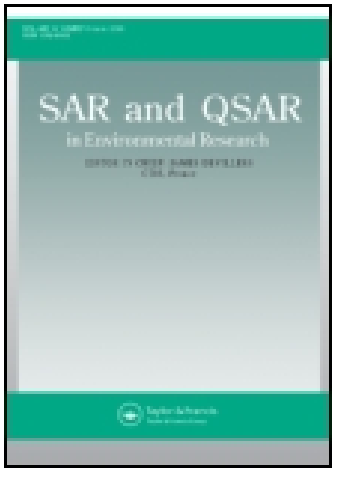

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S. Yilmaz[a], G. Altinkanat-Gelmez[b], K. Bolelli[a], D. Guneser-Merdan[b], M.U. Over-Hasdemir[b], I. Yildiz[a], E. Aki-Yalcin[a] & I. Yalcin[a]

[a] Pharmaceutical Chemistry Dept., Faculty of Pharmacy, Ankara University, Ankara, Turkey

[b] Medical Microbiology Dept., Faculty of Medicine, Marmara University, Istanbul, Turkey

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Pharmacophore generation of 2-substituted benzothiazoles as AdeABC efflux pump inhibitors in *A. baumannii*

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[a]*Pharmaceutical Chemistry Dept., Faculty of Pharmacy, Ankara University, Ankara, Turkey;* [b]*Medical Microbiology Dept., Faculty of Medicine, Marmara University, Istanbul, Turkey*

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RND family efflux pumps are important for multidrug resistance in Gram-negative bacteria. To date no efflux pump inhibitors for clinical use have been found, so developing the specific inhibitors of this pump system will be beneficial for the treatment of infections caused by these multidrug-resistant pathogens. A set of BSN-coded 2-substituted benzothiazoles were tested alone and in combination with ciprofloxacin (CIP) against the RND family efflux pump AdeABC overexpressor *Acinetobacter baumannii* SbMox-2 strain. The results indicated that the BSN compounds did not have antimicrobial activity when tested alone. However, if they were applied in combination with CIP, it was observed that the antibiotic had antimicrobial activity against the tested pathogen, possessing a minimum inhibitory concentration value that could be utilized in clinical treatment. A 3D-common features pharmacophore model was applied by using the HipHop method and the generated pharmacophore hypothesis revealed that the hydrogen bond acceptor property of nitrogen in the thiazole ring and the oxygen of the amide substituted at the second position of the benzothiazole ring system were significant for binding to the target protein. Moreover, three hydrophobic aromatic features were found to be essential for inhibitory activity.

Keywords: benzothiazoles; *A. baumannii* SbMox-2; AdeABC; ciprofloxacin; HipHop; pharmacophore generation

1. Introduction

Acinetobacter baumannii is a ubiquitous non-fermentative Gram-negative bacterium, which is a multidrug-resistant (MDR) opportunistic human pathogen [1–3]. It is most often responsible for a wide spectrum of nosocomial infections such as bloodstream infections, ventilator-associated pneumonia, urinary tract infections and wound infections [4–7]. Sporadic cases of peritonitis, endocarditis, meningitis, osteomyelitis and arthritis have also been reported [8]. In Europe, *A. baumannii* accounts for as many as 10% of all infections caused by Gram-negative bacteria seen in intensive care units [6,9], and in the USA it accounts for 2.5% [6,10]. In addition, *A. baumannii* is recognized as an increasingly important cause of community-acquired pneumonia, with a high mortality rate of 40–64% [6,11,12].

A. baumannii constitutes a major public health problem due to its propensity to develop resistance to numerous drugs, and isolates exhibiting multidrug resistance are emerging in clinical settings [13–15]. A spectacular increase in its resistance to all antimicrobial agents

*Corresponding author. Email: yalcin@ankara.edu.tr

has been observed in the last decade, and nowadays the resistance of *A. baumannii* to ceftazidime is >85%, while that for ciprofloxacin (CIP) is >90% [16–18].

