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Anti-quorum Sensing, Oxidative Stress and Anti-Biofilm Inducing Abilities of Vitamin K3 Analogues Against Chronic Respiratory Infection of Mucoid Variants *Pseudomonas aeruginosa* Strain PAO1

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A B S T R A C T

Almost all antibiotics target either bacterial function or biofilms but do not consider the damage due to host responses, thus, even if the bacteria are eliminated, the tissue damage cannot be prevented. The mucoid phenotype and oxidative stress severely limit the efficacy of current therapeutics for chronic respiratory infections (CRI) of *Pseudomonas aeruginosa* in cystic fibrosis (CF) patients. In the present study, we examined the activity of a novel set of VitaminK_3 derivatives on biofilms of the mucoid variants of *P.aeruginosa*. Acyl-homoserine lactones (AHLs) an important signaling molecules in the quorum sensing gene regulatory processes found in numerous gram-negative species of bacteria were evaluated. Further, the radical scavenging ability and antibiofilm activities were studied. The compounds were also screened for cytotoxic activity by the MTT assay on HeLa cell lines. This work highlights the aggravation of the CF lung caused due to biofilm formation, and exacerbation occurring due to oxidative stress, which is a host response. This study thus presents VitaminK_3 derivatives as antimicrobial compounds which also prevent oxidative stress and thus serve a dual function. Consequently, further development and use of these compounds is likely to complement current CF therapeutics.

Introduction

Pseudomonas aeruginosa is a Gram-negative bacterium responsible for several opportunistic infections in humans, such as the chronic respiratory infection (CRI) in cystic fibrosis (CF). *Pseudomanas aeruginosa* is capable of forming matrix-encased multicellular communities called biofilms. Resistance to antibiotics and environmental stress is often attributed to biofilm formation. It has also been observed that *P.aeruginosa* biofilms are 1000-fold more antibiotic tolerant than planktonic cells to single and combination antibiotics (Nickel *et al.,* 1985; Ceri *et al.,* 1999; Aaron *et al.,* 2002). A typical outcome of CRI is the switch from a classical

non-mucoid phenotype to an atypical alginate-overproducing mucoid phenotype (Hoiby *et al.,* 2001; Hoffman *et al.,* 2007). The ability of *P.aeruginosa* to adapt and grow as a mucoid biofilm is considered as a survival strategy and an important aspect of the pathogenesis in the CF lung disease (Hoffman *et al.,* 2007). This mucoid character also serves to protect the bacterium against antibiotics and immune responses (Hoffman *et al.,* 2007).

There is considerable lung tissue damage in CF owing to immune complex mediated inflammation dominated by the release of oxygen radicals by polymorphonuclear neutrophils (PMNs) (Hoiby *et al.,* 2001; Hoffman *et al.,* 2007; Dosanjh, 2008). Superoxide radicals are the major products of these reactions (Dosanjh, 2008). The hazardous effects of oxygen radicals such as superoxide include: fatty acid oxidation, oxidative inactivation of proteins, lipid peroxidation and DNA damage. Some studies suggest that oxygen radicals thus released are also responsible for inducing mutations in the *mucA* gene, leading to the mucoid phenotype (Mathee *et al.,* 1999; Hoffman *et al.,* 2007).

The extracellular matrix of *P.aeruginosa* biofilms includes extracellular DNA (Whitchurch *et al.,* 2002; Allesen-Holm *et al.,* 2006). Genomic DNA has been shown to localize in the biofilms, surrounding the mushroom shaped microcolonies (Allesen-Holm *et al.,* 2006). DNA in the biofilm matrix is also likely released by dead bacterial cells or by host immune system cells. The possible roles of this DNA as a polymeric substance, a nutrient and a gene transporter have been considered (Finkel *et al.,* 2001). It also aids in bacterial adhesion to biotic and abiotic surfaces and promotes surface aggregation (Das *et al.,* 2010). It has been demonstrated that this DNA is essential for initial establishment of the biofilm (Whitchurch *et al.,* 2002) and it also functions to maintain the 3D biofilm architecture by acting as a cell-cell interconnecting compound (Whitchurch *et al.,* 2002). Once a *P.aeruginosa* infection is established in the CF lung, its treatment by current protocols and by conventional therapeutics is extremely difficult (Leichtzin *et al.,* 2006; Merlo *et al.,* 2007). One of the current treatment protocols includes antibiotic treatment, which is switched to a rotation of various antibacterial agents but ultimately fails due to decreased lung function via bacterial consumption (Adonizio, 2008). The bacterial intercellular communication mechanism defined as Quorum sensing (QS), has emerged as an attractive target for novel anti-infective agents as against the use of antibiotics. QS inhibitory compounds possess the ability to inhibit bacterial pathogenicity. Phytochemicals due to the myriad of secondary metabolites such as phenolics, quinones, flavonoids, alkaloids, terpenoids, and polyacetylenes are been screened for its effective QS inhibitory activity (Asfour, 2018). Vitamin K_3 analogues such as Plumbagin (5-hydroxy,2-methyl–1,4 naphthoquinone) and shikonin (5,8-dihydroxy-2-(1-hydroxy-4-methyl-pent-3-enyl)-1,4-naphthoquinone) have been known for their antimicrobial activity against a variety of bacteria pathogenic to plants, such as *Erwinia ssp., Agrobacterium ssp.* (Travis S. Walker *et al.,* 2005) as various other strains pathogenic to animals such as *Streptococcus ssp*. In the present studies VitaminK_3 analogues are derived from parent compound Vitamin K_3 (menadione) by substitution on the quinonoidal scaffold, at positions C1 and C4. The properties of interest of these compounds include redox cycling; their highly conjugated planar ring structure enables them to exhibit properties such as curing, intercalation, fluorescence etc. Furthermore, their amphipathic nature suggests their potential as future drugs, which has been evaluated in the present investigation.

