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Bioorganic & Medicinal Chemistry 16 (2008) 3941–3958
Bioorganic & Medicinal Chemistry

Novel cyclohexene derivatives as anti-sepsis agents: Synthetic studies and inhibition of NO and cytokine production

Masami Yamada,* Takashi Ichikawa, Masayuki Ii, Katsumi Itoh,† Norikazu Tamura and Tomoyuki Kitazaki

Pharmaceutical Research Division, Takeda Pharmaceutical Co., Ltd, 2-17-85, Jusohonmachi, Yodogawa-ku, Osaka 532-8686, Japan

Received 6 December 2007; revised 16 January 2008; accepted 17 January 2008
Available online 24 January 2008

Abstract—In order to develop an anti-sepsis agent, a series of cyclohexene derivatives were synthesized and evaluated for their biological activities. Through modification of the sulfonamide spacer moiety depicted by formula **II**, it was found that the benzylsulfone derivative **10a** had potent inhibitory activity against the production of NO. Further modifications of the phenyl ring, ester moiety, and benzyl position of benzylsulfone derivatives **III** were carried out. Among these compounds, (*R*)-(+)-**10a** and (6*R*, 1*S*)-(+)-**22a** showed strong inhibitory activity not only against NO production but also against inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) in vitro. Furthermore, (*R*)-(+)-**10a** and (6*R*, 1*S*)-(+)-**22a** protected mice from LPS-induced lethality in a dose-dependent manner.
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1. Introduction

Sepsis and septic shock are the main causes of death in intensive care units.[1] The incidence of sepsis has increased along with the higher occurrence of chronic disease states, permanent catheters, and resistance to antimicrobial agents, among other causes.[2] The condition of sepsis is caused by bacterial infection, which prompts the host immune cells to activate and stimulate the production of various types of mediators.[3] Although a number of inflammatory modifiers have been developed for the treatment of sepsis over the last 20 years, most have not demonstrated significant efficacy in clinical trials.[4] In 2001, Drotrecogin-α (Xigris™),[5] a recombinant human activated protein C, was launched onto the market as the first anti-sepsis agent; however, the usefulness of this drug is limited due to its efficacy and safety issue. Therefore, there is still a major unmet medical need for more effective and safer new anti-sepsis agents. We have recently reported the synthesis of novel cyclohexene derivatives and their inhibitory activities of nitric oxide (NO) and cytokine production in vitro.[6a] Among these derivatives, ethyl (6*R*)-6-[*N*-(2-chloro-4-fluorophenyl)sulfamoyl]cyclohex-1-ene-1-carboxylate [TAK-242, (*R*)-**1**] was found to exhibit potent inhibitory activity for the production of not only NO but also of various cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) in vitro and in vivo including potent protective effects in the mouse endotoxin shock model.[6] This compound was selected as a clinical candidate for development as a new class of anti-sepsis agent.

As an extension of our study on the cyclohexene derivatives depicted by the general formula **I** in Figure 1, we next focused on the synthesis of analogs with substitutions of the *N*-phenylsulfamoyl moiety, such as phenylsulfonylamino, phenyloxysulfonyl, benzylsulfide, and benzylsulfone derivatives with the general formula **II**. Furthermore, we planned the substitution of various (hetero)aryl groups (Ar), modification of ester moiety (Z) and introduction of substituents (R^1) at the benzyl position of benzylsulfone derivatives with the general formula **III**. Compounds of type **III** were expected to exhibit potent activity because of their structural similarity to compounds of type **I**.

We herein describe the synthesis, biological activities, and structure–activity relationships (SAR) of new cyclohexene derivatives, **II** and **III**.

Keywords: Sepsis; Cyclohexene; Benzylsulfone; TAK-242.
* Corresponding author. Tel.: +81 6 6300 6651; fax: +81 6 6300 6306; e-mail: Yamada_Masami1@takeda.co.jp
† Present address: Kyoto Factory, Riken Vitamin Co. Ltd, 1-2 Kawarajiri-Higashikakiuchi, Kawarabayashicho, Kameoka, Kyoto 621-0007, Japan.

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doi:10.1016/j.bmc.2008.01.030

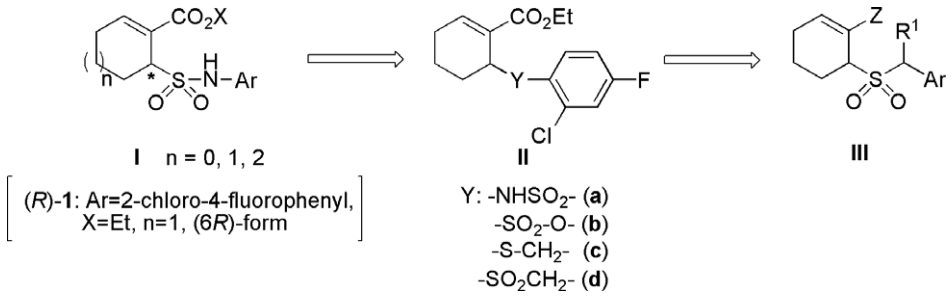


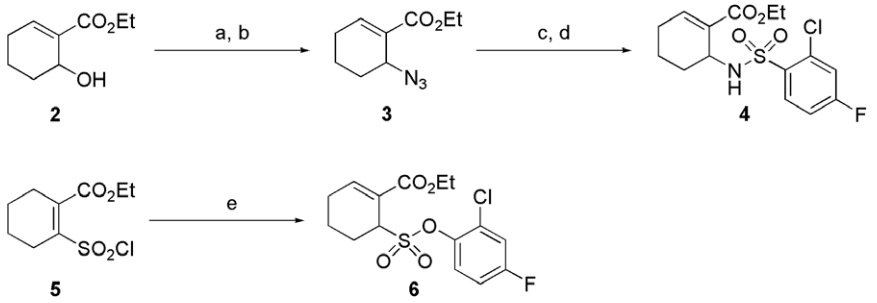
Figure 1. Design of cyclohexene derivatives with a sulfone linker.

2. Chemistry

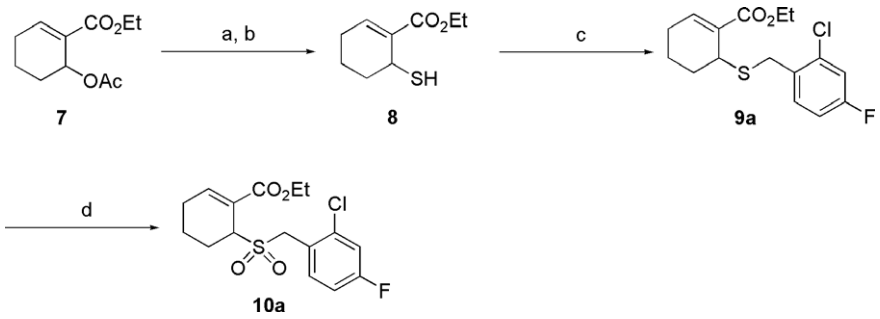
First, in order to examine the effect of linker (Y) on the inhibition of NO production, we chose functional groups such as sulfonyl or sulfide in replacing the sulfonamide moiety of compound **I**. The synthesis of phenylsulfonylamino and phenyloxysulfonyl derivatives is shown in Scheme 1. Bromination of hydroxy cyclohexene **2**[7] by Villieras' method[8] followed by reaction with sodium azide in *N*,*N*-dimethylformamide (DMF) afforded azide **3**. Subsequent reduction of **3** with zinc powder under acidic conditions gave the amine, which was treated with 2-chloro-4-fluorobenzenesulfonylchloride in the presence of triethylamine (Et_3N) and pyridine in tetrahydrofuran (THF) to give phenylsulfonylamido product **4**. The phenyloxysulfonyl derivative **6** was prepared by coupling of sulfonyl chloride **5**[6a] with 2-chloro-4-fluorophenol in the presence of 1,4-diazabicyclo[2.2.2]octane (DABCO).

The synthetic route for sulfide and sulfone derivatives is shown in Scheme 2. Thioacetylation of the acetoxy group of **7** followed by deacetylation under the acidic conditions gave thiol **8**. Sulfide **9a** was prepared by the coupling reaction of thiol **8** and 2-chloro-4-fluorobenzylbromide. Subsequently, the sulfide was oxidized with *m*-chloroperbenzoic acid (*m*CPBA) to yield the sulfone **10a**.

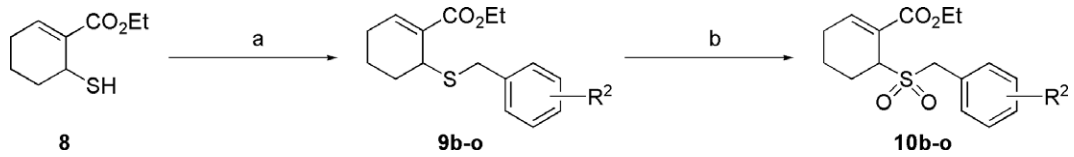
Substitution on the phenyl ring in compounds of type **III** (Z = CO_2Et, R^1 = H) was performed according to the synthetic route shown in Scheme 3. The target benzylsulfone derivatives **10b–o** were prepared by oxidation of the corresponding sulfides **9b–o** using *m*CPBA. Compounds **9b–o** were synthesized by the condensation of thiol **8** and benzylbromides **11b–o** in the presence of an appropriate base [Et_3N, 1,8-diazabicyclo [5.4.0]undec-7-ene (DBU) or potassium carbonate (K_2CO_3)].



Scheme 1. Reagents: (a) NBS, PPh_3, CH_2Cl_2 (48%); (b) NaN_3, DMF (48%); (c) Zn, HCl, acetone; (d) 2-chloro-4-fluorobenzenesulfonylchloride, pyridine, Et_3N, THF (23%, 2 steps); (e) 2-chloro-4-fluorophenol, DABCO, AcOEt (5.3%).



Scheme 2. Reagents: (a) AcSK, Et_3N (81%); (b) 4 N HCl/AcOEt, EtOH (94%); (c) 2-chloro-4-fluorobenzylbromide, DBU, DMF (87%); (d) mCPBA, AcOEt (88%).

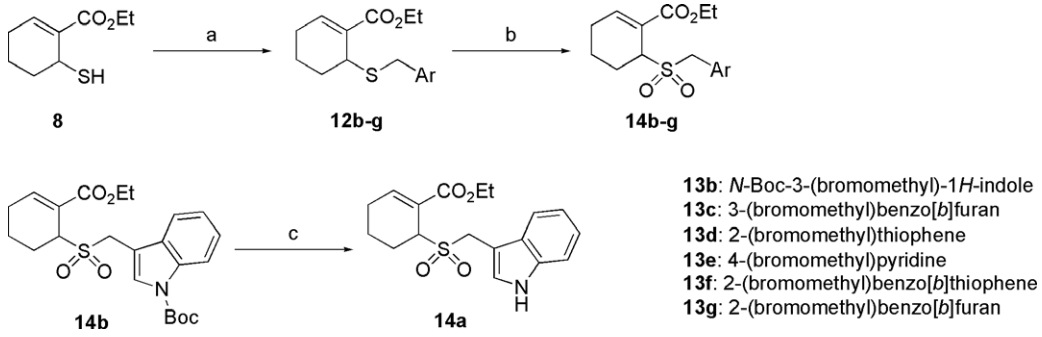


Scheme 3. Reagents: (a) $BrCH_2$–Ar **(11b–o)**, base; (b) mCPBA, AcOEt.

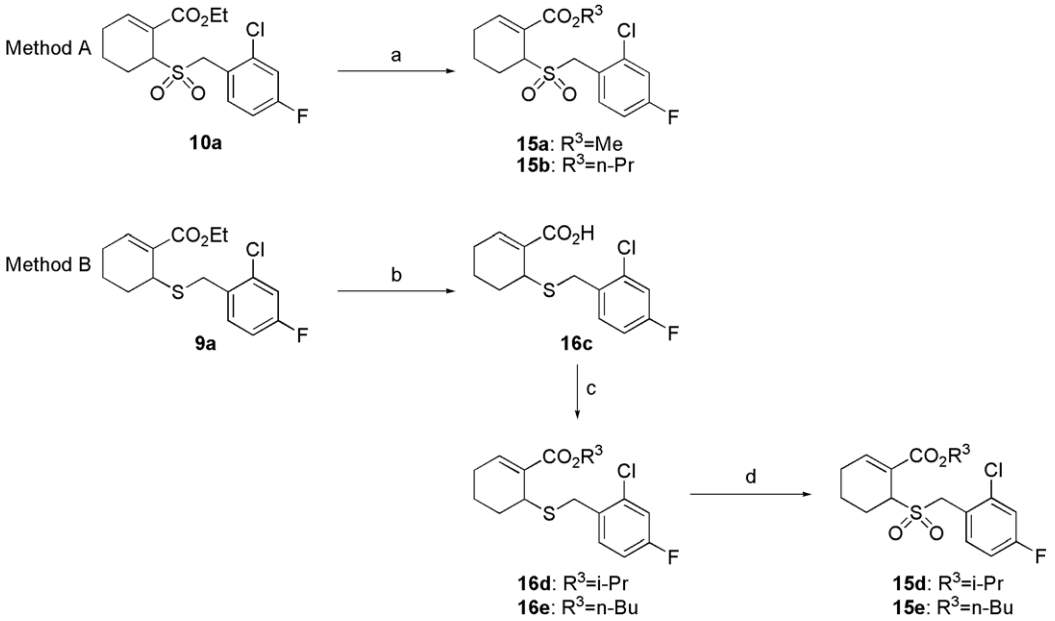
Sulfones containing a heteroaryl ring in place of the phenyl ring were synthesized as shown in Scheme 4. As described above, the condensation between thiol **8** and heteroarylmethylbromides **13b–g**[9–13] was carried out under the presence of DBU to give sulfides **12b–g**. Oxidation with *m*CPBA yielded the heteroarylmethyl sulfones **14b–g**. The deprotection of **14b** was achieved with trifluoroacetic acid (TFA) to give indole derivative **14a**.

