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Novel chalcone-thiazole Hybrids as Potent Inhibitors of Drug Resistant *Staphylococcus Aureus*

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KEYWORDS: Chalcone- thiazole hybrids, *Staphylococcus aureus*, antibacterial activities, MIC

Abstract: A series of novel hybrids possessing chalcone and thiazole moieties were synthesized and evaluated for their anti-bacterial activities. In general this class of hybrids exhibited potency against *Staphylococcus aureus* and in particular compound **27** exhibited potent inhibitory activity with respect to other synthesized hybrids. Furthermore, the hemolytic and toxicity data demonstrated that the compound **27** was non-hemolytic and non-toxic to mammalian cells. The *in vivo* studies utilizing a *S. aureus* septicemia model, demonstrated that the compound **27** was as potent as vancomycin. The results of antibacterial activities underscore the potential of this scaffold that can be utilized for developing a new class of novel antibiotics.

Staphylococcus aureus is an opportunistic pathogen that can cause wide spectrum of infections, ranging from severe to potentially fatal or invasive diseases. Recent data suggested that the incidence of infection due to *S. aureus* seems to be the highest in neonates, immune compromised and/or hospitalized individuals with a mortality rate as high as 50%. In USA alone, *S. aureus* has been accounted for 80,461 infections and 11,285 deaths per year, indicating the seriousness of the problem.¹ Currently to treat *S. aureus* infections, wide range of FDA approved drugs are available ranging from ciprofloxacin to oritavancin. Unfortunately, acquiring resistance to the antibiotics by *S. aureus* has recently increased the number of untreatable infections.² Recent strategies to develop a vaccine for *S. aureus* have not been very successful as data suggested that vaccination with bacterial cell-surface antigens increases disease severity instead of offering protection.³ Given this, Centers for Disease Control, USA have listed *S. aureus* as microorganism of serious threat level. (<http://www.cdc.gov/drug-resistance/threat-report-2013/>).

Regrettably, many pharmaceutical companies have abolished their programs on new antibiotic discovery and development long ago^{4, 5} and recent search for novel targets by genomic and proteomic based approaches also have yielded only limited success.^{6, 7} Given the alarming situation of drug-resistant *S. aureus*, the search for

new antimicrobial agents against *S. aureus*, especially strains resistant to multiple antibiotics is an urgent and unmet medical need.

Natural and synthetic chalcones have been reported to exhibit broad spectrum of pharmacological activity.⁸ They are of special interest due to their pronounced antimicrobial activities.^{9, 10} On the other side, thiazole ring is

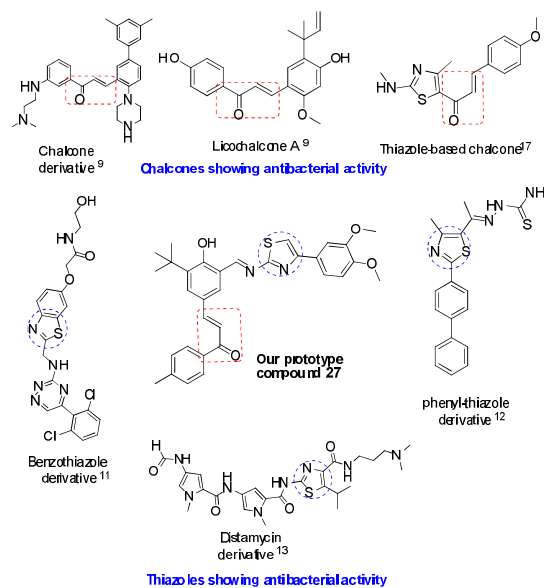


Figure 1. Designing of chalcone-thiazole hybrids by pharmacophore hybridization.

