

Microbial siderophore-based iron assimilation and therapeutic applications

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Abstract Siderophores are structurally diverse, complex natural products that bind metals with extraordinary specificity and affinity. The acquisition of iron is critical for the survival and virulence of many pathogenic microbes and diverse strategies have evolved to synthesize, import and utilize iron. There has been a substantial increase of known siderophore scaffolds isolated and characterized in the past decade and the corresponding biosynthetic gene clusters have provided insight into the varied pathways involved in siderophore biosynthesis, delivery and utilization. Additionally, therapeutic applications of siderophores and related compounds are actively being developed. The study of biosynthetic pathways to natural siderophores augments the understanding of the complex mechanisms of bacterial iron acquisition, and enables a complimentary approach to address virulence through the interruption of siderophore biosynthesis or utilization by targeting the key enzymes to the siderophore pathways.

Keywords Siderophore · Biosynthetic pathways · Iron assimilation · Therapeutic application

Introduction

Iron is an essential element for all microorganisms and an important cofactor required for key cellular, metabolic and biosynthetic processes (Sutak et al. 2008; Saha et al. 2012). The specific acquisition of elemental iron is critical for a microbe's survival and frequently virulence (Miethke and Marahiel 2007; Weinberg 2009). Under common aerobic and neutral pH conditions, environmental ferric ion has a low bioavailability concentration (10^{-9} – 10^{-18} M), which is usually below the requirement for optimal growth (Braun et al. 1997). As a consequence, bacteria and fungi have evolved diverse strategies to import and utilize iron under iron-deficient conditions exemplified as: the direct extracellular reduction of ferric compounds of low solubility to soluble ferrous iron (Deneer et al. 1995); the acquisition of iron-bound heme or heme-containing proteins (Tong and Guo 2009); the removal of metal from host iron-bound transferrin, lactoferrin or ferritin via specific outer membrane receptors (Braun 2001); and the synthesis, secretion and utilization of iron specific chelators, ferric-binding siderophores (Hider and Kong 2010).

Siderophores are low molecular weight compounds (typically ~400–1200 Da) with extraordinary high affinity for ferric (less so for ferrous) ion, and tend to use negatively charged oxygen as coordinating, donor atoms (Hider and Kong 2010; Saha et al. 2012). Broadly, siderophores can be classified into three categories, depending on the ligand moiety for Fe^{3+}

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coordination: catecholate, hydroxamate/carboxylate, and mixed-type, exemplified by enterobactin (**1**, *Escherichia coli*), aerobactin (**2**, *E. coli*)/rhizoferrin (*Rhizopus microsporus*) and mycobactin (**3**, *Mycobacterium tuberculosis*) respectively (Fig. 1). The different subclasses typically contain multi-dentate ligands, and show strong affinity for the higher oxidation state of iron with association constants of $\sim 10^{30}$ or greater. Siderophores help maintain the intracellular iron concentration between 10^{-7} and 10^{-5} M necessary for survival and replication (Braun et al. 1997). In addition to iron, siderophores also have the ability to seize and transport other metals (Johnstone and Nolan 2015).

General approaches of siderophore biosynthesis

Siderophores are commonly produced in the cytosol, or sometimes in peroxisomes (Miethke and Marahiel 2007; Gründlinger et al. 2013). A large variety of siderophores have been discovered in the past decades with two biosynthetic pathways resulting in structurally distinct siderophores. Most peptide-based siderophores are biosynthesized via well-

characterized, non-ribosomal peptide synthetases (NRPSs). NRPSs are large multi-enzyme complexes responsible for the synthesis of diverse peptide-based secondary metabolites (Concurso and Bruner 2012). NRPSs consist of common domains responsible for peptide synthesis, adenylation (A), condensation (C), peptidyl carrier protein (PCP) and thioesterase (Te), along with other specific functional domains including epimerization (E), oxidation (Ox), methylation (Mt), and cyclization (Cy) (Lazos et al. 2010; Concurso and Bruner 2012). NRPS functional modules carry out steps of monomer selection (A-PCP), modification (E, Ox, Mt), peptidyl chain elongation (C), and cyclization/termination (Cy, Te). Siderophore NRPS assemblies frequently initiate the peptidyl chain with a non-canonical amino acid building block derived from an aryl acid, such as salicylic acid (SA) or 2,3-dihydroxybenzoic acid (DHB) functioning as catecholate chelating moiety. The presence of metal chelating thiazoline or oxazoline rings in siderophore pathways is also common, installed by NRPS embedded Cy/Ox domains acting on cysteine and serine residues. Post assembly-line modification of siderophore NPRs is less usual, a typical example is enterobactin C-glycosylation catalysed by the glycosyltransferase, IroB, in

