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Design, synthesis, and biological activities of madindoline analogues

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Abstract—A research program is under way to develop a series of madindoline-based inhibitors targeting interleukin 6. Such inhibitors will have potential use in fighting a variety of diseases for which no effective therapeutic drugs currently exist. Madindoline is no longer available from natural sources. Consequently, we have developed a purely synthetic route to ensure a supply of the compound. The synthesis of a range of analogues is described, all of which were evaluated for their inhibitory activity against the growth of IL-6-dependent 7TDI cells. From these assays, several synthetic madindoline analogues were identified as highly promising candidates for further development.

Interleukin 6 (IL-6) is a multifunctional cytokine involved in control of antibody production, T cell activation, hematopoiesis, and acute responses. Moreover, uncontrolled IL-6 activity is known to cause various serious diseases.[1] It has been reported that excess IL-6 production is closely associated with cancer cachexia,[2] Castleman's disease,[3] rheumatoid arthritis,[4] hypercalcemia,[5] and multiple myeloma.[6] No effective therapeutic drugs for these diseases have been developed, but our research shows that a low molecular weight compound could be developed for therapeutic use that modulates function of IL-6 via a new mode of action.

In 1996, we reported the isolation of the two novel indole alkaloids from a culture broth of *Streptomyces nitrosporeus* K93-0711, madindolines A (+)-**1** and B (+)-**2** (Fig. 1). Both are selective inhibitors of IL-6.[7,8] The biological activity profiles of (+)-**1** and (+)-**2** were exceptional. Both (+)-**1** and (+)-**2** specifically inhibited the growth of the IL-6-dependent MH60 cell line (IC_{50} values of 8 and 30 μM, respectively), but they did not affect the IL-6-independent MH60 cell line.

Detailed biological studies of the compound have showed that (+)-**1** competitively binds to gp130, but does not inhibit formation of the IL-6/IL-6R/gp130 complex.

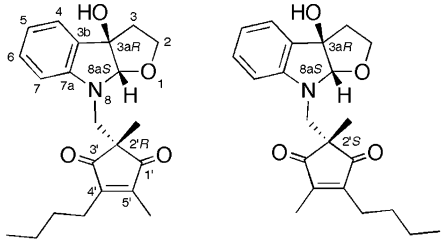

Figure 1. Structures of (+)-madindolines A ((+)-**1**) and B ((+)-**2**).

Furthermore, oral administration of (+)-**1** to ovariectomized (OVX) mice significantly suppressed the decrease in bone mass and increase in serum Ca^{2+} level after ovariectomy. This suppression mechanism was distinct from that of 17β-estradiol.[9]

Unfortunately, the scarcity of natural material has precluded further evaluation. Madindolines are no longer available from natural sources due to mutation of the originating bacterial strain.

Intrigued by the novel architecture, the significant IL-6 inhibitory activity, and the scarcity of these natural

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products, we devised a process which achieved their total synthesis in 2000.[10] Although the synthetic route defined for the first time their relative and absolute configurations, it did not produce enough material to allow extensive biological studies. We therefore developed a more practical (i.e., scalable) total synthesis to produce (+)-madindolines, thereby generating a source of material for further studies and biological development. Our initial approach to (+)-madindoline synthesis has been refined into a practical and effective route for total synthesis[11] (Scheme 1). The process has so far led to the generation of over 1 g of the synthetic natural product.