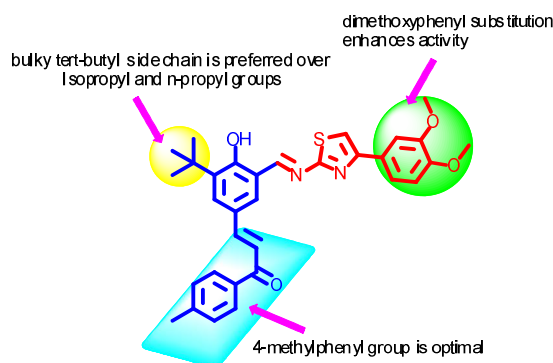


Figure 2. SAR of synthesized hybrids.

found to be a common structural component in a large number of biological agents.¹¹ Thiazoles have also been extensively studied for their antibacterial activities.^{12–15} Though in some cases combining two pharmacophores may lead to inactive and molecular obese compounds, the design of hybrid molecules has generated immense interest in medicinal chemists as it can lead to compounds with superior efficacy while circumventing the problem of resistance.^{16, 17} Increasingly in literature examples of hybrid molecules, incorporating chalcone and thiazoles are reported as being active as antimicrobials.¹⁸ Thus keeping in view the antimicrobial potential of chalcones and thiazoles, it was envisaged that the synthesis and antibacterial evaluation of hitherto novel chalcone-thiazole hybrids is worth the attempt. Figure 1 shows the representative structures of potent antibacterial compounds that contain either chalcone or thiazoles and form the basis of our designed prototype.

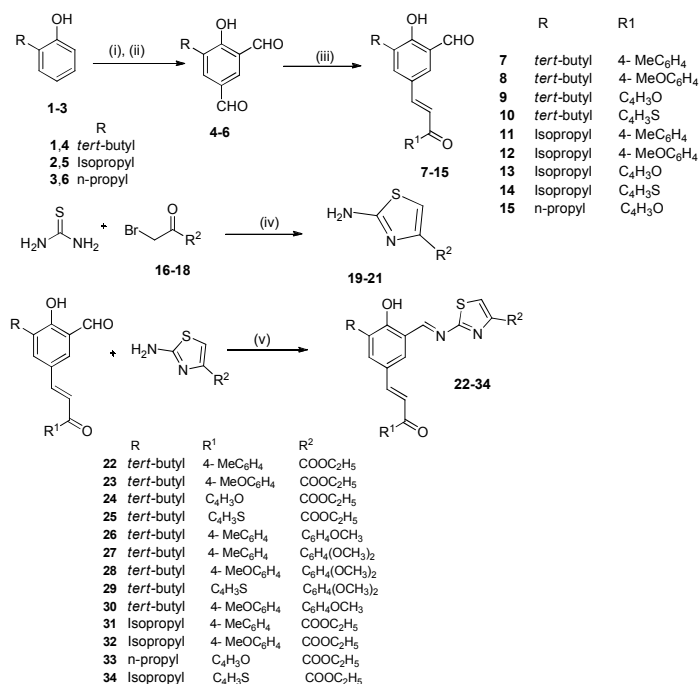
The synthetic routes for the preparation of the target chalcone-thiazole hybrids are depicted in the scheme 1. The synthesis of aromatic dicarbaldehydes (**4–6**) were achieved by the Duff formylation reaction on 2- substituted phenols with hexamethylenetetramine (HMTA) and trifluoroacetic acid (TFA) at 120 °C followed by hydrolysis using 10% aqueous H₂SO₄.¹⁹ The regioselective synthesis of chalcones (**7–15**) was achieved using acid catalyzed condensation between aromatic dicarbaldehydes and substituted acetophenones.²⁰ The 2-aminothiazole derivatives (**19–21**) were synthesized by the reaction between thiourea and phenacylbromide derivatives in presence of ethanol as solvent. Finally, the target compounds were synthesized via formation of the Schiff's bases between chalcones (**7–15**) and 2-aminothiazole derivatives (**19–21**) in presence of ethanol. All the new synthesized compounds were characterized using NMR, IR and mass spectrometry (Supporting Information).

Initially, we screened all the synthesized hybrids for their antibacterial activity *in vitro* with

norfloxacin and gentamycin as reference drugs. The *in vitro* antibacterial results confirmed that some of the chalcone-thiazole hybrids exhibited antibacterial activity against various strains of *Staphylococcus aureus* as shown in Table 1 with compounds **22**, **25** and **27** being the most potent in the series. A pictorial representation of the preliminary Structure–activity relationships (SARs) could be summarized as depicted in Figure 2. The presence of bulky *tert* butyl side chain (**22**, **25**, **27**) is preferred over isopropyl and *n*-propyl groups at ortho position to hydroxyl group. In the chalcone part though both aromatic and heteroaromatic groups are tolerated, the presence of 4-methylphenyl group is optimum. While in the thiazole core phenyl with dimethoxy substitution (**27**) is preferred over monomethoxy (**26**) and ethyl ester (**23**, **24**, **31**).