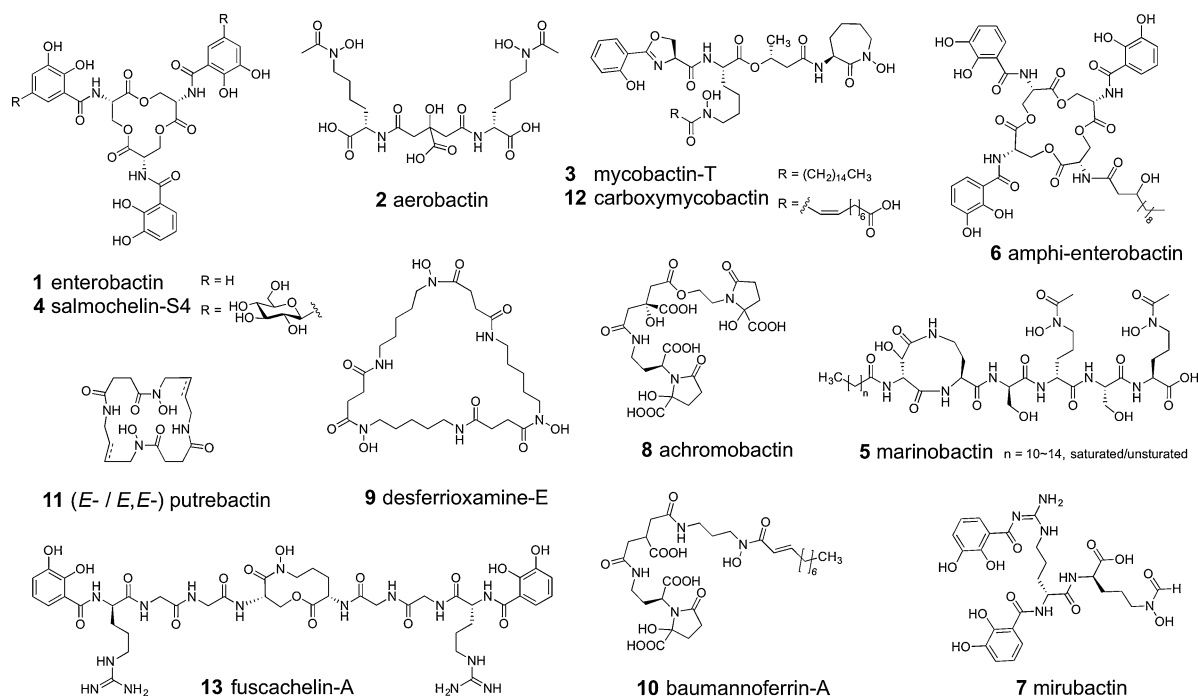


Fig. 1 Microbial siderophores with diverse scaffolds

salmochelin (**4**) production (Fischbach et al. 2005). In addition, a suite of marine siderophores have a peptidic head group for ferric chelation, as well as fatty acid tailoring that varies in length and saturation to tune the overall molecular amphiphilicity (Kem and Butler 2015). Marinobactin (**5**) is produced by *Marinobacter sp.* largely using NRPS machinery and is further acylated with saturated or unsaturated C12–18 fatty acids (Gauglitz et al. 2014; Kem et al. 2015). Similarly, amphi-enterobactin (**6**) produced in *Vibrio harveyi* BAA-1116 also contains a fatty acid moiety. The amphi-enterobactin biosynthesis pathway contains a unique bi-functional C-domain, which can accept amino acyl-phosphopantothetyl-PCP, as well as acyl-CoAs as substrates (Zane et al. 2014). Other recently described novel NRPS siderophore scaffolds include mirubactin (**7**) from *Actinosynnema mirum* (Giessen et al. 2012; Kishimoto et al. 2015).

