Structure pre-requisites for isoflavones as effective antibacterial agents

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Submitted: 03-06-2010

ABSTRACT

Recent reports reveal that there is increasing incidence of infections of multidrug-resistant bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE). Flavonoids and related compounds have been shown to possess potent antimicrobial activities. Most of the flavonoids are considered as constitutive antimicrobial substances recently termed as "Phytoanticipins," especially those belonging to prenylated flavonoids and isoflavones. The current review highlights the structure prerequisites for isoflavones as antibacterial agents. Structure–activity relationship (SAR) conclusions have been drawn by comparing the reported minimum inhibitory concentration values for the various isoflavones against *S. aureus* and MRSA. There exists a significant co-relationship between the presence of certain functional groups (prenyl group, phenolic hydroxyl) at particular positions and antibacterial activity of the compounds. These trends have been postulated with a view of assisting better drug designing of future next-generation antiinfectives, particularly against the bothersome multidrug-resistant microbes. The SAR of these isoflavones has also proved to be a basis to explore the mechanism of antibacterial action. Thus, the study would prove extremely useful to synthesize antibacterial isoflavones in future, which would eventually be beneficial for optimizing the lead molecule for the antibacterial action

Key words: Antibacterial activity, Isoflavones, SAR of isoflavones

INTRODUCTION

In recent times, microbial infections have once emerged as the leading cause of concern globally. The increasing prevalence of multidrug-resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the specter of untreatable bacterial infections, which adds urgency to the search for novel infection-fighting strategies.

Infections caused by methicillin-resistant *S. aureus* (MRSA) in compromised hosts pose a serious problem all over the world, because MRSA strains are resistant to numerous antibiotics and can be transmitted from patient to patient via transiently colonized hands of hospital personnel. Although vancomycin is the most effective antibiotic for MRSA infections, clinical use often results in unexpected side effects and development of infections by vancomycin -resistant enterococci (VRE).[1] Rational drug design does not always yield effective antimicrobials. In the past, potent enzyme inhibitors have been successfully designed and synthesized but they had only modest antibacterial activity, probably owing to the complex issue of drug uptake by cells. Broad empirical screening of a chemical entity for antimicrobial activity represents an alternative strategy for the development of novel drugs.

Natural products have been a particularly rich source of antiinfective agents, yielding, for example, the penicillins in 1940, the tetracyclines in 1948, and the glycopeptides in 1955.[2] Naturally occurring products from plants have played an important role in the discovery of new therapeutic agents since ancient times, for example, quinine obtained from *Cinchona* has been successfully used to treat malaria. Substantial attention has been focused on exploration and utilization of secondary metabolites of plants (phytochemicals) as an alternative to and/or in combination with traditional antibiotics for treating MRSA infections.[3-5] Among the phytochemicals, flavonoids, isoflavonoids, and related compounds seem to be the most potentially useful candidates because they are widely distributed in edible plants and possess broad pharmacologic activity.[6]

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Access this article online

Quick Response Code:

Website: www.phcogrev.com

DOI: 10.4103/0973-7847.79095

The antimicrobial properties showed by various plants could be due to the presence of mixtures of active constituents (mostly prenylated flavonoids), which show a broad spectrum of biological and pharmacologic activities. Most of the flavonoids [Figures 1-2] are considered as constitutive antimicrobial substances recently termed as "Phytoanticipins," especially those belonging to prenylated flavonoids and isoflavones, but not excluding other classes of compounds.[7]

The current report highlights the structural peculiarities of isoflavones in various plant species. Special focus lies on the antibacterial activity of isoflavones and an attempt to draw the Structure–Activity Relationships are made.

ANTIBACTERIAL ACTIVITY OF ISOFLAVONES

Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted secondary metabolites. In many cases, these substances serve as plant defense mechanisms against predation by microorganisms, insects, and herbivores. Thus, keeping in mind the possibility of these secondary metabolites having an inherent capability to be antimicrobial or antibacterial agents, the current focus is on identification and screening of such secondary metabolites.

