An Introduction to Bacterial Efflux Pump Inhibitor (EPI) and Analytical Methods for Studying Efflux Pump Inhibitors for Drug Development

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Abstract:- Bacterial diseases are becoming a more severe problem around the world. Treatment of multidrug-resistant pathogens has become a major concern in the present world. Bacterial efflux pumps are present in all microorganisms and play a key role in both innate and acquired bacterial drug resistance. Therefore, discovering small molecule efflux pump inhibitors (EPIs) that are capable of restoring antibiotic effectiveness is a hot topic in the scientific world. Seeking new EPIs has taken a lot of effort over the past two decades. None of them, however, has yet to be approved for medicinal use. To introduce these inhibitors for escalating the responsiveness of bacterial pathogens to antibiotics, we should have proper knowledge of drug detection and the mechanism of efflux pumps. This review of EPIs is a good place to start looking for new anti-bacterial drugs that are safe.

Keywords:- Efflux Mechanisms, Efflux Pump Inhibitor (EPI), Antibiotic Resistance, Multidrug Resistant (MDR), Bacteria.

INTRODUCTION

Antibiotic Resistance can be described as the impotence of the antibiotics to reach the target in adequate concentration for impeding the activity of the target. The reason behind the acquiring resistance is due to: decrease in the affinity of the target for the antibiotic, or, reducing the concentration of the antibiotic in the cell. Bacteria may be intrinsically immune to antibiotics due to systemic or functional differences. Weak permeability of the bacterial cell wall and constitutive expression of efflux pumps are examples of these characteristics that are encoded by house-keeping genes in the bacterial genome [1].

The most common form of resistance factor, efflux pumps, can be found in all species, from bacteria to mammals [2,3]. The function of the efflux pump is to extrude out drugs and chemicals outside the cell wall. The majority of bacterial efflux systems are non-drug-specific proteins that can identify and pump out a wide variety of chemically and structurally unrelated compounds in an energy-dependent manner [4]. Presence of these multidrug efflux mechanisms in pathogenic bacteria has become a major reason for the discovery of the efflux pump inhibitors(EPIs) that helps in blocking the efflux pumps.

MECHANISM OF THE ACTIVITY OF EFFLUX PUMP INHIBITORS (EPIS)

The most promising method for inhibiting multidrug efflux pumps is to use Efflux pump inhibitors (EPIs). This can be accomplished by interacting with the functional assemblies of efflux pump components, and efflux inhibition can help prevent bacterial pathogens from developing resistance. In reality, EPIs function in a variety of ways, depending on their properties and mechanisms, can interfere with efflux function, assembly, and pumping. However, due to the decline in antibiotic expansion, it is important to look for and classify compounds that restore the antibacterial activity of older antibiotics. The efflux pump inhibitor-antibiotic combination is intended to increase the intracellular concentration of antibiotics expelled by efflux pumps, minimize intrinsic bacterial resistance to antibiotics, reverse acquired resistance associated with efflux pump overexpression, and reduce the frequency of resistant mutant strains.
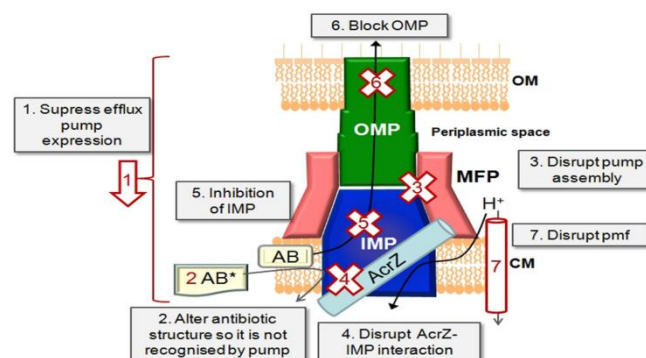

Figure 1: Schematic representation of a tripartite drug efflux complex in complex with a small protein such as AcrZ [5].

Figure 1 shows the action of EPI on a tripartite drug efflux complex, which contain three proteins which span the inner membrane (CM), the outer membrane (OM), and the periplasmic space [5]. The inner-membrane protein (IMP), e.g., AcrB or MexB is responsible for substrate specificity and catalyzes ΔpH dependent drug transport [5]. Examples of the outer membrane protein (OMP) are TolC or OprM [5]. The periplasmic membrane fusion protein (MFP), e.g., AcrA or MexA connects the IMP and the OMP [5].

