives exhibited less than 50% growth inhibition against A549 cell line.[19] The Lithium-Acetate-Mediated Biginelli compounds required mostly more than 100µM to exert 50% Cytotoxic activity against the A549 cell line.[20] In all these studies the IC50 values are greater than we achieved in the current work.

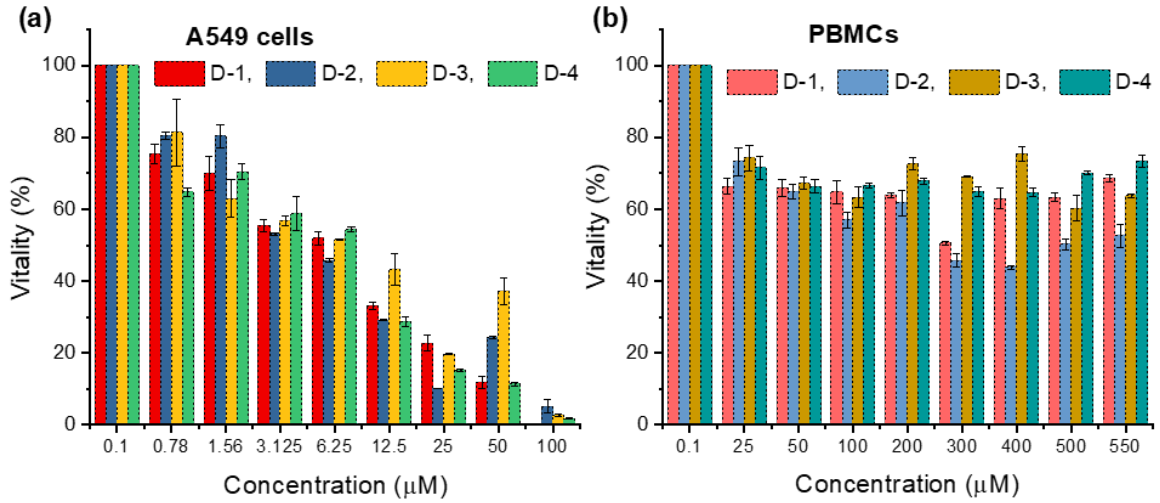


Figure 3: (a)Dose dependent cytotoxicity of DHPM derivatives against A549 cell lines. (b) Dose dependent cytotoxicity of DHPM derivatives against PBMC cell lines.

Antibacterial activity:

The DHPM derivatives were tested for their antibacterial activity against *Staphylococcus aureus*, Methicillin--resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, Colistin Resistance *Escherichia coli* (CREC) and *Pseudomonas aeruginosa*. The results for antibacterial testing of the compounds are reported as minimum inhibitory concentration (MIC) (**Table 3**). 'MIC' is the lowest quantity of the antibacterial agent which can completely impede growth of the concerned bacteria within an overnight. Thus, a lower value of MIC is better since a less amount of the antibiotic could be used to kill the bacteria and stop bacterial infection. The gram-positive and

gram-negative bacteria have been chosen not only due to they are common but also, they have long history of infections in humankind. As for example, *Staphylococcus aureus* or Staph can cause a range of diseases starting from minor skin infections to deadly pneumonia, meningitis, endocarditis etc. Staph is one of the most five reasons for hospital-related-infections like wound infections or post-surgery infections. MRSA is a more lethal variant of *S. aureus* since it is resistant to common beta-lactam class of antibiotic - which makes them to severity of the infections or sepsis. *Psuedomonas aeruginosa* is an opportunistic pathogen infects mostly immune-compromised individuals. However, treatment of *P. aeruginosa* is tricky due to its natural resistance to common antibiotics. On the other hand, though *Escherichia coli* is known as mostly benign and non-pathogenic but CREC becomes a threat since it is resistant to polymyxin drugs which are the only commercially available antibiotic to treat multi-drug resistant gram-negative bacteria pathogens.

Our DHPM derivates showed excellent activity against *Psuedomonas aeruginosa* with MIC values at 62.5 µg/mL when compared to other organisms. The results showed that all the compounds exhibited a moderate to potent antibacterial activity. The activity of the compounds was also compared with the standard positive control gentamycin. Gentamycin belongs to aminoglycoside antibiotic class and is a well-known antibiotic used for several different bacterial infections; it has inhibitory effect against a broad band of bacteria including both gram-positive and gram-negative strains.

Table 3: Antibacterial screening of the DHPM derivatives compounds**.** (*S. aureus* -*Staphylococcus aureus,* MRSA – Methicillin-resistant *Staphylococcus aureus, E. coli-Escherichia coli,* CREC - Colistin-resistant *Escherichia coli, P. aeruginosa - Pseudomonas aeruginosa*).