Isoflavonoids are a subclass of the more ubiquitous flavonoids, which are naturally occurring polyphenols. Isoflavonoids differ from other classes of flavonoids by their greater structural variability, their frequent presence in plants in their free form, rather than as a glycoside, and by the greater frequency of isoprenoid substitution. They are divided into subclasses depending on the oxidation level of the central pyran ring. Isoflavonoids constitute a specific branch of flavonoid metabolism, differing from the other flavonoids by the position of the phenolic ring B.[8]

STRUCTURE–ACTIVITY RELATIONSHIPS OF ISOFLAVONES WITH ANTIBACTERIAL ACTIVITY

An exhaustive literature survey was done and isoflavones possessing antibacterial activity were identified from various plants.[9-17] The antibacterial activities of these different isoflavones were compared with respect to their minimum inhibitory concentration (MIC) values. There existed a significant co-relationship between the presence of certain functional groups (prenyl group, phenolic hydroxyl) at particular positions and antibacterial activity of the compounds. These trends have been studied keeping in mind the activity of the isoflavones against *S. aureus* and MRSA. Thus, the present study draws an attempt to predict the structure–activity relationship (SAR) of isoflavones with good antibacterial activity against the susceptible as well as resistant forms of bacteria.

Following conclusions with respect to structural prerequisites of isoflavones with lethal activity against the susceptible and resistant forms of bacteria have been drawn.

Prenyl group

Presence of the Prenyl group at C-6 produces isoflavones with potent activity. For example, Genistein with no prenyl group has very less activity (MIC values against *S. aureus* and MRSA: >128 μg/mL).[17]

Removal of Prenyl group or movement to C-8 decreases activity. For example, Isoangustone (MIC values against *S. aureus* and MRSA: 16 μg/mL),[17] FS 1(MIC values against *S. aureus* and MRSA: 28 and 36 μg/mL, respectively)[16] to Glycyrrhizoflavone (MIC values against *S. aureus* and MRSA: 32 and 32–64 μg/mL, respectively).[17]

Prenyl group on the fused pyran ring system governs activity. There is no mandatory requirement of presence of the prenyl group on aryl substitution. For example, 8-(γ,γ-dimethylallyl)-wighteone (MIC values against *S. aureus* and MRSA: 8 μg/mL),[17] and Ganaconin (MIC values against *S. aureus* and MRSA: 16 μg/mL)[17] show potent activity, although there are no prenyl groups on aryl substitution.

Phenolic hydroxyl groups

Hydroxyl group on fused pyran ring system as well as the aryl substitution is very important for biological activity. Hydroxyl group should be present at positions C-7 and/or C-5 position. For example, DS 5 (MIC values against *S. aureus* and MRSA: 2 and 4 μg/mL, respectively),[10] and Isoangustone A (MIC values against *S. aureus* and MRSA: 16 μg/mL, respectively).[17]

Removal of hydroxyl group or conversion of hydroxyl group to –CHO abolishes activity. For example, DS 1.[10]

Methylation of fused pyran ring hydroxyl group decreases anti-*S. aureus* activity, but sharply increases anti-MRSA activity. For example, DS 6 (MIC values against *S. aureus* and MRSA: 128 and 2 μg/mL, respectively), and DS 8 (MIC values against *S. aureus* and MRSA: 128 and 2 μg/mL, respectively).[10]

Methylation of flavonoid hydroxyl group coupled with absence of prenyl group further decreases antibacterial activity. For example, Glisoflavone (MIC values against *S. aureus* and MRSA: 64 μg/mL).[17]

Cyclization between prenyl and hydroxyl groups

Cyclization between prenyl and hydroxyl groups on the aryl substitution decreases activity, but not greatly. For example, Semilicoisoflavone (MIC values against *S. aureus* and MRSA: 32 and 32–64 μg/mL, respectively).[17]

Cyclization between C-6 prenyl and C-7 hydroxyl group drastically decreases activity. For example, EV 1 (reported to have "No significant activity"),[12] but presence of a prenyl group at 3' position of aryl substitution with this cyclization retains activity.

