Review article

Hydroxamate siderophores: Natural occurrence, chemical synthesis, iron binding affinity and use as Trojan horses against pathogens

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ABSTRACT

Hydroxamic acids are an important class of molecules, in particular because of their metal-chelating ability. Microorganisms, including pathogenic bacteria, use hydroxamate-based entities (siderophores), among others, to acquire Fe (III). The “Trojan horse” strategy exploits the need of bacteria for this metal by using Fe (III) active transporters to carry antibacterial or bactericidal moieties into the bacterial cell. Many natural Trojan horses (sideromycins) are derived from hydroxamic acids, thereby reflecting their potency. Various artificial sideromycins and their antibacterial activities have been reported. This review discusses the structural aspects of the hydroxamate-siderophores isolated in the last two decades, the chemical synthesis of their building blocks, their binding affinity towards Fe (III), and their application as Trojan horses (weaknesses and strengths).

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1. Introduction

Iron is an essential nutrient for most living organisms [1]. Although accounting for about 35% of Earth's mass [2], its bioavailability to microorganisms is limited. This is attributed to the fact that under anaerobic conditions, iron is present as Fe (III), which in aqueous media is either precipitated out (trapped) as insoluble hydroxides ($K_{sp} = 10^{-18}$ M) or is bound to host proteins such as transferrin, ferritin and lactoferrin leaving the free Fe (III) at concentrations as low as 1×10^{-24} M [1,3].

To acquire iron in iron-deficient environments, microorganisms (such as bacteria and fungi) produce “Siderophores” [1,4], the Greek term meaning iron carriers. Siderophores have specific and high affinity towards iron, thus allowing microorganisms to scavenge this coveted nutrient from their environment and ensure their survival.

Fe (III) forms octahedral complexes with six electron-donating atoms symmetrically spatially distributed around the central iron. Maximum binding affinity is achieved when all six atoms interacting with

iron are present in the same molecule, thus emphasizing the “chelate effect” in the multiple denticity. Natural siderophores are mostly hexadentates, in which the binding units vary between catecholates, α -hydroxy carboxylates, hydroxamates and a mixture of these functional groups [5] (Fig. 1).

The microbial iron uptake cycle starts with the production and secretion of “endogenous” siderophores (those produced by the cell itself) into the surroundings. These siderophores solubilize Fe(III) from its hydroxide or capture it from host proteins [6]. Ferrisiderophores (siderophore-Fe(III) complexes) are usually more than 500 Da, which means that they require certain transport systems to transfer them into the cell. Specific membrane (outer membrane in gram-negative bacteria) protein receptors recognize the complex via key features [6,7]. Recognition triggers the transport system and subsequent uptake occurs. Some outer membrane protein receptors are binding unit-specific and can recognize a wide spectrum of siderophores with same type of chelating units, such as FhuA (ferric hydroxamate outer membrane receptor) and FecA (ferric citrate). This capacity allows some species to use the siderophores produced by other organisms (xenosiderophores) [8]. Other receptors are specific for only a given siderophore, such as FptA (ferric pyochelin transport) and FpvA (ferric pyoverdine) [9].

Although outer membrane receptors for ferrisiderophore mediate the nourishment (feeding) of the cell, they can also poison it. Some microorganisms hybridize the siderophore with a bactericidal moiety

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Fig. 1. Representative natural siderophores with different binding units (hydroxamic groups are shown in purple, catechol in blue, α -hydroxy carboxylic acid in green, and other groups in maroon). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

(sideromycin = siderophore + antibiotic) to attack other species and kill them. This microbial strategy (Trojan horse) has inspired the research community to develop a new approach to increase the permeability of the drug into the multi-drug resistant cell [10–12]. In this regard, increasing bacterial resistance to almost all known antibiotics has come about from the overuse of antibiotics for the treatment of bacterial infections [13,14]. The discovery of only a handful of novel antibiotics over the last few decades has made it necessary to devise innovative strategies to combat pathogenic bacteria [15], the Trojan horse strategy emerging as a promising approach.

Among the five known families of natural sideromycins, three—albomycins, ferrimycins and salmycins—use the hydroxamate binding moiety (the other two families are salmochelin and microcin). Albomycins (3, Fig. 2) are the most widely known natural sideromycins and they have a proven efficiency against many organisms with the FhuA receptor in their outer membrane (see section 2.4.1.1). This activity emphasises the role of this family of chelators in overcoming the permeability barriers of the microbial cell wall [16].

In this review we discuss hydroxamate siderophores in terms of their natural occurrence, chemical synthesis, binding affinity towards Fe(III) and potency as antimicrobial drug conjugates.

2. Hydroxamate-based siderophores

Hydroxamic acids have the general formula $RC(O)N(OH)R'$. They can be defined as *N*-hydroxy amides. This structure gives the proton of the hydroxyl some acidic properties depending on the solvent, with pKa values of about 9 [17]. When deprotonated, hydroxamate ion can strongly bind to various positively charged metal ions (Fig. 2). In a

wide spectrum of natural siderophores, the chelating unit of hydroxamic acid is found either within the backbone or as pendants (Fig. 2).

2.1. Natural occurrence of hydroxamate siderophores

Hydroxamate siderophores are produced mainly by fungi and some strains of bacteria. The first known families were ferrichrome (1, Fig. 2), which was isolated from smut fungus *Ustilago sphaerogana* [18], and coprogen (5) [19], both isolated and reported in 1952. The structure of ferrichrome was characterized later in 1960 [20]. Another hydroxamate siderophore is desferrioxamine B (DFO B, 6), the most famous member of the ferrioxamine family. Nobel laureate Vladimir Prelog and co-workers isolated DFO B from *Streptomyces pilosus* and first reported it in 1960 [21]. Ciba-Geigy (now Novartis) introduced DFO B to the market as a strong metal chelator and this compound was later approved by US Food and Drug Administration (FDA) in 1968 as “Desferal” for the treatment of iron poisoning (iron overloading) [22].

Albomycins are hydroxamate sideromycins. They were reported previously as grisein [23] and were first isolated in 1947, but their structures were not fully characterized till 1982 [24] (see section 2.4.1.1).

Many other hydroxamate siderophores have since been isolated and reported [25]. In the last two decades (2000-to date), more than 135 new members have been added to the family (entries 10–61, Table 1), many extracted mainly from bacterial species collected from the Atlantic Ocean [26]. Most hydroxamate siderophores are hydrophilic. In contrast, most marine siderophores are amphiphilic, having both hydrophilic and hydrophobic parts in their structure, the latter enabling the siderophore to form micelles when complexed to Fe(III), thereby in-

Siderophores with hydroxamate pendants: **Siderophores with backbone hydroxamate:** **Representative Hydroxamate-Fe(III) complex**

Fig. 2. Representative hydroxamate-derived siderophores.

creasing the availability of the complex to the cell in the seawater environment [27]. Ferrioxamine E and G are among the most soluble marine siderophores [26]. Table 1 shows most of the hydroxamate siderophores isolated in the period 2000–2020, their structures and the species that produce them (see Table 2).