It has been thoroughly illuminated that the active efflux-medicated drug resistance in *A. baumannii* plays a major role in MDR strains of this pathogen [7,13,16,19]. In general, efflux constitutes a resistance mechanism that involves the extrusion of antimicrobial agents (as well as other compounds) from the inner side of bacterial membranes to the external environment by means of specific proteins, typically named efflux pumps [20]. These pumps may also be involved in the early stages of infection, such as adhesion to host cells and colonization [19]. Importantly, they remove from the cell antibiotics commonly used in the therapy of infections caused by these bacteria. Efflux pumps exemplify a unique phenomenon in drug resistance: a single mechanism causing resistance against several different classes of antibiotics. According to amino acid sequence similarities, energy source, number of components, number of transmembrane-spanning regions and types of substrates, efflux pumps in bacteria are classified into up to five distinct families: the ATP-binding cassette (ABC) super family, the multidrug and toxic compound extrusion (MATE) family, the small multidrug resistance (SMR) family, the major facilitator (MFS) super family and the resistance–nodulation–cell division (RND) family [19,20]. The RND family is usually predominant in Gram-negative bacteria and it is the most prevalent mechanism in multiply resistant *A. baumannii* [13,19]. RND pumps typically exhibit a wide substrate range that can include antibiotics, dyes, biocides, detergents and antiseptics [13]. Members of this family consist of a tripartite system including a transporter protein (RND pump) embedded within the inner membrane, an outer membrane protein (OMP) channel and a membrane fusion protein (MFP) that links the other two [13,19,20].

Studies reported by Magnet et al. in 2001 identified a proton motive force-dependent RND-type efflux pump in *A. baumannii*, named AdeABC [21], possessing a tripartite structural organization as shown in Figure 1 [19,22]. In *A. baumannii* the efflux transporter protein (AdeB) captures its substrates either from within the phospholipid bilayer of the inner membrane or the cytoplasm and then transports them into the extracellular medium via AdeC, and the periplasmatic protein AdeA mediates in the cooperation between the AdeB and AdeC components [19,22]. Drug transport is driven by the transmembrane electrochemical proton gradient, exchanging one proton for one drug molecule [19,20]. Inactivation of this pump revealed that it was responsible for resistance to a wide variety of antimicrobials including kanamycin, gentamicin, tobramycin, netilmicin, amikacin, erythromycin, tetracycline,

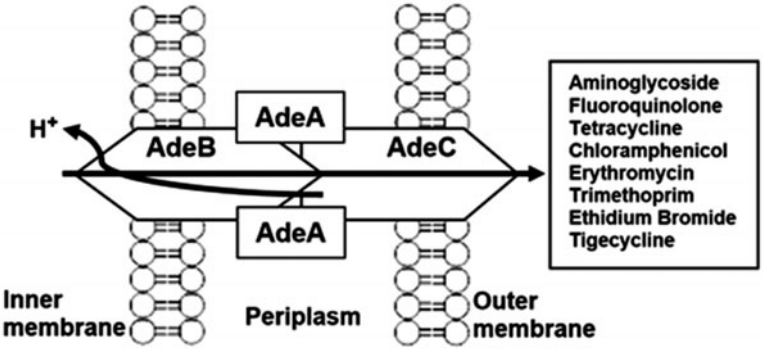


Figure 1. The bacterial RND family tripartite structural organization of MDR AdeABC efflux pump in *A. baumannii.*

chloramphenicol, trimethoprim, rifampin, ciprofloxacin, sparfloxacin, ofloxacin, perfloxacin, norfloxacin, ethidium bromide and, more recently, tigecycline and meropenem [19,20,22]. The prevalence of these AdeABC overexpressor MDR *A. baumannii* strains leaves limited clinical options for treatment, underscoring the need to develop novel antibiotics for bacterial pathogens in general and Gram-negative pathogens in particular [6].

Pharmacological inhibition of the AdeABC efflux pump might be an attractive goal to reverse MDR in *Acinetobacter* species and to improve therapy options. A few putative bacterial RND efflux pump inhibitors (EPIs) have been described to date [23]. A recognized example is phenyl-arginine-β-naphthylamide (PAβN), which was reported to be a broad-spectrum EPI capable of reversing the MDR phenotype of MexAB-OprM RND-type efflux pump over expressed in *P. aeruginosa* and several other Gram-negative bacteria [24]. It has also been tested in AdeABC overexpressor *A. baumannii* clinical isolates, and a 4-fold or greater reduction in the minimum inhibitory concentration (MIC) of nalidixic acid after PAβN addition was observed in approximately half of the tested isolates, although there was no significant effect of PAβN addition on the susceptibility to CIP [25,26]. In one study, it was reported that the effect of another EPI, 1-(1-naphthylmethyl)-piperazine (NMP), on drug susceptibility in *A. baumannii* reveals that NMP partially reversed MDR, and differs in its activity from that of PAβN in this species [25,27].

