Arch. Pharm. Chem. Life Sci. 2015, *348*, 463–474

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Full Paper

Discovery of Benzylidene Derivatives as Potent Syk Inhibitors: Synthesis, SAR Analysis, and Biological Evaluation

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Four scaffolds of varied benzylidene derivatives were synthesized and evaluated as Syk inhibitors for the treatment of rheumatoid arthritis (RA). Among these 31 compounds, 3-benzylidene pyrrolidine-2,5-dione derivatives (including **12k**) universally showed good Syk inhibitory activities in the low micromolar to submicromolar range. In the cellular profiling, compound **12k**, the most efficient compound, showed excellent antiproliferative activity against fibroblast-like synoviocytes (FLS)-RA, and demonstrated potencies for suppression of IL-6 and MMP-3 secretion almost equal to R406 (positive control). The oral efficacy of **12k** in the murine collagen-induced arthritis model was significant, despite being weaker than R406. Taken together, all preliminary pharmacological results supported **12k** as a potential small-molecule inhibitor targeting Syk for the treatment of RA.

Keywords: Antiproliferation / Benzylidene derivatives / Biological evaluation / Rheumatoid arthritis / Syk inhibitors

Received: March 16, 2015; Revised: April 17, 2015; Accepted: April 17, 2015

DOI 10.1002/ardp.201500096

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Introduction

Spleen tyrosine kinase (Syk), a 72-kDa multiple-domain intracellular cytosolic non-receptor protein tyrosine kinase, serves as a key mediator of Fc receptor- and B-cell receptor-mediated signaling in inflammatory cells [1], such as B-cells, T-cells, monocytes, macrophages, mast cells, neutrophils, basophils, dendritic cells (DC), as well as noninflammatory cells [2], such as osteoclasts and synoviocytes. Syk is located upstream in the cell signaling pathway, and possesses

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Abbreviations: ATP, adenosine triphosphate; **Brij-35,** polyoxyethyleneglycol dodecyl ether; **CCK-8,** cell counting kit-8; **CFA,** complete Freund's adjuvant; **CIA,** collagen-induced arthritis; **DTT,** dithiothreitol; **EDTA,** ethylene diamine tetraacetic acid; **EI,** electric ionization; **ELISA,** enzyme-linked immunoassay; **ESI,** electrospray ionization; **FAM,** fast auxiliary memory; **FBS,** fetal bovine serum; **FLS,** fibroblast-like synoviocytes; **H-DMEM,** high-glucose Dulbecco's modified Eagle's medium; **HEPES,** 4-2-hydroxyethyl-1-piperazineethanesulfonic acid; **HRMS,** high resolution mass spectrometer; **IC_{50},** half maximal inhibitory concentration; **IgA,** immunoglobulin A; **IL-6,** interleukin-6; **MMP-3,** matrix metalloproteinase-3; **MTT,** 3-4,5-dimethyl-2-thiazolyl-2,5-diphenyl-2-*H*-tetrazolium bromide; **PAINS,** pan-assay interference compounds; **RA,** rheumatoid arthritis; **SAR,** structure–activity relationship; **Syk,** spleen tyrosine kinase; **TNFα,** tumor necrosis factor α; **TZD,** thiazolidinedione; **UV,** ultraviolet; **YLD,** years lived with disability.

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multiple phosphorylation sites, several of which serve as docking sites for downstream signaling proteins that orchestrate overall cellular process [3]. Therefore, therapies targeting Syk might be more advantageous than that acting on a single downstream event. Despite the compelling result, blocking the activation of inflammatory cells (e.g., macrophages and B cells) may raise the possibility of side effects owing to the shutting down of the whole inflammatory process.

Rheumatoid arthritis (RA) is a chronic and multifactorial autoimmune disorder that is associated with the release of autoantibodies, pannus formation, the erosion of cartilage and bone, synovial hyperplasia, and various extra-articular manifestations. A report from the World Health Organization releases that RA is the 31st leading reason of years lived with disability (YLD) accounting for 0.8% of total global YLD [4]. Several biologic agents targeting at proinflammatory cytokines have had a high effect on the treatment of RA [5]. However, these therapies are expensive and will produce significant side effects. The majority of patients relapse when the treatment is withdrawn [6]. In addition, approximately one-third of the patients fail to respond adequately to these agents [7]. Consequently, the use of small molecule inhibitors (e.g., Syk-targeting inhibitors) against the signaling pathways has been a recent focus for development of drugs for RA.

Nowadays, numerous Syk chemtypes (Fig. 1) including **1** (R406) [8], **2** (fostamatinib disodium) [9], **3** (R112) [10], **4** (GS-9973) [11], **5** (BAY 61-3606) [12], **6** (P505-15) [13], and **7** (GSK-143) [14] have been reported by famous pharmaceutical companies such as Rigel, Gilead Sciences, Bayer, Portola, and GlaxoSmithKline. Fostamatinib disodium (**2**) is the disodium phosphate prodrug form of R406 (**1**) and is the best-characterized Syk inhibitor in patient studies. With good solubility but poor selectivity, **2** has completed phase II trials in human patients for RA, but failed in phase III trials [15]. The drug is currently in phase I for IgA nephropathy and phase II for idiopathic thrombocytopenic purpura (ITP) [16]. R112 (**3**) is an intranasal inhibitor tested for the alleviation of seasonal allergies and now under phase II for allergic rhinitis. GS-9973 (**4**) failed in phase I for RA and now is in phase II as a potential indication for hematological malignancies. BAY 61-3606 (**5**) is an imidazopyrimdine analog that selectively inhibits Syk activity to treat allergic diseases *in vitro*. Diaminopyrimidine carboxamides P505-15 (**6**) and GSK-143 (**7**) are both highly selective Syk inhibitors. Compound **6** is currently in phase II for allergic asthma and other inflammatory disorders, while **7** is

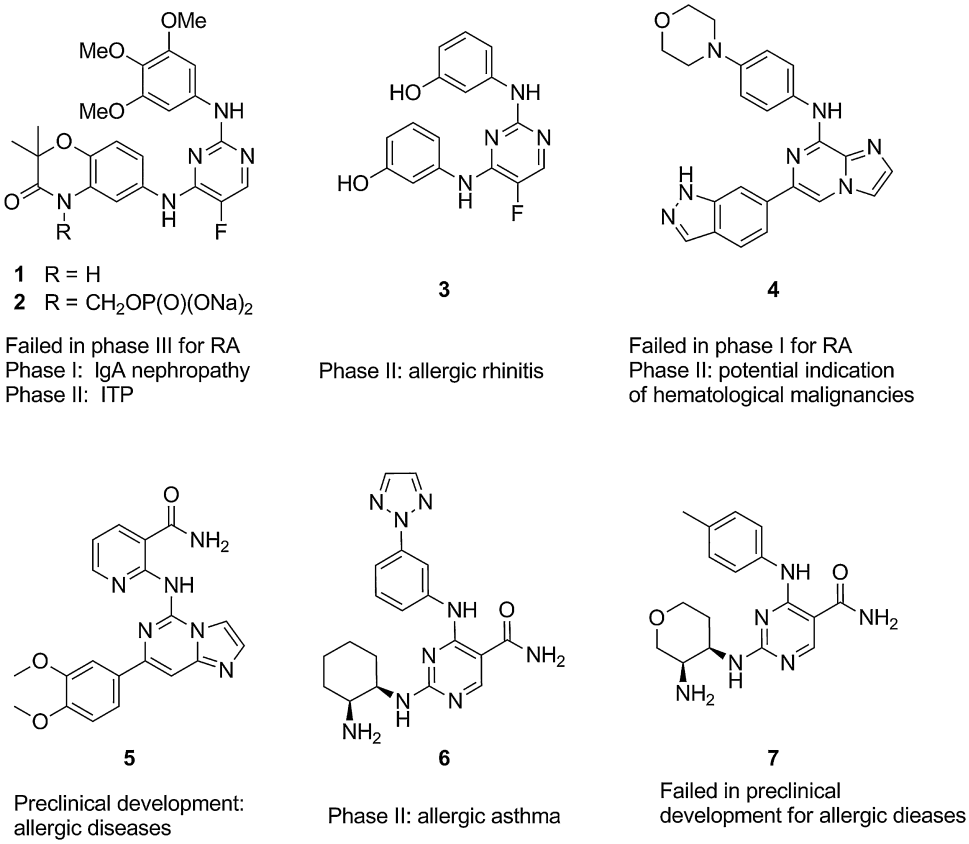
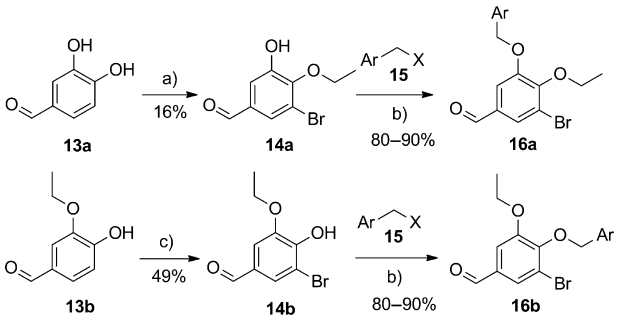


Figure 1. Examples of Syk inhibitors. R406 (**1**), fostamatinib disodium (**2**), R112 (**3**), GS-9973 (**4**), BAY 61-3606 (**5**), P505-15 (**6**), and GSK-143 (**7**).