From a structural point of view, all the siderophores in Table 1 contain the hydroxamate chelating unit, either alone as in entries 10–29 and 34–38, or mixed with other binding units as in entries 30–33 and 39–61. The hydroxamate parts are derived from Orn, Lys, or N-hydroxydiaminoalkane [17]. In Orn-derived hydroxamates, the δ -N of the Orn is hydroxylated and acylated. The acylation moieties vary between

formyl, as in 13, 17, 23–26, 30, 31, and 33, acetyl as in 10–12 and 14–15, malonyl as in 16, or anhydro-mevalonyl as in 20–22 and 28, 3-hydroxy-butanoyl as in 27, 3-methyl-glutaconyl as in 29 (this acyl is an intermediate in leucine metabolism [84]), fatty acid as in 56–57, α -COOH of the Orn itself forming a six membered cycle as in 23–26, or the α -COOH of another amino acid within the backbone (Arg, Asn, or Ser) as in 17–19 and 24–26. Some siderophores show two or more different acylating moieties for the distinct hydroxamate groups within the same molecule as in 17–19 and 23–26.

Table 1
Hydroxamate siderophores isolated and reported in 2000–2020.

#	Siderophore	Structure	Secreted by	Ref
Ornithine-derived siderophores				
10	Amphibactins B-I, S, and T	<p>Amphibactins</p> <p> $R = \text{---}(\text{CH}_2)_m\text{---C(=O)CH}_2\text{---}$, $R = \text{---}(\text{CH}_2)_m\text{---CH=CH---}(\text{CH}_2)_n\text{---C(=O)CH}_2\text{---}$ </p> <p> B, m=10, X=OH D, m=10, X=H F, m=12, X=OH H, m=12, X=H T, m=8, X=H C, m=5, n=5, X=OH E, m=5, n=5, X=H G, m=7, n=5, X=OH I, m=7, n=5, X=H S, m=5, n=3, X=H </p>	Amphibactin B-I: <i>Proteobacteriu</i> , <i>Vibrio</i> sp. <i>R-10</i> Amphibactin S and T: <i>Vibrio</i> sp. <i>HC0601C5</i>	[28,29]
11	Marinobactins A, B, C, D1, D2, E, and F	<p>Marinobactins</p> <p> $R = \text{---}(\text{CH}_2)_m\text{---C(=O)CH}_2\text{---}$, $R = \text{---}(\text{CH}_2)_m\text{---CH=CH---}(\text{CH}_2)_n\text{---C(=O)CH}_2\text{---}$ </p> <p> A, m=10 C, m=12 E, m=14 B, m=5, n=5 D1, m=5, n=7 D2, m=7, n=5 F, m+n=14 </p>	<i>Marinobacter</i> sp. strains <i>DS40M6</i> and <i>DS40M8</i>	[30,31]
12	Moanachelins	<p>Moanachelins</p> <p> $R_2 = \text{---}(\text{CH}_2)_m\text{---C(=O)CH}_2\text{---}$, $R_2 = \text{---}(\text{CH}_2)_m\text{---CH=CH---}(\text{CH}_2)_n\text{---C(=O)CH}_2\text{---}$ </p> <p> Ala-B, $R_1 = \text{CH}_3$, m=10 Ala-D, $R_1 = \text{CH}_3$, m=12 Gly-B, $R_1 = \text{H}$, m=10 Gly-D, $R_1 = \text{H}$, m=12 Gly-C, $R_1 = \text{H}$, m+n=10 Gly-E, $R_1 = \text{H}$, m=7, n=5 </p>	Marine bacterial isolate <i>Vibrio</i> sp. <i>Nt1</i>	[32]
13	Coelichelin	<p>Coelichelin</p>	<i>Nocardia tenerifensis</i> NBRC 101015	[33]

#	Siderophore	Structure	Secreted by	Ref
14	AS2524371, and AS2488059	<p>AS2488059, R= </p> <p>AS2524371, R= </p>	<i>Acremonium persicinum</i> MF-347833	[34]
15	Acremonpeptides A– D	<p>Acremonpeptide</p> <p>A, R=CH₂OH B, R=CH₃</p> <p>C, R= D, R= </p>	Marine-Derived <i>Acremonium persicinum</i> SCSIO 115	[35]
16	Desmalonichrome	<p>Desmalonichrome</p>	Fungus <i>Fusarium oxysporum</i>	[36]
17	Peucechelin	<p>Peucechelin</p>	<i>Streptomyces. peucetius</i>	[37]
18	Thermochelin	<p>Thermochelin</p>	<i>Thermocrisum agreste</i> DSM 44070	[38]
19	Erythrochelin	<p>Erythrochelin</p>	Strain <i>S. erythraea</i>	[39]

#	Siderophore	Structure	Secreted by	Ref																														
20	Metachelins 1, 2, and 3	<p>Metachelin 1, R₁=R₂=A, R₃=B 2, R₁=R₂=A, R₃=C 3, R₁=R₂=H, R₃=B</p> <p>A= B= C= </p>	<i>Metarhizium robertsii</i>	[40]																														
21	Beauverichelin A	<p>Beauverichelin A R= </p>	<i>Beauveria bassiana</i>	[41]																														
22	Talarazines A–E	<p>Talarazine</p> <table border="1"> <thead> <tr> <th></th> <th>R</th> <th>X</th> <th>Y</th> <th>Z</th> </tr> </thead> <tbody> <tr> <td>A</td> <td>H</td> <td>-</td> <td>OH</td> <td></td> </tr> <tr> <td>B</td> <td></td> <td>H</td> <td></td> <td>H</td> </tr> <tr> <td>C</td> <td></td> <td></td> <td>H</td> <td></td> </tr> <tr> <td>D</td> <td></td> <td>OH</td> <td></td> <td>OH</td> </tr> <tr> <td>E</td> <td></td> <td></td> <td>OH</td> <td>H</td> </tr> </tbody> </table>		R	X	Y	Z	A	H	-	OH		B		H		H	C			H		D		OH		OH	E			OH	H	Fungus, <i>Talaromyces</i> sp. (CMB-W045)	[42]
	R	X	Y	Z																														
A	H	-	OH																															
B		H		H																														
C			H																															
D		OH		OH																														
E			OH	H																														
23	Albisporachelin	<p>Albisporachelin</p>	<i>Amycolatopsis</i>	[43]																														
24	Tsukubachelin	<p>Tsukubachelin</p>	<i>Streptomyces</i> sp. TM-74	[44]																														

