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## Synthesis of 2-amino-9*H*-chromeno[2,3-*d*]thiazol-9-ones with anti-inflammatory activity *via* cascade reactions of 2-amino-3 iodochromones with amines and carbon disulfide<sup>†</sup>

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A simple and efficient synthetic approach to 2-amino-9*H*-chromeno[2,3-*d*]thiazol-9-ones *via* copper-promoted cascade reactions was developed. The reaction employed easily available 2-amino-3-iodochromones and amines as substrates and the targeting tricyclic compounds could be obtained with moderate to good yields. Even more important, several synthesized compounds exhibited potent anti-inflammatory activities, which suggested that this protocol may provide valuable hits for drug development in the future.

## Introduction

Chromones (4*H*-chromen-4-ones) are an important class of oxygen-containing heterocyclic compounds with a benzopyrone scaffold that are ubiquitous in nature, particularly in plants.<sup>1,2</sup> Chromone derivatives exhibit various pharmacological activities, such as anti-inflammatory,<sup>3,4</sup> antimicrobial,<sup>5</sup> antifungal,<sup>6</sup> antiviral,<sup>7</sup> anti-cancer,<sup>8</sup> anti-oxidant,<sup>9</sup> and so on. Chromones are also useful building blocks for constructing diverse and complex heterocycles.<sup>10–17</sup> Among the chromone containing scaffolds, tricyclic chromone fused heterocycle compounds are quite important. Some of the tricyclic compounds are recurrent drugs or drug candidates, for example, the anti-inflammatory and anti-allergic drug amlexanox (**1**),<sup>18</sup> the anti-tubercular compound **2**,<sup>19</sup> the antimicrobial compound **3**,<sup>20</sup> and the anti-tumor compounds **4** (ref. 21) and **5** (ref. 22) (Fig. 1). Although there has been extensive research on chromone-fused six-membered heterocycles, the synthesis and pharmaceutical activities of five-membered heterocycle fused chromones have been seldomly reported so far.

Thiazole is a common heterocyclic skeleton in drug molecules that exhibits various biological activities and excellent

physical and chemical properties.<sup>23</sup> Among the thiazole derivatives, 2-aminothiazoles are unique and significant in the field of bio-active compounds and drugs, such as the antibacterial drug sulfathiazole,<sup>24</sup> the antifungal drug dimazole,<sup>25</sup> the anti-tumor drug dasatinib,<sup>26</sup> and the non-steroidal anti-inflammatory drug meloxicam<sup>27</sup> (Fig. 2). Based on the broad applications of tricyclic heterocycle-fused chromone and 2-aminothiazole scaffolds in pharmaceutical researches, we aimed to synthesize novel chromone-fused 2-aminothiazole compounds and tried to discover their pharmaceutical activity from these 2-amino-9*H*-chromeno[2,3-*d*]thiazol-9-ones (Scheme 1).

2-Aminobenzothiazoles, as simple 2-aminothiazole-fused derivatives, are commonly synthesized in two ways, employing phenylthiourea<sup>28–31</sup> and 2-halogenated aniline<sup>32,33</sup> as starting materials. The synthesis of 2-amino-9*H*-chromeno[2,3-*d*]thiazol-9-ones using the first method requires the prior synthesis of the

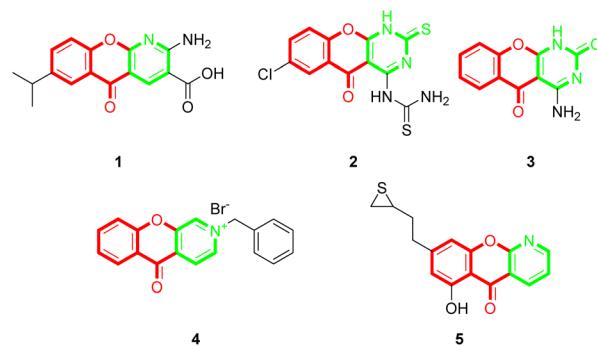


Fig. 1 Representative examples of chromone-fused tricyclic derivatives with pharmaceutical activities.