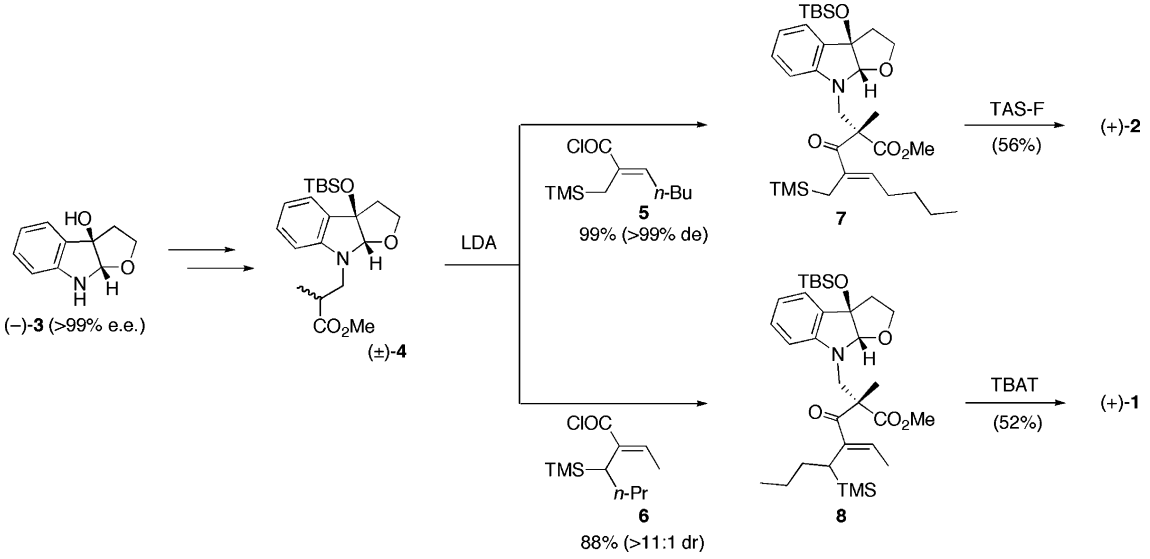
In parallel with our efforts to develop a reliable source of product for testing, we broadened our interest to include the production of analogues. This allowed us to investigate structure–activity relationship, as well as to identify more stable madindolines which retained their ability to inhibit IL-6.

Our strategy for synthesis of madindoline analogues focused on two distinct aspects: (1) length of the side chains of the cyclopentene ring and (2) functionalization of the 3a-hydroxyfuroindoline ring.

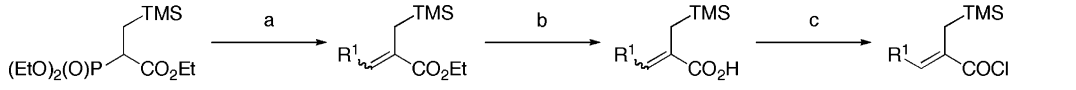
Access to the side-chain modified madindoline analogues was secured by modifying the alkyl group to the aldehyde, as depicted in Scheme 2. Thus, Wittig–Horner reaction[12] of the appropriate aldehyde with **9** produced moderate yields of the methyl allylsilane (**10a**), ethyl allylsilane (**10b**), heptyl allylsilane (**10c**), nonyl allylsilane (**10d**), undecyl allylsilane (**10e**), heneicosyl allylsilane (**10f**), and benzyl allylsilane (**10g**). All were used in the synthesis of analogues (**15a–g**). Although *Z*/*E* mixtures were obtained, these diastereomers generated (*Z*)-α,β-unsaturated acyl chlorides as a single isomer (**12a–g**). Finally, the desired (*Z*)-α,β-unsaturated acyl chlorides (**12a–g**) were incorporated into our efficient and shorter synthesis system to produce the side-chain modified analogues (**15a–g**) with sufficient quantities for in vitro studies.

The growth inhibition activity of the three series (two of them: vide infra) of analogues investigated was assayed against IL-6-dependent 7TDI cells and the bioactivity of the analogues compared against the natural compound. The degree of cell growth inhibition was measured in the presence of 10 μM of each of the tested analogues. The level of inhibition using 10 μM madindoline A was defined as 1.0. Tables 1–4 show the relative impact of the analogues compared with madindoline A.

It is interesting to note that while the shorter and the longest side chain analogues (Table 1, compounds **15a**,



Scheme 1. Summary of short total synthesis of (+)-**1** and (+)-**2**.