Next, modification of the ester group (Z) in benzylsulfone derivatives of type **III** was carried out as outlined in Schemes 5 and 6. The esters **15a** and **15b** were prepared by transesterification of **10a** with the appropriate alcohol in the presence of a catalytic amount of concentrated sulfuric acid (Method A). The other esters were synthesized from **9a** in three steps: ester hydrolysis, followed by Mitsunobu reaction with the appropriate alcohol, and subsequent oxidation of sulfides **16d** and **16e** to give **15d** and **15e**, respectively (Method B, Scheme 5).

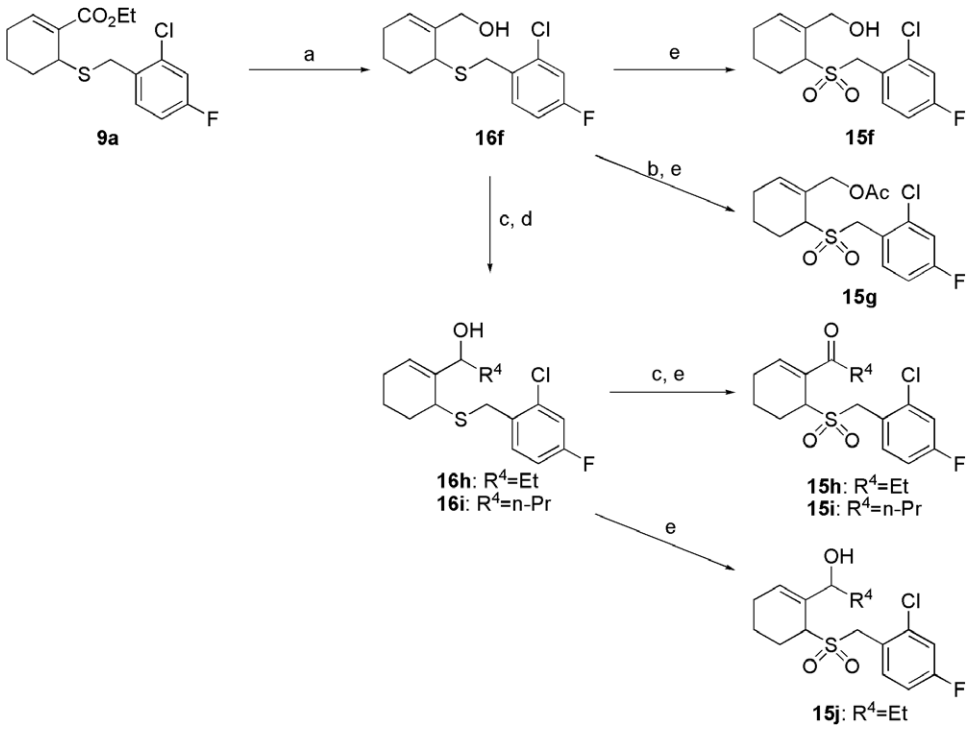
Conversion of the ester moiety to hydroxymethyl or ketone is shown in Scheme 6. Reduction of sulfide **9a** with diisobutylaluminum hydride (DIBAL-H) gave the hydroxymethyl derivative **16f**, which was oxidized with



Scheme 4. Reagents: (a) heteroarylmethylbromides (**13b–g**); DBU, DMF; (b) mCPBA, AcOEt; (c) TFA (74%).



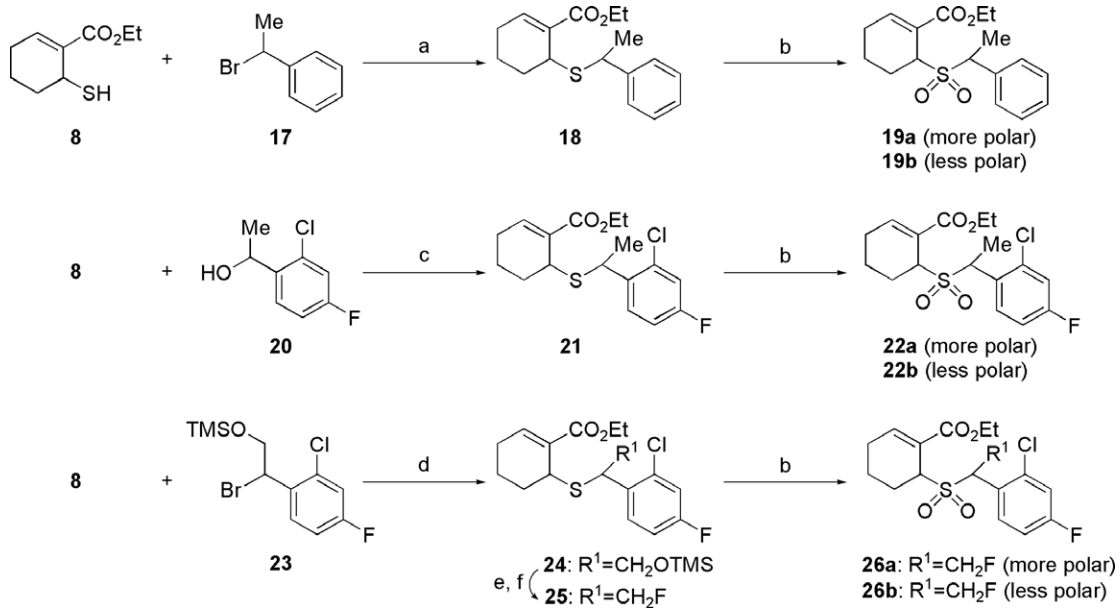
Scheme 5. Reagents and condition: (a) R^3OH, concd H_2SO_4; (b) 1 N NaOH, EtOH, 40 °C (58%); (c) R^3OH, DEAD, PPh_3, EtOH-THF; (d) mCPBA, AcOEt.



Scheme 6. Reagents: (a) DIBAL-H, CH_2Cl_2 (86%); (b) AcCl, Py; (c) TPAP, NMO, CH_2Cl_2; (d) R^4MgBr, THF; (e) mCPBA, AcOEt.

*m*CPBA to prepare the sulfone derivative **15f**. On the other hand, the reaction of **16f** with acetyl chloride followed by oxidation gave acetoxy derivative **15g**. Furthermore, oxidation of **16f** with tetra-*n*-propylammonium perruthenate (TPAP) followed by Grignard reaction with ethyl or *n*-propylmagnesium bromide gave **16h** and **16i** as a diastereomeric mixture, respectively. Each of these compounds was oxidized with TPAP and *m*CPBA in two steps to provide ketones **15h** and **15i**. 1-Hydroxypropyl compound **16h** was also converted into sulfone **15j** by *m*CPBA oxidation.

Compounds possessing an alkyl group in the benzyl position of compound type **III** (Z = CO_2Et) were synthesized as described in Scheme 7. Condensation of thiol **8** and (1-bromoethyl)benzene (**17**) according to the conditions shown above in Scheme 2 gave sulfide derivative **18** as a diastereomeric mixture. On the other hand, the



Scheme 7. Reagents: (a) DBU, DMF (61%); (b) mCPBA, AcOEt; (c) $Bu_3P{=}CHCN$, toluene; (d) Et_3N, DBU, DMF (23%); (e) 6 N HCl, MeOH (89%); (f) DAST, CH_2Cl_2 (55%).

condensation of **8** and 1-(2-chloro-4-fluorophenyl)ethanol (**20**) was successfully carried out under Mitsunobu conditions with cyanomethylenetributylphosphorane (CMBP) to give sulfide **21**. The reaction of **8** and α-substituted benzylbromide **23** proceeded under the basic conditions to give sulfide **24**. Furthermore, **24** was treated with acid followed by diethylaminosulfurtrifluoride (DAST) to afford α-fluoromethyl sulfide **25**. The sulfides **18**, **21**, and **25** thus prepared were oxidized in the usual manner with *m*CPBA to afford the sulfone derivatives **19a**, **19b**, **22a**, **22b**, **26a**, and **26b**, which were isolated as a single diastereomer through each separation of a diastereomeric mixture using silica gel column chromatography.

3. Results and discussion

The compounds were evaluated for their suppressive activity against NO production using a murine macrophage cell line, RAW264.7 induced by LPS as the primary in vitro screen. The results are summarized in Tables 1–6 as IC_{50} values.

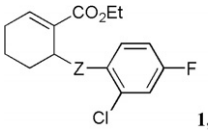
We initially examined the effect of the linker between cyclohexene ring and 2-chloro-4-fluorophenyl ring. As shown in Table 1, the aminosulfone (–$NHSO_2$–) derivative **4** showed a loss of potency. Changing to the sulfonyloxy (–SO_2–O–) or sulfanylmethyl (–S–CH_2–) linker (compounds **6** and **9a**, respectively) resulted in a remarkable decrease in activity. In contrast, benzylsulfone compound **10a** with the sulfonylmethyl linker (–SO_2CH_2–) showed clear activity (IC_{50} = 10 nM) compared with that for the sulfonamide linker (–SO_2NH–). We therefore selected the benzylsulfone type of compound as our next synthetic target, and investigated the effect of the substitution on the phenyl ring of **10a**.

As shown in Table 2, removal of the two halogen atoms on the phenyl ring resulted in a 10-fold decrease in potency (**10b**, IC_{50} = 101 nM). Introduction of a chlorine atom at the 2- or 3-position on the phenyl ring resulted in a sevenfold enhancement in potency (**10e**, **f**) with an IC_{50} value of 14 nM, while 4-chloro derivative **10g** did not show increased activity compared to unsubstituted compound **10b**. In the case of 2,3-, 3,4- and 2,4-dichlorinated derivatives (**10i**, **j**, **k**), all compounds showed a twofold improvement in potency compared to **10b**, but these potencies were less than those of 2- and 3-chloro derivatives (**10e**, **f**). Substitution at the 2-position of the phenyl ring of **10b** with a fluorine atom did not increase potency (**10d**). Introduction of a fluorine atom at the 3-position also did not influence activity, as shown by the IC_{50} value of 2,3-difluorinated derivative **10o** being nearly equal to that of 2-fluorinated compound **10d**. In contrast, the potency of 2,4-difluorophenyl derivative **10h** showed sevenfold stronger potency than that of 2-fluorinated compound **10d**, indicating that introduction of a fluorine atom at the 4-position was preferred for activity. 3-Chloro-4-fluoro and 3-chloro-2-fluoro compounds (**10l**, **10m**) were equipotent to **10a**. On the other hand, introduction of chlorine at the 4-position of the 2- or 3-halogenophenyl ring reduced potencies (**10c**, **j**, **k**). Comparison of the substituted phenyl derivatives indicated that the compounds having 2, 4-dihalogeno or 3-chloro substituents on the phenyl ring had strong activity. Overall, these results suggest that inhibitory potency depends significantly on the position and electronic properties of the phenyl ring substituents-nearly identical to our findings for the sulfonamide compounds.[6a]

Subsequently, we investigated the replacement of phenyl ring moiety with the heteroaryl groups shown in Table 3. All heteroaryl compounds (**14a**, **c**–**g**) exhibited lower potencies than the benzyl derivative **10a**.

Next, modification of the ester group on the cyclohexene ring of 2-chloro-4-fluorophenyl derivatives was carried out (Table 4). Replacement of the ethyl ester group with other alkyl esters resulted in a 10-fold reduction in activity, although *n*-propyl ester **15b** maintained potency comparable with **10a**. Preparation of reduced compounds **15f**, **15g**, and **15j** led to a substantial loss of activity (IC_{50} = >10,000 nM), even though the effect of reducing the ethyl ester into the hydroxymethyl derivatives on inhibitory activity was of interest to us. On the other hand, ketones **15h** and **15i**, though slightly weaker, exhibited potencies comparable to that of **10a** (**15h**, IC_{50} = 64 nM; **15i**, IC_{50} = 11 nM). Thus, it would

Table 1. Physicochemical properties and inhibitory activities against NO production of compounds **1**, **4**, **6**, **9a**, and **10a**

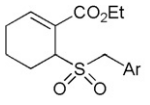


1, 4, 6, 9a and 10a

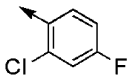
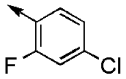
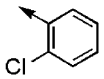
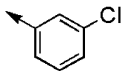
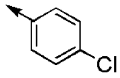
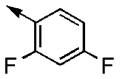
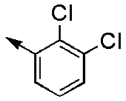
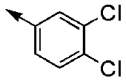
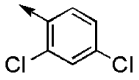
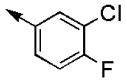
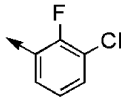
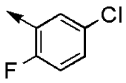
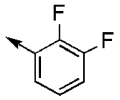
Compound	Z	IC_{50}[a] (nM)	Mp (°C) (solv.)	Formula	Anal.[b]
1	–SO_2NH–	4.5	112–113	$C_{15}H_{17}ClFNO_4S$	CHN
4	–$NHSO_2$–	>10,000	127.0–128.0 (hexane)	$C_{15}H_{17}ClFNO_4S$	CHN
6	–SO_2O–	904	Amorphous	$C_{15}H_{16}ClFO_5S$	—
9a	–SCH_2–	5645	Oil	$C_{16}H_{18}ClFO_2S$	—
10a	–SO_2CH_2–	10	110.0–111.0 (hexane)	$C_{16}H_{18}ClFO_4S$	CH

[a] Inhibitory activities are shown as IC_{50} values, the concentration of test compound required to suppress the production of NO by 50% of control.
[b] All compounds gave satisfactory elemental analysis (0.4%) for C, H, and N.

