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Inhibitory effect of 4,4′-dihydroxy-α-truxillic acid derivatives on NO production in lipopolysaccharide-induced RAW 264.7 macrophages and exploration of structure–activity relationships

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ABSTRACT

The inhibitory activity of 4,4′-dihydroxy-α-truxillic acid and its derivatives (**5-1a**–**5-35a**) on nitric oxide (NO) release was evaluated in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages. Compounds **5-3a**, **5-4a**, **5-5a**, **5-10a**, **5-24a**, **5-26a** and **5-30a** exhibited significant inhibitory effects on NO production, with IC_{50} values of 19.8, 21.1, 16.4, 17.5, 20.8, 22.6 and 17.6 μM, respectively. Their cytotoxicities were also estimated using a CCK-8 assay. Among them, compound **5-10a** showed no cytotoxic effect on cells up to a concentration of 50 μM. The structure–activity relationships of the compounds are also discussed.

4,4′-Dihydroxy-α-truxillic acid was isolated from the cell walls of *Lolium multiflorum*.[1] Cyclobutane dimers derived from *p*-coumaric acid and ferulic acid in the cell walls of tropical grasses have been identified by Ford and Hartley.[2] These dimers can be formed by photodimerising *p*-coumaric and ferulic acid units in grass cell walls under the influence of sunlight[3] and may function as cross-linking units between cell wall macromolecules, lowering the biodegradability of cell wall polysaccharides and reducing their suitability for consumption by foraging species.[2] A novel monoterpene alkaloid, incarvillateine, which shares the same dimeric structure as that of α-truxillic acid, was isolated from the aerial parts of *Incaruillea sinensis*, commonly known by the Chinese name 'jiao hao', which has been used to treat rheumatism and relieve pain in Traditional Chinese Medicine.[4] Incarvillateine displays significant antinociceptive activity, comparable to that of morphine, in a formalin-induced pain model in mice.[5] However, the mechanism of action differs from that of morphine. 4,4′-Dihydroxy-α-truxillic acid exhibited significant anti-inflammatory activity in the formalin test, while its monomer component, (*E*)-*p*-coumaric acid, showed no activity.[6] This result indicates that the dimeric structure plays an important role in the expression of anti-inflammatory activity. In our study, 4,4′-dihydroxy-α-truxillic acid was found to inhibit NO production, while its monomeric components, compounds **1** and **2** (Scheme 1), showed no inhibitory activity (see the Supplementary data). This result also displayed that the dimeric structure was important in the expression of anti-NO activity.

Inflammation is the response of an organism to infestation by foreign bodies such as parasites, bacteria and viruses. The inflammatory response is an important protective reaction to injury, irritation and infection and is characterised by redness, heat, swelling, loss of function and pain.[7] The inflammatory response involves many categories of tissues and cells. The common modulators produced by many of these cells are eicosanoids, cytokines, reactive oxygen species and nitrogen intermediates.[8] The production of nitric oxide (NO), a short-lived radical gas with physiological or pathophysiological effects in almost every organ system, is catalysed by nitric oxide synthase (NOS).[9] Inducible NOS (iNOS) produces a high level of NO that modulates inflammation and plays an important role in the regulation of immune reactions. However, overproduction of NO by iNOS is involved in tissue destruction and immunological and inflammatory diseases, such as the nonspecific host defence, ischemia, reperfusion injury, chronic or acute inflammation, rheumatoid arthritis and onset of colitis.[9] Therefore, substances that inhibit NO release have potential therapeutic effects for inflammatory diseases.

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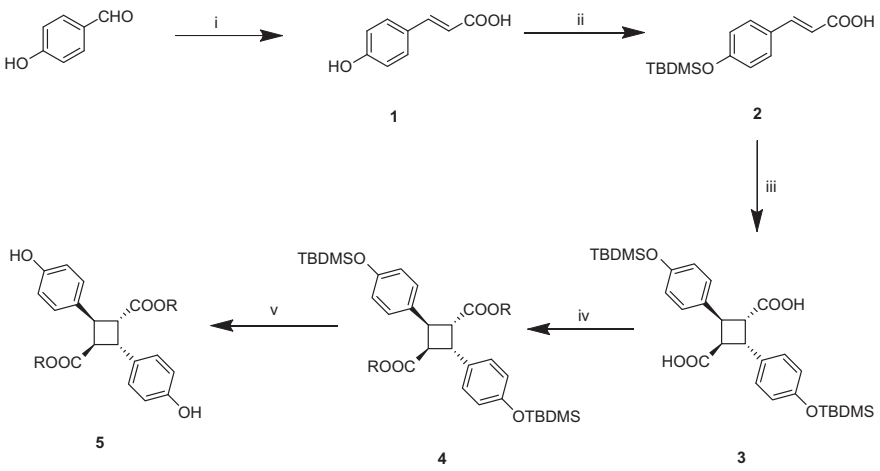

