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# Chemical scaffolds with structural similarities to siderophores of nonribosomal peptide–polyketide origin as novel antimicrobials against *Mycobacterium tuberculosis* and *Yersinia pestis*

Julian A. Ferreras [a,†], Akash Gupta [b,‡], Neal D. Amin [a,§], Arijit Basu [c], Barij N. Sinha [c], Stefan Worgall [d], Venkatesan Jayaprakash [c,*], Luis E.N. Quadri [b,*]

[a] *Department of Microbiology and Immunology, Weill Medical College of Cornell University, New York, NY 10021, USA*
[b] *Department of Biology, Brooklyn College, City University of New York, 2900 Bedford Avenue, Brooklyn, NY 11210, USA*
[c] *Department of Pharmaceutical Sciences, Birla Institute of Technology, Mesra, Ranchi 835 215, India*
[d] *Department of Pediatrics and Genetic Medicine, Weill Medical College of Cornell University, New York, NY 10021, USA*

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ABSTRACT

*Mycobacterium tuberculosis* (Mtb) and *Yersinia pestis* (Yp) produce siderophores with scaffolds of nonribosomal peptide–polyketide origin. Compounds with structural similarities to these siderophores were synthesized and evaluated as antimicrobials against Mtb and Yp under iron-limiting conditions mimicking the iron scarcity these pathogens encounter in the host and under standard iron-rich conditions. Several new antimicrobials were identified, including some with increased potency in the iron-limiting condition. Our study illustrates the possibility of screening compound libraries in both iron-rich and iron-limiting conditions to identify antimicrobials that may selectively target iron scarcity-adapted bacteria and highlights the usefulness of building combinatorial libraries of compounds having scaffolds with structural similarities to siderophores to feed into antimicrobial screening programs.

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*Mycobacterium tuberculosis* (Mtb), the causative agent of tuberculosis, and *Yersinia pestis* (Yp), the etiologic agent of plague, are bacterial pathogens with serious impacts on global public health. Multidrug-resistant (MDR) tuberculosis is an emerging pandemic, and the more recent emergence of extensively drug-resistant (XDR) tuberculosis poses a new global threat.[1] Plague is a re-emerging disease for which the documented occurrence of MDR Yp strains and self-transferable Yp plasmids conferring antibiotic resistance raises concerns about future plague control.[2] These grim scenarios underscore the need for expanding the anti-tuberculosis and anti-plague drug armamentarium.

Many bacteria utilize secreted, small (<1000 Da) $Fe^{3+}$-chelating compounds (compds) ($K_d < 10^{-25}$ M) called siderophores to scavenge $Fe^{3+}$ from their microenvironments and transport it into the cell.[3] The Mtb siderophore (mycobactin/carboxymycobactin) and the Yp siderophore (yersiniabactin) are based on substituted

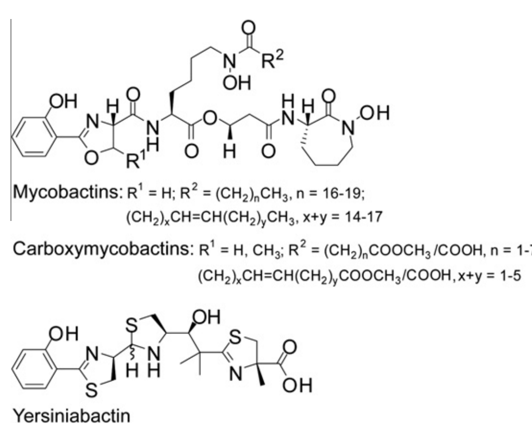


**Figure 1.** Structures of *M. tuberculosis* and *Y. pestis* siderophores.

* Corresponding authors. Tel.: +91 651 2275843 (V.J.); +1 718 951 5000x6254 (L.E.N.Q.).

*E-mail addresses:* venkatesanj@bitmesra.ac.in (V. Jayaprakash), LQuadri@brooklyn.cuny.edu (L.E.N. Quadri).

† Present address: Department of Genetics, Universidad Nacional de Misiones, Posadas (3300), Misiones, Argentina.

‡ Present address: Yale University School of Medicine, New Haven, CT 06510, USA.

§ Present address: University of California San Diego, School of Medicine, La Jolla, CA 92093, USA.