Table 2. Physicochemical properties and inhibitory activities against NO production of compounds **10a–o**

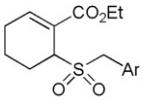


10a-o

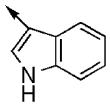
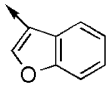
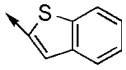
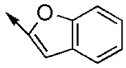
Compound	Ar	IC_{50}[a] (nM)	Mp (°C) (solv.)	Formula	Anal.[b]
10a		10	110–111 (hexane)	$C_{16}H_{18}ClFO_4S$	CH
10b		101	44.5–45.5 (hexane)	$C_{16}H_{20}O_4S{\cdot}1/2H_2O$	CH
10c		104	88–89 (hexane)	$C_{16}H_{18}ClFO_4S$	CH
l0d		97	77–78 (*i*-Pr_2O–hexane)	$C_{16}H_{19}FO_4S$	CH
l0e		14	58–59 (AcOEt–hexane)	$C_{16}H_{19}ClO_4S$	CH
l0f		14	61–62 (*i*-Pr_2O–hexane)	$C_{16}H_{19}ClO_4S$	CH
l0g		101	74.5–76 (hexane)	$C_{16}H_{19}ClO_4S$	CH
l0h		14	97.5–98.5 (AcOEt–hexane)	$C_{16}H_{18}F_2O_4S$	CH
l0i		65	116–117 (hexane)	$C_{16}H_{18}Cl_2O_4S$	CH
10j		53	89–91 (*i*-Pr_2O–hexane)	$C_{16}H_{18}Cl_2O_4S$	CH
10k		60	104–105 (hexane)	$C_{16}H_{18}Cl_2O_4S$	CH
10l		13	Oil	$C_{16}H_{18}ClFO_4S$	—
10m		12	90–91 (*i*-Pr_2O–hexane)	$C_{16}H_{18}ClFO_4S$	CH
l0n		23	75.5–76.5 (*i*-Pr_2O–hexane)	$C_{16}H_{18}ClFO_4S$	CH
l0o		100	84–85 (*i*-Pr_2O–hexane)	$C_{16}H_{18}F_2O_4S$	CH

[a,b] See the corresponding footnotes in Table 1.

Table 3. Physicochemical properties and inhibitory activities against NO production of compounds **14a** and **14c–g**

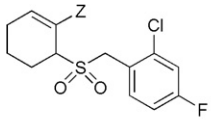


14a, 14c-g

Compound	Ar	IC_{50}[a] (nM)	Mp (°C) (solv.)	Formula	Anal.[b]
14a		1009	115–117 (dec AcOEt–hexane)	$C_{18}H_{21}NO_4S$	CHN
14c		106	123–124 (AcOEt–hexane)	$C_{18}H_{20}O_5S$	CH
14d		105	87–88 (hexane)	$C_{14}H_{18}O_4S_2$	—
14e		1412	Oil	$C_{15}H_{19}NO_4S$	—
14f		101	74–75 (*i*-Pr_2O–hexane)	$C_{18}H_{20}O_4S_2$	CH
14g		134	104–106 (*i*-Pr_2O–hexane)	$C_{18}H_{20}O_5S$	CH

[a,b] See the corresponding footnotes in Table 1.

Table 4. Physicochemical properties and inhibitory activities against NO production of compounds **10a** and **15a–j**



10a, 15a-j

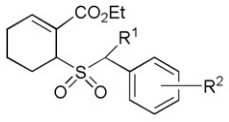
Compound	Z	IC_{50}[a] (nM)	Mp (°C) (solv.)	Formula	Anal.[b]
10a	CO_2Et	10	110.0–111.0 (hexane)	$C_{16}H_{18}ClFO_4S$	CH
15a	CO_2Me	99	94–95 (*i*-Pr_2O–hexane)	$C_{15}H_{16}ClFO_4S$	CH
15b	CO_2n-Pr	14	77–78 (*i*-Pr_2O–hexane)	$C_{17}H_{20}ClFO_4S$	CH
15d	CO_2i-Pr	100	91–93 (*i*-Pr_2O–hexane)	$C_{17}H_{20}ClFO_4S$	CH
15e	CO_2n-Bu	103	74–76 (*i*-Pr_2O–hexane)	$C_{18}H_{22}ClFO_4S$	CH
15f	CH_2OH	>10,000	78.5–79.5 (*i*-Pr_2O)	$C_{14}H_{16}ClFO_3S$	CH
15g	CH_2OAc	>10,000	70–71 (AcOEt–hexane)	$C_{16}H_{18}ClFO_4S$	CH
15h	C(=O)Et	64	133.5–134.5 (*i*-Pr_2O–hexane)	$C_{16}H_{18}ClFO_3S$	CH
15i	C(=O)*n*-Pr	11	89–91 (*i*-Pr_2O–hexane)	$C_{17}H_{20}ClFO_3S$	CH
15j	CH(OH)Et	>10,000	148–149 (*i*-Pr_2O–hexane)	$C_{16}H_{20}ClFO_3S$	CH

[a,b] See the corresponding footnotes in Table 1.

appear that the carbonyl group at 1-position of the cyclohexene ring is essential for activity.

We examined the effects of substitution at the benzylic position (R^1), as shown in Table 5. Initially, we introduced the methyl group into the benzylic position of compound **10b** to investigate its effect, in particular the difference in potencies between diastereomers (**19a**, **19b**). Previous studies[6a] indicated that alkylation of amide moiety caused a decrease in potency. In the present benzylsulfone case, however, placing the methyl substituent at the benzylic position of **10b** caused relatively little change in activity. Regarding the diastereomers, the more polar product **19a** exhibited substantially higher potency than the less polar product **19b** and was almost equal to **10b** (**19a**, IC_{50} = 100 nM; **19b**, IC_{50} = 1325 nM). Based on these results, we examined 2-chloro-4-fluorophenyl derivatives. Upon introduction of the methyl group a similar outcome was seen, with the more polar product **22a** showing sevenfold stronger

Table 5. Physicochemical properties and inhibitory activities against NO production of compounds **10a**, **19**, **22**, and **26**

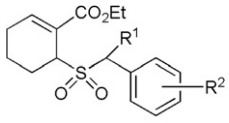


10a, 19a,b, 22a,b and 26a,b

Compound	R^1	R^2	IC_{50}[a] (nM)	Mp (°C) (solv.)	Formula	Anal.[b]
10a	H	2-Cl, 4-F	10	110–111 (hexane)	$C_{16}H_{18}ClFO_4S$	CH
19a more polar	Me	H	100	74–76 (AcOEt–hexane)	$C_{17}H_{22}O_4S$	CH
19b less polar	Me	H	1325	95.5–96.5 (hexane)	$C_{17}H_{22}O_4S$	CH
22a more polar	Me	2-Cl, 4-F	14	127.5 (*i*-Pr_2O)	$C_{17}H_{20}ClFO_4S$	CH
22b less polar	Me	2-Cl, 4-F	102	100.4 (*i*-Pr_2O)	$C_{17}H_{20}ClFO_4S$	CH
26a more polar	CH_2F	2-Cl, 4-F	435	92.1 (*i*-Pr_2O)	$C_{17}H_{19}ClF_2O_4S$	CH
26b less polar	CH_2F	2-Cl, 4-F	729	68.2–68.3 (*i*-Pr_2O)	$C_{17}H_{19}ClF_2O_4S$	CH

[a,b] See the corresponding footnotes in Table 1.

Table 6. Conditions of optical resolution with chiral HPLC and inhibitory activities against NO production of both enantiomers of **10a**, **10e**, and **22a**



10a, 10e and 22a

Compound	R^1	R^2	HPLC condition[b]	(−)-form		(+)-form	
				IC_{50}[a] (nM)	% ee	IC_{50}[a] (nM)	% ee
10a	H	2-Cl, 4-F	CHIRALPAK AD hexane/EOH = 8:2	10,000	99.8	5.7–8.2	>99.9
10e	H	2-Cl	CHIRALCEL OJ hexane/EOH = 3:7	1200	>99.9	11	99.9
22a	Me	2-Cl, 4-F	CHIRALCEL OJ hexane/EOH = 3:7	1300	>99	4.0	>99

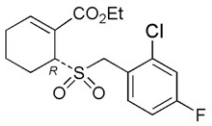
[a] See the corresponding footnote in Table 1.
[b] UV: 230 nm.

potency than that of the less polar product **22b** (**22a**, IC_{50} = 14 nM; **22b**, IC_{50} = 102 nM), but had little increase of their potency compared to that of **10a**. Whereas the introduction of a fluoromethyl moiety gave compounds **26a** and **26b**, potency was substantially reduced for both diastereomers (**26a**, IC_{50} = 435 nM; **26b**, IC_{50} = 729 nM).

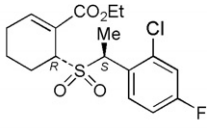
In accordance with the results of the above SAR study, it would appear that activity depends on at least two parts of the molecule (the ester and phenyl moieties) interacting with the protein, presumably in two different pockets. This is similar to our findings for the sulfonamide derivatives, since it seemed that each moiety was strictly recognized, as mentioned above.

Finally, we selected three compounds (**10a**, **10e**, **22a**), which exhibited potent suppression of NO production and potent in vivo efficacy in preliminary study, and separated each into its two enantiomers using chiral HPLC in order to evaluate the stereochemical requirements for activity. The results are summarized in Table 6. As expected from previous report,[6a] the (+)-enantiomers presented 15–300 times more potent activity than the (−)-enantiomers, as with the sulfonamide derivatives. Furthermore, the suppressive effects of the (+)-enantiomers of **10a** and **22a** for the production of cytokines from LPS-stimulated RAW264.7 cells were examined. The (+)-enantiomers showed potent inhibitory activities of TNF-α and IL-6 production with IC_{50} values [(+)-**10a**, 8.3 and 1.5 nM; (+)-**22a**, 20 and 1.8 nM, respectively], which were two- to threefold stronger than those of the racemic compounds (Table 7). Based on these results, we then selected (+)-**10a** and (+)-**22a** as candidates

Table 7. In vitro inhibitory activities against TNF-α and IL-6 production of compounds **10a**, (*R*)-(+)-**10a**, **22a**, and (6*R*, 1*S*)-(+)-**22a**



(*R*)-(+)-10a



(6*R*, 1*S*)-(+)-22a

Compound	IC_{50}[a] (nM)	
	TNF-α	IL-6
10a	21	4.8
(*R*)-(+)-**10a**	8.3	1.5
22a	42	5.8
(6*R*, 1*S*)-(+)-**22a**	20	1.8

[a] Inhibitory activities are shown as IC_{50} values, the concentration of test compound required to suppress the production of TNF-α or IL-6 by 50% of control. Values are means ± SD of three or four experiments.

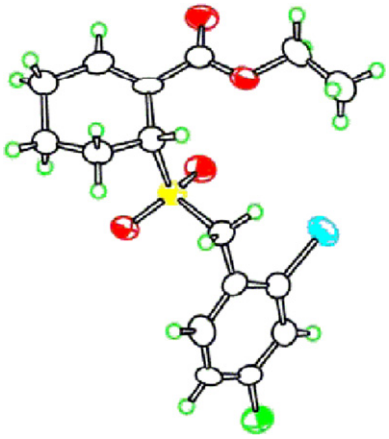


Figure 2. X-ray crystal structure of (*R*)-(+)-**10a**.

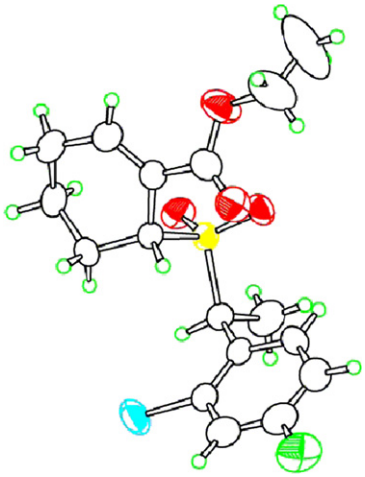


Figure 3. X-ray crystal structure of (6*R*, 1*S*)-(+)-**22a**.

for further in vivo evaluation. These enantiomers were submitted for X-ray crystallographic analysis and the absolute configuration of (+)-**10a** and (+)-**22a** was confirmed to be (*R*)- and (6*R*, 1*S*)-form, respectively. The X-ray crystal structures of both compounds are shown in Figures 2 and 3.

Table 8. Effects of compounds (*R*)-(+)-**10a** and (6*R*, 1*S*)-(+)-**22a** on LPS-induced serum NO_x levels and lethality in mice[a]

Compound	Dose (mg/kg)	% Inhibition of NO production	ED_{50} (mg/kg)	Survival/ total
Normal		—	—	8/8
Vehicle (CyD soln)		0	—	0/8
(*R*)-(+)-**10a**	1	27.0		2/10
	3	54.0	1.85	6/10
	10	76.0		10/10
(6*R*, 1*S*)-(+)-**22a**	1	−10.1		0/7
	3	55.7	5.6	5/7
	10	78.0		7/7

[a] Compounds (*R*)-(+)-**10a** and (6*R*, 1*S*)-(+)-**22a** (1–10 mg/kg) were administered intravenously 1 h before LPS (7 mg/kg, ip) challenge.

