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Emerging Therapeutic Discovery Against *Escherichia coli* Infection by Rational Inhibition of Fatty Acid Responsive Transcription Factor: Theoretical Molecular Investigations of Some Metabolites as Inhibitors of Fatty Acid Metabolism Protein

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Abstract

For the discovery of effective agent for overcoming resistance against Escherichia coli (E. coli) bacteria by modulating Fatty Acid Metabolism Protein (FadR)—a Fatty Acid Responsive Transcription Factor. The present study involved discovery of some metabolites of furan, furan-2-yl, macrolactin, and chromene scaffold via in silico structure-based drug design (SBDD) technique by utilizing the Maestro 9.1 software. The study will provide clues, motivate, and throw light on imperative aspects for the globally working medicinal chemists in the rational designing of anti-infective drugs in the near future.

Keywords: *Chromene, FadR, furan, furan-2-yl, macrolactin*

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INTRODUCTION

Escherichia coli (*E. coli*) bacteria normally live in the intestines of healthy people and animals. Most varieties of *E. coli* are harmless or cause relatively brief diarrhea. But a few particularly nasty strains, such as *E. coli* O157:H7, can cause severe abdominal cramps, bloody diarrhea and vomiting. In contrast, the other strains that cause intestinal sickness are called Shiga toxin–producing *E. coli* (STEC) after the toxin that they produce [1]. In *E. coli*, a number of antimicrobial targets have been identified by the researchers, from which fatty acid metabolism protein (FadR) is an emerging and attractive biological target to develop drug candidates [2].

Fatty acids are essential components of membranes and are important sources of metabolic energy in all organisms. Thus, fatty acid degradation and biosynthesis pathways must be switched on and off according to the availability of fatty acids to maintain membrane lipid homeostasis [3]. The regulation of these pathways has been mainly studied in a model prokaryote, *E. coli*. The FadR protein of *E. coli* has been shown to play a dual role in the transcription of the genes of bacterial fatty acid metabolism [4]. The protein acts as a repressor of β-oxidation and an activator of unsaturated fatty acid synthesis. FadR DNA binding is antagonized by long-chain acyl-CoAs, and thus FadR acts as a sensor of fatty acid availability in the environment [5]. Inhibition of *E. coli* FadR represents an effective way of controlling the infection.

The goal of the present investigation was to identify molecules that could inhibit the activity of bacterial proteins, ultimately causing decreased growth of the bacteria. This was attempted by identifying certain metabolic processes or functions within the bacterial cell that, if inhibited, could slow growth, interrupt gene expression, or inhibit protein function. We screened various furan, furan-2-yl, macrolactin, and chromene-based compounds via *in silico* structure-based drug design (SBDD) technique by utilizing the Maestro 9.1 software.

MATERIALS AND METHODS

Preparation of Protein

The protein target FadR was selected based on the availability of crystal structures and as per the reports from the previous docking studies. The crystal structures of the FadR enzyme with ligand-bound X-ray structures available in the Protein Data Bank (PDB ID: 1H9G, 2.20 Å X-ray resolution) was retrieved. The PDB file contained a single unit of the FadR enzyme cocrystallized with a native acyl-COA inhibitor. The protein target was prepared using the protein preparation tool in Maestro 9.1. The hydrogens were added to the protein at a pH 7 and the water molecules were removed beyond 5 Å distance. The energetically accessible ionization and tautomeric states was generated using Epik tool. The optimization of hydrogen bonding network in the complex was performed and the hydroxyl torsions were optimized. The RMSD cutoff of 0.3 Å was set and finally, the protein–ligand complex was subjected to minimization using "inpreff" tool in the protein preparation wizard [6].

Ligand Preparation

The 2D structures of the inhibitor compounds were drawn using ChemDraw®Ultra and saved as Mol files. Both the ligands were prepared for docking using the LigPrep program of Schrodinger® Maestro software. A protonated form of each molecule at pH 7.0 was calculated by using LigPrep. All the hydrogen atoms were added and the ligands were submitted in full structure optimization. The minimization procedure was carried out by using energy function OPLS 2006 force field. The minimized ligand file was prepared by LigPrep program. All the structures were checked to be correct and ionization states were generated for all the inhibitors by taking into account the metal mode of Epik ionizer [7].

