ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

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Molecular docking study on quercetin derivatives as inhibitors of Pantothenate Synthetase (PanC) of *Mycobacterium tuberculosis*

Premalatha E[1], Dineshraj R[1], Iyanar Kannan[*1], Bhaarath KS[2], Sharavanan TKV[3]

[1]Department of Microbiology, Tagore Medical College and Hospital, Rathinamangalam, Chennai – 600127, Tamil Nadu, India
[2]CRRI, Tagore Medical College and Hospital, Rathinamangalam, Chennai – 600127, Tamil Nadu, India
[3]Department of General Medicine, Tagore Medical College and Hospital, Rathinamangalam, Chennai – 600127, Tamil Nadu, India

Article History:

Received on: 21 Mar 2020
Revised on: 18 Apr 2020
Accepted on: 22 Apr 2020

Keywords:

Tuberculosis, molecular docking, Pantothenate Synthetase (PanC), Quercetin derivatives, AutoDock

ABSTRACT

The anti-TB drugs currently in the use are insufficient to address these major health challenges. Hence, it is imperative to discover and develop new and efficient drugs against TB. The enzyme pantothenate synthetase (PS or PanC), necessary for the production of pantothenate (vitamin B5), critical components of fatty acid synthesis, when inhibited will in turn affect the cell wall synthesis of bacilli. In the present study, an attempt will be made to find the drug like molecules from quercetin derivatives prepared *in silico* to find out possible inhibitors of PanCof *M. tuberculosis*. The 3D structure of PanC was obtained from RCSB database and quercetin from ZINC database. The derivatives of quercetin were prepared and were docked initially with iGEMDOCK docking tool. The final docking was done in AutoDock vina software. The ADMET properties of the selected ligands were done in admetSAR online server tool. The present study revealed that four derivatives of quercetin has excellent binding with Pantothenate Synthetase (PanC) of *M. tuberculosis.* These derivatives can be taken for *in vitro* enzymatic assays for its inhibitory property in the search for new anti-TB drugs

[*]Corresponding Author

Name: Iyanar Kannan
Phone: 044-9840520950
Email: dr.ikannan@tagoremch.com

ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v11i3.2529

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INTRODUCTION

The tuberculosis (TB) caused by *Mycobacterium tuberculosis* is one of the important bacterial infections that lead to millions of deaths all around the world (KJ *et al.*, 2004). The TB is seen high in many developing countries where, with AIDS and immunocompromised diseases are common. The current treatment for TB has a prolonged course of antibiotics, which normally associated with poor patient compliance (Tomioka and Namba, 2006). This has now let to emergence of multi-drug resistant (MDR) and extensively drug resistant (XDR) strains of *M. tuberculosis* (WHO, 2014). In 2009, a new strain of "Totally drug resistant Tuberculosis" (TDR) emerged in some parts of the world (Velayati *et al.*, 2009). These TDR strains showed *invitro* resistance to all first- and second-line drugs. In 2012, the first TDR strain was reported in India (Udwadia *et al.*, 2012). The anti-TB drugs currently in the use are insufficient to address these major health challenges. Hence, it is imperative to discover and develop new and efficient drugs against TB (Zumla *et al.*, 2013). The characteristics feature of *M. tuberculosis* is its lipid rich cell wall, which helps the bac-

teria of its intracellular survival and its pathogenicity. The cell wall synthesis pathway is a promising target for new anti-TB drug discovery (Jackson *et al.*, 2013; Cole *et al.*, 1998). The enzyme pantothenate synthetase (PS or PanC), is a key enzyme needed for the biosynthesis of coenzyme A (CoA). It produces pantothenate (vitamin B5), which is aprecursor for the biosynthesis of CoA. The CoA is a critical component of fatty acid synthesis (Abrahams *et al.*, 2012). If this enzyme is inhibited, the fatty acid synthesis of *M. tuberculosis* will be affected which in turn will affect the cell wall synthesis (Sambandamurthy *et al.*, 2002).