Scheme 1. Reagents and conditions: a) (i) Br_2, acetic acid, rt, 2 h; (ii) CH_3CH_2I, Cs_2CO_3, DMF, 40°C, overnight; b) K_2CO_3, DMF, 40°C, 4 h; c) Br_2, acetic acid, rt, 2 h.

under further modification due to a mutagenicity risk [17]. However, none of them is put into practical use. Thus, research on Syk inhibitors for the treatment of autoimmune (RA) is necessary and has a great significance.

Herein, we carried out a random screening of our internal chemical library and identified compound **9a** which had a promising inhibitory activity in Syk kinase inhibition assay. Further design and synthesis led to four structural classes of benzylidene compounds, and the biological activities of them were evaluated *in vitro* and *in vivo*. A number of 3-benzylidene pyrrolidine-2,5-dione analogs were found to be potent Syk inhibitors with single-digit micro-molar inhibitory activity except **12b**. A promising compound **12k** demonstrated almost equal potencies for the suppression of IL-6 and MMP-3 to R406 in the cellular assay. Oral efficacy in the murine collagen-induced arthritis (CIA) model supported **12k** as a good lead compound for potential treatment for RA.

Results and discussion

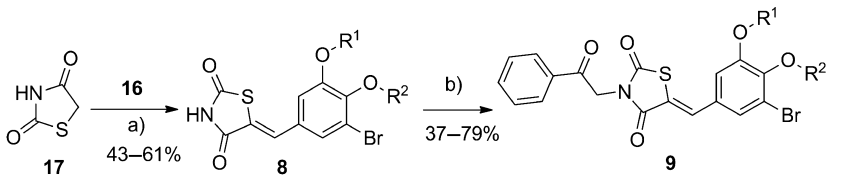
Chemistry

Four structural classes of compounds (**8–9**, **10–12**) were prepared by Knoevenagel reaction or Wittig reaction starting from the intermediates **16** (see Scheme 1), as shown in Schemes 2–5. The exocyclic double bond was *Z*-configuration in series of 2,4-thiazolidinedione (TZD, **8a–g**, **9a–f**), 6,7-dihydro-2*H*-thiazolo[3,2-*a*]pyrimidin-3(5*H*)-one (**10a–d**) and 2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one (**11a,b**) or *E*-configuration in the system of pyrrolidine-2,5-dione **(12a–l)** which may be ascribed to the thermodynamic stability of these configurations. The key intermediates **16** were synthesized in one or two steps (Scheme 1). 3-Bromo-4-ethoxy-5-hydroxybenzaldehyde (**14a**) was obtained by halogenation following *O*-alkylation of commercially available 3,4-dihydroxybenzaldehyde (**13a**). In parallel, 3-bromo-5-ethoxy-4-hydroxybenzaldehyde (**14b**) was acquired by halogenation of the commercial 3-ethoxy-4-hydroxybenzaldehyde (**13b**). Subsequent *O*-alkylation coupling reaction of **14** with varied benzyl halide **15** afforded **16**. All of the heterocycle parts (**17**, **19**, **20**, **22**) of our structural classes were either purchased from reagent companies or prepared according to the literature procedures.

SAR analysis

The 5-benzylidene thiazolidine-2,4-dione derivative **9a**, which was discovered by a random screening of our internal chemical library, exhibited promising Syk inhibitory activity, with an IC_{50} value of 3.6 μM. Maintaining the structure of 5-benzylidene thiazolidine-2,4-dione (**8**, **9**) or replacing the thiazolidine-2,4-dione with other heterocycle moieties (**10–12**), four structural classes of small molecules were designed and synthesized.

For the first series of compounds, we focused on the structure of 5-benzylidene thiazolidine-2,4-dione relating to the parent compound **9a** and synthesized five derivatives (**9b–f**). Initially we sought to vary the R^2 substituent while fixing the R^3 group of the TZD part and the R^1 group on oxygen atom. Compound **9b** without the carboxyl substituent at the R^2 group did not show any Syk inhibitory activity. Similarly, esterification of carboxyl group with methanol or benzyl alcohol (**9e,f**) resulted in the loss of potency. Replacement of the carboxylbenzyl group with a methyl (**9c**) produced a slight decrease (IC_{50} = 7.8 μM, Table 1). In addition, removal of the whole 4-carboxylbenzyl group (**9d**) was about threefold less potent compared with the hit. Compound **8a**, which was



	R^1	R^2
8a	ethyl	4-carboxylbenzyl
8b	ethyl	pyridin-2-yl
8c	ethyl	2-chlorobenzyl
8d	ethyl	3,5-dimethylbenzyl
8e	ethyl	3,4,5-trimethoxybenzyl
8f	ethyl	2,4-dichlorobenzyl
8g	2,4-dichlorobenzyl	ethyl

	R^1	R^2
9a	ethyl	4-carboxylbenzyl
9b	ethyl	benzyl
9c	ethyl	methyl
9d	ethyl	H
9e	ethyl	4-MeOCO-benzyl
9f	ethyl	4-BnOCO-benzyl

Scheme 2. Reagents and conditions: a) piperdine, EtOH, reflux, 36 h; b) 2-bromo-1-phenylethanone, K_2CO_3, acetone, reflux, 8 h.

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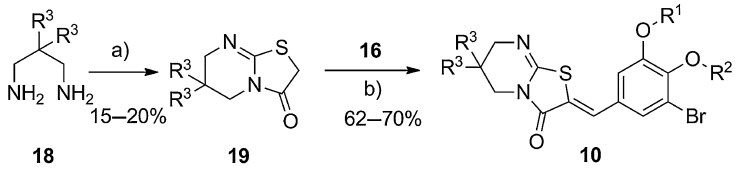


	R^3	R^1	R^2
10a	H	ethyl	2,4-dichlorobenzyl
10b	methyl	ethyl	2,4-dichlorobenzyl
10c	H	2,4-dichlorobenzyl	ethyl
10d	methyl	2,4-dichlorobenzyl	ethyl

Scheme 3. Reagents and conditions: a) (i) carbon disulphide, EtOH, 40°C, overnight; (ii) H_2O, 40°C, 10 h; (iii) $BrCH_2COOH$, absolute ethanol, reflux, 6 h; b) piperdine, EtOH, reflux, 48 h.

obtained by removing the 2-oxo-2-phenylethyl part of **9a**, exerted a comparative activity with an IC_{50} value of 2.9 μM.

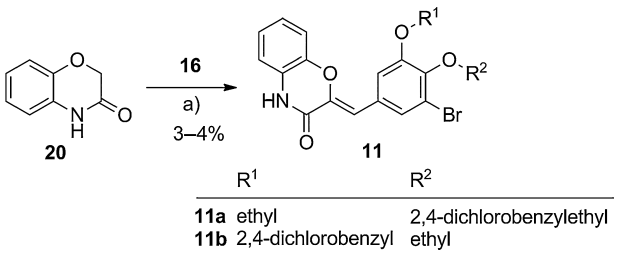
Compounds **8b–f**, the truncated TZDs bearing a pyridin-2-yl, 2-chlorobenzyl, 3,5-dimethylbenzyl, 3,4,5-trimethoxybenzyl, or 2,4-dichlorobenzyl groups at the R^2 position, exhibited inhibitory activity in a range of 0.5–50.0 μM. Notably, analog **8f**, which possessed a 2,4-dichlorobenzyl group at the R^2 position, displayed an excellent activity by near sevenfold more potent compared with the hit. Interestingly, compound **8g**, in which the position of the ethyl and 2,4-dichlorobenzyl groups of **8f** was transposed, was tolerated with a moderate increase (IC_{50} = 0.4 μM, Table 1). Considering "pan-assay interference compounds (PAINS)" feature of TZD moiety [18], we hoped to replace the TZD with other heterocycle moieties.