By using human RBCs, hemolytic activity for all the test compounds was evaluated and the results showed that all the compounds were non-hemolytic (Figure 3A). Among all the compounds, **22** and **27** were selected to screen in the MTT assay as these molecules showed a lower MIC values and no hemolytic activity (Figure 3A).

Scheme 1. Synthesis of chalcone-thiazole hybrids.



Reagents and conditions: (i) HMTA, TFA, 120 °C, 4h; (ii) aq. H₂SO₄, 100 °C, 2h; (iii) conc. HCl, *p*-R¹C₆H₄COCH₃, 1, 4-dioxane, 80–90 °C, 2.5–3.5 h; (iv, v) Ethanol, reflux, 3h.

Table 1. Antibacterial activity of chalcone-thiazole hybrids

Compound	Minimum inhibitory conc.(MIC) in μ M												
	Bacteria							Fungi					
	1	2	3	3a	3b	3c	4	5	6	7	8	9	10
22	>104.9	>104.9	6.5	13.1	6.5	3.3	>104.9	>104.9	>104.9	>104.9	>104.9	>104.9	>104.9
23	>101.5	>101.5	12.7	12.7	12.7	3.2	>101.5	>101.5	>101.5	>101.5	>101.5	>101.5	>101.5
24	>110.5	>110.5	13.8	13.8	13.8	13.8	>110.5	>110.5	>110.5	>110.5	>110.5	>110.5	>110.5
25	>106.7	>106.7	13.3	13.3	6.7	3.3	>106.7	>106.7	>106.7	>106.7	>106.7	>106.7	>106.7
26	>97.9	>97.9	12.2	12.2	12.2	12.2	>97.9	>97.9	>97.9	>97.9	>97.9	>97.9	>97.9
27	>92.5	>92.5	5.8	11.6	5.8	1.4	>92.5	>92.5	>92.5	>92.5	>92.5	>92.5	>92.5
28	>89.8	>89.8	11.2	11.2	11.2	5.6	>89.8	>89.8	>89.8	>89.8	>89.8	>89.8	>89.8
29	>93.9	>93.9	23.5	11.7	11.7	11.7	>93.9	>93.9	>93.9	>93.9	>93.9	>93.9	>93.9
30	>94.9	>94.9	11.9	11.9	11.9	3.0	>94.9	>94.9	>94.9	>94.9	>94.9	>94.9	>94.9
31	>108.1	>108.1	27.0	13.5	13.5	13.5	>108.1	>108.1	>108.1	>108.1	>108.1	>108.1	>108.1
32	>104.5	>104.5	26.1	26.1	26.1	26.1	>104.5	>104.5	>104.5	>104.5	>104.5	>104.5	>104.5
33	>102.4	>102.4	51.2	51.2	51.2	51.2	>102.4	>102.4	>102.4	>102.4	>102.4	>102.4	>102.4
34	>110.0	>110.0	27.5	27.5	27.5	13.7	>110.0	>110.0	>110.0	>110.0	>110.0	>110.0	>110.0
Gen	6.5	1.6	1.6	104.9	3.3	104.9	3.3	>104.9	104.9	104.9	104.9	104.9	104.9
Nor	0.0	1.0	0.5	33.6	1.0	0.5	0.1	>33.6	33.6	33.6	33.6	33.6	33.6
Van	>156.6	>156.6	2.4	9.8	1.2	2.4	>156.6	>156.6	156.6	156.6	156.6	156.6	156.6
Flu	>34.5	>34.5	>34.5	>34.5	>34.5	>34.5	>34.5	0.7	1.4	1.4	22.1	22.1	1.4
AmB	>54.1	>54.1	>54.1	>54.1	>54.1	>54.1	>54.1	0.0	0.1	0.3	0.3	0.5	0.0

1. *E. coli* (ATCC 9637), 2. *P. aeruginosa* (ATCC BAA-427), 3. *S. aureus* (ATCC 25923), 3a. *S. aureus* (ATCC 700699 Methicillin Resistant), 3b. *S. aureus* (ATCC 29213), 3c. *S. aureus* (ATCC 33592 Methicillin & Gentamicin Resistant), 4. *K. pneumonia* (ATCC 27736), 5. *C. albicans*, 6. *C. neoformans*, 7. *S. schenckii*, 8. *T. mentagrophytes*, 9. *A. fumigates*, 10. *C. parapsilosis* (ATCC 22019); Gen. Gentamicin, Nor. Norfloxacin, Van. Vancomycin, Flu. Fluconazole; AmB, Amphotericin-B.

