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Controllable conformation and reactivity of bicyclic α-methylene cyclopentanones and their NF-κB pathway inhibitory activity†

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Tuning the electrophilicities of Michael acceptors is important for the development of targeted covalent drugs. To this end, the electronic effects of electrophilic structures have been well investigated, but not the steric effects. In this work, we synthesized ten α-methylene cyclopentanones (MCPs), screened them for NF-κB inhibitory activity, and analyzed their conformations. We found that **MCP-4b**, **MCP-5b**, and **MCP-6b** are novel NF-κB inhibitors, whereas the corresponding diastereomers **MCP-4a**, **MCP-5a**, and **MCP-6a** are inactive. Conformational analysis suggested that the stereochemistry of the side chain (R) on MCPs dictates the stable conformation of the core bicyclic 5/6 ring system. The conformational preference seemed to influence their reactivity toward nucleophiles. Consequently, a thiol reactivity assay showed that **MCP-5b** has higher reactivity than **MCP-5a**. The results indicate that the conformational switching of MCPs may control reactivity and bioactivity in the presence of steric effects.

Introduction

Tuned electrophilic fragments are valuable motifs for the development of targeted covalent drugs.[1] For example, acryl amides show moderate reactivity and are frequently used as a warhead to anchor a target protein. The acryl amide of ibrutinib acts as an electrophile that binds to Burton's tyrosine kinase.[2,3] In addition, the acryl amide of sotorasib binds to KRAS G12C-mutated protein (Fig. 1a).

It has also been reported that the reactivity of electrophilic fragments can be tuned by introducing an electron-withdrawing or -donating group[4–9] (Fig. 1b). Accumulated knowledge on the electronic effects is useful for the fine-tuning of covalent drugs. Compared with the electronic effects, however, the steric effects tuning the reactivity have not been investigated comprehensively.[10] Such steric effects were only accidentally found in some natural products and their derivatives containing sterically unique electrophiles.[11,12] In this context, we were curious to know whether the steric effect of electrophiles tunes both reactivity and biological activity.

Some electrophilic compounds are known to inhibit transcription factor nuclear factor-κB (NF-κB).[7] The phosphorylation and activation of NF-κB, which are induced by inflammatory cytokines, regulate immune responses; however, abnormal NF-κB activation is observed in various diseases including cancer.[13] Therefore, the inhibition of NF-κB activation is con-

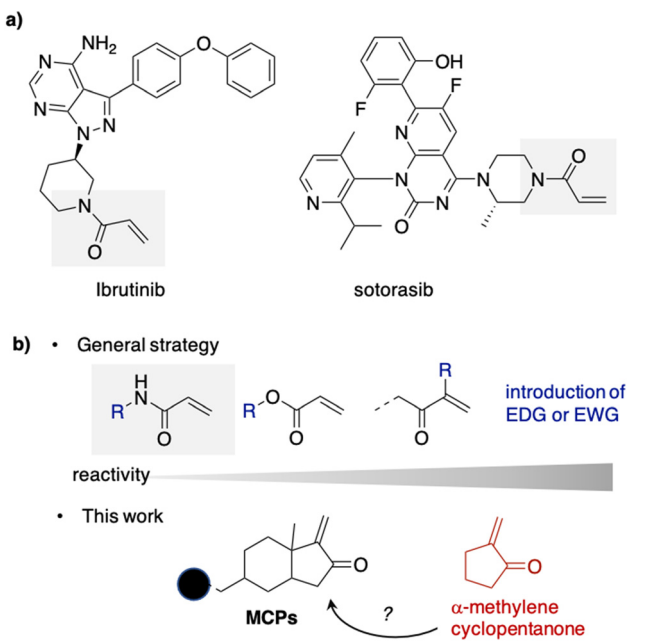

Fig. 1 (a) Approved targeted covalent drugs. (b) Tuning the reactivity of α,β-unsaturated carbonyl compounds.

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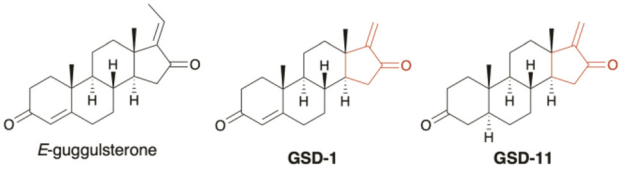


Fig. 2 Guggulsterone and its derivatives (GSDs).

sidered to be an effective strategy to suppress cancer progression.