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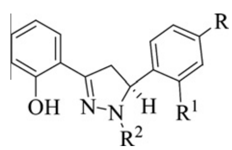
scaffolds of nonribosomal peptide–polyketide origin (Fig. 1).[4] Studies in cellular and animal models of infection have established the relevance of the mycobactin/carboxymycobactin and yersiniabactin siderophore systems in these pathogens.[5] The siderophores are believed to facilitate iron scavenging inside the host, where free iron is scarce ($10^{-25}$ to $10^{-15}$ M) and the pathogens experience and must adapt to iron-limiting conditions.[6] These observations suggest that the Mtb and Yp siderophore systems represent potential in vivo conditionally essential target candidates for the development of alternative therapeutics against tuberculosis and plague.[7]

We hypothesize that screening compds with structural features resembling Mtb and Yp siderophores for growth inhibitory activity against these pathogens may lead to the discovery of novel antimicrobial scaffolds. Such novel antimicrobials could illuminate alternative paths to drug development and/or be useful as small-molecule tools to assist in the elucidation of new target candidates for drug development. Compds with structural features resembling Mtb and Yp siderophores may impair the siderophore systems (e.g., by inhibiting siderophore biosynthesis or transport) and halt bacterial growth in the host's iron-limiting environments. Alternatively, these compds might gain access to the intracellular environment using siderophore transport systems and inhibit essential functions unrelated to iron acquisition. Consistent with these views, we have recently demonstrated potent antimicrobial activity against Mtb and Yp for novel diaryl-carbothioamide-pyrazoline derivatives with structural features resembling the hydroxyphenyl-oxazoline/thiazoline-containing half of Mtb and Yp siderophores.[8]

In an effort to identify additional novel inhibitors of Mtb and Yp growth, we synthesized and evaluated the antimicrobial activity of new 3,5-diaryl-substituted pyrazoline (DAP) derivatives (compds **1**–**22**,[9a] Table 1). In addition, we synthesized and tested the activity of a group of (2*E*)-2-benzylidene-*N*-hydroxyhydrazine carbo(ox/thio/oximid)-amide (BHHC) derivatives (compds **23**–**32**,[9b] Table 2) with hydroxyphenyl-cap functionalities resembling that of the siderophores. The compds were tested for growth inhibitory activity against Mtb and Yp in iron-limiting media, which mimic the iron-scarcity condition that the pathogens encounter in the host, and in standard iron-rich media.[10a] We also assessed selected compds for mode of action (bactericidal or bacteriostatic) in iron-limiting media and for cytotoxicity toward mammalian cells.[10b]

Testing against Mtb revealed that 17 compds (**1**, **2**, **5**, **8**–**15**, **24**, **27**–**31**) had $IC_{50}$s and MICs (3–222 μM range, Table 3) within the concentration series tested in the iron-limiting medium, GASTD. Of these 17 compds, 15 (**1**, **2**, **5**, **9**–**12**, **14**, **15**, **24**, **27**–**31**) also had determinable $IC_{50}$s and MICs (2–132 μM range) in the iron-rich medium, GASTD+Fe. Examination of $IC_{50GASTD+Fe}/IC_{50GASTD}$ and $MIC_{GASTD+Fe}/MIC_{GASTD}$ ratios revealed that the inhibitors had no noteworthy increased potency in the iron-limiting medium within the concentration series tested. This suggests that interference with iron acquisition, or any other bacterial process differentially required for growth under the iron-limiting condition, is not a property that significantly contributes to the compds' antimicrobial activity against Mtb. Interestingly, **14** is 5-fold more potent against Mtb cultured in GASTD+Fe, as judged by MIC values. This phenomenon might suggest that the mechanism(s) of action of **14** against Mtb might be potentiated by an elevated production of cytotoxic hydroxyl radicals originated through increased levels