10a (R^1 = CH_3) (99%)
10b (R^1 = CH_3CH_2) (66%)
10c (R^1 = $CH_3(CH_2)_6$) (66%)
10d (R^1 = $CH_3(CH_2)_8$) (99%)
10e (R^1 = $CH_3(CH_2)_{10}$) (85%)
10f (R^1 = $CH_3(CH_2)_{20}$) (71%)
10g (R^1 = Bn) (57%)

11a (R^1 = CH_3) (99%)
11b (R^1 = CH_3CH_2) (99%)
11c (R^1 = $CH_3(CH_2)_6$) (99%)
11d (R^1 = $CH_3(CH_2)_8$) (58%)
11e (R^1 = $CH_3(CH_2)_{10}$) (85%)
11f (R^1 = $CH_3(CH_2)_{20}$) (31%)
11g (R^1 = Bn) (48%)

12a (R^1 = CH_3)
12b (R^1 = CH_3CH_2)
12c (R^1 = $CH_3(CH_2)_6$)
12d (R^1 = $CH_3(CH_2)_8$)
12e (R^1 = $CH_3(CH_2)_{10}$)
12f (R^1 = $CH_3(CH_2)_{20}$)
12g (R^1 = Bn)

Scheme 2. Reagents and conditions: (a) NaH, DME, 0 °C; (b) KOH, EtOH–H_2O, rt; (c) $SOCl_2$, Δ.

