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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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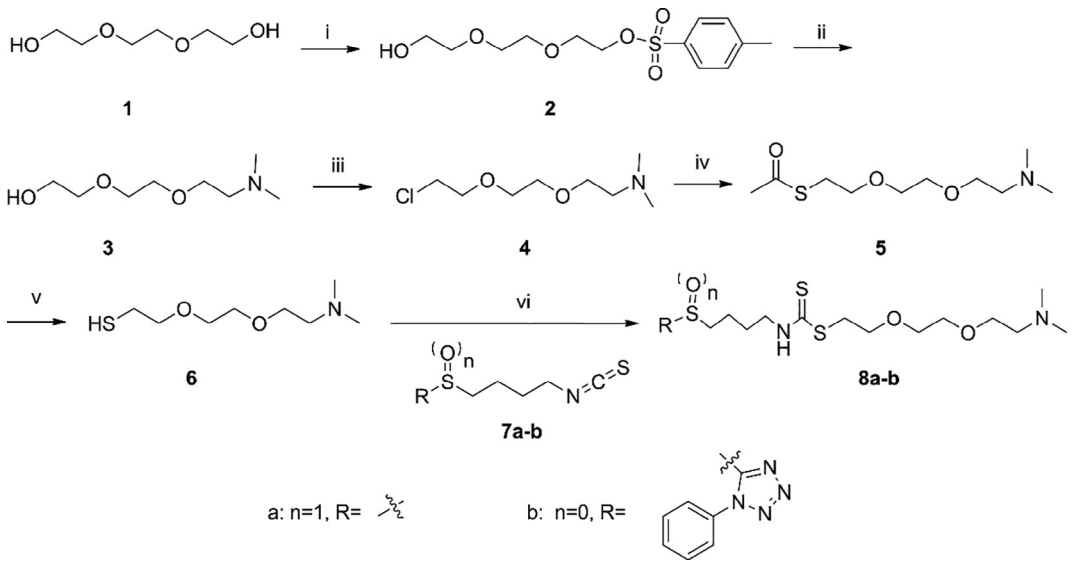

Scheme 1. Synthesis of isothiocyanate prodrugs **8a**–**b**. Reagents and conditions: (i) DMAP, PTSC, Et_3N, THF, 0 °C to r.t, 44.4%; (ii) dimethylamine aqueous, r.t, 62.9%; (iii) $SOCl_2$, $CHCl_3$, 0 °C to 70 °C, 95%; (iv) Potassium thioacetate, DMF, 75 °C, 62.6%; (v) Hydrazine hydrate, MeOH, r.t,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

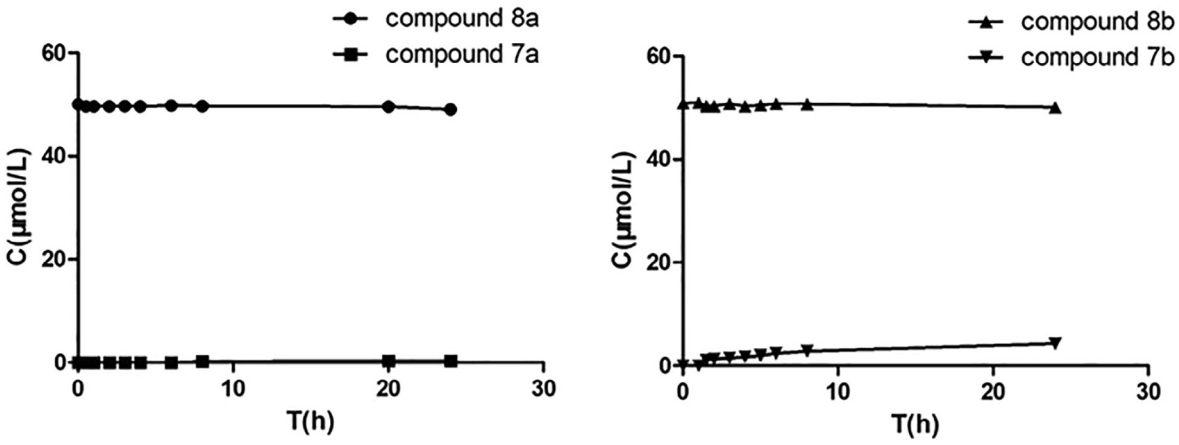

Fig. 1. Concentration–time curve of **8a** (●), **7a** (■), **8b** (▲), and **7b** (▼) in HEPES buffer of pH 4.4.

