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PII: S0960-894X(14)01374-2
DOI: http://dx.doi.org/10.1016/j.bmcl.2014.12.066
Reference: BMCL 22311

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 12 July 2014
Revised Date: 8 December 2014
Accepted Date: 19 December 2014

Please cite this article as: Ashok, D., Kumar, A.V., Gandhi, D.M., Srinivas, G., Kumari, K.M., Vijjulatha, M., Balasubramanian, S., Ernala, P., Synthesis, antimicrobial activity and molecular docking of novel tetracyclic scaffolds incorporating a flavonoid framework with medium sized oxygen heterocycles, *Bioorganic & Medicinal Chemistry Letters* (2014), doi: http://dx.doi.org/10.1016/j.bmcl.2014.12.066

# Synthesis, antimicrobial activity and molecular docking of novel tetracyclic scaffolds incorporating a flavonoid framework with medium sized oxygen heterocycles

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A convenient approach for the synthesis of novel tetracyclic scaffolds incorporating a flavonoid framework with medium sized heterocyclic rings ( eight-, nine-, ten- and eleven-membered rings ) containing two oxygen atoms from flavonols through alkylation using different dibromoalkanes was described. The synthesized compounds were established based on the spectral data and X-ray crystal structure for **6c**. The synthesized compounds were evaluated for their *in vitro* antimicrobial activity. Docking studies were carried out for most active two compounds **6f** and **6i**.

Keywords: Flavonoid framework, Medium sized rings, Antimicrobial activity, Molecular docking

Medium sized heterocyclic rings are commonly found structural units within the frame work of a variety of natural products and is the main reason for the growing importance of such class of compounds.[1] In particular synthesis of medium sized heterocyclic rings bearing oxygen or nitrogen atom(s) are important synthetic targets for organic chemists as they are integral parts of many medicinally interesting synthetic compounds. However synthesis of medium sized heterocyclic rings continues to be one of the fascinating endeavours in organic chemistry because of challenges involved in their synthesis.[2] Moreover for an equal number of atoms, cyclic analogues inherently possess a lower number of rotatable bonds than their acyclic analogues. As a result cyclic counterparts are more conformationally restricted than their acyclic analogues, which potentially can impart higher target binding and selectivity and improved oral bioavailability.[3] Flavonoid framework is medicinally important structural organization present in many bioactive molecules showing various activities like antiviral, antibacterial, antiprotozoal, oestrogenic, anti-inflammatory, mutagenic, antimutagenic and antineoplastic activities, and is also capable of inhibiting many types of enzymes.[4-6] Therefore there has been increasing interest in the synthesis of diverse classes of compounds containing flavonoid frame work.

Among numerous known heterocycles containing flavonoid framework, artelastocarpin and carpelastofuran (Figure 1) have attractive frameworks because of their reported cytotoxic properties.[7]

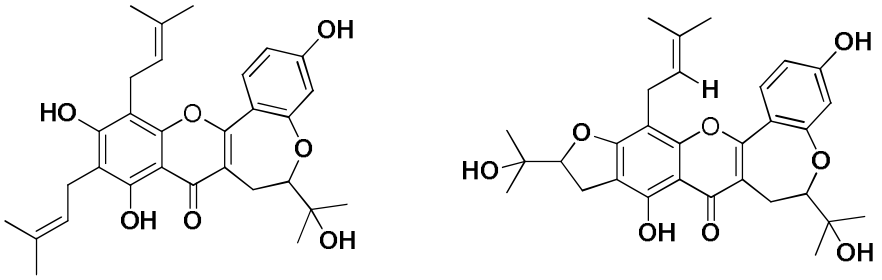



**Figure 1** Bioactive molecules of interest

Inspired by the growing importance of medium sized heterocyclic rings and the wide range of biological importance of heterocycles containing flavonoid framework, we have devised a synthesis for the construction of novel tetracyclic ring systems incorporating a flavonoid framework with medium sized oxygen heterocycles and further evaluated their antimicrobial activity.

