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ORIGINAL RESEARCH

Molecular docking studies on the activity of naturally occurring pyranochalcones on the transcriptional regulator enzyme of *Pseudomonas putida*

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Satya B Paul[1]
Sudip Choudhury[2]

[1]Department of Chemistry, Assam University, Silchar-788011, Assam, India; [2]Department of Chemistry, Gurucharan College, Silchar-788004, Assam, India

Abstract: The paper reports the *in-silico* docking results of 10 naturally occurring pyranochalcones on the transcriptional regulator (TtgR) enzyme, which is a key efflux pump TtgABC operon repressor in the Gram-negative bacteria *Pseudomonas putida* (DOT-T1E strain) as the receptor. TtgR is a multidrug-binding protein and regulates one of the key mechanisms of its antibiotic resistance by active extrusion of toxic compounds through the membrane-bound efflux pumps. Although the bacteria exhibits resistance against a number of antibiotics, one natural pyranochalcone *Pongachalcone I* has been reported to be active against it. The presence of alkoxy moiety in the aromatic side unit of the pyranochalcones seems to be instrumental in binding

Keywords: TtgR, docking, *in-silico*, antibiotic resistance

Introduction

The growing population of antibiotic resistance strains of bacteria has become one of the major challenges to be addressed in the arena of research in drug design and discovery. Resistance to antimicrobials is as a result of three main strategies namely enzymatic inactivation of the drug, modification of target sites and extrusion by efflux.[1–3] The active efflux of toxic compounds is one of the common mechanisms employed by bacteria to protect themselves against the deleterious effects of toxic molecules they encounter in the environment.[4]

The DOT-T1E strain is interesting for its particularly high resistance to toxic organic solvents and three RND efflux pumps, TtgABC, TtgDEF, and TtgGHI, were found to be essential for this resistance. TtgABC has been shown to play an important role in the intrinsic tolerance of *Pseudomonas putida* DOT-T1E to organic solvents.[4]

Pyranochalcones are widely distributed naturally occurring flavonoids. A number of pyranochalcones have been reported to exhibit antimutagenic, antimicrobial, anti-ulcer and antitumor activities.[5] *Pongachalcone I* was isolated from *Tephrosia deflexa*, and it has been shown to have antibacterial activity against the bacteria *P. putida*.[5,6] This wide range of biological properties has stimulated interest in the synthesis of naturally occurring pyranochalcones.

Motivated by that, investigation on the interaction of different naturally occurring pyranochalcones on the transcriptional regulator (TtgR) enzyme of *P. putida* were carried out by the authors, as the enzyme seems to be the key component in sensing

Correspondence: Sudip Choudhury
Department of Chemistry, Gurucharan College, Silchar-788004, Assam, India
Tel +91-9435883166
Email sudipch1@gmail.com

and active expulsion of chemicals toxic to the bacterium, which is reported in the present work.

Material and methods

The substrate

The HTH-type transcriptional regulator TTgR (from Protein Data Bank code: 2UXI) in *P. putida* (bound with phloretin) was taken as the substrate for docking (Figure 1). This substrate was chosen because

- TtgR is a multidrug-binding protein which represses the transcription of TtgABC. It triggers the pumping out of the toxic materials making the organism resistant to antibiotics, solvents and toxic plant secondary products.
- Searches of the Protein Data Bank databases reported only TtgR to bind with the plant-derived flavonoid, quercetin. Interestingly, pyranochalcones possess the structural features of both quercetin, the flavonoid and phloretin, the plant antimicrobial.

The X-ray crystal structures were downloaded from RCSB Protein Data Bank[7] for TtgR bound with phloretin (2UXI),[7,8] quercetin (2UXH),[7,8] chloramphenicol (2UXP),[7,8] and naringenin (2UXU).[7,8]

The ligands

Pseudomonas putida is resistant to toxic substances or antibiotics, yet the pyranochalcone *Pongachalcone I* (*Tephrosia deflexa*), exhibited inhibitory effect on it. Keeping that in mind, a number of natural and synthetic pyranochalcones reported in various literatures are considered as ligand.