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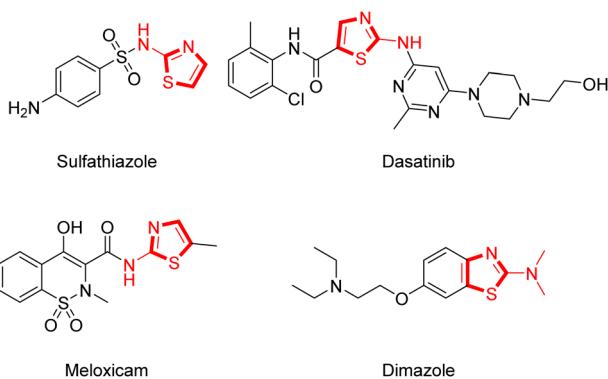
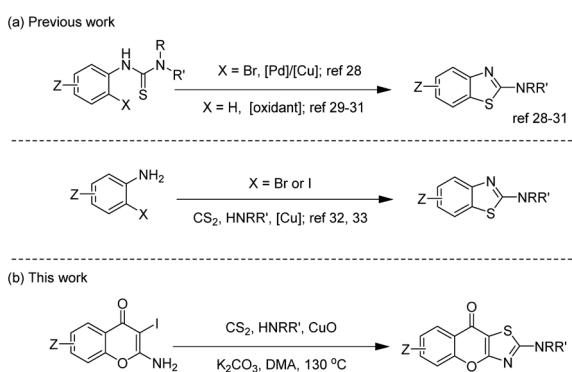


Fig. 2 Representative drugs with 2-aminothiazole scaffold.

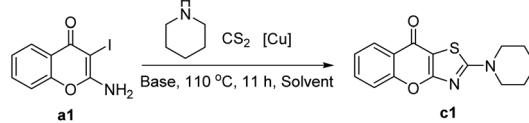


Scheme 1 (a) Synthetic strategies of 2-aminothiazole derivatives. (b) Our strategy for synthesis of 2-amino-9H-chromeno[2,3-d]thiazol-9-ones.

starting materials 1-(4-oxo-4*H*-chromen-2-yl)thioureas, which involves tedious procedures. In 2011, Ma's group<sup>32</sup> reported an efficient method for preparing 2-*N*-substituted benzothiazole derivatives through a copper-mediated three-component reaction of *ortho*-iodoanilines with carbon disulfide and amines. In 2014, inspired by Ma's work, our group reported the synthesis of 2-*C*-substituted benzothiazoles *via* a copper-promoted domino reaction.<sup>34</sup> Based on the research mentioned above, we proposed using 2-amino-3-iodo-4*H*-chromen-4-ones which are easy for preparation as substrates to synthesize 2-amino-9*H*-chromeno[2,3-*d*]thiazol-9-ones. After successfully constructing this privileged scaffold, we conducted anti-inflammatory tests due to the similar structure of amlexanox. Actually, the results showed several 2-amino-9*H*-chromeno[2,3-*d*]thiazol-9-ones had good anti-inflammatory activity.

## Results and discussion

We initially chose 2-amino-3-iodo-4*H*-chromen-4-one **a1** and piperidine **b1** as the model substrates using optimal reaction condition reported by Ma.<sup>32</sup> Although we were able to detect the target product **c1**, the yield was only 25% (Table 1, entry 1). Later, several copper salts were screened, namely  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ ,  $\text{CuO}$ ,  $\text{CuBr}_2$ ,  $\text{Cu}(\text{AcO})_2 \cdot \text{H}_2\text{O}$ ,  $\text{Cu}_2\text{O}$  and  $\text{CuI}$  (Table