Materials and Methods

Media preparation, *Pseudomonas aeruginosa* strains and Vitamin K_3 analogues

The PAO1 laboratory strain of Pseudomonas was used in this study, which was kindly provided by the Microbial Biotechnology Lab, IBB, University of Pune. For inoculating these cultures into the Microtitre Plates (MTP) and further studies, they were grown overnight on shaker at 120 rpm in Luria Bertanni-broth (0.5gm Yeast extract, 1gm Caesine enzyme Tryptone type I,1gm Sodium chloride in 100ml distilled water & 7.2 pH) This ensured proper aeration of the culture. Vitamin K_3 derivatives and their metal complexes with 0.5mM stock solutions of each compound in 1% DMSO (Dimethyl sulphoxide) were prepared.

Determination of Minimum Inhibitory concentration (MIC) and Sub-Inhibitory concentration (SIC) of the given compounds on an overnight culture of *P.aeruginosa*

MIC and SIC were performed as previously described (CMI, 9, 2003). MICs of the Vitamin K_3 derivatives were determined using the broth microdilution method.

Briefly, the test compounds was diluted with LB medium (Sigma) by serial dilutions. The final concentration of bacterial cultures was about 10^5 cfu/ml. After incubation at 37°C for 24 h, the absorbance at 620 nm was measured using a microplate reader (Thermo Electron Corporation) to assess the cell growth. The blank readings of each compound were subtracted from the test readings and a graph of concentration vs. absorbance was plotted and the MIC value for each compound was determined from this graph. The value preceding the MIC was the SIC i.e. Sub-Inhibitory concentration value was used for further studies.

Biofilm Assay

Static biofilm formation was carried out in 96-well microtiter plates. Briefly, overnight grown cultures were diluted to about $1x10^6$ cfu/ml with fresh sterile medium and each well of microtiter plates was filled with 100 µl aliquots of the diluted cultures. The plates were incubated at 37°C for 24 h without shaking for biofilm growth, and non-adherent bacteria were washed by 0.9% (w/v) NaCl for three times. After incubation, the culture was aspirated out of each well. The wells were given a wash with 200µl 1X PBS. Plate was air dried for 15min and 200µl crystal violet was added in each well and kept for 15 min. The stain was discarded and 200µl 95% ethanol was added in each well. Absorbance was measured at 570nm using an ELISA Plate reader (Thermo electron corporation).

Addition of compounds in the biofilm plates

The MIC concentrations obtained for each of the compounds were added in the biofilm plates along with 200µl mixture of culture + medium. The biofilm was then allowed to form at 37°C for 24 hrs and the biofilm assay was done according to the above described method. The readings thus obtained were compared with the control biofilm readings (Without the compounds).

Preparation of Samples for the Superoxide dismutase Assay

One of the objectives of this study was to examine the intracellular antioxidant potential of the given compounds on the superoxide anion production, mediated by pyocyanin. For this purpose, aliquots of 1.5ml of culture + 200µl (5 µM) of each compound were prepared and incubated overnight at 37°C. After incubation, the mixtures were homogenized using a sonicator (LABSONIC ® M 230V/50Hz Ultrasonic homogenizer, Sartorius). Aliquot with culture was used as control.

Superoxide Dismutase Activity

Superoxide dismutase activity was measured by the Nitroblue tetrazolium (NBT) method with alkaline DMSO as the radical source (Bhirud and Shrivastav, 1990). Calculation of the percentage inhibition of the Superoxide anions was calculated using the formula: {[control (slope) – test (slope)]/control (slope)} x 100.