It is an established fact that the phytochemicals present in the plants are important source for the discovery and development of anti-microbial drugs (Zandi *et al.*, 2009, 2011; Chiu *et al.*, 2012). Medicinal Plants contains naturally occurring phytochemicals (Calixto, 2000). Medicinal plants are rich in many types of phytochemicals like polyphenols and flavonoids proven to have many medical properties with drug like properties (Jassim and Naji, 2003). Quercetin, a flavonoid present in many medicinal plants, has been proved to have many medicinal properties and drug likeliness (Parasuraman *et al.*, 2016).

Molecular docking is the *insilico* technique that looks for the best drug like molecules that can fit in the binding site of the drug targets and thus helps in drug development for various diseases (Rajamani and Good, 2007). The first *in silico* aided drug that came commercially is dorzolamide in 1996 (Kubinyi, 1999). Since then many drugs are developed successfully by this method and are being used as the treatment for various diseases including cancer.In the present study, an attempt will be made to find the drug like molecules from quercetin derivatives prepared *in silico* to find out possible inhibitors of Pantothenate Synthetase (PanC) of *M. tuberculosis*.

MATERIALS AND METHODS

Target Protein preparation

The three-dimensional structure of Pantothenate Synthetase (PanC) (Figure 1) of *M. tuberculosis*was retrieved from RCSB-PDB database (www.rcsb.org) and was saved in the .pdb format. Its PBD code is 1N2H.

Active site prediction

As there is no three-dimensional structure of protein-ligand complex in the RCSB database for PanC, the 3DLIGANDSITE online server tool was used to determine the possible binding sites. The protein was uploaded in the server which gave various binding sites in the order of ranking (Wass *et al.*, 2010). The binding site which was ranked first was chosen for the study.

Figure 1: Structure of Pantothenate Synthetase (PanC)

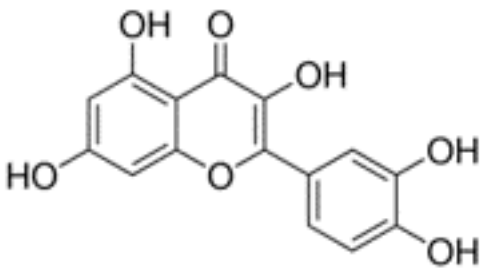

Figure 2: Structure of Quercetin

Generation and optimization of Ligand

The structure of quercetin was obtained from ZINC database (Figure 2). Its zinc ID is ZINC-33980813. Its IUPAC NAME is (2*S*)-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-2*H*-chromene-3,4-dione with a molecular weight of 302.2357 g/mol. The ACD chemsketch software tool was used to prepare the derivatives of quercetin.

The quercetin was opened by the software and its side chains were modified to prepare the derivatives. The central ring structure was not changed during the preparation of the derivatives. The derivatives were saved in *.mol* format. Later, the derivatives are converted to *.pdb* format in Open Babel software.

Table 1: Docking results of Quercetin derivatives by iGEMDOCK

S.No	Ligand	Total binding energy (kcal/mol)	Vander waals force (kcal/mol)	H bond (kcal/mol)	Electrostatic bond (kcal/mol)
1.	(2S)-5,7-dihydroxy-2-{3-hydroxy-4-[(4-methylphenyl) sulfonyl] phenyl}-2H-chromene-3,4-dione	-131.572	-112.779	-18.7922	0
2.	3-amino-4-{5-[(2)-5,7-dihydroxy-3,4-dioxo-3,4-dihydro-2H-chromen-2-yl]-2,3-dihydroxyphenyl}-4-oxobutanamide	-123.464	-100.274	-23.1897	0
3.	(2S)-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3,4-dioxo-3,4-dihydro-2H-chromene-6-sulfonic acid	-130.351	-93.0331	-37.3177	0
4.	Benzyl (2S)-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3,4-dioxo-3,4-dihydro-2H-chromene-6-carboxylate	-129.767	-105.356	-24.4109	0

