Antimicrobial Efficacy of Synthetic Pyranochromenones and (Coumarinyloxy)acetamides

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Abstract Four (1, 2, 4 and 6) synthetic quaternary ammonium derivatives of pyranochromenones and (coumarinyloxy)acetamides were synthesized and investigated for their antimicrobial efficacy on MRSA (Methicillin-resistant *Staphylococcus aureus*), and multi-drug resistant *Pseudomonas aeruginosa*, *Salmonella enteritidis* and *Mycobacterium tuberculosis* H37Rv strain. One of the four compounds screened i.e. *N,N,N*-triethyl-10-((4,8,8-trimethyl-2-oxo-2,6,7,8-tetrahydropyrano[3,2-*g*]chromen-10-yl)oxy)decan-1-aminium bromide (1), demonstrated significant activity against *S. aureus*, *P. aeruginosa* and *M. tuberculosis* with MIC value of 16, 35, and 15.62 µg/ml respectively. The cytotoxicity evaluation of compound 1 on A549 cell lines showed it to be a safe antimicrobial molecule, TEM study suggested that the compound led to the rupture of the bacterial cell walls.

Keywords Antibacterial · Antimycobacterial · Chromenones · Cytotoxicity · Pyranochromenones · Transmission electron microscopy

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The emergence of bacterial strains characterized by multiple antibiotic resistance outlines the legacy of past decades of antimicrobial use and misuse that led to thousands of deaths every year [1, 2]. The remarkable antibacterial activity offered by quaternary ammonium derivatives has been well documented in the literature [3, 4]. In pursuit of new antimicrobials with improved efficacy, our research group has been actively involved in the synthesis of chromenones and quinolinones with the successful incorporation of a quaternary ammonium group which have been identified as promising antimicrobial agents [5–7]. Furthermore, investigation of pyranochromenones and chromenones functionalized with quaternary ammonium (1–3) and amido group (4–6) respectively, against various pathogenic strains of bacteria and fungus, too, have revealed them as potential antimicrobial ingredients (Fig. 1) [8].

The present study deals with the synthesis of the four quaternary ammonium derivatives of pyranochromenones and (coumarinyloxy)acetamides (1, 2, 4 and 6) that were studied for their antimicrobial efficacy against resistant strains of *Staphylococcus aureus* (ATCC 43300), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella enteritidis* (ATCC 13076) and *Mycobacteria* (H37Rv). Also, these compounds were subjected to the assessment of their cytotoxicity profile, and transmission electron microscopic details were used to explore the mechanism of action of the most potent compound.

The compounds used in the present study were synthesized by following a literature procedure [8] and well characterized from their physical and spectral data. *p*-Iodonitrotetrazolium chloride (INT) used in the determination of MIC was obtained from Sigma-Aldrich Chemicals, USA. TEM grid (3.05 mm (d) 200-mesh copper grid) was procured from Icon Analytical Equipment Pvt. Ltd.,

Fig. 1 Active antimicrobial compounds [8] **Table 1** MIC (μg/ml) of compounds **1**, **2**, **4** and **6** against resistant pathogenic bacterial strains

Compound	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Salmonella enteritidis</i>	H37Rv
1	16	35	100	15.62
2	50	80	250	62.50
4	200	110	90	> 250
6	180	90	95	62.50
Gentamicin	100	20	20	–
Neomycin	250	35	35	–
Vancomycin	1.5	250	250	–
Isoniazid	–	–	–	0.30

Mumbai, India. Alamar blue used in anti-mycobacterial activity was obtained from AbD Serotec, Oxford, UK. A549 cell lines used to determine the cytotoxicity of compound **1** were procured from ATCC. Kirby-Bauer disc susceptibility test was used to determine the bacterial growth inhibitory potency of the synthesized compounds [9, 10]. The growth inhibitory potency of compounds **1**, **2**, **4** and **6** against *M. tuberculosis* strain H37Rv was assessed by MDA as described by Swaroop et al. [11]. TEM study was carried out in order to ascertain the morphological changes in bacteria, following the protocol of Yadav et al. [12] and Yuanqing et al. [13]. MTT colorimetric assay as described by Riss et al. [14] was utilized to evaluate the in vitro cell cytotoxicity of compound **1** using lung epithelial cell line (A549).