The in vivo efficacy of compounds (*R*)-(+)-**10a** and (6*R*, 1*S*)-(+)-**22a**, which showed high inhibitory potency in our in vitro assays, was examined using a mouse endotoxin shock model, the results of which are summarized in Table 8. When administrated intravenously 1 h before lethal LPS challenge, NO level in serum increased significantly after LPS challenge and both compounds (1–10 mg/kg) suppressed the production of NO induced by LPS in a dose-dependent manner. Both (*R*)-(+)-**10a** and (6*R*, 1*S*)-(+)-**22a** demonstrated efficacy against the LPS-induced cytokines with a high survival rate in mice in our endotoxin shock model; at a dose of 10 mg/kg all mice survived.

4. Conclusion

Based on our previous experience,[6a] we designed and synthesized new cyclohexene derivatives with modification of sulfonamide group of compound **1** to sulfonylmethyl group. Further investigations of the ester, substituted phenyl, and sulfonylmethyl moieties were carried out, and we discovered several compounds with high activities. As a result of our investigation of the structure–activity relationship for these compounds, we succeeded in finding the novel benzylsulfone compounds **10a** and **22a**, which showed potent inhibitory activity against NO production from mouse macrophages stimulated with LPS. In particular, (*R*)-(+)-**10a** and (6*R*, 1*S*)-(+)-**22a** exhibited highly potent inhibition of inflammatory cytokines production such as TNF-α, IL-6, and NO, in addition to showing significant in vivo efficacy in our mouse endotoxin shock model, although the activity of TAK-242 is a little superior compared with those of (*R*)-(+)-**10a** and (6*R*, 1*S*)-(+)-**22a**. These compounds suppressed the increase in serum levels of NO and protected mice from lethal septic shock with ED_{50} values of 1.85–5.6 mg/kg. These results suggest that benzylsulfone inhibitors may be promising agents for the treatment of sepsis.

5. Experimental

5.1. Chemistry

Melting points were determined on a Yanagimoto micro melting point apparatus or Buchi, and are uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Varian Gemini-200 (200 MHz) or Mercury 300 (300 MHz) spectrometer. Chemical shifts are given in parts per million (ppm) with tetramethylsilane as an internal standard, and coupling constants (*J* values) are given in Hertz (Hz). The following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Optical resolutions were recorded with Jasco DIP-370 or P-1030 digital polarimeter. Elemental analyses were carried out by Takeda Analytical Research Laboratories, Ltd, and results obtained were within ±0.4% of the theoretical values. Reactions were carried out at room temperature unless otherwise noted and followed by TLC on silica gel 60 F254 precoated TLC plates (E. Merck) or by HPLC using an octadecyl

silica (ODS) column (A-303, 4.6 mm i.d. ×250 nm, YMC Co., Ltd). Standard work-up procedures were as follows. The reaction mixture was partitioned between the indicated solvent and water. Organic extracts were combined and washed in the indicated order using the following aqueous solutions: water, saturated aqueous sodium carbonate solution (aqueous $NaHCO_3$), saturated sodium chloride (NaCl) solution (brine), and 1 N hydrochloric acid (1 N HCl). Extracts were dried over anhydrous magnesium sulfate ($MgSO_4$), filtered, and evaporated in vacuo. Chromatographic separations were carried out on Silica Gel 60 (0.063–0.200 mm, E. Merck) or ODS (CPO-273 L^R, pre-packed column, 22 mm × 300 mm, Kusano Kagaku Kikai Co.) using the indicated eluents. Yields are unoptimized.

5.2. Ethyl 6-azidocyclohex-1-ene-1-carboxylate (3)

(1) A mixture of compound **2**[7] (500 mg, 2.94 mmol) and CH_2Cl_2 (4.33 mL) was cooled to 0 °C, PPh_3 (1.0 g, 3.82 mmol) and *N*-bromosuccinimide (860 mg, 3.82 mmol) added, then the reaction stirred for 30 min at 0 °C. The reaction mixture was concentrated under reduced pressure, and the residue was purified by flash silica gel column chromatography (hexane/AcOEt = 20:1, v/v) to give ethyl 6-bromocyclohex-1-ene-1-carboxylate[8] (488 mg, 48%) as a colorless oil.

(2) To a mixture of above bromide (1.01 g, 4.33 mmol) and DMF (4.33 mL), sodium azide (1.13 g, 17.3 mmol) was added and heated at 60 °C for 3 h, then the reaction stirred during overnight at room temperature. The reaction mixture was diluted with AcOEt and worked up (water, brine). The residue was purified by silica gel column chromatography (hexane/AcOEt = 12:1, v/v) to give **3** (404 mg, 48%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 1.33 (3H, t, J = 7.2 Hz), 1.56–1.76 (3H, m), 1.94–2.10 (1H, m), 2.19–2.41 (2H, m), 4.26 (2H, q, J = 7.2 Hz), 4.54 (1H, s), 7.23–7.45 (1H, m).

5.3. Ethyl 6-{[(2-chloro-4-fluorophenyl)sulfonyl]amino} cyclohex-1-ene-1-carboxylate (4)

To a mixture of compound **3** (205 mg, 1.05 mmol) and acetone (16 mL) were added concd HCl (1.0 mL) and zinc powder (1.0 g) over 10 min, then the reaction stirred for 15 min at room temperature. The mixture was diluted with AcOEt and worked up (brine). The residue was dissolved with THF (8.0 mL), added 2-chloro-4-fluorobenzenesulfonylchloride (241 mg, 1.05 mmol) and cooled to 0 °C. To the mixture, pyridine (85 μL) was added and the reaction stirred for 4 h at room temperature. The mixture was diluted with AcOEt and washed with 0.5 N HCl and brine, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography (hexane/AcOEt = 10:1–3:1, v/v) and crystallized with hexane to give **4** (88 mg, 23%) as a white powder. Mp 127–128 °C. IR (KBr): 3289, 1713, 1588, 1468, 1429, 1331, 1260, 1238, 1215, 1157, 1059 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 1.09 (3H, t, J = 7.2 Hz), 1.33–1.55 (2H, m), 1.71–1.78 (2H, m), 1.96–2.29 (2H, m), 3.71–4.06 (2H, m), 4.09 (1H, br s), 6.96 (1H, t, J = 4.4 Hz), 7.37 (1H, dt, J = 8.0, 2.6 Hz), 7.60 (1H, dd, J = 8.8, 2.6 Hz), 8.05 (1H, dd, J = 8.8, 8.0 Hz), 8.12 (1H, br s). Anal. Calcd for $C_{15}H_{17}ClFNO_4S$: C, 49.79; H, 4.74; N, 3.87. Found: C, 49.75; H, 4.51; N, 3.87.

5.4. Ethyl 6-[(2-chloro-4-fluorophenoxy)sulfonyl]cyclohex-1-ene-1-carboxylate (6)

To a mixture of 2-chloro-4-fluorophenol (0.2 mL, 1.89 mmol) and AcOEt (6.0 mL) was added DABCO (353 mg, 3.15 mmol) and compound **5**[6a] (398 mg, 1.58 mmol) dissolved in AcOEt (2.0 mL) at 0 °C, then the reaction stirred for 4 h at room temperature. The mixture was diluted with AcOEt and worked up (water, 0.5 N HCl and brine). The residue was purified by flash silica gel column chromatography (hexane/AcOEt = 8:1, v/v) and crystallized with hexane to give **6** (30 mg, 5.3%) as a white powder. ^{1}H NMR ($CDCl_3$) δ: 1.26 (3H, t, J = 7.0 Hz), 1.71–1.99 (2H, m), 2.13–2.78 (4H, m), 4.23 (2H, q, J = 7.0 Hz), 4.87 (1H, br d, J = 5.4 Hz), 6.96–7.06 (1H, m), 7.21 (1H, dd, J = 7.8, 3.0 Hz), 7.37 (1H, t, J = 3.0 Hz), 7.47 (1H, dd, J = 9.2, 5.0 Hz).

5.5. Ethyl 6-mercaptocyclohex-1-ene-1-carboxylate (8)

(1) To a solution of **7**[14] (5.22 g, 23.1 mmol) in EtOH (40 mL) were added potassium thioacetate (3.04 g, 26.6 mmol) and Et_3N (3.56 mL, 25.7 mmol), then the reaction mixture was stirred for 4 days at room temperature and concentrated. The residue was diluted with *i*-Pr_2O and worked up (water, brine). The residue was purified by silica gel column chromatography (AcOEt/hexane = 1:5, v/v) to give the thioacetyl intermediate (5.11 g, 91%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 1.26 (3H, t, J = 7.0 Hz), 1.65–2.27 (6H, m), 2.31 (3H, s), 4.07–4.21 (2H, m), 4.70 (1H, br s), 7.10–7.14 (1H, m).

(2) To the above product (510 g, 21.1 mmol) in EtOH (20 mL) was added 4 N HCl–AcOEt soln (25 mL) and the mixture was stirred for 4 days at room temperature. The reaction mixture was concentrated and the residue was purified by silica gel column chromatography (AcOEt/hexane = 1:20, v/v) to give **8** (3.59 g, 94%) as a colorless oil. SIMS: 186 (M^+). ^{1}H NMR ($CDCl_3$) δ: 1.30 (3H, t, J = 7.2 Hz), 1.62–1.82 (2H, m), 1.86–2.10 (2H, m), 2.13–2.22 (2H, m), 3.89 (1H, s), 4.09 (1H, br s), 4.22 (2H, q, J = 7.2 Hz), 6.89–6.97 (1H, m).

5.6. Synthesis of sulfide derivatives: typical procedure for the preparation of compounds 9a–o and 12b–h

5.6.1. Ethyl 6-[(2-chloro-4-fluorobenzyl)sulfanyl]cyclohex-1-ene-1-carboxylate (9a). To a solution of **8** (40 mg, 0.21 mmol) in DMF (0.9 mL) maintained under a nitrogen atmosphere were added **11a** (58 mg, 0.26 mmol) and DBU (0.04 mL, 0.26 mmol). The mixture was then stirred for 1 h and worked up (AcOEt; water, brine). The residue was purified by silica gel column chromatography (AcOEt/hexane = 1:50–1:10, v/v) to give **9a** (61 mg, 87%) as a colorless oil. EI-MS: 328 (M^+). IR (KBr): 2938, 1715, 1489, 1260, 1242 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 1.26 (3H, t, J = 7.0 Hz), 1.56–2.36 (6H, m), 3.82 (1H,

m), 3.94 (2H, s), 4.19 (2H, q, J = 7.0 Hz), 6.96 (1H, dt, J = 8.6, 2.6 Hz), 7.12 (1H, dd, J = 8.6, 2.6 Hz), 7.46 (1H, dd, J = 8.6, 6.0 Hz).

5.6.2. Ethyl 6-(benzylsulfanyl)cyclohex-1-ene-1-carboxylate (9b). Yield 67%. Colorless oil. IR (KBr): 2946, 1713, 1260, 1242, 1094, 1063 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.27 (3H, t, J = 7.0 Hz), 1.55–2.36 (6H, m), 3.76 (1H, m), 3.86 (2H, s), 4.19 (2H, q, J = 7.0 Hz), 6.95 (1H, t, J = 4.0 Hz), 7.22–7.39 (5H, m).

5.6.3. Ethyl 6-[(4-chloro-2-fluorobenzyl)sulfanyl]cyclohex-1-ene-1-carboxylate (9c). Yield 88%. Colorless oil. IR (KBr): 2937, 1714, 1489, 1259, 1244, 1093, 1062 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.25 (3H, t, J = 7 Hz), 1.6–2.3 (6H, m), 3.77 (1H, m), 3.84 (2H, s), 4.17 (2H, q, J = 7 Hz), 6.9–7.5 (4H, m).

5.6.4. Ethyl 6-[(2-fluorobenzyl)sulfanyl]cyclohex-1-ene-1-carboxylate (9d). Yield 92%. Colorless oil. ^1H NMR ($CDCl_3$) δ: 1.24 (3H, t, J = 7.2 Hz), 1.63–1.74 (2H, m), 1.83–2.33 (4H, m), 3.80 (1H, br s), 3.88 (2H, ABq, J = 14 Hz), 4.16 (2H, q, J = 7.2 Hz), 6.94–6.97 (1H, m), 6.99–7.12 (2H, m), 7.18–7.23 (1H, m), 7.41 (1H, dd, J = 7.8, 1.5 Hz).

5.6.5. Ethyl 6-[(2-chlorobenzyl)sulfanyl]cyclohex-1-ene-1-carboxylate (9e). Yield 83%. Colorless oil. ^1H NMR ($CDCl_3$) δ: 1.24 (3H, t, J = 7.2 Hz), 1.59–2.32 (6H, m), 3.84 (1H, br s), 3.96, 3.97 (2H, ABq, J = 14 Hz), 4.16 (2H, q, J = 7.2 Hz), 6.95–6.97 (1H, m), 7.16–7.25 (2H, m), 7.35 (1H, dd, J = 7.5, 2.1 Hz), 7.45 (1H, dd, J = 7.5, 2.1 Hz).