Focusing on the NF-κB signaling and electrophilic moiety, we conducted a structure–activity relationship (SAR) study of the natural Michael acceptor guggulsterone (GS) and its derivatives (GSDs).[14–18] In the course of the screening experiments, we found that GSD-1 and GSD-11 showed the most potent NF-κB inhibitory activity at a concentration of 25 μM (ref. 19) (Fig. 2).

These compounds feature a powerful electrophilic α-methylene cyclopentanone structure that has not been further studied as a practical electrophilic fragment because of its high reactivity, in contrast to the moderately reactive acrylamide. Thus, we address the question of whether the reactivity can be controlled without any direct structural modifications of enones (Fig. 1b). Herein, we report the SARs of truncated α-methylene cyclopentanones (**MCPs**) as NF-κB inhibitors. **MCPs** are inspired by **GSD-1** and **GSD-11** and designed as non-steroidal and small derivatives bearing a hydroindane framework and an alkyl side chain. The structural simplification enables easy access to a variety of derivatives.

Results and discussion

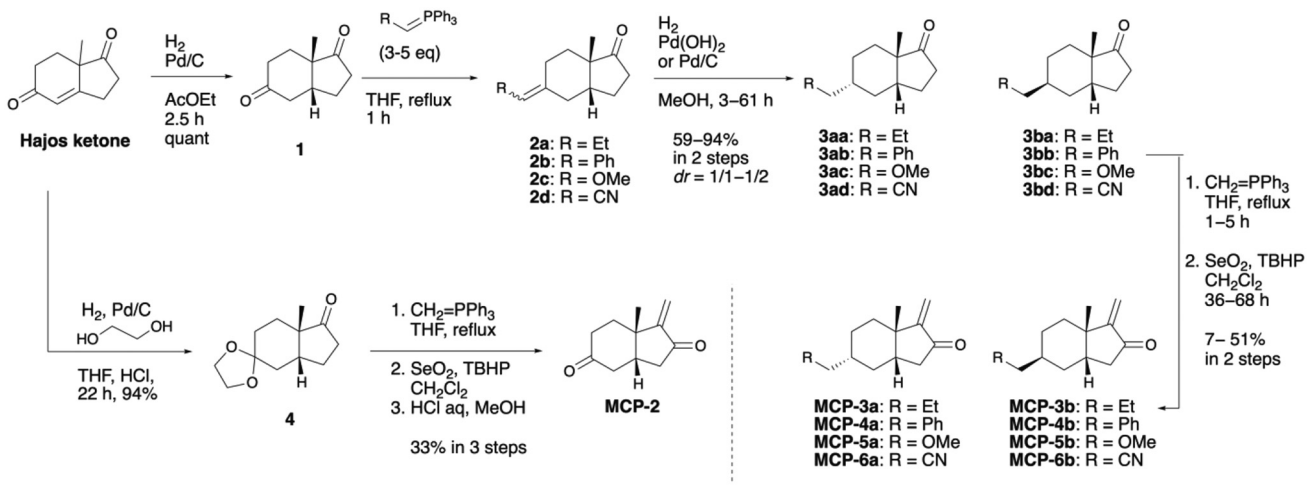
Synthesis of α-methylene cyclopentanones

The synthesis commenced with the hydrogenation of racemic Hajos ketone to afford diketone **1** (Scheme 1). The Wittig reaction of diketone **1** proceeded regioselectively to provide the corresponding alkenes **2** as a mixture of *E* and *Z* isomers, respectively. Various alkyl side chains could be introduced into **1** with the corresponding Wittig reagents. Alkenes **2** were hydrogenated to afford alkanes **3**. The two diastereomers thus formed could be separated at this stage. Finally, cyclopentanones **3aa–3ad** and **3ba–3bd** were elaborated to give **MCP-3a–6a** and **MCP-3b–6b** in a two-step sequence entailing the Wittig reaction and the following allylic oxidation. **MCP-2** without an alkyl side chain was also synthesized similarly *via* monoprotected ketone **4**.

