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An efficient synthesis and bioactivity evaluation of oxazole-containing natural hinduchelins A–D and their derivatives†

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Oxazoles are an important class of biologically active metabolites from nature, and exhibit broad biological activities as the lead for drug discovery. Hinduchelins are a class of unusual natural products with an oxazole unit, isolated from *Streptoalloteichus hindustanus*, and with a potential iron-chelating ability. These compounds are the first identified naturally occurring unusual oxazole derivatives to possess a catechol unit. However, some of these compounds are not abundant in nature, and thus, the efficient syntheses of these compounds are advantageous in exploring their potential applications. This paper reports the efficient synthesis and bio-evaluation of hinduchelins A–D and their derivatives with convenient procedures and high yields.

Introduction

Natural products have been widely used as lead molecules for the discovery of novel drugs[1–3] and agrochemicals[4–7] over the past century, and they possess enormous structural and chemical diversity that can afford an opportunity to explore novel candidates with different mechanisms of action and unique biological properties from the existing agents.

In particular, these small molecules of natural products containing heterocyclic rings have attracted a great deal of attention due to their extensive biological properties.[8] Among them, oxazole skeletons with five-membered heterocycles arouse many researchers' interest,[9,10] and many oxazole-type natural products[11–28] have been elucidated as potential antibacterial, antifungal, anticancer, antiviral, and antioxidation agents, monoamine oxidase inhibitors *etc.* Indolyl-oxazole derivatives[11–18] are an important class of oxazole-type natural products, and these natural compounds and their derivatives have been demonstrated to exhibit broad biological and pharmaceutical activities. 2,5-Disubstituted oxazole alkaloids[19–23] are another kind of oxazole natural product with special structures, and isolated from different plants such as *Amyris texana etc.* These diaryloxazole alkaloids show broad bioactivity such as antimycobacterial activity, anticancer activity *etc.* Meanwhile, several of the novel oxazole-amide derivatives (Fig. 1) have also been isolated and characterized as potential antitumor, anti-tuberculosis, and anti-proliferative agents.[24–27] Very recently, several unusual aryl-oxazole alkaloids have also been isolated from *Streptoalloteichus hindustanus* by Abe *et al.*,[28] and these compounds (hinduchelins A–D in Fig. 1) have a special structural moiety similar to the unit in amamistatin. The test of iron-binding properties of these natural products was performed, and some molecules showed moderate ability to induce pyoverdine production at 50 μM.

Besides the aforementioned natural oxazole alkaloids, many synthetic methods for various oxazole derivatives have been explored,[14,18,29,30] and many synthetic derivatives have also been investigated and developed as potential drugs or agrochemicals,[31–35] and therefore the unique structure and important bioactivity of oxazole heterocyclic derivatives have generated significant interest in the total synthesis of such compounds. Thus, we intend to develop an effective synthetic strategy for the study of hinduchelins A–D and their derivatives. Afterwards, process development for the novel hinduchelin analogues with potential pharmacological or agroactivities involving structural optimization and bioassay screening helps us to explore the mechanism of action for the biological activity of hinduchelin derivatives. Herein, we report the first total synthesis of hinduchelins A–D and their derivatives, and the *in vitro* cytotoxicity, and antibacterial, fungicidal and insecticidal activities of these compounds have also been evaluated.

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†Electronic supplementary information (ESI) available: The synthetic methods for target compounds, and ^{1}H NMR, ^{13}C NMR and ESI-MS spectra for all the intermediates and target compounds. See DOI: 10.1039/c9ob00352e