5.6.6. Ethyl 6-[(3-chlorobenzyl)sulfanyl]cyclohex-1-ene-1-carboxylate (9f). Yield 85%. Colorless oil. ^1H NMR ($CDCl_3$) δ: 1.27 (3H, t, J = 7.2 Hz), 1.60–1.95 (4H, m), 2.04–2.34 (2H, m), 3.73 (1H, s), 3.81 (2H, ABq, J = 14 Hz), 4.19 (2H, q, J = 7.2 Hz), 6.94–6.97 (1H, m), 7.20–7.25 (3H, m), 7.36 (1H, d, J = 1.5 Hz).

5.6.7. Ethyl 6-[(4-chlorobenzyl)sulfanyl]cyclohex-1-ene-1-carboxylate (9g). Yield 93%. Colorless oil. IR (KBr): 2942, 1715, 1644, 1489, 1260, 1242, 1094, 1063 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.27 (3H, t, J = 7.0 Hz), 1.58–2.32 (6H, m), 3.72 (1H, m), 3.81 (2H, s), 4.18 (2H, q, J = 7.0 Hz), 6.93–6.96 (1H, m), 7.24–7.32 (4H, m).

5.6.8. Ethyl 6-[(2,4-difluorobenzyl)sulfanyl]cyclohex-1-ene-1-carboxylate (9h). Yield 66%. Colorless oil. EI-MS: M^+ = 312. IR (KBr): 2938, 1715, 1505, 1260, 1244 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 1.26 (3H, t, J = 7.0 Hz), 1.56–2.36 (6H, m), 3.78 (1H, m), 3.84 (2H, s), 4.18 (2H, q, J = 7.0 Hz), 6.76–6.88 (2H, m), 6.97 (1H, t, J = 4.4 Hz), 7.34–7.46 (1H, m).

5.6.9. Ethyl 6-[(2,3-dichlorobenzyl)sulfanyl]cyclohex-1-ene-1-carboxylate (9i). Yield 91%. Colorless oil. IR (KBr): 2941, 1714, 1504, 1259, 1242, 1097, 1062 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.25 (3H, t, J = 7 Hz), 1.5–2.4 (6H, m), 3.82 (1H, m), 3.99 (2H, s), 4.17 (2H, q, J = 7 Hz), 6.6–7.5 (4H, m).

5.6.10. Ethyl 6-[(3,4-dichlorobenzyl)sulfanyl]cyclohex-1-ene-1-carboxylate (9j). Yield 79%. Colorless oil. ^1H NMR ($CDCl_3$) δ: 1.27 (3H, t, J = 7.2 Hz), 1.62–1.95 (4H, m), 2.05–2.33 (2H, m), 3.71 (1H, s), 3.79 (2H, ABq, J = 14Hz), 4.19 (2H, q, J = 7.2 Hz), 6.95–6.97 (1H, m), 7.20–7.23 (1H, m), 7.37 (1H, d, J = 8.1 Hz), 7.46 (1H, d, J = 2.4 Hz).

5.6.11. Ethyl 6-[(2,4-dichlorobenzyl)sulfanyl]cyclohex-1-ene-1-carboxylate (9k). Yield 78%. Colorless oil. IR (KBr): 2937, 1712, 1471, 1259, 1242, 1095, 1062 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.26 (3H, t, J = 7 Hz), 1.64–2.34 (6H, m), 3.81 (1H, m), 3.92 (2H, s), 4.18 (2H, q, J = 7 Hz), 6.97 (1H, m), 7.19–7.43 (3H, m).

5.6.12. Ethyl 6-[(3-chloro-4-fluorobenzyl)sulfanyl]cyclohex-1-ene-1-carboxylate (9l). Yield 75%. Colorless oil. IR (KBr): 2934, 1713, 1499, 1244, 1094, 1061, 764 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.26 (3H, t, J = 7.2 Hz), 1.64–1.99 (4H, m), 2.05–2.34 (2H, m), 3.82 (1H, s), 3.93 (2H, ABq, J = 14 Hz), 4.19 (2H, q, J = 7.2 Hz), 6.90 (1H, dt, J = 8.1, 3.0 Hz), 6.97–6.99 (1H, m), 7.24–7.33 (1H, m).

5.6.13. Ethyl 6-[(2-fluoro-3-chlorobenzyl)sulfanyl]cyclohex-1-ene-1-carboxylate (9m). Yield 80%. Colorless oil. ^1H NMR ($CDCl_3$) δ: 1.24 (3H, t, J = 7.2 Hz), 1.64–1.97 (4H, m), 2.05–2.33 (2H, m), 3.79 (1H, s), 3.88 (2H, ABq, J = 14 Hz), 4.16 (2H, q, J = 7.2 Hz), 6.95–6.98 (1H, m), 7.04 (1H, td, J = 7.8, 1.2 Hz), 7.25–7.35 (2H, m).

5.6.14. Ethyl 6-[(2-fluoro-5-chlorobenzyl)sulfanyl]cyclohex-1-ene-1-carboxylate (9n). Yield 79%. Colorless oil. ^1H NMR ($CDCl_3$) δ: 1.24 (3H, t, J = 7.2 Hz), 1.64–1.72 (2H, m), 1.77–1.96 (2H, m), 2.06–2.34 (2H, m), 3.78 (1H, s), 3.82 (2H, ABq, J = 14 Hz), 4.18 (2H, q, J = 7.2 Hz), 6.94–7.00 (2H, m), 7.14–7.18 (1H, m), 7.41 (1H, dd, J = 6.6, 2.7 Hz).

5.6.15. Ethyl 6-[(2,3-difluorobenzyl)sulfanyl]cyclohex-1-ene-1-carboxylate (9o). Yield 74%. Colorless oil. ^1H NMR ($CDCl_3$) δ: 1.25 (3H, t, J = 7.2 Hz), 1.62–1.97 (4H, m), 2.05–2.33 (2H, m), 3.79 (1H, br s), 3.84–3.94 (2H, m), 4.17 (2H, q, J = 7.2 Hz), 6.96–7.07 (3H, m), 7.14–7.18 (1H, m).

5.6.16. *tert*-Butyl 3({[2-(ethoxycarbonyl)cyclohex-2-en-1-yl]sulfanyl}methyl)-1*H*-indole-1-carboxylate (12b). Yield 96%. Colorless oil. ^1H NMR ($CDCl_3$) δ: 1.20 (3H, t, J = 7 Hz), 1.60–2.33 (6H, m), 1.67 (9H, s), 3.84 (1H, br), 3.99 (2H, s), 4.04–4.25 (2H, m), 6.95 (1H, t, J = 4 Hz), 7.20–7.40 (2H, m), 7.60 (1H, s), 7.68 (1H, d, J = 8 Hz), 8.12 (1H, d, J = 8 Hz).

5.6.17. Ethyl 6-[(1-benzofuran-3-ylmethyl)sulfanyl]cyclohex-1-ene-1-carboxylate (12c). Yield 77%. Colorless oil. ^1H NMR ($CDCl_3$) δ: 1.19 (3H, t, J = 7 Hz), 1.62–2.30 (6H, m), 3.81 (1H, br), 3.89–4.20 (4H, m), 6.95 (1H, t, J = 4 Hz), 7.21–7.31 (2H, m), 7.45 (1H, d, J = 7 Hz), 7.64–7.69 (2H, m).

5.6.18. Ethyl 6-[(2-thienylmethyl)sulfanyl]cyclohex-1-ene-1-carboxylate (12d). Yield 98%. Pale-pink oil. ^1H NMR

($CDCl_3$) δ: 1.28 (3H, t, J = 7.2 Hz), 1.63–1.74 (2H, m), 1.81–1.95 (2H, m), 2.05–2.34 (2H, m), 3.81 (1H, s), 4.60, 4.81 (2H, ABq, J = 14 Hz), 4.20 (2H, q, J = 7.2 Hz), 6.90–7.00 (3H, m), 7.19 (1H, dd, J = 4.8, 1.2 Hz).

5.6.19. Ethyl 6-[(4-pyridylmethyl)sulfanyl]cyclohex-1-ene-1-carboxylate (12e). The reaction was carried out by using of 4-(bromomethyl)pyridine hydrobromide. Yield 93%. Colorless oil. IR (KBr): 2939, 1712, 1599, 1413, 1259, 1242, 1093, 1062 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.27 (3H, t, J = 7 Hz), 1.5–2.4 (6H, m), 3.73 (1H, m), 3.81 (2H, s), 4.18 (2H, q, J = 7 Hz), 6.9 (1H, m), 7.31 (2H, d, J = 6 Hz), 8.54 (2H, d, J = 6 Hz).

5.6.20. Ethyl 6-[(1-benzothiophen-2-ylmethyl)sulfanyl] cyclohex-1-ene-1-carboxylate (12f). Yield 93%. Colorless oil. ^1H NMR ($CDCl_3$) δ: 1.25 (3H, t, J = 7 Hz), 1.60–2.36 (6H, m), 3.85 (1H, br), 4.05–4.24 (4H, m), 6.97 (1H, t, J = 4 Hz), 7.23–7.35 (3H, m), 7.66–7.79 (2H, m).

5.6.21. Ethyl 6-[(1-benzofuran-2-ylmethyl)sulfanyl]cyclohex-1-ene-1-carboxylate (12g). Yield 75%. Colorless oil. ^1H NMR ($CDCl_3$) δ: 1.21 (3H, t, J = 7 Hz), 1.60–2.35 (6H, m), 3.89 (1H, br), 3.92 (1H, d, J = 15 Hz), 4.03 (1H, d, J = 15 Hz), 4.14 (2H, q, J = 7 Hz), 6.65 (1H, s), 6.98 (1H, t, J = 4 Hz), 7.16–7.28 (2H, m), 7.43–7.53 (2H, m).

5.7. 6-[(2-Chloro-4-fluorobenzyl)sulfanyl]cyclohex-1-ene-1-carboxylic acid (16c)

To a mixture of **9a** (780 mg, 2.37 mmol) and EtOH (24 mL) was added 1 N NaOH (24 mL) and the reaction mixture was stirred for 5 h at 40 °C. After cooling, 1 N HCl (24 mL) was added slowly and the mixture was diluted with water (200 mL), then the whole was worked up (AcOEt, brine). The residue was purified by silica gel column chromatography (hexane/AcOEt = 3:1–1:2, v/v) to give **16c** (410 mg, 58%) as a yellow powder. ^1H NMR ($CDCl_3$) δ: 1.59–1.87 (4H, m), 2.07–2.28 (2H, m), 3.77 (1H, s), 3.90 (2H, m), 6.81 (1H, t, J = 4.0 Hz), 7.15 (1H, td, J = 8.4, 2.6 Hz), 7.36 (1H, dd, J = 8.8, 2.6 Hz), 7.54 (1H, dd, J = 8.4, 6.6 Hz), 12.19 (1H, s).

5.7.1. Isopropyl 6-[(2-chloro-4-fluorobenzyl)sulfanyl] cyclohex-1-ene-1-carboxylate (16d). To a mixture of **16c** (116 mg, 0.39 mmol), triphenylphosphine (202 mg, 0.77 mmol), and 2-PrOH (59 mL, 0.77 mmol) in THF (3.0 mL) was added diethyl azodicarboxylate (DEAD; 40 wt% toluene solution, 0.35 mL, 0.77 mmol) at 0 °C. The reaction mixture was then stirred for 23 h under a nitrogen atmosphere and worked up (AcOEt, water, brine). The residue was purified by silica gel column chromatography (AcOEt/hexane = 1:10, v/v) to give **16d** (170 mg, 90%) as a colorless oil. ^1H NMR ($CDCl_3$) δ: 1.24 (6H, d, J = 6.2 Hz), 1.61–2.38 (6H, m), 3.83 (1H, s), 3.93 (2H, s), 5.08 (1H, septet, J = 6.2 Hz), 6.91–7.00 (2H, m), 7.12 (1H, dd, J = 8.4, 2.6 Hz), 7.45 (1H, dd, J = 8.8, 6.0 Hz).

5.7.2. Butyl 6-[(2-chloro-4-fluorobenzyl)sulfanyl]cyclohex-1-ene-1-carboxylate (16e). Compound **16e** was synthesized from **16c** by the same procedure described above for **16d**. Yield 98%. ^1H NMR ($CDCl_3$) δ: 0.94 (3H, t, J = 7.2 Hz), 1.34–1.45 (2H, m), 1.57–1.75 (4H, m), 1.83–1.98 (2H, m), 2.05–2.33 (2H, m), 3.82 (1H, s), 3.93 (2H, s), 4.13 (2H, t, J = 6.9 Hz), 6.91–6.98 (2H, m), 7.10 (1H, dd, J = 8.4, 2.7 Hz), 7.44 (1H, dd, J = 8.4, 6.0 Hz).

5.8. {6-[(2-Chloro-4-fluorobenzyl)sulfanyl]cyclohex-1-en-1-yl}methanol (16f)

To a solution of **9a** (75 mg, 0.23 mmol) in CH_2Cl_2 (1.5 mL) was added DIBAL-H (0.56 mL, 0.9 M solution in hexane) at −78 °C. After stirring for 1 h under a nitrogen atmosphere, Et_2O (4.0 mL) and water (0.56 mL) were added to the reaction mixture. The mixture was stirred for 2 h, then filtered through Celite, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (AcOEt/hexane = 1:4, v/v) to give **16f** (56 mg, 86%) as a colorless oil. ^1H NMR ($CDCl_3$) δ: 1.59–1.68 (2H, m), 1.81–2.07 (5H, m), 3.39 (1H, s), 3.85 (2H, ABq, J = 14 Hz), 3.96–4.17 (2H, m), 5.81–5.83 (1H, m), 6.95 (1H, td, J = 8.4, 2.7 Hz), 7.12 (1H, dd, J = 8.4, 2.7 Hz), 7.37 (1H, dd, J = 8.7, 6.0 Hz).