#	Siderophore	Structure	Secreted by	Ref
25	Albachelin	<p>Albachelin</p>	<i>Amycolatopsis alba</i>	[45]
26	Scabichelin and Turgichelin	<p>Scabichelin, R=CH₂CH₂NH₂ Turgichelin, R=OH</p>	Scabichelin: <i>Streptomyces antibioticus</i> NBRC 13838/ <i>Streptomyces scabies</i> JCM 7914 Turgichelin: <i>Streptomyces turgidiscabies</i> JCM 10429	[46]
27	Vicibactin	<p>Vicibactin</p>	<i>Rhizobium leguminosarum</i> ATCC 14479 bv. <i>Trifolii</i>	[47]
28	Fusigen	<p>Fusigen</p>	<i>Aureobasidium pullulans</i> HN6.2	[48]
29	Basidiochrome	<p>Basidiochrome</p>	<i>Mycorrhizal fungi Ceratobasidium</i> and <i>Rhizoctonia</i> spp.	[49]
30	Lysine-derived hydroxamate siderophores Amamistatins A, and B	<p>Amamistatin: A, R=OCH₃ B, R=H</p>	<i>Actinomycete Nocardia asteroides</i> SCRC-A2359	[50,51]

#	Siderophore	Structure	Secreted by	Ref
31	Brasilibactin A	<p>Brasilibactin A</p> <p>Brasilibactin A is a stereoisomer of Nocardimicin H (#33)</p> <p>Brasilibactin A is a stereoisomer of Nocardimicin H (#33)</p>	<i>Actinomycete Nocardia brasiliensis IFM 0995</i>	[52]
32	Nocardimicins A-F	<p>Nocardimicin</p> <p>A: $R_1=OH$, $m=8$ B: $R_1=OH$, $m=10$ C: $R_1=O$, $m=10$ D: $R_1=OH$, $m=12$ E: $R_1=H$, $m=12$ F: $R_1=OH$, $m=14$</p>	<i>Nocardia</i> sp. TP-A0674	[53]
33	Nocardimicins G-I	<p>Nocardimicin</p> <p>G: $m=12$ H: $m=14$ I: $m=16$</p> <p>Nocardimicin H is a stereoisomer of Brasilibactin A (#31)</p> <p>Nocardimicin H is a stereoisomer of Brasilibactin A (#31)</p>	<i>Nocardia nova</i> JCM 6044	[54]
N-Hydroxy-diaminoalkane-derived hydroxamate siderophores				
34	Nocardichelins A and, B	<p>Nocardichelin</p> <p>A, $n=12$ B, $n=10$</p>	<i>Nocardia</i> Strain Acta 3026	[55]
35	Tenacibactins A, and B	<p>Tenacibactins:</p> <p>A, $n=1$, $R=OCH_3$ B, $n=1$, $R=OH$ C, $n=2$, $R=OH$ D, $n=2$, $R=NH_2$</p>	<i>Marine-derived bacterium Tenacibaculum</i> sp. A4K-17	[56]

#	Siderophore	Structure	Secreted by	Ref
36	Legonoxamine A1, and B2	<p>Legonoxamine A1</p> <p>Legonoxamine B2</p>	The soil bacterium, <i>Streptomyces</i> sp. MA37	[57]
37	Bisucaberin	<p>Bisucaberin</p>	<i>Salmonicida</i>	[58]
38	Avaroferrin	<p>Avaroferrin</p>	<i>Shewanella</i> algae B516	[59]
Mixed 39	Rhodochelin	<p>Rhodochelin</p>	<i>Rhodococcus</i> <i>jostii</i> RHA1	[60]
40	Lystabactins A, B, and C	<p>Lystabactin A, R=OH C, R=OCH₃</p> <p>Lystabactin B</p>	Marine <i>Pseudoalteromonas</i> sp	[61]

#	Siderophore	Structure	Secreted by	Ref
41	Rhodobactin		<i>Rhodococcus erythropolis</i> PR4	[62]
42	Heterobactins A, S ₁ , and S ₂	<p>Rhodobactin</p> <p>Heterobactin</p> <p>A, R₁=R₂=H S₁, R₁=H, R₂=SO₃H S₂, R₁=SO₃H, R₂=H</p>	<i>Rhodococcus erythropolis</i> PR4	[63]
43	JBIR-16		<i>Nocardia tenerifensis</i> NBRC 101015	[64]
44	Fuscachelins A-C	<p>JBIR-16</p> <p>Fuscachelins A</p> <p>Fuscachelin: B, R = OH C, R = NH₂</p>	<i>Thermobifida fusca</i>	[65]
45	Anguibactin		<i>Vibrio</i> sp. DS40M4	[66]
46	Fimsbactins A-C	<p>Anguibactin</p> <p>Fimsbactin</p> <p>A : R₁ = H, R₂= H B : R₁ = CH₃, R₂= H C : R₁ = CH₃, R₂= CH₃</p>	<i>Acinetobacter baumannii</i> and <i>Acinetobacter baylyi</i>	[67]

#	Siderophore	Structure	Secreted by	Ref
47	Aquachelins A, B, C, D, I, and J	<p>Aquachelins</p> <p>R = $\begin{matrix} X \\ \\ \text{---} \text{C} \text{---} \text{CH}_2 \text{---} \text{CH}_2 \text{---} \text{CH}_2 \text{---} \end{matrix}$, R = $\begin{matrix} \text{---} \text{CH}_2 \text{---} \text{CH}_2 \text{---} \text{CH}_2 \text{---} \text{CH}_2 \text{---} \text{CH}_2 \text{---} \end{matrix}$</p> <p>B, X=H, m=8 D, X=H, m=10 I, X=OH, m=8 J, X=H, m=6</p> <p>A, m=5, n=3 C, m=5, n=5</p>	<p>For A-D: <i>Halomonas aquamarina</i> strain DS40M3</p> <p>For I, J: <i>Halomonas meridiana</i> str. HC4321C1</p>	[30]
48	Variochelins A, and B	<p>Variochelins</p> <p>A, n=10 B, n=12</p>	<i>Variovorax boronicumulans</i>	[68]
49	Imaqobactin	<p>Imaqobactin</p>	<i>Arctic Marine Bacterium Variovorax Species RKJM285</i>	[69]
50	Variobactins A, and B	<p>Variobactins</p> <p>A, n=11 B, n=9</p>	<i>Variovorax paradoxus P4B</i>	[70]
51	Vacidobactins A, and B	<p>Vacidobactin</p> <p>A, R=OH B, R=H</p>	<i>Variovorax</i>	[70]