Table 2: Docking results of Quercetin derivatives using Auto Dock vina

S.No	Ligand	Total binding energy (kcal/mol)	RMSD lower bond	RMSD upper bond
1.	(2S)-5,7-dihydroxy-2-{3-hydroxy-4-[(4-methylphenyl) sulfonyl] phenyl}-2H-chromene-3,4-dione	-9.0	0.0	0.0
2.	3-amino-4-{5-[(2S)-5,7-dihydroxy-3,4-dioxo-3,4-dihydro-2H-chromen-2-yl]-2,3-dihydroxyphenyl}-4-oxobutanamide	-8.0	0.0	0.0
3.	(2S)-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3,4-dioxo-3,4-dihydro-2H-chromene-6-sulfonic acid	-8.3	0.0	0.0
4.	Benzyl (2S)-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3,4-dioxo-3,4-dihydro-2H-chromene-6-carboxylate	-8.0	0.0	0.0

Rough docking

The rapid docking of the derivatives with the protein was performed in softwarei GEMDOCK v2.0 (Yang and Chen, 2004). Under docking option, the rough docking was selected with used a population size of 150 with 70 generation. After docking the binding energy and binding pose of each derivatives were analysed. The best derivatives in terms of binding energy and pose were further taken for docking in Autodock vina.

Protein-ligand docking

The docking of ligands was performed using AutoDockvina software using PyRx as GUI (Morris *et al.*, 2009). First the protein was loaded in software and it was prepared for docking. During this process, the protein will be converted from *.pdb* format to *.pdbqt* format, a format needed for AutoDockvina for docking. Similarly, the derivatives (ligands) were also loaded and was converted to *.pdbqt* format. Before docking, the grid box was placed around the binding site so as enable the ligands binds only inside the grid box ie., in the binding site. After the grid box was set, the docking simulations were run for all the ligands. The AutoDock vina calculates the energy values using the Lamarckian Genetic Algorithm (LGA) algorithm. A total of eight binding configurations was generated for each ligand and were arranged according to their root mean square deviation (RMSD) values.

Table 3: ADMET properties of Quercetin derivatives

S.no	Ligand	Drug like-ness	Mutagenic	Tumorigenic	Irritant
1.	(2S)-5,7-dihydroxy-2-{3-hydroxy-4-[(4-methylphenyl)sulfonyl]phenyl}-2H-chromene-3,4-dione	7.131	No	No	No
2.	3-amino-4-{5-[(2S)-5,7-dihydroxy-3,4-dioxo-3,4-dihydro-2H-chromen-2-yl]-2,3-dihydroxyphenyl}-4-oxobutanamide	1.208	No	No	No
3.	(2S)-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3,4-dioxo-3,4-dihydro-2H-chromene-6-sulfonic acid	0.079	No	No	No
4.	benzyl (2S)-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3,4-dioxo-3,4-dihydro-2H-chromene-6-carboxylate	0.170	No	No	No

The binding confirmation ranked one was selected as the best in terms of binding energy and pose.

Visualization

The docking pose of Quercetin derivatives were visualized and analysed using software pymol software.

ADMET properties

The adsorption, distribution, metabolism, excretion and toxicity of the derivatives were analysed by admetSAR - 2.0 server tool.

RESULTS

Among several flavonoids, Quercetin was chosen and its 500 derivatives were created using Chemsketch software. The initial quick docking was done with software iGEMDOCK. Four derivatives were selected based on the binding energy and docking pose (Table 1). The derivates with a high total binding energy with good H bond strength were selected. The (2*S*)-5,7-dihydroxy-2-{3-hydroxy-4-[(4-methylphenyl) sulfonyl] phenyl}-2*H*-chromene-3,4-dione showed a total binding energy of -131.572 kcal/mol with H bond energy of -18.7922 kcal/mol. The derivative 3-amino-4-{5-[(2)-5,7-dihydroxy-3,4-dioxo-3,4-dihydro-2H-chromen-2-yl]-2,3-dihydroxyphenyl}-4-oxobutanamide produced a total binding energy of -123.464 kcal/mol and H bond energy of -23.1897 kcal/mol. Another derivative, (2*S*)-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3,4-dioxo-3,4-dihydro-2*H*-chromene-6-sulfonic acid too showed a good total binding energy of -130.351 kcal/mol with H bond energy of -37.3177 kcal/mol. Benzyl (2*S*)-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3,4-dioxo-3,4-dihydro-2*H*-chromene-6-carboxylate also showed a high total binding energy of -129.767 kcal/mol and a good H bond energy of -24.4109 kcal/mol. For the validation of the results, the *z-score* value was used which measures the interaction conservation between the interacting groups and the screening derivatives.