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

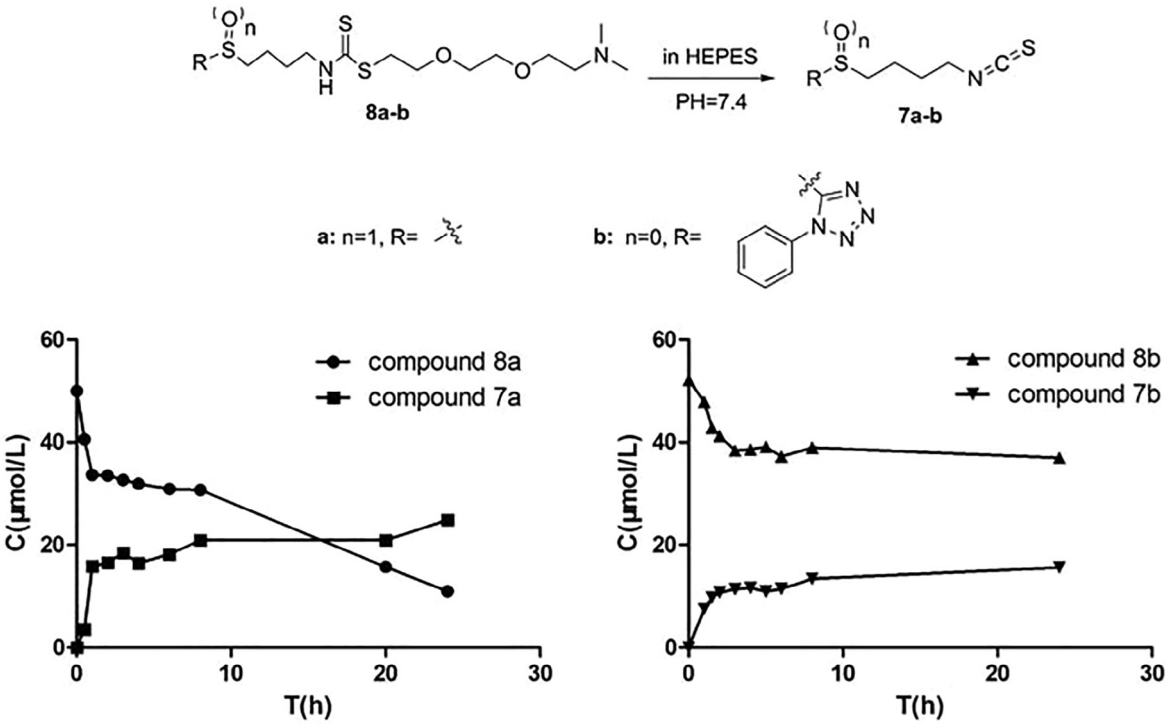

Fig. 2. Concentration–time curve of **8a** (●), **7a** (■), **8b** (▲), and **7b** (▼) in HEPES buffer of pH 7.4.

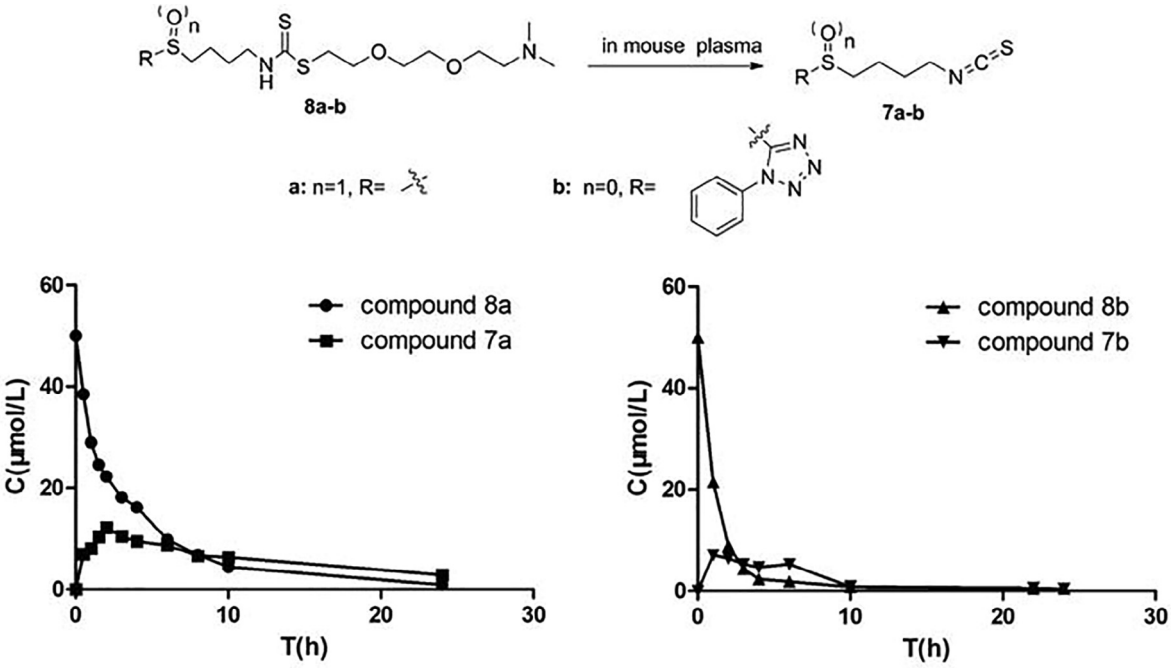

Fig. 3. Concentration–time curve of **8a** (●), **7a** (■), **8b** (▲), and **7b** (▼) in mouse plasma.