Fluorescence Microscopy

Glass slides were cleaned thoroughly with soap and alcohol and made grease free. Biofilms were scraped from the bottom and walls of polystyrene plates (by the process described in method 2.3), and suspended in 0.9% NaCl. This mixture was homogenized using a cyclomixer. 40µl of this solution was transferred on to a slide using a micropipette, to this; 5µl of each compound in its sub-inhibitory concentration was added. Step 5 was repeated for all the compounds and the slide was observed under the 100X objective lens of a Zeiss™ Scope A1 Fluorescence Microscope, by observing their fluorescence under excitation wavelength of 485nm and emission wavelength of 535 nm (green).

Anti-quorum sensing activity

Chromobacterium violaceum was used as the biomonitor organism for this assay. In this bacterium, production of violacein, a purple pigment, is under QS control. Anti-QS compounds inhibit the production of violacein, making this bacterium ideal for screening of anti-QS activity. Standard disk-diffusion assay was used to detect the anti-QS activity of the compounds (Bauer *et al.*, 1966). 10µl of each compound was loaded onto sterile paper disks (6 mm diameter) and placed onto prepared LB plates spread with overnight culture of *C.violaceum*. Plates were incubated at 30ºC overnight. QS inhibition was detected by a ring of colourless but viable cells around the disk. Measurements were made from the outer edge of the disks to the edge of the zones of anti-QS inhibition. Sterile disks loaded with the antibiotic gentamycin and sterile distilled water were used as negative controls.

AHL analysis

Pseudomonas aeruginosa produces two autoinducer signalling molecules i.e. two AHLs viz. N-(3-oxo-dodecanoyl)-L-homoserine lactone (OdDHL) and BHL (Kumar *et al.,* 2022). In this study OdDHL was extracted. Aliquots of overnight cultures and compounds were prepared.

In each aliquot, culture and one of the compounds were mixed in the ratio 9:1. The aliquots were then incubated overnight. For extraction of OdDHLs, the supernatants were used which were obtained by centrifuging the aliquots at 10,000 rpm for 10 minutes at 4ºC on a cooling centrifuge (Kubota, Japan). OdDHL was extracted from culture supernatants using the methods of Makemson *et al.,* (2006).

Extraction of OdDHL

The AHL lactone ring can be opened by alkaline hydrolysis at room temperature or can remain closed and stabilized under mild acidic conditions (Eberhard and Schineller *et al.,* 2000). Therefore, to measure AHLs in bacterial media, aliquots of cultures + compounds were acidified by the addition of glacial acetic acid (20 µl/ml medium).

Each aliquot was extracted with ethyl acetate three times (1:1, v/v), the extracts were combined and evaporated and the remainder was dissolved in methanol. These extracts were stored at 4ºC until further analysis. This method has an extraction efficiency of >99% (Makemson, 2006).

Detection of OdDHLs by TLC

OdDHLs have a polar lactone ring and a hydrophobic 12 carbon chain. TLC detection is based upon UV fluorescence. 2 µl each of the ethyl acetate extracts were spotted onto TLC plates (silica gel 60 F254; Merck). An OdDHL standard (purchased from Sigma-Aldrich) was also included as a reference. After development in methanol-ethyl acetate-hexane (1:2:1) solvent system, the TLC plates were observed for UV detection.

Culture and maintenance of cell lines

The cell lines Glioma were obtained from National Centre for Cell Sciences Repository, University of Pune, Pune. The cells were maintained in RPMI (Roswell Park Memorial Institute) media, with 10 % FBS and 2 % antibiotic solution at 37ºC in the steri-cycle CO_2 incubator (Thermo Electron Corporation) with HEPA Class 100 filters at 5 % CO_2, splitting at 95 % confluency was attained.

Quantification of cell death via MTT assay

To evaluate the ability of carbon dots synthesized by Flow injection method and Hydrothermal method to cell death for cancerous (glioma) cell lines by following protocol. The solution of 5 mg/mL MTT was dissolved in PBS and then the solution gets filter-sterilized using a syringe filter. After incubation for the stipulated time, 20 µL of MTT solution was added to 200 µL of cell content solution.

The plate was incubated for 2 h in the CO_2 incubator at ambient temperature. After incubation, the media was removed and 500 µL of DMSO was added to each well to dissolve the crystals.

The plate was put into the incubator at 37°C for 5 minutes. Measure the absorbance at 540 nm and 620 nm on the plate reader (SYNERGY/ H1 microplate reader) for a period of 24 h.

Statistics

Each experiment was performed in independent triplicates and was repeated three times. The averages and standard deviations were calculated using Microsoft Excel.