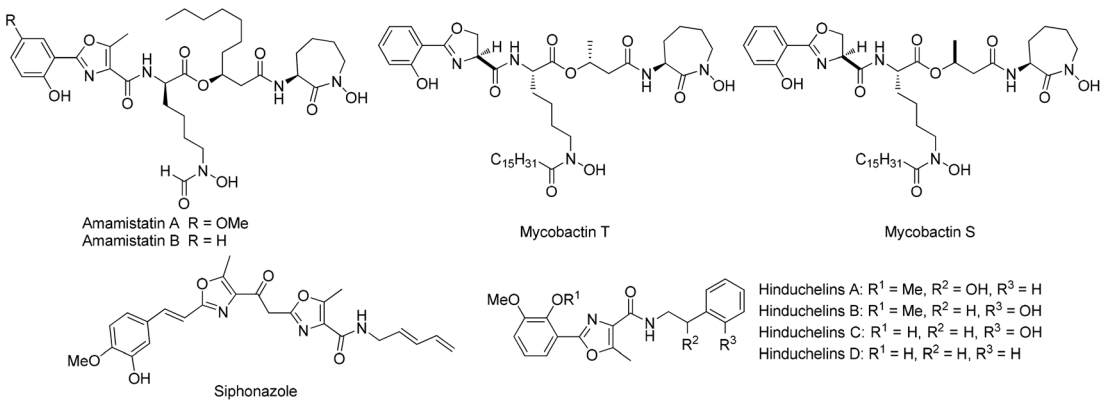


Fig. 1 Biologically active naturally occurring oxazole-amide alkaloids.

Results and discussion

Retrosynthetic analysis

The retrosynthetic analysis for hinduchelins A–D is described in Scheme 1. From Scheme 1, we can find that these hinduchelins A–D can be divided into two parts including carboxylic acid and substituted phenethylamine. This relies on an initial disconnection of the amide bond to give the corresponding oxazole-containing carboxylic acid and substituted phenethylamine. The synthesis of the oxazole-containing carboxylic acid could be processed from aldehydes (*o*-veratraldehyde and *o*-vanillin) and ethyl 2-(hydroxyimino)-3-oxobutanoate *via* the heterocyclization reaction.

General synthesis of hinduchelins A–D

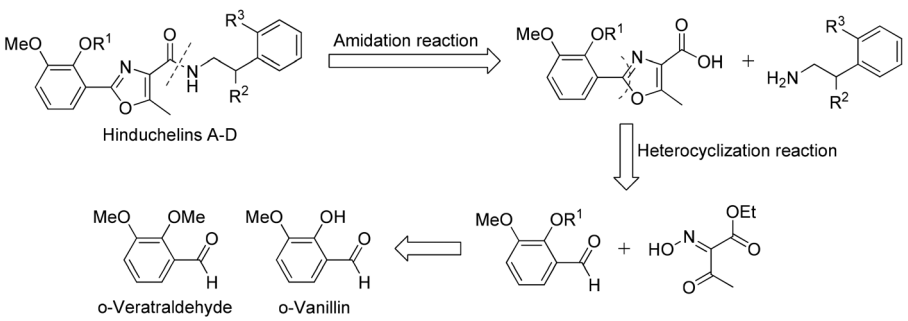
According to the retrosynthetic analysis, the following total synthesis procedures for hinduchelins A–D were provided and investigated (Scheme 2).

