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Total Synthesis of Hinduchelins A–D, Stereochemical Revision of Hinduchelin A, and Biological Evaluation of Natural and Unnatural Analogues

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Abstract

Here, we report the first total synthesis of hinduchelins A–D, a family of nontoxic catechol derivatives from *Streptoalloteichus hindustanus*, possessing a druglike chemotype and modest iron-chelating ability. A concise synthesis was developed employing methyl 5-methyloxazole-4-carboxylate as a single starting material to provide hinduchelins A–D (and unnatural analogues) in only four steps and 5–15% overall yields; moreover, the stereochemistry of hinduchelin A was reassigned from (*S*) to (*R*). Biological evaluation confirmed that natural and unnatural hinduchelins are weak iron chelators (siderophores).

Graphical Abstract

Recently, small heterocyclic natural products, exemplified by **1–6**, have been proven to possess unique biological activity at a variety of therapeutic targets, and further elaboration

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Supporting Information

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Natural and synthetic compound comparison tables, supplemental figures, ¹H and ¹³C NMR spectra for new compounds, and LCMS traces (PDF)

The authors declare no competing financial interest.

into unnatural analogs has increased their desired biological activities (Figure 1).^{1–6} Importantly, these small heterocyclic natural products show little to no cytotoxicity, engender druglike physicochemical properties, and can be accessed in less than 10 synthetic steps, increasing their attractiveness as leads for discovery efforts. Beyond biological activity, synthetic efforts aimed at such natural products have led to structural and/or stereochemical reassignments benefiting the natural products community while also further augmenting structure–activity relationships (SAR).^{1–6}

As part of this ongoing total synthesis and biological evaluation of small, heterocyclic natural products in our laboratory, we were attracted to the recent disclosure of four new heterocyclic catechol derivatives, hinduchelins A–D (**7–10**), isolated from *Streptoalloteichus hindustanus* (Figure 2).⁷ The hinduchelins were of interest due to their druglike structures and properties (e.g., a notable structural similarity to reported estrogen receptor- β ligands^{8,9}), a lack of cytotoxicity in multiple tumor cell lines (HL-20, A549, SMMC-7721, MCF-7, and SW-480), and weak iron-chelating ability (siderophore activity), for **10**, in *Pseudomonas aeruginosa*. Iron-chelating agents have been reported to exhibit antibacterial and biofilm-eradicating capabilities.^{10,11} The absolute configuration of hinduchelin A (**7**) as the (*S*)-enantiomer was established by quantum-chemical ECD calculations.⁷

Scheme 1 highlights our retrosynthetic analysis of hinduchelins A–D (**7–10**), providing a concise synthetic strategy to rapidly construct all four members of this natural product family starting from commercial oxazole **13**. From **13**, a regiospecific bromination, subsequent Suzuki coupling with catechol boronic acids, ester hydrolysis, and a HATU-mediated amide coupling with an appropriately functionalized phenethylamine delivered **7–10**.

To evaluate the route, we first focused our attention on the synthesis of hinduchelin A (**7**). Bromination of commercial methyl 5-methyloxazole-4-carboxylate **13** provided the desired 2-bromo congener **12** in 24% isolated yield (Scheme 2).¹² A Suzuki coupling reaction between bromide **12** and (2,3-dimethoxyphenyl)boronic acid **14** under standard conditions provided **15** in 88% yield. Hydrolysis of the ester proceeded in near-quantitative yield (99%) to the acid **16**, followed by a HATU-mediated amide coupling with (*S*)-2-amino-1-phenylethanol **17** in good isolated yield to afford hinduchelin A (**7**) as the reported (*S*)-enantiomer at C7''. The overall yield for the synthesis of **7** was 15.2%. While the ¹H and ¹³C spectra and Hi-Res MS all confirmed the structure of **7** (and were in agreement with the literature data),^{7,12} the optical rotation differed (reported $[\alpha]_{\text{D}}^{25} = -2.7$ (*c* 0.1, MeOH); synthetic material, $[\alpha]_{\text{D}}^{25} = +9.8$ (*c* 0.1, MeOH)), suggesting the absolute stereochemistry, which was assigned on the basis of quantum-chemical ECD calculations, might be incorrect.¹²