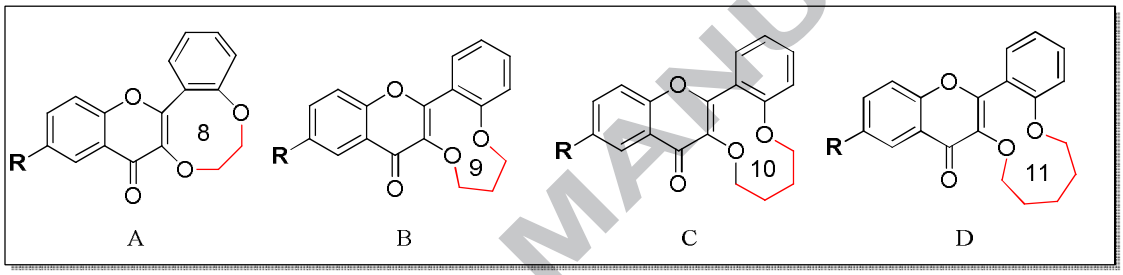
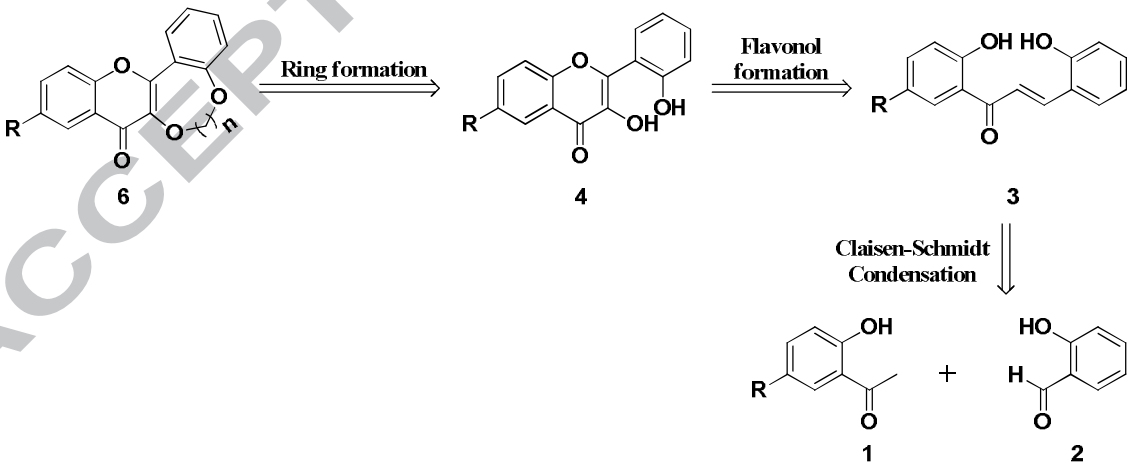


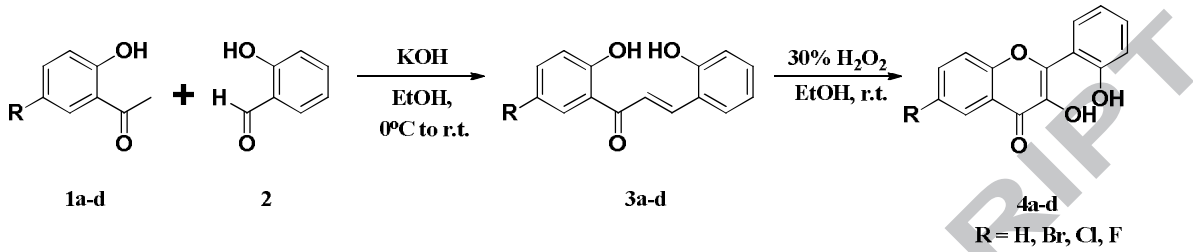

**Figure 2** Proposed tetracyclic scaffolds

The retro-synthetic sequence of required tetracyclic medium sized heterocyclic rings is shown in Scheme 1.