Induced-Fit Docking Study

Molecular docking is a method used to model the three-dimensional structure of a protein target, and members of large libraries of compounds whose chemical–physical properties are known are fit into the targeted structure. The flexibility of both ligand and receptor from the docking study can be possible through induced-fit docking (IFD) studies using Schrodinger Maestro®. In the IFD protocol, ligands were docked into the flexible protein using the Glide tool; it provides the exact orientation of ligand–protein complex. The Van der Waals radii scaling of 0.8 was set for the proteins. Ten poses of each ligand were used to interact with the protein. The flexibility of protein was considered by selecting residues having at least one atom within 5 A° of any of the 10 ligand poses which were subject to a conformational search and energy minimization process. The residues outside this zone were fixed and the resulted best scored pose was taken forward for redocking and Glide XP (extra-precision) was used for all the docking calculations. The binding affinity of each protein–ligand complex was reported in terms of Glide Score [8].

RESULTS AND DISCUSSION

The molecular docking method was applied to the inhibitors to build a binding affinity model. Glide score, hydrogen bonds, Van der Waal's interactions, π-cation interaction, and π-π stacking interactions were the main parameters used for the prediction of results. The affinity of ligands towards proteins was calculated based on the above parameters. The more negative value of the G-score indicates good binding affinity. The docking results demonstrated several key features: higher Van der Waals interactions represented that the ligands have a large number of bulky groups, the presence of more hydrogen bonding interaction signifies that the inhibitor have a good binding affinity with the molecular target to exhibit antagonist activity. Hence, the IFD module could be applied to predict the binding interactions between inhibitors and protein.

Furan-Based Compounds

While coming to the results obtained from IFD, the generated model was found to be very close to the native ligand in the crystal 1H9G. The best pose of compound **1** expressed the potent FadR inhibition with Glide score of -12.57 kcal/mol whereas the score of compound **2** was found to be -10.20 kcal/mol. Based on the crystal structure of the molecular target, the compound **1** formed a strong hydrogen bond with Asp145 via oxo-group of the furan moiety in docking complex. The

compound also illustrated hydrophobic residues of π-π stacking interactions with Phe180 and Tyr172 with the furan ring. The positively charged binding site amino acid residue Arg105 of the crystal structure was involved in the formation of π-cation interaction. The interaction of compound **1** and FadR are described in Figure 1. By the docking study of the compound **2**, the top scored pose was selected. The structures and interaction modes of compound **2** are also shown in Figure 1. The IFD scores of compound **2** (IFD score of -10.20 kcal/mol) was observed to be lower than compound **1**, which indicates that from the selected inhibitors, compound **1** would have higher FadR inhibition activity in practical *in vivo* studies. The inhibitor interacted with the Tyr172 with furan ring forming a π-π stacking interaction. The oxygen group of furan moiety was also detected linked to the positively charged binding site amino acid residue Arg105 through π-cation interaction. As compared with the compound **1**, the compound **2** seemed to be less active and the reason for the higher activity of the former molecule was due to the hydrogen bonding, hydrophobic, polar, and charged interactions. The surface of protein around the ligand surface is also portrayed in Figure 1. Both the identified compounds were found to *in silico* inhibit the molecular target FadR protein of *E. coli* using the molecular docking technique which may be correlated with reducing the growth of the microbe. From Figure 1, it was clear that both the compounds were surrounded by the hydrophobic amino acid residues. There was no solvent exposure can be seen which state that both the compounds were able to penetrate deeper into the active site binding cleft of protein. Additionally, the smaller size of the ligands may enable them to penetrate deeper into the cavity. The visualization of ligand at the cavity was not possible while applying surface representation because the amino acid residues masked fully around the ligand. The stable fitting of ligands provides maximum stability through Van der Waals contacts. Therefore, it may be concluded that these compounds had a wide range of activity throughout the *E. coli* species as proved by the docking studies. The results obtained from the docking studies of both the furan metabolites are depicted in Table 1.