To explore this possibility, we coupled **16** to (*R*)-2-amino-1-phenylethanol **18** under HATU-mediated conditions to deliver **19** (Scheme 3) in 73% isolated yield (and again 15.2% overall). As expected, the ¹H and ¹³C spectra and Hi-Res MS all confirmed the structure of **7**, but now the optical rotation of synthetic **19**, $[\alpha]_{\text{D}}^{25} = -11.9$ (*c* 0.1, MeOH), more closely

aligned with that of natural **7** (reported $[\alpha]_D^{25} = -2.7$ (c 0.1, MeOH). Based on these data, we feel that the stereochemistry of **7** was misassigned, and it has been corrected to reflect (*R*)-stereochemistry at C7''.¹² Thus, the correct structure of hinduchelin A is **19**, and **7** represents the unnatural enantiomer.

As the original isolation/characterization paper only profiled **10** as a weak siderophore, we needed to make the remaining members of the family in order to assess bioactivity and potential SAR. Hinduchelins B–D (**8–10**) did not possess the C7'' hydroxyl but instead displayed alternate substitution on the eastern phenyl ring and/or free phenols on the western catechol ring. In the event, hinduchelin B (**8**) was prepared from intermediate **16**, employing a HATU-mediated amide coupling with 2-(2-aminoethyl)phenol to deliver **8** in 75% yield (Scheme 4).¹²

The remaining hinduchelins, C and D (**9** and **10**, respectively), differed in that the C1 position bore an –OH rather than the –OMe moiety of **7** and **8**. Here (Scheme 5), a Suzuki coupling reaction between bromide **12** and (2-hydroxy-3-methoxyphenyl)boronic acid **21** provides ester **22** in 61% yield. Hydrolysis of the ester proceeded in 84% yield to acid **23**. A HATU-mediated amide coupling with amine **20** generated hinduchelin C (**9**) in 44% yield, whereas amine **24** delivered hinduchelin D (**10**) in 71% yield for overall yields of 5.4% and 8.7%, respectively, for **9** and **10**. Hinduchelins B–D (**8–10**) were identical in all aspects to that of the reported natural products.^{7,12}

Prior to biological studies, we employed the chemistries developed herein and quickly prepared additional unnatural analogues of **25** and **26** to probe SAR (Figure 3), following the synthetic routes previously described.¹²

Hinduchelin D (**10**) was previously reported to moderately induce iron-dependent fluorescence in *Pseudomonas aeruginosa*, an activity which was attributed to iron chelation by the compound.⁷ Under iron restriction, *P. aeruginosa* is known to secrete two fluorescent siderophores, pyoverdine (excitation 400 nm, emission 460 nm)¹³ and pyochelin (excitation 355 nm, emission 440 nm),¹⁴ which serve to bind iron for subsequent capture by the bacteria. Perturbations to iron homeostasis in *P. aeruginosa* can alter the production of these siderophores and thus the detectable fluorescence serves as a proxy for assessing iron restriction.¹⁵ We repeated these fluorescence assays but did not observe a reproducible increase in fluorescence in the presence of these compounds and, if anything, observed a trend toward decreased overall fluorescence (see the Supporting Information).¹² The inconsistencies in these results may be attributable to small differences in the availability of free iron between assays, as the regulation of siderophore production in *P. aeruginosa* is highly sensitive to iron and often multifactorial.¹⁵

To further investigate the role of hinduchelins in iron acquisition by *P. aeruginosa*, we performed agar plate bioassays whereby siderophore-deficient *P. aeruginosa*¹⁶ were supplied with hinduchelin A and its derivatives as a sole iron source. If *P. aeruginosa* is capable of utilizing hinduchelins directly, we would expect to see growth in the presence of these compounds. No growth was observed in the presence of any of the compounds tested,

suggesting that *P. aeruginosa* is not capable of utilizing hinduchelin or its derivatives as xenosiderophores (Figure 4).¹²