#	Siderophore	Structure	Secreted by	Ref
52	Loihichelins A-F	<p>Loihichelins</p> <p> $R = \begin{matrix} X \\ \\ \text{---} \end{matrix}$, $\begin{matrix} X \\ \\ \text{---} \end{matrix}$, $\begin{matrix} X \\ \\ \text{---} \end{matrix}$ A, X=H, m=6 B, X=OH, m=8 D, X=H, m=8 F, X=H, m=10 C, m=5, n=3 E, m=5, n=5 </p>	Marine bacterium <i>Halomonas</i> sp. LOB-5	[71]
53	Pacifibactin	<p>Pacifibactin</p>	<i>Alcanivorax pacificus</i>	[72]
54	Serobactins (A-C)	<p>Serobactin</p> <p> A, n=8 B, n=10 C, n=12 </p>	Grass endophyte <i>Herbaspirillum seropedicae</i>	[73]
55	Cupriachelin	<p>Cupriachelin n=8</p>	Bioplastic Producer <i>Cupriavidus necator</i> H16	[74]
56	Woodybactins A-D	<p>Woodybactin</p> <p> $R = \begin{matrix} X \\ \\ \text{---} \end{matrix}$ A, X=H, n=5 B, X=CH₃, n=5 C, X=H, n=6 D, X=H, n=7 </p>	A marine <i>Shewanella</i>	[75]
57	Ochrobactins A, B, and C	<p>Ochrobactin</p> <p> A, R= --- B, R= --- C, R= --- </p>	Coastal α -proteobacterium <i>Ochrobactrum</i> sp. SP18	[76]

#	Siderophore	Structure	Secreted by	Ref
58	Synechobactins A, B, and C	<p>Synechobactin</p> <p>R=</p> <p>A, n=10 B, n=8 C, n=6</p>	Coastal marine cyanobacterium <i>Synechococcus</i> sp. PCC 7002	[77]
59	Schizokinen, and Schizokinen A	<p>Schizokinen, R=</p> <p>Schizokinen A, R=</p>	<i>Rhizobium leguminosarum</i> IARI 917	[78]
60	Baumannoferrins A and B	<p>Baumannoferrin A</p> <p>Baumannoferrin B</p>	<i>Acinetobacter baumannii</i>	[79]
61	Catenulobactins B		<i>Catenuloplanes</i> sp. RD067331	[80]
Other Interesting				
62	Mirubactin	<p>Mirubactin</p>	<i>Actinosynnema mirum</i>	[81]
63	Gramibactin	<p>Gramibactin</p>	Rhizosphere bacteria (<i>Paraburkholderia graminis</i>)	[82]
64	Strepturidin	<p>Strepturidin</p>	<i>Streptomyces albus</i> DSM 40763	[83]

Also, ϵ -hydroxamate Lys residues are found in amamistatins (**30**), brasilibactin (**31**), nocardimicins (**32** and **33**), with the acylating moieties varying as in the Orn siderophores.

Other hydroxamate siderophores are derived from N-hydroxy-1,5-diaminopentane as in **34–38** and are usually acylated with a succinic moiety. N-hydroxy-1,5-diaminobutane is found in **38**.

Many of these hydroxamate siderophores are peptides. They show a variety of structures. In agreement with the previously known representative siderophores, siderophores **10–13** are linear with hydroxamate appendages, as in albomycin (**3**) (although **11** has a cyclic part but the chelating unit is linear). Siderophores **14–16** are cyclic peptides (lactams) with exocyclic hydroxamate appendages as in ferrichrome **1**.

Entries **17–22** resemble coprogen (**5**), a tris hydroxamate siderophore with a cyclic di-Orn part with hydroxamate side chains. The denticity is extended to hexadentate through the acyl (peptidyl) part of the hydroxamate moieties to include an additional functionalized Orn as in **17–19**, or other hydroxamate containing acyl as in **20–22**.

The hydroxamate in the non-peptidyl siderophores are found only with the backbone (not as appendages) and the backbones are either linear as in **35–37** or cyclic (endo cyclic hydroxamate) as in **38–39**.

2.2. Chemical synthesis of hydroxamate residues in siderophores

Several synthetic pathways can be used to prepare hydroxamic acids. In this regard, these acids can be synthesized from carboxylic acids [85], esters [86], aldehydes [87], alkyl halides [86], amines [88,89], and nitro compounds [90,91]. All reported methods can be briefly outlined as one of the five possible routes (either full or sub-route) shown in Scheme 1, which illustrates the most general ap-

proaches for the synthesis of hydroxamic acids from alkyl amines, hydroxyl amine or simple amides.

2.2.1. Synthesis of δ -acyl- δ -N-Hydroxy-Orn building block

To prepare the δ -acyl- δ -N-hydroxy-ornithine building unit, most of the reported procedures involved the conversion of the alkylamine (in this case the Orn side chain) to N-hydroxyl alkylamine and then to N-acyl-N-hydroxyl alkylamine (the red route in Scheme 1). The hydroxylation of the primary amine (δ -NH₂) is the crucial step in the synthesis, and various approaches for this reaction have been reported in the literature. The syntheses start with Orn, with suitable protecting groups for the α -NH₂ and COOH. Next, δ -N is hydroxylated mostly with benzoyl peroxide in a biphasic mixture at pH = 10.5 [88,89,92] (Scheme 2). Although this is the most widely used protocol, it is hampered by low yield and requires critical purification due to the formation of the δ -benzamide by-product. Another peroxide, dimethyl dioxirane (DMD) with acetone, was used for the oxidation of the amine to provide the nitron (the N-oxide of the imine) and then the hydroxamic acid in two steps [87] (Scheme 3). In other cases, and following the same imine-nitron approach, the hydroxylation step was done by reacting the δ -N of Orn with benzaldehyde in basic medium, followed by the addition of an oxidizing agent (m-CPBA) and then a treatment with acid to obtain N-OH. The advantage of this functionalization is that it can be done either to the building block (Scheme 3) or to the Orn residue side chains after the peptide has been built [87].

Once the δ -NH₂ has been hydroxylated, the acylation step is carried out using strong amide formation strategies, such as acyl halides [89,92], acyl anhydrides [87] and HATU [88], due to lower nucleophilicity of the hydroxylated amine compared to the free amine.

Scheme 1. Possible routes for the preparation of hydroxamic acid from different starting compounds (coloured arrows represent routes reported for siderophore synthesis, all arrows (black and coloured) represent reported routes for hydroxamic acid preparation in general).

Scheme 2. Synthesis of 'PG₁-Orn(acyl,OBz)-OPG₂' building block, using benzoyl peroxide (for the hydroxylation step). (R = H, X = EDC active ester) in pyoverdine D. [88], (R = CH₃, X = Cl) in amphibactin-T [92] and albomycin [89].

Scheme 3. Synthesis of 'PG₁-Orn(acyl,OBz)-OPG₂' building block through the imine-nitrone intermediates and using DMD or PhCHO [87].

