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A New Synthesis of Cytoxazone and Its Diastereomers Provides Key Initial SAR Information

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Abstract—A short, enantioselective, and diastereoselective synthesis of cytoxazone, a Th2-selective immunomodulator from *Streptomyces*, is described. The route was readily adapted to the synthesis of the three other stereoisomers of natural cytoxazone. Evaluation of these compounds revealed that the stereochemical configuration of the oxazolidinone ring did not influence their biological activity.

In 1998, Osada and co-workers reported the isolation of cytoxazone (**1**, Fig. 1) from a soil sample of *Streptomyces* sp,[1] and identified it as a selective modulator of T_H2 cytokine secretion.[1] The structure of **1** was definitively determined using X-ray crystallographic analysis,[2] and the absolute stereochemistry was assigned via comparison of the circular dichroism spectrum of **1** with the spectra of (*S*)- and (*R*)-4-phenyl-oxazolidin-2-ones. The structure was later confirmed by the Sharpless AD-mediated synthesis of **1** from 4-methoxycinnamate by T. Nakata and co-workers.[3] Since T_H2 cells play a role in mediating the immune response to allergens,[4] we reasoned that cytoxazone might be a useful lead compound for the development of therapeutic agents for atopic dermatitis and asthma, and we designed a new synthesis of **1** that utilized an aldol/Curtius sequence (Fig. 1).[5] The brevity and convergency of our synthesis make it ideal for analogue generation.

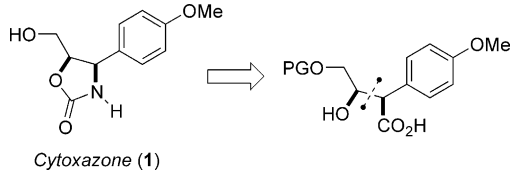
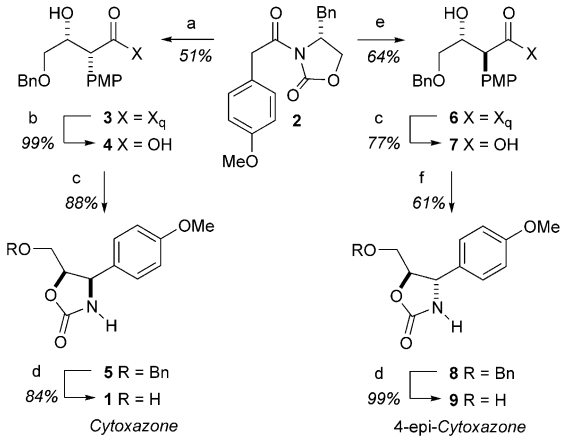


Figure 1. Retrosynthesis of cytoxazone **1**.

The syntheses of cytoxazone **1** and epi-cytoxazone **9** are shown in Scheme 1. Acylation of the commercially available (*R*)-4-phenylmethyloxazolidin-2-one with 4-methoxyphenylacetic acid provided the known imide **2**.[6] Subsequent reaction of the dibutylboron enolate of **2** with the commercially available benzyloxyacetaldehyde according to the pioneering work of Evans[7] provided the aldol **3** in good *syn*-diastereoselectivity (>95:5, ^{1}H NMR). Removal of the chiral auxiliary from **3** using



Scheme 1. Synthesis of cytoxazone **1** and *epi*-cytoxazone **9**. Notes: PMP = *para*-methoxyphenyl; X_q = (*R*)-4-benzyloxazolidin-2-one. Reagents and conditions: (a) Bu_2BOTf, *i*-Pr_2EtN, −78 °C, 20 min; $BnOCH_2CHO$, −78 °C to 0 °C, 1.5 h; (b) 4:1 THF:H_2O, H_2O_2, LiOH, 0 °C, 1 h; $NaHSO_3$; (c) $(PhO)_2PON_3$, $PhCH_3$, 23 °C, 40 min; 110 °C, 3 h; (d) 1 atm H_2, $Pd(OH)_2$, MeOH, 23 °C, 24 h; (e) Bu_2BOTf, *i*-Pr_2EtN, 0 °C, 30 min; add $BnOCH_2CHO$ precomplexed w/0.5 equiv $SnCl_4$, −78 °C, 3 h; (f) $(PhO)_2PON_3$, CH_2Cl_2, 23 °C, 40 min; 45 °C, 12 h.