Table 1. Activities of the side-chain modified analogues

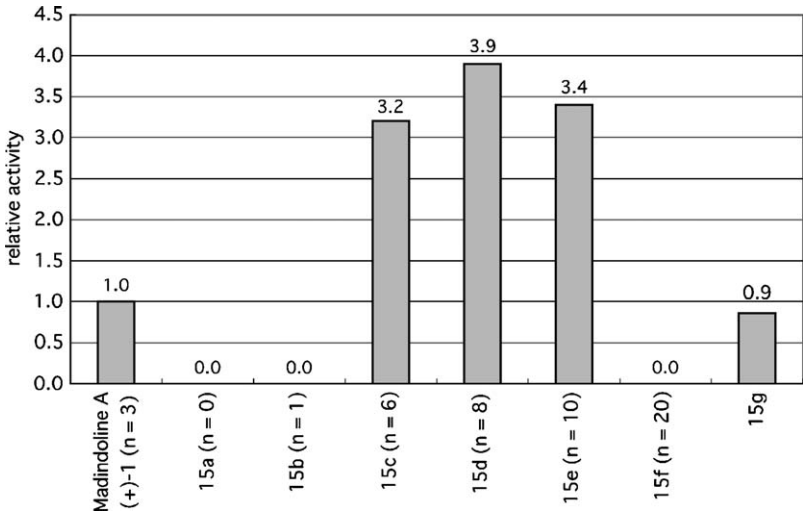


Table 2. Activities of the 5-halo sector analogues

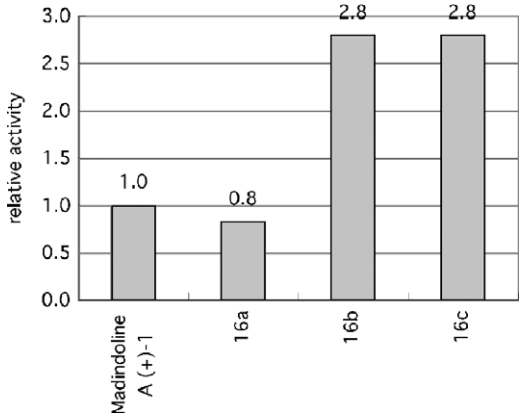
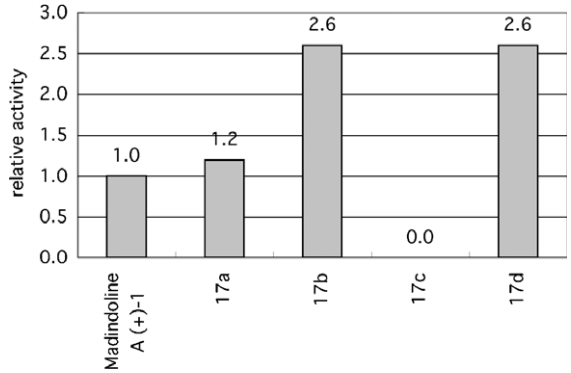
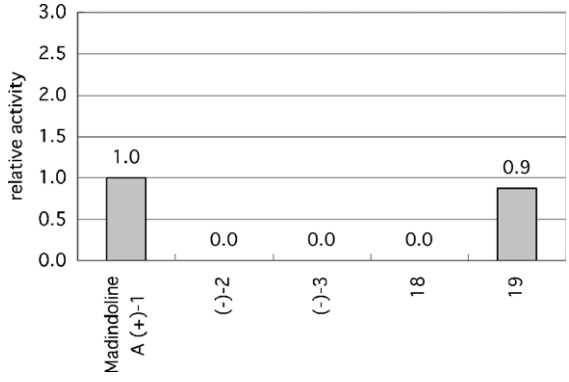


Table 3. Activities of the acyl sector analogues



15b, and **15f**) possess no activity as compared to madindoline A, the appropriately longer side-chain analogues (Table 1, compounds **15c**, **15d**, and **15e**) exhibited significantly greater potency.

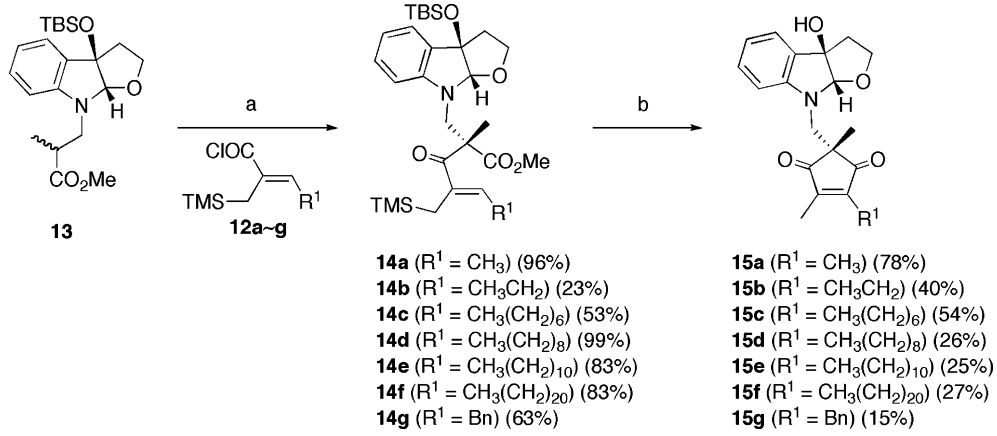
Table 4. Activities of madindoline analogues



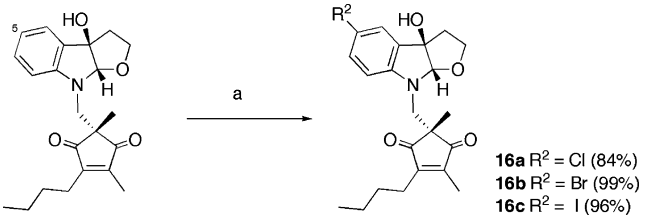
Given the structural information (Fig. 1), this seems to identify an oxidation at the 5-position. Indeed, it appears that (+)-**1** and (+)-**2** are easily metabolized and subsequently produce 5-hydroxy madindolines in plasma. Consequently, we were concerned that the 5-halo analogues might increase tolerances of oxidation in a living body. We therefore focused on a careful assessment of the action of the drug. The more efficient second-generation synthesis system we had developed caused us to examine whether we could access clinical candidate madindolines by this new method. Attainment of this goal would involve a functionalization of the 3a-hydroxyfuroindoline moiety (see Scheme 3).