Other syntheses reported the preparation of the hydroxamate Orn starting from different amino acids. A full synthesis of ferrichrome in 1969 described the conversion of δ -nitro norvaline (non-branched Val) into δ -N-hydroxy ornithine by reducing the nitro group with zinc in an aqueous ammonium chloride solution [90,91] (Scheme 4). Another protocol included the conversion of the COOH side chain of glutamic

acid to OH then its attachment to a protected simple hydroxamic acid (blue route in Scheme 1) using diisopropyl azodicarboxylate and triphenyl phosphine [85] (Scheme 4) (see Scheme 5).

Scheme 4. Synthesis of 'PG₁-Orn(acyl,OBn)-OPG₂' starting from 3 different amino acids.

Scheme 5. Synthesis of N-5-aminopentyl-N(hydroxy)-succinic acid building block in desferrioxamine B [93] and bisucaberin [94].

2.2.2. Synthesis of N-5-aminopentyl-N(hydroxy)-succinic acid building block

Reported syntheses of N-5-aminopentyl-N(hydroxy)-succinamic acid building unit started with the protected hydroxylamine, followed by an alkylation step with a cyano aldehyde (4-cyanobutanal) as in the preparation of desferrioxamine B [93], or a haloalkane nitrile (5-chloro valeronitrile) as in the synthesis of bisucaberin [94] (see scheme 5). The prepared cyanoalkylhydroxylamine is divided into two parts, the nitrile group in one of them is reduced to an amine group and acylation is done using succinic anhydride (the route followed is shown by green dashed lines, Scheme 1). The same route was reported for the synthesis of alcaligin but with a shorter alkyl part to prepare the N-5-aminobutyl-N(hydroxy)-succinic acid [95].

2.2.3. Synthesis of other hydroxamate building blocks

In some artificial siderophores, the hydroxamate moiety was embedded in the backbone through α -N-hydroxy amino acids prepared indirectly from other closely related acids. For example, α -benzyloxyglycine was obtained by reacting bromoacetic acid tert-butyl ester and O-benzylhydroxylamine [96]. N-benzyloxyalanine (or N-benzyloxyphenylalanine) was prepared starting from D-lactic acid (or D-phenyllactic acid) *t*-butyl ester by converting the α -OH to a triflate intermediate, followed by the reaction with O-benzylhydroxylamine [97]. The S_N2 attack of the benzyloxyamine resulted with inverted configuration of α -carbon. The amide bond was then formed by condensing the hydroxylated α -N with an α -COOH of another amino acid using an effective form of activated acid [96,98]. All these methods followed the green route of Scheme 1 by introducing the alkyl part into a previously prepared benzyl protected-hydroxyl amine (Scheme 6).

A more recent study hydroxylated the α -N of the amino acid with m-CPBA after alkylating it with bromoacetonitrile (converting it temporarily into a secondary amine prior to hydroxylation, making the latter much more efficient) [99] (Scheme 6).

2.2.4. Incorporating the hydroxamate building block into the backbone

2.3. Binding affinity towards iron

Siderophores, in general, form strong complexes with Fe (III), with thermodynamic formation constants β up to 10^{49} for the tris-catecholate enterobactin (Fig. 1), compared to 10^{25} for EDTA, a widely used complexing agent for metal ions. Tris hydroxamate siderophores also show high formation constants of $\sim 10^{30}$. In the fully deprotonated forms of ligands (at highly basic conditions), catecholate siderophores show greater binding to Fe(III) than hydroxamates (the difference in $\log \beta$ values can go up to 19 units, which means that the concentration of the catecholate-Fe complex is 10^{19} times that of hydroxamate-Fe under same conditions). However, at physiological pH (pH = 7.4), the binding affinity (reflected by pFe^{3+} , which equals $-\log[Fe^{3+}]_{free}$ at pH = 7.4, $[Fe^{3+}]_{total} = 10^{-6}$ M and $[Ligand]_{total} = 10^{-5}$ M) of the two siderophores is comparable (the difference in binding affinities is much lower). This observation is attributed to the acidic character of the hydroxamates, a feature that increases the proportion of deprotonated molecules (ready to bind to Fe(III)) of hydroxamate compared to catecholate.

For comparative purposes, Table 3 shows the formation constants and iron affinity for several siderophores with different chelating groups.

Scheme 6. Reported syntheses of α -N-benzyloxy amino acids through two routes: a) alkylation of hydroxylamine [97] and b) hydroxylation of alkylamine [99].

Table 2

Reported strategies used for the incorporation of hydroxamate building blocks into the siderophore backbone.

Hydroxamate Building unit	Siderophore	Entry No.	Synthetic Strategies		Ref.
Ornithine-derived hydroxamate siderophores	Pyoverdin	Fig. 1	Fmoc/tBu	Super acid-labile Rink acid resin (solid phase)	[88]
	Amphibactin-T	10	Fmoc/tBu	CTC-polystyrene resin (solid phase)	[92]
	Albomycin	3	Fmoc/tBu	Solution phase	[89]
	Ferrichrome	1	Boc/Bzl	Solution phase	[90]
	DIG-siderophore (conj.)	–	Functionalizing the Orn as building block or its tripeptide with free side chains		[87]
N-hydroxy diaminoalkane derived sidrophores	Desferrioxamine	6	Metal-templated Cyclic Trimerization	Solution phase	[100]
	Bisucaberin	37	Boc/Bzl	Solution phase	[101]
	Alcaligin	7	Diphenylphosphoryl azide (DPPA) + triethyl amine	Solution phase	[95]

2.4. Applications of hydroxamate molecules and hydroxamate siderophores

Hydroxamic acids have found many applications in medical and agricultural practices [109] and as probes [1,3]. Their medical use can be therapeutic or diagnostic [110]. The presence of hydroxy substituents on the amide bonds of an active peptide enhances its stability against enzymatic degradation [96]. Furthermore, hydroxamic acids inhibit methionine aminopeptidase (MetAP), an essential enzyme in protein synthesis in a variety of organisms. This inhibition enables the use of hydroxamic acids as potent antibacterial or antitumor agents [96,111]. Also, these acids have the potential to inhibit metalloproteases and HIV protease, and they show activity in immune suppression [96].

As metal chelators, Desferal, the brand name of desferrioxamine B (6), was the first drug used for the treatment of metal poisoning, namely iron overloading [22,112]. Also, the hydroxamate groups have shown unprecedented activity against the growth of *P. aeruginosa* biofilms (which confer cells extra resistance to antibiotics by adhering in the form of a film) when they were combined with glycol motifs in a bifunctional molecule, in which the glycol targeted the adhesion of cells and hydroxamate deprived cells of iron [113].