With the optimized R^1 and R^2 substituents, subsequently, we worked on the optimization of the heterocycle moieties selected from 6,7-dihydro-2*H*-thiazolo[3,2-*a*]pyrimidin-3(5*H*)-one, 2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one, or pyrrolidine-2,5-dione. Finally, pyrrolidine-2,5-dione (**12a,b**, Table 1) as preferred heterocycle moiety was selected to do further structural modification (IC_{50} up to 1.3 μM, Table 1), whereas 6,7-dihydro-2*H*-thiazolo[3,2-*a*]pyrimidin-3(5*H*)-ones (**10a–d**, Table 1) showed no better beneficial effect on Syk inhibition (IC_{50} up to 3.7 μM, Table 1), and 2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-ones (**11a,b**, Table 1) abolished activities (IC_{50} up to 50 μM, Table 1). Furthermore, we varied the positions of the chlorine atoms or increased the number of chlorine atoms, and gained compounds **12c–l** (Table 1). All of them showed significant inhibition against Syk. Especially, **12d**, **12f**, and **12g** exerted IC_{50} values of 0.8, 1.0, and 0.7 μM, respectively (Table 1). Based on the good results, the positions of ethyl and substituted benzyl in compounds **12d**, **12f**, and **12g** were transposed to form analogs **12j–l** (Table 1), which also showed comparable activities (**12j**, IC_{50} = 2.9 μM; **12f**, IC_{50} = 1.3 μM; **12g**, IC_{50} = 4.6 μM, Table 1).

Biology

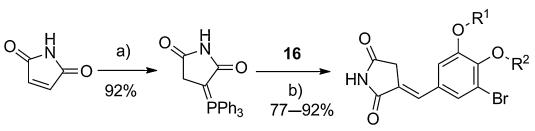
Effects of compounds on FLS cells

Compounds including **9a** (the hit), **8f**, **8g**, **12a**, **12d**, **12f**, **12g**, and **12k**, which showed high Syk inhibitory activities (expressed as IC_{50} under 1.5 μM), were selected for a preliminary test in cellular profiling. Firstly, the antiproliferative activity against fibroblast-like synoviocytes (FLS) was evaluated with CCK-8 assay (Fig. 2a), and then **9a**, **12a**, **12g**, and **12k** emerged as excellent compounds showing promising cell inhibition efficiencies. These four compounds were further evaluated with MTT-assay. The results are shown in Fig. 2b. R406 was included as a comparator in MTT-assay with an IC_{50} value of 18.1 μM. Compounds **12a** and **12g** resulted in less-potent activities with IC_{50} values of 21.3 and 32.2 μM, respectively (Table 1). While compounds **9a** and **12k** were able to inhibit FLS-RA proliferation with more superior activities (**9a**, IC_{50} = 14.8 μM, ns; **12k**, IC_{50} = 11.8 μM, $p < 0.01$, Fig. 2b) compared with R406. As a member of PAINS [18], compound **9a** was no longer an alternative despite its excellent



	R^1	R^2
11a	ethyl	2,4-dichlorobenzylethyl
11b	2,4-dichlorobenzyl	ethyl

Scheme 4. Reagents and conditions: a) Et_3N, acetic anhydride, reflux, 9 h.



	R^1	R^2
12a	ethyl	2,4-dichlorobenzyl
12b	2,4-dichlorobenzyl	ethyl
12c	ethyl	2,3-dichlorobenzyl
12d	ethyl	2,5-dichlorobenzyl
12e	ethyl	3,5-dichlorobenzyl
12f	ethyl	2,3,5-trichlorobenzyl
12g	ethyl	2,3,6-trichlorobenzyl
12h	ethyl	3,4-dichlorobenzyl
12i	ethyl	2,6-dichlorobenzyl
12j	2,5-dichlorobenzyl	ethyl
12k	2,3,5-trichlorobenzyl	ethyl
12l	2,3,6-trichlorobenzyl	ethyl

Scheme 5. Reagents and conditions: a) acetone, reflux, 4 h; b) MeOH, reflux, 6 h.

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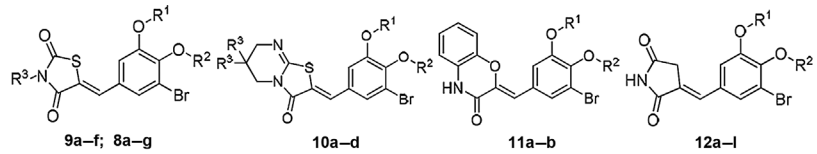
Table 1. Structures and Syk inhibitory activities of benzylidene derivatives.



Compounds	R^3	R^1	R^2	IC_{50} (μM)[a)]
9a	2-oxo-2-Phenylethyl	Ethyl	4-Carboxylbenzyl	3.6
9b	2-oxo-2-Phenylethyl	Ethyl	Benzyl	>50.0
9c	2-oxo-2-Phenylethyl	Ethyl	Methyl	7.8
9d	2-oxo-2-Phenylethyl	Ethyl	H	10.0
9e	2-oxo-2-Phenylethyl	Ethyl	4-MeOCO-benzyl	>50.0
9f	2-oxo-2-Phenylethyl	Ethyl	4-BnOCO-benzyl	>50.0
8a	H	Ethyl	4-Carboxylbenzyl	2.9
8b	H	Ethyl	Pyridin-2-yl	2.4
8c	H	Ethyl	2-Chlorobenzyl	1.6
8d	H	Ethyl	3,5-Dimethylbenzyl	2.0
8e	H	Ethyl	3,4,5-Trimethoxybenzyl	>50.0
8f	H	Ethyl	2,4-Dichlorobenzyl	0.5
8g	H	2,4-Dichlorobenzyl	Ethyl	0.4
10a	H	Ethyl	2,4-Dichlorobenzyl	4.7
10b	Methyl	ethyl	2,4-Dichlorobenzyl	>50.0
10c	H	2,4-Dichlorobenzyl	Ethyl	3.7
10d	Methyl	2,4-Dichlorobenzyl	Ethyl	>50.0
11a		Ethyl	2,4-Dichlorobenzyl	>50.0
11b		2,4-Dichlorobenzyl	Ethyl	>50.0
12a		Ethyl	2,4-Dichlorobenzyl	1.3
12b		2,4-Dichlorobenzyl	Ethyl	22.0
12c		Ethyl	2,3-Dichlorobenzyl	2.2
12d		Ethyl	2,5-Dichlorobenzyl	0.8
12e		Ethyl	3,5-Dichlorobenzyl	1.6
12f		Ethyl	2,3,5-Trichlorobenzyl	1.0
12g		Ethyl	2,3,6-Trichlorobenzyl	0.7
12h		Ethyl	3,4-Dichlorobenzyl	1.5
12i		Ethyl	2,6-Dichlorobenzyl	2.1
12j		2,5-Dichlorobenzyl	Ethyl	2.9
12k		2,3,5-Trichlorobenzyl	Ethyl	1.3
12l		2,3,6-Trichlorobenzyl	Ethyl	4.6

[a)] All data listed represent the mean of $n = 3$ independent experiments.

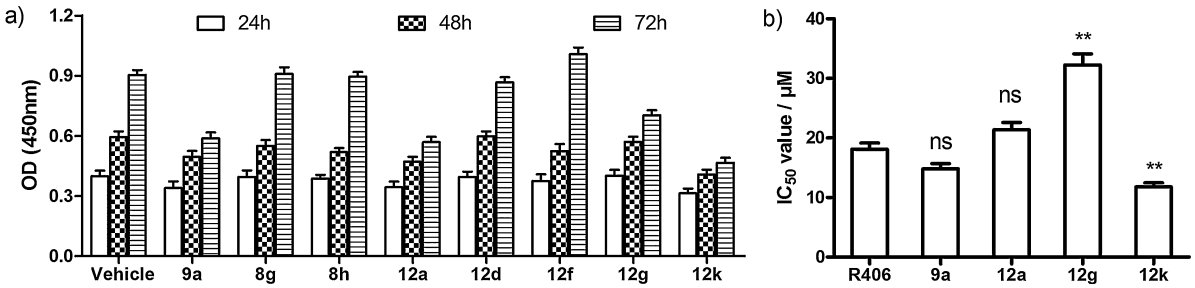


Figure 2. Effect of compounds on FLS-RA proliferation ($n = 3$). a) Preliminary test of eight compounds was determined by the difference of the absorbance with CCK-8. FLS-RA cells were cultured in 96-well plates and treated with Syk inhibitors for 24, 48, and 72 h at a concentration of 10 μM. b) The IC_{50} values were determined by MTT assay and fitted by nonlinear regression analysis to calculate. Statistical comparisons with control were performed by *t*-tests using GraphPad Prism 5.0 software. Data are the mean ± SEM, $^{**}p < 0.01$ versus R406, ns, not significant.

antiproliferative activity. From this refinement, compound **12k** which showed the highest antiproliferative activity was chosen to be a lead candidate. The levels of IL-6 and MMP-3 production suppressed by compound **12k** then were tested. IL-6 and MMP-3 play key roles in the pathogenesis of RA, and are the representative RA-related proinflammatory cytokines. *In vitro*, compound **12k** promoted the reduction of IL-6 and MMP-3 secretion from FLS-RA stimulated from by TNFα (Fig. 3). The IL-6 levels in FLS-RA cells treated with different concentrations of compound **12k** (15 μM or 50 μM) were decreased by 80.9 and 85.7%, respectively (Fig. 3a), compared with the vehicle group. Similarly, the MMP-3 levels in FLS-RA cells treated with compound **12k** (15 or 50 μM) were decreased by 42.0 and 46.6%, respectively (Fig. 3b), compared with those treated with vehicle alone. Compound **12k** demonstrated almost equal potencies for suppression of IL-6 and MMP-3 production to R406 dose dependently.