Consequently, research into new therapeutic solutions as specific EPIs, including AdeABC inhibitors, could enable suppression of the activity of RND-type efflux pumps and restore the sensitivity to commonly used antibiotics of important bacteria such as *A. baumannii*. However, there has been little research done on the RND active efflux pump structure of AdeABC in *A. baumannii*, and no X-ray crystal structure of the AdeABC efflux pump protein has been registered in the Protein Data Bank (PDB) archive to date. If the understanding of the pump structure and mechanism of this RND active efflux pump can be increased, it may help us to decipher its natural function and the mechanism of substrate recognition and extrusion, and provide clues to block or alter this efflux mechanism.

The aim of this present study was (i) to evaluate novel lead compounds that are active as RND-type AdeABC EPIs in *A. baumannii* to reverse the antibacterial activity of antibiotics, particularly CIP, in the AdeABC overexpressor *A. baumannii* clinical isolate; and (ii) to analyse the structure–activity relationships of active sites of protein–ligand interactions by generating a pharmacophore hypothesis to increase the knowledge of this type RND active efflux pump structure and mechanism.

2. Materials and methods

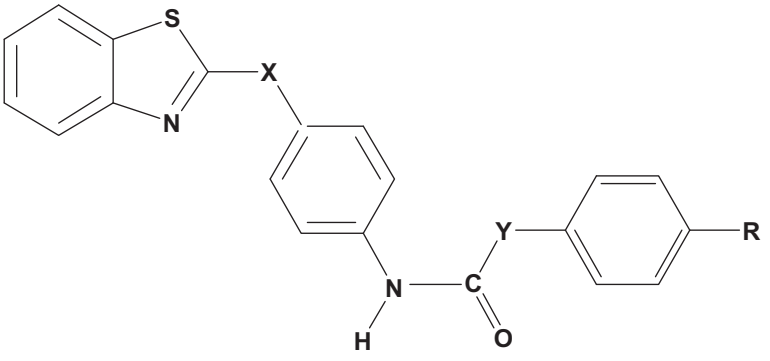
2.1 *Tested compounds*

In order to investigate the bacterial RND family AdeABC efflux pump inhibitory activity in *A. baumannii*, we tested 23 previously synthesized novel BSN-coded fused heterocyclic compounds that include the benzothiazole ring system in their structure holding different atoms and/or atom groups on X, Y and R positions, as given in Table 1 [28, 29].

2.2 *Bacterial strain*

A RND-type AdeABC efflux pump overexpressor clinical isolate, *A. baumannii* SbMox-2 [25], which is a second-step clinically isolated mutant differing in dye accumulation and adeB gene expression, but which had no new mutations in the quinolone resistance-determining

Table 1. Training set of tested BSN-coded 2-substituted benzothiazoles.



Compound Code	*R*	*X*	*Y*
BSN1	H	-	-
BSN2	$OCH(CH_3)C_2H_5$	-	-
BSN3	C_2H_5	-	-
BSN4	OCH_3	-	CH_2
BSN5	F	-	CH_2
BSN6	CH_3	-	CH_2
BSN7	NO_2	-	CH_2
BSN8	H	-	CH_2
BSN9	Cl	CH_2	CH_2
BSN10	F	CH_2	CH_2
BSN11	Br	CH_2	CH_2
BSN12	NO_2	CH_2	CH_2
BSN13	CH_3	CH_2	CH_2
BSN14	OCH_3	CH_2	CH_2
BSN15	H	CH_2	CH_2
BSN16	F	CH_2	-
BSN17	Br	CH_2	-
BSN18	NO_2	CH_2	-
BSN19	C_2H_5	CH_2	-
BSN20	H	CH_2	-
BSN21	H	CH_2	C_2H_4
BSN22	OCH_3	CH_2	C_2H_4
BSN23	H	-	C_2H_4

regions (QRDR) of topoisomerase genes gyrA and parC, was tested. The test strain was grown overnight at 37°C in Mueller Hinton Broth (MHB) (Oxoid,UK).