Given the aforementioned results, we went on to directly assess whether hinduchelin and its derivatives are capable of chelating iron. To this end, we assessed the ability of the products to mobilize iron from the colorimetric iron-binding dye, Chrome Azurol S (CAS). CAS assays are commonly employed to assess total siderophore activity, regardless of the iron-coordinating moiety. Using a modified version of the universal CAS assay (Figure 5),^{17,18} we found none of the compounds to be CAS active, suggesting that they do not function as strong iron chelators. We confirmed the aforementioned results through UV-vis spectroscopy, where none of the compounds exhibited the characteristic spectra for Fe(III)-ligand interaction, or the hallmark color change of a catechol substrate from colorless to brown upon iron-binding. Altogether, our findings suggest that it is unlikely the predominant biological function of hinduchelin involves iron acquisition.¹²

In conclusion, we have completed the first total synthesis of hinduchelins A–D (7–10) and reassigned the stereochemistry of hinduchelin A from (*S*) to (*R*). The facile synthetic route also allowed for the preparation of several unnatural analogues. Biological evaluation confirmed that all of the natural and unnatural hinduchelins were only weak iron chelators (siderophores). Although this may limit the utility of hinduchelins as antibacterial agents, further investigation into biofilm inhibition and biofilm eradication is warranted and will be forthcoming. The druglike properties of the hinduchelins, coupled with their lack of cytotoxicity, positions them as intriguing potential lead compounds in other therapeutic fields. Further biological evaluation across large panel screens (e.g., estrogen receptor modulation) is underway, and results will be reported in due course.

EXPERIMENTAL SECTION

Evaluation of Siderophore Activity.

The siderophoric activity of the synthesized compounds was determined using a previously published method.^{12,19}

Agar Plate Bioassays.

Iron-starved siderophore-deficient *P. aeruginosa* PA01 were seeded into Tris minimal succinate (TMS) agar plates supplemented with 20 μ M of the nonmetabolizable iron chelator ethylenediamine-*N,N'*-bis(2-hydroxyphenylacetic acid) (EDDHA). Sterile paper disks were preloaded with the iron substrate indicated (10 μ L of 10 mM stock, unless otherwise indicated) and placed on the agar plates. Growth promotion of the bacteria by the sole iron source was assessed by measuring the growth diameter about the disk at 24 h. Apo- indicates siderophores were supplied in iron-free form, and holo- indicates siderophores were preloaded with one-third the saturating concentration of iron (~3.3 mM). Holo-DHBA, holo-DFO, and holo-coelichelin were all provided as positive controls, while ddH₂O was provided as a negative control. FeCl₃ (3 mM) is a comparator for the iron-loaded siderophores. The limit of detection (LOD) for this assay is set at 6 mm, which is the diameter of the sterile paper disks. Statistical differences are given relative growth

promotion by ddH₂O. Data are representative of three independent experiments with three replicates each.

General Methods.

All reactions were carried out employing standard chemical techniques under inert atmosphere. Solvents used for extraction, washing, and chromatography were HPLC grade. All reagents were purchased from commercial sources and were used without further purification. Analytical HPLC was performed on an Agilent 1200 LCMS with UV detection at 215 nm along with ELSD detection and electrospray ionization, with all final compounds showing >95% purity and a parent mass ion consistent with the desired structure. All NMR spectra were recorded on a 400 MHz Bruker AV-400 instrument. ¹H chemical shifts are reported as δ values in ppm relative to the residual solvent peak (CDCl₃ = 7.26). Data are reported as follows: chemical shift, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, m = multiplet), coupling constant (Hz), and integration. ¹³C chemical shifts are reported as δ values in ppm relative to the residual solvent peak (CDCl₃ = 77.16). High-resolution mass spectra were obtained on an Agilent 6540 UHD Q-TOF with ESI source. Automated flash column chromatography was performed on an Teledyne ISCO Combi-Flash system. Melting points were recorded on an OptiMelt automated melting point system by Stanford Research Systems.