EPI Against Gram Positive Bacteria

Gram-positive bacteria are one of the most common human pathogens linked to clinical infections ranging from minor skin infections to sepsis. Gram-positive bacteria (such as those belonging to the genera Staphylococcus, Streptococcus, and Enterococcus) are among the most common causes of clinical infection. Although the problem of multi-drug resistance (MDR) in Gram-negative bacteria has received a lot of attention recently, Gram-positive Antimicrobial resistance is also a serious concern [6]. MRSA (methicillin-resistant Staphylococcus aureus) is perhaps the most well-known example, and it is a major cause of community-acquired and healthcare-associated infection worldwide [7].

Vancomycin (VANC) and teicoplanin (TEIC) are glycopeptide antimicrobials that are only active against Gram-positive bacteria [6]. Since all glycopeptides currently available have low oral bioavailability, they must be administered parenterally for the treatment of systemic infections, typically by intravenous (IV) injection. TEIC and the newer lipophilic glycopeptides are playing an increasingly important role in the delivery of OPAT [6].

Daptomycin, a cyclic lipopeptide, has a wide range of action against Gram-positive bacteria, including GRE and MRSA [8]. DAPT is a hydrophobic polypeptide with a lipophilic side chain that has 13 members [8]. This structure is thought to have a one-of-a-kind mechanism of action. The lipophilic area is thought to insert into the bacterial cell membrane, oligomerizing into pore-like structures and allowing substantial potassium ion efflux. Following membrane depolarization, DNA, RNA, and protein synthesis are immediately halted, resulting in rapid bacterial cell death.

The oxazolidinones (OXAs) are the most recent antimicrobial class to receive human approval. OXAs have bacteriostatic activity against a wide range of bacteria, mostly Gram-positive bacteria like MRSA and vancomycin-resistant enterococci (VRE). Cefazoline and ceftobiprole are fifth-generation Cephalosporins with a distinct bacteriological spectrum.

EPIs Against Gram-Negative Bacteria Identified So Far

The presence of lipophilicity and an additional outer membrane makes it impossible for amphipathic compounds to enter gram-negative bacteria. As a result, there are very few antibiotics that are specifically designed for gram-negative bacteria [9]. The proton motive force (PMF) drives the operation of Resistance-nodulation-division (RND) efflux pumps, which are proton antiporters. Antibiotic hybrids are a brand-new class of antimicrobials and adjuvants for Gram-

negative pathogens. Aminoglycoside conjugates, for example, have been designed to deliver EPIs into cells. When used in combination with tetracycline, the EPIs 1-(1-naphthylmethyl)-piperazine and paroxetine conjugated to tobramycin decreased the electrical portion of the PMF, enhancing their adjuvant properties [10].

Further research revealed that when used against multidrug-resistant P. aeruginosa, tobramycin–EPI conjugates have a synergistic effect with fluoroquinolones, rifampicin, and Fosfomycin [10]. Blocking tripartite efflux pumps' outer membrane factor (OMF) can also render them inactive [11]. The indole derivatives 3-amino-6-carboxyl-indolenin and 3-nitro-6-amino-indole have been shown to be able to block TolC, the OMF of various Enterobacteriaceae efflux pumps, increasing bacterial susceptibility to various antibiotics [12]. This group contains pyranopyridine derivatives. One of them, MBX2319, has broad antibacterial activity against E. coli and potentiates the antibacterial activity of a variety of AcrAB efflux pump substrates [13]. RND efflux pumps, in particular, use competitors that can bind to the efflux pump's substrate pocket, preventing other substrates from binding. These inhibitors have been shown to bind more closely to the AcrB binding pocket than antibiotic substrates, resulting in successful inhibition even at low concentrations [13]. The crystal structures of AcrB from E. coli and MexB from P. aeruginosa bound to the pyridopyrimidine derivative D13–D900 has recently been released, revealing the structural basis for RND transporter inhibition [13].