2.3 *Susceptibility testing*

A standard microdilution assay was used to determine the MIC of BSN-coded compounds (Table 1) and CIP (Sigma-Aldrich, US) [30]. The BSN-coded compounds were dissolved in dimethyl sulphoxide (DMSO) and the two-fold serial dilutions were prepared using cation-adjusted MHB at a concentration range between 512 μg/ml and 0.0625 μg/ml. Direct colony suspension of the tested bacteria from the fresh cultures was prepared in order to have a turbidity equivalent to 0.5 McFarland standard. The inoculum suspension was then diluted in

MHB to give a final cell number of 5×10^5 cfu/ml. The final concentration of the inoculum was added to two-fold serial dilution of the compounds. The microdilution trays were incubated at 35±2°C for 16–20 h in an ambient air incubator.

MICs of CIP were determined in the presence and absence of the BSN-coded compounds. Half the concentration of the observed MIC values of the tested BSN-coded compounds was added into broth microdilution wells in the combinations with CIP considered as having a non-inhibitory effect on bacterial growth. A 16-fold or greater reduction in the MIC value of CIP after addition of BSN-coded fused heterocyclic compounds, which comprises a MIC value of 1 μg/ml providing below the European Committee on Antimicrobial Susceptibility Testing (EUCAST) MIC susceptibility breakpoint for CIP versus *Acinetobacter spp* for use in clinical treatment [31], was considered as a potential EPI. All susceptibility tests were performed twice.

2.4 *Pharmacophore generation*

Because of the absence of crystallographic structures of RND-type AdeABC efflux pump proteins in the PDB archive for which the active site for receptor binding is clearly identified, a 3D-common feature pharmacophore hypothesis was generated for these recognized BSN-coded AdeABC efflux pump inhibitor compounds by applying the HipHop method through the work flow given in Figure 2 by using the Accelrys Discovery Studio 3.5 software [32] in order to analyse the structure–activity relationships of active sites of protein–ligand interactions.

The chemical structures of the molecules were constructed using the Discovery Studio Visualizer, and standard 3D structures were generated and the geometry of all molecules was optimized with Dreiding Minimization. Accelrys Discovery Studio 3.5 software automatically generated conformational models for each compound using the Poling Algorithm [33–35]. The 'best conformer generation' procedure was applied to provide the best conformational coverage for a maximum number of conformers generated, defaulted to 250 in a 0–25 kcal mol^{-1} range from the global minimum. The generated conformations were used to align

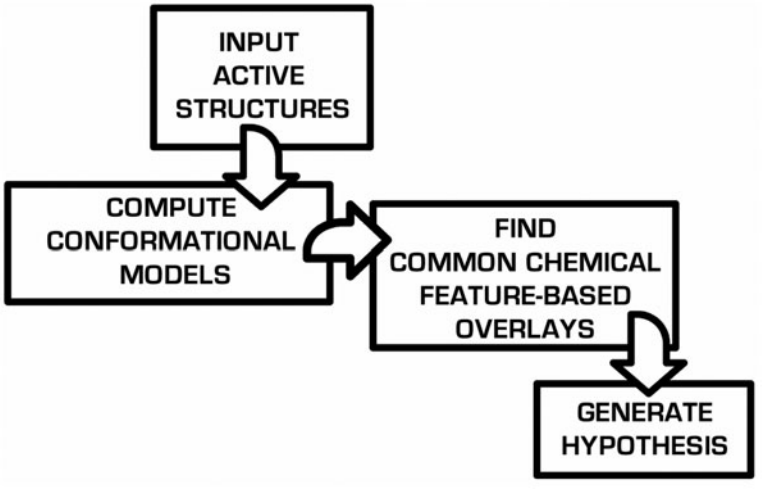


Figure 2. Work flow of HipHop common feature-based generated pharmacophore hypothesis.

common molecular features and generate a pharmacophore hypothesis. The HipHop method was used in the conformations generated to align chemically important functional groups common to the molecules in the study set. A pharmacophoric hypothesis then was generated from these aligned structures [32,36,37].