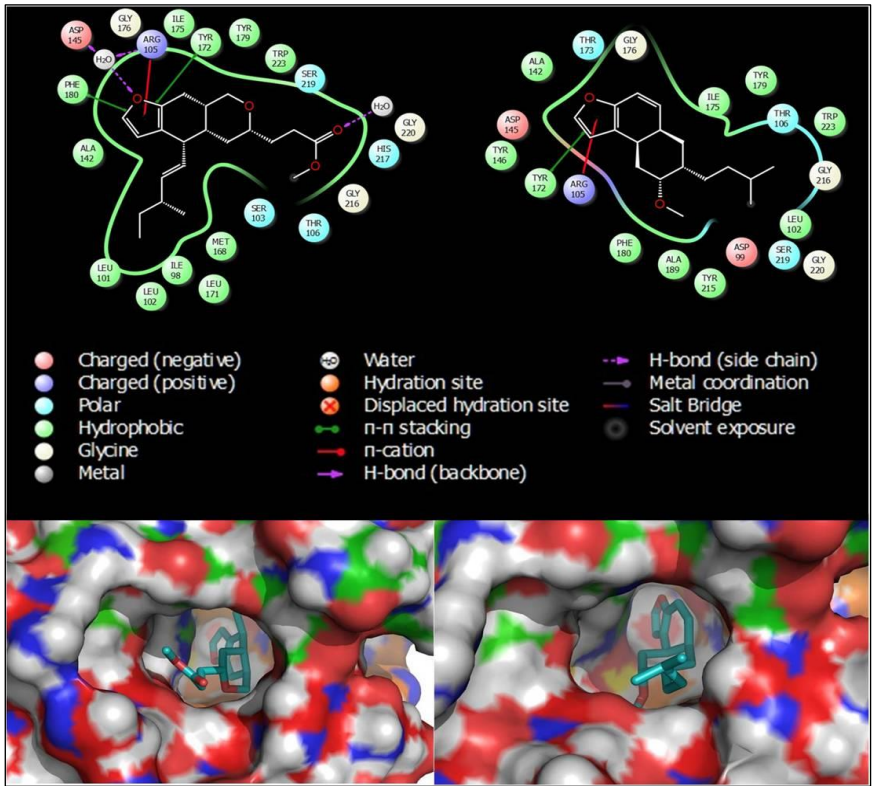

Fig. 1: *The Structures and Interaction Modes of Compound 1 and 2 with FadR (Above). IFD-Generated Surface Model of Compound 1 and 2 at the Active Site Binding Cleft of FadR (Below).*

Table 1: *Details Obtained from the IFD Studies of Various Compounds.*

Inhibitors	Glide score (kcal/mol)	Hydrogen bond interacting residues	Number of VdW contact
Compound 1	- 12.57	Asp145, Arg105, Phe180 and Tyr172	2003
Compound 2	- 10.20	Arg105 and Tyr172	2369
Compound 3	-13.23	Asp145	1819
Compound 4	-13.68	Asp145 and Leu165	1733
Compound 5	-9.32	Arg213, Asn100, and His217	702
Compound 6	-9.87	Arg213, Tyr179, and Gln96	565
Compound 7	-12.83	Tyr215 and Asp145	2634
Compound 8	-9.29	Thr106	2391

Glidescores, number of H-bond interacting residues, residues involved in π-cation interaction, residues involved in π-π staking interaction, and number of VdW contacts of the best pose for each compounds are demonstrated only.

Furan-2-yl Based Compounds

The molecular docking revealed that both the compounds formed hydrogen bonding successfully with the active site of FadR. The *in silico* docking study demonstrated notable inhibition of the bacterial metabolic target by the furan-2-yl containing compounds. The best pose of compound **3** stated the most effective FadR inhibition with Glide score of -13.68 kcal/mol whereas the score was found to be -13.23 kcal/mol (Table 1). Based on the crystal structure of the molecular target, the compound **4** formed a strong hydrogen bond with Asp145 via hydroxyl-group of the furan moiety in docking complex whereas the branched oxygen atom present in the hydroxyl group of compound **4** made hydrogen bonding with the amino acid residue Leu165. The water molecule present in the protein also formed hydrogen bonding with the Asp145 (Figure 2). This interaction might give flexibility as well as the force the ligands to penetrate at the active site cavity. The Van der Waals contacts provided maximum stability by offering steady fitting of the ligands in the active site. The molecule **3** seemed to be less active and the reason for the higher activity of the former molecule was due to the hydrogen bonding, hydrophobic, polar, and charged interactions. No solvent exposure were detected which reflected that the compounds penetrated into the active site of the protein.