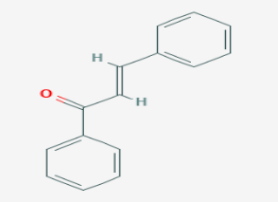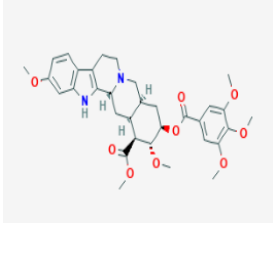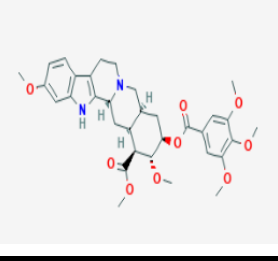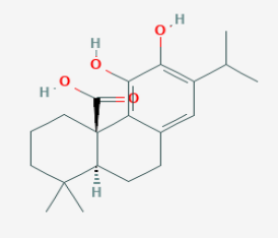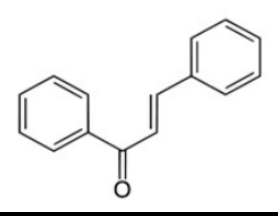## OVERVIEW OF RECENT UPDATED COMPUTATIONAL TOOLS FOR EFFLUX PUMPS AND THEIR INHIBITORS

The structural aspects of Multidrug resistant (MDR) pumps have been greatly enhanced by crystallographic structures, but this is insufficient to help structure-based drug design [14]. Computational methods, which can detect functional dynamics of biological systems, are a great resource for addressing mechanistic knowledge gaps.
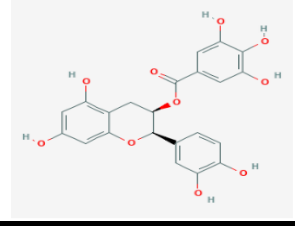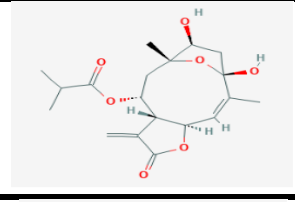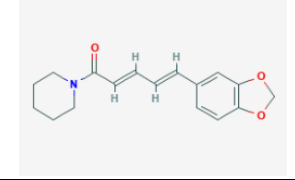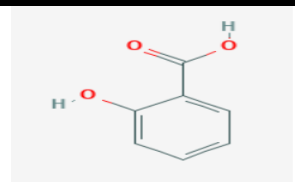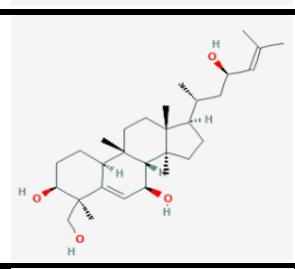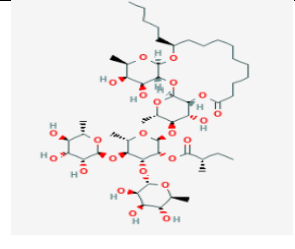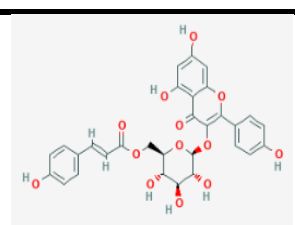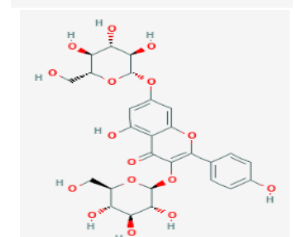
Molecular docking, in particular, is useful in the silico method for identifying drug-binding sites, and Molecular dynamics (MD) simulations are comparable [14]. Becoming popular computational methods for both rationalizing existing data and making numerous predictions, such as drug recognition and binding, interaction-free energies, and translocation mechanism [14]. They are often used to verify homology models [14]. Furthermore, normal mode and functional mode analysis of protein movements allow for comparison of simulations of the apo and hollo structures, potentially leading to the discovery of factors closely linked to the efflux mechanism's first steps. Significant conformational changes that would usually be inaccessible by standard MD techniques due to large free energy barriers between such conformations and correspondingly unaffordable computational time have recently been sampled using 'coarse-grained simulations, in which generally four atoms are fused into one particle, and biased MD simulations [15]. Various computational studies on the EPIs of MDR pumps, predominantly RND and ATP-binding cassette (ABC)

transporters, have focused on identifying successful EPIs through high-throughput screening of compound databases and determining the mechanism of inhibition by inspecting molecular level inhibitor–pump interactions and coupled conformational changes in the transporters [16].

Although there has only been one published structure of an RND protein bound to an inhibitor, docking and molecular simulation studies were used to investigate the putative binding modes of other inhibitors such as PAN and N-Methylpyrrolidone (NMP), and in silico screening provided information on the binding of putative EPIs [5].

Table 1: Plant-derived EPIs with their structure, antibiotics, and complex efflux pumps.