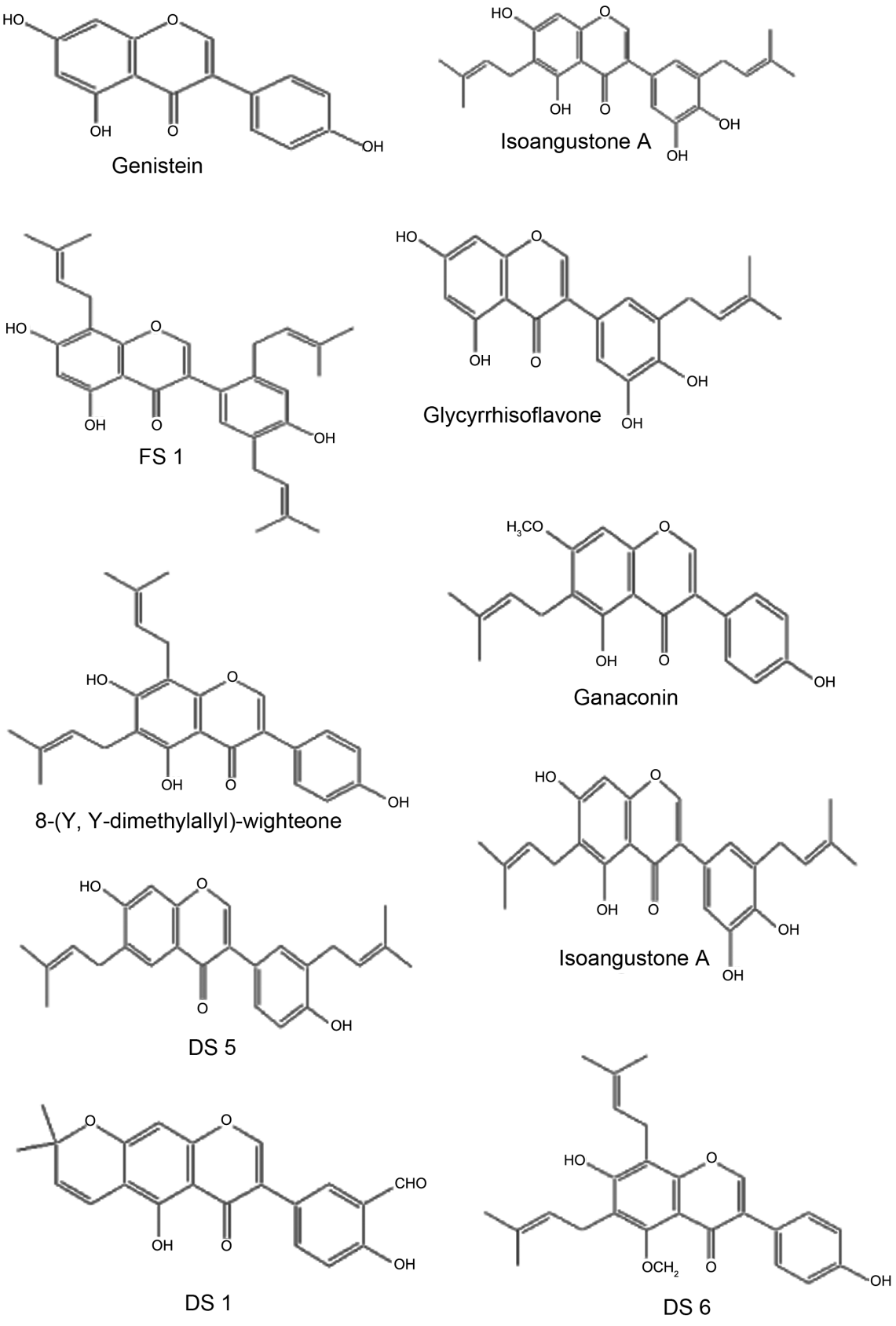

Figure 1: Some of the isolated flavonoids

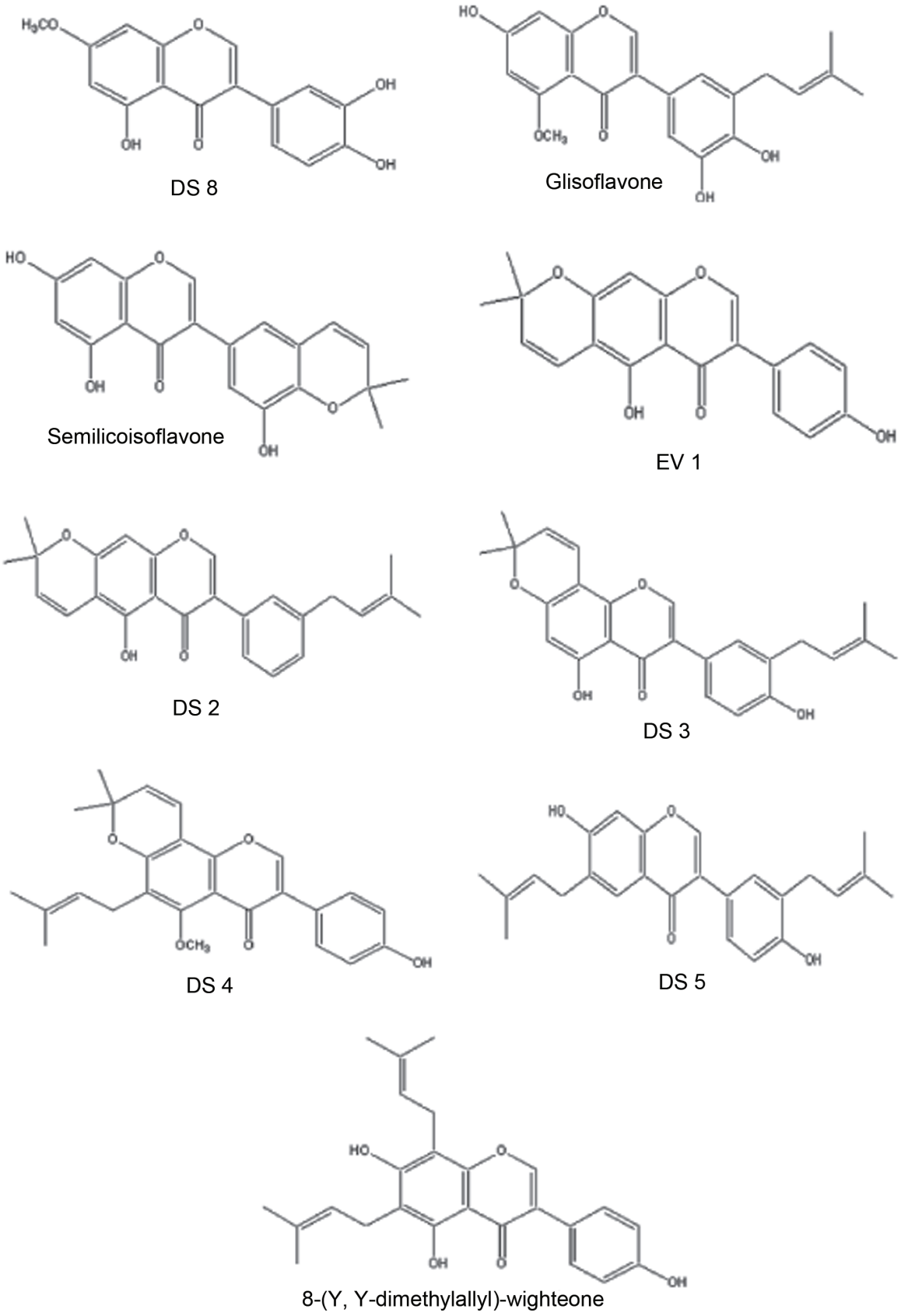

Figure 2 : Some of the isolated flavonoids

For example, DS 2 (MIC values against *S. aureus* and MRSA: 128 and 16 μg/mL, respectively).[10]

Cyclization between C-8 prenyl and C-7 hydroxyl group abolishes activity, but presence of a prenyl group at C-6 or 3' position with this cyclization also does not retain much activity. For example, DS 3 and DS 4.[10]

The antimicrobial effect of isoflavones is also attributed to the presence of phenolic hydroxyl groups that have affinity for proteins and therefore act as inhibitors of microbial enzymes as well as their biosynthetic pathways. In addition, substitution of the flavonoid ring system with prenyl groups is thought to increase their lipophilicity and consequently, enhances their antibacterial activity through interaction with cellular membranes.[18]

The lipophilicity of the aliphatic groups (prenyl) seems to be required to disturb the bacterial cell membrane or its function, in a way analogous to the supposed mechanisms related to the antibacterial activities of catechin derivatives and alkyl gallates. The requirements of the phenolic hydroxyl groups may be correlated with the damage on the bacterial tissues through some pro-oxidant effects in the participation of the phenolic hydroxyl groups, in accordance with the reported role of hydroxyl groups in the antibacterial effects of catechins.[19]