*Effects of **12k** on CIA model*

The efficient inhibitory activity of **12k** on the cell proliferation assay as well as inflammatory mediators prompted us to examine the compound for the effect on murine CIA model, which recapitulated the clinical development of human RA. Once-daily dosing was initiated on day 14 when chronic inflammation was well established, and **12k** was highly effective at reversing established ankle swelling with a dose of 10 mg/kg (Fig. 4). From macroscopic evidences, administration of **12k** ameliorated the typical signals of acute inflammation such as swelling and edema compared with vehicle group (Fig. 4a). Ankle thickness of mice was markedly reduced from day 25 through day 43 (Fig. 4b) after administration of **12k**. However, the efficacy of R406 was more potent than that of **12k**, and paws from mice treated with R406 were similar to those of disease-free mice.

Experimental

Chemistry

Synthetic starting materials, reagents, and solvents were purchased from Alfa Aesar, Acros, Adamas-beta, Energy Chemical, J&K, Shanghai Chemical Reagent Co. and TCI at the highest commercial quality and used without further purification. Melting points were determined on a SGW X-4 melting point apparatus without correction. Analytical thin-layer chromatography (TLC) was performed on HSGF 254 (150–

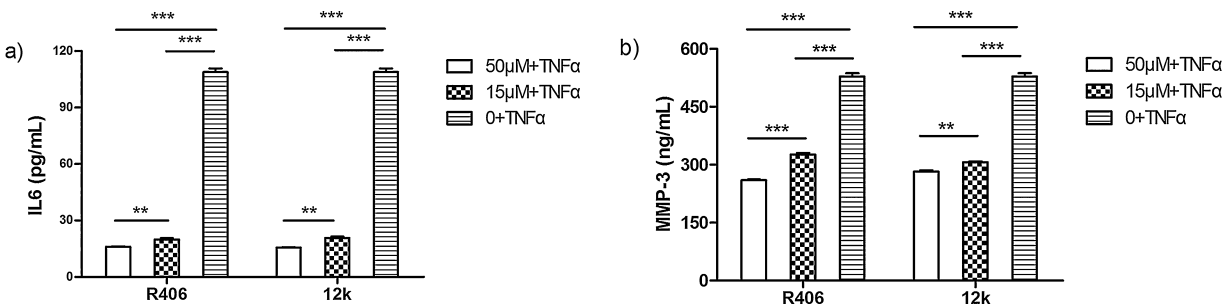


Figure 3. Effects of compound **12k** on inflammatory cytokine production ($n = 3$). a) Effects on IL-6. b) Effects on MMP-3. Inflammatory cytokines in the cultured supernatants were measured with ELISA. Data are the mean ± SEM, $^{**}p < 0.01$, $^{***}p < 0.001$ versus vehicle control.

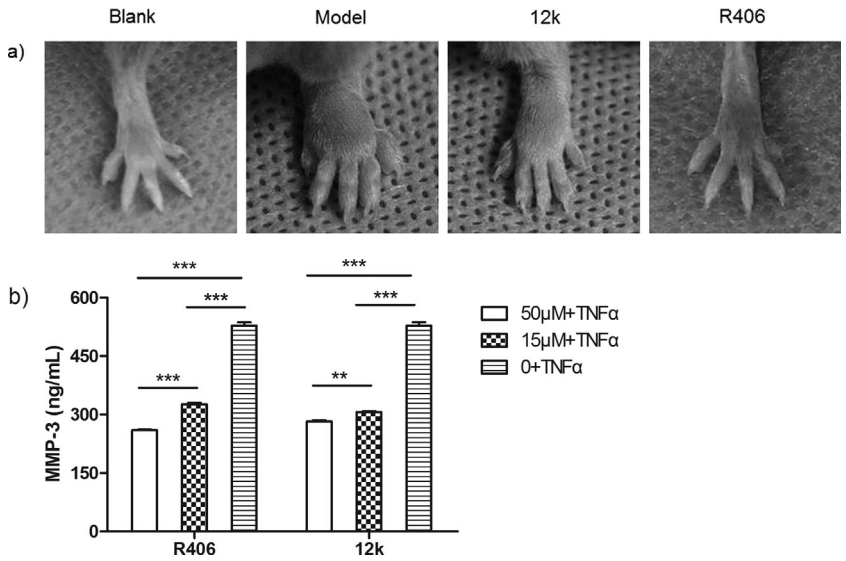


Figure 4. Effects of compound **12k** and R406 in murine collagen-induced arthritis model ($n = 5$). Collagen-induced arthritis was induced in young male DBA/1 mice as described in the Experimental section. a) Macroscopic evidences of arthritis such as edema and swelling were markedly observed in model mice (day 43), while dose of 10 mg/kg/day compound **12k** or positive control (R406) significantly attenuated arthritis severity. b) Once-daily oral dosing with vehicle, R406, or compound **12k** was commenced from days 14 to 43 after the onset of the disease. Calipers were used to measure ankle thickness every three days. Data are the mean ± SEM, $^{**}p < 0.01$, $^{***}p < 0.001$ versus vehicle control.

200 μm thickness; Yantai Huiyou Co., China), and components were visualized by observation under UV light (254 and 365 nm). The products were purified by column chromatography on silica gel (200–300 mesh). Reaction yields were not optimized.

Low- and high-resolution mass spectra (LRMS and HRMS) were given with electric and electrospray ionization (EI and ESI) produced by a Finnigan MAT-95 and a LCQ-DECA spectrometer. ^{1}H NMR spectra were performed on a Bruker AMX-400 spectrometer in $CDCl_3$ or DMSO-d_6 solutions, and chemical shifts were reported in parts per million (ppm) downfield from tetramethylsilane (TMS). Proton coupling patterns were described as singlet (s), doublet (d), triplet (t), quartet (q), doublet of doublets (dd), triplet of doublets (td), and multiplet (m). The purity of compounds used for biological evaluation was determined to be higher than 95% using Agilent-1100 with an Agilent Eclipse plus-C18 column (4.6 mm × 250 mm, 5 μM particle size, column temperature: 20°C), quaternary pump, and diode-array detector (DAD). The compounds were eluted with CH_3OH/H_2O in ratios of 80:20 or 90:10 v/v at a flow rate of 0.5 mL/min (Supporting Information Table S1).

*3-Bromo-4-ethoxy-5-hydroxybenzaldehyde (**14a**)*

(i) Bromine (2.78 mL, 54.3 mmol) was added dropwise to a solution of commercial 3,4-dihydroxybenzaldehyde **13a** (10.00 g, 72.4 mmol) in acetic acid (30 mL) at room temperature. After completion of addition, the solution was stirred for 2 h. Then, the yellow solids (7.25 g, 46%) that had formed were removed by filtration and washed using acetic acid (30 mL), which were used for the next step without further purification. (ii) Cs_2CO_3 (1.13 g, 3.4 mmol) was added to a solution of 3-bromo-4,5-dihydroxybenzaldehyde (0.50 g, 2.3 mmol) in dry DMF (10 mL), and then the mixture was heated to 40°C. After stirring over 30 min, iodoethane (0.18 mL, 2.3 mmol) was added portionwise and the mixture was stirred overnight. After cooling to room temperature, the mixture was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, filtered, and concentrated. The crude material was then purified via flash chromatography (silica gel, ethyl acetate/petroleum ether = 1:2 to 1:1) to afford **14a** as a white solid (0.20 g, 35%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.83 (s, 1H), 7.62 (d, $J = 2$ Hz, 1H), 7.42 (d, $J = 2$ Hz, 1H), 5.98 (s, 1H), 4.24 (q, $J = 7.2$ Hz, 2H), 1.48 (t, $J = 7.2$ Hz, 3H).

*3-Bromo-5-ethoxy-4-hydroxybenzaldehyde (**14b**)*

Bromine (2.32 mL, 45.2 mmol) was added dropwise to a solution of commercial 3-ethoxy-4-hydroxybenzaldehyde **13b** (10.00 g, 60.2 mmol) in acetic acid (30 mL) at room temperature. After completion of addition, the solution was stirred for 2 h. Then, the yellow solids that had formed were removed by filtration and washed with acetic acid (30 mL). After dried in vacuum, crude **14b** (7.23 g, 49%) was acquired. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.78 (s, 1H), 7.63 (s, 1H), 7.35 (s, 1H), 6.56 (s, 1H), 4.22 (q, $J = 7.0$ Hz, 2H), 1.50 (t, $J = 7.0$ Hz, 3H).