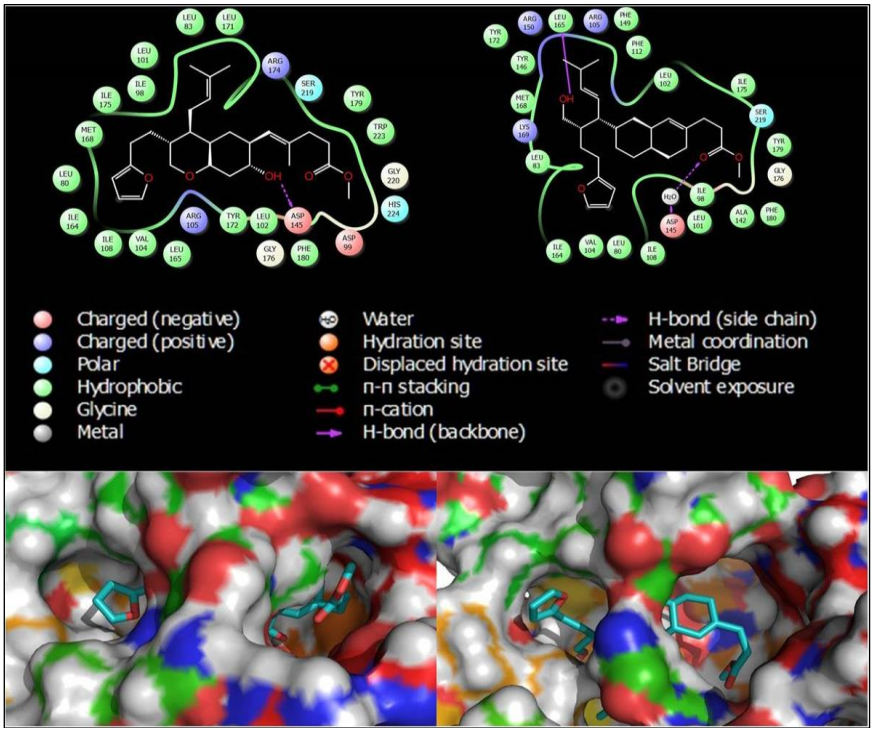

Fig. 2: *The Structures and Interaction Modes of Furan-2-yl Containing Compounds 1 and 2 with FadR (Above). IFD-Generated Surface Model of Compound 1 and 2 at the Active Site Binding Cleft of FadR (Below).*

Macrolactin-Based Compounds

The IFD docking method was applied to the inhibitors in order to build a binding affinity model. The purpose of this docking was to generate theoretical binding scores and to predict the possible interaction of the macrolactin structures (compounds **5** and **6**) with FadR. These two compounds seem to be structurally very similar and the established automated molecular docking with the biological target. Mainly, three parameters for the prediction of results were considered; the G-score, hydrogen bonds, and Van der Waals interactions. Both the macrolactins were docked at the enzyme active site to predict the inhibitory activity which displayed the IFD binding scores of -9.32 kcal/mol and -9.87 kcal/mol (Table 1), respectively, as calculated by the Glide module. Primarily, three hydrogen bond interactions were found between FadR with ligand **5**. The two oxygen atoms of the ligand made the hydrogen bond with amino acid residues Arg213 and Asn100. The hydroxyl group also participated in the hydrogen bond formation with His217 residue through a water molecule placed near the enzyme active site (Figure 3). The attention has been focused on the most prominent binding pose from the characteristic receptor–ligand interactions for macrolactin**6** as it is the most active compound. The hydroxyl groups of the ligand demonstrated two prominent interactions with the Arg213 and Tyr179 residues along with the formation of one hydrogen bond by oxygen atom with Gln96 residue. Additionally, one π-cation interaction with the aromatic ring present with the Arg105 was displayed. From the results, it was clear that there was slight variation in the Glide scores and energies of the complexes.

Chromene-Based Compounds

In order to validate and provide understandable evidence for the antibacterial activity of the compounds, the induced-fit molecular docking studies had been carried out. It is a well-established technique used to determine the interaction of molecules with their target protein and find the best orientation of ligand would form a complex with overall minimum energy. Both the compounds were docked at the centroid of the protein structure of 1H9G in complex with acyl-COA. The *in silico* studies revealed that the compounds demonstrated a good Glide score toward the target protein, i.e., -12.83 kcal/mol and -9.29 kcal/mol, respectively for the ligand **7** and ligand **8**. Compound **7** showed higher inhibitory activity than compound **8** based on the *in silico* docking scores. In the first case, Tyr215 and Asp145 made water-mediated hydrogen bonds with the keto group present in the aromatic ring. Additionally, Trp223 and Tyr179 made π-π interaction with the same aromatic ring (Figure 4). Compound **7** showed higher Glide score, which enter deep into the cavity and fully surrounded by the hydrophobic residues.