Synthesis of oxazole-containing carboxylic acid fragments

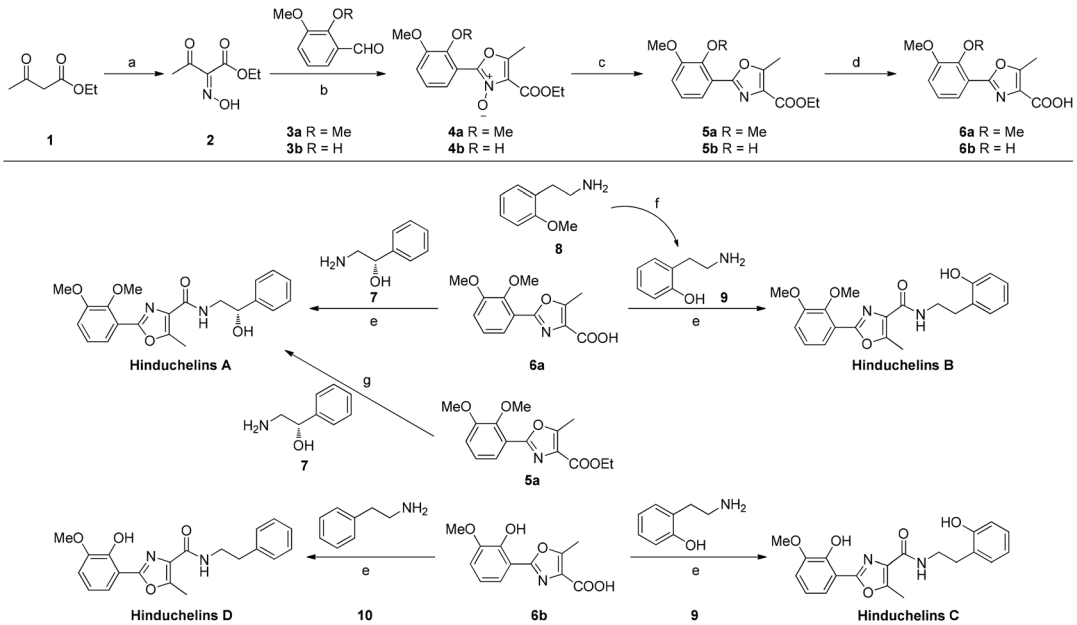
According to the retrosynthetic analysis, the substituted oxazole-containing carboxylic acids are the key molecular fragments, and can be prepared from the commonly available reagent ethyl acetoacetate. The ethyl acetoacetate **1** was transformed into ethyl 2-(hydroxyimino)-3-oxobutanoate **2** when treated with sodium nitrite in the presence of acetic acid. Then the obtained intermediate **2** was treated with the corresponding *o*-veratraldehyde or *o*-vanillin *via* a heterocyclization reaction[29] to afford the heterocyclic intermediates **4a–b**, which were reduced with zinc powder in the presence of acetic acid to obtain the key intermediates aryl-oxazole carboxylates **5a–b**. After this, the intermediates aryl-oxazole carboxylic acids **6a–b** were conveniently obtained by the hydrolysis of the corresponding aryl-oxazole carboxylates **5a–b** in the presence of sodium hydroxide, and the corresponding spectra for these intermediates **5a–b** and **6a–b** are described in the ESI.†

Synthesis of hinduchelins A and B

For the synthesis of hinduchelin A, the corresponding (*S*)-2-amino-1-phenylethanol (**7**) and intermediate **5a** were first used to explore the transformation. The corresponding aryl-oxazole **5a** was treated with an amine (**7**) in refluxing ethanol to give the target hinduchelin A in 18% isolated yield. The direct coupling reaction between aryl-oxazole **6a** and amine **7** was also investigated using a coupling reagent. Fortunately, hinduchelin A was produced in 64% yield (isolated yield). In addition, further UPLC-MS analyses also confirmed that the compounds obtained *via* these two methods are consistent. With 2-(2-methoxyphenyl)ethanamine **8** being available, the required 2-(2-aminoethyl)phenol **9** was synthesized by demethylation using HBr in the presence of acetic acid (Scheme 2). Hinduchelin B was prepared using a similar coupling reaction to that of hinduchelin A with a yield of 65%.



Scheme 1 Retrosynthetic analysis for hinduchelins A–D.



Scheme 2 Synthesis of hinduchelins A–D. Reagents and conditions: (a) $NaNO_2$, AcOH, H_2O, 0–5 °C, 1.5 h, 95%; (b) HCl(g), AcOH, 5–10 °C, 2–3 h; (c) Zn, AcOH, 45–50 °C, 3–4 h, 88% for **5a**, and 82% for **5b**; (d) NaOH, MeOH, H_2O, rt, overnight, 83% for **6a**, and 76% for **6b**; (e) HOBt, EDCI, Et_3N, DMF, rt, 20–45 h, 64–82%; (f) HBr, AcOH, reflux, 4 h; and (g) EtOH, reflux, 42 h, 18%.

Synthesis of hinduchelins C and D

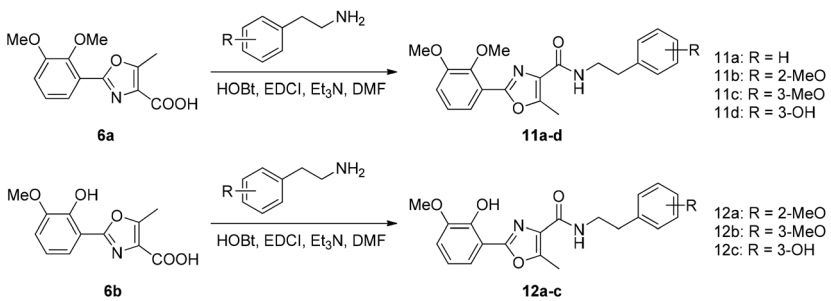
Based on the aforementioned investigation of the synthesis of hinduchelins A and B, and with the intermediate **6b** in hand, the different substituted 2-phenylethanamines **9** or **10** can be efficiently transformed into the corresponding target hinduchelins C and D with the yields of 74% and 82%, respectively.