5.8.1. 1-{6-[(2-Chloro-4-fluorobenzyl)sulfanyl]cyclohex-1-en-1-yl}propan-1-ol (16h). (1) A solution of **16f** (497 mg, 1.73 mmol), molecular sieves (4Å, 482 mg), 4-methylmorpholine N-oxide (482 mg, 4.10 mmol) in CH_2Cl_2 (10 mL) was added TPAP (31 mg, 86.7 μmol) then the reaction stirred for 1 h at 0 °C under a nitrogen atmosphere. The reaction mixture was submitted to silica gel column chromatography (Et_2O) to give 6-[(2-chloro-4-fluorobenzyl)sulfanyl]-1-cyclohexene-1-carbaldehyde (332 mg, 67%) as a white amorphous material. ^1H NMR ($CDCl_3$) δ: 1.61–2.48 (6H, m), 3.76 (1H, s), 3.98 (2H, s), 6.82 (1H, t, J = 3.3 Hz), 6.96 (1H, td, J = 8.4, 2.7 Hz), 7.11 (1H, dd, J = 8.4, 2.7 Hz), 7.50 (1H, dd, J = 8.7, 6.0 Hz), 9.38 (1H, s).

(2) To a solution of the aldehyde (178 mg, 0.63 mmol) prepared above in THF (3.5 mL), ethylmagnesium bromide (3.0 M solution in Et_2O, 417 μL, 1.25 mmol) was added dropwise at −20 °C. The mixture was stirred for 1.5 h and worked up (Et_2O; water, brine). The residue was purified by silica gel column chromatography (AcOEt/hexane = 14:86–26:74, v/v) to afford **16 h** (130 mg, 66%) as a colorless oil. The isolated **16h** was a diastereomeric mixture in the ratio of ca. 3:1. ^1H NMR ($CDCl_3$) δ: 0.80 (0.75H, t, J = 7.2 Hz), 0.82 (2.25H, t, J = 7.2 Hz), 1.26–2.15 (9H, m), 3.12 (0.76H, s), 3.40 (0.24H, s), 3.73–3.93 (2H, m), 3.95 (1H, br s), 5.80 (0.24H, br), 5.89 (0.76H, br), 6.97 (1H, td, J = 8.4, 2.6 Hz), 7.14 (1H, dd, J = 8.4, 2.6 Hz), 7.38 (1H, dd, J = 8.4, 6.0 Hz).

5.8.2. 1-{6-[(2-Chloro-4-fluorobenzyl)sulfanyl]cyclohex-1-en-1-yl}butan-1-ol (16i). Compound **16i** was prepared from the above aldehyde derivative by a similar procedure to that described for the synthesis of **16h**. The isolated **16i** was a diastereomeric mixture in the ratio of ca. 65:35. Yield 54%. Colorless oil. ^1H NMR ($CDCl_3$) δ: 0.81–0.90 (3H, m), 1.12–1.45 (4H, m), 1.60–2.08 (7H, m), 3.11 (0.65H, s), 3.38 (0.35H, s), 3.79, 3.86 (1.3H,

ABq, J = 13 Hz), 3.83, 3.89 (0.7H, ABq, J = 13 Hz), 3.95–4.08 (1H, m), 5.79–5.90 (1H, m), 6.93–7.01 (1H, m), 7.11–7.17 (1H, m), 7.33–7.41 (1H, m).

5.8.3. Ethyl 6-[(1-phenylethyl)sulfanyl]cyclohex-1-ene-1-carboxylate (18). Compound **18** was prepared in the same manner described for compound **9a**. The isolated **18** was a diastereomeric mixture in the ratio of ca. 1:1. Yield 61%. Colorless oil. ^{1}H NMR ($CDCl_3$) δ: 1.22 (1.5H, t, J = 7.2 Hz), 1.34 (1.5H, t, J = 7.2 Hz), 1.41–1.80 (4H, m), 1.90–2.32 (2H, m), 3.54 (0.5H, s), 3.65 (0.5H, s), 4.06–4.31 (3H, m), 6.87–6.93 (1H, m), 7.19–7.40 (5H, m).

5.8.4. Ethyl 6-{[1-(2-chloro-4-fluorophenyl)ethyl]sulfanyl}cyclohex-1-ene-1-carboxylate (21). To a solution of **8** (186 mg, 1.0 mmol) in toluene (3.0 mL) maintained under a nitrogen atmosphere were added **20** (175 mg, 1.0 mmol) and CMBP (362 mg, 1.5 mmol), and the reaction stirred for 3 h at 100 °C. The mixture was cooled to room temperature and concentrated under reduced pressure, then the residue was purified by silica gel column chromatography (AcOEt/hexane = 1:40–1:20, v/v) to give **21** (diastereomeric mixture; 123 mg, 36%) as a colorless oil. Yield 40%. IR (KBr): 2560, 1715, 1489, 1242 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 1.24 (1.73H, t, J = 7.0 Hz), 1.33 (1.27H, t, J = 7.0 Hz), 1.48 (1.73H, d, J = 7.0 Hz), 2.05–2.57 (6H, m), 3.53 (0.58H, br s), 3.83 (0.42H, br s), 4.06–4.31 (2H, m), 4.66 (1H, quintet, J = 7.0 Hz), 6.92–7.06 (3H, m), 7.55 (0.42H, dd, J = 8.8, 6.2 Hz), 7.73 (0.58H, dd, J = 8.0, 5.8 Hz).

5.9. Ethyl 6-({1-(2-chloro-4-fluorophenyl)-2-[(trimethylsilyl)oxy]ethyl}sulfanyl)-cyclohex-1-ene-1-carboxylate (24)

To a solution of **8** (373 mg, 2.0 mmol) in DMF (5.0 mL) maintained under a nitrogen atmosphere were added **23** (782 mg, 2.4 mmol), Et_3N (0.56 mL, 4.0 mmol), and DBU (0.3 mL, 2.0 mmol) in DMF (5.0 mL), and the reaction stirred for 1 h at 0 °C. The mixture was worked up (AcOEt; water, brine), then the residue was purified by silica gel column chromatography (AcOEt/hexane = 0.5:99.5–5:95, v/v) to give **24** (diastereomeric mixture; 198 mg, 23%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: −0.06 to −0.02 (9H, m), 1.18 (1.8H, t, J = 7.0 Hz), 1.29 (1.2H, t, J = 7.0 Hz), 1.57–2.23 (6H, m), 3.60–4.25 (5H, m), 4.52–4.68 (0.4H, m), 4.63 (0.6H, t, J = 4.6 Hz), 6.88–7.22 (3H, m), 7.41 (0.4H, dd, J = 8.8, 6.2 Hz), 7.61 (0.6H, dd, J = 8.8, 6.2 Hz).

5.10. Ethyl 6-{[1-(2-chloro-4-fluorophenyl)-2-fluoroethyl]sulfanyl}cyclohex-1-ene-1-carboxylate (25)

(1) 6 N HCl (275 μL, 1.65 mmol) was added dropwise to a solution of **24** (198 mg, 0.46 mmol) in MeOH (3.0 mL). The mixture was stirred for 0.5 h, then worked up (AcOEt; water, brine) to give ethyl 6-{[1-(2-chloro-4-fluorophenyl)-2-hydroxyethyl]sulfanyl}-1-cyclohexene-1-carboxylate (diastereomeric mixture; 147 mg, 89%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 1.25 (1.8H, t, J = 7.0 Hz), 1.33 (1.2H, t, J = 7.0 Hz), 1.52–2.36 (6H, m), 2.90 (0.4H, t, J = 6.4 Hz), 3.81–4.33 (5.6H, m), 4.45–4.55 (1H, m), 6.96–7.18 (3H, m), 7.37 (0.4H, dd, J = 8.8, 6.2 Hz), 7.66 (0.6H, dd, J = 8.8, 6.2 Hz).

(2) A solution of above product (145 mg, 0.40 mmol) in CH_2Cl_2 (10 mL) was cooled to −78 °C and DAST (107 mL, 0.81 mmol) in CH_2Cl_2 (10 mL) added dropwise. The mixture was stirred for 1 h at −78 °C to −60 °C, further stirred at −10 °C for 2 h. The whole was worked up (CH_2Cl_2; aqueous $NaHCO_3$, water, brine) and the residue was purified by silica gel column chromatography (AcOEt/hexane = 1:99–15:5, v/v) to give **25** (diastereomeric mixture; 80 mg, 55%). ^{1}H NMR ($CDCl_3$) δ: 1.15–1.37 (3H, m), 1.62–2.54 (6H, m), 2.76–3.22 (1H, m), 3.53–4.32 (4H, m), 4.61–4.88 (1H, m), 6.86–7.22 (3H, m), 7.29–7.74 (1H, m).

5.11. Synthesis of sulfone derivatives: typical procedure for the preparation of compounds 10a–o (Table 2), 14b–g (Table 3), 15d, e, f, j (Table 4), and 19a, b, 22a, b, and 26a, b (Table 5)

5.11.1. Ethyl 6-[(2-chloro-4-fluorobenzyl)sulfonyl]cyclohex-1-ene-1-carboxylate (10a). A mixture of compound **9a** (896 mg, 2.73 mmol) and AcOEt (27 mL) was added *m*CPBA (1.48 g, 6.00 mmol) at 0 °C and the reaction stirred for 2 h. The mixture was diluted with AcOEt and worked up (aqueous $NaHCO_3$, water, and brine). The residue was purified by silica gel column chromatography (hexane/AcOEt = 6:1–4:1, v/v) and crystallized with AcOEt–hexane to give **10a** (869 mg, 88%) as a white powder. IR (KBr): 2942, 1709, 1605, 1493, 1314, 1250, 1128, 1061 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 1.32 (3H, t, J = 7.0 Hz), 1.55–2.62 (6H, m), 4.25 (2H, q, J = 7.0 Hz), 4.41 (1H, d, J = 5.6 Hz), 4.59 (2H, s), 7.03 (1H, dt, J = 8.4, 2.6 Hz), 7.21 (1H, dd, J = 8.4, 2.6 Hz), 7.42 (1H, t, J = 4.0 Hz), 7.62 (1H, dd, J = 8.6, 6.0 Hz). Anal. Calcd for $C_{16}H_{18}ClFO_4S$: C, 53.26; H, 5.03. Found: C, 53.08; H, 4.95.

5.11.2. Ethyl 6-(benzylsulfonyl)cyclohex-1-ene-1-carboxylate (10b). Yield 95%. White powder. IR (KBr): 1705, 1306, 1250, 1119, 1098, 1061 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 1.34 (3H, t, J = 7.2 Hz), 1.41–2.50 (6H, m), 4.28 (2H, q, J = 7.2 Hz), 4.29 (1H, d, J = 13.8 Hz), 4.35 (1H, m), 4.55 (1H, d, J = 14 Hz), 7.37–7.45 (4H, m), 7.50–7.55 (2H, m). Anal. Calcd for $C_{16}H_{20}O_4S{\cdot}1/2H_2O$: C, 60.55; H, 6.67. Found: C, 60.96; H, 6.32.

5.11.3. Ethyl 6-[(4-chloro-2-fluorobenzyl)sulfonyl]cyclohex-1-ene-1-carboxylate (10c). Yield 50%. IR (KBr): 2941, 1714, 1489, 1313, 1249, 1126, 1097, 1060 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 1.32 (3H, t, J = 7 Hz), 1.6–2.6 (6H, m), 4.26 (2H, q, J = 7 Hz), 4.36 (1H, m), 4.39, 4.53 (2H, ABq, J = 13 Hz), 7.1–7.2 (2H, m), 7.4–7.6 (2H, m). Anal. Calcd for $C_{16}H_{18}ClFO_4S$: C, 53.26; H, 5.03. Found: C, 53.28; H, 5.10.

5.11.4. Ethyl 6-[(2-fluorobenzyl)sulfonyl]cyclohex-1-ene-1-carboxylate (10d). Yield 28%. White powder. ^{1}H NMR ($CDCl_3$) δ: 1.31 (3H, t, J = 7 Hz), 1.56–2.45 (6H, m), 4.26 (2H, q, J = 7 Hz), 4.39–4.43 (1H, m), 4.43 (1H, d, J = 14 Hz), 4.57 (1H, d, J = 14 Hz), 7.09–7.60 (5H, m). Anal. Calcd for $C_{16}H_{19}FO_4S$: C, 58.88; H, 5.87. Found: C, 58.99; H, 6.05.

5.11.5. Ethyl 6-[(2-chlorobenzyl)sulfonyl]cyclohex-1-ene-1-carboxylate (10e). Yield 86%. White powder. ^{1}H

NMR ($CDCl_3$) δ: 1.30 (3H, t, J = 7.2 Hz), 1.59–1.78 (2H, m), 1.94–2.54 (4H, m), 4.25 (2H, q, J = 7.2 Hz), 4.46 (1H, br d, J = 6 Hz), 4.625, 4.632 (2H, ABq, J = 14 Hz), 7.29–7.47 (4H, m), 7.59–7.64 (1H, m). Anal. Calcd for $C_{16}H_{19}ClO_4S$: C, 56.05; H, 5.59. Found: C, 55.88; H, 5.63.