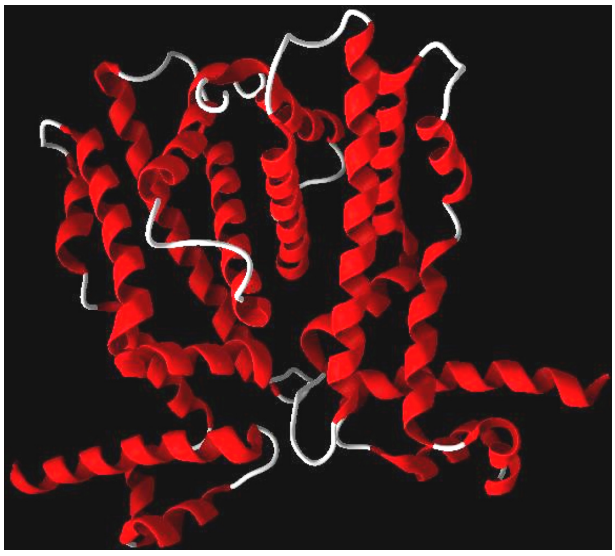

Figure 1 Dimeric structure of TtgR.

Pongachalcone I was isolated from *Tephrosia tunicate*. Glabrachromene II and glabrachalcone were both isolated from *Pongamia glabra* and *Millettia pachycarpa*. Glaychalcones A and B were isolated from *Glycosmis citrifolia*, which is used in folk medicine for the treatment of skin itch, scarbies, and ulcers.[5] *Licoagrochalcone B* with the pyranochalcone moiety was isolated from *Patrinia villosa*[9] (BaiJiangCao in China) and *Glycyrrhiza glabra*.[10] *Licoagrochalcone B* shows potent anticancer activity against human cancer cell such as A549, BEL-7402, SGC-7901, MCF-7, HT-29, K562, and A 498. Harborne and Williams provided on excellent review on pyranochalcones.[11]

The structures and sources for the pyranochalcones studied in the present work as ligands are presented in Figure 2 and Table 1, respectively. The ligands were optimized

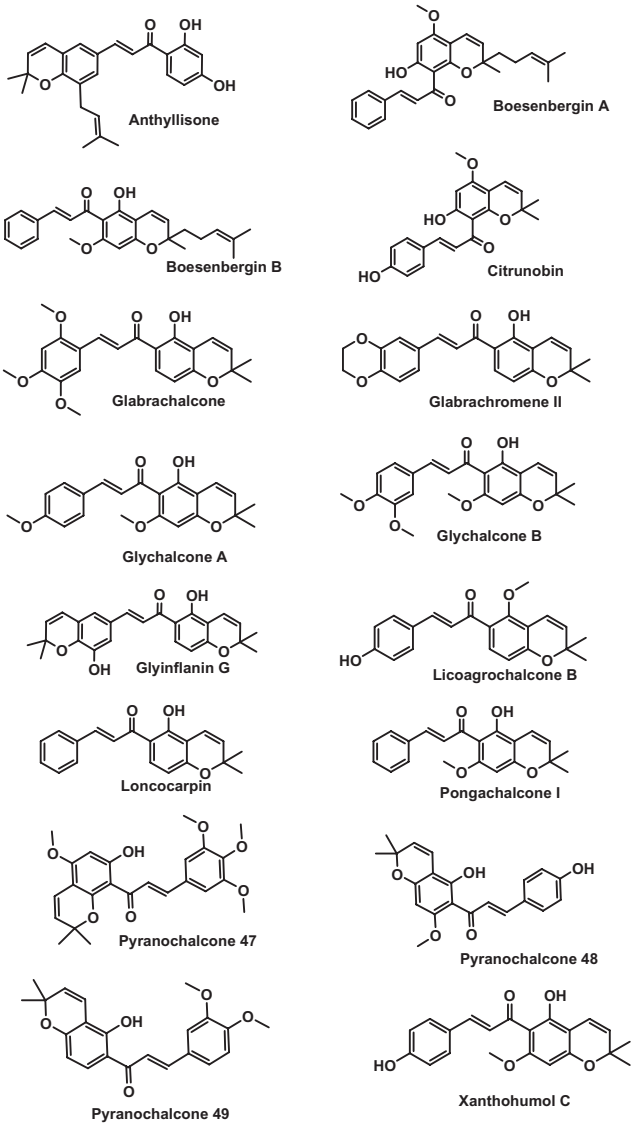

Figure 2 Naturally occurring pyranochalcones used in this study.