Results and Discussion

Quantitative Biofilm assay

The quantitative biofilm assay were performed in the 96 well microtiter plates. Vitamin K3 derivatives and their copper complexes displayed a dose-dependent inhibitory effect on biofilm formation of *P. aeruginosa* (Figure 2) in the presence of the compounds added.

The control bar without addition of any compound shows maximum absorbance at 570 nm. While addition of compounds (C1-C8) there is a considerable decrease in the absorbance indicating significant anti-biofilm properties. The compounds were evaluated for the

biofilm disruption activity in their 1X and 4X MIC concentrations. The 1X MIC concentrations however, yielded unsatisfactory results; hence results obtained after treatments with the compounds at their 4X MIC concentrations are shown in all the proceeding assays.

Superoxide dismutase activity

The compounds (1-8) evaluated for their superoxide scavenging ability by the NBT assay, are compared in Figure 3.

The radicals generated within the experimental set-up could be the reason for its antibiofilm activity. Since Vitamin K3 is known for redox cycling, it was essential to evaluate the radical interaction abilities of compound 1-8.

The compounds (1-8) were found to possess superoxide scavenging ability by the NBT assay. Thus, the compounds are able to combat with the superoxide anions produced by pseudomonas as suggested by Dosanjh (2008).

Fluorescence Microscopy

Vitamin K_3 analogues have a highly conjugated ring structure, having pi bonds which distribute the electrons in the outermost orbitals over a wide area; These electrons, in their ground state, on absorbing incident light energy undergo alterations in their electronic, vibrational and rotational states.

These compounds are optimal for fluorescence microscopy as the energy difference between their excited states and ground state orbitals is small enough that relatively low energy photons in the visible part of the electromagnetic spectrum can be used to excite electrons to the excited state.

Specific sites were seen fluorescing under the microscope immediately after addition of the compounds, but after 10-15min, nothing was seen fluorescing. No fluorescence indicates cell death.

When glass slides were prepared by method 2.6 and immediately observed under the microscope, thick clouds of biofilms were seen as in Figure 4 (a). After 30 min, these clouds started fragmenting, and the fluorescence diminished.

Anti-quorum sensing activity

Chromobacterium violaceum was used as the biomonitor organism for this assay. In this bacterium, production of violacein, a purple pigment, is under quorum sensing (QS) control. Anti-QS compounds inhibit the production of violacein, making this bacterium ideal for screening of anti-QS activity.

From the figure 5.2 (b) formation of a ring of cells still viable but lacking the purple pigment can be seen. In the figures corresponding to the compounds C-2, C-6, C-4, C-8, the zone has both sparsely pigmented cells and non-pigmented cells.

Loss of purple pigment in *C. violaceum* is indicative of QS inhibition by all the VitaminK_3 derivatives. Disks containing the antibiotic gentamicin and distilled water were also included as controls. As expected, a zone of growth inhibition was observed with gentamycin and no inhibition was apparent with distilled water.

The inhibition zones measured are tabulated in Table 2.

AHL analysis

TLC detection under UV light

AHL production was strongly inhibited by treatment with VitaminK_3 derivatives. In Figure 6 (a) a spot corresponding to the OdDHL standard was seen and an OdDHL spot was also seen in the control (b, without sample) which indicates the presence of OdDHL in the control sample. However, no/ or slight OdDHL spots were seen for treated samples C1-C8.

Cytoxicity on human cell lines

HeLa and THP-1 cell lines were taken to evaluate the cytotoxicity of the menadione drivatives. The THP-1 normal leukemic macrophages exhibited no cell death with the compounds (1-8) at concentrations upto 30 µM. However at 25 µM considerable cell death was observed against human cervical cell line (HeLa).

Further the antioxidant status after treatment to the cell lines was evaluated by homogenizing the cell. It was found the radical scavenging abilities was increased inside the cell. While the copper complexes became pro-oxidants on internalization.

Table.1 The following table represents the MIC and SIC values of VitaminK3 derivatives and its copper complexes (C1-C8).

compound	MIC (mM)	SIC(mM)
MSCZ (C1)	0.458	0.381
Cu-MSCZ (C2)	0.229	0.183
MTSC (C3)	0.458	0.381
Cu-MTSC (C4)	0.458	0.381
CLAMG (C5)	0.458	0.381
Cu-CLAMG (C6)	0.458	0.381
CLAH (C7)	0.265	0.229
Cu-CLAH (C 8)	0.19	0.153

Table.2 QS inhibition zone diameters for all the compounds.

Compound	Inhibition zone (mm)
C-7	1.1
C-3	1.2
C-8	1.1
C-4	0.9
C-5	1
C-1	1.2
C-6	1.2
C-2	0.8

Table.3 % of viable cells after incubation with compounds 1-8. The cells were freeze thawed and the supernatant of the cell extracts were evaluated for their radical scavenging ability by SOD assay.