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lithium hydroperoxide provided the acid **4** in good yield; notably, **4** underwent retro-aldol reaction upon silica gel chromatography, and so it was purified with a carefully-controlled acid/base extractive workup. Acid **4** was transformed into the oxazolidinone **5** in a one-pot, three-step (acyl azide formation, Curtius rearrangement, isocyanate trapping) procedure that was initiated using diphenylphosphoryl azide (toluene, 23 °C) and then completed at elevated temperature (110 °C, 3 h). Ether **5** was not purified, but rather directly debenzylated using Pearlman's catalyst to provide cytoxazone **1** as a white powder after silica gel chromatography. The spectral characteristics (^{1}H and ^{13}C NMR, IR, HRMS, and optical rotation) of synthetic **1** were the same as those reported in the literature for **1**.[1–3] The enantiomer of **1** was prepared in identical fashion to cytoxazone itself (Scheme 1), beginning with (*S*)-4-phenylmethyl-oxazolidin-2-one.[8]

The synthesis of 4-*epi*-cytoxazone **9** required the use of an *anti*-selective aldol protocol (Scheme 1). Addition of a precomplexed solution of benzyloxyacetaldehyde and 0.5 equiv of $SnCl_4$ to the dibutylboryl enolate of **2** according to the procedure of Heathcock[9] proceeded smoothly to give the desired aldol **6** in reasonable *anti*-diastereoselectivity (75:25, ^{1}H NMR); chromatography provided diastereomerically-pure **6** in 64% yield. Notably, no detectable product was obtained when imide **2** was used with the alternative Et_2AlCl- or Mg(II)-based *anti*-selective protocols.[9,10] Hydrolysis of **6** (H_2O_2, LiOH; $NaHSO_3$) provided the carboxylic acid **7**, which was then transformed into oxazolidinone **8** with diphenylphosphoryl azide. Due to the poor solubility of **7** in toluene, the rearrangement was conducted in methylene chloride, which highlights the low temperature requirements of this particular Curtius rearrangement (complete conversion at 45 °C after 12 h). Hydrogenation of the benzyl ether **8** as before provided 4-*epi*-cytoxazone **9**, which had spectral characteristics (^{1}H and ^{13}C NMR, IR, HRMS, and optical rotation) consistent with those reported in the literature.[3] The enantiomer of **9** (5-*epi*-cytoxazone) was synthesized in identical fashion, beginning with (*S*)-4-phenylmethyloxazolidin-2-one. The *anti*-disposition of the oxazolidinone ring of *ent*-**9** was shown via comparison to **1** in one-dimensional nuclear Overhauser enhancement (NOE) experiments (Fig. 2).

We first confirmed the reported biological selectivity of **1** by demonstrating that synthetic **1** preferentially blocked the secretion of IL-10 relative to IL-2 in pokeweed mitogen (PWM)-stimulated murine splenocytes (Fig. 3A).[11] The IC_{50} for this inhibition was approximately 10 μM, which was consistent with the work of Osada.[1] We also examined the effects of **1** on stimulated human peripheral blood mononuclear cells, and found that 30 μM concentrations of **1** selectively inhibited the production of the prototypical T_H2 cytokines IL-4 (73%), IL-5 (66%) and IL-10 (94%) relative to the secretion of the T_H1 cytokines IL-2 (19%) and interferon-γ (7%).[12] To our surprise, ent-**1**, **9**, ent-**9**, and **5** all showed equivalent effects in this system (Fig. 3B). Likewise, *ent*-**1**, **9**, *ent*-**9**, and **5** were equipotent to **1** for the inhibition of cytokine secretion of PWM-stimulated murine splenocytes (data not shown). To confirm that these effects were not due to some type of toxicity, we also examined **1**, *ent*-**1**, **9**, *ent*-**9**, and **5** for cytotoxicity in the human Ramos cell line, but none of these compounds were cytotoxic at concentrations up to 50 μM in a standard 24 h assay.[13] Furthermore, these compounds were not potent anti-proliferative agents in PWM-stimulated splenocytes (<15% inhibition of ^{3}H-thymidine incorporation at 10 μM; see Figure 3A for data obtained with **1**).[11b]

In summary, our work has provided a new synthetic approach to the natural product cytoxazone and its stereoisomers. These syntheses represent the shortest published routes to cytoxazone[14–18] and *epi*-cytoxazone.[16,18,19] In addition, they are the first routes that offer a fully convergent approach to the cytoxazone core.[14b] The biological data described above also extend our understanding of **1** in several key ways: (1) its T_H2-selective inhibition of cytokine secretion is effective on human T-cells; (2) it is neither an anti-proliferative nor cytotoxic agent; and (3) its biological

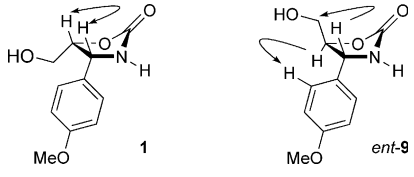

Figure 2. Stereochemical proof of the synthetic sequence via NOE studies. Note that a weak NOE between the two oxazolidinone ring protons was also observed in the *trans* diastereomer *ent*-**9**.