*General procedure for the synthesis of **16***

K_2CO_3 (1.5 equiv) was added to a solution of **14** (1 equiv) in dry DMF (10 mL). The mixture was stirred for 30 min, and then the commercial substituted benzyl chloride **15** (1 equiv) was added and stirred for another 4 h. Finally, the reaction mixture was poured into water (30 mL) and the precipitate was isolated by filtration, washed with water (20 mL), and dried in vacuum resulting in **16**.

*General procedure for the synthesis of **8b–g***

Thiazolidine-2,4-dione **17** (1 equiv) and piperidine (1.5 equiv) were added to a solution of **16** (1 equiv) in ethanol (15 mL), and then the solution was refluxed for 36 h. When the mixture was cooled to room temperature, the products formed were removed by filtration to give homogeneous compounds **8b–g**.

*(Z)-4-((2-Bromo-4-((2,4-dioxothiazolidin-5-ylidene)-methyl)-6-ethoxyphenoxy)methyl)benzoic acid (**8a**)*
(*Z*)-Methyl-4-((2-bromo-4-((2,4-dioxothiazolidin-5-ylidene)-methyl)-6-ethoxyphenoxy)methyl)benzoate was obtained in the manner described above. Li_2CO_3 (0.03 g, 0.4 mmol) was added to a stirred solution of methyl **8a** (0.13 g, 0.3 mmol) in the solution (10 mL) of tetrahydrofuran/methol/water (3:3:1, v/v/v). The mixture was stirred at 40°C for 4 h, and then the mixture was concentrated in vacuum and the residue resuspended in tetrahydrofuran (5 mL). The mixture was acidified to pH 3–4. The resulting brown precipitate **8a** (0.11 g, 87%) was collected by filtration and washed with water (10 mL) and air dried; mp: 275–277°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.98 (s, 1H), 12.67 (s, 1H), 7.97 (d, J = 8.2 Hz, 2H), 7.75 (s, 1H), 7.62 (d, J = 8.2 Hz, 2H), 7.41 (s, 1H), 7.28 (s, 1H), 5.16 (s, 2H), 4.16 (q, J = 6.9 Hz, 2H), 1.40 (t, J = 6.9 Hz, 3H); HRMS (EI): *m/z* [M^+] calcd. for $C_{20}H_{16}BrNO_6S$: 476.9882, found 476.9883.

*(Z)-5-(3-Bromo-5-ethoxy-4-(pyridin-2-ylmethoxy)-benzylidene)thiazolidine-2,4-dione (**8b**)*
White solid (43%); mp: 225–227°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.70 (s, 1H), 8.54 (d, J = 4.8 Hz, 1H), 7.88 (td, J_1 = 7.6 Hz, J_2 = 1.6 Hz, 1H), 7.74 (d, J = 4.8 Hz, 1H), 7.72 (s, 1H), 7.42 (d, J = 1.6 Hz, 1H), 7.39–7.35 (m, 1H), 7.30 (d, J = 2.0 Hz, 1H), 5.16 (s, 2H), 4.15 (q, J = 6.8 Hz, 2H), 1.35 (t, J = 6.8 Hz, 3H); HRMS (EI): *m/z* [M^+] calcd. for $C_{18}H_{15}BrN_2O_4S$: 435.9915, found 435.9938.

*(Z)-5-(3-Bromo-4-((2-chlorobenzyl)oxy)-5-ethoxybenzylidene)thiazolidine-2,4-dione (**8c**)*
Beige solid (60%); mp: 213°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.68–7.71 (m, 1H), 7.48–7.51 (m, 1H), 7.44 (s, 1H), 7.39–7.41 (m, 2H), 7.36 (d, 1H), 7.27 (d, 1H), 5.17 (s, 2H), 4.14 (q, 2H), 1.37 (t, 3H); HRMS (ESI): *m/z* [$M-H]^-$ calcd. for $C_{19}H_{14}BrClNO_4S$: 465.9515, found 465.9520.

*(Z)-5-(3-Bromo-4-((3,5-dimethylbenzyl)oxy)-5-ethoxybenzylidene)thiazolidine-2,4-dione (**8d**)*
Yellow solid (46%); mp: 224°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.67 (s, 1H), 7.74 (s, 1H), 7.41 (d, J = 1.6 Hz, 1H), 7.27 (d, J = 1.7 Hz, 1H), 7.11 (s, 2H), 6.98 (s, 1H), 4.97 (s, 2H), 4.16 (q, J = 6.8 Hz, 2H), 2.28 (s, 6H), 1.42 (t, J = 6.8 Hz, 3H); HRMS (EI): *m/z* [M^+] calcd. for $C_{21}H_{20}BrNO_4S$: 463.0254, found 463.0295.

*(Z)-5-(3-Bromo-5-ethoxy-4-((3,4,5-trimethoxybenzyl)oxy)-benzylidene)thiazolidine-2,4-dione (**8e**)*
Yellow solid (50%); mp: 165–167°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.67 (s, 1H), 7.74 (s, 1H), 7.41 (s, 1H), 7.28 (s, 1H), 6.80 (s, 2H), 5.04 (s, 2H), 4.17 (q, J = 6.8 Hz, 2H), 3.78 (s, 6H), 3.66 (s, 3H), 1.44 (t, J = 6.8 Hz, 3H); HRMS (EI): *m/z* [M^+] calcd. for $C_{22}H_{22}BrNO_7S$: 525.0280, found 525.0270.

*(Z)-5-(3-Bromo-4-((2,4-dichlorobenzyl)oxy)-5-ethoxybenzylidene)thiazolidine-2,4-dione (**8f**)*
Yellow solid (43%); mp: 245–247°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.75 (s, 1H), 7.69 (s, 2H), 7.51 (d, J = 7.9 Hz, 1H), 7.42 (s, 1H), 7.29 (s, 1H), 5.19 (s, 2H), 4.15 (d, J = 6.8 Hz, 2H), 1.38 (t, J = 6.8 Hz, 3H); HRMS (EI): *m/z* [M^+] calcd. for $C_{19}H_{14}BrCl_2NO_4S$: 502.9184, found 502.9200.

*(Z)-5-(3-Bromo-5-((2,4-dichlorobenzyl)oxy)-4-ethoxybenzylidene)thiazolidine-2,4-dione (**8g**)*
Yellow solid (61%); mp: 170–172°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.75 (s, 1H), 7.70–7.66 (m, 2H), 7.50 (dd, J_1 = 7.6Hz, J_2 = 1.6 Hz, 1H), 7.41 (s, 1H), 7.29 (s, 1H), 5.18 (s, 2H), 4.14 (q, J = 7.2 Hz, 2H), 1.37 (t, J = 6.8 Hz, 3H); HRMS (EI): *m/z* [M^+] calcd. for $C_{19}H_{14}BrCl_2NO_4S$: 502.9184, found 502.9167.

*General procedure for the synthesis of **9b–f***
K_2CO_3 (1.2 equiv) was added to a solution of **8** (1 equiv) in acetone (10 mL). The mixture was stirred for 15 min, and then the commercial 2-bromo-1-phenylethanone (1.5 equiv) was added and refluxed for another 8 h. The mixture was concentrated in vacuum and the residue was purified by flash column chromatography (silica gel, tetrahydrofuran/petroleum ether = 1:4) to afford **9b–f**.

*(Z)-4-((2-Bromo-4-((2,4-dioxo-3-(2-oxo-2-phenylethyl)-thiazolidin-5-ylidene)methyl)-6-ethoxyphenoxy)methyl)-benzoic acid (**9a**)*
(*Z*)-Methyl-4-((2-bromo-4-((2,4-dioxo-3-(2-oxo-2-phenylethyl)-thiazolidin-5-ylidene)methyl)-6-ethoxyphenoxy)methyl)benzoate was obtained in the manner described above. A stirred solution of methyl **9a** (0.15 g, 0.2 mmol) in hydrobromic acid (5 mL) was refluxed overnight. The mixture was concentrated in vacuum to afford **9a** (0.07 g, 52%); mp: 247–249°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.09 (d, 4H), 8.00–8.01 (m, 2H), 7.46–7.55 (m, 6H), 5.17 (s, 2H), 4.52 (d, 2H), 3.74 (q, 2H), 1.24 (t, 3H); HRMS (ESI): *m/z* [$M-H]^-$ calcd. for $C_{28}H_{21}BrNO_7S$: 594.0222, found 594.0208.

*(Z)-5-(4-(Benzyloxy)-3-bromo-5-ethoxybenzylidene)-3-(2-oxo-2-phenylethyl)thiazolidine-2,4-dione (**9b**)*
Yellow solid (71%); mp: 173–174°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.12–8.08 (m, 1H), 7.97 (s, 1H), 7.75 (m, 1H), 7.61 (t, J = 8.0 Hz, 2H), 7.53–7.49 (m, 3H), 7.44–7.34 (m, 5H), 5.34 (s, 2H), 5.12 (s, 2H), 4.20 (q, J = 6.9 Hz, 2H), 1.44 (t, J = 6.9 Hz, 3H); HRMS (EI): *m/z* [M^+] calcd. for $C_{27}H_{24}BrNO_5S$: 553.0559, found 553.0392.