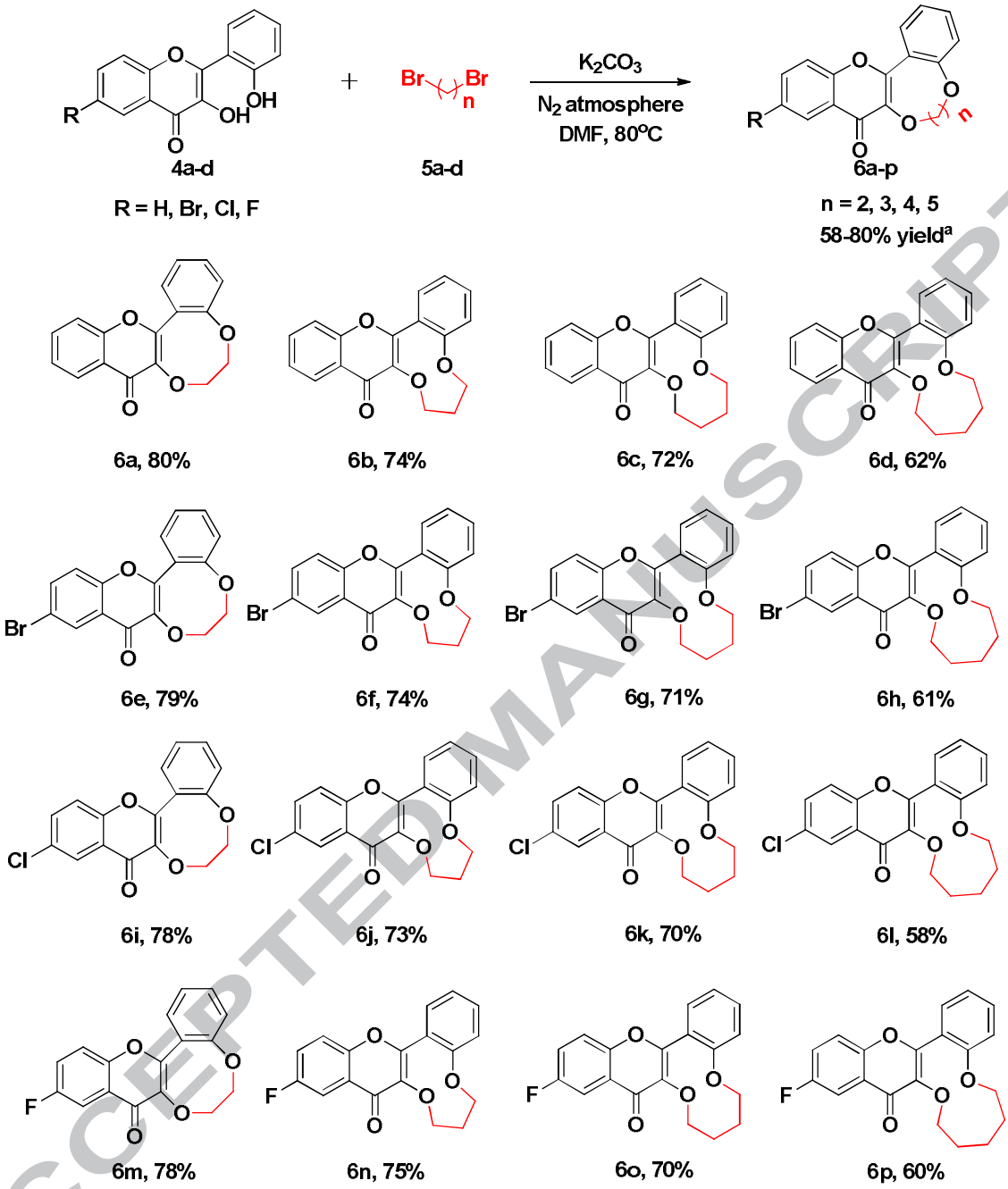

**Scheme 1** Retro-synthetic strategy

Accordingly the required tetracyclic scaffolds were synthesized in three steps. Claisen-Schmidt condensation of substituted 2-hydroxyacetophenones **1a-d** and salicylaldehyde **2** in presence of potassium hydroxide gave corresponding chalcones **3a-d** (Scheme 2).[8] Chalcones were converted to corresponding flavonols **4a-d** by treatment with alkaline hydrogen peroxide.[9, 10]