**Table 1**
3,5-Diarvl-substituted pyrazoline (DAP) derivatives (**1**–**22**)



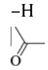
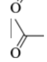
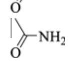
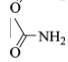
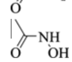
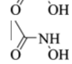
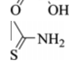
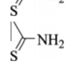
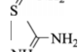
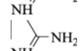
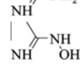
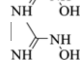
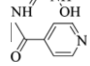
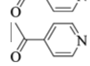
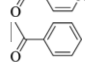
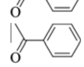
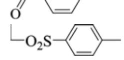
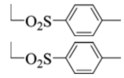
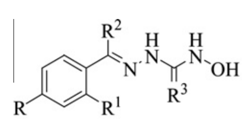
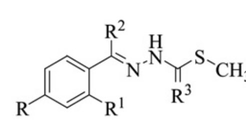
| Compound | R | R′ | R |
|---|---|---|---|
| **1** | –H | –OH | –H |
| **2** | –OH | –H | –H |
| **3** | –H | –OH |  |
| **4** | –OH | –H |  |
| **5** | –H | –OH |  |
| **6** | –OH | –H |  |
| **7** | –H | –OH |  |
| **8** | –OH | –H |  |
| **9** | –H | –OH |  |
| **10** | –H | $-CH_3$ |  |
| **11** | –H | –OH |  |
| **12** | –OH | –H |  |
| **13** | –H | –OH |  |
| **14** | –OH | –H |  |
| **15** | –H | –Oh |  |
| **16** | –OH | –H |  |
| **17** | –H | –OH |  |
| **18** | –OH | –H |  |
| **19** | –H | –OH |  |
| **20** | –OH | –H |  |
| **21** | –H | –OH |  |
| **22** | –OH | –H |  |

**Table 2**
(2*E*)-2-Benzylidene-*N*-hydroxyhydrazine carbo(ox/thio/oximid)-amide (BHHC) derivatives (**23**–**32**)



Cmpds **23-26**, **30**, **31**



Cmpds **27-29**

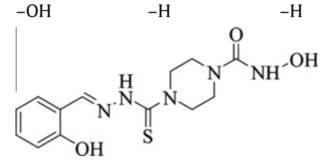
| Compound | R | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|---|
| **23** | –H | –OH | –H | =O |
| **24** | –OH | –H | –H | =O |
| **25** | –H | –OH | –CH, | =O |
| **26** | –OH | –H | –CH3 | =O |
| **27** | –H | –OH | –H | =S |
| **28** | –OH | –H | –H | =S |
| **29** | –H | –OH | –CH3 | =S |
| **30** | –H | –OH | –H | –NH |
| **31** | –OH | –H | –H | –NH |
| **32** |  | | | |

**Table 3**
Antimicrobial activity against *M. tuberculosis*

| Compound | $IC_{50}$[a] (μM) | | Ratio | $MIC_{90}$[b] (μM) | | Ratio | Mode of action[c] |
|---|---|---|---|---|---|---|---|
| | GASTD+Fe | GASTD | | GASTD+Fe | GASTD | | |
| *DAP series* | | | | | | | |
| **1** | 18 | 25 | 1 | 59 | 66 | 1 | BC (2 × MIC) |
| **2** | 37 | 46 | 1 | 125 | 125 | 1 | BC (I × MIC) |
| **3** | >16 | >31 | nd | >16 | >32 | nd | nd |
| **4** | >8 | >8 | nd | >8 | >8 | nd | nd |
| **5** | 64 | 77 | 1 | 104 | 208 | 0.5 | BC (2 × MIC) |
| **6** | >32 | >63 | nd | >63 | >63 | nd | nd |
| **7** | >32 | 54 | nd | >31 | >63 | nd | nd |
| **8** | 40 | 42 | 1 | >31 | 125 | nd | BC (1 × MIC) |
| **9** | 50 | 53 | 1 | 125 | 125 | 1 | BC (2 × MIC) |
| **10** | 7 | 7 | 1 | 16 | 16 | 1 | BC (2 × MIC) |
| **11** | 23 | 44 | 1 | 66 | 66 | 1 | BC (2 × MIC)[d] |
| **12** | 26 | 31 | 1 | 76 | 125 | 1 | BC (2 × MIC)[d] |
| **13** | 32 | 37 | 1 | >125 | 125 | >l | BC (2 × MIC) |
| **14** | 22 | 47 | 0.5 | 31 | 125 | 0.2 | BC (2 × MIC) |
| **15** | 8 | 8 | 1 | 16 | 16 | 1 | BC (2 × MIC) |
| **16** | >4 | >8 | nd | >4 | >8 | nd | nd |
| **17** | 98 | 84 | 1 | >500 | >500 | nd | nd |
| **18** | >16 | >63 | nd | >63 | >63 | nd | nd |
| **19** | >16 | >63 | nd | >31 | >63 | nd | nd |
| **20** | >3I | >63 | nd | >31 | >63 | nd | nd |
| **21** | >31 | >63 | nd | >31 | >63 | nd | nd |
| **22** | >3I | >63 | nd | >31 | >63 | nd | nd |
| *BHHC series* | | | | | | | |
| **23** | >16 | >16 | nd | >16 | >16 | nd | nd |
| **24** | 40 | 97 | 0.4 | 97 | 146 | 1 | BC (2 × MIC) |
| **25** | >8 | >8 | nd | >8 | >8 | nd | nd |
| **26** | >500 | >500 | nd | >500 | >500 | nd | nd |
| **27** | 2 | 3 | 1 | 6 | 7 | 1 | BC (4 × MIC) |
| **28** | 33 | 31 | 1 | 59 | 59 | 1 | BC (2 × MIC) |
| **29** | 4 | 4 | 1 | 9 | 10 | 1 | BC (2 × MIC) |
| **30** | 15 | 11 | 1 | 26 | 222 | 1 | BC (2 × MIC) |
| **31** | 43 | 43 | 1 | 132 | 222 | 1 | BC (2 × MIC) |
| **32** | >8 | 20 | nd | >8 | >31 | nd | nd |
| Isoniazid | 0.09 | 0.07 | 1 | 0.2 | 0.2 | 1 | BC (2 × MIC) |