*(Z)-5-(3-Bromo-5-ethoxy-4-methoxybenzylidene)-3-(2-oxo-2-phenylethyl)thiazolidine-2,4-dione (**9c**)*
Yellow solid (52%); mp: 156–158°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.00 (d, J = 7.2 Hz, 2H), 7.79 (s, 1H), 7.65 (t, J = 7.2 Hz, 1H), 7.53 (t, J = 7.2 Hz, 2H), 7.33 (d, J = 2.0 Hz, 1H), 7.00 (d, J = 2.0 Hz, 1H), 5.18 (s, 2H), 4.14 (q, J = 7.0 Hz, 2H), 3.94 (s, 3H), 1.51 (t, J = 7.0 Hz, 3H); HRMS (EI): *m/z* [M^+] calcd. for $C_{21}H_{20}BrNO_5S$: 477.0246, found 477.0068.

*(Z)-5-(3-Bromo-5-ethoxy-4-hydroxybenzylidene)-3-(2-oxo-2-phenylethyl)thiazolidine-2,4-dione (**9d**)*
Yellow solid (79%); mp: 200–201°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.32 (s, 1H), 8.09 (d, J = 7.7 Hz, 2H), 7.91 (s, 1H), 7.75 (t, J = 7.3 Hz, 1H), 7.61 (t, J = 7.5 Hz, 2H), 7.45 (s, 1H), 7.24 (s, 1H), 5.33

(s, 2H), 4.17 (q, $J = 6.8$ Hz, 2H), 1.40 (t, $J = 6.8$ Hz, 3H); HRMS (EI): *m/z* [M^+] calcd. for $C_{20}H_{18}BrNO_5S$: 463.0089, found 462.9919.

*(Z)-Methyl-4-((2-bromo-4-((2,4-dioxo-3-(2-oxo-2-phenylethyl)thiazolidin-5-ylidene)methyl)-6-ethoxyphenoxy)methyl)benzoate (**9e**)*
Brown oil (40%); ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.09 (d, 2H), 8.02 (d, 2H), 7.83 (s, 1H), 7.63–7.70 (m, 3H), 7.56 (t, 2H), 7.36 (m, 1H), 7.04 (m, 1H), 5.21 (d, 5H), 4.16 (q, 2H), 3.96 (s, 2H), 1.52 (t, 3H); HRMS (EI): *m/z* [M^+] calcd. for $C_{29}H_{24}BrNO_7S$: 611.0436, found 611.0452.

*(Z)-Benzyl-4-((2-bromo-4-((2,4-dioxo-3-(2-oxo-2-phenylethyl)thiazolidin-5-ylidene)methyl)-6-ethoxyphenoxy)methyl)benzoate (**9f**)*
Brown oil (37%); ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.12 (d, 2H), 8.02 (d, 2H), 7.82 (s, 1H), 7.66–7.70 (m, 3H), 7.56–7.64 (m, 2H), 7.39–7.50 (m, 2H), 7.36–7.38 (m, 4H), 7.03 (s, 1H), 5.40 (s, 2H), 5.12 (d, 4H), 4.16 (q, 2H), 1.51(t, 3H); HRMS (EI): *m/z* [M^+] calcd. for $C_{35}H_{30}BrNO_7S$: 687.0926, found 687.0748.

*General procedure for the synthesis of **19***
(i) Carbon disulfide (4 equiv) was added dropwise to a solution of propane-1,3-diamine or 2,2-dimethylpropane-1,3-diamine **18** (1 equiv) in ethanol (4 mL) below 40°C. The reaction was stopped when the solid was no longer produced and filtrated. (ii) The cake was dissolved in water (4 mL) and reacted for another 10 h under 40°C. The precipitate was filtered and used for the next step without further purification. (iii) Bromoacetic acid (2 equiv) was added to a solution of appropriate tetrahydropyrimidine-2(1*H*)-thione (1 equiv) in ethanol (5 mL). The reaction mixture was refluxed for 6 h. The precipitate was filtered and the cake was washed with ethanol (5 mL). The crude was extracted with dichloromethane (3 × 15 mL), washed with brine (10 mL), dried over Na_2SO_4, and concentrated to give **19**.

*General procedure for the synthesis of **10a–d***
2,4-Dichlorobenzyl substituted **16** (1 equiv) and piperidine (1 equiv) were added to a solution of **19** (1 equiv) in ethanol (10 mL), and then the solution was refluxed for 48 h. When the mixture was cooled to room temperature, the resulting precipitate was collected by filtration and washed with ethanol (10 mL) and air dried. The residue was purified by flash chromatography (silica gel, tetrahydrofuran/petroleum ether = 1:3) to afford **10a–d**.

*(Z)-2-(3-Bromo-4-((2,4-dichlorobenzyl)oxy)-5-ethoxybenzylidene)-6,7-dihydro-2H-thiazolo[3,2-a]-pyrimidin-3(5H)-one (**10a**)*
Yellow solid (70%); mp: 144–145°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.73 (d, $J = 8.0$ Hz, 2H), 7.43 (s, 1H), 7.34–7.32 (m, 2H), 7.02 (s, 1H), 5.24 (s, 2H), 4.15 (q, $J = 7.2$ Hz, 2H), 3.94 (t, $J = 4.4$ Hz, 2H), 3.81 (t, $J = 4.4$ Hz, 2H), 2.22–2.10 (m, 2H), 1.47 (t, $J = 6.8$ Hz, 3H); HRMS (EI): *m/z* [M^+] calcd. for $C_{22}H_{19}BrCl_2N_2O_3S$: 541.9656, found 541.9656.

*(Z)-2-(3-Bromo-4-((2,4-dichlorobenzyl)oxy)-5-ethoxybenzylidene)-6,6-dimethyl-6,7-dihydro-2H-thiazolo[3,2-a]pyrimidin-3(5H)-one (**10b**)*
Yellow solid (68%); mp: 145–157°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.73 (d, $J = 7.9$ Hz, 2H), 7.43 (s, 1H), 7.33 (d, $J = 5.7$ Hz, 2H), 7.02 (s, 1H), 5.23 (s, 2H), 4.15 (dd, $J_1 = 13.8$ Hz, $J_2 = 6.7$ Hz, 2H), 3.58 (s, 2H), 3.48 (s, 2H), 1.47 (t, $J = 6.9$ Hz, 3H), 1.14 (s, 6H); HRMS (EI): *m/z* [M^+] calcd. for $C_{24}H_{25}BrCl_2N_2O_3S$: 570.0146, found 569.9935.

*(Z)-2-(3-Bromo-5-((2,4-dichlorobenzyl)oxy)-4-ethoxybenzylidene)-6,7-dihydro-2H-thiazolo[3,2-a]-pyrimidin-3(5H)-one (**10c**)*
White solid (62%); mp: 146°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.76–7.71 (m, 2H), 7.43 (s, 1H), 7.33 (t, $J = 7.2$ Hz, 2H), 7.02 (s,1H), 5.23 (s, 2H), 4.15 (dd, $J_1 = 14.1$ Hz, $J_2 = 6.9$ Hz, 2H), 3.97–3.90 (m, 2H), 3.81 (dd, $J_1 = 7.8$ Hz, $J_2 = 3.1$ Hz, 2H), 2.18–2.09 (m, 2H), 1.47 (t, $J = 6.9$ Hz, 3H); HRMS (EI): *m/z* [M^+] calcd. for $C_{22}H_{19}BrCl_2N_2O_3S$: 541.9656, found 541.9642.

*(Z)-2-(3-Bromo-5-((2,4-dichlorobenzyl)oxy)-4-ethoxybenzylidene)-6,6-dimethyl-6,7-dihydro-2H-thiazolo[3,2-a]pyrimidin-3(5H)-one (**10d**)*
White solid (66%); mp: 179–180°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.82–7.75 (m, 1H), 7.73 (d, $J = 8.0$ Hz, 1H), 7.43 (s, 1H), 7.34–7.31(m, 2H), 7.02 (s, 1H), 5.24 (s, 2H), 4.15 (q, $J = 6.8$ Hz, 2H), 3.61 (s, 2H), 3.50 (s, 2H), 1.47 (t, $J = 6.8$ Hz, 3H), 1.16 (s, 6H); HRMS (EI): *m/z* [M^+] calcd. for $C_{24}H_{25}BrCl_2N_2O_3S$: 570.0146, found 569.9971.