Bacteria	EPI	Efflux Pump	Antibiotic	Plant Source	Structure	References
Bacillus subtilis	Reserpine	Bmr	Tetracycline	*Rauwolfia vomitoria*		[17]
Bacillus cereus	Chalcone	NorA	Berberine, erythromycin and tetracycline	*Nicotiana tobacum, Dalea versicolor*		[18]
Streptococcus pneumoniae	Reserpine	NorA	Ciprofloxacin	*Rauwolfia vomitoria*		[17]
Staphylococcus aureus	Reserpine	TetK, NorA	Norfloxacin, Tetracycline	*Rauwolfia vomitoria*		[17]
Staphylococcus aureus	Carnosic acid & Carnosol	Tet (K) & Msr (A)	Tetracycline and erythromycin	*Rosmarius officinalis*		[19]
Staphylococcus aureus	Chalcone	NorA	berberine, erythromycin and tetracycline	*Dalea versicolor*		[20]

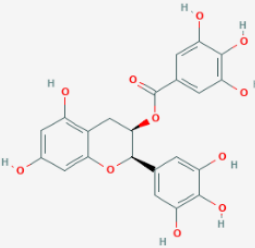| Bacteria	EPI	Efflux Pump	Antibiotic	Plant Source	Structure	References
Staphylococcus aureus	Epicatechin gallate & Epigallocatechin gallate	NorA	Norfloxacin	*Camellia sinensis*		[21]
Staphylococcus aureus	Orizabin	Nor A	Norfloxacin	*Ipomoea violacea*		[22]
Staphylococcus aureus	Piperine	MdeA & Nor A	Ciprofloxacin	*Piper nigrum, Piper longum*		[23]
Staphylococcus aureus	Salicylic acid	SarA	Ciprofloxacin, Ethidium bromide	*Salix alba*		[24]
Staphylococcus aureus	Balsaminol, Balsaminagenin, Karavilagenin	NorA	AcrAB-TolC	*Momordica balsamnia*		[22]
Staphylococcus aureus	Murucoidins	Nor A	Norfloxacin	*Ipomoea murucoides*		[12]
Staphylococcus aureus	Kaempferol Glycoside, Tiliroside	Nor A	Ciprofloxacin	*Herissantia tiubae*		[25]
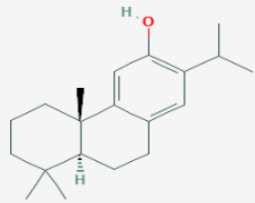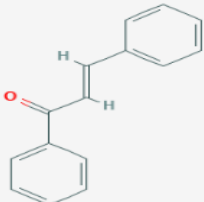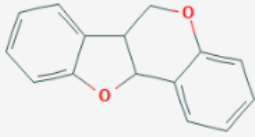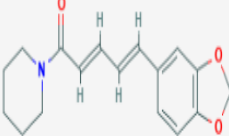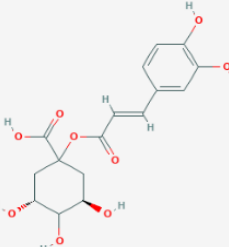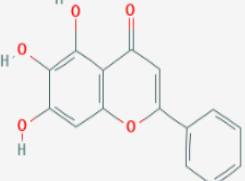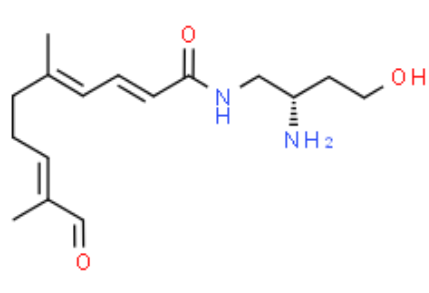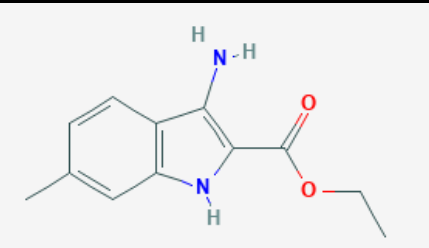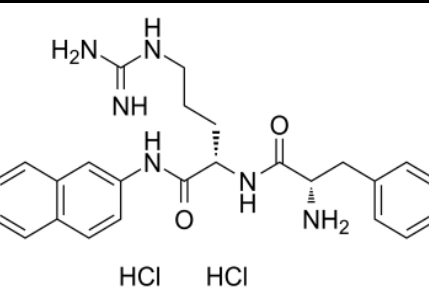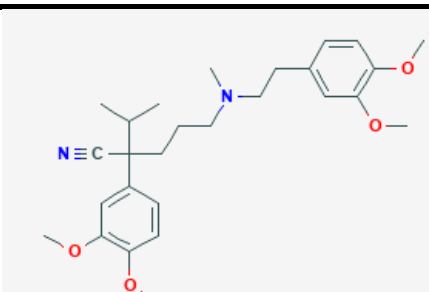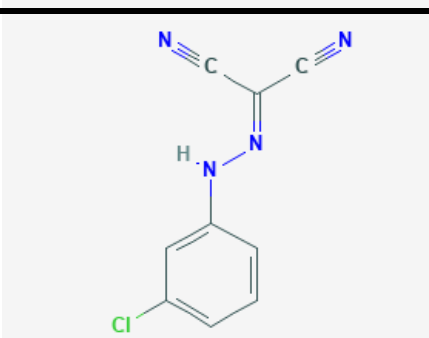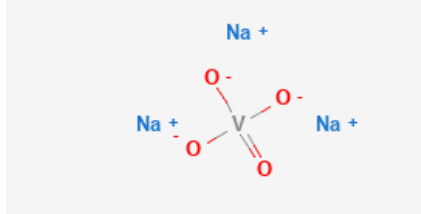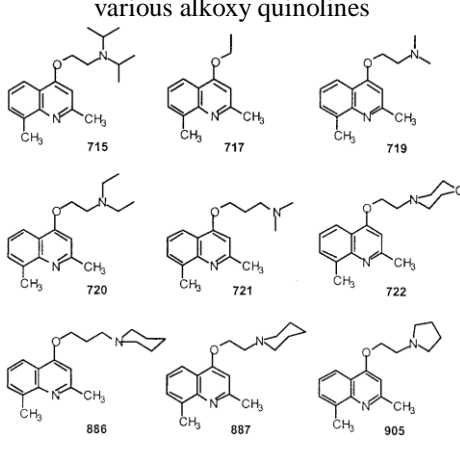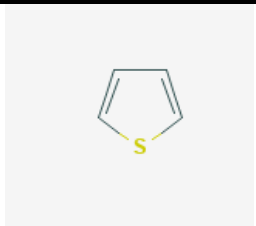
Bacteria	EPI	Efflux Pump	Antibiotic	Plant Source	Structure	References
Staphylococcus aureus	Ferruginol	NorA	Norfloxacin, oxacillin	Chamaecyparis lawsoniana		[26]
Staphylococcus aureus	Chalcone	Nor A	Ethidium bromide	Nicotiana tobacum		[20]
Staphylococcus aureus	Pterocarpan	Nor A	Berberine	Dalea spinosa		[27]
Staphylococcus epidermidis	Epigallocatechin Gallate	Tet(K)	Tetracycline	Camellia sinensis		[28]
Mycobacterium spp.	Piperine	Rv1258c	Ethidium bromide	Piper nigrum, Piper longum		[29]
Enterococcus faecalis	Caeffeoyl quinic acid	NorA	Berberine	Artemisia absinthium		[4]
E. coli	Baicalein	Tet K	Tetracycline	Thymus vulgaris		[30]