**Scheme 2** Synthesis of 3-Hydroxy-2-(2-hydroxyphenyl)-4*H*-chrome-4-ones

Thus obtained substituted 3-Hydroxy-2-(2-hydroxyphenyl)-4*H*-chrome-4ones **4a-d** were treated with dibromoalkanes **5a-d** in the presence of anhydrous potassium carbonate at 80°C for 8 hours in DMF under $N_2$ atmosphere provided required tetracyclic compounds **6a-p** in moderate to good yields (58-80%).




**Scheme 3** Synthesis of tetracyclic compounds. [a]Isolated yields

All the compounds synthesized were well characterized by spectral (NMR, MS, IR) data, the molecular structure of **6c** was characterized by X-ray crystallography (CCDC 972844), [11] as shown in Figure 3.

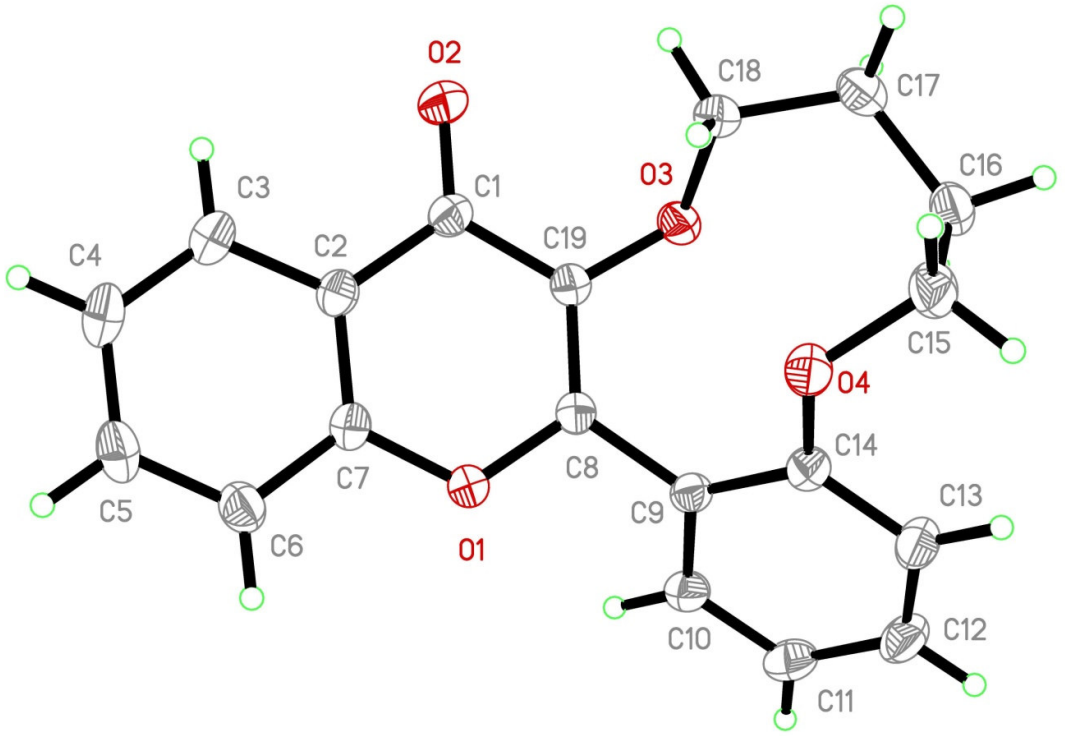
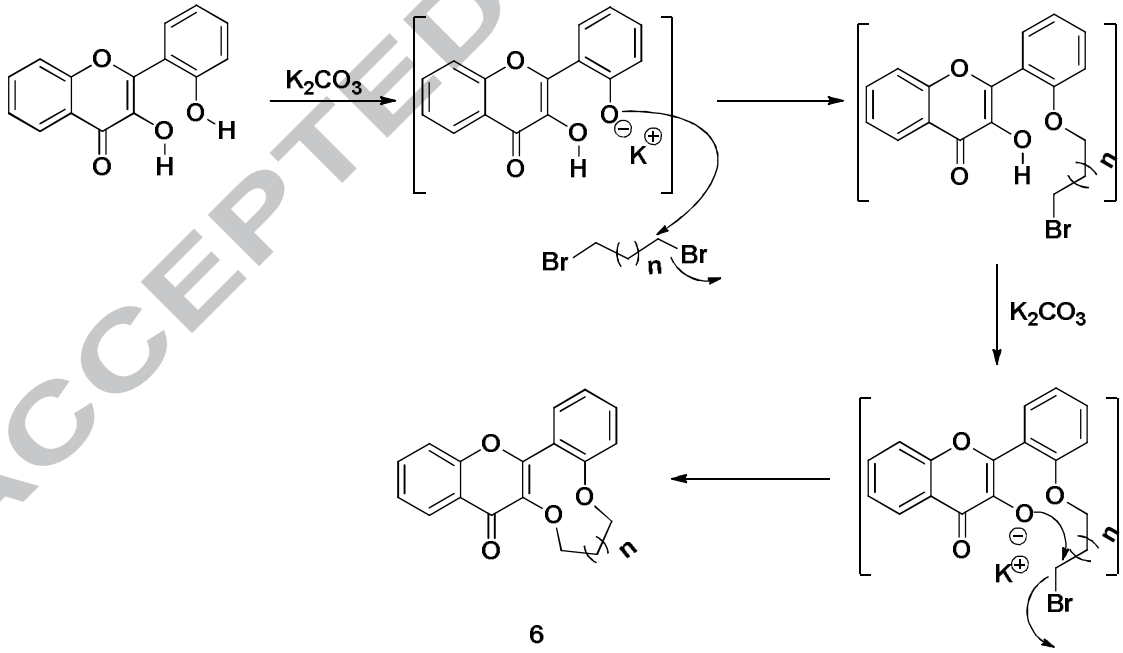