S.No.	DHPM Compounds	Minimum Inhibitory Concentration (µg/mL)				
		Gram positive organisms		Gram negative organisms		
		S.aureus	**MRSA**	***E.coli***	**CREC**	***P.aeruginosa***
1	D1	500	500	250	250	62.5
2	D2	250	250	125	250	62.5

3	D3	250	250	125	125	62.5
4	D4	250	250	125	125	62.5
5	Gentamycin	25	25	25	25	25

Antifungal activity:

The DHPM derivatives were tested for their antifungal activity against clinical pathogens *Candida albicans* and *Aspergillus niger*. Though *Candida albicans* live with human as friendly parasite for long, but it become pathogenic to individuals with poor health condition. Along with other Candida species, *C. albicans* is responsible for 50-90% of all candidiasis in human beings with a mortality rate 40% of the candidiasis globally only due to *C. albicans*.[21, 22] Aspergillosis is kind of fungal infection in lungs and often spreads through blood circulation to other internal organs. This disease is caused by *Aspergillus niger* in immune-compromised patients or patients who has gone through lung surgery or organ transplantation. If not diagnosed in time, Aspergillosis becomes fatal specifically to those who has other lethal disease like AIDS, cancer etc. Chronic pulmonary Aspergillosis one of the prevalent strains affecting around 3 million people worldwide per year.[23]

The antifungal competency of the DHPM derivatives have been estimated using Zone of Inhibition (ZOI) test. The ZOI test is also known as Kirby-Bauer test or Agar Diffusion test; it is measured by the radius of almost circular region around the spot of the antifungal agent where the target fungus can not sprout. DHPM derivatives exhibited moderate antifungal activities at the concentration of 1000µg/mL. However, it has been found that the DHPM derivatives have better antifungal efficacy than DHPM as similar ZOI achieved with lower concentration of DHPM derivatives.[15] The antifungal ZOI test results of DHPM derivatives along with a control drug, itraconazole, have been demonstrated in **Table 4**. Itraconazole belong to azole molecular group and used for treatment of wide variety of fungal infections including aspergillosis, candidiasis etc.

However, for the current study itraconazole concentration is taken as 10 µg/mL. It can be seen that D1, DHPM substituted with ethylamine, and D3, DHPM substituted with diethanolamine, are the only two compounds have antifungal activity against both *Candida albicans* and *Aspergillus niger* like itraconazole. D1 has ZOI of 6 mm and 9 mm where ZOI for D3 are 8mm and 11mm respectively against *Candida albicans* and *Aspergillus niger*. The other compounds e.g., ethanol substituted DHPM and triethanolamine substituted DHPM have antifungal activities specific to either *Candida albicans* or *Aspergillus niger*.

Table 4: Antifungal screening of CQD-DHPM nanocomposites and DHPM derivatives compounds.

S.No.	**DHPM Compounds [1000 µg/mL]**	**Zone of inhibition (mm)**	
		Candida albicans	***Aspergillus niger***
1	D1 (ethylamine)	6	9
2	D2 (ethanolamine)	12	-
3	D3 (diethanolamine)	8	11
4	D4 (triethanolamine)	-	10
5	Itraconazole [10 µg/mL]	38	20

Discussion:

In this study, we conducted an *in vitro* examination of cytotoxicity induced by four DHPM derivatives with different subgroups (ethylamine, ethanolamine, diethanolamine and triethanolamine) against A549 cell lines. Our results indicate that the drugs induce a dose dependent cell death. The cell viability decreased gradually as the concentration has been increased. DHPM analogues were non-toxic to PBMCs compared their activity on A549 cells. In the published work of Mostafa *et. al.*, the DHPM derivatives exhibited lower toxicity against MRC-5 cells at IC50 value of 44.16 and 32.04µM.[10] But the toxicity of our novel synthesized DHPM derivatives against PBMCs remained low even at the half maximal inhibitory concentration (<30%) as more than 70% of cells remained viable after the treatment of 72 hrs. In the research work published by Venugopala *et al*., the DHPM derivatives exhibited over 80% of

cell growth inhibition with IC50 value around 6 to 35µM.[18] The monastrol mimic Biginelli DHPM derivatives exhibited cytotoxicity against HepG2 with half maximal inhibitory concentration of 120.62µg/mL and it exhibited weak toxicity towards HeLa cell lines with IC50 200µg/mL.[24, 25] Interestingly, our newly synthesized DHPM derivatives with four new subgroups found to have potent activity against A549 cells with their IC50 values less than 10µM. Even at the higher concentrations, the compounds exhibited very low toxic effect on normal cells (PBMCs).