5.11.6. Ethyl 6-[(3-chlorobenzyl)sulfonyl]cyclohex-1-ene-1-carboxylate (10f). Yield 36%. White powder. ^{1}H NMR ($CDCl_3$) δ: 1.34 (3H, t, J = 7.0 Hz), 1.47–2.55 (6H, m), 4.22–4.34 (4H, m), 4.53 (1H, d, J = 14 Hz), 7.32–7.46 (4H, m), 7.56 (1H, s). Anal. Calcd for $C_{16}H_{19}ClO_4S$: C, 56.05; H, 5.59. Found: C, 55.82; H, 5.42.

5.11.7. Ethyl 6-[(4-chlorobenzyl)sulfonyl]cyclohex-1-ene-1-carboxylate (10g). Yield 57%. White powder. IR (KBr): 2980, 2939, 1705, 1493, 1305, 1249, 1120, 1095, 1060 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 1.34 (3H, t, J = 7 Hz), 1.4–2.6 (6H, m), 4.2–4.4 (3H, m), 4.54 (1H, d, J = 14 Hz), 7.3–7.6 (5H, m). Anal. Calcd for $C_{16}H_{19}ClO_4S$: C, 56.05; H, 5.59. Found: C, 56.08; H, 5.41.

5.11.8. Ethyl 6-[(2,4-difluorobenzyl)sulfonyl]cyclohex-1-ene-1-carboxylate (10h). Yield 75%. White powder. ^{1}H NMR ($CDCl_3$) δ: 1.32 (3H, t, J = 7.0 Hz), 1.59–2.50 (6H, m), 4.27 (2H, q, J = 7.0 Hz), 4.35 (1H, d, J = 14.0 Hz), 4.39 (1H, m), 4.51 (1H, d, J = 14.0 Hz), 6.83–6.96 (2H, m), 7.42 (1H, t, J = 4.0 Hz), 7.49–7.61 (1H, m). Anal. Calcd for $C_{16}H_{18}F_2O_4S$: C, 55.80; H, 5.27. Found: C, 55.95; H, 5.40.

5.11.9. Ethyl 6-[(2,3-dichlorobenzyl)sulfonyl]cyclohex-1-ene-1-carboxylate (10i). Yield 50%. White powder. IR (KBr): 2941, 1699, 1427, 1311, 1249, 1122, 1097, 1060 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 1.32 (3H, t, J = 7 Hz), 1.6–2.6 (6H, m), 4.25 (2H, q, J = 7 Hz), 4.44 (1H, m), 4.69 (2H, s), 7.2–7.3 (1H, m), 7.4–7.6 (3H, m). Anal. Calcd for $C_{16}H_{18}Cl_2O_4S$: C, 50.94; H, 4.81. Found: C, 50.94; H, 4.76.

5.11.10. Ethyl 6-[(3,4-dichlorobenzyl)sulfonyl]cyclohex-1-ene-1-carboxylate (10j). Yield 79%. White powder. IR (KBr): 2944, 1707, 1472, 1310, 1250, 1117, 1098, 1061 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 1.35 (3H, t, J = 7.2 Hz), 1.49–1.63 (1H, m), 1.73–2.00 (2H, m), 2.16–2.53 (3H, m), 4.25–4.28 (1H, m), 4.25, 4.49 (2H, ABq, J = 14 Hz), 4.29 (2H, q, J = 7.2 Hz), 7.38 (1H, t, J = 1.8 Hz), 7.42–7.46 (3H, m). Anal. Calcd for $C_{16}H_{18}Cl_2O_4S$: C, 50.94; H, 4.81. Found: C, 51.16; H, 4.63.

5.11.11. Ethyl 6-[(2,4-dichlorobenzyl)sulfonyl]cyclohex-1-ene-1-carboxylate (10k). Yield 71%. White powder. IR (KBr): 2943, 1712, 1473, 1311, 1251, 1128, 1097, 1060 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 1.32 (3H, t, J = 7 Hz), 1.5–2.6 (6H, m), 4.26 (2H, q, J = 7 Hz), 4.44 (1H, m), 4.60 (2H, s), 7.2–7.6 (4H, m). Anal. Calcd for $C_{16}H_{18}Cl_2O_4S$: C, 50.94; H, 4.81. Found: C, 50.81; H, 4.98.

5.11.12. Ethyl 6-[(3-chloro-4-fluorobenzyl)sulfonyl]cyclohex-1-ene-1-carboxylate (10l). Yield 98%. Colorless oil. IR (KBr): 2944, 1705, 1501, 1312, 1250, 1125, 1098, 1063, 781 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 1.35 (3H, t, J = 7.2 Hz), 1.49–1.62 (1H, m), 1.72–2.02 (2H, m), 2.16–2.31 (1H, m), 2.38–2.53 (2H, m), 4.19–4.32 (4H, m), 4.51 (1H, d, J = 14 Hz), 7.15 (1H, t, J = 8.7 Hz), 7.40–7.45 (2H, m), 7.61 (1H, dd, J = 6.9, 2.4 Hz).

5.11.13. Ethyl 6-[(2-fluoro-3-chlorobenzyl)sulfonyl]cyclohex-1-ene-1-carboxylate (10m). Yield 91%. White powder. IR (KBr): 2944, 1705, 1462, 1312, 1250, 1128, 1098, 1061 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 1.32 (3H, t, J = 7.2 Hz), 1.59–1.80 (2H, m), 1.92–2.51 (4H, m), 4.27 (2H, q, J = 7.2 Hz), 4.40 (1H, d, J = 5.4 Hz), 4.48, 4.54 (2H, ABq, J = 13.5 Hz), 7.12 (1H, t, J = 7.8 Hz), 7.40–7.49 (3H, m). Anal. Calcd for $C_{16}H_{18}ClFO_4S$: C, 53.26; H, 5.03. Found: C, 53.02; H, 5.03.

5.11.14. Ethyl 6-[(2-fluoro-5-chlorobenzyl)sulfonyl]cyclohex-1-ene-1-carboxylate (10n). Yield 94%. White powder. IR (KBr): 2946, 1705, 1489, 1312, 1248, 1127, 1100, 1061 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 1.33 (3H, t, J = 7.2 Hz), 1.57–1.80 (2H, m), 1.92–2.09 (1H, m), 2.17–2.33 (1H, m), 2.42–2.53 (2H, m), 4.27 (2H, q, J = 7.2 Hz), 4.38 (1H, s), 4.43, 4.49 (2H, ABq, J = 13.8 Hz), 7.07 (1H, t, J = 9.0 Hz), 7.30–7.35 (1H, m), 7.43 (1H, t, J = 3.6 Hz), 7.55 (1H, dd, J = 6.0, 2.7 Hz). Anal. Calcd for $C_{16}H_{18}ClFO_4S$: C, 53.26; H, 5.03. Found: C, 53.31; H, 5.12.

5.11.15. Ethyl 6-[(2,3-difluorobenzyl)sulfonyl]cyclohex-1-ene-1-carboxylate (10o). Yield 88%. White powder. IR (KBr): 2944, 1709, 1495, 1314, 1250, 1125, 1061 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 1.33 (3H, t, J = 7.2 Hz), 1.60–1.79 (2H, m), 1.91–2.11 (2H, m), 2.42–2.52 (2H, m), 4.27 (2H, q, J = 7.2 Hz), 4.40 (1H, br d, J = 4.8 Hz), 4.49, 4.55 (2H, ABq, J = 14 Hz), 7.06–7.33 (3H, m), 7.41–7.44 (1H, m). Anal. Calcd for $C_{16}H_{18}F_2O_4S$: C, 55.80; H, 5.27. Found: C, 55.59; H, 5.37.

5.11.16. *tert*-Butyl 3({[2-(ethoxycarbonyl)cyclohex-2-en-1-yl]sulfonyl}methyl)-1*H*-indole-1-carboxylate (14b). Yield 85%. White powder. ^{1}H NMR ($CDCl_3$) δ: 1.34 (3H, t, J = 7.0 Hz), 1.42–2.52 (6H, m), 1.68 (9H, s), 4.29 (2H, q, J = 7.0 Hz), 4.42 (1H, d, J = 14 Hz), 4.44 (1H, br), 4.76 (1H, d, J = 14 Hz), 7.27–7.41 (2H, m), 7.79 (1H, d, J = 7.0 Hz), 7.88 (1H, s), 8.14 (1H, d, J = 8.8 Hz). Anal. Calcd for $C_{23}H_{29}NO_6S$: C, 61.72; H, 6.53; N, 3.13. Found: C, 61.61; H, 6.32; N, 2.92.

5.11.17. Ethyl 6-[(1-benzofuran-3-ylmethyl)sulfonyl]cyclohex-1-ene-1-carboxylate (14c). Yield 88%. White powder. IR (KBr): 1705, 1306, 1250, 1123 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 1.32 (3H, t, J = 7.0 Hz), 1.46–2.49 (6H, m), 4.23–4.38 (2H, m), 4.54 (1H, d, J = 5.1 Hz), 4.95 (2H, q, J = 6.9 Hz), 7.41 (1H, t, J = 3.9 Hz), 7.46–7.59 (3H, m), 7.72 (1H, d, J = 3.9 Hz), 7.85–7.89 (2H, m), 8.22 (1H, d, J = 9.0 Hz). Anal. Calcd for $C_{18}H_{20}O_5S$: C, 62.05; H, 5.79. Found: C, 62.12; H, 5.87.

5.11.18. Ethyl 6-[(3-thienylmethyl)sulfonyl]cyclohex-1-ene-1-carboxylate (14d). Yield 69%. White powder. ^{1}H NMR ($CDCl_3$) δ: 1.38 (3H, t, J = 7.0 Hz), 1.4–2.5 (6H, m), 4.28 (2H, q, J = 7.0 Hz), 4.39 (1H, m), 4.42 (1H, d, J = 17 Hz), 4.86 (1H, d, J = 17 Hz), 7.05 (1H, m), 7.2–7.5 (3H, m).

5.11.19. Ethyl 6-[(4-pyridylmethyl)sulfonyl]cyclohex-1-ene-1-carboxylate (14e). Yield 55%. Pale-yellow oil. IR (KBr): 2939, 1712, 1599, 1413, 1259, 1242, 1093, 1062 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.27 (3H, t, J = 7 Hz), 1.5–2.4 (6H, m), 3.73 (1H, m), 3.81 (2H, s), 4.18 (2H, q, J = 7 Hz), 6.9 (1H, m), 7.31 (2H, d, J = 6 Hz), 8.54 (2H, d, J = 6 Hz).

5.11.20. Ethyl 6-[(1-benzothiophen-2-ylmethyl)sulfonyl]cyclohex-1-ene-1-carboxylate (14f). Yield 68%. White powder. ^1H NMR ($CDCl_3$) δ: 1.35 (3H, t, J = 7.0 Hz), 1.27–2.55 (6H, m), 4.30 (2H, q, J = 7.0 Hz), 4.46 (1H, br), 4.49 (1H, d, J = 14 Hz), 4.98 (1H, d, J = 14 Hz), 7.31–7.44 (3H, m), 7.56 (1H, s), 7.77–7.85 (2H, m). Anal. Calcd for $C_{18}H_{20}O_4S_2 \cdot 0.2H_2O$: C, 58.74; H, 5.59. Found: C, 58.47; H, 5.49.

5.11.21. Ethyl 6-[(1-benzofuran-2-ylmethyl)sulfonyl]cyclohex-1-ene-1-carboxylate (14g). Yield 85%. White powder. IR (KBr): 1705, 1306, 1250, 1123 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.32 (3H, t, J = 7.0 Hz), 1.46–2.49 (6H, m), 4.23–4.38 (2H, m), 4.54 (1H, d, J = 5.1 Hz), 4.95 (2H, q, J = 6.9 Hz), 7.41 (1H, t, J = 3.9 Hz), 7.46–7.59 (3H, m), 7.72 (1H, d, J = 3.9 Hz), 7.85–7.89 (2H, m), 8.22 (1H, d, J = 9.0 Hz). Anal. Calcd for $C_{18}H_{20}O_5S$: C, 62.05; H, 5.79. Found: C, 62.12; H, 5.87.

5.11.22. Isopropyl 6-[(2-chloro-4-fluorobenzyl)sulfonyl]cyclohex-1-ene-1-carboxylate (15d). Yield 82%. White powder. ^1H NMR ($CDCl_3$) δ: 1.29 (3H, d, J = 6.3 Hz), 1.31 (3H, d, J = 6.3 Hz), 1.60–1.76 (2H, m), 1.95–2.10 (1H, m), 2.16–2.30 (1H, m), 2.41–2.51 (2H, m), 4.42 (1H, d, J = 4.8 Hz), 4.58 (2H, s), 5.11 (1H, dt, J = 6.3 Hz), 7.02 (1H, td, J = 8.4, 2.7 Hz), 7.19 (1H, dd, J = 8.4, 2.7 Hz), 7.38 (1H, t, J = 4.2 Hz), 7.58 (1H, dd, J = 8.7, 6.0 Hz). Anal. Calcd for $C_{17}H_{20}ClFO_4S$: C, 54.47; H, 5.38. Found: C, 54.46; H, 5.29.