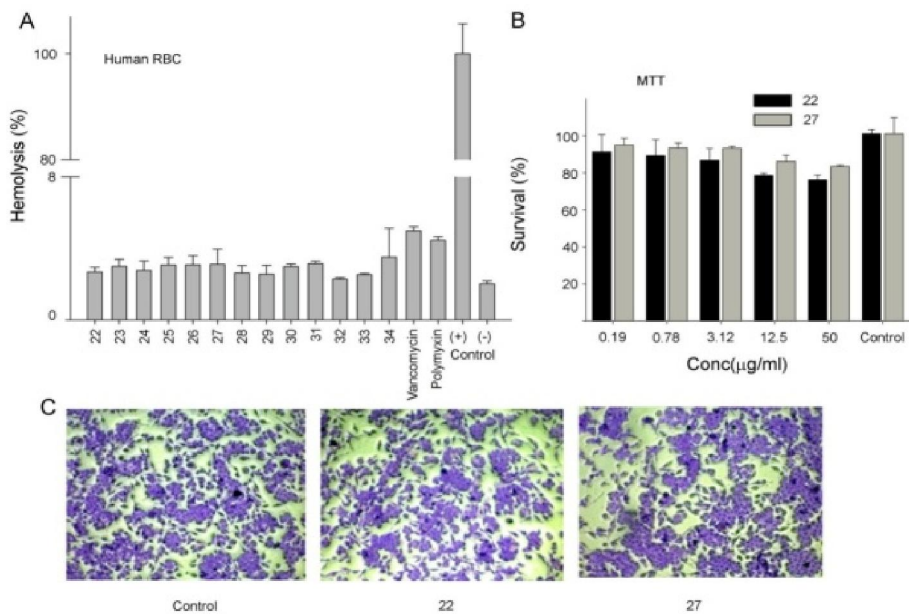


Figure 3. Effect of chalcone-thiazole hybrids on eukaryotic cells. (A) Influence of test compounds (22-36) on erythrocytes in the absence of human serum. Hemolytic effects of the test compounds were investigated and the corresponding data for vancomysin and polymyxin B-sulphate are included for comparison. RBC was incubated at 100 μ g/mL and 2 % Triton X-100 was used as positive control. Hemoglobin released was measured at 540 nm and is expressed as a percentage of Triton X-100 induced hemolysis. (B) The MTT assay (right top panel) was used to measure the viability of Mammalian fibroblast cell line, L929 in the presence of compounds 22 and 27. In the assay, enzymes associated with metabolic activity will modify the MTT into a dye, blue formazan which is measured at 550 nm. (C) Morphological changes on the L929 were investigated by using phase contrast microscopy. Cells were incubated for 24 hours at 1x MIC for 24 hours and stained with Giemsa stain before observing under the phase contrast microscope for the morphological changes.