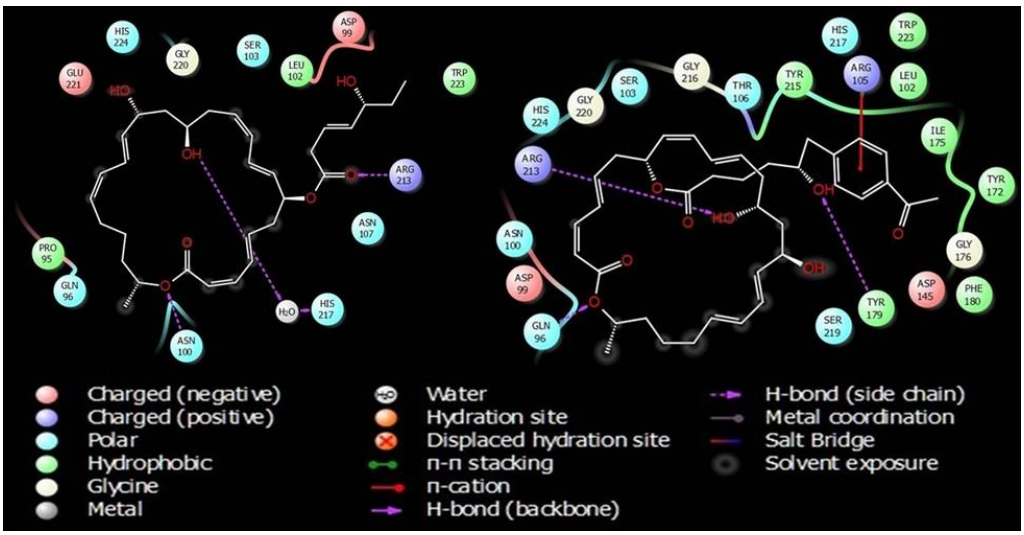

Fig. 3: *Interaction of Macrolactin Compounds at the Active Site of FadR. The Hydrogen Bonding Interactions are Indicated by the Violet Lines.*

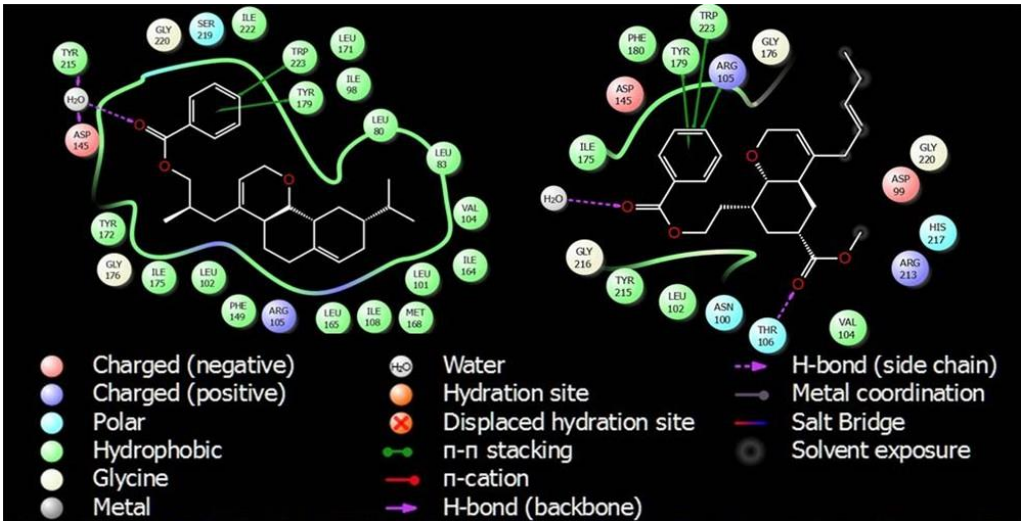

Fig. 4: *Docking Poses of Substituted Chromene Derivatives as FadR Inhibitors.*

In the case of Compound **8**, Thr106 made a hydrogen bond (side chain) directly with the keto group present in it. In addition to it, Tyr179, Trp223, and Arg105 made π-π interactions with the aromatic ring provided structural stability to the inhibitor. Therefore, the influence of hydrophobic interactions was encountered by both the chromene-based inhibitors. Furthermore, the presence of a large number of Van der Waals contact of 2634 (ligand **7**) and 2391 (ligand **8**) provided better stability of the molecular complex. It may also be believed that the hydrophilic, steric, hydrophobic, polar, and charged interactions do contribute high stability to the docking complex. An idea regarding the type of substituents and their positions was derived essentially from the study to design better antibacterial compounds of this scaffold.

CONCLUSION

The present study involved identification of novel furan, furan-2-yl, macrolactin, and chromene-based compounds as potent antibacterial or more specific anti *E. coli* agents. It was clear from this research that both the compounds perfectly bound with the active site cavity of the biological target. The inhibitors demonstrated hydrogen bonding interactions directly as wells as through water molecules with the amino acid residues, and thus they could penetrate deeper into the active site cavity. These may provide the compounds a better orientation and can inhibit them complementary to the active site. Therefore, the research will undeniably motivate the modern day (medicinal) chemists and biologists to further explore and study the better halves of applications.

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