The amine group in the side chain of a molecule can have a number of bio-chemical effects. It can a) provide a site for hydrogen bonding to other molecules, such as DNA or proteins,[26] b) functioning as a chelating agent, though binding to metal ions that promote the proliferation of cancer cells,[27] c) undergoing oxidation process to form a reactive aldehyde group, which can damage DNA molecules in the target cells,[28] and d) be metabolized to form molecules with anticancer activity.[29] The ability of the amine groups to form hydrogen bond is particularly significant. Despite the fact that hydrogen bonds are weak by nature, they play an important role in the interactions that can occur between molecules with containing polar groups. Hydrogen bonds can work in two ways, i) helping the molecule to persist in a stable state in solution, making it more likely to reach its target and ii) help the molecule to bind to other molecules, such as DNA or proteins, which could inhibit the growth of cancer cells. In addition, the amine group has the capacity to function as a chelating agent. Chelation is the process of forming a complex between a metal ion and a molecule that contains multiple electron-donor groups. It has been already shown that metal ions play a role in promoting the growth of cancer cells.[30] The amine group in the side chain of a molecule can bind to these metal ions and preventing them from interacting with cancer cells, which will inhibit the growth of the cancer cells. Furthermore, the amine group in the

side chain of a molecule can be oxidized to form a reactive aldehyde group. These highly reactive aldehyde groups can damage DNA, leading to the cell death.[31] The oxidation of the amine group can be catalyzed by enzymes in cancer cells, making this a particularly effective mechanism for killing cancer cells. However, the roles of amines for anticancer activity have not been fully understood yet. Thus, further studies should be carried out in order to elucidate the molecular pathways in NSCLC cancer cell line death induced by DHPM compound.

The DHPM derivatives with the addition of four different subgroups (ethylamine, ethanolamine, diethanolamine and triethanolamine) all exerted a potent antibacterial activity against Pseudomonas aeruginosa. In the article published by Bhalgat and Ramesh, 2014, the DHPM derivatives treated against the *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* required mostly higher than 750µg/mL – 1000µg/mL.[12] Fluorine Containing Pyrazole-clubbed Dihydropyrimidinones tested against MRSA exhibited inhibitory activity with the maximum MIC of more than 50µg/mL.[32] Based on the antifungal results obtained, we have concluded that the DHPM compounds with different substitutions alone had antifungal activity and it also shows that substitution of ethyl and amino groups to the DHPM compound enhances its activity overall. The third derivative with diethanolamine substitution shown to have good to moderate activity against *Candida albicans* and *Aspergillus niger.*

The specific mechanism of action of a molecule with an amine group in its side chain will depend on the structure of the molecule and the type of microbes being targeted.[33, 34] However, in general, the amine group can contribute to the molecule's ability to inhibit microbial growth and replication. We would like to discuss some examples of molecules with amine groups in their side chains that have been shown to have antimicrobial activity. Chloramphenicol binds to the 23S ribosomal subunit of bacteria.[35] This prevents the binding of aminoacyl-tRNA to the ribosome,

which is essential for protein synthesis. Tetracycline binds to the 30S ribosomal subunit of bacteria.[36] This prevents the binding of mRNA to the ribosome, which is also essential for protein synthesis. Nystatin binds to the sterols in the cell membranes of yeast and fungi.[34, 37] This disrupts the membranes and allows for the leakage of essential cellular components, leading to cell death. It is important to note that not all molecules with amine groups in their side chains have antimicrobial activity. The structure of the molecule and the type of microbe being targeted are important factors that determine whether or not a molecule will have antimicrobial activity. The research on the role of amine groups in antimicrobial activity is ongoing.[38] Scientists are working to identify new molecules with amine groups that have potent antimicrobial activity. Dihydropyrimidinone can be one of these molecules – which could have multifaceted feature to fight against cancers.

Conclusion

In this study, we have found that amine group substitutions to dihydropyrimidinone have improved the growth inhibition against A549 cell line with respect to their precursors. Also, we have noticed that these substitutes are more specific only to cancer cells even at lower concentration as minimum as IC50 value 3.6 μM for D2. On the other hand, at the higher concentrations, the compounds exhibited very low toxic effect on normal cells (PBMCs), more than 60% vitality after 72 hrs incubation.

In addition to these, the substituted DHPM molecules have shown interesting antimicrobial activities particularly against *Pseudomonas aeruginosa* and *Candida albicans*. Both of these microbes have significant infective roles when human immunity is compromised.

However, there is a variance of activity, both anticancer and antimicrobial, for different substituted molecules. From our viewpoint it seems D2 i.e. ethanolamine substituted DHPM is more effective

than its other counterparts. In all these substituted molecules the secondary amine group conformation has been varied. Though we are not sure, but that may be reason of different efficacies. Further studies are in order to elucidate the molecular pathways in NSCLC cancer cell line death as well as antimicrobial activities induced by DHPM compound. To summarize the novel synthesized DHPM and its derivatives might be useful as a potential antibacterial, antifungal, and anti-cancer agent in future.

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Supplementary Information

Supplementary Information of the article would be available from the journal website.

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