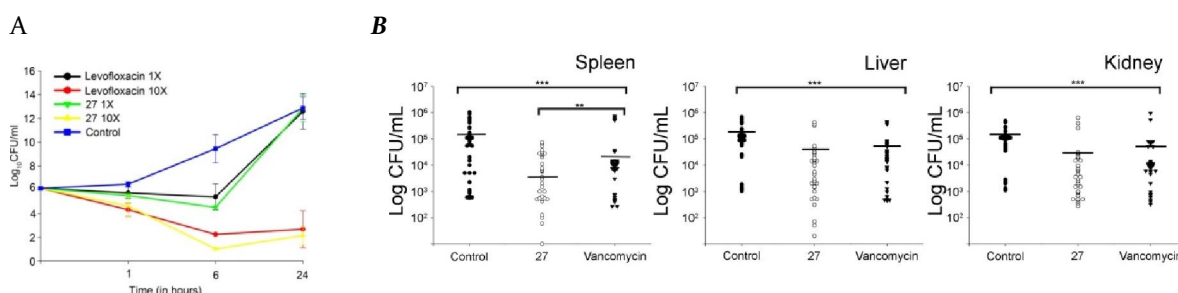


Figure 4. Static and cidal curve with different concentrations of compound 27 (A). Compound 27 (1X and 10X of MIC concentration) were incubated with *S. aureus* for 24h and at regular time points aliquot was taken and CFU was calculated. *In vivo* antimicrobial activity of the compound 27 (B). BALB/C mice were initially made neutropenic by injecting cyclophosphamide (150 mg/Kg of body weight), followed by *S. aureus* ATCC 29213 injection. After 3 hour of post infection, compound 27 and vancomycin at 15 mg/Kg of body weight was injected into mice for 2 times at an interval of 3 hours between injection. After 24 hours, the mice were sacrificed and the organs (spleen, kidney, liver) were crushed before plating them on the TH plates for CFU determination. The data from three independent experiments were pooled and the P-value was determined using the ANOVA on ranks by using Sigma stat software (**P<0.05).

These results clearly demonstrated that the tested molecules do not have any toxicity issue at tested (10X MIC) concentrations. In order to prove that the test molecules are indeed non-toxic, MTT assay was carried out to measure the toxicity of 22 and 27 against mammalian fibroblast cell line, L929 (Figure 3B). Results showed that the tested compounds did not show any toxicity against L929 cells at MIC and lower concentrations. Even though the test compounds did not have any immediate toxic effects, we were interested to investigate whether the molecules induced any morphological changes over 24h (Figure 3C). L929 cells were exposed to test compounds at 1X MIC, followed by staining and observed under the phase contrast microscope to see any changes in the morphology of the cells. Results demonstrated that the test compounds have no immediate toxicity nor induced any morphological changes. In comparison to control, compound treated L929 cells were normal in appearance, transparent and attached to the surface of the wells of tissue culture plate (Figure 3C).

Next, in order to determine whether the compounds exhibited time-dependent or concentration-dependent antimicrobial activity, compound 27 was tested at 1X and 10X MIC against *S. aureus* ATCC 29213 and aliquots were plated at regular intervals. As can be seen in Figure 4A, the compound exhibits concentration dependent activity with a 10 log₁₀ reduction in cfu in comparison to no-drug control at 24h, which is comparable to levofloxacin. Based upon the above results, we were of opinion that the developed molecule could be tested *in vivo* conditions.

In order to investigate the possible *in vivo* efficacy of compound 27, we injected the compound into mice infected intraperitoneally with *S. aureus* ATCC 29213. In the control group, mice injected with bacteria developed severe signs of sepsis whereas the treatment group mice showed symptoms of mild to average sick behavior, but later recovered upon second dose of injection. Compared to the controls, treatments with compound 27 yielded a increase in survival and significantly lower bacterial numbers in the spleen and liver of the animals as compared to kidney (Figure 4B). We believe that the lower activity of the drug in the kidney may be due to excretion of the drug through urine or drug metabolism.²¹

In conclusion, we have designed and synthesized a new class of hybrids by using pharmacophore hybridization approach. The majority of these hybrid compounds, especially, 27, exhibited promising *in vitro* and *in vivo* antibacterial activity against *S. aureus*. Furthermore, these compounds were not showing the hemolytic activity against human erythrocytes at 50µg/ml concentration. Further studies to unravel the mechanism of action and structural optimization of this lead are currently underway in our laboratory. It is intended that this study will help to discover and develop new class of next generation antibacterial agents.

ASSOCIATED CONTENT

Supporting Information

Experimental procedures, characterization of all compounds together with protocols for biological assays are available free of charge via the Internet at <http://pubs.acs.org>.

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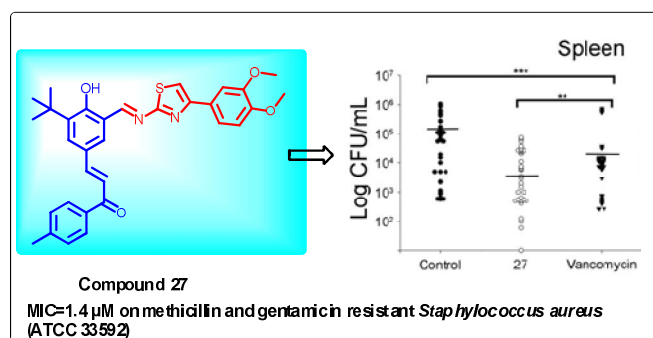
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