The relative configurations of **3ac** and **3bc** were determined from the ^{1}H NMR coupling constants as follows. After the assignment of all protons on the basis of ^{1}H NMR, ^{13}C NMR, HMQC, and HMBC spectra (ESI, S-13†), the *J* values (>10 Hz) of H-3 in **3ac** and H-1 in **3bc** were analyzed and compared with those of the possible conformers, respectively (Fig. 3). H-3 in **3ac** was assigned to an axial proton in conformer **I** because its NMR signals appeared as a doublet of doublets of doublets with *J* values of 12.8, 12.5, and 12.5 Hz, requiring two axial–axial couplings. Next, H-3 in **3bc** was assigned to an axial proton in conformer **IV** because its NMR signals also appeared as a double double doublet with *J* values of 13.5, 13.5, and 13.5 Hz. In addition, the chemical shifts of both H-1 and H-3 in **3ac** appeared upfield (<1 ppm), whereas the hydrogens in **3bc** did not. These results are consistent with the fact that only **3ac** (conformer **I**) has hydrogens (H-1 and H-3) shielded by the carbonyl group in the axial direction. The two upfield hydrogens were observed only in **3aa**, **3ab**, **3ac**, **3ad**, and **MCP-3a–6a** (ESI, Fig. S2†).[20] Therefore, these distinctive chemical shifts were used to determine the relative stereochemistry of **MCPs**.

Biological evaluation

MCP-2, **MCP-3a–6a**, and **MCP-3b–6b** were screened for NF-κB inhibitory activity by evaluating the phosphorylation level of p65, the major component of NF-κB.[21] To this end, HeLa cells were stimulated with TNF-α after treatment with 25 μM test



Scheme 1 Synthesis of **MCP-2**, **MCP-3a–6a**, and **MCP-3b–6b**.

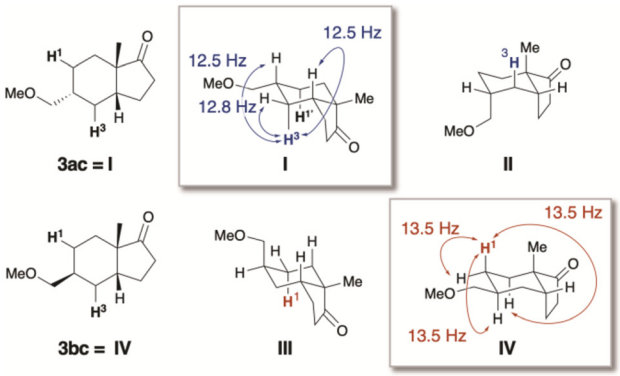


Fig. 3 Possible conformations I–IV of **3ac** and **3bc**.

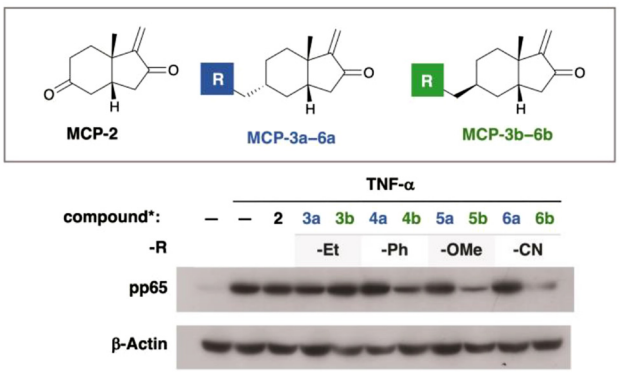


Fig. 4 **MCP-4b**, **MCP-5b**, and **MCP-6b** inhibit TNF-α-induced p65 phosphorylation. HeLa cells were pre-treated with 25 μM **MCP-3a–6a** or **MCP-3b–6b** for 30 min and then stimulated with 20 ng mL^{-1} TNF-α. Whole-cell lysates were immunoblotted with anti-phospho-p65 (Ser-536) and β-actin antibodies. *Compound names are shown without "MCP-".

compounds for 30 min. Fig. 4 shows that **MCP-4b**, **MCP-5b**, and **MCP-6b** inhibited TNF-α-induced p65 phosphorylation and the activation of p65 compared with control. On the other hand, **MCP-2** and **MCP-3b** were inactive. These observations suggest that a functionalized side chain on the cyclohexane ring is necessary for the NF-κB inhibitory activity. Interestingly, the corresponding diastereomers **MCP-4a**, **MCP-5a**, and **MCP-6a** were all inactive.

In an additional experiment, the enantiomers of the most active compounds **MCP-5b** and **MCP-6b** were synthesized from optically pure (7*S*)- or (7*R*)-Hajos ketone, respectively, and their biological activities were evaluated (ESI, Fig. S3-1†). As a result, each enantiomer of **MCP-6b** showed NF-κB inhibitory activity at the same level. On the other hand, (7*R*)-**MCP-5b** showed slightly higher activity than (7*S*)-**MCP-5b**, suggesting that the absolute stereochemistry of **MCP-5b** was distinguished in the inhibition mechanism.