Synthesis of hinduchelin A–D derivatives

In order to investigate the possible structure–activity relationships, some derivatives of natural hinduchelins A–D have been prepared according to the aforementioned method, and the general synthetic route is described in Scheme 3.

Spectroscopy studies

All the structures of target compounds were demonstrated by their ^{1}H NMR, ^{13}C NMR and mass spectroscopy, and all these spectral data were in good agreement with the proposed structures. For ^{1}H NMR studies of hinduchelin A, the typical signal for the proton of CH attached OH was resonated as a triplet at 4.89 ppm, and the two sets of signals that emerged in their ^{1}H NMR spectrum in the ranges 3.76–3.72 and 3.50–3.46 ppm were assigned to the protons of the methylene group linked to the amino group. In addition, the signal peaks for three methyl groups are very obvious, and the signals at lower fields in the corresponding ^{1}H NMR spectrum were attributed to the NH and aromatic protons. In the original spectra, hinduchelins B–D has similar spectral characteristics to those of hinduchelin A. The ^{13}C NMR spectra of compounds hinduchelins A–D display obvious peaks in the alkyl region indicating the presence of the methyl, methylene and methine groups, and other peaks appearing at lower fields were assigned to the heterocyclic and aromatic moiety. The electrospray ionization mass spectra (ESI-MS) for hinduchelins A–D were measured on a WATERS ACQUITY UPLC® H-CLASS PDA (Waters®) instrument (Xevo TQD), and the ion peak or adduct ions of the synthesized



Scheme 3 Synthesis of hinduchelin A–D derivatives.

compounds were investigated. Experimentally, in the positive ion mode, the ESI-MS of these compounds exhibit the obvious molecular peaks of $[M + H]^+$ and $[M + Na]^+$ with relative abundance. All the characteristic peaks observed in the ^1H NMR and ^{13}C NMR spectra for hinduchelins A–D and their derivatives are described in the ESI,† and they are almost consistent with the reported data from ref. 28. In particular, the optical rotation for hinduchelin A was also determined on an Autopol IV (Serial #83376, Rudolph Research Analytical, USA), and the value is $[\alpha]_D^{25} = -11.0$ (*c* 0.1, MeOH), which demonstrated that the configuration of synthesized hinduchelin A is consistent with the reported structure.

Biological assays

With these compounds in hand, in order to explore their potential applications, all the *in vitro* cytotoxicity, and antibacterial, fungicidal and insecticidal activities have been evaluated. However, none of the compounds were cytotoxic toward the SGC-7901, A875, HepG2, and MARC cell lines at 40 μg mL^{-1}, and the antibacterial activities of these compounds were also very poor on the Gram-positive and negative organisms such as *Staphylococcus aureus* subsp. *aureus Rosenbach* ATCC25923, *Erysipelothrix rhusiopathiae* ATCC 19414, *Escherichia coli* ATCC25922, and *Pasteurella multocida* subsp. *multocida* ATCC 43137. In addition, their potential antifungal activities against seven kinds of plant pathogenic fungi commonly found in agricultural systems, including *Ralstonia solanacearum*, *Botrytis cinerea*, *Septoria nodorum*, *Alternaria solani*, *Fusarium culmorum*, *Rhizoctonia solani*, and *Uromyces fabae*, have also been evaluated, but all these compounds exhibited a low antifungal activity at 50 μg mL^{-1}. The insecticidal activity screening against *Helicoverpa armigera* and *Aphis craccivora Koch* indicated that these compounds did not show potential insecticidal activity at 100 μg mL^{-1}.

Conclusions

In summary, the first total syntheses of the natural products hinduchelins A–D and their derivatives were investigated, and an efficient approach involving five or six steps to access these molecules has been developed. The attempted synthetic route was achieved using commercially available materials, and the application of this synthetic methodology for the construction of new structural analogues is well under way.

Conflicts of interest

The authors declare no competing financial interest.

Acknowledgements

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