HipHop provides feature-based alignment of a collection of active compounds and without considering the activity. It matches the chemical features of a molecule against drug candidate molecules. HipHop takes a collection of conformational models of molecules and a selection of chemical features, and produces a series of molecular alignments in a variety of standard file formats. HipHop begins by identifying configurations of features common to a set of molecules. A configuration consists of a set of relative locations in 3D space and associated feature types. A molecule matches the configurations if it possesses conformations and structural features that can be superimposed within a certain tolerance from the corresponding ideal locations. HipHop also maps partial features of molecules in the alignment set. This provision gives the option to use partial mapping during the alignment. Partial mapping allows identification of larger, more diverse, more significant hypotheses and alignment models without the risk of missing compounds that do not have to map to all of the pharmacophore features [32,36,37].

3. Results and discussion

3.1 *Microbiological activity*

The dramatically rising prevalence of MDR microbial infections in the past few decades has become a serious healthcare problem. In particular, the emergence of MDR strains of Gram-negative bacteria pathogens such as AdeABC efflux pump overexpressor clinical isolate of *A. baumannii* is a problem of ever-increasing significance. In order to prevent this problem, new efforts to develop new antibacterial agents are urgently needed.

Substituted benzothiazoles and their analogues such as benzoxazoles and benzimidazoles, which are the structural isosteres of nucleotides owing to fused heterocyclic nuclei in their structure, have been the aim of many researchers for many years, because they constitute an important class of heterocyclic compounds with antitumour [38], antiviral [39], and antimicrobial activities [40]. Recent observations suggest that these fused heterocyclic compounds interact easily with biopolymers and possess potential activity with lower toxicities for chemotherapeutic approaches in man [41–44].

Over the last few years, we have reported the synthesis of several 2-substituted benzothiazole derivatives as chemotherapeutic agents [45–48]. According to these studies, the compounds were found to have an inhibitory effect against some Gram-positive, Gram-negative bacteria and the yeast *C. albicans* [45, 46]. Moreover, they were found to be very potent eukaryotic topoisomerase II inhibitors, exhibiting better activity than the reference drug etoposide [47,48].

These observations provide us with some predictions to design and evaluate novel lead compounds that are active as the RND-type EPIs, in order to reverse the antibacterial activity of antibiotics against MDR Gram-negative bacteria such as AdeABC efflux pump overexpressor *A. baumannii.*

For the antibacterial activity test against *A. baumanii* SbMox-2 clinical isolate, which is an AdeABC efflux pump overexpressor, BSN-coded 2-substituted benzothiazoles were first tested alone to observe their intrinsic antibacterial affinity. When they were tested alone against the AdeABC efflux pump overexpressor *A. baumannii* SbMox-2 they did not exhibit

Table 2. Observed MIC values of ciprofloxacin (CIP) and BSN coded 2-substituted benzothiazoles as tested alone and in combinations against the AdeABC efflux pump overexpressor *A. baumannii* SbMox-2 strain.

Compound Code	*A. baumannii SbMox-2*		
	MIC(μg/ml)[a]	Combination with CIP	MIC(μg/ml)[b]
CIP	128		
BSN1	128	CIP + BSN1	64
BSN2	128	CIP + BSN2	2
BSN3	128	CIP + BSN3	64
BSN4	128	CIP + BSN4	0.125
BSN5	64	CIP + BSN5	32
BSN6	>512	CIP + BSN6	0.125
BSN7	128	CIP + BSN7	64
BSN8	256	CIP + BSN8	0.5
BSN9	128	CIP + BSN9	128
BSN10	64	CIP + BSN10	128
BSN11	128	CIP + BSN11	0.5
BSN12	256	CIP + BSN12	0.5
BSN13	64	CIP + BSN13	128
BSN14	128	CIP + BSN14	128
BSN15	128	CIP + BSN15	64
BSN16	128	CIP + BSN16	64
BSN17	64	CIP + BSN17	64
BSN18	128	CIP + BSN18	64
BSN19	64	CIP + BSN19	64
BSN20	64	CIP + BSN20	8
BSN21	256	CIP + BSN21	2
BSN22	64	CIP + BSN22	2
BSN23	>512	CIP + BSN23	0.125