Table 1 Optimization of reaction conditions<sup>a</sup>

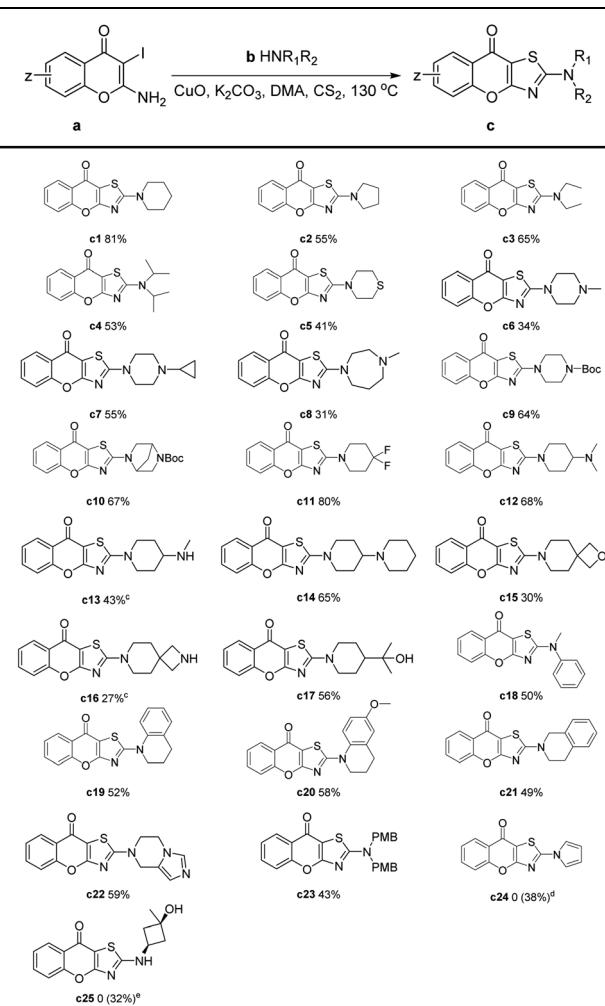
Entry	[Cu]	Base	Solvent	Yield <sup>b</sup> (%)
1	$\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$	$\text{K}_2\text{CO}_3$	DMF	25
2	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	$\text{K}_2\text{CO}_3$	DMF	15
3	$\text{CuO}$	$\text{K}_2\text{CO}_3$	DMF	50
4	$\text{CuBr}_2$	$\text{K}_2\text{CO}_3$	DMF	24
5	$\text{Cu}(\text{AcO})_2 \cdot \text{H}_2\text{O}$	$\text{K}_2\text{CO}_3$	DMF	18
6	$\text{Cu}_2\text{O}$	$\text{K}_2\text{CO}_3$	DMF	28
7	$\text{CuI}$	$\text{K}_2\text{CO}_3$	DMF	0
8	$\text{CuO}$	$\text{CS}_2\text{CO}_3$	DMF	27
9	$\text{CuO}$	$\text{Na}_2\text{CO}_3$	DMF	36
10	$\text{CuO}$	$\text{KHCO}_3$	DMF	39
11 <sup>c</sup>	$\text{CuO}$	$\text{K}_2\text{CO}_3$	DMF	49
12 <sup>d</sup>	$\text{CuO}$	$\text{K}_2\text{CO}_3$	DMF	56
13 <sup>e</sup>	$\text{CuO}$	$\text{K}_2\text{CO}_3$	DMF	59
14	$\text{CuO}$	$\text{K}_2\text{CO}_3$	NMP	43
15	$\text{CuO}$	$\text{K}_2\text{CO}_3$	DMSO	39
16	$\text{CuO}$	$\text{K}_2\text{CO}_3$	DMA	55
17 <sup>d,e</sup>	$\text{CuO}$	$\text{K}_2\text{CO}_3$	DMA	81

<sup>a</sup> Reaction conditions: **a** (0.35 mmol), **b** (0.52 mmol),  $\text{CuO}$  (0.35 mmol),  $\text{K}_2\text{CO}_3$  (1.05 mol), and  $\text{CS}_2$  (0.42 mmol) at 110 °C for 11 h. <sup>b</sup> Isolated yields. <sup>c</sup>  $T = 110$  °C, time = 1 h. <sup>d</sup>  $T = 130$  °C, time = 1 h. <sup>e</sup>  $\text{CS}_2$  (0.7 mmol), piperidine (1.05 mmol).

1, entries 2–7). Among these copper salts,  $\text{CuO}$  was optimal and provided a 50% yield of the desired compound **c1**. Subsequently, we investigated the influence of several common inorganic bases (Table 1, entries 8–10) in the reaction under the  $\text{CuO}$  condition. Compared to potassium carbonate, it was found that stronger and weaker bases all gave negative impacts on the reaction. Therefore, potassium carbonate was chosen to be the optimal base. There was only a slight change in yield when the reaction time was reduced from 11 hours to 1 hour (Table 1, entries 11). Notably, increasing the reaction temperature to 130 °C resulted in an increased yield of 56% (Table 1, entries 12). Moreover, by increasing the equivalent of carbon disulfide and piperidine (Table 1, entries 13), the yield was also obviously improved. We next screened several polar aprotic solvents (entries 14–16) and observed a slight reduction when using *N*-methylpyrrolidone (NMP) and dimethyl sulfoxide (DMSO) as solvents. In contrast, the yield was improved when the *N,N*-dimethylacetamide (DMA) was used. After combining these optimal conditions, the yield of target compound **c1** reached 81% (Table 1, entries 17).