Table 1 Pyranochalcones used in the screening

Sl	Compound	Source	Reference
1	Anthyllisone	Isolated from *Anthyllis hermanniae* aerial parts	20
2	Boesenbergin A	Isolated from *Boesenbergia rotunda* rhizomes	21
3	Boesenbergin B	Isolated from *Boesenbergia pandurata* rhizomes	22
4	Citrunobin	Isolated from *Citrus sinensis* and *Citrus nobilis*	23
5	Glabrachalcone	Isolated from *Pongamia glabra* and *Millettia pachycarpa*	5
6	Glabrachromene II	Isolated from *Pongamia glabra* and *Millettia pachycarpa*	5
7	Glychalcone A	Isolated from *Glycosmis citrifolia* leaves	11
8	Glychalcone B	Isolated from *Glycosmis citrifolia* leaves	11
9	Glyinflanin G	Isolated from *Glycyrrhiza inflata* roots	11
10	Licoagrochalcone B	Isolated from *Patrinia villosa*	24
11	Lonchocarpin	Isolated from *Derris sericea* roots	24
12	Pongachalcone I	Isolated from *Tephrosia tunicate*	5
13	Pyranochalcone 47	Isolated from *Neoraputia magnifica* stems	11
14	Pyranochalcone 48	Isolated from *Humulus lupulus* resin	11
15	Pyranochalcone 49	Isolated from *Lonchocarpus subglaucescens* roots	11
16	Xanthohumol C	Isolated from *Humulus lupulus*	25

by density functional theory (DFT) at the level of B3LYP using GAUSSIAN 03 package.[12]

Virtual screening

The docking of the above compounds on the TtgR enzyme was performed on the Molegro® Virtual Docker (MVD) software package[13] and the top 5 compounds with the best docking energies were obtained.

The structure of the protein was corrected for missing atoms or unknown units using MVD. 2UXI has a dimeric structure (A and B) with identical sequence of residues. So to simplify the simulation, the unit A was taken. All other residue, water, etc were removed.

MVD performs flexible ligand docking, so the optimal geometry of the ligand will be determined during the docking.[14–16] The identification of ligand-binding modes in MVD is done by iteratively evaluating a number of candidate solutions (ligand conformations) and estimating the energy of their interactions with the macromolecule. The docking scoring function, E_{score}, is defined by

$$E_{score} = E_{inter} + E_{intra}$$

Where E_{inter} is the ligand–protein interaction energy:

$$E_{inter} = \sum_{i \in ligand} \sum_{j \in protein} E_{PLP}\left(r_{ij}\right) + 33_2 \frac{q_i q_j}{4 r_i^2 j}$$

and

- E_{PLP} is a piecewise linear potential,
- q_1 and q_2 are the charges on two atoms being considered,
- r_{ij} is the distance between two atoms being considered, and
- the second term describes the electrostatic interactions between the two charged atoms.

The electrostatic interaction is assumed to be a Coulomb potential with a distance-dependent dielectric constant, $D(r) = 4r$. To ensure that no energy contribution can be higher than the clash penalty, the electrostatic energy is set to a cut-off level corresponding to a distance of 2.0 Å for distances less than 2.0 Å.

The binding modes of the best docking pose for each of the ligands were investigated on the Ligandscout 2.0 software package.[17,18]

Results

Docking

The protein unit from 2UXI [A] was taken as the substrate for docking. The substrate had only one cavity (Figure 3). The grid resolution for the binding site was kept at 0.30 Å.

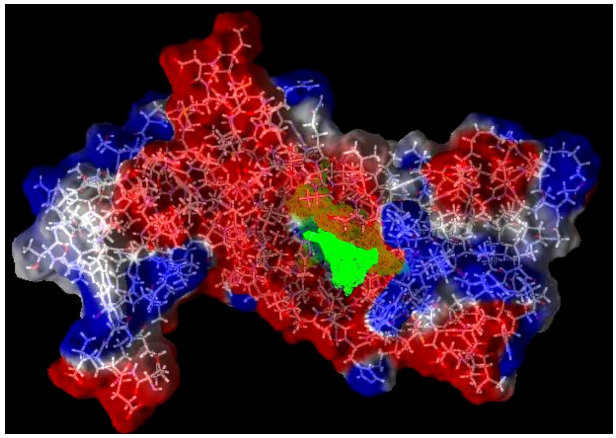

Figure 3 Electrostatic surface and active site cavity (green grid) of sequence A of TtgR (2UXI).