% Cell Viability				%SOD Activity	%SOD Activity
HeLa					
	24hrs	48hrs	72hrs	in cell lines	Neat compd
CLAH	78.45	72.19	67.25	62.58	66.13
CLAMG	62.18	56.84	49.25	58.26	57.25
M-SCZ	55.24	49.87	41.29	63.58	61.87
M-TSC	63.85	57.54	51.32	67.25	66.98
Cu-CLAMG	33.21	24.98	19.68	PROXIDANT	85.21
Cu-CLAH	57.84	43.85	37.21	PROXIDANT	75.25
Cu-MSCZ	38.54	39.57	22.14	PROXIDANT	86.21
Cu-MTSC	41.2	35.7	29.57	PROXIDANT	79.24

Figure.1 Derivatives and their metal complexes of Vitamin K3 derivatives evaluated for their antimicrobial properties. (* The above compounds have been synthesized and characterized in Prof. Sandhya Rane Lab, Savitribai Phule Pune University, Pune 411007)

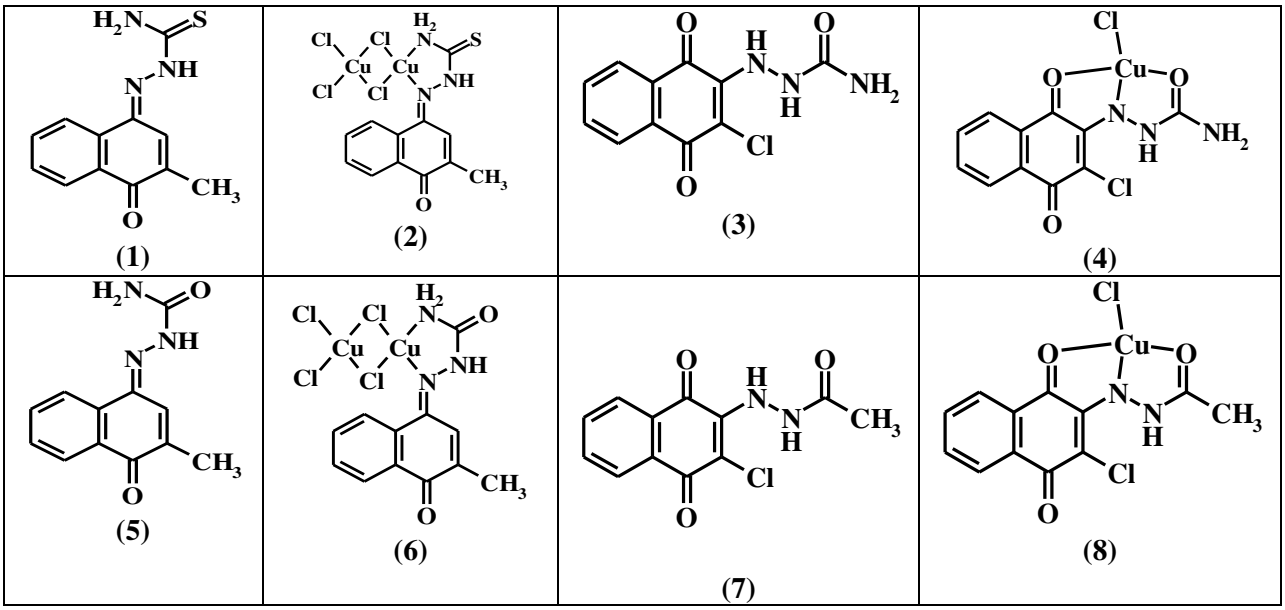


Figure.2 Effect of treatment of VitaminK_3 derivatives on pre-formed biofilms (in AP medium) by *P.aeruginosa*. As can be seen from the figure, the OD of control sample (in which no compounds were added) is around 1.3 and the OD of the treated samples decreases rapidly to values in the range of 0.5-0.7. This activity can be attributed to the biofilm disruption activity of the compounds which causes a rapid decrease in the biofilm biomass.

