

Binding site description of 2-substituted benzothiazoles as potential RND efflux pump inhibitors

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Introduction

The resistance-nodulation-cell division family (RND) efflux pumps exemplify a unique phenomenon with drug resistance in different gram negative bacterial strains as a single mechanism causing resistance against several different classes of antibiotics. In *Escherichia coli* AG102 and *Acinetobacter baumannii* SBMox2 strains the well characterized RND efflux pumps are AcrAB-TolC and the AdeABC respectively. Most of the antibiotics were found to be substrates for these pumps by increasing the expression of the efflux pump genes, leading to multidrug resistance (MDR) and the treatment failure and death caused by these gram negative bacterial infections or underlying diseases are common (Sun et al., 2014). Consequently, the need of searching new therapeutic solutions that suppress the activity of efflux pumps and restore the sensitivity of commonly used antibiotic is essential.

RND efflux pumps, which are only found in Gram-negative bacteria, have a tripartite composition. RND type efflux pumps contain an inner membrane transporter protein (RND pump), an outer membrane protein (OMP) channel, and a periplasmic membrane fusion protein (MFP). They are allowed direct extrusion of various antibiotics from the cytosol or periplasmic space to the outside of the bacterial cell, and have been found to be associated extensively with clinically significant antibiotic resistance (Sun et al., 2014).

Recent studies reported that RND type efflux pumps, which are named AcrAB-TolC in *E. coli* and AdeABC in *A. baumannii*, comprise a transporter protein (RND pump) AcrB in *E. coli* or AdeB in *A. baumannii* acting as a pro-

ton/drug antiporter, an outer membrane channel protein TolC in *E. coli* or AdeC in *A. baumannii*, and a periplasmic membrane fusion protein AcrA, which serves as a linker between TolC and AcrB in *E. coli* or AdeB, which serves as a linker between AdeC and AdeB in *A. baumannii* (Sun et al., 2014).

The emergence of MDR strains of Gram-negative bacteria pathogens is a problem of ever increasing significance (Sun et al. 2014). Interestingly, these RND efflux pumps decrease the antibacterial activity of dissimilar antibiotic structures, which can be considered a MDR mechanism. Because of bacteria become insensitive to different classes of antibiotic therapy, new therapeutic approaches must be looked for, searching for new molecules to block efflux, to restore drug susceptibility to resistant clinical strains.

The goal of this study is (i) to define the potential RND efflux pump inhibitor (EPI) activity of our previously synthesized BSN coded 2-substituted benzothiazoles by observing the reversal antibacterial activity of antibiotics particularly to chloramphenicol (CHL) and/or ciprofloxacin (CIP) in the AdeABC efflux pump overexpressor *Acinetobacter baumannii* SbMox2 and/or AcrAB-TolC efflux pump overexpressor *E. coli* AG102 clinical isolates, and (ii) to examine the structure activity relationships by describing the binding site features of these tested compounds and to analyze the active site protein-ligand interactions of RND efflux pump AdeABC in *A. baumannii* by generating pharmacophore hypothesis.

Materials and methods

A well-known standard microdilution assay was used to determine the minimum inhibitory concentration (MIC) of our previously synthesized BSN coded 2-substituted

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benzothiazoles derivatives (Yilmaz et al. 2013), CHL and CIP and MICs of CHL and CIP were determined in the presence and absence of the BSN coded compounds.

A common feature pharmacophore hypothesis, HipHop method, was generated by using the Accelrys Discovery Studio 2.1 software to explain the specification of the structure activity relationships of pharmacophoric sites of the tested BSN coded 2-substituted benzothiazoles in the targeted AdeABC efflux pump. This tool builds pharmacophore hypotheses (overlying common features) for which the fit of individual molecules to a hypothesis could be correlated with activity of the molecule.

A set of potential AdeABC efflux pump inhibitors of BSN coded 2-substituted benzothiazoles, which exhibited 16-folds or greater reduction in the MIC value of CHL after used in combination in *A. baumannii* SbMox-2, was selected as the EPI active training set to use in the HipHop pharmacophore generation method. Among the tested BSN coded compounds, the most active molecules, BSN4, BSN6, and BSN23, were used to derive common feature-based alignments and considered as "reference compounds" specifying a principal value of 2 and a maximum omitting features value of 0.

Results and discussion

For the antibacterial activity test against *A. baumannii* SbMox-2 and/or *E. coli* AG102 clinical isolates, BSN coded 2-substituted benzothiazoles were first tested alone to observe their intrinsic antibacterial affinity. However, when they were tested alone they did not exhibit any significant intrinsic antibacterial activity. But, when they were tested in combinations with CHL or CIP against the AdeABC overexpressor *A. baumannii* SbMox-2 mutant, a reversal in the antibacterial activity of 22, 20 fold double dilution better MIC values were observed respectively for CHL and CIP. Moreover, the combinations of the tested compounds with CHL or CIP against the AcrAB-TolC overexpressor *E. coli* AG102 strain were exhibited a reversal antibacterial activity of 6, 10 fold double dilution better MIC values respectively for CHL and CIP. Among the tested BSN coded benzothiazoles, BSN4, BSN6, and BSN23 reversal the antibacterial activity of CHL revealing a MIC values of 0.125 µg/ml against the AdeABC overexpressor *A. baumannii* SbMox-2 strain.

The generated 3D-common feature pharmacophore hypothesis containing two Hydrogen Bond Acceptors

(HBA) and three Hydrophobic Aromatics (HpAr) was anticipated as the common-feature functions to explain the pharmacophoric site specifications of the EPI activity of BSN coded 2-substituted benzothiazole compounds. The generated pharmacophore model reveals that the two HBA and three HpAr features are found significant for binding to the active site of the target protein. Three HpAr features demonstrate the appropriate active shape of the molecule, displaying the required place of bulky aromatic moieties. Two HBA atoms or groups at the given positions are necessary in the molecule to bind to the target protein.

Conclusion

The generated pharmacophore model revealed that when the tested compounds substituted by a benzyl group instead of phenyl ring attached to the benzothiazole nucleus then, they could not be able to show any match with the hydrogen bond acceptor feature of nitrogen atom in the thiazole ring at the fused ring system. Therefore, these compounds showed lower fit value and were not able to match with all the mapped pharmacophore common-features in the anticipated model. This observation explains why 2-phenylbenzothiazole structure is more favourable than 2-benzylbenzothiazole for increasing potency in this set of compounds.

In conclusion, the generated 3D-common feature pharmacophore hypothesis reveals that the conformational properties of the compounds are significant for the AdeABC efflux pump inhibitor activity against the multi-drug resistant *A. baumannii* SbMox-2 strain and compounds possessing 2-[4-(4-substituted-2-phenyl-acetamido)phenyl]benzothiazole and/or 2-[4-(4-substituted-3-phenylpropionamido)phenyl]benzothiazole structures are important for improving the AdeABC efflux pump inhibitor potency.

References

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