Table 2: Synthetic EPIs with structure, antibiotics, and specific efflux pumps.

EPI	Efflux pump	Antibiotic	Synthetic Source	Structure	References
5,9-dimethyl-deca-2,4,8-trienoic acid amides	EPI against *Staphylococcus aureus*	ciprofloxacin	Amide derivatives		[31]
3-amino-6-carboxyl - indole	EPI against *E. coli* YD2 and FJ307 overexpressing AcrAB-TolC efflux pump	chloramphenicol, tetracycline, erythromycin and ciprofloxacin	Indole derivatives		[32]
PAβN	RND efflux pumps (MexAB-OprM, MexCD-OprJ, MexEF-OprN, and MexXY-OprM).	chloramphenicol and macrolides	Valinomycin & Dinitrophenol (DNP		[33]
EPI	**Efflux pump**	**Antibiotic**	**Synthetic Source**	**Structure**	**References**
Verapamil	EPI against *Mycobacterium tuberculosis* AND EPI of LmrA efflux pump of *Lactococcus lactis*	isoniazid, rifampin and pyrazinamide.	Verapamil		[34]
CCCP	MFS efflux pump in *Mycobacterium smegmatis* 33 and in *Mycobacterium fortuitum*	Carbapenem	Carbonyl cyanide m-chlorophenylhydrazone (CCCP)		[35]