Possible mechanisms of antibacterial activity

Antibacterial activity of many of the synthetic and natural agents has been thought to be mediated *via* interference with the bacterial topoisomerase activity. Two bacterial type II topoisomerases have been identified: topoisomerase IV (topo IV) and DNA gyrase. Topo IV catalyzes the separation of two double-stranded covalently closed circular DNA molecules that are intertwined in a chain. DNA gyrase is a type II topoisomerase that introduces negative supercoils (or relaxes positive supercoils) into DNA by looping the template so as to form a crossing, then cutting one of the double helices and passing the other through it before releasing the break, changing the linking number by two in each enzymatic step. This process occurs in prokaryotes (particularly in bacteria), whose single circular DNA is cut by DNA gyrase and the two ends are then twisted around each other to form supercoils. The unique ability of gyrase to introduce negative supercoils into DNA is what allows bacterial DNA to have free negative supercoils. The ability of gyrase to relax positive supercoils comes into play during DNA replication. Several inhibitors of DNA gyrase and/or topo IV have been developed for clinical use as antimicrobials. Synthetic antibiotics target both DNA gyrase and topo IV by stabilizing the topo II–DNA reaction intermediate, known as the cleavable complex. By trapping this complex, they introduce lesions into the intracellular DNA. Isoflavone genistein inhibits the catalytic activity of the enzyme by stabilizing the covalent topoisomerase II–DNA cleavage complex. Genistein inhibits bacterial growth in vitro by the inhibition of topo IV, based on several lines of evidence. First, genistein inhibits the activity of topo IV rather than that of DNA gyrase. Second, topo IV in *S. aureus* is the primary target of topo II-targeted drugs, whereas in *Escherichia coli* the primary target is DNA gyrase. Our results demonstrate genistein-mediated growth inhibition of *S. aureus* but not of *E. coli*.[11]

Many of the isoflavones have potent activity against gram-positive bacteria and have no activity or negligible activity against gram-negative bacteria. It has been postulated that the protoplasmic membrane of the bacterial cell is the operative target of isoflavones and related compounds, and thus they exhibit antibacterial activity by interfering with the incorporation of metabolites and nutrients into bacterial cells. As stated earlier, these compounds show a broad spectrum of antibacterial activity on gram-positive bacteria, including *Staphylococci*, *Streptococci*, *Actinomyces*, and *Lactobacillus* species, however, they fail to inhibit gram-negative bacteria, such as *E. coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* even at relatively high concentrations, suggesting that the outer membrane of gram-negative bacteria interferes with the accessibility of the compounds to the protoplasmic membrane. The second possibility is that the nucleic acids in MRSA cells get affected. In addition to complete inhibition of metabolite incorporation of bacterial cells, the observed intense bactericidal action of these compounds bears some resemblance to that induced by 4-quinolone antimicrobial agents, which inhibit DNA synthesis.[1] This is in agreement with the fact that isoflavones bear some structural resemblance with 4-quinolone antibacterial agents.

CONCLUSIONS

Newer antiinfectives are being continuously designed and synthesized by researchers world over, but the problem of antibiotic resistance is on the rise. Thus, there is a dire need for the discovery of new compounds with antibiotic activity against the resistant forms of bacteria. The current study is an attempt to evaluate isoflavones as potential antibacterial agents. The SAR of isoflavones with antibacterial activity revealed a co-relationship between the presence of prenyl group and hydroxyl group at certain positions of the isoflavone ring structure and anti-*S. aureus* and anti-MRSA activity. Of the compounds included in this study, DS 5 and 8-(γ,γ-dimethylallyl)-wighteone were found to be the most active compounds. DS 5 has the C-6 prenyl group as well as the C-7 hydroxyl group. On the other hand, 8-(γ,γ-dimethylallyl)-wighteone has C-6 and C-8 prenyl group and C-5 and C-7 hydroxyl group. These structural peculiarities possessed by these two compounds are very important for anti-*S. aureus* and anti-MRSA activity as revealed by this review. Thus, these two compounds can act as future candidates to develop newer synthetic antibacterials in due course.

The current study would be useful to design and synthesize newer antibacterial agents. These agents can either be used alone or in combination with other antibiotics to combat infections of *S. aureus* and MRSA. Use of these agents in combination would

reduce the effective dose to be administered and also reduce the incidence of resistance to antibiotics.

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Source of Support: Nil, **Conflict of Interest:** None declared