**Figure 3** A view of AT78, showing the atom-labelling Scheme of compound **6c**. Displacement ellipsoids are drawn at the 30% probability level and H atoms are represented by circles of arbitrary radii.




**Scheme 4** Representative mechanism of ring closure reaction for synthesis of compound **6**[12]

The antibacterial activity of the precursor's **4a-d** and synthesized tetracyclic compounds **6a-p** were tested for their *in vitro* antibacterial activity against *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 10536), *Pseudomonas aeruginosa* (ATCC 10145) and *Klebsiella pneumonia* (ATCC 10031). The results of the antibacterial screening were compared with the standard antibacterial drug gentamicin sulfate. The minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) are reported in **Table 1** and **Table 2** respectively. As observed from **Table 1**, the inhibitory efficiency of tetracyclic compounds was higher than their acyclic precursor's. Among newly synthesized compounds, the inhibitory efficiency of compound **6f** against Gram-negative bacteria, *Klebsiella pneumonia,* compounds **6f, 6h** and **6k** against *Escherichia coli* were close to those of standard (MIC = 0.025-0.032 µM). In the case of Gram-positive bacteria, the inhibitory efficiency of compounds **6e** and **6l** against *Bacillus subtilis*, compounds **6e** and **6i** against *Staphylococcus aureus* were close to those of standard (MIC = 0.027-0.033 µM). It was observed that compound **6f** exhibited broad spectrum of antibacterial activity as the observed MIC values against all the tested strains were below 0.050 µM. All other compounds showed slightly higher MIC values. The antibacterial activity study results revealed that tetracyclic compounds with bromo substitution showed promising antibacterial activity compared to other substituted tetracyclic compounds.