[a]Observed MIC values of compounds tested alone.
[b]Observed MIC values of CIP tested in combination with BSN compounds.

any significant intrinsic antibacterial activity, showing MIC values between 64–512 μg/ml. However, when they were tested in combination with CIP against the same bacterial mutant, a reversal in the antibacterial activity of CIP – up to 20-fold double dilution better MIC values – was observed, as seen in Table 2. When the tested BSN-coded 2-substituted benzothiazole derivatives were compared, it was found that the compounds holding a phenyl group on the second position of the benzothiazole ring, such as BSN2, BSN4, BSN6, BSN8, and BSN23, provided more a significant contribution to the reversal of antibacterial activity of CIP, rather than having a benzyl group at that position.

Several molecules of BSN-coded compounds supplied a reversal of the antibacterial activity of CIP against the AdeABC overexpressor *A. baumannii* SbMox-2 strain, contributing sensitivity on the MIC values of CIP between 2 and 20-fold double dilution better antibacterial activity. Among the tested combinations, the compounds BSN4, BSN6, and BSN23 exhibited the most significant reversal of antibacterial activity of CIP against *A. baumannii* SbMox-2, providing a MIC value of 0.125 μg/ml, which was below the EUCAST susceptibility MIC breakpoint for CIP versus *Acinetobacter spp.* for use in clinical treatment [31].

Consequently, the observed microbiology results revealed that the tested BSN-coded 2-substituted benzothiazoles, which provided the reversal of multidrug resistance in