Thiol reactivity assay

In the first screening, the two diastereomers of **MCP-4–6** exhibited different biological activities despite sharing the same functional groups and a two-dimensional framework. These interesting results encouraged us to investigate whether the thiol reactivity is correlated with the NF-κB inhibitory activity.[8] To compare the reactivities of two diastereomers, the electrophilic reactivities of **MCP-5a** and **MCP-5b** as Michael acceptors were evaluated in a ^{1}H NMR assay: the reaction mixtures of each compound and methyl thioglycolate were monitored by ^{1}H NMR spectroscopy (ESI, Fig. S1†). As expected, **MCP-5a** did not react even after 24 h, whereas **MCP-5b** reacted with the thiol and thiol adduct **5b** was observed after 24 h (Fig. 5).[22] These results imply that **MCP-3b–6b** have higher Michael reactivity than **MCP-3a–6a**, consistent with the trend of biological activity.

Computational analysis

To discuss these results from the perspective of conformation, the stable conformations of **MCPs** were calculated with MacroModel. The analysis classified the **MCPs** into two types of conformations, conformer A and conformer B (Fig. 6). It should be noted that the alkyl side chain is oriented to the equatorial position in both conformers. Thus, in conformer A (**MCP-3a–6a**), the methyl substituent at the ring juncture is oriented pseudo axial to the cyclopentane ring,[23] whereas in conformer B (**MCP-3b–6b**), the methyl substituent at the ring juncture is oriented pseudo equatorial to the cyclopentane ring. With this difference in mind, we consider that the β-carbon in conformer A is more hindered than the β-carbon in conformer B because the methyl substituent of conformer A appears to hamper easy access of thiol nucleophiles to β-carbon in addition to the hindered concave face. That is why the α-methylene cyclopentanone of **MCP-5b** is more reactive toward thiol than that of **MCP-5a**.

We conducted density functional theory (DFT) calculations to understand further the reaction of α-methylene cyclopentanone with thiols. All calculations were performed on the additions of methane thiolate to **MCP-5a** or **MCP-5b** using the ωB97XD/6-31+G* condition because the ωB97XD functional is reported to give more reliable values than other functionals in the calculation of the Michael addition of thiolates.[24] Fig. S5†

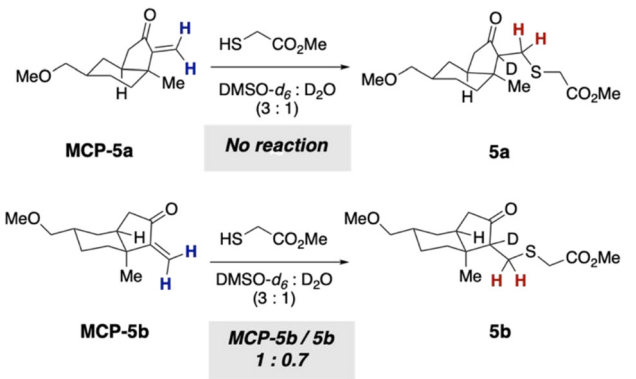


Fig. 5 Thia-Michael reaction of **MCP-5a** or **MCP-5b** with methyl thioglycolate. Highlighted protons were used to monitor the reaction.