*General procedure for the synthesis of **11a,b***
2,4-Dichlorobenzyl substituted **16** (1 equiv) and triethylamine (1 equiv) were added to a solution of commercial 2*H*-benzo[*b*]-[1,4]oxazin-3(4*H*)-one **20** (1 equiv) in acetic anhydride (3 mL). The reaction mixture was refluxed for 9 h. After stirring at room temperature overnight, the mixture was poured into crushed ice. The crude product was collected by filtration and extracted with dichloromethane (3 × 15 mL), washed with brine (10 mL), dried over Na_2SO_4, and concentrated to give **11a**,**b**.

*(Z)-2-(3-Bromo-4-((2,4-dichlorobenzyl)oxy)-5-ethoxybenzylidene)-2H-benzo[b][1,4]oxazin-3(4H)-one (**11a**)*
Beige solid (4%); mp: 225–227°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.19 (s, 1H), 7.72–7.67 (m, 3H), 7.64 (s, 1H), 7.51 (dd, $J_1 = 8.0$ Hz, $J_2 = 2.0$ Hz, 1H), 7.21–7.13 (m, 1H), 7.07–7.04 (m, 2H), 6.99 (dd, $J_1 = 6.8$ Hz, $J_2 = 2.4$ Hz, 1H), 6.75 (s, 1H), 5.15 (s, 2H), 4.18 (q, $J = 6.8$ Hz, 2H), 1.39 (t, $J = 6.8$ Hz, 3H); HRMS (ESI): *m/z* [M+Na]$^+$ calcd. for $C_{24}H_{18}BrCl_2NNaO_4$: 555.9694, found 555.9713.

*(Z)-2-(3-Bromo-5-((2,4-dichlorobenzyl)oxy)-4-ethoxybenzylidene)-2H-benzo[b][1,4]oxazin-3(4H)-one (**11b**)*
White solid (3%); mp: 213–215°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.20 (s, 1H), 7.79–7.74 (m, 2H), 7.72 (s, 1H), 7.67 (d, $J = 8.4$ Hz, 1H), 7.55 (dd, $J_1 = 8.3$ Hz, $J_2 = 2.0$ Hz, 1H), 7.09–6.96

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(m, 4H), 6.75 (s, 1H), 5.30 (s, 2H), 4.07 (q, $J = 7.2$ Hz, 2H), 1.30 (t, $J = 7.2$ Hz, 3H); HRMS (ESI): *m/z* $[M+Na]^+$ calcd. for $C_{24}H_{18}BrCl_2NNaO_4$: 555.9694, found 555.9670.

*3-(Triphenylphosphoranylidene)pyrrolidine-2,5-dione (**22**)*
Triphenylphosphine (2.70 g, 10.3 mmol) was added to a solution of maleimide (1.00 g, 10.3 mmol) in anhydrous acetone (10 mL) and refluxed for 4 h. After cooling, the precipitates formed were filtered and the filter cake was washed with cold acetone (10 mL). Drying under reduced vacuum afforded **22** (3.40 g, 92%) as a white solid. The solid **22** was used for the next step without further purification.

*General procedure for the synthesis of **12a–l***
Varied **16** (1 equiv) was added to a solution of **22** (1 equiv) in methanol (10 mL), and the solution was refluxed for 6 h. When the mixture was cooled to room temperature, the product precipitated out of the solution to afford **12a–l**.

*(E)-3-(3-Bromo-4-((2,4-dichlorobenzyl)oxy)-5-ethoxybenzylidene)pyrrolidine-2,5-dione (**12a**)*
White solid (90%); mp: 273–274°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.48 (s, 1H), 7.71–7.66 (m, 2H), 7.50 (dd, $J_1 = 8.0$ Hz, $J_2 = 2.0$ Hz, 1H), 7.44 (d, $J = 1.6$ Hz, 1H), 7.33 (t, $J = 2.0$ Hz, 1H), 7.29 (d, $J = 1.6$ Hz, 1H), 5.15 (s, 2H), 4.17 (q, $J = 6.8$ Hz, 2H), 3.71 (d, $J = 2.4$ Hz, 2H), 1.36 (t, $J = 6.8$ Hz, 3H); HRMS (EI): *m/z* $[M^+]$ calcd. for $C_{20}H_{16}BrCl_2NO_4$: 484.9619, found 484.9613.

*(E)-3-(3-Bromo-5-((2,4-dichlorobenzyl)oxy)-4-ethoxybenzylidene)pyrrolidine-2,5-dione (**12b**)*
White solid (88%); mp: 233–234°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.48 (s, 1H), 7.74 (d, $J = 2.0$ Hz, 1H), 7.66 (d, $J = 8.4$ Hz, 1H), 7.56–7.51 (m, 1H), 7.49 (s, 1H), 7.42 (s, 1H), 7.34 (s, 1H), 5.28 (s, 2H), 4.05 (q, $J = 7.0$ Hz, 2H), 3.70 (d, $J = 2.0$ Hz, 2H), 1.26 (t, $J = 7.0$ Hz, 3H); HRMS (EI): *m/z* $[M^+]$ calcd. for $C_{20}H_{16}BrCl_2NO_4$: 482.9640, found 482.9646.

*(E)-3-(3-Bromo-4-((2,3-dichlorobenzyl)oxy)-5-ethoxybenzylidene)pyrrolidine-2,5-dione (**12c**)*
White solid (91%); mp: 257–259°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.48 (s, 1H), 7.74 (d, $J = 2.0$ Hz, 1H), 7.66 (d, $J = 8.4$ Hz, 1H), 7.53 (dd, $J_1 = 8.4$ Hz, $J_2 = 2.0$ Hz, 1H), 7.49 (s, 1H), 7.42 (s, 1H), 7.34 (s, 1H), 5.28 (s, 2H), 4.05 (q, $J = 6.8$ Hz, 2H), 3.70 (d, $J = 2.0$ Hz, 2H), 1.26 (t, $J = 7.2$ Hz, 3H); HRMS (EI): *m/z* $[M^+]$ calcd. for $C_{20}H_{16}BrCl_2NO_4$: 484.9619, found 484.9621.

*(E)-3-(3-Bromo-4-((2,5-dichlorobenzyl)oxy)-5-ethoxybenzylidene)pyrrolidine-2,5-dione (**12d**)*
White solid (92%); mp: 270–272°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.48 (s, 1H), 7.60 (m, 3H), 7.46 (s, 1H), 7.34 (s, 1H), 7.30 (s, 1H), 5.08 (s, 2H), 4.18 (q, $J = 6.8$ Hz, 2H), 3.72 (d, $J = 2.0$ Hz, 2H), 1.38 (t, $J = 6.8$ Hz, 3H); HRMS (EI): *m/z* $[M^+]$ calcd. for $C_{20}H_{16}BrCl_2NO_4$: 484.9619, found 484.9622.

*(E)-3-(3-Bromo-4-((3,5-dichlorobenzyl)oxy)-5-ethoxybenzylidene)pyrrolidine-2,5-dione (**12e**)*
White solid (88%); mp: 270–271°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.48 (s, 1H), 7.77 (d, $J = 2.3$ Hz, 1H), 7.55 (d, $J = 8.6$ Hz, 1H), 7.51–7.44 (m, 2H), 7.34 (s, 1H), 7.30 (s, 1H), 5.16 (s, 2H), 4.17 (q, $J = 6.9$ Hz, 2H), 3.72 (d, $J = 1.7$ Hz, 2H), 1.36 (t, $J = 6.9$ Hz, 3H). HRMS (EI): *m/z* $[M^+]$ calcd. for $C_{20}H_{16}BrCl_2NO_4$: 484.9619, found 484.9615.

*(E)-3-(3-Bromo-5-ethoxy-4-((2,3,5-trichlorobenzyl)oxy)-benzylidene)pyrrolidine-2,5-dione (**12f**)*
White solid (85%); mp: 279°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.48 (s, 1H), 7.89 (d, $J = 2.4$ Hz, 1H), 7.76 (d, $J = 2.4$ Hz, 1H), 7.46 (s, 1H), 7.34 (s, 1H), 7.30 (s, 1H), 5.20 (s, 2H), 4.16 (q, $J = 6.8$ Hz, 2H), 3.72 (s, 2H), 1.33 (t, $J = 6.8$ Hz, 3H); HRMS (ESI): *m/z* $[M-H]^-$ calcd. for $C_{20}H_{14}BrCl_3NO_4$: 515.9172, found 515.9157.

*(E)-3-(3-Bromo-5-ethoxy-4-((2,3,6-trichlorobenzyl)oxy)-benzylidene)pyrrolidine-2,5-dione (**12g**)*
White solid (79%); mp: 279–280°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.48 (s, 1H), 7.89 (d, $J = 2.4$ Hz, 1H), 7.76 (d, $J = 2.4$ Hz, 1H), 7.46 (s, 1H), 7.34 (s, 1H), 7.30 (s, 1H), 5.20 (s, 2H), 4.16 (q, $J = 6.8$ Hz, 2H), 3.72 (s, 2H), 1.33 (t, $J = 6.8$ Hz, 3H); HRMS (ESI): *m/z* $[M-H]^-$ calcd. for $C_{20}H_{14}BrCl_3NO_4$: 515.9172, found 515.9158.

