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Therapeutic effects of isothiocyanate prodrugs on rheumatoid arthritis

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ABSTRACT

Isothiocyanates **7a** and **7b** have poor stability and aqueous solubility. To address these problems, prodrugs **8a** and **8b** were synthesized. Prodrugs **8a** and **8b** were stable in HEPES buffer at pH 4.4, but released the active compounds **7a** and **7b** in HEPES buffer at pH 7.4 and in mouse plasma, respectively. Compound **8a** and especially compound **8b** showed anti-inflammatory effects. Compound **8b** demonstrated significant efficacy in animal models of traumatic inflammation, acute inflammation and rheumatoid arthritis. Compound **8b** also did not cause appreciable toxicity in mice after 5 weeks at a daily dose of 200 mg/kg.

Rheumatoid arthritis (RA) is a heterogeneity, chronic, systemic autoimmune diseases, characterized by symmetrical polyarthritis. Women are approximately three times more likely to develop RA than men.[1] Although the exact etiology of RA is unclear, it is generally believed that this is a genetic disease driven by multiple factors. Individuals with a family history of RA have a 3–9-fold higher risk of developing the disease than the general population.[2] The collagen-induced arthritis model[3,4] and the adjuvant arthritis model[5,6] are the most widely used animal models of RA.

Isothiocyanate compounds, such as sulforaphane (SFN), erucin, phenethyl isothiocyanate and benzyl isothiocyanate, are abound in plants, especially cruciferous plants. Previous studies have shown that isothiocyanate compounds have anti-inflammatory effect, represented by SFN.[7–15] But isothiocyanates have poor stability and solubility, for example, the half-life of SFN is 2.2 h in rats[16] and 1.77 ± 0.13 h in one human study.[17]

Herein, **8a** and **8b**, prodrugs of isothiocyanates **7a** and **7b**, were synthesized and evaluated for stability and efficacy. Preliminary toxicity data was also presented.

Triethylene glycol was treated with 4-dimethylaminopyridine (DMAP) and *p*-toluenesulfonyl chloride (PTSC) to obtain compound **2**, which was further converted to compound **3** in aqueous dimethylamine. Treatment of compound **3** with $SOCl_2$ then gave compound **4**, which was treated with potassium thioacetate to provide compound **5**. Hydrolysis of compound **5** in methanol containing hydrazine hydrate then afforded compound **6**. Treatment of isothiocyanates **7a** and **7b** with compound **6** gave prodrugs **8a** and **8b**, respectively (Scheme 1).

Transformation from prodrugs **8a** and **8b** to active drug was determined in HEPES buffer and plasma to simulate stomach, intestines and blood environment *in vivo*. In HEPES buffer at pH 4.4, compounds **8a** and **8b** were stable, and no significant amount of **7a** or **7b** was released within 24 h (Fig. 1). In HEPES buffer at pH 7.4, compounds **8a** and **8b** were slowly converted to the active drugs, **7a** and **7b**. Compound **8a** achieved balance at 1 h and then continued to transform to **7a**. Compound **8b** achieved balance at 2 h and then remained unchanged (Fig. 2). In plasma, compounds **8a** and **8b** were transformed to **7a** and **7b**, and had almost completely decomposed after 24 h.

In mouse plasma, compounds **8a** and **8b** were also slowly converted to the active drugs, **7a** and **7b**. Tmax were 2 h. Compounds **8a** and **8b** almost completely decomposed in 24 h and 10 h, respectively. The half-lives of compounds **8a** and **8b** in mouse plasma were about 6 h *in vitro*, so compounds **8a** and **8b** were thus likely to be stable *in vivo* (Fig. 3).

Pharmacodynamics of the isothiocyanate prodrugs were evaluated by the neutrophilic inflammation model in EGFP transgenic zebrafishes, the carrageenan-induced paw edema model in rats

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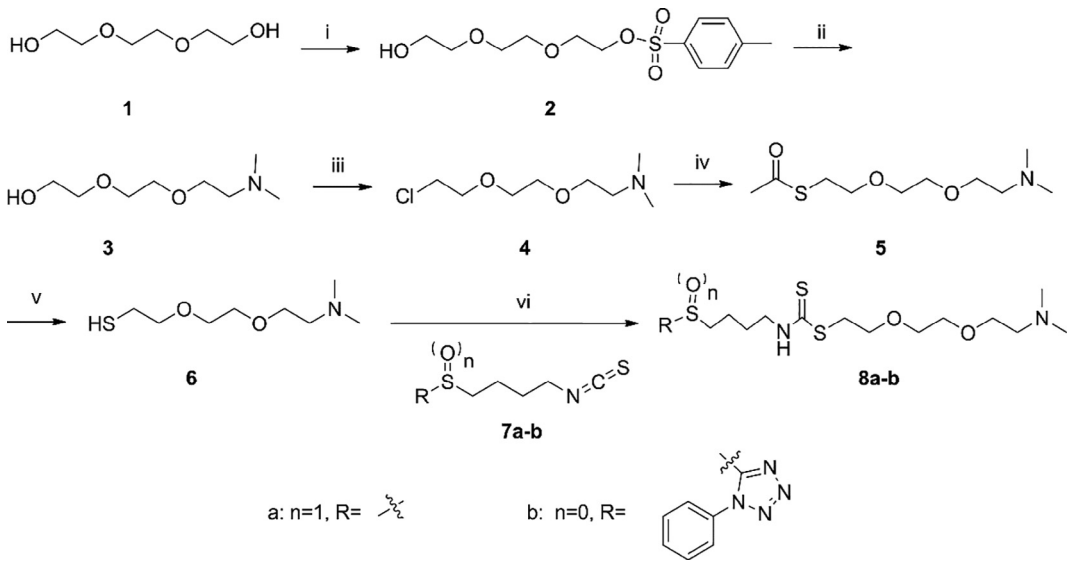