comparable with that in the MTX group (58.2 vs 59.2 g) (Fig. 6A). In the model group, the degree of paw swelling was noticeably increased. The degree of paw swelling was significantly decreased after treatment with MTX or **8b** for 4 days ($P < .05$, compared with the model group). From day 10, the reduction of paw swelling in animals treated with **8b** (10 and 20 mg/kg) was comparable with that in the MTX group, but the high dose of **8b** (40 mg/kg) was less effective. (Fig. 6B). Low dose **8b** (10 mg/kg) thus markedly relieved arthritis-induced inflammation, with minimal effects on body weight.

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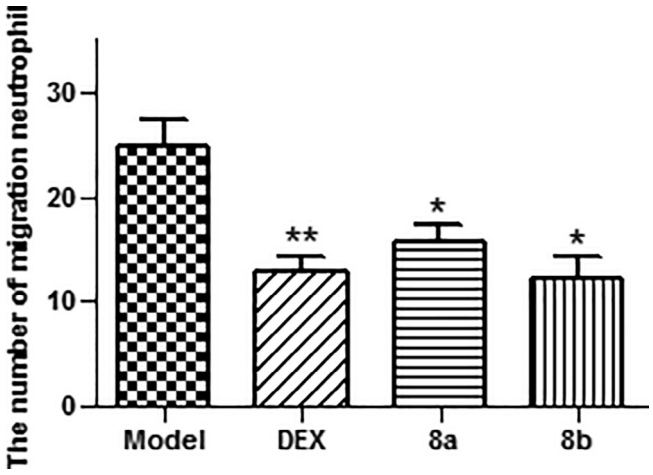

Fig. 4. Effect of compounds **8a** and **8b** on the progress of traumatic inflammation in EGFP transgenic zebrafish. 24 EGFP transgenic zebrafishes of 72 h development period were divided into 4 groups of control group, positive control DEX, compounds **8a** and **8b**. 2 ml positive control DEX (30 μg/mL), compounds **8a** (5 μg/mL) and **8b** (0.5 μg/mL) were added to cultured zebrafishes well for 3 h, respectively. $^{*}P < .05$, $^{**}P < .01$, compared with the model group, SPSS 14.0 was used to analyze the data. EGFP: enhanced green fluorescent protein; DEX: dexamethasone.

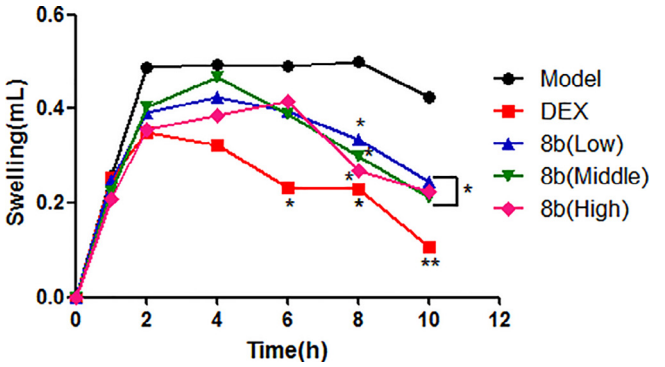

Fig. 5. Effect of compound **8b** on the progress of acute inflammation induced by CG in Sprague-Dawley rats. 30 rats were randomly divided into 5 groups, and orally administered with positive control DEX (5 mg/kg) and compound **8b** (10, 20 and 50 mg/kg) for 2 times at 2 h and 6 h after finishing injected carageen glue, respectively. $^{*}P < .05$, $^{**}P < .01$, compared with the model group, SPSS 14.0 was used to analyze the data. CG: Carrageen glue; DEX: dexamethasone.

Toxicology of the compound **8b** were evaluated in mice. Mice treated for 5 weeks with compound **8b** (200 mg/kg) survived, and body weights in both the control group and the animals treated with compound **8b** increased significantly. Body weight increased more slowly in animals treated with compound **8b** group, but from day 30, body weights in the treatment with compound **8b** group were comparable with those in the control group. Therefore, treatment with compound **8b** at the tested dose (200 mg/kg) for 5 weeks did not cause appreciable toxicity, as judged by body weight (Fig. 7).