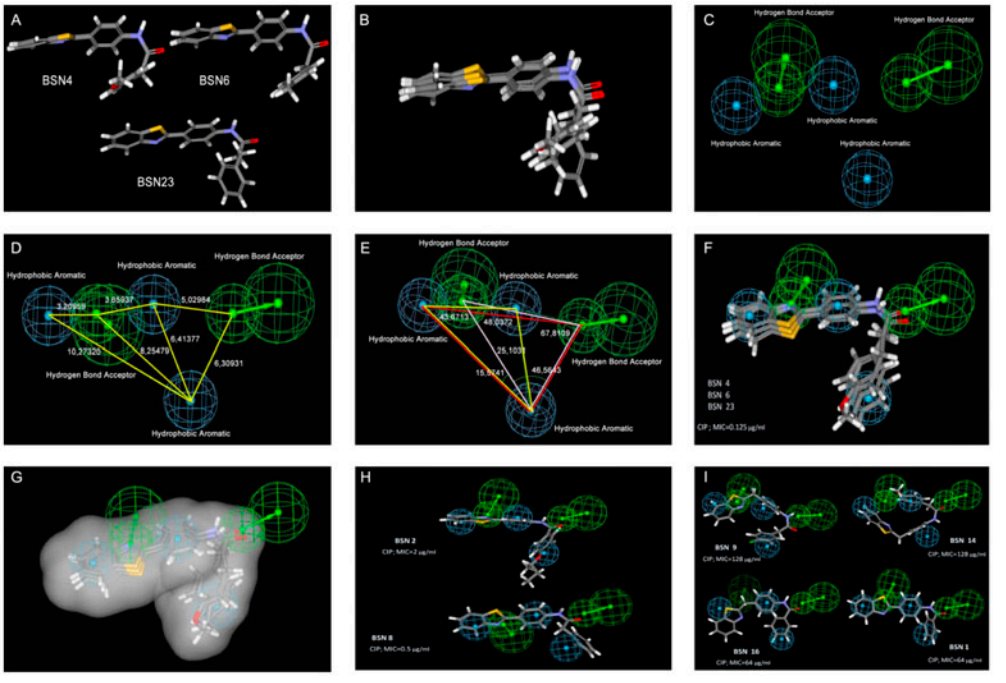


Figure 3. A. The generated conformer 3D shapes of the most active AdeABC efflux pump inhibitor BSN-coded compounds. B. Alignment of the 3D generated conformer structures of BSN4, BSN6 and BSN23 as the reference AdeABC efflux pump inhibitors. C. Anticipated pharmacophore model generated for AdeABC efflux pump inhibitor activity of tested BSN compounds. D. Distances between the generated common features calculated in the participated pharmacophore model. E. Angles between the generated common features calculated in the participated pharmacophore model. F. Pharmacophore mapping of the reference BSN4, 6, and 23 coded compounds. G. The generated slab of the anticipated pharmacophore model with the reference BSN4, 6, and 23 coded compounds. H. Pharmacophore mapping of tested BSN coded compounds less active than BSN4, 6, and 23. I. Pharmacophore mapping of not active AdeABC efflux pump inhibitor BSN-coded compounds.

combination with CIP, exhibited their effects by inhibiting the AdeABC multidrug efflux pump affinity in the *A. baumannii* SbMox-2 strain.

3.2 *Pharmacophore hypotheses generated by the HipHop method*

In this research, the HipHop method [32,36,37] was used to generate pharmacophore hypotheses 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 (overlaying 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 EPIs of BSN-coded 2-substituted benzothiazoles from Table 1, which exhibited 16-fold or greater reduction in the MIC value of CIP when used in combination in *A. baumannii* SbMox-2, was selected as the EPI active training set for use in the HipHop pharmacophore generation method. Among the tested BSN-coded compounds, the most active molecules, BSN4, BSN6, and BSN23, given in Figure 3(a) and 3(b), were used to derive common feature-based alignments and considered as 'reference compounds', specifying

a principal value of 2 and a maximum omitting features (MaxOmitFeat) value of 0. Five pharmacophoric hypotheses then were generated from these aligned structures using the Common Feature Pharmacophore Generation protocol. A preparative test was performed with hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), hydrophobic (Hp), hydrophobic aromatic (HpAr), hydrophobic aliphatic (HpAl), negative ionisable (NI), positive ionisable (PI) and ring aromatic (R). NI and PI were used, rather than negative charge and positive charge, in order to broaden the search for deprotonated and protonated atoms or groups at physiological pH. By using conformational poling, a representative family of conformers was generated, within a 25 kcal mol^{-1} range of the computed minimum, for each molecule. Potential hypothesis models were produced with the minimum permitted interfeature spacing of 2.00 Å generating alignments of common features that included the projected points of HpAr and HBA. Within the generated five hypotheses, the hypothesis Figure 3(c) has been chosen for the further evaluation as the anticipated pharmacophore model having five features containing two HBAs and three HpArs, and possessing the highest ranking score.

The generated 3D common feature pharmacophore hypothesis containing two HBAs and three HpArs, as shown in Figure 3(c–e), was projected as the common feature functions to explain the pharmacophoric site specifications of the EPI activity of BSN-coded 2-substituted benzothiazole compounds. Figures 3(f) and 3(g) represent the mapping of BSN4, BSN6, and BSN23 on the anticipated pharmacophore model meant for the most active molecules.

The generated pharmacophore model reveals that the two HBA and three HpAr features are found to be 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 placement of bulky aromatic moieties. Two HBA atoms or groups at the given positions are necessary in the molecule to bind to the target protein.

When the calculated distances and angles between the features within the geometry of anticipated pharmacophore model are examined, it is found that:

- the hydrogen bond acceptor property of nitrogen atom in the thiazole ring at the fused ring system,
- the hydrogen bond acceptor property of carbonyl oxygen in the amide function substituted on second position at the benzothiazole ring system,
- the hydrophobic aromatic property of the benzene ring in the fused ring system,
- the hydrophobic aromatic property of the phenyl group directly attached to the second position at the benzothiazole ring system, and
- the hydrophobic aromatic property of the phenyl ring in the 2-phenylacetamide and/or 3-phenylpropionamide moiety

are the essentials for the specification of inhibitory activity of BSN-coded compounds.

All the tested BSN-coded 2-substituted benzothiazoles with their conformational models were mapped onto the generated HipHop pharmacophore model using a ‘rigid’ fitting method and ‘best mapping only’ option to obtain the bioactive conformation of each molecule in the Ligand Pharmacophore Mapping protocol in Discovery Studio 3.5. The results of mapping of features onto the compounds are given in Table 3, and the BSN23, BSN6, and BSN4-coded compounds, which were experimentally found as the most active 2-substituted benzothiazole derivatives, indicated the best fit value of 4.999, 4.021, and 3.477, respectively, for the generated pharmacophore hypothesis, fitting to all the mapped common features in the anticipated model a specified ‘Pharmprint’ value of ‘11111’ (see Figure 3(f)).