In this review, we focus on and discuss the use of hydroxamate siderophores as Trojan horses to overcome bacterial resistance to antibiotics.

2.4.1. HA siderophores-antibiotic conjugates (application as Trojan horses)

After being excreted and complexed to Fe(III), the loaded siderophores are recognized by specific protein receptors present on the microbial cell membrane. They are then transferred through specified cycles to their target inside the cell (cytoplasm or sometimes periplasm in some gram-negative bacteria). Fe(III) is then released from the siderophore through certain mechanisms [11,12], reduced to Fe(II) and metabolized [8].

The microorganisms that produce hydroxamate siderophores have a special protein receptor, FhuA, embedded in their membranes to recognize and mediate the uptake of the iron-siderophore complex

[11,16,114]. FhuA may also be present in the membrane of other microorganisms that do not produce endogenous hydroxamate siderophores yet can take up the hydroxamate siderophores produced by other organisms [13].

Some studies have suggested that the protein receptors recognize and identify key points in the siderophore backbone. Others propose that recognition occurs via identification of the steric configuration of the e-donating atoms around the central iron in the inner coordination sphere [13,17]. The latter notion may explain how some species can take up iron from **xenosiderophore** complexes. A study by Shanzher's group suggested that the recognition takes place in two domains: first, the configuration of the complex around iron; and second, the spatial orientation of the functional groups (C=O) with respect to the inner coordination sphere [115,116]. It is likely that microorganisms have two types of receptors—specific ones to identify endogenous siderophore-iron complexes and more general ones that identify the core of the xenosiderophore-iron complexes.

Some bacterial strains exploit the greed for theft of other species by producing and attaching a siderophore chelator to a **microbiocidal** moiety. The resulting conjugate is called a “sideromycin”. These molecules are then released into the matrix as Trojan horses to poison competing opponent cells.

2.4.1.1. Natural conjugates (sideromycins) Natural sideromycins are highly specific against certain types of bacteria and are taken up through the same pathway as the corresponding type of siderophores with respect to the binding unit. The first reported sideromycin was grisein, discovered by Waksman's group in 1947 [23]. It was isolated from a strain of *Streptomyces griseus*. It showed antimicrobial activity against many strains of bacteria but its structure was not elucidated then and was partially characterized in 1951 [117]. Grisein was later reported as albomycin (3) [112]. Albomycins are the most widely known natural sideromycins. In 1951, Gause and Brazhnikova isolated an albomycin from *Actinomyces subbropicus*, a species of streptomycetes [118]. It was found to strongly inhibit the growth of some gram-positive bacteria, such as *S. aureus*, *S. pneumoniae* and *B. subtilis*, as well as some gram-negative bacteria [112]. The activity of this albomycin was found to be ten times stronger than that of penicillin [118], and it was

Table 3
Comparison of the thermodynamic constants for various representative tris-hydroxamate siderophores and their Fe(III) complexes with that of enterobactin.

Siderophores	Entry no.	pKa	Logβ ₁₁₀	^a pFe ³⁺	Ref
Enterobactin (triscatecholate)	Fig. 1	6.00	49	34.3	[102,103]
		7.50			
		8.55			
Ferrichrome	1	8.11	29.07	25.2	[104,105]
		9.00			
		9.83			
Ferricrocin	2	8.14	30.4	26.5	[106]
		9.01			
		9.92			
Ferrioxamine B	6	8.39	30.6	26.6	[104–106]
		9.03			
		9.70			
Basidiochrome	30	Not evaluated	27.8	25	[107]
Aquachelin-C (bi-hydroxamate)	40	3.79 (COOH)	31.4	–	[108]
		8.73 (CONOH)			
		9.52 (CONOH)			
Marinobactin-D (bi-hydroxamate)	11	3.75 (COOH)	Not evaluated	–	[108]
		8.47 (CONOH)			
		9.40 (CONOH)			
Marinobactin-E (bi-hydroxamate)	11	3.85 (COOH)	31.8	–	[108]
		8.89 (CONOH)			
		9.52 (CONOH)			

^a pFe³⁺ = -log[Fe³⁺]_{free} at pH = 7.4, [Fe³⁺]_{total} = 10^{−6} M, [Ligand]_{total} = 10^{−5} M.

used in the Soviet Union as an antibiotic. Its structure was firmly established by Benz and coworkers in 1982 [24]. Similarities between grisein and albomycin that led to conclude that they are the same compound have been discussed in the literature [119]. Other examples of hydroxamate sideromycins are salmycins and ferrimycins (Table 4).All bacteria susceptible to the microbicidal activity of these hydroxamate sideromycins were found to have the FhuA receptor in their membranes. These sideromycins function better under iron starvation conditions than under conditions of sufficiency [3]. Iron starvation can be achieved by using biostatic agents (strong chelators that bind iron tightly).In addition to these sideromycins, another seryl tRNA synthetase inhibitor, SB-217452 [122], was isolated from a *Streptomyces* species. Although structural characterization showed that SB-217452 contains the same nucleoside moiety present in albomycin, it showed very weak antibacterial activity compared to the latter. This can be explained by the fact that SB-217452 does not contain the chelator moiety that mediates the uptake of albomycin [13,122]. This example and many others clearly demonstrate the role played by the chelator moiety in overcoming the low permeability of the pathogenic cell wall towards the bactericidal moiety alone.Albomycin (3) consists of a linear Orn-derived tri hydroxamate. The tri peptide is linked through its C-terminus to a glycoside entity by a di-seryl linker. When the competing bacteria internalize the conjugate-iron complex, peptidases release the warhead, thus inducing bacterial death [13] (Fig. 3-A). Ferrimycin (66) resembles the ferrioxamine family in its chelating unit, which is a linear non-

peptidyl tris-hydroxamate derived from alternative N-hydroxy-di-aminopentane and succinic acid with a 4-amino-5-hydroxybenzamide linkage to an iminoester-substituted lactam antibiotic [112]. Salmycin consists of danoxamine siderophore (9) attached to an aminoglycoside through a succinic linker [121]. When the hydroxamate moieties are engaged with iron complexation, the payload remains attached to the conjugate. Once the reductase of the cell frees the iron, the protonated hydroxamate attacks the ester group causing a cyclization reaction that results in the release of the antibiotic. This only happens after the competing cell obtains the iron from the stolen complex [13,123]. The mechanism of drug release is shown in Fig. 3-B [123].Of note, the sideromycin-producing organism itself is not affected by the conjugate because it does not have a target for the warhead or the related enzyme that causes drug release [13]. Also, vulnerable bacteria can readily develop resistance against these sideromycins through the deletion of the hydroxamate membrane receptor [14].