Methyl 2-Bromo-5-methyl-oxazole-4-carboxylate (13).—To a round-bottom flask were added methyl 5-methyloxazole-4-carboxylate (3.54 mmol) and THF (17.7 mL). The mixture was cooled to –78 °C, and then lithium bis(trimethylsilyl)amide (5.32 mmol) was added dropwise. The mixture was allowed to stir at –78 °C for 15 min, and then bromine (7.03 mmol) was added dropwise. The mixture was allowed to stir for 1 h at –78 °C. Upon completion, as determined by LCMS, the mixture was allowed to warm to rt, and the reaction was quenched with satd NaS₂O₃, diluted with brine, and extracted with DCM. The organics were dried with Na₂SO₄ and then filtered and concentrated to dryness. The sample was purified via Teledyne ISCO Combi-Flash system (solid loading, 40G column, 0–30% EtOAc, 25 min run) to afford 190.6 mg (25% yield) of the desired product as an off-white solid. ¹H NMR (400 MHz, CDCl₃): δ 3.90 (s, 3H), 2.63 (s, 3H). ¹³C{¹H} NMR (100 MHz, CDCl₃): 161.6, 160.1, 132.0, 130.0, 52.3, 12.1. LCMS: t_R = 0.623 min (>98%); m/z = 222.0 [M + H]⁺. HRMS (TOF, ES⁺): C₆H₇BrNO₃ [M + H]⁺ calcd mass 219.9609, found 219.9604. Mp: 78.4–81.1 °C.

General Procedure for Suzuki Coupling.

To a round-bottom flask were added boronic acid (0.30 mmol), methyl 2-bromo-5-methyl-oxazole-4-carboxylate (0.27 mmol), tetrakis(triphenylphosphine)palladium(0) (0.03 mmol), and potassium carbonate, 0.33 mmol in 1,4-dioxane (3.4 mL) and water (1.1 mL). The reaction flask was purged with nitrogen for 5 min, and then the reaction was stirred for 4 h at 90 °C. Upon completion, as determined by LCMS, the reaction was cooled to rt, and then solvent was removed in vacuo. The crude solid was taken up in DCM, syringe filtered and then purified via Teledyne ISCO Combi-Flash system (solid loading, 40G column, 10–60% EtOAc, 25 min run) to afford pure products.

Methyl 2-(2,3-Dimethoxyphenyl)-5-methyloxazole-4-carboxylate (15).—

Compound **15** (262.7 mg, 88% yield) was obtained as a colorless amorphous solid. ^1H NMR (400 MHz, CDCl_3): δ 7.53 (dd, J 7.9, 1.5 Hz, 1H), 7.09 (dd, J 7.9 Hz, 7.9 Hz, 1H), 6.99 (dd, J 7.9 Hz, 1.5 Hz, 1H), 3.90 (s, 3H), 3.90 (s, 3H), 3.87 (s, 3H), 2.69 (s, 3H). $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3): δ 163.0, 158.3, 156.6, 153.6, 147.9, 128.2, 124.2, 121.9, 121.4, 114.8, 61.4, 56.1, 51.9, 12.2. LCMS: t_R = 0.815 min (>98%); m/z 278.4 $[\text{M} + \text{H}]^+$. HRMS (TOF, ES+): $\text{C}_{14}\text{H}_{16}\text{NO}_5$ $[\text{M} + \text{H}]^+$ calcd mass 278.1023, found 278.1026.