We have also tested the antifungal activity of these tetracyclic compounds against two fungal strains, *Candida albicans* (ATCC 10231) *and Candida tropicalis* (ATCC 750). The results of the antifungal screening were compared with standard antifungal drug fluconazol. However all the tetracyclic compounds were ineffective against the tested fungal strains up to a concentration of 200 µg/mL.

**Table 1** Antimicrobial activity (MICs in μM[a])

| | Bacteria | | | | | | |
|---|---|---|---|---|---|---|---|
| | Gram-positive | | Gram-negative | | | Fungus | |
| Compound | *B. subtilis* | *S. aureus* | *E. coli* | *P. aeruginosa* | *K. pneumoniae* | *C. albicans* | *C. tropicalis* |
| **4a** | 0.218 | 0.198 | 0.177 | 0.157 | 0.177 | >0.787 | >0.787 |
| **4b** | 0.167 | 0.152 | 0.135 | 0.120 | 0.135 | >0.602 | >0.602 |
| **4c** | 0.173 | 0.190 | 0.140 | 0.157 | 0.138 | >0.694 | >0.694 |
| **4d** | 0.167 | 0.259 | 0.220 | 0.275 | 0.202 | >0.735 | >0.735 |
| **6a** | 0.071 | 0.142 | 0.108 | 0.125 | 0.107 | >0.714 | >0.714 |
| **6b** | 0.085 | 0.272 | 0.051 | 0.136 | 0.102 | >0.680 | >0.680 |
| **6c** | 0.064 | 0.097 | 0.146 | 0.113 | 0.064 | >0.649 | >0.649 |
| **6d** | 0.186 | 0.155 | 0.063 | 0.062 | 0.077 | >0.621 | >0.621 |
| **6e** | **0.027** | **0.027** | 0.097 | 0.097 | 0.083 | >0.558 | >0.558 |
| **6f** | 0.040 | 0.053 | **0.032** | 0.040 | **0.026** | >0.537 | >0.537 |
| **6g** | 0.066 | 0.079 | 0.038 | 0.051 | 0.090 | >0.518 | >0.518 |
| **6h** | 0.063 | 0.051 | **0.025** | 0.062 | 0.037 | >0.500 | >0.500 |
| **6i** | 0.065 | **0.033** | 0.047 | 0.063 | 0.047 | >0.636 | >0.636 |
| **6j** | 0.077 | 0.092 | 0.045 | 0.060 | 0.106 | >0.609 | >0.609 |
| **6k** | 0.073 | 0.204 | **0.029** | 0.146 | 0.146 | >0.584 | >0.584 |
| **6l** | **0.028** | 0.140 | 0.057 | 0.070 | **0.028** | >0.561 | >0.561 |
| **6m** | 0.117 | 0.134 | 0.167 | 0.100 | 0.117 | >0.671 | >0.671 |
| **6n** | 0.081 | 0.129 | 0.160 | 0.064 | 0.112 | >0.641 | >0.641 |
| **6o** | 0.092 | 0.153 | 0.062 | 0.076 | 0.046 | >0.613 | >0.613 |
| **6p** | 0.117 | 0.132 | 0.102 | 0.073 | 0.088 | >0.588 | >0.588 |
| **Gentamycin sulfate** | **≤0.017** | **≤0.017** | **≤0.017** | **≤0.017** | **≤0.017** | --- | --- |
| **Fluconazole** | --- | --- | --- | --- | --- | **≤0.033** | **≤0.033** |

[a] The MIC values are interpreted as an average of triplets. MIC: minimum inhibitory concentration (the lowest concentration that inhibited the bacterial growth)