After determining the optimal reaction conditions, we investigated the scope of the substrates by employing various amines (Table 2). From the results, most target compounds could be obtained with moderate to good yields. Compared with piperidine, the yields of pyrrolidine and alkyl amines were decreased to moderate level (Table 2, **c2–c4**). For thiomorpholine, the yield was significantly reduced to 41%. While most



Table 2 Scope of amines<sup>a,b</sup>

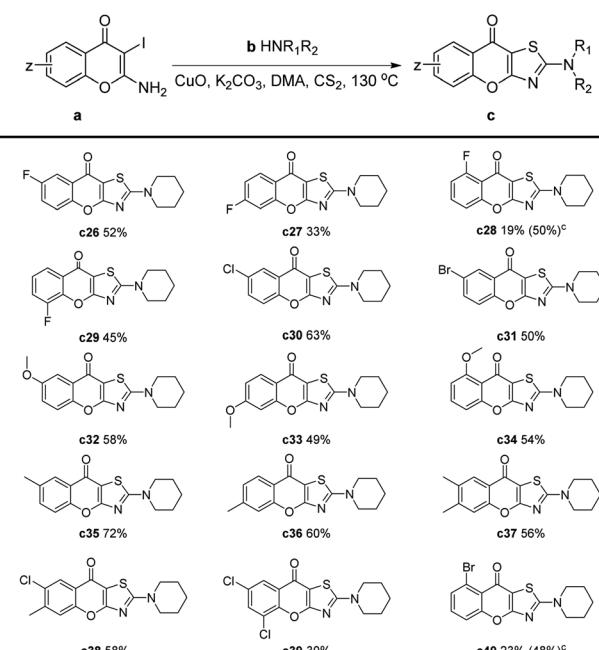
<sup>a</sup> Reaction conditions: **a** (0.35 mmol), **b** (1.05 mmol), CuO (0.35 mmol), K<sub>2</sub>CO<sub>3</sub> (1.05 mol) and CS<sub>2</sub> (0.7 mmol) in DMA (2.5 mL) at 130 °C for 1 h.  
<sup>b</sup> Isolated yields. <sup>c</sup> Total yield after deprotection of -Boc and alkalinizing. <sup>d</sup> NaOH was used instead of K<sub>2</sub>CO<sub>3</sub>. <sup>e</sup> Total yield after the deprotection of -PMB.

piperazines were smoothly transferred under the reaction condition with acceptable results (Table 2, **c6–c10**). For substituted piperidine, 4,4-difluoropiperidine gave an excellent yield of 80% (Table 2, **c11**). Meanwhile, most substituents on piperidine like amino, dimethyl hydroxyl, and phenyl resulted in moderate yields (Table 2, **c12–c14**, **c17**, **c19–c21**). However, the spiro compounds were obtained with relatively low yields (Table 2, **c15** and **c16**). This may be attributed to the instability of spiro oxacyclobutyl and azacyclobutyl groups under high temperatures with transition metal. Notably, when pyrrole was employed as the substrate, the desired compound could not be obtained. Our LC-MS analysis revealed that amount of pyrrole did not react, leading us to speculate that the relatively low density of electron clouds on the nitrogen atom of pyrrole resulted in decreased nucleophilic performance. After the replacement of potassium carbonate to sodium hydroxide, compound **c24** was synthesized with a yield of 38%. We tried to

expand substrates from secondary amines to primary amines, but only a trace amount of the target compound was detected. Nevertheless, the tandem reaction proceeded smoothly when *p*-methoxybenzyl (-PMB) was utilized as protecting group (Table 2, **c25**). After removing -PMB, the target compound **c25** was finally obtained with a total yield of 32%. In summary, the yields of different substrates in Table 2 varied due to many factors including nucleophilicity, steric hindrance and stability of the amines.