Methyl 2-(2-Hydroxy-3-methoxyphenyl)-5-methyloxazole-4-carboxylate (22).—

Compound **22** (61.6 mg, 61% yield) was obtained as a white solid. ^1H NMR (400 MHz, CDCl_3): δ 10.95 (s, 1H), 7.41 (dd, 8.0, 1.4 Hz, 1H), 6.98 (dd, J = 8.0, 1.4 Hz, 1H), 6.90 (dd, J = 8.0, 8.0 Hz, 1H), 3.93 (s, 3H), 3.92 (s, 3H), 2.72 (s, 3H). $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3): δ 162.3, 159.5, 15.2, 148.8, 147.7, 127.0, 119.3, 117.7, 114.6, 110.7, 56.4, 52.1, 12.1. LCMS: t_R = 1.068 min (>98%); m/z = 264.2 $[\text{M} + \text{H}]^+$. HRMS (TOF, ES+): $\text{C}_{13}\text{H}_{14}\text{NO}_5$ $[\text{M} + \text{H}]^+$ calcd mass 264.0872, found 264.0866. Mp: 158.7–162.0 °C.

General Procedure for Ester Hydrolysis.

To a mixture of the desired isoxazole ester (0.05 mmol) in THF (1.1 mL) was added 250 μL of 2 N NaOH. The reaction was brought to 50 °C and allowed to stir for 3 h. Upon completion of this reaction (as determined by LCMS; 5–95% MeCN in water), the solvent was partially evaporated. The aqueous mixture was diluted with water, neutralized with 2 N of HCl, and then extracted with 3:1 chloroform/2-propanol. The organics were passed through a phase separator and then concentrated to dryness to afford pure product.

2-(2,3-Dimethoxyphenyl)-5-methyloxazole-4-carboxylic Acid (16).—Compound **16** (13.1 mg, >99% yield) was obtained as a white solid. ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 7.38 (dd, J 7.1, 2.3 Hz, 1H), 7.25–7.18 (m, 2H), 3.86 (s, 3H), 3.80 (s, 3H), 2.64 (s, 3H). $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, $\text{DMSO}-d_6$): δ 163.6, 157.4, 156.4, 153.9, 147.6, 129.0, 125.1, 121.4, 121.3, 115.6, 61.3, 56.4, 12.4. LCMS: t_R = 0.768 min (>98%); m/z = 264.2 $[\text{M} + \text{H}]^+$. HRMS (TOF, ES+): $\text{C}_{13}\text{H}_{14}\text{NO}_5$ $[\text{M} + \text{H}]^+$ calcd mass 264.0866, found 264.0870. Mp: 143.6–145.5 °C.

2-(2-Hydroxy-3-methoxyphenyl)-5-methyloxazole-4-carboxylic Acid (23).—

Compound **23** (31.7 mg, 84% yield) was obtained as a white solid. ^1H NMR (400 MHz, CD_3OD): δ 7.43 (dd, J = 8.0, 1.1 Hz, 1H), 7.92 (s, 1H), 7.10 (dd, 8.0, 1.1 Hz, 1H), 6.94 (dd, J = 8.0, 8.0 Hz, 1H), 3.90 (s, 3H), 2.72 (s, 3H). $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CD_3OD): δ 164.8, 160.6, 156.7, 149.9, 148.3, 128.6, 120.7, 119.0, 115.9, 111.9, 56.7, 12.0. LCMS: t_R = 0.870 min (>98%); m/z = 250.2 $[\text{M} + \text{H}]^+$. HRMS (TOF, ES+): $\text{C}_{12}\text{H}_{12}\text{NO}_5$ $[\text{M} + \text{H}]^+$ calcd mass 250.0716, found 250.0709. Mp: 250.6–252.2 °C.

General Procedure for HATU Amide Coupling.

To a mixture of desired isoxazole carboxylic acid (0.04 mmol) in DMF (1 mL) was added DIPEA (0.08 mmol). After 10 min, HATU (0.04 mmol) was added, and the reaction was allowed to stir for an additional 30 min. After this time, the desired amine (0.05 mmol) was added, and the reaction was allowed to stir for 3 h. Upon completion of this reaction (as

determined by LCMS; 5–95% MeCN in water), the reaction contents were purified using Gilson semipreparative HPLC (Basic, 30 × 100 mm column, 15–65% ACN/0.05% aqueous ammonium hydroxide, 12 min run). Fractions containing product were dried to afford pure amide products.