**Table 2** Antimicrobial activity (MBCs in μM)

| Compound | Gram-positive | | Gram-negative | |
|---|---|---|---|---|
| | *B. subtilis* | *S. aureus* | *E. coli* | *P. aeruginosa* |
| **6a** | 0.071 | 0.142 | 0.085 | 0.071 |
| **6b** | 0.034 | 0.136 | 0.052 | 0.084 |
| **6c** | 0.064 | 0.059 | 0.072 | 0.082 |
| **6d** | 0.159 | 0.154 | 0.059 | 0.089 |
| **6e** | 0.023 | 0.023 | 0.061 | 0.061 |
| **6f** | 0.025 | 0.025 | 0.027 | 0.037 |
| **6g** | 0.053 | 0.024 | 0.027 | 0.024 |
| **6h** | 0.035 | 0.040 | 0.020 | 0.050 |
| **6i** | 0.035 | 0.033 | 0.027 | 0.063 |
| **6j** | 0.075 | 0.051 | 0.030 | 0.030 |
| **6k** | 0.024 | 0.154 | 0.028 | 0.058 |
| **6l** | 0.014 | 0.088 | 0.034 | 0.028 |
| **6m** | 0.073 | 0.093 | 0.096 | 0.033 |
| **6n** | 0.032 | 0.116 | 0.096 | 0.032 |
| **6o** | 0.040 | 0.081 | 0.055 | 0.030 |
| **6p** | 0.039 | 0.110 | 0.074 | 0.035 |
| **Gentamycin sulfate** | ≤0.008 | ≤0.008 | ≤0.008 | ≤0.008 |

MBC: minimum bactericidal concentration (the lowest concentration at which no bacterial growth was observed).

In order to give an explanation and understanding of the most potent compounds **6f** against bacteria *Escherichia coli* and **6i** against bacteria *Staphylococcus aureus*, docking studies into the crystal structure of *Escherichia coli* KAS III and *Staphylococcus aureus* KAS III was performed. β-ketoacyl carrier protein synthase (KAS) III is a condensing enzyme that initiates fatty acid biosynthesis in most bacteria and is a key target enzyme to overcome the antibiotic resistance problem. Molecular basis of interactions between target enzyme and synthesized ligands can be understood with the help of docking analysis and interactions as represented in figures 4 and 5. It is pertinent to note that the more active compounds **6f** and **6i** showed good docking interactions figures 4 and 5 for *Escherichia coli* KAS III and *Staphylococcus aureus* KAS III respectively. Glide predictions revealed that the flavonoid derivatives are having greater binding affinity with the KAS III. These in silico findings are well supported by results of antibacterial activity. The binding patterns of compound **6f** in *Escherichia coli* KAS III and compound **6i** in *Staphylococcus aureus* KAS III were depicted in figures 4 and 5 respectively and clearly revealed that the aliphatic ring with oxygens and the aromatic moiety with –C=O showed binding interactions with Arg151 as seen for *Escherichia coli* KAS III with compound **6f** and the same aliphatic ring with oxygen of compound **6i** showed binding interaction with Ser152 of *Staphylococcus aureus* KAS III.