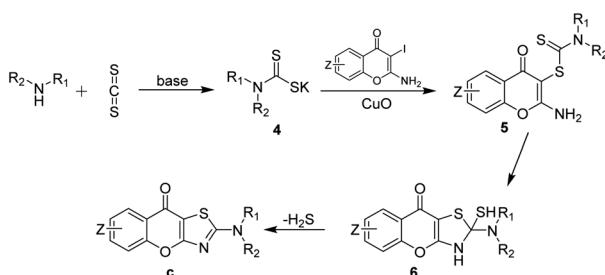
Later, we investigated the impact of different substituents on the chromones for this reaction (Table 3). The results showed that electron-withdrawing substituents, such as fluorine, chlorine, and bromine, had an adverse effect on the reaction (Table 3, **c26–c31**, **c38–c40**). Specifically, when these substituents were located at the C-5 position, the yield of -F and -Br substitution was only 19% and 23%, respectively (Table 3, **c28** and **c40**). This may be attributed to the side reaction of the amines with halogen atoms which were on the *ortho*-position of a ketone. Therefore, we performed the reaction at 90 °C and extended the reaction time to 4 hours, the yields of **c28** and **c40** were significantly increased to 50% and 48%, respectively. Substrates with an electron-donating group reacted smoothly to give products in moderate to good yields. In the cases of methyl or methoxy substituents, slightly decreased yields could be obtained compared to the unsubstituted chromones (Table 3, **c32–c37**).

According to the available literature,<sup>32,33</sup> a plausible reaction mechanism was outlined in Scheme 2. Initially, the amines react with carbon disulfide in the presence of base, resulting in the formation of dithiocarbamate salts **4**. Subsequently, the

Table 3 Scope of 2-amino-3-iodo-4H-chromen-4-ones<sup>a,b</sup>

<sup>a</sup> Reaction conditions: **1** (0.35 mmol), **2** (1.05 mmol), CuO (0.35 mmol), K<sub>2</sub>CO<sub>3</sub> (1.05 mol) and CS<sub>2</sub> (0.7 mmol) in DMA (2.5 mL) at 130 °C for 1 h.

<sup>b</sup> Isolated yields. <sup>c</sup> The reactions were performed at 90 °C for 4 hours.



Scheme 2 Proposed mechanism.

dithiocarbamate salts act as a coupling agent and participate in Ullmann coupling reaction with 2-amino-3-iodo-4*H*-chromen-4-ones **a** mediated by copper(II) oxide, leading to the generation of dithiocarbamates **5**. Then, the amino at the C-2 position of the chromones undergoes intramolecular nucleophilic addition to form intermediates **6**. Finally, the target product, chromone[2,3-*d*]thiazole derivatives **c**, are obtained by intramolecular elimination of hydrogen sulfide. To verify this hypothesis, we conducted a series of stepwise experiments. Initially, we employed piperidine, carbon disulfide, and potassium carbonate in DMA at room temperature for 0.5 hours under Ar to verify the formation of intermediate **4-1** by LC-MS. After that, we added copper oxide and **a1** to the mixture and kept the reaction at room temperature for another 12 hours. The intermediate **5-1** was also successfully detected by LC-MS.

We next tested the anti-inflammatory activity of these compounds as planned. Lipopolysaccharide (LPS) is widely used to establish inflammation models due to the ability of stimulating various cell types to release inflammatory cytokines, such as IL-1 $\beta$ , IL-6, and TNF- $\alpha$ .<sup>35</sup> We assessed our compounds on the level of inflammatory cytokine IL-1 $\beta$  on LPS induced RAW264.7 cells. Among these compounds, **c1**, **c3**, **c12**, **c14**, **c30**, and **c39** showed good anti-inflammatory activity (Table 4). The IC<sub>50</sub> values of these compounds were lower than the positive control diacerein which was known as a typical IL-1 $\beta$  inhibitor. Compound **c12** exhibited the most potent activity (IC<sub>50</sub> = 8.19  $\mu$ M) and showed excellent safety with extremely low cytotoxicity (CC<sub>50</sub> = 84.64  $\mu$ M, Fig. 3a and b). Subsequently, we further deepened the investigation of the anti-inflammatory mechanism of compound **c12**. The results showed that **c12** also performed potent suppression on the production of IL-6 (IC<sub>50</sub> =