[a] $IC_{50}$ values were calculated from sigmoidal curves fitted to triplicate sets of dose–response data.
[b] $MIC_{90}$ values are means of triplicates. Ratio, GASTD+Fe/GASTD. All values >l, <l, >0.5, and ⩽0.5 were rounded to the nearest whole number, to 1, and to one significant digit, respectively.
[c] Mode of action was evaluated in GASTD in duplicate. The concentration at which each compound was tested for mode of action is indicated between parentheses.
[d] Some bactericidal activity detected, yet below the 99% killing criterion set for defining bactericidal mode of action. BS, bacteriostatic; BC, bactericidal; nd, not determined.

of Fenton reaction in the iron-rich medium. Such a potentiation would be in line with recent findings of Collins and co-workers regarding antibiotic-induced cell death.[16] Alternatively, **14** might inhibit an oxidative stress protection function(s) more critically needed in the iron-rich medium. Testing for cytotoxicity at the MIC against Mtb (125 μM) revealed that **14** had no significant cytotoxicity at short cell–compd contact time (4 h), yet cell viability was reduced by 70% relative to untreated controls after prolonged contact time (24 h) (Supplementary data, Fig. S1).

Among the active DAP derivatives, **10** and **15** were the most potent against Mtb ($IC_{50}$ = 7–4 μM, MIC = 16 μM, Table 3). Of these two compds, only **10** displayed significant cytotoxicity at the MIC against Mtb (16 μM). Compd **10** had a modest impact on cell viability, which was reduced only by 24% after 24 h of cell–compd contact (Supplementary data, Fig. S1). Encouragingly, these and most other inhibitors in the DAP derivatives series examined for mode of action against Mtb were bactericidal (>99% killing relative to inoculum) at concentrations of 1–2 × $MIC_{GASTD}$ (Table 1). This finding is significant since bactericidal activity is a desirable property in any early lead compd evaluated for antibacterial drug development programs. It is worth noting that the only two compds (**11** and **12**) defined as bacteriostatic in Table 1 showed significant bactericidal activity, yet below the 99% killing criterion set in this study for defining bactericidal mode of action. Among the compds of the BHHC derivatives series with defined $IC_{50}$ and MIC values, **27** and **29** were the most active against Mtb ($IC_{50}$ = 2–4 μM, MIC = 6–10 μM, Table 3). These two compds displayed no significant cytotoxicity in mammalian cells at their respective MIC values determined against Mtb (Supplementary data, Fig. S1). Gratifyingly, **27**, **29** and other active compds in this series displayed bactericidal mode of action against Mtb at concentrations of 1.7–4 × $MIC_{GASTD}$ (Table 3).