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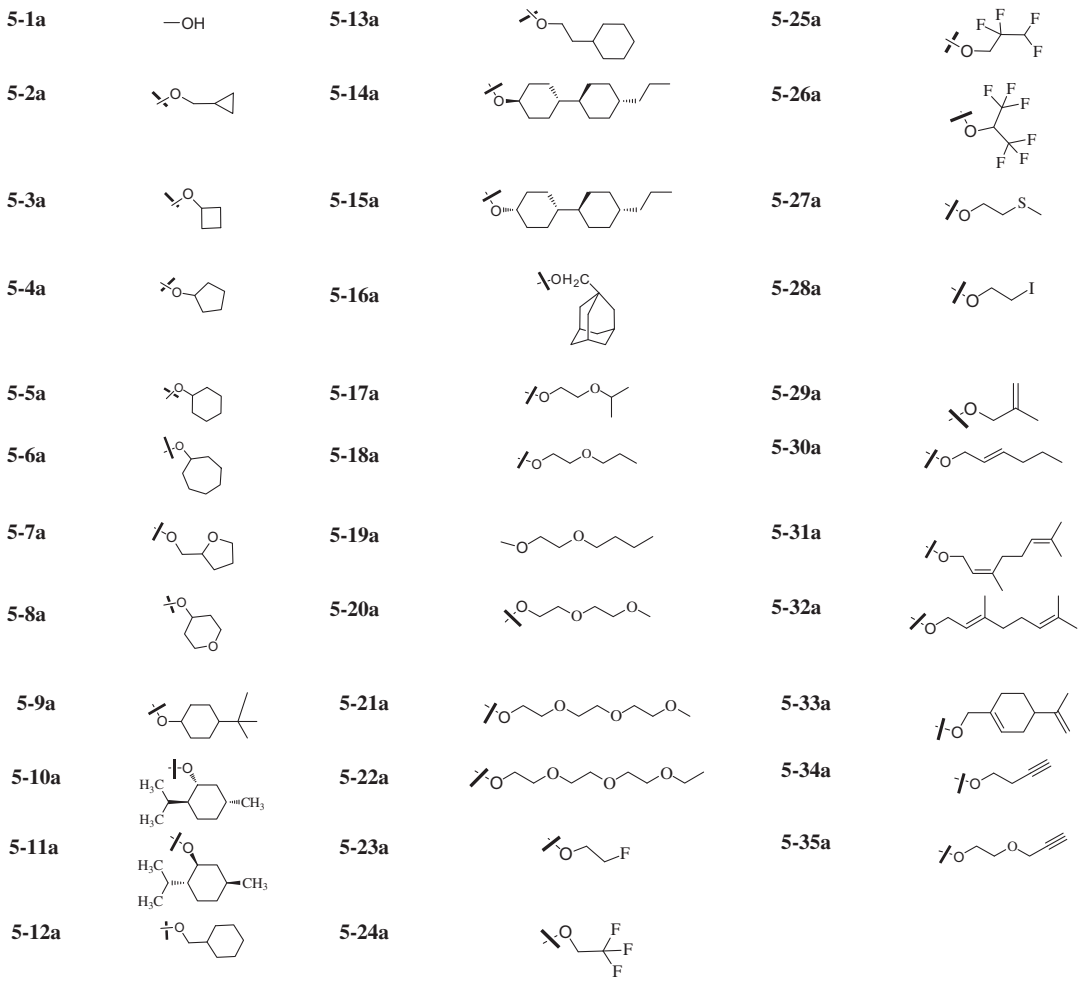

Scheme 1. General route for the synthesis of 4,4′-dihydroxy-α-truxillic acid and its derivatives. Reagents and conditions: (i) β-alanine, malonic acid, DBU, EtOH, 75%; (ii) TBDMS triflate, triethylamine, CH_2Cl_2, HCl, 85%; (iii) 400 W high-pressure mercury lamp, hexane, 70%; (iv) (a) EDCI, DMAP, CH_2Cl_2, 85%, (b) PPh_3, DIAD, THF, 70%; (v) tetrabutylammonium fluoride, AcOH, THF, 90%.

Although intense research efforts had been devoted to design and develop of NOS inhibitors, there were still lots of factors that hindered them to be the clinical candidates.[10] In order to search for more and more clinical requirements, it required the researchers to find more and novel NOS inhibitors. To the best of our knowledge, no existing literature focuses on the inhibitory effects of NO production by 4,4′-dihydroxy-α-truxillic acid and its derivatives. Furthermore, structural diversity is important for the