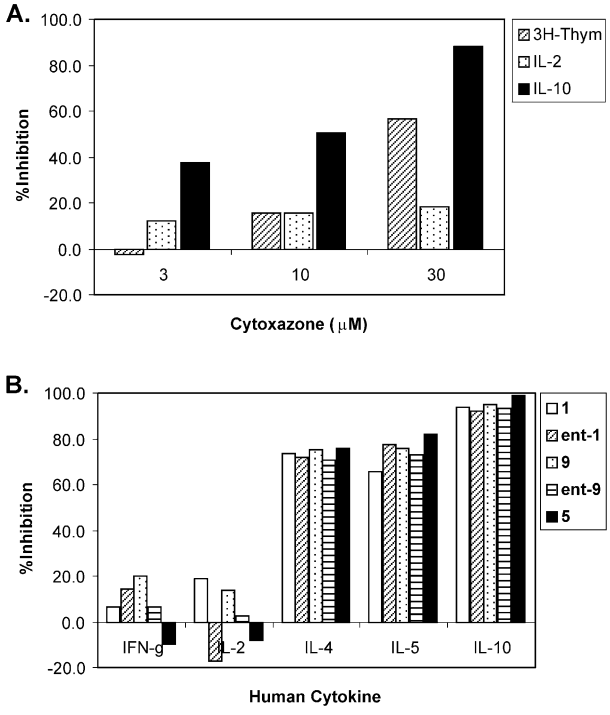

Figure 3. (A) The dose-dependent inhibition of IL-10 production by **1** from PWM-stimulated murine splenocytes is compared with its effects on IL-2 production and ^{3}H-thymidine uptake (see ref 11). (B) Shown is the selective inhibition of T_H2 cytokine release from stimulated human PBMCs by cytoxazone and its stereoisomers (see ref 12).

activity is shared by its three stereoisomers and the *O*-benzylated derivative **5**. These initial SAR data suggest that the oxazolidinone ring of **1** — which is a rare component of bacterial natural products[1] — does not function simply to hold the aryl and hydroxymethyl groups in rigid, stereo-defined arrangement. They may also indicate that the hydroxymethyl group of **1** can be replaced with more lipophilic moieties. Our studies on other analogues of **1** and on our attempts to define its biological target are in progress and will be reported in due course.

Acknowledgements

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11. (a) Splenocytes were isolated from 6–8 week old female Balb/c mice (Charles River). Following lysis of red blood cells with NH_4Cl, lymphocytes were plated in 96-well plates (Costar) at 5×10^5 cells/well in RPMI (Gibco BRL) +10% fetal calf serum (HyClone) +50 μM 2-ME. Compounds were dissolved in DMSO and serially diluted in media to give a final concentration of 0.3% DMSO. Compounds were added to cells 5 min prior to stimulating the cells with pokeweed mitogen (5 μg/mL). Cells were cultured in a final volume of 200 μL at 37 °C for 48 h. Supernatants were then harvested and cytokine levels measured by ELISA according to the manufacturer's instructions (R&D Systems). (b) Alternatively, cells were pulsed for 18 h with ^{3}H-thymidine (1 mCi/well) and then harvested and counted.
12. Human PBMCs were isolated by centrifugation over Ficoll-Hypaque (Pharmacia). Cells were plated in AIM V media (Gibco BRL) in 96-well plates (Costar) at 2×10^5 cells/well. Compounds were dissolved in DMSO and serially diluted in media to give a final concentration of 0.3% DMSO. Compounds were added to cells 5 min prior to stimulating the cells with anti-CD3 (R&D Systems) at 1 mg/mL and anti-CD28 (Caltag Laboratories) at 200 μg/mL. Cells were cultured in a final volume of 200 μL at 37 °C for 24 h. Supernatants were then harvested and cytokine levels measured by ELISA according to the manufacturer's instructions (Pharmingen).
13. Ramos cells were seeded at 3×10^4 cells/well in serum free AIM V media (Gibco BRL) in 96-well plates with various concentrations of compound. After incubation for 24 h at 37 °C, MTS (Promega) was added to each well at a final concentration of 333 μg/mL. Cell viability was determined after an additional 3 h incubation at 37 °C by reading the plates at 490 nM absorbance. Inhibition was determined relative to cells cultured with 1% DMSO.
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