Sodium orthhovanadate (Na3VO4)	ABC efflux pumps of *Streptococcus pneumoniae*	ciprofloxacin & Ethidium bromide.	Sodium orthhovanadate (Na3VO4		[36]
2,8-dimethyl-4-(2'-pyrrolidinoethyl)-oxyquinoline	efflux pumps in *E. aerogenes* and *K. pneumoniae*	chloramphenicol, norfloxacin, tetracycline and cefepime	Alkoxy quinolone derivatives	various alkoxy quinolines	[37]
Thiophene	NorA efflux pump of *Staphylococcus aureus*	ciprofloxacin	Thiophene		[38]

FUTURE PERSPECTIVE

Efflux pump inhibitors have so far been discovered by screening synthetic chemical agents or plant metabolites at random [39]. Researchers can classify the pharmacophores of a putative inhibitor that know the unique binding site of an efflux pump using advanced 3D structure resolution, molecular modeling, and molecular dynamics simulation. The ability of inhibitors to cross the outer membrane barrier is important in Gram-negative bacteria. Combining noncompetitive and competitive inhibitors and/or selective blockers to saturate the biological transport pathway is one way to achieve optimum pump inhibition. MDR transporters indicate that by manipulating molecular interactions that differentiate substrate transport from inhibitor binding, it may be possible to create inhibitors that can substantially and directly reduce efflux in bacteria [40]. Recent structures will certainly increase the chances of predicting a compound's export susceptibility, and this can be supplemented by using quantitative structure-activity relationship data to establish medicinal chemistry programs. These molecular docking studies aim to gain insight into the drug-protein interactions that differentiate between compounds [41]. The ability of computational biology to examine substrate recognition has been demonstrated; a combination of MD simulations and docking experiments offered a physicochemical explanation for the observed differences in the transport of various antibiotics or other drugs[42]. One of the most difficult aspects of developing EPIs

for Gram-negative bacteria is creating compounds that can cross the outer membrane (OM) and bind to the RND pumps with high affinity [5]. These developments could help solve some of the major obstacles in the discovery and production of clinically beneficial EPIs when taken together.

I. CONCLUSION

In the last two decades, EPIs has become a popular research subject. A very few EPI is available till now for the treatment of Gram-negative infections due to their low stability, selectivity, and high cytotoxicity for eukaryotic cells. The interactions between antibacterial compounds and efflux pumps are complicated, making the development of successful EPIs difficult [43]. Since porins regulate the outer membrane's penetration, which favors zwitterionic and smaller hydrophilic molecules, developing novel EPIs in Gram-negative bacteria remains a difficult job. The main RND efflux pumps, on the other hand, prefer larger hydrophobic molecules. In this case, a logical design in which charged groups are attached where they will increase bacterial penetration can be used to identify a suitable location on EPIs. Nonetheless, the synthesis of these compounds will increase antibiotic resistance (including some antibiotics for which Gram-negative bacteria are intrinsically resistant) and, possibly for some particular efflux pumps, will reduce bacterial pathogen virulence [44].

ACKNOWLEDGMENT

REFERENCES

[1].Andraž Lamut, Lucija Peterlin Mašič, Danijel Kikelj, Tihomir Tomašič. Efflux pump inhibitors of clinically relevant multidrug resistant bacteria. 2019 Nov;39(6):2460-2504.

[2].Martinez, J.L.; Sanchez, M.B.; Martinez-Solano, L.; Hernandez, A.; Garmendia, L.; Fajardo, A.; Alvarez-Ortega, C. Functional role of bacterial multidrug efflux pumps in microbial natural ecosystems. FEMS Microbiol. Rev. 2009, 33, 430–449. [CrossRef] [PubMed]

[3].Martinez, J.L.; Fajardo, A.; Garmendia, L.; Hernandez, A.; Linares, J.F.; Martinez-Solano, L.; Sanchez, M.B. A global view of antibiotic resistance. FEMS Microbiol. Rev. 2009, 33, 44–65. [CrossRef] [PubMed])

[4].Tanuja Rana, Sunity Singh, Navroop Kaur, Kajal pathania, Umar Farooq. A Review on Efflux Pump Inhibitors of Medically Important Bacteria from Plant Sources. May – Jun 2014. Article No. 19. Pg: 101-111

[5].Henrietta Venter, Rumana Mowla, Thelma Ohene-Agyei and Shutao Ma. RND-type drug efflux pumps from Gram-negative bacteria: molecular mechanism and inhibition. 28 April 2015.M. Young, The Technical Writer's Handbook. Mill Valley, CA: University Science, 1989.