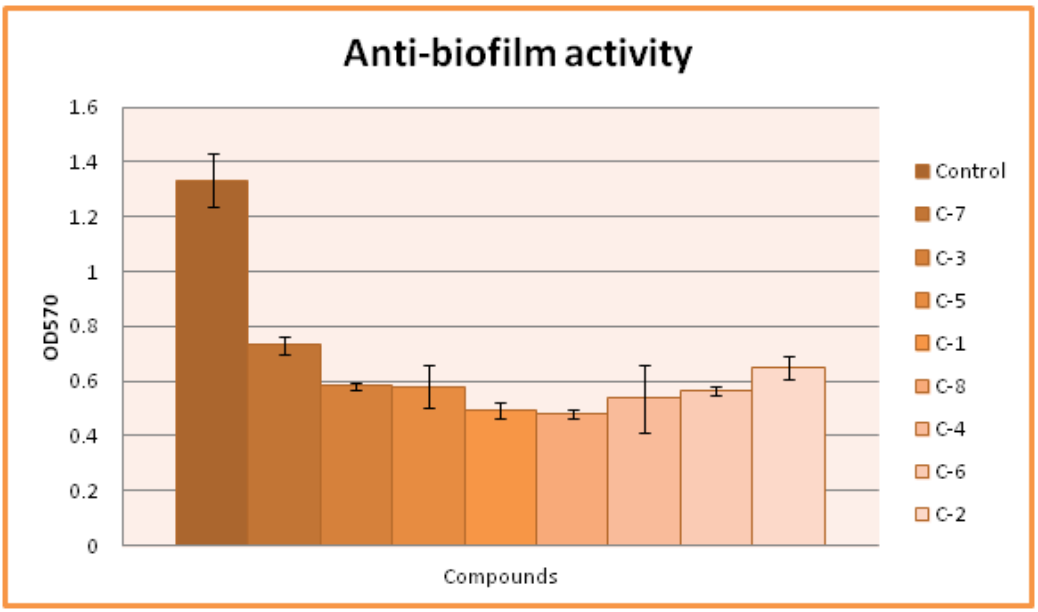


Figure.3 % inhibition values for the compounds. As can be seen from the figure, all the compounds possessed antioxidant activity. The minimum activity is 40%, which is displayed by compound 8.

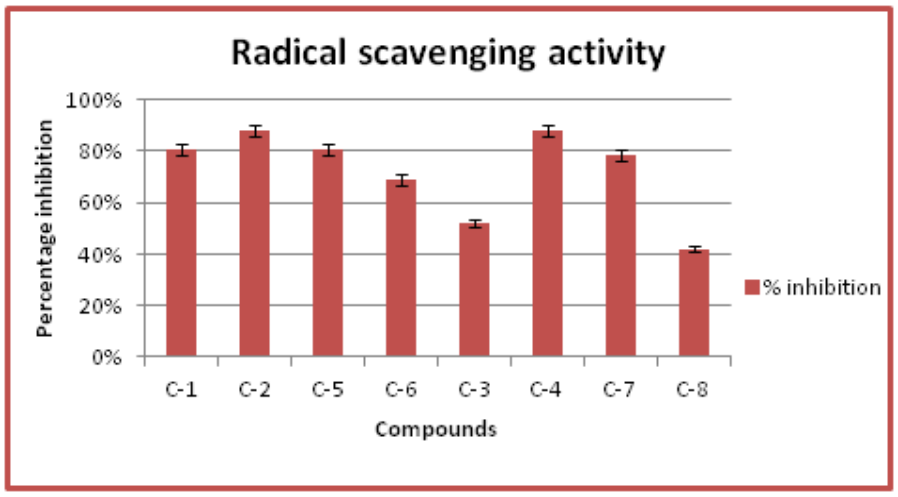


Figure.4(a) Samples viewed immediately after addition of the compounds.

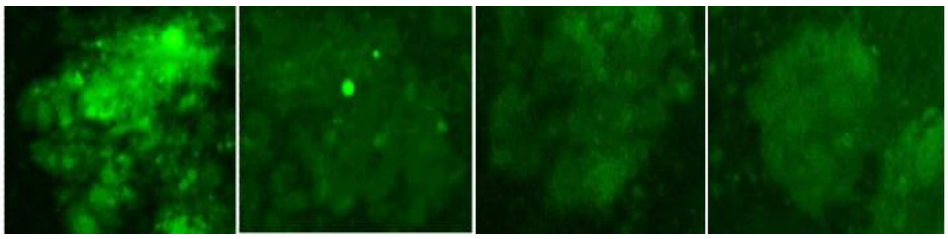


Figure.4(b) Samples viewed 10-15 minutes after addition of the compounds : (clockwise from left) C-1, C-2, C-3, C-4, C-5, C-6, C-7, C-8.