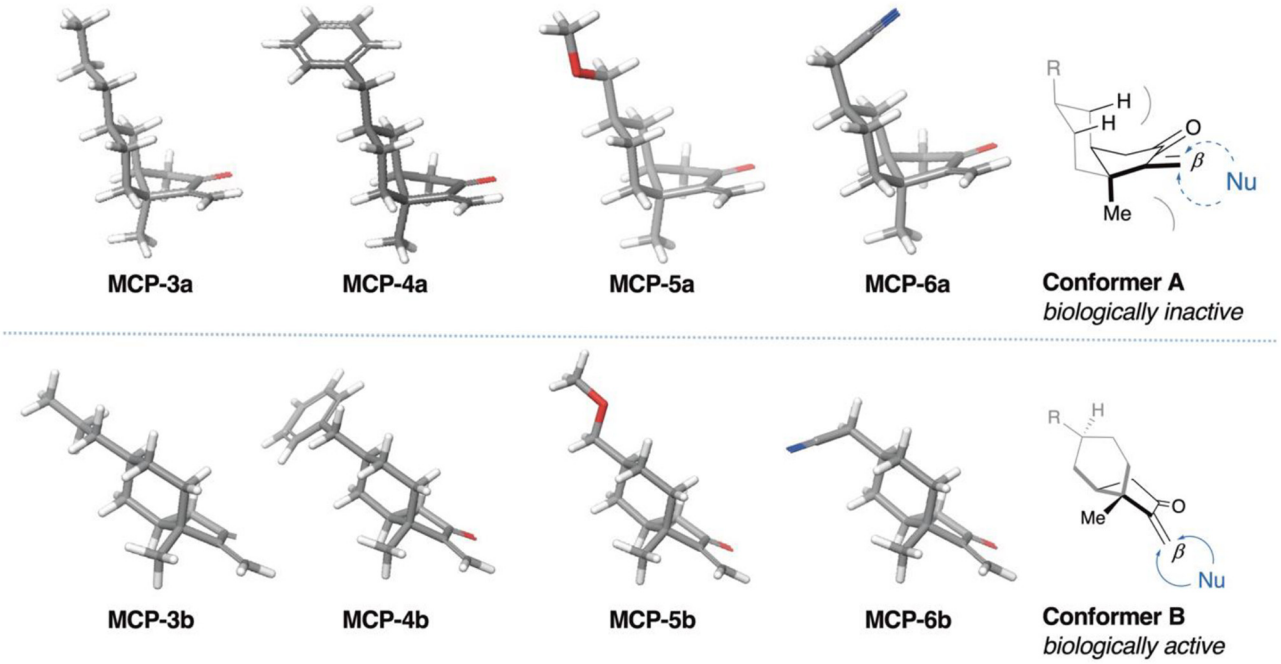


Fig. 6 Lowest energy conformers of **MCP-3a**, **MCP-4a**, **MCP-5a**, **MCP-6a**, **MCP-3b**, **MCP-4b**, **MCP-5b**, and **MCP-6b**. Conformer A and conformer B, and accessibility to the enone.

shows the free energy profile of additions of methane thiolate to Michael acceptors **MCP-5a** and **MCP-5b**. Although no significant difference was observed in the activation free energies ($\Delta G^{\ddagger}$) of **MCP-5a** and **MCP-5b**, the free energies of enolate intermediates (Int) were 1–2 kcal more stable for **MCP-5b** than for **MCP-5a**. Increased free energies of Int promote the reverse reaction to eliminate thiolate. The above results obtained from MacroModel and DFT calculations suggested that the difference in conformation between **MCP-5a** and **MCP-5b** affects the reactivity of Michael addition and the stability of enolate intermediates.

On the basis of the conformational analogy, it is assumed that other MCPs have similar thiol reactivity to **MCP-5a** and **MCP-5b**: **MCP-3a–6a** have a biologically inactive conformer A with the sterically shielded enone moiety showing low thiol reactivity. On the other hand, **MCP-3b–6b** have a biologically active conformer B with the unshielded enone moiety showing high thiol reactivity. 8-Methyl hydroindanes have two conformations,[25] and our synthesized MCPs should also have such conformational flexibility. Therefore, the stereochemistry of the side chain on *cis*-hydroindane dictates the conformation, the steric environment of the enone moiety, the thiol reactivity, and the NF-κB inhibitory activity. In short, the biological activity of MCPs can be controlled by the relative configuration of the side chain with minor exceptions.

Conclusions

In conclusion, we identified **MCP-4b**, **MCP-5b**, and **MCP-6b** as novel NF-κB inhibitors. Importantly, their corresponding diastereomers **MCP-4a**, **MCP-5a**, and **MCP-6a** were inactive. Spectral analysis identified the [5,6]-bicyclic conformation of MCPs as being controllable by the stereochemistry of the side chain. The differences between the two diastereomers involved their reactivity toward thiols, which contributed to the NF-κB inhibitory activity. These comprehensive results suggest the possibility of utilizing MCPs as controllable electrophilic fragments by tuning the steric environment.

Author contributions

A. K. supervised the project, prepared the manuscript, and performed the NMR assay and conformational analysis. A. S. performed the chemical syntheses and experimental data collection. Y. Z. performed the biological experiments and the experimental data collection. M. T. performed the computational analysis and the experimental data collection. K. S. performed the NMR assay and the experimental data collection. H. S. was responsible for research activity planning and execution. Y. M. supervised the project and conceived the manuscript.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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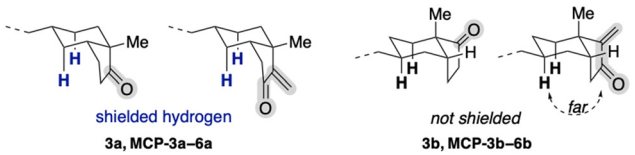

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