[6].Christopher Eades, Stephen Hughes, Katie Heard & Luke SP Moore. Antimicrobial therapies for Gram-positive infections. 12 September 2017

[7].Klevens RM, Morrison MA, Nadle J et al. Invasive methicillin- resistant Staphylococcus aureus infections in the United States. JAMA 2007;298(15):1763–1771.

[8].Andres Beiras-Fernandez, Ferdinand Vogt, Ralf Sodian and Florian Weis. Daptomycin: a novel lipopeptide antibiotic against Gram-positive pathogens. 2010; 3: 95–101.

[9].Shanqing Li, Zhijun Han, et.al. Society for Translational Medicine expert consensus on the use of antibacterial drugs in thoracic surgery. Vol 10, No 11 (November 2018).

[10].Xuan Yang, Ronald Domalaon, Yinfeng Lyu. Tobramycin-Linked Efflux Pump Inhibitor Conjugates Synergize Fluoroquinolones, Rifampicin and Fosfomycin against Multidrug-Resistant Pseudomonas aeruginosa. June 2018. 7(7):158.

[11].João Anes, Matthew P. McCusker, Séamus Fanning, and Marta Martins. The ins and outs of RND efflux pumps in Escherichia coli. 2015; 6: 587.

[12].Atin Sharma, Vivek Kumar Gupta, and Ranjana Pathania. Efflux pump inhibitors for bacterial pathogens: From bench to bedside. 2019 Feb; 149(2): 129–145.

[13].Robert L. Marshall, Georgina S. Lloyd, et.al. New Multidrug Efflux Inhibitors for Gram-Negative Bacteria. DOI: 10.1128/mBio.01340-20

[14].Shirin Jamshidi, J Mark Sutton & Khondaker M Rahman. An overview of bacterial efflux pumps and computational approaches to study efflux pump inhibitors. 2015.

[15].Timothy J. Opperman and Son T. Nguyen. Recent advances toward a molecular mechanism of efflux pump inhibition. 2015

[16].Venkata Krishnan Ramaswamy, Pierpaolo Cacciotto, Giuliano Malloci, Attilio V. Vargiu and Paolo Ruggerone. Computational modelling of efflux pumps and their inhibitors. 2017 (141–156).

[17].F R Stermitz 1, P Lorenz, J N Tawara, L A Zenewicz, K Lewis. Synergy in a medicinal plant: antimicrobial action of berberine potentiated by 5'-methoxyhydnocarpin, a multidrug pump inhibitor. 2000 Feb 15;97(4):1433-7.

[18].L Ralston 1, S T Kwon, M Schoenbeck, J Ralston, D J Schenk, R M Coates, J Chappell. Cloning, heterologous expression, and functional characterization of 5-epi-aristolochene-1,3-dihydroxylase from tobacco (Nicotiana tabacum). 2001 Sep 15;393(2):222-35.

[19].Isabel Borrás-Linares, Zorica Stojanović, et.al. Rosmarinus officinalis leaves as a natural source of bioactive compounds. 2014 Nov 10;15(11):20585-606.

[20].Ramya Kuber Banoth and Ashwini Thatikonda. A REVIEW ON NATURAL CHALCONES AN UPDATE.

[21].Daneel Ferreira, Riaan Bekker. Carbohydrates and Their Derivatives Including Tannins, Cellulose, and Related Lignings. 1999

[22].Armel JacksonSeukep, Victor Kuete, Lutfun Nahar, Satyajit D.Sarker, Mingquan Guo. Plant-derived secondary metabolites as the main source of efflux pump inhibitors and methods for identification. Volume 10, Issue 4, August 2020, Pages 277-290

[23].Anshuly Tiwari, Kakasaheb R.Mahadik, Satish Y.Gabhe. Piperine: A comprehensive review of methods of isolation, purification, and biological properties. Volume 7, September 2020.

[24].Agnieszka E. Laudy, Agnieszka Mrowka, Joanna Krajewska and Stefan Tyski. The Influence of Efflux Pump Inhibitors on the Activity of Non-Antibiotic NSAIDS against Gram-Negative Rods. 2016; 11(1).