Scheme 1. Synthesis of isothiocyanate prodrugs **8a**–**b**. Reagents and conditions: (i) DMAP, PTSC, Et_3N, THF, 0 °C to r.t, 44.4%; (ii) dimethylamine aqueous, r.t, 62.9%; (iii) $SOCl_2$, $CHCl_3$, 0 °C to 70 °C, 95%; (iv) Potassium thioacetate, DMF, 75 °C, 62.6%; (v) Hydrazine hydrate, MeOH, r.t, Ar, 51.1%; (vi) DCM, Et_3N, r.t, 64.5% for **8a** and 70.3% for **8b**.

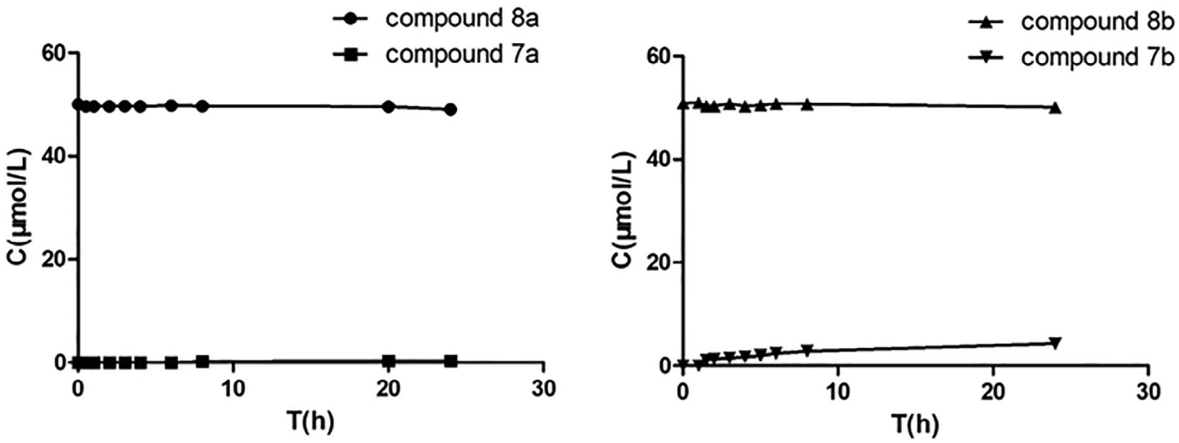

Fig. 1. Concentration–time curve of **8a** (●), **7a** (■), **8b** (▲), and **7b** (▼) in HEPES buffer of pH 4.4.

and the Freund 's complete adjuvant model in rats. These models were traumatic inflammation, acute inflammation and RA, respectively.

Dexamethasone (DEX) and methotrexate (MTX) were used as positive controls. (Figs. 4–6). The animal experiments have been approved by ethics committee of Tianjin International Joint Academy of Biomedicine.

In the zebrafish neutrophilic inflammation model, the number of migrating neutrophils is an important evaluation index, with fewer migrating cells indicating better efficacy (Fig. 4). The numbers of migrating neutrophils in zebrafish treated with the positive control DEX, compound **8a** and compound **8b** (13, 16 and 12, respectively) were smaller than the control group (25), indicating that compounds **8a**, **8b** and DEX had comparable anti-inflammatory effects in EGFP transgenic zebrafish.

In the rat carrageenan-induced paw edema model, animals treated with the positive control DEX had less swelling than those in the model group at each time point, especially at 6, 8, and 10 h (Fig. 5). Animals in the groups treated with compound **8b** (low, middle and high doses) also had noticeably less swelling than animals in the model group, but more than animals in the DEX group at the same time points. Compared with the model group, animals treated with compound **8b** showed significant differences at 8 and 10 h. Compound **8b** thus produced an anti-inflammatory effect at all three doses and was comparable in efficacy to the positive control DEX.

In rat Freund 's complete adjuvant model, the body weights of animals in the model group, and in all three groups treated with compound **8b**, increased significantly, whereas the body weights of animals in the positive control MTX group started to fall after day 29. Body weights increased by 80.8, 70.8, and 58.2 g, respectively, for animals treated with low, middle and high doses of compound **8b**. The increase of body weight in the low dose group was comparable with that in the model group (80.8 vs 84.6 g) and the increase of body weight in the high dose group was