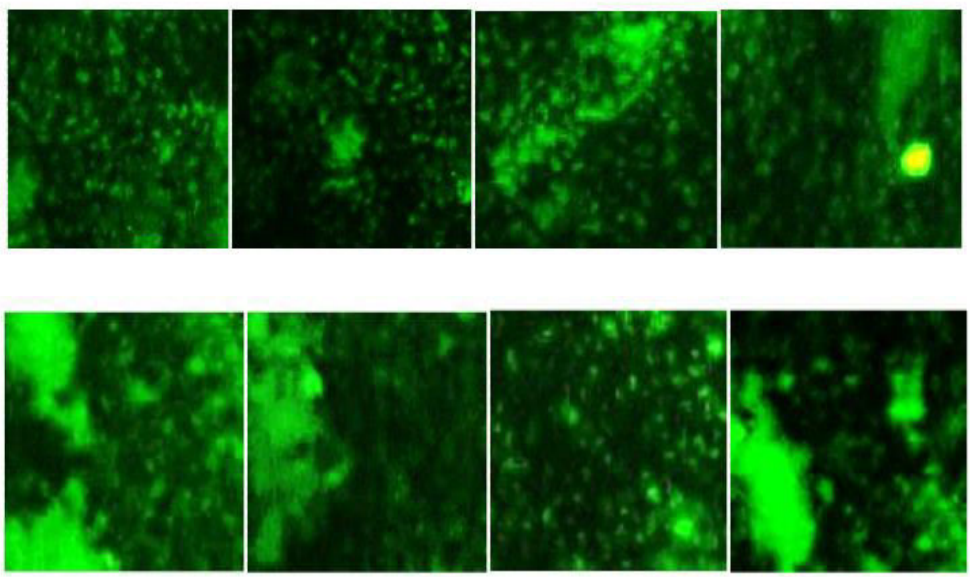


Figure.5(a) Photographs of the disk with the negative controls: distilled water (left) and gentamicin (right).

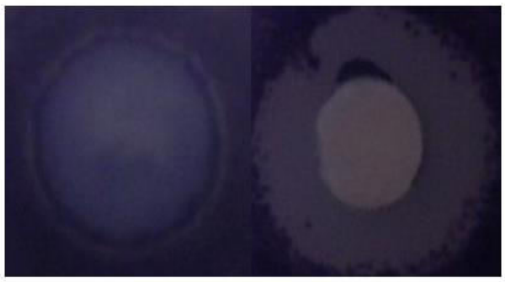


Figure.5(b) Photographs of disks with the compounds. Clockwise from left, C-7, C-3, C-5, C-1, C-2, C-6, C-4, C-8.

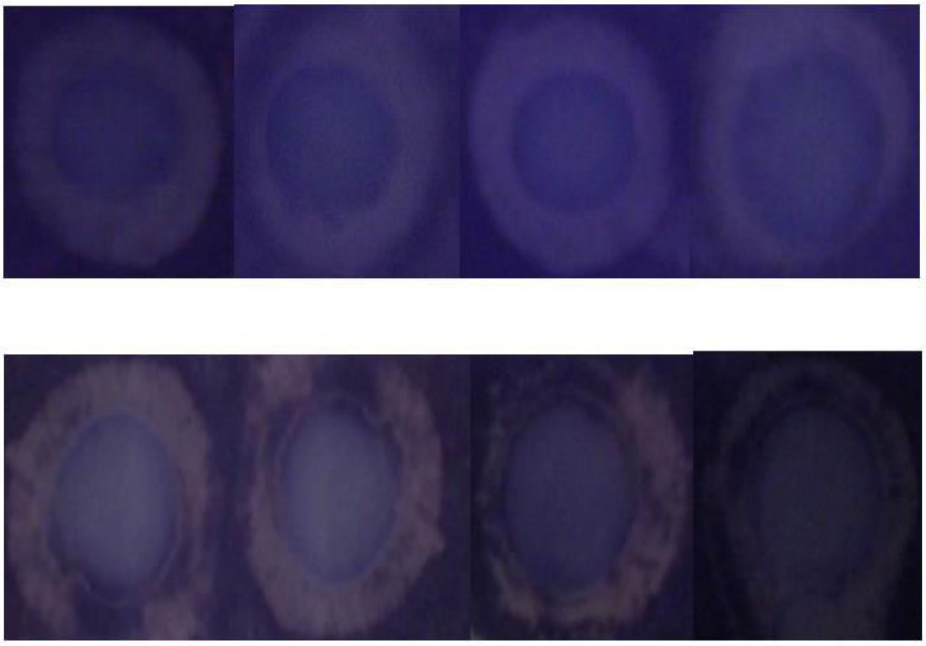
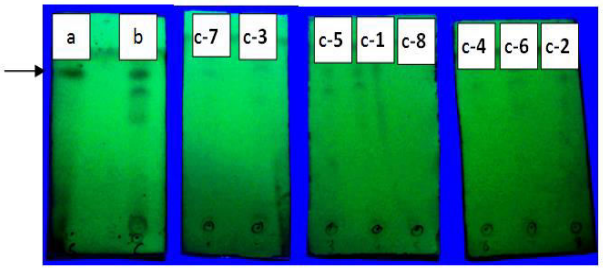


Figure.6 Effect of treatment of VitaminK_3 derivatives on AHL production by *P.aeruginosa*.