[25].Sandro Cid-Ortega and José Alberto Monroy-Rivera. Extraction of Kaempferol and Its Glycosides Using Supercritical Fluids from Plant Sources: A Review. 2018 Dec; 56(4): 480–493.

[26].Guoqing Luo, Jingjing Zhou, et.al. Ferruginol Diterpenoid Selectively Inhibits Human Thyroid Cancer Growth by Inducing Mitochondrial Dependent Apoptosis, Endogenous Reactive Oxygen Species (ROS) Production, Mitochondrial Membrane Potential Loss and Suppression of Mitogen-Activated Protein Kinase (MAPK) and PI3K/AKT Signaling Pathways.

[27].Chelliah Selvam, Brian C Jordan, Sandhya Prakash, Daniel Mutisya, Ramasamy Thilagavathi. Pterocarpan scaffold: A natural lead molecule with diverse pharmacological properties. 2017 Mar 10;128:219-236.

[28]. Chelliah Selvam, Brian C Jordan, Sandhya Prakash, Daniel Mutisya, Ramasamy Thilagavathi. Pterocarpan scaffold: A natural lead molecule with diverse pharmacological properties. 2017 Mar 10;128:219-236.

[29]. Anshuly Tiwari, Kakasaheb R.Mahadik, Satish Y.Gabhe. Piperine: A comprehensive review of methods of isolation, purification, and biological properties. Volume 7, September 2020.

[30]. Adelina Jiménez-Arellanes, et.al. Thymus vulgaris as a potencial source of antituberculous compounds. January 2006

[31]. Niranjan Thota, Surrinder Koul, et.al. Citral derived amides as potent bacterial NorA efflux pump inhibitors. 2008 Jul 1

[32]. D.W. SullivanJr., S.E. Gad. Indole. 2014

[33]. Ryan P. Lamers, Joseph F. Cavallari, Lori L. Burrows. The Efflux Inhibitor Phenylalanine-Arginine Beta-Naphthylamide (PAβN) Permeabilizes the Outer Membrane of Gram-Negative Bacteria. March 27, 2013.

[34]. Shashank Gupta, Sandeep Tyagi, et.al. Acceleration of Tuberculosis Treatment by Adjunctive Therapy with Verapamil as an Efflux Inhibitor. 2013 Sep 1; 188(5): 600–607.

[35]. J Kasianowicz, R Benz, S McLaughlin. The kinetic mechanism by which CCCP (carbonyl cyanide m-chlorophenylhydrazone) transports protons across membranes. 1984;82(2):179-90.

[36]. Qingan Yu, Wenjing Jiang, et.al. Sodium orthovanadate inhibits growth and triggers apoptosis of human anaplastic thyroid carcinoma cells in vitro and in vivo. 2019 May; 17(5): 4255–4262.

[37]. Jacqueline Chevalier, Jerome Bredin, Abdallah Mahamoud, Monique Malle´, Jacques Barbe, and Jean-Marie Pages. Inhibitors of Antibiotic Efflux in Resistant Enterobacter aerogenes and Klebsiella pneumoniae Strains. Mar. 2004, p. 1043–1046.

[38]. Rashmi Shah and Prabhakar Kumar Verma. Therapeutic importance of synthetic thiophene. 2018; 12: 137.

[39]. Jean-Marie Pagès, Leonard Amaral, Séamus Fanning. An Original Deal for New Molecule: Reversal of Efflux Pump Activity, A Rational Strategy to Combat Gram-Negative Resistant Bacteria. June 2011.

[40]. Monique Putman, Hendrik W. van Veen, and Wil N. Konings. Molecular Properties of Bacterial Multidrug Transporters. 2000 Dec; 64(4): 672–693.

[41]. Xuan-Yu Meng, Hong-Xing Zhang, Mihaly Mezei and Meng Cui. Molecular Docking: A powerful approach for structure-based drug discovery. 2012 Jun 1.

[42]. Gregory Sliwoski, Sandeepkumar Kothiwale, Jens Meiler, and Edward W. Lowe, Jr. Computational Methods in Drug Discovery. 2014 Jan; 66(1): 334–395.

[43]. Dreier J., Ruggerone P. Interaction of antibacterial compounds with RND efflux pumps in Pseudomonas aeruginosa. Front. Microbiol. 2015;6:660.

[44]. Jose M. Munita and Cesar A. Arias. Mechanisms of Antibiotic Resistance. 2016