(S)-2-(2,3-Dimethoxyphenyl)-N-(2-hydroxy-2-phenylethyl)-5-methyloxazole-4-carboxamide (7).—Compound **7** (10.6 mg, 73% yield) was obtained as a colorless amorphous solid. ¹H NMR (400 MHz, CDCl₃): δ 7.58 (t, *J* = 5.8 Hz, 1H), 7.45–7.41 (m, 3H), 7.38–7.33 (m, 2H), 7.30–7.27 (m, 1H), 7.12 (dd, *J* = 8.1, 8.1 Hz, 1H), 7.02 (dd, *J* = 8.1, 1.7 Hz, 1H), 4.95 (ddd, *J* = 8.0, 2.5, 2.5 Hz, 1H), 3.90 (s, 3H), 3.88 (s, 3H), 3.85–3.77 (m, 2H), 3.53 (ddd, *J* = 14.0, 8.1, 5.4 Hz, 1H), 2.73 (s, 3H). ¹³C{¹H} NMR (100 MHz, CDCl₃): δ 163.6, 156.9, 153.9, 153.4, 147.9, 142.1, 130.0, 128.6, 127.9, 126.0, 124.5, 121.5, 121.3, 114.7, 74.2, 61.4, 56.2, 47.5, 12.0. LCMS: *t*_R = 1.105 min (>98%); *m/z* 383.2 [M + H]⁺. HRMS (TOF, ES⁺): C₂₁H₂₃N₂O₅ [M + H]⁺ calcd mass 383.1601, found 383.1602. Specific rotation [α]_D²³ = +9.8 (*c* = 0.1, MeOH).

(R)-2-(2,3-Dimethoxyphenyl)-N-(2-hydroxy-2-phenylethyl)-5-methyloxazole-4-carboxamide (19).—Compound **19** (59.8 mg, 82% yield) was obtained as a colorless amorphous solid. ¹H NMR (400 MHz, CDCl₃): δ 7.71 (t, *J* = 5.9 Hz, 1H), 7.42–7.37 (m, 3H), 7.33–7.28 (m, 2H), 7.26–7.22 (m, 1H), 7.07 (dd, *J* = 8.0, 8.0 Hz, 1H), 6.98 (dd, *J* = 8.3, 1.4 Hz, 1H), 4.93 (dd, *J* = 8.2, 2.6 Hz, 1H), 4.29 (br s, 1H), 3.87 (s, 3H), 3.85 (s, 3H), 3.83–3.78 (m, 1H), 3.47 (ddd, *J* = 14.1, 8.5, 5.4 Hz, 1H), 2.69 (s, 3H). ¹³C{¹H} NMR (100 MHz, CDCl₃): δ 163.2, 156.7, 153.8, 153.2, 147.7, 142.2, 130.0, 128.4, 127.7, 125.9, 124.4, 121.3, 121.1, 114.6, 73.7, 61.2, 56.1, 47.2, 11.8. LCMS: *t*_R = 1.102 min (>98%); *m/z* = 383.2 [M + H]⁺. HRMS (TOF, ES⁺): C₂₁H₂₃N₂O₅ [M + H]⁺ calcd mass 383.1601, found 383.1602. Specific rotation [α]_D²³ = –11.9 (*c* = 0.1, MeOH).