2.4.1.2. Artificial conjugates mimicking the natural ones The activity of the natural sideromycins opened the door for research into new conjugates. In this regard, attention was turned to tuning the structure of the chelators and varying the antimicrobial moiety, thereby providing promising tools for tackling fatal multidrug-resistant bacteria.The first synthetic conjugate 85 was reported by Zahner in 1977 [13]. He hybridized DFO B with a sulfonamide directly through an amide bond (no linker) between the available primary amine of the chelator moiety and the carboxyl of the sulfonamide. Many artificial hybrids containing hydroxamate chelators have been reported to date (Table 5), with cargos varying between cephalosporines, fluoroquinolones and others. However, none of the hydroxamate-drug hybrids have yet been approved as a drug. Cefiderocol (S-649266) is the first and only siderophore-drug conjugate to be authorized by the FDA (November 2019) [124]. This drug consists of a catechol chelating unit and a cephalosporin antibiotic cargo [125] (see section 3).In summary, conjugates 68–73 are semi synthetic sideromycins that share the same chelator moiety as albomycin (3) which is a linear Orn-derived tri hydroxamate. The antibiotic moieties in these conjugates varies between β-lactams as in 68 and 69, and fluoroquinolones as in 70, attached to the C-terminal of the chelating peptide backbone. In conjugates 71–73, the antibiotics digoxigenin, biotin and virgimycin, respectively, are attached to the tripeptide backbone via different linkers through its N-terminals.Conjugates 74–77 are totally synthetic; the chelator moiety is an iso cyanuric acid-based tri Orn hydroxamate, inspired by the natural bi hydroxamate siderophore rhodotorulic (4). The antibiotic is directly attached to the acyl part of the hydroxamate, enabling the preparation of multi-warhead conjugates [142].Conjugates 78–82 were synthesized to illustrate the effect of linker on overall activity. All are containing the same chelator moiety, desferrioxamine B, and ciprofloxacin antibiotic. The linkers vary from stable uncleavable succinic moiety 78 to esterase-cleavable 79, phosphatase-cleavable 80 and reductase-cleavable 81, ending with thiol-maleimide linker 82. The mechanisms of drug release are shown in Fig. 4 [132]. Conjugates 82–84 share the same desferrioxamine B chelator, the same linker (thiol maleimide) and three different antibiotics.Conjugates 86–88 contain danoxamine analogue chelators with differing denticity (mono, bi and tri hydroxamate) and three different antibiotics attached through a stable linker.Conjugates 89–102 contain mixed chelators. Compounds 89–98 are biscatecholate-mono hydroxamate, with various antibiotic warheads, while 99–102 are pyoverdine-derived (bis hydroxamate, mono catecholate) chelators attached to a range of drugs through different linkers at distinct positions of the pyoverdine scaffold.Conjugate 106 is mycobactin chelator (employing lysine residue as the hydroxamate-bearing moiety) attached to the antibiotic artemisinin.

2.4.1.2.1. Summary of the antibacterial activity of these conjugates Most of these conjugates had moderate to low antibacterial activity. Conjugates with β-lactams showed better performance than those with non

Table 4

Natural hydroxamate sideromycins, chelator moieties are shown in black and purple, linkers in green and antibiotic moiety in red.

#	Sideromycin	Structure	Produced naturally by	Year/ref.
	General structure	<p>the driving moiety the toxic drug</p>		
65	Grisein	Structure not fully elucidated	<i>Streptomyces griseus</i>	1947 [23,120]
3	Albomycin	Albomycin ϵ , X=O Albomycin δ_1 , X=NH Albomycin δ_2 , X=NCONH ₂	<i>Actinomyces subitropicus</i>	1951 [118]
66	Ferrimycin	Ferrimycin A ₁		1960 [112]
67	Salmycin	<p>Salmycin A n=5, X= NOH B n=5; X= O (or OH₂) C n=4; X=O (or OH₂) D n=4; X= NOH</p>	<i>Streptomyces violaceus</i>	1995 [121]

Fig. 3. Possible mechanisms for drug release from sideromycin: a) albomycin and b) salmycin.

β -lactams [14]. The activity of the conjugates under iron-deficient conditions was enhanced but was lower than that of the parent antibiotic alone in most cases. This observation may be attributable to premature release, non-suitable substrates for enzymatic cleavage, or readily de-

veloped resistance [135]. The modification of linkers (trimethyl lock) in 79–80 did not achieve better results. Here are some examples with the main conclusions learned from their study: Conjugates 86–88 showed that the denticity of the chelator is crucial, where it should

Table 5
Artificial hydroxamate siderophore-drug (SD) conjugates, chelator moieties are shown in black and purple, linkers in green and antibiotic moiety in red.

#	Structure	Antibiotic	ref.
68		Loracarbef	[126,127]
69		Oxamazin	[128]
70		Flouroquinoline	[129]
71		Digoxigenin	[87]
72		Biotin	[87]
73		Virgimycin	[130]

#	Structure	Antibiotic	ref.
74			[131]
75			[131]
76		Nucleoside	[14]
77		Macrolide	[14]
78			[132]
79		Esterase release	[132]
80		Phosphatase release	[132]
81		Reductase cleave	[112]
82			[133]

#	Structure	Antibiotic	ref.
83		Sulfonamide	[133]
84			[133]
85			[112]
86		β -Lactam Lorabide	[134]
87		Triclosan	[135]
88		Fluroquinoline Ciprofloxacin	[134]
89		Ampicillin	[136]

#	Structure	Antibiotic	ref.
90			[136]
91		Glycopeptide Vancomycin	[14,112]
92		Loracarbef	[13,112,137,138]
93		Cephalosporin	[13]
94			[137]
95			[112,138]
96		Daptomycin	[112,139]
97		Cephem	[13,14]

#	Structure	Antibiotic	ref.
98		Vancomycin	[13]
99		Norofloxacin cleavable	[112]
100		Norofloxacin non cleavable	[112]
101		Ampicillin	[112,140]
102			[141]
103		Artemisinin	[13]

form a 1:1 complex with Fe(III) to actively transport the conjugate [134,135]. Conjugate **92** employs a bis catecholate-mono hydroxamate chelator that is analogous to fimsbactin (a siderophore produced by *A. baumannii*), the chelator is conjugated to loracarbef. It shows high activity against *A. baumannii*, again demonstrating the vulnerability of a bacterium against a hybrid of its own siderophore (advantage of endogenous over xenogenous siderophores). Furthermore, **92** mediates uptake through two receptors in *E. coli*, namely Cir (for the catechol) and FhuA (for the hydroxamate) [14,112]. The same mixed bis-catecholate mono-hydroxamate chelator hybridized with a ciprofloxacin warhead as in **95**, shows no activity against *A. baumannii* [112,134],

emphasizing the importance of harmony between the uptake cycle of the chelator and the target of the antibiotic. The cycle of this conjugate ends in the periplasm, while the target of ciprofloxacin (unlike the loracarbef in **92**) is the cytoplasm, thereby explaining the lack of activity of **95**. A strong promising example is conjugate **96**, which conferred the gram-positive antibiotic daptomycin activity against some gram-negative bacteria [138,139]. This conjugate revealed that that large molecules (such as daptomycin >500 Da which cannot diffuse into the cell like the smaller molecules) can also be transported into the cell through specific iron-mediated receptors [112]. Conjugate **103** was highly specific to *M. tuberculosis*. The chelating moiety in conjugate **103** resem-

Fig. 4. "Trimethyl lock" mechanism of drug release in conjugates: a) **79** b) **80** [132] and c) **81** [13].

bles mycobactin, the siderophore produced by *M. tuberculosis*. However, **103** did not attack other gram-positive or -negative bacteria [13,112]. Again, this observation indicates that the use of the pathogen's own siderophore may be useful. The same conclusion was drawn from pyoverdine-derived conjugate **101** against the pyoverdine producer *P. aeruginosa* [140].