Quantitative Structure activity relationship (QSAR) studies need to be carried out to explain the exact mechanism of drug action. The compounds exhibit no cytotoxicity against THP-1 cell lines. Our future work involves a novel drug delivery system/formulation for these compounds.

Removal of *P.aeruginosa* from the respiratory tract of cystic fibrosis (CF) patients by early aggressive antimicrobial therapy might be possible during initial colonization (Tacetti *et al.,* 2005; Ratjen *et al.,* 2010; Riera *et al.,* 2010), but when the infection is fully established, this is no longer possible.

Moreover, the mucoid phenotype hampers the success of antimicrobial treatments and even if antimicrobial treatments are successful, they fail to prevent the lung tissue damage inflicted by the oxidative stress. Thus, the hypothesis of this study was that the selected VitaminK_3derivatives would combat the mucoid biofilms of *P.aeruginosa* and prevent lung tissue damage due to oxidative stress. Certain intriguing physico-chemical properties of VitaminK_3 derivatives such as green fluorescence, redox cycling, DNA intercalation (Fuji *et al.,* 1992) and plasmid curing (Lakshmi *et al.,* 1996) activities led to the hypothesis of the current study.

We first evaluated the biofilm bactericidal activity of the compounds and obtained that all the compounds were effective antibiofilm agents at their 4X MIC concentrations. This is evident from the data shown in Figure 2 where the absorbance at 570nm of biofilms (Pitts *et al.,* 2003; Molobela *et al.,* 2010) is represented. Increasing the concentrations from 4X to 16X resulted in increased clearance of the biofilms (data not shown). Further, fluorescence microscopy confirmed the antibiofilm activity as changes were observed in the morphologies of the bacterial communities after 24hrs of treatment (Figure 4). The control biofilms illustrated in Figure 4 (a) show confluent microcolonies while the microcolonies in the treated biofilms were not confluent and their sizes and numbers had decreased considerably. Imbibition of the compounds by the biofilm is observed as green fluorescent patches are observed within the acridine orange-stained biofilm cells.

The green fluorescence observed cannot be attributed to acridine orange as acridine orange emits green fluorescence when excited at 502 nm. Hence the spectrophotometric as well as the microscopy data supports potent biofilm disruption by the compounds. This property can be put to use against a mucoid *P.aeruginosa* biofilm which is already established in a CF patient.

Patients with CF experience a combination of chronic systemic oxidative stress, generation of free radicals in the lungs due to a hyperimmune response of polymorphonuclear neutrophils (PMNs) and a diminished ability to scavenge free radicals (McGrath *et al.,* 2000). A major role for oxygen derived free radicals has been proposed in the damage to the lung tissue in CF (Salh *et al.,* 1989; McGrath *et al.,* 2000). The dominant sources of these radicals are the PMNs, as discussed earlier. While these are important in killing the pathogens they may also damage the pulmonary epithelium by way of peroxidative destruction of cell membranes (Davis, 1993; McGrath *et al.,* 2000). Further, PMNs and thus oxidative stress are also held responsible to mount an active respiratory burst, leading to lung tissue deterioration (Kolpen *et al.,* 2010). We hence, evaluated the radical scavenging activity for the compounds.

All the compounds were proved to be antioxidant as is evident from figure 3. But, when the intracellular antioxidant potential was evaluated through *P.aeruginosa* cells, the copper complexes turned out to be pro-oxidant (Table 3). A possible reason for this behaviour could be the redox cycling by the metal complexes.

Three severe complications occurring during CRI in CF have been highlighted in this study viz. mucoid biofilms, initial establishment, aggregation and CAP resistance of the biofilms catalysed by the extracellular DNA and lung tissue damage due to the stress induced by oxygen radicals. As is evident from our discussion, all these three complications were successfully tackled by VitaminK_3derivatives; the only exception being the pro-oxidant nature of the copper complexes which would need further investigation.

In conclusion, Vitamin K_3 derivatives show encouraging properties for the treatment of CRI caused by mucoid variants of *P.aeruginosa* in CF patients, in which the mucoid nature of biofilms and severe lung tissue injury are the limiting factors for the success of current therapeutics. This inspiring perspective will be further evaluated by tests on diverse clinical strains recovered from CF patients as well as pertinent clinical trials.

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Author Contributions

Dr. Rohan Chipalkatti and Dr. Ayesha Khan: Conceived the original idea and designed the model and wrote the manuscript. Dr. Rohan Chipalkatti: Completed all the microbiological assays. Dr. Ayesha Khan: Completed all the animal tissue culture and superoxide dismutase assays. Dr. Kirti Badave and Prof. Sandhya Rane: Designed the Vitamin K3 derivatives. Dr. Kirti Badave: Synthesized and characterized the C1-C8 compounds.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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