2-(2,3-Dimethoxyphenyl)-N-(2-hydroxyphenethyl)-5-methyloxazole-4-carboxamide (8).—Compound **8** (10.9 mg, 75% yield) was obtained as a colorless amorphous solid. ¹H NMR (400 MHz, CDCl₃): δ 7.96 (br.s, 1H), 7.67 (t, *J* = 5.8 Hz, 1H), 7.47 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.17–7.07 (m, 3H), 7.04 (dd, *J* = 8.2, 1.5 Hz, 1H), 6.90 (dd, *J* = 8.0, 0.9 Hz, 1H), 6.81 (ddd, *J* = 7.4, 1.2, 1.2 Hz, 1H), 3.92 (s, 3H), 3.92 (s, 3H), 3.60–3.53 (m, 2H), 2.99–2.94 (m, 2H), 2.74 (s, 3H). ¹³C{¹H} NMR (100 MHz, CDCl₃): δ 163.6, 156.9, 155.4, 153.9, 153.4, 147.9, 130.5, 130.0, 128.3, 124.9, 124.6, 121.4, 121.3, 120.1, 116.5, 114.7, 61.4, 56.2, 40.2, 31.1, 12.0. LCMS: *t*_R = 1.155 min (>98%); *m/z* 383.2 [M + H]⁺. HRMS (TOF, ES⁺): C₂₁H₂₃N₂O₅ [M + H]⁺ calcd mass 383.1601, found 383.1601.

2-(2-Hydroxy-3-methoxyphenyl)-N-(2-hydroxyphenethyl)-5-methyloxazole-4-carboxamide (9).—Compound **9** (10.5 mg, 71% yield) was obtained as an off-white solid. ¹H NMR (400 MHz, (CD₃)₂SO): δ 10.31 (s, 1H), 9.39 (s, 1H), 8.83 (t, *J* = 5.8 Hz, 1H), 7.38 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.13 (dd, *J* = 8.1, 1.4 Hz, 1H), 7.08 (dd, *J* = 7.4, 1.6 Hz, 1H), 7.02 (td, *J* = 7.7, 1.6 Hz, 1H), 6.94 (dd, *J* = 8.0, 8.0 Hz, 1H), 6.80 (dd, *J* = 8.0, 1.0 Hz, 1H), 6.72 (td, *J* = 7.4, 1.0 Hz, 1H), 3.84 (s, 3H), 3.42 (m, 2H), 2.79 (t, *J* = 8.0 Hz, 2H), 2.67 (s, 3H). ¹³C{¹H} NMR (100 MHz, (CD₃)₂SO): 160.4, 157.3, 15.4, 151.6, 148.4, 146.3, 130.2, 128.8, 127.3, 125.5, 119.4, 119.0, 117.7, 115.0, 114.9, 110.5, 56.0, 30.2, 11.3. LCMS: *t*_R =

0.949 min (>98%); m/z = 369.3 $[M + H]^+$. HRMS (TOF, ES+): $C_{20}H_{21}N_2O_5$ $[M + H]^+$ calcd mass 369.1451, found 369.1445. Mp: 235.3–237.5 °C.

2-(2-Hydroxy-3-methoxyphenyl)-5-methyl-N-phenethylloxazole-4-carboxamide (10).—Compound **10** (6.2 mg, 44% yield) was obtained as an off-white solid. 1H NMR (400 MHz, $CDCl_3$): δ 10.33 (s, 1H), 7.42 (dd, J = 7.9, 1.5 Hz, 1H), 7.37–7.31 (m, 2H), 7.25–7.22 (m, 2H), 6.99 (dd, 8.1, 1.5 Hz, 1H), 6.92 (t, J = 8.0 Hz, 1H), 6.73 (t, J = 5.3 Hz, 1H), 3.94 (s, 3H), 3.68 (td, J = 7.0, 6.2 Hz, 2H), 2.92 (t, J = 7.0 Hz, 2H), 2.75 (s, 3H). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): 161.1, 158.6, 152.4, 148.7, 147.1, 138.6, 128.9, 128.8, 126.9, 119.7, 118.0, 114.5, 110.9, 56.4, 40.5, 36.0, 11.8. LCMS: t_R = 1.007 min (>98%); m/z = 353.3 $[M + H]^+$. HRMS (TOF, ES+): $C_{20}H_{21}N_2O_4$ $[M + H]^+$ calcd mass 353.1501, found 353.1496. Mp: 119.1–121.7 °C.