The four derivatives were further subjected to docking with AutoDock vina with PyRx GUI. TheRMSDvalue was taken for validation for selecting best binding energy and docking pose among eight combinations of output given by AutoDock for a single ligand. The docking energies of four ligands were found to be excellent (Table 2). The derivatives, (2*S*)-5,7-dihydroxy-2-{3-hydroxy-4-[(4-methylphenyl) sulfonyl] phenyl}-2*H*-chromene-3,4-dione, 3-amino-4-{5-[(2*S*)-5,7-dihydroxy-3,4-dioxo-3,4-dihydro-2*H*-chromen-2-yl]-2,3-dihydroxyphenyl}-4-oxobutanamide, (2*S*)-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3,4-dioxo-3,4-dihydro-2*H*-chromene-6-sulfonic acid and Benzyl (2*S*)-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3,4-dioxo-3,4-dihydro-2*H*-chromene-6-carboxylate showed the binding energy values of -9.0 kcal/mol, -8.0 kcal/mol, -8.3 kcal/mol and – 8.0 kcal/mol respectively. The RMDS values of all the selected derivatives were zero.

The docking poses of the derivatives with the PanC is shown in Figure 3. All selected ligands were shown to fit into the binding pocket of the drug target. The ADMET properties the ligands were analysed byadmetSAR server tool. All the selected four derivatives satisfied important properties (Table 3). The treatment and management of drug sensitive TB is successful and brought down the TB cases under control.