Syntheses of the 5-halo madindolines were achieved, accompanied by each *N*-halo succinimide reagent (Scheme 4). Moreover, esterification of the 3a-hydroxyl group was achieved by treatment with several acid anhydrides, DMAP, and pyridine, leading to the desired acyl derivatives (Scheme 5).

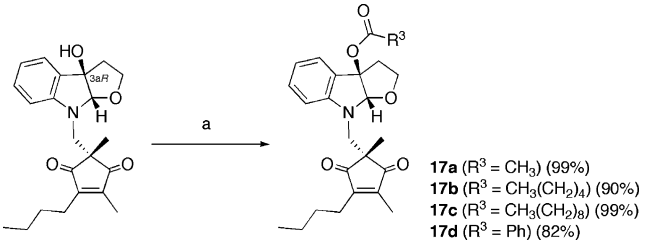
Table 2 and 3 depict the 5-halo and the acyl sector analogues in order of potency in comparison with the natural compound. Although increased activity of



Scheme 3. Reagents and conditions: (a) LDA, THF, −78 °C; (b) TBAT, DMF, Δ.



Scheme 4. Reagents: (a) NCS or NBS or NIS, CH_2Cl_2.



Scheme 5. Reagents: (a) $(R^3CO)_2O$, DMAP, pyridine.

5-chloro madindoline **16a** was not observed, 5-bromo and iodo madindolines (compounds **16b** and **16c**) showed moderate levels of activity. The unprecedented result would mean that enhanced activities of the 5-halo sector analogues depend on their hydrophobicity rather than improvement of stabilities against oxidation for metabolism. Metabolic studies of **16a**, **16b**, and **16c** would be continued in the future. Interestingly, hexanoate and benzoate analogues (compounds **17b** and **17d**) exhibited much higher efficacy than the natural compound, however, decanoate analogue (compound **17c**) possessed no activity.

Finally, our investigation of the madindoline series (Fig. 2) examined the effect of stereochemistry and parts of the system on a compound's IL-6 inhibitory properties. Although (−)-**3**, cyclopentendione **18**,[13] and the inversion of stereochemistry of the 3a-furoindoline moiety (−)-**2** do not invoke the activities as compared to madindoline A, the reductive 3a-hydroxyfuroindoline moiety **19** appeared to retain its potency.

In summary, preliminary studies of the totally synthetic madindoline analogues indicated enhanced activities, reinforcing initial chemical biology studies that revealed a number of unexpected structure–activity relationships within the madindoline class. Notably, promising candidates **15c**, **15d**, and **15e** demonstrated the importance of side chain length. The 5-halo and some of the acyl sector analogues also exhibit enhanced activities. Using madindolines as a lead structure, the collection of synthetic analogues we have produced offer promising leads to the discovery of a series of potent IL-6 inhibitors. Further in vitro and in vivo studies on madindoline derivatives are in progress.

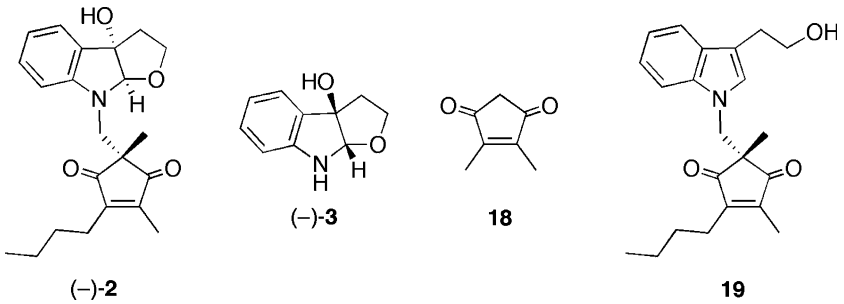


Figure 2. Structures of madindoline analogues.

Acknowledgments

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