Isothiocyanate prodrugs **8a** and **8b** were successfully synthesized. Stability studies indicated that **8a** and **8b** were stable under acidic solution but were gradually converted to the parent drugs at higher pH. This suggests that the prodrugs should be stable in gastric juice but should slowly release the active substances in blood. Compounds **8a** and **8b** produced anti-inflammatory effects in the neutrophilic inflammation model in EGFP transgenic zebrafishes. Compound **8b** showed comparable anti-inflammatory activity with DEX, at a dose 300-fold lower than that of DEX. In the carrageenan-induced paw edema model in rats, compound **8b** showed anti-inflammatory effects at low, medium and high doses, but displayed more adverse effects than DEX. At the lowest dose (10 mg/kg), **8b**

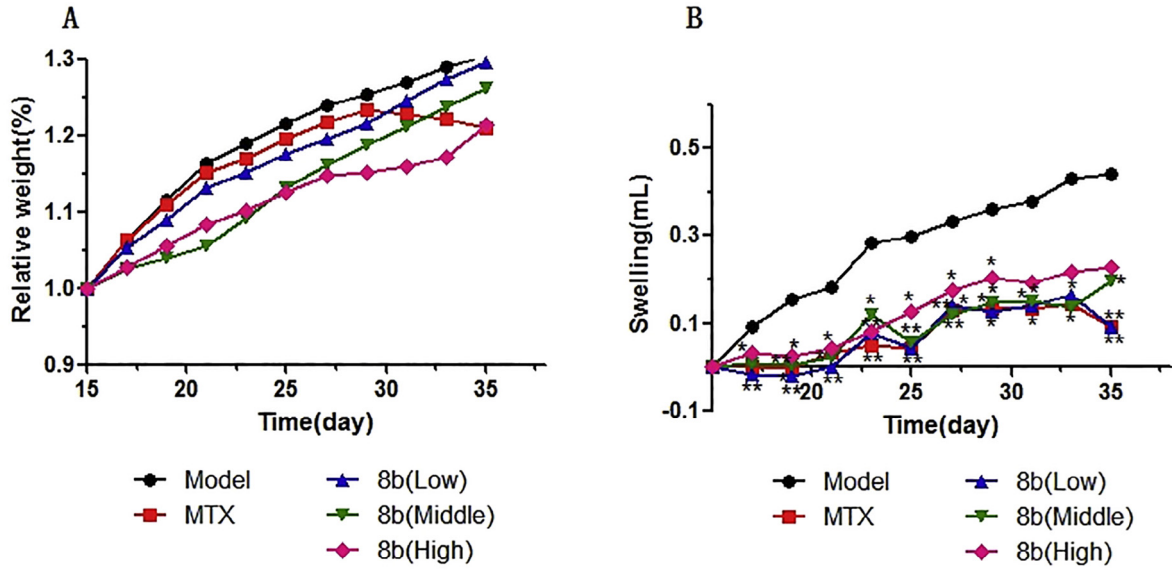

Fig. 6. Effect of compound **8b** on the progress of Rheumatoid arthritis induced by FCA in Wistar rats. 30 Wistar rats were randomly divided into 5 groups, and orally administered with positive control MTX (1 mg/kg) and compound **8b** (10, 20 and 40 mg/kg) from day 16 after the initial inflammation, respectively. (A) The effect of compound **8b** on the increase of the body weight. (B) The effect of compound **8b** on the paw swelling degree. the paw swelling degree was evaluated at two days intervals. $^{*}P < .05$, $^{**}P < .01$, compared with the FCA group, SPSS 14.0 was used to analyze the data. FCA: Freund's complete adjuvant; MTX: methotrexate.

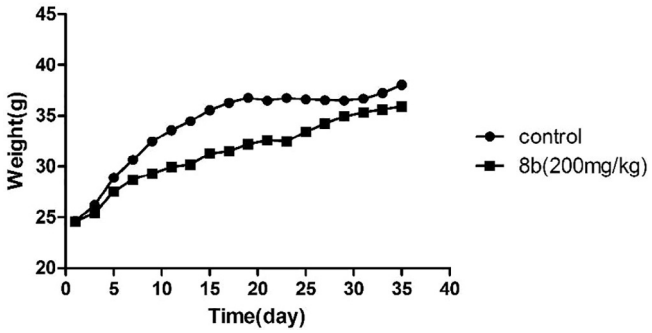

Fig. 7. The effect of compound **8b** on the body weight in Kunming white mouse. Mice were orally administered with compound **8b** (200 mg/kg) everyday for 5 weeks.

low group markedly reduced arthritis-induced inflammation and had the smallest effect on body weight. At the tested dose (200 mg/kg), treatment with compound **8b** for 5 weeks did not cause appreciable toxicity in mice.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bmcl.2018.01.009.

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