Testing against Yp revealed that 14 compds (**1**, **2**, **7**–**9**, **11**, **13**, **14**, **19**, **27**–**29**, **31**, **32**; Table 1) reached $IC_{50}$s (0.04–181 μM range, Table 4) within the concentration series tested in the iron-limiting medium, PMHD. Nine of these compds (**1**, **11**, **13**, **14**, **27**–**29**, **31**, **32**) also reached MICs (0.2–388 μM range) in PMHD. Only **29** and **30** had determinable activity in the iron-rich medium, PMHD+Fe (**29**: $IC_{50}$ = 156 μM, MIC = 233 μM; **30**: $IC_{50}$ = 305 μM). Interestingly, examination of the $IC_{50PMHD+Fe}/IC_{50PMHD}$ ratios revealed a number of inhibitors (**7**, **11**, **13**, **14**, **19**, **27**, **29**, **31**, **32**) with increased potency (>3-fold) against Yp cultured under iron scarcity. Compd **32**, with >100-fold and >20-fold higher potency in PMHD based on $IC_{50}$ and MIC values, respectively, stood out in this group. The higher potency of these compds in the iron-limiting medium raises the possibility that interference with an iron acquisition function, or other function more critically required for growth under the iron-limiting condition, is a property that significantly contributes to the compds' antimicrobial activity against Yp. One of the possible mechanisms of action of these compds could be related to iron-binding properties. An iron-binding ability strong enough to outcompete the powerful iron chelating capacity of

**Table 4**
Antimicrobial activity against *Y. pestis*

| Compound | $IC_{50}$[a] (μM) | | Ratio | $MIC_{90}$[b] (μM) | | Ratio | Mode of action[c] |
|---|---|---|---|---|---|---|---|
| | PMHD+Fe | PMHD | | PMHD+Fe | PMHD | | |
| *DAP series* | | | | | | | |
| **1** | >8 | 9 | nd | >8 | 31 | nd | BC (1 × MIC) |
| **2** | >31 | 20 | >2 | >31 | >31 | nd | nd |
| **3** | >31 | >31 | nd | >31 | >31 | nd | nd |
| **4** | >16 | >16 | nd | >16 | >16 | nd | nd |
| **5** | >250 | >250 | nd | >250 | >250 | nd | ml |
| **6** | >31 | >31 | nd | >31 | >31 | nd | nd |
| **7** | >31 | 8 | >4 | >3l | >31 | nd | nd |
| **8** | >31 | 13 | >2 | >31 | >31 | nd | nd |
| **9** | >63 | 181 | nd | >63 | >250 | nd | nd |
| **10** | >31 | >62 | nd | >31 | >63 | nd | nd |
| **11** | >63 | 7 | >9 | >63 | 42 | >l | BC (1 × MIC) |
| **12** | >63 | >125 | nd | >63 | >125 | nd | nd |
| **13** | >16 | 4 | >4 | >16 | 16 | >1 | BC (2 × MIC) |
| **14** | >63 | 5 | >13 | >63 | 21 | >3 | BC (2 × MIC) |
| **15** | >16 | >16 | nd | >16 | >16 | nd | nd |
| **16** | >8 | >8 | nd | >8 | >8 | nd | nd |
| **17** | >63 | >31 | nd | >63 | >31 | nd | nd |
| **18** | >16 | >31 | nd | >16 | >3l | nd | nd |
| **19** | >31 | 8 | >4 | >31 | >31 | nd | nd |
| **20** | >31 | >16 | nd | >31 | >16 | nd | nd |
| **21** | >16 | >31 | nd | >16 | >31 | nd | nd |
| **22** | >16 | >31 | nd | >16 | >31 | nd | nd |
| *BHHC series* | | | | | | | |
| **23** | >4 | >16 | nd | >4 | >16 | nd | nd |
| **24** | >250 | >250 | nd | >250 | >250 | nd | nd |
| **25** | >2 | >4 | nd | >2 | >4 | nd | nd |
| **26** | >500 | >500 | nd | >500 | >500 | nd | nd |
| **27** | >59 | 4 | >15 | >59 | 15 | >4 | BC (2 × MIC) |
| **28** | >233 | 137 | >2 | >233 | 388 | nd | BC (1 × MIC) |
| **29** | 156 | 61 | >3 | 233 | 116 | 2 | BC (2 × MIC) |
| **30** | 305 | 70 | 4 | >500 | 250 | >2 | BC (2 × MIC) |
| **31** | >500 | >500 | nd | >500 | >500 | nd | nd |
| **32** | >4 | 0.04 | >100 | >4 | 0.2 | >20 | BC (2 × MIC) |
| Streptomycin | 1 | 1 | 1 | 2 | 1 | 2 | BC (2 × MIC) |