In addition to NRPS systems, a number of microbial siderophores are constructed by NRPS-independent synthetase (NIS) pathways (Barry and Challis 2009). NIS-based siderophores include aerobactin (**2**), achromobactin (**8**, *Pseudomonas syringae*) and desferrioxamine (**9**, *Streptomyces griseus*). These siderophores are usually assembled from alternating dicarboxylic acid and diamine/amino alcohol building blocks linked by amide or ester bonds. Recently described examples of NIS-siderophores include baumannoferrin, (**10**) isolated from an acinetobactin (the preliminary NRPS siderophore)-deficient *Acinetobacter baumannii* AYE strain under iron-limiting conditions (Penwell et al. 2015); and putrebactins (**11**), the first identified unsaturated macrocyclic dihydroxamic acid siderophores in *Shewanella putrefaciens*, synthesized by a precursor-directed mechanism (Soe and Codd 2014).

Siderophore secretion

After biosynthesis, apo-siderophores are secreted into the media to scavenge iron (Fig. 2) (Miethke and Marahiel 2007). Siderophore secretion is one of the least understood steps of the iron acquisition process in microorganisms. Several different secretion systems have been identified as being implicated in the process, comprising transporters from the major facilitator superfamily (MFS) and the efflux pumps

of the resistance, nodulation, and cell division superfamily (RND).

Many NRPS-based siderophore gene clusters contain an MFS transporter-encoding gene. MFS is one of the largest groups of transporters, conserved from bacteria to human, with a wide spectrum of substrates including ions, carbohydrates, lipids, peptides, nucleosides, and other primary and secondary metabolites (Reddy et al. 2012). The majority of MFS members contain 12-transmembrane α -helices (TMs), with some having 14-TMs or more (Reddy et al. 2012). Several MFS proteins from different subfamilies have been structurally elucidated, but none known to be associated with siderophore secretion (Yan 2015). In Gram-negative *E. coli*, apo-enterobactin is transported by EntS, a 43-kDa archetype MFS transporter encoded by the gene *ybdA* within the Fur-regulated *ent-fep* gene cluster (Furrer et al. 2002). EntS exports enterobactin across the cytoplasmic membrane. Mutant Δ entS (Δ ybdA) shows significant reduction of enterobactin secretion with an increased release of biosynthetic byproducts. In the Gram-positive *Bacillus subtilis*, the MFS protein YmfE was identified to participate in siderophore bacillibactin secretion from screening mutants (Miethke et al. 2008). The YmfE mutant strain is deficient in bacillibactin secretion and does not grow in iron deficient medium.

In addition to the MFS class, the RND superfamily is a ubiquitous group of proton antiporters, especially common among Gram-negative bacteria. The class promotes the active efflux of heavy metals, xenobiotics and siderophores (Nikaido and Pagès 2012). The iron-regulated MexAB-OprM system in *Pseudomonas aeruginosa* was the first RND identified in siderophore (pyoverdine) secretion (Li et al. 1995). In *E. coli*, complementary with MFS transporters, TolC is responsible for the siderophore enterobactin efflux across the outer membrane (Bleuel et al. 2005; Pei et al. 2011). TolC is a trans-outer membrane protein and is an essential component of the RND pump AcrAB-TolC. The complex is a general transporter for bacteria antibiotics and imparts tolerance to toxic compounds, and the whole complex has been structurally characterized (Du et al. 2014). The AcrAB-TolC pump consists of AcrB:AcrA:TolC in a 3:6:3 ratio. AcrA interacts with TolC through a hairpin domain and connects with AcrB through β -barrel and membrane-proximal domains. The role of AcrAB in *E. coli* enterobactin secretion is not entirely clear, the