The initial screening results revealed that compound **1** developed the highest zone of inhibition against resistant *S. aureus* and *P. aeruginosa* in comparison to *S. enteritidis* (Table S1, SI). To get a vivid picture about the antibacterial potential of these compounds, minimum inhibitory concentration (MIC) was evaluated using broth dilution assay. Compound **1** was observed to inhibit the growth of pathogenic bacterial strains *S. aureus* (MRSA strain) and *P. aeruginosa* at a concentration of 16 and 35 μg/ml, respectively. However, on going to a shorter alkyl chain, from ten carbons to six carbons (in compound **2**), MIC value increased and a concentration of 50 and 80 μg/ml was required to inhibit the growth of resistant *S. aureus* and *P. aeruginosa*, respectively. On the other hand, moderate

activity could be observed for (coumarinyloxy)acetamides (**4/6**) against the above mentioned microbes (Table 1).

Encouraged by the antibacterial results of **1**, **2**, **4** and **6**, the inhibitory potency of these compounds against *Mycobacterium tuberculosis* strains H37Rv was also examined using the protocol as described by Swaroop et al. [11]. The results revealed that the compound **1** too, displayed highest antimycobacterial activity (MIC: 15.62 μg/ml) in comparison to compounds **2**, **4** and **6** (Table 1).

To have insight into the mechanism of antimicrobial action of the most potent compound **1** from all the assays, morphological changes of untreated and treated resistant *S. aureus* and *P. aeruginosa* with compound **1** at 5X MIC for 15 and 30 min was investigated using transmission electron microscopy (TEM) (Fig. 2).

The results unraveled that the untreated *S. aureus* and *P. aeruginosa* displayed an even and smooth surface, however incubation of the bacterial strains with compound **1** led to the rupture of cell walls and ultimately to cell death. This substantiated that there was an efficient interaction of compound **1** with the bacterial cell wall (Fig. 2). These evidences suggest that quaternary ammonium compounds (QACs) instigate the disruption of cell membrane with subsequent leakage of intracellular components and cell death due to electrostatic and hydrophobic interactions with phospholipids and hydrophobic membrane core, respectively.

In vitro cytotoxicity of the most active compound **1** was examined using lung epithelial cell line (A549). The cell

Fig. 2 TEM images of untreated and treated *S. aureus* and *P. aeruginosa* with compound **1** after 15 min and 30 min

lines were treated with compound **1** at different multiple concentrations of its MIC: 1X (16 µg/ml), 2X (32 µg/ml), 4X (64 µg/ml), 6X (96 µg/ml), 8X (128 µg/ml) and 10X (160 µg/ml) and the viability was evaluated using MTT assay. The cells were found to be ~95–97% viable after 24 h of treatment at 1X, 2X and 4X concentration of its MIC. However, on further increasing the concentration of compound **1** (6X), the cell viability reduced to ~60–80%, and even higher reduction observed at 8X. At 10X concentration, the cell viability was reduced by a greater fold (Figure S1, SI).

Thus, the compound **1** not only exhibit highest antibacterial activity against resistant strains of *S. aureus* and *P. aeruginosa* with MIC value of 16 and 35 µg/ml, respectively, but also it was observed to be a safe antimicrobial candidate at its MIC value. Furthermore, the compound **1** was observed to be a good candidate for antimycobacterial activity due to its capacity to inhibit pathogenic *M. tuberculosis* H37Rv strain with MIC value of 15.62 µg/ml. The inhibitory effect of compound **1** on *S. aureus* and *P. aeruginosa* could be seen through TEM studies, wherein the morphological changes in bacterial cells were evident after 15 and 30 min of incubation at 5X MIC.

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Compliance with Ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

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