the Major Facilitator Superfamily

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Bacterial pathogens are serious causative agents of infectious disease. Such microorganisms are resistant to multiple antimicrobial agents, thereby compromising the therapeutic efficacy of treatment. Multidrug-resistant pathogens harbor antimicrobial efflux pumps, many transporters of which are members of the extensive major facilitator superfamily of proteins. These bacterial multidrug efflux pumps are good molecular targets for modulation and possible inhibition. This entry briefly discusses several current developments for drug efflux pump modulation.

Keywords: antimicrobial agents ; multidrug resistance ; bacteria ; pathogens ; major facilitator superfamily ; transporters ; sequence motifs ; infection

1. Introduction

Due to their widespread occurrence among cells from across all known living taxa and because of their ability to confer multiple antimicrobial resistance, bacterial multidrug efflux pumps from the major facilitator superfamily make suitable targets for resistance modulation[1][2][3]. A variety of efflux pump modulators have been discovered, such as naturally-occurring bioactive agents[4][5], synthetic agents[6], and synergistic modulator combinations[7]. Table 1 lists some examples of various modulators of antimicrobial efflux pumps belonging to the major facilitator superfamily, which are discussed in detail elsewhere[1].

Table 1. Some examples of various modulators of antimicrobial efflux in some bacterial efflux pumps from the major facilitator superfamily.

Efflux Pump Targeted	Modulators	References
EmrB from *Escherichia coli*	Phenylalanine arginyl β-naphthylamide (PAβN) and 1-(1-naphthyl methyl)-piperazine (NMP)	[8]
EmrD-3 from *Vibrio cholerae*	Garlic, allyl sulfide	[9]
LmrP from *Lactococcus lactis*	Verapamil and quinine Nicardipine and vinblastine Tetraphenyl phosphonium	[10]
QacA from *Staphylococcus aureus*	Hydantoin, silybin	[11][12]
MdfA from *Escherichia coli*	Reserpine	[13]

QacB from *Staphylococcus aureus*	Silybin	[11]
LmrS from *Staphylococcus aureus*	Cumin seed oil, cumin aldehyde, reserpine	[14]

NorA from *Staphylococcus aureus*	3-aryl piperidines	[15]
	Berberine	[16]
	Reserpine	[17]
	Omeprazole, lansoprazole	[18]
	GG918, tariquidar (primary active transport inhibitors)	[19][20]
	Verapamil, ciprofloxacin, ofloxacin	[21]
	5,9′dimethyl-deca-2,4,8-trienoic acid, 9-formyl-5-methyl-deca-2,4,8-trienoic acid	[22]
	Chlorpromazine, thioridazine, and prochlorperazine	[23][24][25]
	Kaempferol rhamnoside	[26]
	Chalones	[27]
	COX-2 inhibitor analog, 3-(4-chlorophenyl)-1-(4-nitrophenyl)-1,4-dihydropyrazolo[4,3-c] [1,2] benzothiazine 5,5-dioxide	[28]
	Coumarin	[29]
	Genistein (flavonoid compound)	[12]
	Ginsenoside 20(S)-Rh2	[30]
	Boronic acid molecules, 6-(3-phenylpropoxy) pyridine-3-boronic acid and 6-(4-phenylbutoxy) pyridine-3-boronic acid	[31]
	Silybin	[32]
	5′-methoxy-hydnocarpin, pheophorbide A, 5′-MHC, curcumin, kaempferol, silibinin, isoflavone, orizabins, capsaicin, tannic acid,	[33]
	nerol, dimethyl octanol, estragole	[34]
	Riparin B	[35]

Olaanolic acid, ulvaol	[36]
Brachydins: BR-A, BR-B	[37]

2. Modulation of Multidrug Efflux Pumps of the Major Facilitator Superfamily

One of the earliest clear examples of modulation upon a major facilitator superfamily antimicrobial efflux pump was that of the energy uncoupler carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) and the TetA(C) tetracycline efflux pump[38], demonstrating that the pump was a secondary active transporter. Since this groundbreaking study, CCCP has been used as a means of establishing the ion-driven process of energization for most newly discovered secondary active transport systems[39][40]. Furthermore, CCCP has been shown to be effective, albeit in an indirect manner, as an inhibitor of antimicrobial efflux in a great variety of major facilitator superfamily transporters by collapsing the proton motive force[1][2][3]. Along these lines, reserpine and piperine have served as general inhibitors for many efflux pumps, independent of the mode of energy, substrates, and superfamily membership[41][42][43].

A universal target for a multitude of efflux pump inhibitors is the NorA transporter from the critical pathogen *S. aureus* and is considered in further detail elsewhere[44][45]. Similarly, the QacA efflux pump from *S. aureus* represents another well-studied target for modulation by a large number of inhibitors, which have been extensively reviewed[5][46][47]. In our laboratory, we discovered that the non-toxic cumin spice extract and its bioactive agent cuminaldehyde inhibited resistance and efflux, respectively, which were mediated by the multidrug efflux pump LmrS from *S. aureus*[14][48]. More recently, brachydin-based compounds extracted from extracts of *Arrabidaea brachypoda* were shown to inhibit both the growth of *S. aureus* and NorA drug efflux[37]. As clinical infection by *S. aureus* is a critical public health concern and because the genome encodes over a dozen distinctive antimicrobial efflux pumps, this bacterium will continue to be a target of intensive study for resistance modulation[49][50][51].

We also evaluated the efficacy of the garlic extract and its bioactive agent allyl sulfide towards multidrug resistance conferred by the EmrD-3 multidrug efflux pump from the *Vibrio cholerae* pathogen[9]. We found a direct effect upon antimicrobial transport across EmrD-3 by garlic extract at low concentrations but an indirect effect on resistance at higher garlic extract amounts, probably through modulation at the level of the respiratory chain[9]. Correspondingly, we observed similar modulatory effects with cumin and drug transport through LmrS and with the energetics of the respiratory chain in *S. aureus*[14]. We anticipate that similar direct effects on antimicrobial transport at low modulator concentrations and indirect effects at relatively higher modulator amounts will continue to be observed with other bacterial pathogens that harbor multidrug efflux pumps that constitute members of the major facilitator superfamily.

Previously known as CmlA and Cmr, and now as MdfA, the protein structure of this multidrug efflux pump from *E. coli* was determined at high resolution in which one of its substrates, chloramphenicol, plus two substrate analogs and putative efflux pump inhibitors *n*-dodecyl-*N,N*-dimethylamine-*N*-oxide and deoxycholate, were bound to MdfA[52]. Interestingly, chloramphenicol makes contact with the conserved and negatively-charged residues Glu-26 and Asp-34, which are located in α-helix one of MdfA and are encircled by conserved members of motif C, namely, Val-149, Ala-150, Ala-153, and Pro-154, constituting the so-called domain interface between the two global bundles[52]. In more recent studies, it was discovered that not only is the α-helical structure formed by the motif C kinked, as predicted[53], but the fifth helix also rotationally twists during substrate translocation across the membrane[54]. Thus, because of its presence in efflux pumps of the major facilitator superfamily, it is anticipated that the domain interface component of the molecular hinge is a desirable target for the development of potent efflux pump inhibitors[55].

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