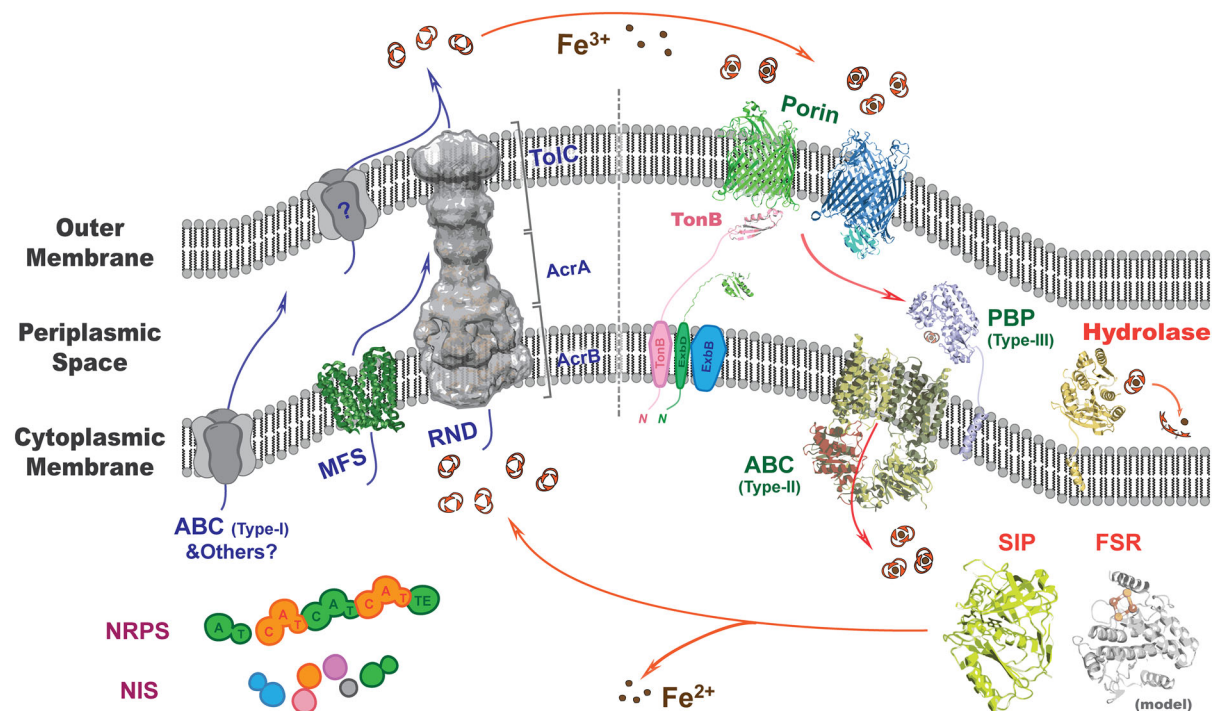


Fig. 2 Schematic of typical siderophore pathways found in Gram-negative bacteria as discussed in the text. Figure was prepared with PyMol (<https://www.pymol.org/>) with PDB

entries used listed: 3WDO, 2XMN, 4DK0, 4C48, 1F11, 2W76, 2GSK, 2PFU, 5DH0, 2GZR, 4GIU and 4YHB

triple mutant $\Delta acrB/\Delta acrD/\Delta mdtABC$, but not any individual deletions, results in decreased enterobactin excretion (Horiyama and Nishino 2014). AcrAD and MdtABC are two additional TolC-dependent RND pumps. Additionally, the MmpS5-MmpL5 (or 4) RND efflux pump, a homolog of AcrAB, has been identified as an critical siderophore export system in *M. tuberculosis* (Wells et al. 2013). MmpS5-MmpL5 is regulated by Rv0678, an MarR-like transcriptional regulator (Radhakrishnan et al. 2014). Mutants lacking the *mmpS4* and *mmpS5* genes do not affect the uptake of external carboxymycobactin (12), but stops cell growth under low iron conditions (Jones et al. 2014). The MmpS5-MmpL5 efflux system also results in azole resistance in *M. tuberculosis* (Milano et al. 2009).

Outside of the MFS and RND subclasses, IroC, a type-I ATP-binding cassette (ABC) transporter, similar to eukaryotic multidrug resistance (MDR) proteins, was previously suggested as responsible for the uptake of ferric-salmochelin in *E. coli*, and is also proposed to be an active enterobactin/salmochelin exporter (Caza et al. 2008; Crouch et al. 2008). The

double mutant $\Delta entS/\Delta iroC$ has a severely compromised growth rate, while IroC does not impact the ferric-enterobactin/salmochelin utilization in the growth promotion (Crouch et al. 2008; Caza et al. 2011).