Table 2 Docking energies and interactions

Name	Interaction energy between ligand and protein (arbitrary unit)	H-Bond Energy (arbitrary unit)	ASN110	CYS137	ALA74 SER77 GLU78	VAL96 ILE175 VAL171
Anthyllisone	–110.41	–9.27	x	x	x	
Boesenbergin A	–116.99	0	–	–	–	x
Boesenbergin B	–113.46	0	–	–	–	–
Citrunobin	–103.57	–4.45	x	x	–	x
Glabrachalcone	–114.96	–7.11	x	x	–	x
Glabrachromene II	–105.72	–2.29	x	x		x
Glychalcone A	–79.62	–6.66	x	x	–	x
Glychalcone B	–118.33	–2.77	x	–	–	–
Glyinflanin G	–91.40	–4.28	–	x	–	x
Licoagrochalcone B	–103.46	–7.03	x	–	x	x
Lonchocarpin	–85.08	0	–	–	–	x
Pongachalcone I	–90.88	0	–	–	–	–
Pyranochalcone 47	–114.79	–2.31	x	x	–	–
Pyranochalcone 48	–108.18	–6.30	x	x	–	x
Pyranochalcone 49	–107.37	–5.00	x	x	–	x
Xanthohumol C	–110.25	–6.95	–	–	x	–
Naringenin	–88.91	–6.89	x	x		x
Phloretin	–83.45	–10.51	x	x	x	x
Quercetin	–82.91	–5.71	x	x	–	x

"x" represents presence of interaction.

The compounds phloretin, naringenin, and quercetin which are already reported to bind with TtgR and subsequently effluxes out by the bacterial system were first docked on the 2UXI[A] substrate taking phloretin (already bound in 2UXI as ligand) as a template.

The orientations of the compounds (naringenin and quercetin) in their respective best poses were found to be in excellent agreement with that found in their crystal structures (PDB Code: 2UXH, 2UXP, 2UXU for naringenin and quercetin complexed with TtgR, respectively). As alignment and bioactive conformation selection are important factors for obtaining meaningful models, this step ensured that, all the pyranochalcones could be docked in the substrate taking the already bound phloretin as a template.

Table 3 Pharmacophore results

Name	Pharm. score	CLogP	TPSA [P Ertl]	Acceptors	Rel. TPSA	Donors
PHLORETIN-12 [# 12]	59.39	2.09	97.99	5	2.88	4
Anthyllisone-1 [# 1]	48.74	5.46	66.76	4	1.21	1
Licoagrochalcone_B-11 [# 11]	46.92	4.25	55.76	4	1.24	1
Glabrachromene II-7 [# 7]	37.97	4.01	64.99	5	1.38	1
Glabrachalcone-6 [# 6]	37.90	4.27	74.22	6	1.40	1
QUERCETIN-18 [# 18]	37.80	1.99	131.36	6	4.11	5
Pyranochalcone_48-16 [# 16]	37.76	3.95	75.99	5	1.65	2
NAR 1211 [A] [# 27]	37.63	1.99	86.99	5	2.72	3
Pyranochalcone_49-17 [# 17]	37.61	4.26	64.99	5	1.33	1
Citrunobin-4 [# 4]	37.13	3.95	75.99	5	1.65	2
Glychalcone_A-8 [# 8]	36.98	4.26	64.99	5	1.33	1
Glyinflanin G-10 [# 10]	35.35	5.13	75.99	5	1.41	2
Boesenbergin_A-2 [# 2]	0.00	5.98	55.76	4	0.96	1
Xanthohumol_C-24 [# 24]	0.00	3.95	75.99	5	1.65	2
Pongachalcone_I-14 [# 14]	–1.00	4.25	55.76	4	1.24	1
Boesenbergin_B-3 [# 3]	–1.00	5.98	55.76	4	0.96	1
Loncocarpin-25 [# 25]	–1.00	4.24	46.53	3	1.13	1
Pyranochalcone_47-15 [# 15]	–1.00	4.28	83.45	7	1.46	1
Pongachalcone_I-13 [# 13]	–1.00	4.25	55.76	4	1.24	1
Glychalcone_B-9 [# 9]	–1.00	4.27	74.22	6	1.40	1

Docking results between the substrate and the Pyranochalcones as well as with the drug molecules are shown in Table 2.

Ligand-substrate interaction

The binding modes of the best docking pose for each of the ligands were investigated on Ligandscout 2.0 software package,[18] and are shown in Table 3.