The fitting and ‘Pharmprint’ values of the BSN-coded compounds considered for the pharmacophoric features in the anticipated model given in Table 3 showed that the compounds

Table 3. BSN coded compounds for HipHop pharmacophore modelling and results of mapping features.

Compound	*Fit Value*	*HpAr-1*[a]	*HpAr-2*[b]	*HpAr-3*[c]	*HBA-1*[d]	*HBA-2*[e]	*Pharmprint*[f]
BSN01	3.773	1	1	1	0	1	"11101"
BSN02	2.929	1	1	1	1	1	"11111"
BSN03	3.566	1	1	1	0	1	"11101"
BSN04	3.477	1	1	1	1	1	"11111"
BSN05	3.805	1	0	1	1	1	"10111"
BSN06	4.021	1	1	1	1	1	"11111"
BSN07	3.978	1	0	1	1	1	"10111""
BSN08	2.111	1	1	1	1	1	"11111"
BSN09	1.290	1	1	1	0	0	"11100"
BSN10	2.685	1	1	0	0	1	"11001"
BSN11	2.308	1	1	1	1	1	"11111"
BSN12	2.142	1	1	1	1	1	"11111"
BSN13	1.219	1	1	0	0	1	"11001"
BSN14	0.131	1	1	0	0	1	"11001"
BSN15	2.443	1	1	1	0	1	"11011"
BSN16	2.544	1	1	1	0	1	"11101"
BSN17	2.131	1	1	1	0	1	"11101"
BSN18	1.942	1	1	1	0	1	"11101"
BSN19	1.821	1	1	1	0	1	"11101"
BSN20	2.331	1	1	1	1	1	"11111"
BSN21	3.013	1	1	1	1	1	"11111"
BSN22	2.731	1	1	1	1	1	"11111"
BSN23	4.999	1	1	1	1	1	"11111"

[a]Hydrophobic aromatic property of the benzene ring in the fused ring system.
[b]Hydrophobic aromatic property of the phenyl group directly attached to the 2nd position at the benzothiazole ring system.
[c]Hydrophobic aromatic property of the phenyl ring in the 2-phenylacetamide and/or 3-phenylpropionamide moiety.
[d]Hydrogen bond acceptor property of nitrogen atom in the thiazole ring at the fused ring system.
[e]Hydrogen bond acceptor property of carbonyl oxygen in the amide function substituted on 2nd position at the benzothiazole ring system.
[f]If the training compound is match onto the mapped pharmacophoric feature in the anticipated model a principal value of each feature specified as 1 and if it is not fit a value of 0 is given.

holding a phenyl group on the second position at the benzothiazole ring, for example BSN2, BSN4, BSN6, BSN8, and BSN23, rather than having a benzyl group at that position, such as BSN9, BSN14, and BSN16, possessed a better match with all the features in the anticipated model (see Figure 3(f), 3(h,i)). This indicates why compounds with a phenyl group at the second position on the benzothiazole ring, such as BSN2, BSN4, BSN6, BSN8, and BSN23, provide more significant activity rather than having a benzyl group at that position.

When the tested compounds were substituted with a benzyl group instead of a phenyl ring at the second position of the benzothiazole nucleus, such as BSN9, BSN14, and BSN16, then were not able to show any match with the hydrogen bond acceptor feature of the nitrogen atom in the thiazole ring at the fused ring system, as given in Figure 3(i). As given in Table 3, these compounds showed a lower fit value and were not able to match with all the mapped common features in the anticipated model (see Figure 3(i)). This observation explains why the 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 AdeABC efflux pump inhibitor activity against the multidrug-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 AdeABC efflux pump inhibitor potency, rather than the 2-[4-(4-substituted-benzamido)benzyl]benzothiazole structure (see Figure 3(h) and 3(i)) in these tested BSN-coded 2-substituted benzothiazoles.

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