Holo-siderophore acquisition

Holo-siderophore acquisition from the extracellular environment is one of the key steps in iron assimilation. Most siderophores have molecular weights in excess of 600 Da, and their ability to permeate the outer membrane, passively, is low (Hider and Kong 2010). Therefore, specific energy-dependent integral, outer membrane porins are essential to deliver an iron-siderophore complex from the extracellular into the periplasmic space. The ferric-ferricrocin receptor, FhuA, is a 22-stranded β -barrel that spans the outer membrane, with an *N*-terminus plug domain accessible to the barrel pore, facilitating ligand recognition and binding (Pawelek et al. 2006). FhuA also includes an *N*-terminus TonB box, which forms a

protein/protein interaction through a four-stranded β -sheet with TonB, a cytoplasmic membrane sigma regulator with receptor specificity (Naikare et al. 2013). The ferric-citrate receptor FecA in *E. coli* is a member of the TonB-transducer family and contains a signaling domain upstream from the TonB box and is able to self-regulate their own synthesis as well as their cognate siderophores (Ferguson et al. 2002). TonB is part of the TonB/ExbB/ExbD energy transduction system. The complex is located in the cytoplasmic membrane, and provides the energy required for the active transport of holo-siderophores through the outer receptor (Krewulak and Vogel 2011). TonB undergoes energized motion in the bacterial cell envelope, interacts with the ExbB₄-ExbD₂ complex to generate an electrochemical gradient and initiate the energization (Jordan et al. 2013; Sverzhinsky et al. 2014). The interactions promote iron uptake through outer membrane transporters using a rotational mechanism (Ollis and Postle 2012a, b).

The ABC transporter superfamily constitutes a group of transmembrane proteins that perform ATP-coupled translocation on a wide range of substrates across cell membranes (Rees et al. 2009). ABC transporters have the general function of delivering environmental ferric-siderophores to the cytosol in Gram-positive bacteria, or from the periplasm to cytosol in Gram-negative. ABC transporters have a common architecture that consists of a pair of transmembrane domains (TMDs) embedded in the membrane lipid bilayer, and a pair of nucleotide-binding domains (NBDs) that are located in the cytoplasm (Rees et al. 2009). Most siderophore ABC transporters belong to the type-II ABC subclass, and a related homolog, the heme transporter HmuUV in *Yersinia pestis* has been structurally characterized and identified to employ a coupling mechanism distinct from that of other ABC transporter subclasses (Woo et al. 2012). Some additional siderophore-specific ABC transporters have been reported to be regulated by iron-dependent regulators (Rodriguez and Smith 2006; Granger et al. 2013). Additionally, siderophore ABC transporters commonly interact with type-III ABC-transporter periplasmic binding proteins (PBPs) to facilitate substrate delivery into the cytosol (Chu and Vogel 2011). Unlike type-I and type-II PBPs, which undergo significant domain transition during interaction with substrate, most siderophore type-III PBPs have a relatively rigid α -helical structure,

-serving as the hinge between the two domains and ensures the overall structural stability. The flexibility also allows domain movement between open/closed states upon specific substrate(s) recognition (Chu et al. 2014; Li and Bruner 2016). ABC transporter mediated siderophore delivery in Gram-positive bacteria, exemplified by the YxeB-dependent system in *Bacillus cereus*, shuttle metal using an iron-exchange mechanism, from ferric-siderophore to apo-siderophore, without utilizing iron reduction (Fukushima et al. 2013, 2014).

In contrast to ABC importers common in bacteria, the MFS transporter, MirB in the fungal pathogen *Aspergillus fumigatus*, is a 14-TMs protein and reported to be responsible for the uptake of hydroxamate siderophore N,N',N''-triacetylfulvarinine-C (TAFC) (Raymond-Bouchard et al. 2012). No bacterial MFS, however, has so far been reported in holo-siderophore acquisition.