Discussion

In previous study it was observed that, in the active site of TtgR, the residues ASN 110 and CYS 137 along with ALA74, SER77, GLU78, VAL96, ILE175 and VAL171 seem to play crucial role in binding with the ligands and triggering the efflux pump.[19] The moieties ASN 110 and CYS 137 probably are the most instrumental in binding through strong H-bonding and sensing the compounds toxic to the organism (Figure 4).

The residues ALA74, SER77, GLU78, VAL96, ILE175 and VAL171 interact with the ligands mostly by hydrophobic interactions with the ligands.

As it has been observed that, the antimicrobials phloretin, naringenin, and quercetin bind strongly with those residues whereas Pongachalcone I, which is active against this bacterium, does not bind with any of those. From this study, therefore, it is hypothesized that, the pyranochalcogen which will exhibit lower tendency to associate with the above mentioned residues and lower interaction with the pharmacophore (in terms of *Pharm Score*) might be considered as a batter candidate against *P. putida*.

Under the above consideration, using the results presented in Table 2 and Table 3, it may be proposed that, among the 16 pyranochalcones studied:

1. Anthyllisone would be the least active one against *P. putida*.
2. The binding affinity of the pyranochalcones is found to increase with the increase in the number on methoxy moiety in the aromatic side part of the ligands, whereas the effect of the methoxy moiety connected to the fused aromatic unit, seems to be less pronounced, might be due to hindrance.
3. Although the experimental evidence of activity of only pongachalcone I against *P. putida* is reported, Boesenbergin A, B and Lonchocarpin seem also to be high potential candidate for the same, which of course, has to be evaluated experimentally. Contrast to that, compounds which have simultaneous H-bonded association with ASN 110 and CYS 137 and high *Pharm Score* (Anthyllisone, Citrunobin, Glabrachalcone, Glabrachromene II, etc, for instance) have low potentiality to be active against this bacteria.

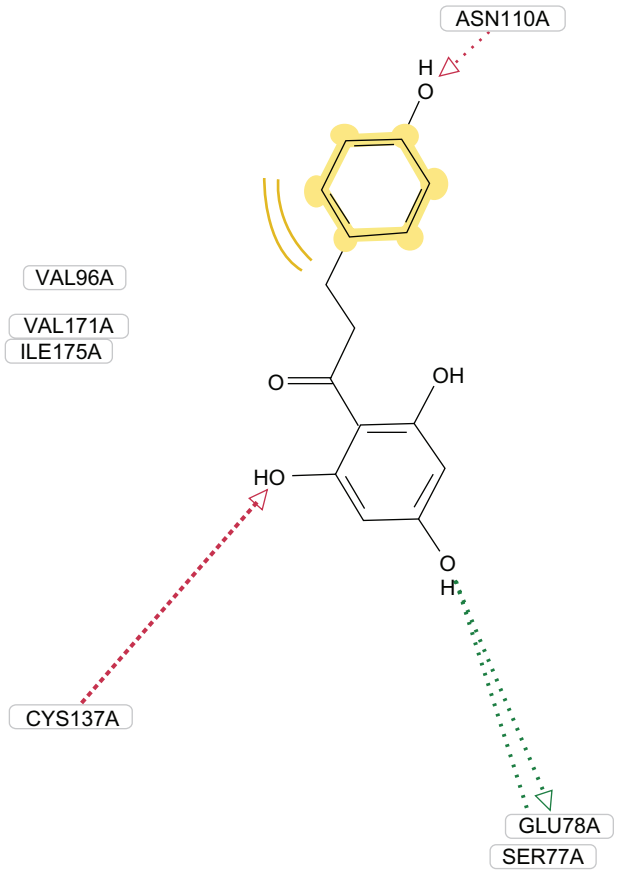

Figure 4 Simplified presentation of interaction of phloretin with TtgR.

Conclusion

In this work, molecular docking has been performed with 16 naturally occurring Pyranochalcones on the transcriptional regulator enzyme (TtgR) of antibiotic resistance strain of the bacteria *P. putida*. The pyranchalones Boesenbergin A, B and Lonchocarpin (along with Pongachalcone I) were projected to be active against the multidrug-resistant strain of the bacteria. Anthyllisone seems to be the least active one. The effect of the number and position of the methoxy group in the pyranochalcones on their binding affinity was also discussed. The results obtained will be helpful in designing of new series of drugs especially for the antibiotic resistant bacteria. Work is in progress to study the interaction of unnatural synthetic pyranochalcones with this bacterium.

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Disclosure

The authors report no conflicts of interest in this work.

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