Table 4 Inhibitory effects of 2-amino-9*H*-chromeno[2,3-*d*]thiazol-9-ones on expression levels of IL-1 $\beta$ 

Compounds	CC <sub>50</sub> ( $\mu$ M)	IC <sub>50</sub> ( $\mu$ M)	
		IL-1 $\beta$	SI
<b>c1</b>	51.40	9.64	5.33
<b>c3</b>	47.81	16.98	2.82
<b>c12</b>	<b>84.64</b>	<b>8.19</b>	<b>10.33</b>
<b>c14</b>	107.50	24.40	4.41
<b>c30</b>	>200	35.43	>5.64
<b>c39</b>	>200	22.22	>9.00
Diacerein	151.4	26.57	5.70

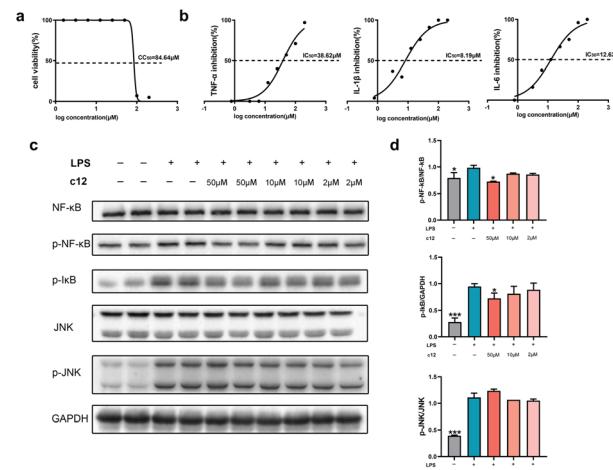


Fig. 3 **c12** inhibits the secretion of IL-1 $\beta$  and IL-6 in LPS-stimulated RAW264.7 cells by suppressing phosphorylation of NF- $\kappa$ B/I $\kappa$ B. (a) Cell viability of RAW264.7 cells cultured with **c12** for 48 h. CC<sub>50</sub> represented the concentration of **c12** that reduced cell viability to 50%. (b) Inhibition of cytokines production by **c12** in the 48 h culture of LPS-stimulated RAW264.7 cells. IC<sub>50</sub> value represented the concentration of **c12** that reduced the cytokine production by 50%. (c) Immunoblotting of NF- $\kappa$ B, p-NF- $\kappa$ B, p-I $\kappa$ B, JNK, p-JNK in RAW264.7 cells following LPS stimulation with or without **c12** treatment. (d) Protein expression levels were normalized against the indicated protein. Data are given as mean values of two independent experiments  $\pm$  SD. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001 vs. cells cultured with LPS only.

12.62  $\mu$ M) but weaker inhibition on TNF- $\alpha$  (IC<sub>50</sub> = 38.62  $\mu$ M). Furthermore, since both NF- $\kappa$ B and c-Jun N-terminal kinase (JNK) signalling are involved in regulating immune responses and the secretion of inflammatory cytokines,<sup>36</sup> we analysed the impact of **c12** on these two pathways. As shown in Fig. 3c and d, **c12** significantly inhibited the phosphorylation of NF- $\kappa$ B and I $\kappa$ B proteins rather than JNK. It meant that, compound **c12** suppressed the release of IL-1 $\beta$  by acting on a specific target through the NF- $\kappa$ B pathway and this result may provide a clue for drug development.

## Conclusions

In conclusion, we have developed a novel copper(II) oxide promoted cascade reaction of 2-amino-3-iodo-4*H*-chromen-4-ones with secondary amines and carbon disulfide in the presence of bases, leading to the formation of 2-amino-9*H*-chromeno[2,3-*d*]thiazol-9-ones. The reaction is simple and efficient, and moderate to good yields can be obtained for most tested substrates. In addition, several compounds with good anti-inflammatory activities were obtained from the synthesized molecules, especially compound **c12**. Therefore, this method can be used for constructing a compound library of natural-like molecules with pharmacological activity.

## Conflicts of interest

There are no conflicts of interest to declare.

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