Siderophore utilization

Imported ferric iron tightly bound to its cognate siderophore must be released upon holo-siderophore delivery to make the metal available to cellular machinery. One strategy for iron release is through hydrolytic destruction of the holo-siderophore by esterases of the α , β -hydrolase family of enzymes, represented by the *E. coli* *iroE* gene product and is a common strategy for macrolactone-based siderophores (Larsen et al. 2006). IroE is a periplasmic trilactone hydrolase that cleaves one of the three esters of enterobactin producing linearized trimers, which still retain a considerable affinity to ferric ion. Thus, in addition to iron assimilation, IroE is likely involved in the production of triscatecholate siderophores (Lin et al. 2005). In contrast, the periplasmic trilactone esterase, Cee, in *Campylobacter* hydrolyses both apo- and holo- forms of enterobactin effectively, and further digests the linearized trimer into dimers and monomers, and this degradation significantly diminishes ferric binding affinity (Zeng et al. 2013). Genomic evidence further demonstrates that Cee is involved in enterobactin mediated iron uptake (Zeng et al. 2013).

For cleavage-independent siderophore paths, iron release is commonly proposed to be facilitated by single-electron reduction of the ferric-siderophore to

the ferrous oxidation state (Li et al. 2015). The relative weaker affinity of the reduced ferrous-siderophore interaction allows kinetic exchange with downstream iron-chelating sites found in cellular proteins or small molecules (Hider and Kong 2010). Two families of proteins have been identified so far involved in the process, termed siderophore-interacting protein (SIP), and ferric-siderophore reductase (FSR). FSR contains a unique C-terminal [2Fe-2S] cluster as C–C-x₁₀–C-x₂–C, and does not show significant similarities to other known [2Fe-2S] proteins. The *E. coli* FSR, FhuF is a part of the siderophore utilization system and has been shown to have a sufficient redox potential to reduce ferric-ferrioxamine (Matzanke et al. 2004). Additional biochemical evidence of function comes from FchR, a FhuF homolog found in *Bacillus halodurans* DSM497 that participates in a three-component electron donor system, along with NADPH and a ferredoxin to reduce various iron chelates with optimal demonstrated reduction activity against ferric-dicitrate (Miethke et al. 2011b). Meanwhile, members of the SIP family are flavoreductases. The most studied example is the *E. coli* YqjH which contains a covalently bound FAD cofactor and can reduce ferric-enterobactin in the presence of NADPH (Miethke et al. 2011a). The *yqjH* gene is regulated by YqjI, a Zn²⁺-dependent regulator (Wang et al. 2011, 2014). The YqjH homolog, FscN in *T. fusca* has been structurally characterized with a FAD-binding domain and an NAD(P)H-interaction domain (Li et al. 2015). FscN contains a non-covalently bound FAD and reduces ferric-fuscachelin-A (13) using NADH as the electron source. Interestingly, the *M. tuberculosis* iron-regulated ABC transporter, IrtA contains an extended N-terminal FAD/NAD(P)H binding domain similar to the SIP family (Ryndak et al. 2010). As a result, IrtA is predicted to have dual function, iron delivery and assimilation.

Siderophores as therapeutic drug leads

Bacterial siderophores have been shown to exhibit specific antifungal or antibiotic activities (Pramanik et al. 2007; Sulochana et al. 2014). Therapeutic iron chelators containing siderophore scaffolds have also been used in the treatments of blood-transfusion requiring diseases (Olivieri et al. 1995). In addition, in the past decade, siderophore-antibiotic conjugates

have been developed as drug leads with reduced permeability-mediated drug resistance using “Trojan Horse”-type mechanisms (Górska et al. 2014). The strategy can impressively reduce permeability-mediated drug resistance with target selectivity, and especially advantageous in multidrug resistance pathogen control (Wencewicz et al. 2009).