*(E)-3-(3-Bromo-4-((3,4-dichlorobenzyl)oxy)-5-ethoxybenzylidene)pyrrolidine-2,5-dione (**12h**)*
White solid (77%); mp: 258–260°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.47 (s, 1H), 7.79 (d, $J = 1.2$ Hz, 1H), 7.67 (d, $J = 8.4$ Hz, 1H), 7.49 (dd, $J_1 = 8.4$ Hz, $J_2 = 1.6$ Hz, 1H), 7.44 (s, 1H), 7.33 (s, 1H), 7.29 (s, 1H), 5.07 (s, 2H), 4.18 (q, $J = 6.8$ Hz, 2H), 3.72 (d, $J = 2.0$ Hz, 2H), 1.39 (t, $J = 6.8$ Hz, 3H); HRMS (ESI): *m/z* $[M-H]^-$ calcd. for $C_{20}H_{15}BrCl_2NO_4$: 481.9562, found 481.9546.

*(E)-3-(3-Bromo-4-((2,6-dichlorobenzyl)oxy)-5-ethoxybenzylidene)pyrrolidine-2,5-dione (**12i**)*
Beige solid (85%); mp: 271–273°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.47 (s, 1H), 7.51 (d, $J = 1.6$ Hz, 1H), 7.49 (s, 1H), 7.45–7.41 (m, 1H), 7.38 (d, $J = 2.0$ Hz, 1H), 7.32 (t, $J = 2.4$ Hz, 1H), 7.25 (d, $J = 1.6$ Hz, 1H), 5.40 (s, 2H), 4.13 (q, $J = 6.8$ Hz, 2H), 3.70 (d, $J = 2.4$ Hz, 2H), 1.37 (t, $J = 6.8$ Hz, 3H); HRMS (ESI): *m/z* $[M-H]^-$ calcd. for $C_{20}H_{15}BrCl_2NO_4$: 481.9562, found 481.9544.

*(E)-3-(3-Bromo-5-((2,5-dichlorobenzyl)oxy)-4-ethoxybenzylidene)pyrrolidine-2,5-dione (**12j**)*
White solid (78%); mp: 189–191°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.48 (s, 1H), 7.74 (d, $J = 2.4$ Hz, 1H), 7.60 (d, $J = 8.4$ Hz, 1H), 7.54–7.48 (m, 2H), 7.45 (s, 1H), 7.35 (s, 1H), 5.27 (s, 2H), 4.06 (q, $J = 6.8$ Hz, 2H), 3.71 (d, $J = 2.0$ Hz, 2H), 1.29 (t, $J = 6.8$ Hz, 3H); HRMS (EI): *m/z* $[M^+]$ calcd. for $C_{20}H_{16}BrCl_2NO_4$: 482.9640, found 482.9644.

*(E)-3-(3-Bromo-4-ethoxy-5-((2,3,5-trichlorobenzyl)oxy)-benzylidene)pyrrolidine-2,5-dione (**12k**)*
White solid (82%); mp: 230–231°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.49 (s, 1H), 7.93 (d, $J = 2.4$ Hz, 1H), 7.73 (d, $J = 2.4$ Hz,

1H), 7.52 (s, 1H), 7.46 (s, 1H), 7.36 (s, 1H), 5.32 (s, 2H), 4.06 (q, $J = 7.2$ Hz, 2H), 3.72 (d, $J = 2.0$ Hz, 2H), 1.31 (t, $J = 7.2$ Hz, 3H); HRMS (EI): *m/z* [M^+] calcd. for $C_{20}H_{15}BrCl_3NO_4$: 516.9250, found 516.9248.

*(E)-3-(3-Bromo-4-ethoxy-5-((2,3,6-trichlorobenzyl)oxy)-benzylidene)pyrrolidine-2,5-dione (**12l**)*
White solid (87%); mp: 226–227°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.48 (s, 1H), 7.81 (d, $J = 8.8$ Hz, 1H), 7.68–7.50 (m, 5H), 7.36 (s, 1H), 5.41 (s, 2H), 3.99 (q, $J = 7.2$ Hz, 2H), 3.77 (s, 2H), 1.18 (t, $J = 7.2$ Hz, 3H); HRMS (EI): *m/z* [M^+] calcd. for $C_{20}H_{15}BrCl_3NO_4$: 516.9250, found 516.9247.

Biology

Syk kinase inhibition assay
The kinase assay was performed in the buffer (50 mM HEPES pH 7.5, 0.015% Brij-35, 10 mM $MgCl_2$, 10 mM $MnCl_2$, 2 mM DTT). A 5 μL volume of tested compounds were prediluted for dose response in 384-well plates. A 10 μL volume of diluted enzyme solution was sequentially added and the assay plates were incubated at room temperature for 10 min. And then a 10 μL volume of a mixture of peptide solution containing FAM-labeled peptide (Cat. No.112396, Lot. No. P100804-XZ112396; GL Biochem, China) and ATP (Cat. No. A7699-1G, CAS No. 987-65-5; Sigma, America) was incubated at 28°C for 25 min. Reaction was stopped with the addition of 50 mM EDTA containing 25 μL of 100 mM HEPES, pH 7.5, 0.015% Brij-35, and 0.2% Coating Reagent #3, and the data were collected on a caliper. Half maximal inhibition (IC_{50}) values were calculated using a nonlinear curve fit with XLfit software.

Primary culture of FLS
FLS from synovial tissues of RA patients were purchased from Shanghai Zhonghua Biological Co. (Shanghai, China). FLS-RA cells were maintained in high-glucose Dulbecco's Modified Eagle's Medium (H-DMEM; Thermo, America) containing 10% fetal bovine serum (FBS; Gibco, America) at 37°C in humidified environment containing 5% CO_2. FLS-RA cells from a homogeneous population (phenotypes $<$1.5% CD55, and $>$94% CD68, as determined by flow cytometry) were cultured for three generations and were used for subsequent experiments.

Antiproliferation examination by CCK-8
Antiproliferation assay was evaluated using the Cell Counting Kit-8 (CCK-8). CCK-8 assays were performed according to the manufactural instructions. FLS-RA cells were seeded at a density of 2×10^3 cells/well into 96-well plates. Tested compounds were added into a 96-well plate (the final concentration was 10 μM). Meanwhile, FLS-RA cells without inhibitors were evaluated as a control. FLS-RA cells were incubated in the medium under 5% CO_2 in an incubator maintained at 37°C for 24, 48, and 72 h, respectively. Then, 10 μL of the CCK-8 was added to each well of a 96-well incubated for additional 4 h. The absorbance was measured at 450 nm by spectrophotometer readings.

Antiproliferation examination by MTT
Based on our previously established CCK-8 assays, an MTT assay was established in a similar format. All wells were incubated for 72 h at 37°C. IC_{50} values were calculated from 10-point dose–response curves using nonlinear regression analysis.

IL-6 and MMP-3 examination by ELISA
FLS-RA cells were seeded at a density of 2×10^5 cells/well into 6-well plate for 48 h, and then incubated with R406, compound **12k** or vehicle for 30 min, and following by stimulation by TNFα. After an 18 h incubation time, supernatants were collected for the determination of the Interleukin-6 (IL-6) and metalloproteinase-3 (MMP-3) levels using enzyme-linked immunoassay (ELISA) kits (Dakewe Biotech Co. Ltd., China) according to kit manufacturer's protocol.

Collagen-induced arthritis
CIA was elicited in Male DBA/1 mice (mean mass 20 g; Beijing HFK Bioscience Co. Ltd., China) by immunization with subcutaneous injection of 100 μL bovine type II collagen (Beijing Biolead Biology SCI & TECH CO. Ltd., China) emulsified with an equal volume complete Freund's adjuvant (CFA) at the base of the tail on day 1 and again on day 22. After the onset of arthritis (on day 15), the mice were randomly divided into three groups ($n = 5$): compound **12k** group (10 mg/kg), R406 group (10 mg/kg), and CIA control group (vehicle) [19]. This dose for R406 as positive control was set according to Ref. [19], in order to facilitate comparison, the dose for **12k** was also set in the same dose. Another normal 5 mice were selected as blank group. Each group was given an oral administration intragastrically using syringe. The treatment was performed once a day from days 15 to 43 after the first immunization. The progression of CIA was evaluated by ankle thickness, which was measured by a caliper.

Financial support of this research provided by the National Natural Science Foundation of China (grant nos. 21222211, 21372001, 91313303), the Program for New Century Excellent Talents in University (grant no. NCET-12-0853), the National Basic Research Program of China (grant no. 2014CB541800), and the Fundamental Research Funds for the Central Universities is gratefully acknowledged.

The authors have declared no conflict of interest.

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