[a] $IC_{50}$ values were calculated from sigmoidal curves fitted to triplicate sets of dose response data.
[b] $MIC_{90}$ values are means of triplicates. Ratio, PMHD+Fe/PMHD. All values >1, <l, >0.5, and ⩽0.5 were rounded to the nearest whole number, to 1 and to one significant digit, respectively.
[c] Mode of action was evaluated in PMHD in duplicate The concentration at which each compound was tested for mode of action is indicated between parentheses. nd, not determined; US, bacteriostatic; BC, bactericidal.

the bacterial siderophore could lead to sequestration of the traces of iron in the iron-limiting medium, thus reducing further iron bioavailability and producing a stronger antimicrobial activity under the iron-scarcity condition. Alternatively, it is possible that these compds gain intracellular access using the iron-scarcity-unregulated yersiniabactin's uptake system,[5e] therefore reducing $IC_{50}$ and MIC values in PMHD.

Compds **13** ($IC_{50PMHD}$ = 4 μM, $MIC_{PMHD}$ = 16 μM) and **14** ($IC_{50PMHD}$ = 5 μM, $MIC_{PMHD}$ = 21 μM) were the most potent among the active DAP derivatives with detectable activity against Yp (Table 4). These two compds displayed no significant cytotoxicity when they were evaluated at their respective MIC values determined against Yp (Supplementary data, Fig. S1). Testing of these and two other active DAP derivatives (**1**, **11**) for mode of action against Yp revealed that the four compds were bacteriostatic at concentrations of 1–2 × $MIC_{PMHD}$ (Table 4). Among the BHHC derivatives with defined $IC_{50}$ and MIC values in at least one condition (iron limiting or iron rich), **32** stood out due to its remarkable potency ($IC_{50PMHD}$ = 0.04 μM, $MIC_{PMHD}$ = 0.2 μM) against Yp cultured under iron scarcity. Encouragingly, **32** displayed no significant cytotoxicity when evaluated in cytotoxicity assays at the MIC determined against Yp (Supplementary data, Fig. S1). Notably, the activity of **32** was significantly higher than that of streptomycin ($IC_{50}$ and MIC ~1 μM), a bactericidal drug used to treat plague and included herein as an anti-Yp activity reference. Five compds (**27**-**29**, **31**, **32**) of the BHHC derivatives series were tested for mode of action against Yp in PMHD at concentrations of 1–2 × $MIC_{PMHD}$. Under the conditions tested, **29** displayed bactericidal activity, whereas **27**, **28**, **31**, and **32** were bacteriostatic.

In sum, 20 of 32 compds synthesized and evaluated herein have detectable antimicrobial against Mtb and/or Yp in at least one condition, iron scarcity or iron sufficient. To our knowledge, these are novel scaffolds not previously shown to have antimicrobial properties. Most active compds identified herein have comparable potency in the low and high iron conditions. This finding suggests that their pharmacological targets are likely to be essential bacterial functions required under both these conditions. In line with our aforementioned hypothesis, however, some of our compds have higher potency under the iron-limiting condition. Under this condition, bacteria depend on siderophores for efficient iron scavenging and engage an adaptive response to tailor their physiology to iron scarcity, thus exposing novel potential in vivo conditional target candidates.[7a] Some of these antimicrobials may impair siderophore system functioning as discussed above, a property that would result in bacteriostatic activity conditional to environmental iron scarcity (e.g., as seen with **27** and **32** against Yp). Overall, our study illustrates the possibility of screening compd libraries in both iron-sufficient and iron-limiting conditions to identify antimicrobials that may selectively target iron scarcity-adapted bacteria and highlights the usefulness of building combinatorial libraries of compds having scaffolds with structural similarities to siderophores to feed antimicrobial screening programs.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.08.052.