Successful design of siderophore-drug conjugates contain a siderophore moiety that can be recognized and imported; a suitable linker stable to the extracellular environment but suitably labile either in the cytoplasm or periplasm; and an effective drug moiety, commonly of the β-lactam drug family (Fig. 3) (de Carvalho and Fernandes 2014). Siderophores-linked lactam antibiotics have an increased penetration through the outer membrane (Kline et al. 2000) and pathogen selectivity can be tuned with altered, conjugated siderophores. Specifically, tris-catecholate siderophore-aminopenicillin conjugates (14) inhibit Gram-negative bacteria such as *Pseudomonas aeruginosa* (Ji et al. 2012). The related biscatecholate-monohydroxamate siderophore-carbacephalosporin conjugates (15) are selective for pathogenic *A. baumannii* (Wencewicz and Miller 2013). Another hexadentate siderophore-mediated drug delivery system has been successfully addressed with enterobactin-cargo conjugates in *E. coli* (and *P. aeruginosa*) (Zheng et al. 2012; Zheng and Nolan 2014). Enterobactin-antibiotic conjugates, including β-lactam antibiotics ampicillin (Amp) and amoxicillin (Amx) conjugates (Ent-Amp/Amx, 16), have a 1000-fold decrease in minimum inhibitory concentration (MIC) value against *E. coli* CFT073 relative to Amp/Amx alone (Zheng and Nolan 2014). In general, the enterobactin-mediated delivery is FepA dependent. Ent-Amp conjugate selectivity can be further constricted with salmochelin modification (GlcEnt-Amp/Amx, 17) to target specific, pathogenic *E. coli* with the *iroN* encoded salmochelin receptor, this modification also lowers mammalian cell toxicity (Chairatana et al. 2015). The lactam-family of drugs can also be delivered with relatively smaller siderophores. The bidentate siderophore-sulfactam BAL30072 (18) has promising activity against multi-resistant Gram-negative *Bacilli*, including strains with multiple drug resistant β-lactamases, such as meropenem-resistance *A. baumannii* (Page et al. 2010; Higgins et al. 2012). Additionally, the antimicrobial efficacy of BAL30072 can be further enhanced through the carbapenem

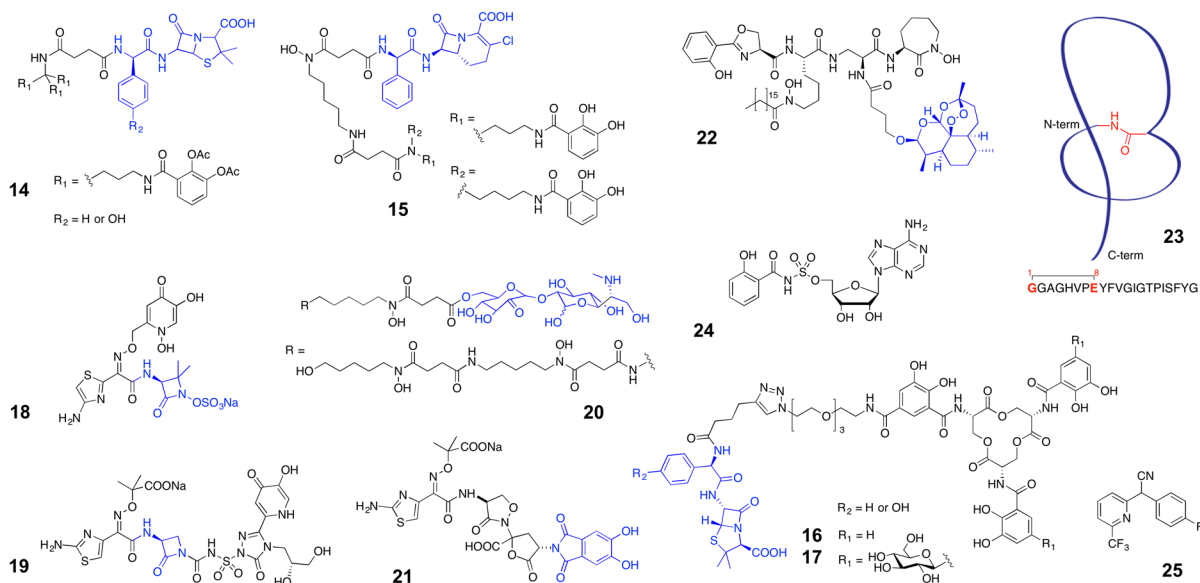


Fig. 3 Examples of siderophore related therapeutic drug leads

combination treatments and BAL30072 was submitted to clinical trials phase-I in 2013 (Hofer et al. 2013; Butler et al. 2013). Comparably, siderophore-mono-carbam conjugates MC-1 (**19**) possess in vitro and predicted in vivo activity against MDR pathogens as *P. aeruginosa* (Flanagan et al. 2011; Murphy-Benato et al. 2015a). The MC-1/penicillin-binding protein co-complex crystal structure established the molecular basis for the recognition specificity and coupling activity (Han et al. 2010; Murphy-Benato et al. 2015b). Similar pyridone-mono-bactam conjugates also possess in vitro antibacterial activity against clinically relevant MDR Gram-negative species (Brown et al. 2013). MC-1 and analogs are most likely to use the TonB-dependent outer membrane siderophore receptor (PiuA, or PirA) as the primary mean of entry (McPherson et al. 2012; Brown et al. 2013).