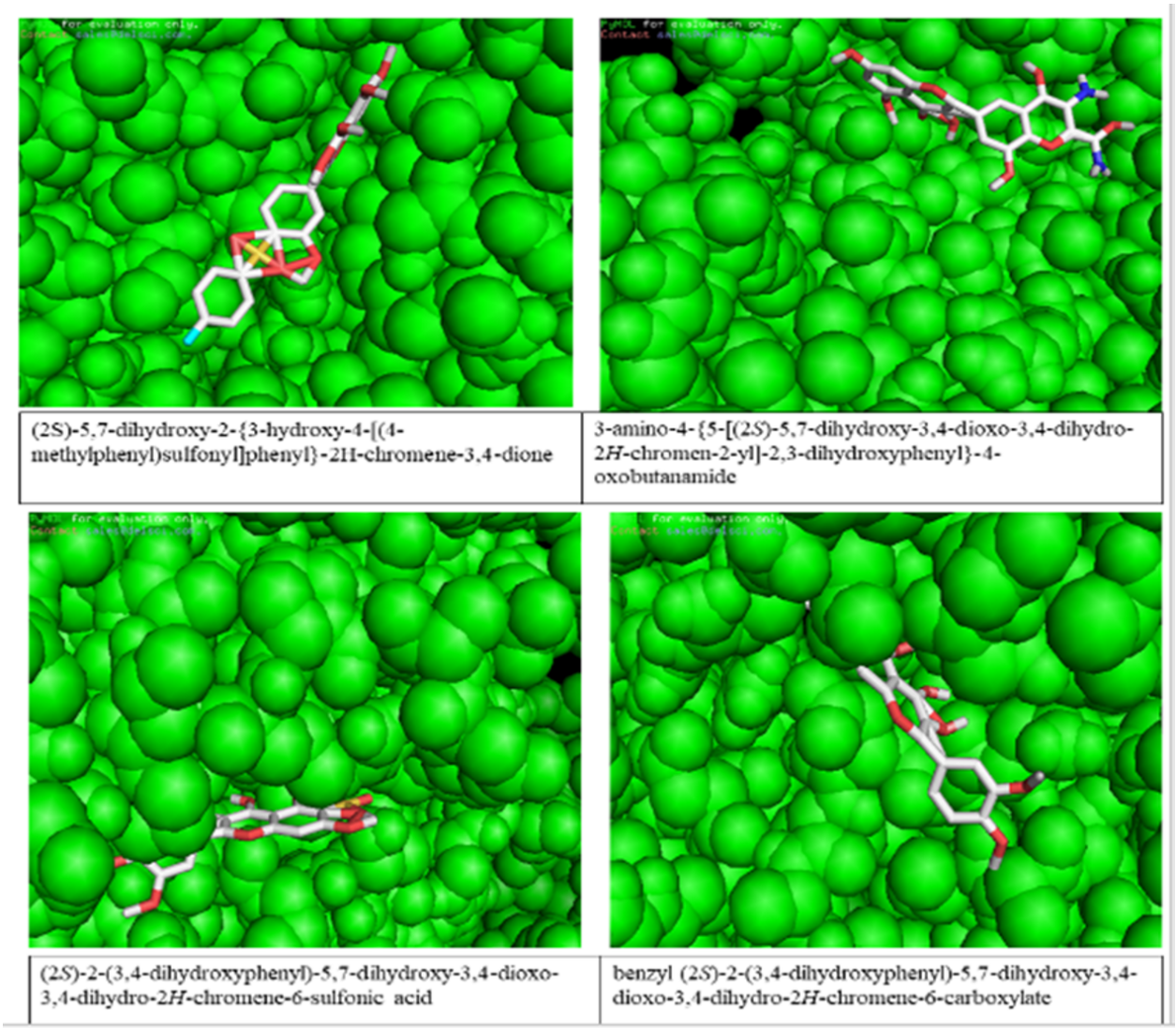

Figure 3: Docking pose of the selected four ligands with drug target PanC seen by Pymol software

However, the continuous emergence of TDR and XDR TB poses threat and challenge in the management of TB caused by drug resistant *M. tuberculosis* (Caminero, 2010). The discovery of streptomycin and subsequent use of it in the treatment of TB is not successful as the bacilli rapidly developed drug resistance (Council, 1948). This led to the strategy of developing combination therapy using at least two active drugs in the treatment of TB (Medical Research Council Investigation , 1952). Soon the multidrug-resistant (MDR) TB started to emerge wherein the TB bacilli showed resistant to isoniazid (INH) and rifampin (Zignol *et al.*, 2006). Further, some MDR bacilli has been found to be resistant for any fluoroquinolone and to at least one of three injectable agents (kanamycin, amikacin, or capreomycin) which are categorised as extensively drug-resistant TB (XDR-TB).

After the drug resistance was identified, the underling mechanisms were explored and was found to be due to spontaneous mutations in the gene of drug targets and failure of binding of drug to the binding site of drug target (Miotto *et al.*, 2015). Hence, various studies are being done to explore new drug targets and discovering potential drug that can bind to them (Abrahams *et al.*, 2012). In the present study, the enzyme pantothenate synthetase has been used as the drug target to derive drug like molecules that can bind and inhibit the activity of the enzyme thereby can kill or inhibit the TB bacilli.

Few similar studies with other drug targets were done. In a study conducted by (Shilpi *et al.*, 2015), it was found that ellagic acid derivatives from *Ludwigiaadscendens* and *Trewianudiflora* showed excellent binding ability with MabA of *M. tuberculosis*.

CONCLUSIONS

The molecular docking technique has paved way to discovery new drug like molecules for the treatment

of TB. In the present study, the quercetin derivatives were prepared and subjected to the molecular docking technique in the quest to find anti-TB drug like molecules. The present study revealed that four derivatives of quercetin has excellent binding with Pantothenate Synthetase (PanC) of *M. tuberculosis.* These derivatives can be further taken for *in vitro* enzymatic assays for its inhibitory property in the search for new anti-TB drugs.

Conflict of Interest

None.

Funding Support

None.

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