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9. (a) Compds **1**–**14** were synthesized from appropriate 2′-hydroxy chalcone derivatives as outlined in Scheme 1 (Supplementary data). Compds **9** and **10** were prepared as we reported earlier.[11] 1*N*-acetyl pyrazolines (**3** and **4**) were obtained by refluxing appropriate 2′-hydroxy chalcones with hydrazine hydrate (80%) in acetic acid.[12] Similarly, **5** and **6** as well as **11** and **12** were obtained by refluxing appropriate 2′-hydroxy chalcones with semicarbazide hydrochloride/aminoguanidine bicarbonate in methanol for 4–6 h. Compds **7** and **8** were synthesized from **1** and **2**, respectively. Ethyl chloroformate was added to a solution of pyrazolines (**1** and **2**) in methanol with constant stirring at room temperature. Triethylamine was added to neutralize the acid liberated. Hydroxylamine in methanol was then added with stirring at room temperature for 2 h to obtain **7** and **8**. Compds **13** and **14** were synthesized from **9** and **9a**, respectively. A slight excess of methyl iodide was added to **9** and **9a** dropwise with stirring (<10 °C, 1 h). Hydroxylamine in methanol was then added and stirring continued (22 °C, 30 min) to obtain **13** and **14**. Compds **15**–**22** were synthesized from appropriate 2′-hydroxy chalcone derivatives as outlined in Scheme 2 (Supplementary data). Compds **15** and **16** were prepared in similar manner to **5** and **6** and by replacing semicarbazide hydrochloride with isoniazide. Compds **17**–**22** were prepared from **1** and **2** as we reported earlier.[13] Reaction of benzoyl chloride with **1** and **2** in pyridine provided **17** and **18**, respectively. Similarly, reaction of sulphonyl chlorides with **1** and **2** in THF provided **19**–**22**. Compds **23**–**32** were synthesized as outlined in Scheme 3 (Supplementary data). (b) Compds **23**–**26** were prepared as reported.[14] *N*-hydroxy semicarbazides were prepared by reaction of hydroxylamine, phenylchloroformate and hydrazine hydrate (80%) in moist ether. The *N*-hydroxy semicarbazides were then condensed with appropriate aldehyde/ketone to yield **23**–**26**. Methyl hydrazinecarbodithioate was prepared as reported[15] and condensed with appropriate aldehyde/ketone to produce **27**–**29**. Compds **30** and **31** were prepared by a strategy similar to that used for **13** and **14**. A slight excess of methyl iodide followed by hydroxylamine to thiosemicarbazones of appropriate aldehydes provided **30** and **31**. Compds **32** was synthesized as we described earlier.[15a] Reaction of **27** with *N*-hydroxypiperidine-4-carboxamide provided **32**. The intermediates were characterized by elemental analysis and FT-IR spectra. Final compds were characterized through $^1$H NMR and FAB-MS spectral data. Synthetic procedures, physicochemical and spectral characteristics of **1**–**32** are presented in Supplementary data.
10. (a) Antimicrobial activity was determined in dose–response experiments using 96-well-plate-based, twofold-microdilution assays as reported.[7b,8,17] Virulent *Mtb* H37Rv was grown in iron-limiting GASTD medium and GASTD supplemented with 100 μM $FeCl_3$ (GASTD+Fe).[7b,17] Avirulent *Yp* KIM6-2082.1+ was grown in iron-limiting PMHD medium and PMHD supplemented with 100 μM $FeCl_3$ (PMHD+Fe).[7b,17] Cultures were started ($OD_{600}$ = 0.001) from deferrated culture stocks prepared as reported.[7b] Growth was assessed as $OD_{600}$ after incubation at 37 °C (*Mtb*: 10 days, stationary condition; *Yp*: 26 h, 200 rpm) using a Spectra Max Plus reader (Molecular Dynamics). Compds were evaluated at up to the highest concentration permitted by their solubility, with a 500 μM upper testing limit, and added as DMSO solutions. DMSO was kept at 0.5% in treated and DMSO-control cultures. $IC_{50}$s were calculated from sigmoidal curves fitted to triplicate dose–response data using KaleidaGraph (Synergy Software) as reported.[7b] $MIC_{90}$s were calculated as the lowest concentration tested that inhibited growth by ⩾90% relative to DMSO controls. Compds were tested at up to the maximum multiple of the MIC permitted by their solubility. (b) After bacterial cultures were treated with compds and incubated as noted above, the mode of action was evaluated by enumerating CFU/mL after plating serial 10-fold dilutions of duplicate *Mtb* and *Yp* cultures on plates of Middlebrook 7H11[18] and plates of TBA,[17] respectively. Plates were incubated at 37 °C for 30 days for *Mtb* and at 30 °C for 3 days for *Yp* before colony counting. Cytotoxicity was assessed against HeLa cells in a 96-well plate platform using a standard ATPLite™ reagent-based cytotoxicity assay (Perkin-Elmer) as previously reported.[8]
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