Besides siderophore-lactam conjugates, several lactam-independent siderophore-drug candidates have been reported. The trihydroxamate siderophore-fluoroquinolone conjugates (**20**) target Gram-positive *S. aureus* SG511 (Wencewicz et al. 2013). The lactivicin analog-phthalimide conjugates (**21**) utilize a wider set of TonB receptors (compare with hydroxypyridone-lactams) to target penicillin-binding proteins and complement the in vitro Gram-negative antibiotics activity (Starr et al. 2014). Lastly the mycobactin-T

analog-artemisinin conjugate (**22**) displays high activity against extensively drug-resistance (XDR) *M. tuberculosis* strains (Miller et al. 2011; Juárez-Hernández et al. 2012).

Targeting siderophore pathways

Specific targeting of siderophore pathways is another applicable therapeutic approach to address the microbial virulence. The disruption of iron recycling constrains the microbial survival and replication, and the interruption of holo-siderophore acquisition is a potential therapeutically approach (Miethke and Marahiel 2007). Most siderophore-drug conjugates do have an added effect of interfering with the native siderophore acquisition. In addition, the reported antibiotic lasso-peptide Microcin-J25 (**23**), and its analogs target the FepA receptor (Mathavan et al. 2014). Lasso-peptides structurally hijack FepA to disrupt ferric-siderophore acquisition and reduce the microbial growth in vitro (Pan and Link 2011). Targeting FepA is an attractive approach as several human pathogens largely acquire iron from host siderophores through FepA (Liu et al. 2014).

Stalling siderophore biosynthesis through small molecule inhibition is an approach to reduce virulence and has received much attention recently. In NRPS-

based siderophore biosynthesis, acyl adenylate intermediates, includes SA- and DHB-AMP analogs interrupt the substrate recognition of the initial adenylation domain, and subsequently inhibit a broad range of microorganisms with aryl-capped siderophores production (Miethke et al. 2006). Salicyl-AMS (**24**) and its analogs inhibit NRPS MbtA in *M. tuberculosis*, and significantly reduce the pathogen growth in mouse lungs in vivo (Ferrerias et al. 2005; Lun et al. 2013). Biaryl nitrile (**25**) and analogs target PvdQ, an essential *N*-terminal nucleophile hydrolase in pyoverdine biosynthesis pathway, and decrease the in vitro siderophore production, thus limit the growth of *P. aeruginosa* under iron-limiting conditions (Wurst et al. 2014).

Targeting the components of siderophore secretion is also a possible therapeutic approach toward pathogenic microbes. Pathogenic *E. coli* requires siderophores for iron acquisition during infection, while the exporters EntS and IroC have been shown to be important for the systemic virulence in a chicken infection model (Caza et al. 2011). Mutation of the outer membrane transporter, TolC, displays a morphological defect in minimal medium, that is likely caused by periplasmic enterobactin accumulation (Vega and Young 2013). Similar approaches and findings have addressed the RND efflux system of *M. tuberculosis* (Jones et al. 2014). Exporter-deficient $\Delta mmpS4/S5$, *M. tuberculosis* has an inhibited growth rate, and the growth defect could not be rescued by supplementing external mycobactin (Jones et al. 2014). Cytoplasmic mycobactin (and carboxymycobactin) accumulation provides a possible self-poisoning inhibition mechanism. In this context, siderophore secretion is a more advantageous target, as the biosynthesis or specific acquisition defect can be overcome by using alternative ferric-carrying heme or siderophores (Jones et al. 2014).

Conclusions

The chemistry and biology of siderophore-mediated iron acquisition is a complex process and a key for step for microbial survival and virulence. Understanding the siderophore-related pathways enables the design and development of siderophore-scaffold drugs, as well as the therapeutic approaches in targeting the pathogenic iron assimilation. Recent progress in

siderophores and their related applications provide a much-improved insight into the complex and important process of iron acquisition. The developing clinical endeavors to target microorganism/pathogen in “iron battle” will continue to stimulate research in the area.

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Compliance with ethical standards

Conflict of interest The authors declare no competing financial interest.

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