2-(2,3-Dimethoxyphenyl)-5-methyl-N-phenethylloxazole-4-carboxamide (25).—Compound **25** (10.9 mg, 78% yield) was obtained as a colorless amorphous solid. 1H NMR (400 MHz, $CDCl_3$): δ 7.37 (dd, J 7.9, 1.5 Hz, 1H), 7.26–7.22 (m, 2H), 7.20–7.13 (m, 3H), 7.10 (t, J 5.8 Hz, 1H), 7.05 (dd, J 7.9, 7.9 Hz, 1H), 6.94 (dd, J 7.9, 1.5 Hz, 1H), 3.83 (s, 3H), 3.79 (s, 3H), 3.61 (td, J 7.1, 5.8 Hz, 2H), 2.85 (t, J 7.1 Hz, 2H), 2.66 (s, 3H). Note: TMS referenced at 0.00 ppm due to $CDCl_3$ overlap. $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ 162.2, 156.7, 153.9, 152.9, 147.9, 139.1, 130.3, 128.9, 128.7, 126.6, 124.4, 121.7, 121.3, 114.6, 61.3, 56.2, 40.3, 36.2, 11.9. LCMS: t_R = 1.275 min (>98%); m/z 367.2 $[M + H]^+$. HRMS (TOF, ES+): $C_{21}H_{23}N_2O_4$ $[M + H]^+$ calcd mass 367.1652, found 367.1656.

(S)-N-(2-Hydroxy-2-phenylethyl)-2-(2-hydroxy-3-methoxyphenyl)-5-methyloxazole-4-carboxamide (26).—Compound **26** (6.9 mg, 58% yield) was obtained as an off-white amorphous solid. 1H NMR (400 MHz, $CDCl_3$): δ 10.41 (s, 1H), 7.44–7.33 (m, 5H), 7.37–7.31 (m, 2H), 7.32–7.27 (m, 1H), 7.24–7.18 (m, 1H), 6.97 (dd, J = 8.0, 1.2 Hz, 1H), 6.73 (dd, J = 8.0, 8.0 Hz, 1H), 4.97 (dd, J = 7.2, 2.2 Hz, 1H), 3.92 (s, 3H), 3.89–3.81 (m, 1H), 3.56–3.46 (m, 1H), 3.39 (br s, 1H), 2.99 (s, 3H) 1.77 (br s, 1H). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ 162.2, 158.6, 152.7, 148.6, 147.0, 141.8, 128.7, 128.1, 125.9, 119.7, 117.9, 114.4, 110.8, 73.7, 56.3, 47.2, 11.8. LCMS: t_R = 0.902 min (>98%); m/z = 369.2 $[M + H]^+$. HRMS (TOF, ES+): $C_{20}H_{21}N_2O_5$ $[M + H]^+$ calcd mass 369.1451, found 369.1447. Specific rotation $[\alpha]_D^{23}$ = + 61.3 (c = 0.1, MeOH).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Recently synthesized heterocyclic natural products **1–6**, which were reported to possess unique biological activity and/or required stereochemical reassignment after synthesis.^{1–6}

Figure 2.
Structures and numbering convention for hinduchelins A-D (**7–10**).

Figure 3. Structures of unnatural hinduchelins **25** and **26** for biological evaluation along with natural hinduchelins A–D (**7–10**).

Figure 4.
Agar plate bioassays assessing the ability of hinduchelin and its derivatives to serve as a sole iron source to *Pseudomonas aeruginosa*.

Figure 5.
Chrome Azurol S (CAS) assay for the detection of iron chelation by hinduchelin derivatives.
Data are expressed as raw absorbance values (A) or as percent activity of 1 mM DFO (B).

Scheme 1.
Retrosynthetic Route for the Hinduchelins A–D (7–10)

Scheme 2.

Synthesis of the Reported Structure of Hinduchelin A (7)

Scheme 3.

Synthesis of the (*R*)-Enantiomer 19 of Hinduchelin A (7) and Stereochemical Reassignment

Scheme 4.
Synthesis of Hinduchelin B (8)

Scheme 5.
Synthesis of Hinduchelins C and D (9 and 10)