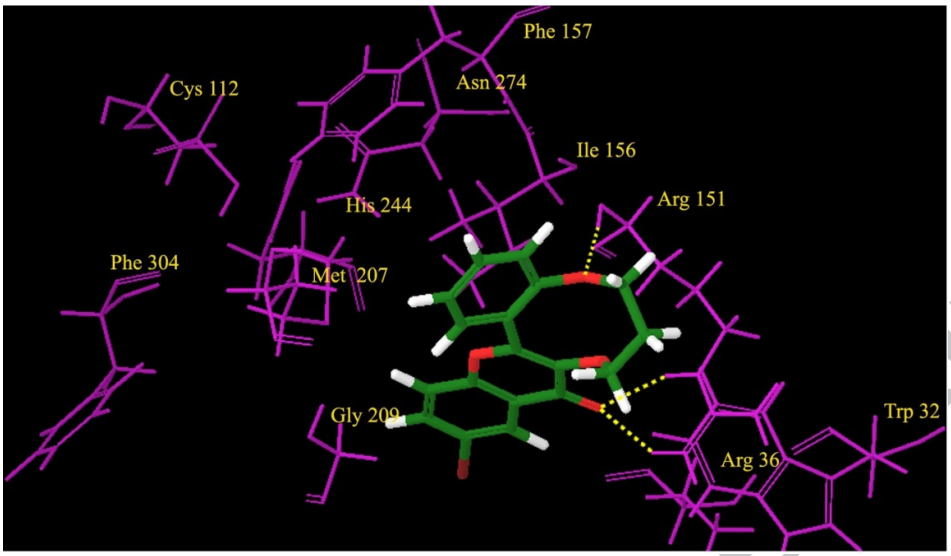



**Figure 4** Binding pose of compound **6f** within the *Escherichia coli* KAS III (1MZS) active site, compound **6f** shown in green colour with oxygens in red

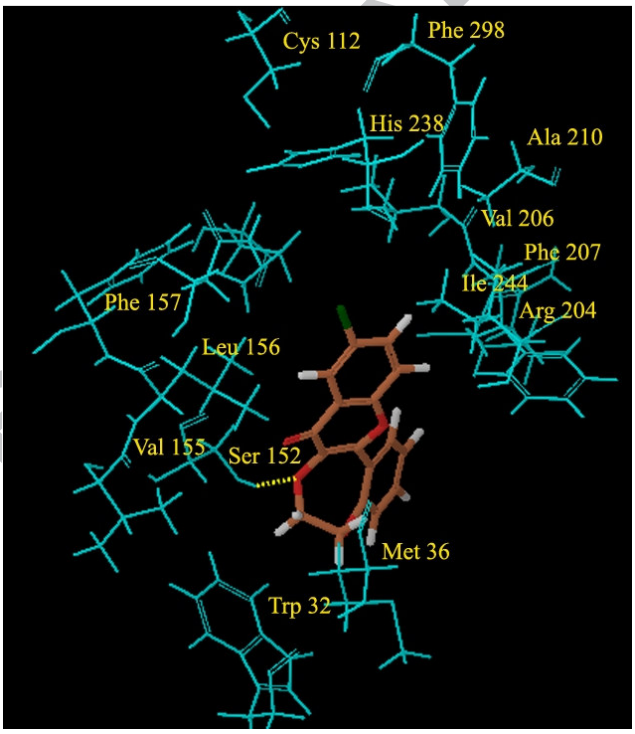



**Figure 5** Binding pose of compound **6i** within the *Staphylococcus aureus* KAS III (1ZOW) active site, compound **6i** shown in orange colour with oxygens in red.

In summary, we have developed an easy synthetic strategy for the synthesis of novel tetracyclic scaffolds incorporating a flavonoid framework with medium sized heterocyclic rings containing two oxygen atoms with moderate to good yields. The reaction protocol requires cheap starting materials and is carried out under mild conditions. The utility of this strategy has been demonstrated by synthesizing a wide variety of tetracyclic derivatives. All the compounds were evaluated for their *in*

*vitro* antimicrobial activity, compounds **6e, 6f, 6h, 6i, 6k** and **6l** were found to be promising with MIC values in the range of 0.027-0.033 µM indicating their potential as novel antibacterial agents. Fitness of most active compounds **6f** against bacteria *Escherichia coli* and **6i** against bacteria *Staphylococcus aureus* into the crystal structure of *Escherichia coli* KAS III and *Staphylococcus aureus* KAS III was studied using in silico tools. Overall, the tetracyclic framework presented here could be an attractive template for the identification of novel antibacterial agents.

**Acknowledgements**

The authors thank The Head, Department of Chemistry, Osmania University, Hyderabad for providing laboratory facilities. AVK thank CSIR for PhD fellowship. We thank CFRD analytical team for providing spectral analysis facilities.

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