2.4.1.3. Rational design for choice of artificial siderophore-drug conjugates Traditional antibiotics are classified into four groups on the basis of their cellular targets. The targets are cell wall assembly (as for β -lactams and glycopeptides), protein synthesis (as for aminoglycosides and macrolides), DNA/RNA replication (as for fluoroquinolones and sulfonamides) and membrane disruption (as for lipopeptides; daptomycin) [112]. Small antibiotics (<500 Da) enter the cell by passive diffusion through the porins in the cell wall of gram-negative bacteria and they are usually broad spectrum. On the other hand, glycopeptides and daptomycin are active only against gram-positive bacteria, with targets present on the outer part of the cell [112]. Hybridizing these antibiotics with siderophores can broaden or narrow the spectrum of activity of the antibiotic warhead.

2.4.1.3.1. Choosing the chelator The chelator of choice depends on the pathogen targeted, its outer membrane protein receptors (what types of siderophores it can recognize), and the iron cycle (where Fe(III) is released—in the cytoplasm or periplasm) [135]. For example, trihydroxamate is suitable to drive cytoplasmic drugs while biscatecholate-hydroxamate is suitable for periplasmic drugs for gram-negative bacteria.

2.4.1.3.2. Role played by the linker Linkers play a critical role in determining the efficiency of the conjugate. Premature release of the warhead or halting it back from attacking the target may fail the whole process. The choice of linker depends on the abovementioned factors. Several types have been reported, some of which have been mentioned before. Another kind of chelator is β -lactam as a linker attached to any type of antibiotic. The release mechanism depends on the attack of β -lactamase to free the other antibiotic payload [13].

2.4.1.3.3. Choosing the antibiotic The antibiotic warhead target determines where the release takes place, and, consequently, the type of linker to be used. Even when taking all factors carefully into consideration, the bacterial fast development of resistance remains the biggest challenge.

2.4.2. Other hydroxamate siderophore conjugates

In all the previous conjugates mentioned, the chelating units are the moiety that drives them towards the target and the payloads are the antibiotics. Hydroxamate chelators can also be used in other conjugates in which an antibody is the driving moiety and a radiolabelled metal-chelator complex is the cargo used for imaging [113,143] (Fig. 5).

3. Cefiderocol (FETROJA®): the first siderophore-drug conjugate in the market

Cefiderocol (previously known S-649266) is a novel catechol-based siderophore that targets gram-negative bacteria, including strains with carbapenem resistance, via a Trojan horse active transport mechanism [125,144]. The aminoacyl catechol moiety is attached with a di-methylene linker to the pyrrolidine ring of the antibiotic [144,145] (Fig. 6).

The structure of the payload in cefiderocol combines the features of the antibiotics ceftazidime and cefepime, thereby boosting activity against gram-negative bacteria, as well as stability. The quaternized N-methyl-pyrrolidine and the carboxylate moieties of cefiderocol form a zwitterion that enhances water solubility, degradation resistance, and capacity to rapidly penetrate the outer cell membrane of gram-negative bacteria [146]. GR69153 (cefetecol), M-14659 and S-9096 are other catechol-cephalosporine conjugates that showed strong antibacterial activity *in vitro*, but were not successful *in vivo* [145].

Cefiderocol was approved by the FDA on November 14, 2019 [124]. It is used for the treatment of complicated urinary tract infections (cUTI), including pyelonephritis caused by susceptible gram-negative microorganisms. It is administered intravenously. It has some reported adverse effects, including diarrhea, infusion site reactions, constipation, rash, candidiasis, cough, elevations in liver tests, headache, hypokalemia, nausea, and vomiting [147].

4. Conclusion

Forty years of research have been devoted to the synthesis and use of sideromycins to tackle pathogens. Among these conjugates, only one has reached the market, namely Cefiderocol, which was approved by the FDA in 2019. Although Cefiderocol contains a catechol (not a hy-

Fig. 5. Hydroxamate chelator-antibody conjugates for imaging applications.

Fig. 6. Chemical structure of Cefiderocol.

droxamic) binding unit—the field remains open for the development of others. No other reported artificial conjugates have been commercialized to date, but they have all contributed to bringing the pieces of the puzzle together. Inspired by albomycins, ferrimycins and salmycins, hydroxamate-based siderophores are well positioned to achieve success. While the full picture of successful conjugate design and development is gaining clarity, more research efforts are required to optimize the technology.

Declaration of competing interest

Developing Multi-drug resistance in bacteria threatens human health when entering the post-antibiotic era. Iron acquiring gates supply bacteria with its nutrient, iron (III) by iron carrying molecules

(Siderophores). Using this strategy, siderophore-antibiotic conjugates (sideromycins) internalize the drug into the bacterial cell through iron gates, release the drug inside the cell and kill it. The most famous natural sideromycins (Albomycin, Salmecin, Ferrimycin) chelate iron (III) through their hydroxamate moieties, which also mediate the recognition and up take. An insisting need to understand the full picture behind their potency lead to the writing of this review. Most recent natural hydroxamate siderophore, chemical synthesis of building blocks, conclusions from the artificial sideromycins trials are all discussed thoroughly in this review.

In our opinion, this is a fully transversal review, which should be of interest to a broad scientific audience. Thus, synthetic chemists can find rich information about natural siderophores and comprehensive synthetic source for building blocks syntheses.

Microbiologists will have access to the detailed structure and producing species for almost all hydroxamate siderophores isolated in the last two decades. It also helps biochemists with the rational relation between the choice of each of the bacteria, chelator, linker, antibiotic and the antibacterial activity, concluded from literature. Finally, drug hunters could find inspiration for the development of new drug discovery programs.

We are confident that our work will be of interest to a large number of colleagues working in related fields and merit publication in *Eur. J. Med. Chem.*

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