5.11.23. Butyl 6-[(2-chloro-4-fluorobenzyl)sulfonyl]cyclohex-1-ene-1-carboxylate (15e). Yield 73%. White powder. IR (KBr): 2961, 1707, 1605, 1493, 1314, 1248, 1128, 1098, 914 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.96 (3H, t, J = 7.2 Hz), 1.36–1.48 (2H, m), 1.60–1.72 (4H, m), 1.96–2.10 (1H, m), 2.16–2.1 (1H, m), 2.43–2.52 (2H, m), 4.13–4.26 (2H, m), 4.15 (1H, d, J = 4.5 Hz), 4.58 (1H, d, J = 1.5 Hz), 7.02 (1H, td, J = 8.4, 2.4 Hz), 7.19 (1H, dd, J = 8.4, 1.8 Hz), 7.39 (1H, t, J = 3.9 Hz), 7.58 (1H, dd, J = 8.4, 6.0 Hz). Anal. Calcd for $C_{18}H_{22}ClFO_4S$: C, 55.59; H, 5.70. Found: C, 55.57; H, 5.74.

5.11.24. {6-[(2-Chloro-4-fluorobenzyl)sulfonyl]cyclohex-1-en-1-yl}methanol (15f). Yield 73%. White powder. IR (KBr): 3480, 2944, 1605, 1582, 1493, 1308, 1235, 1125, 1044, 914 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.64–1.76 (1H, m), 1.84–2.42 (5H, m), 2.45 (1H, t, J = 6.8 Hz), 3.96 (1H, br s), 4.12 (1H, dd, J = 13, 6.8 Hz), 4.38–4.48 (1H, m), 4.48 (2H, s), 6.21 (1H, br), 7.06 (1H, td, J = 8.4, 2.6 Hz), 7.21 (1H, dd, J = 8.4, 2.6 Hz), 7.57 (1H, dd, J = 8.8, 6.0 Hz). Anal. Calcd for $C_{14}H_{16}ClFO_3S$: C, 52.75; H, 5.06. Found: C, 52.51; H, 4.97.

5.11.25. 1-{6-[(2-Chloro-4-fluorobenzyl)sulfonyl]cyclohex-1-en-1-yl}propan-1-ol (15j). Yield 36%. White powder. IR (KBr): 2942, 1605, 1493, 1316, 1235, 1123, 914 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.963 (3H, t, J = 7.2 Hz), 1.34–1.67 (4H, m), 1.75–1.87 (2H, m), 2.04–2.16 (2H, m), 4.28 (1H, d, J = 8.4 Hz), 4.50 (2H, s), 7.06 (1H, td, J = 8.4, 2.4 Hz), 7.20–7.26 (2H, m), 7.57 (1H, d, J = 8.4, 6.0 Hz).

5.11.26. Ethyl 6-[(1-phenylethyl)sulfonyl]cyclohex-1-ene-1-carboxylate (19a, 19b). 19a (More polar product): Yield 26%. ^1H NMR (DMSO-d_6) δ: 1.16 (3H, t, J = 7.0 Hz), 1.51–1.69 (2H, m), 1.68 (3H, d, J = 7.0 Hz), 1.79–2.40 (4H, m), 4.07 (2H, q, J = 7.0 Hz), 4.26 (1H, d, J = 4.6 Hz), 4.70 (1H, q, J = 7.0 Hz), 7.18 (1H, t, J = 3.8 Hz), 7.37–7.45 (5H, m).

19b (Less polar product): Yield 61%. ^1H NMR ($CDCl_3$) δ: 1.17–1.32 (1H, m), 1.35 (3H, t, J = 7.2 Hz), 1.60–1.93 (3H, m), 1.76 (3H, d, J = 7.2 Hz), 2.03–2.43 (2H, m), 4.26–4.33 (1H, m), 4.29 (2H, q, J = 7.2 Hz), 4.64 (1H, d, J = 4.6 Hz), 7.31–7.33 (1H, m), 7.36–7.42 (3H, m), 7.63–7.66 (2H, m). Anal. Calcd for $C_{17}H_{22}O_4S$: C, 63.33; H, 6.88. Found: C, 62.93; H, 6.89.

5.11.27. Ethyl 6-{[1-(2-chloro-4-fluorophenyl)ethyl]sulfonyl}cyclohex-1-ene-1-carboxylate (22a, 22b). 22a (More polar product): Yield 14%. ^1H NMR ($CDCl_3$) δ: 1.29 (3H, t, J = 7.0 Hz), 1.60–1.79 (1H, m), 1.78 (3H, d, J = 7.0 Hz), 2.05–2.64 (4H, m), 4.09–4.30 (2H, m), 4.35 (1H, d, J = 3.8 Hz), 5.15 (2H, q, J = 7.0 Hz), 7.02–7.14 (1H, m), 7.16 (1H, dd, J = 8.6, 2.6 Hz), 7.38 (1H, t, J = 4.0 Hz), 7.67 (1H, dd, J = 8.6, 5.8 Hz). Anal. Calcd for $C_{17}H_{20}ClFO_4S$: C, 54.47; H, 5.38. Found: C, 54.31; H, 5.54.

22b (Less polar product): Yield 5%. ^1H NMR ($CDCl_3$) δ: 1.34 (3H, t, J = 7.0 Hz), 1.27–2.43 (6H, m), 1.73 (3H, d, J = 7.0 Hz), 4.29 (2H, q, J = 7.0 Hz), 4.36 (1H, br s), 5.11 (1H, q, J = 7.0 Hz), 7.03–7.13 (1H, m), 7.22 (1H, dd, J = 8.2, 2.6 Hz), 7.30–7.35 (1H, m), 7.79 (1H, dd, J = 8.2, 6.2 Hz). Anal. Calcd for $C_{17}H_{20}ClFO_4S$: C, 54.47; H, 5.38. Found: C, 54.38; H, 5.35.

5.11.28. Ethyl 6-{[1-(2-chloro-4-fluorophenyl)-2-hydroxyethyl]sulfonyl}cyclohex-1-ene-1-carboxylate (26a, 26b). 26a (More polar product): Yield 46%. IR (KBr): 1713, 1495, 1308, 1250, 1132, 1061 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.31 (3H, t, J = 7.0 Hz), 1.66–2.65 (6H, m), 3.56 (1H, ddd, J = 32.6, 15.0, 2.6 Hz), 3.68–3.87 (1H, m), 4.25 (2H, q, J = 7.0 Hz), 4.44 (1H, d, J = 5.6 Hz), 6.42 (1H, ddd, J = 46.2, 8.8, 2.0 Hz), 7.04–7.19 (2H, m), 7.43 (1H, t, J = 2.8 Hz), 7.53 (1H, dd, J = 8.4, 5.8 Hz). Anal. Calcd for $C_{17}H_{19}ClF_2O_4S$: C, 51.98; H, 4.87. Found: C, 51.97; H, 5.01.

26b (Less polar product): Yield 31%. IR (KBr): 1705, 1495, 1306, 1250, 1128, 1061 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.31 (3H, t, J = 7.0 Hz), 1.58–2.79 (6H, m), 3.37 (1H, ddd, J = 37.8, 15.4, 1.8 Hz), 4.10–4.29 (3H, m), 4.55 (1H, br s), 6.39 (1H, ddd, J = 44.8, 10.8, 1.8 Hz), 7.05–7.16 (2H, m), 7.43 (1H, t, J = 3.6 Hz), 7.59 (1H, dd, J = 8.8, 5.8 Hz). Anal. Calcd for $C_{17}H_{19}ClF_2O_4S$: C, 51.98; H, 4.87. Found: C, 51.86; H, 5.01.

5.12. Ethyl 6-[(1*H*-indol-3-ylmethyl)sulfonyl]cyclohex-1-ene-1-carboxylate (14a)

TFA (2.0 mL) was added dropwise to **14b** (224 mg, 0.5 mmol). The mixture was stirred for 2 h. The mixture was diluted with water and extracted with AcOEt. The organic layer was washed with aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (AcOEt/hexane = 1:2, v/v) followed by crystallization from *i*-Pr_2O to give **14a** (17.5 mg, 10%) as a white powder. ^{1}H NMR ($CDCl_3$) δ: 1.33 (3H, t, J = 7.2 Hz), 1.38–2.47 (6H, m), 4.28 (2H, q, J = 7.2 Hz), 4.42 (1H, br), 4.47 (1H, d, J = 14.4 Hz), 4.80 (1H, d, J = 14.4 Hz), 7.15–7.24 (2H, m), 7.36–7.38 (2H, m), 7.46 (1H, d, J = 2.1 Hz), 7.83 (1H, d, J = 8.4 Hz), 8.38 (1H, br). Anal. Calcd for $C_{18}H_{21}NO_4S$: C, 62.23; H, 6.09; N, 4.03. Found: C, 61.95; H, 5.88; N, 3.85.

5.12.1. Methyl 6-[(2-chloro-4-fluorobenzyl)sulfonyl]cyclohex-1-ene-1-carboxylate (15a). A mixture of **10a** (220 mg, 0.61 mmol), concd H_2SO_4 (0.4 mL), and MeOH (20 mL) was refluxed for 134 h. The mixture was concentrated in vacuo, and then worked up (AcOEt, water, brine). The residue was chromatographed on an ODS column (MeOH/water = 7:3, v/v) followed by crystallization from *i*-Pr_2O–hexane to afford **15a** (120 mg, 57%) as colorless crystals. ^{1}H NMR ($CDCl_3$) δ: 1.58–2.53 (6H, m), 3.79 (3H, s), 4.39–4.42 (1H, m), 4.59 (2H, s), 7.04 (1H, dt, J = 8.4, 2.6 Hz), 7.21 (1H, dd, J = 8.4, 2.6 Hz), 7.41 (1H, t, J = 3.6 Hz), 7.61 (1H, dd, J = 8.4, 6.0 Hz). Anal. Calcd for $C_{15}H_{16}ClFO_4S$: C, 51.95; H, 4.65. Found: C, 51.96; H, 4.62.

5.12.2. Propyl 6-[(2-chloro-4-fluorobenzyl)sulfonyl]cyclohex-1-ene-1-carboxylate (15b). Compound **15b** was prepared from **10a** in the same manner as described above for **15a**. Yield 56%. IR (KBr): 2982, 2940, 1705, 1605, 1495, 1314, 1254, 1128, 1111, 912 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 0.98 (3H, t, J = 7.4 Hz), 1.60–2.53 (8H, m), 4.12–4.20 (2H, m), 4.41 (1H, br), 4.59 (2H, s), 7.03 (1H, dt, J = 8.8, 3.0 Hz), 7.20 (1H, dd, J = 8.8, 3.0 Hz), 7.42 (1H, t, J = 3.8 Hz), 7.59 (1H, dd, J = 8.8, 6.0 Hz). Anal. Calcd for $C_{17}H_{20}ClFO_4S$: C, 54.47; H, 5.38. Found: C, 54.34; H, 5.54.

5.13. {6-[(2-Chloro-4-fluorobenzyl)sulfonyl]cyclohex-1-en-1-yl}methyl acetate (15g)

(1) To a solution of **16f** (200 mg, 0.70 mmol) in pyridine (4.0 mL) maintained under a nitrogen atmosphere was added acetylchloride (74 μL, 1.05 mmol) at 0 °C. The mixture was stirred for 1 h at 0 °C and then room temperature for 17 h, and worked up (AcOEt; 1 N HCl, water and brine). The residue was purified by silica gel column chromatography (AcOEt/hexane = 1:7, v/v) followed by crystallization from hexane to give {6-[(2-chloro-4-fluorobenzyl)sulfanyl]cyclohex-1-en-1-yl}methyl acetate (92 mg, 40%) as white crystals. ^{1}H NMR ($CDCl_3$) δ: 1.60–1.70 (1H, m), 1.76–2.10 (5H, m), 1.97 (3H, s), 3.26 (1H, s), 3.82 (2H, ABq, J = 14 Hz), 4.37 (1H, d, J = 12.3 Hz), 4.68 (1H, dd, J = 12.3, 1.2 Hz), 5.84–5.86 (1H, m), 6.94 (1H, td, J = 8.4, 2.7 Hz), 7.10 (1H, dd, J = 8.4, 2.7 Hz), 7.45 (1H, dd, J = 8.4, 6.0 Hz). Anal. Calcd for $C_{16}H_{18}ClFO_2S$: C, 58.44; H, 5.52. Found: C, 58.49; H, 5.45.

(2) Compound **15g** was prepared from the above product by a similar procedure to that described for the synthesis of **10a**. Yield 80%. White powder. IR (KBr): 2948, 1738, 1605, 1493, 1312, 1235, 1127, 1044, 914 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 1.67–1.76 (1H, m), 1.78–1.94 (1H, m), 1.96–2.11 (1H, m), 2.01 (3H, s), 2.14–2.42 (3H, m), 3.81 (1H, br s), 4.39, 4.48 (2H, ABq, J = 14 Hz), 4.66, 4.76 (2H, ABq, J = 14 Hz), 6.25–6.26 (1H, br), 7.02–7.08 (1H, m), 7.20 (1H, dd, J = 8.4, 2.7 Hz), 7.59 (1H, dd, J = 8.7, 6.0 Hz). Anal. Calcd for $C_{16}H_{18}ClFO_4S$: C, 53.26; H, 5.03. Found: C, 53.14; H, 5.12.

5.13.1. 1-{6-[(2-Chloro-4-fluorobenzyl)sulfonyl]cyclohex-1-en-1-yl}propan-1-one (15h). (1) To a mixture of **16h** (80 mg, 0.25 mmol), molecular sieves (4Å, 71 mg), 4-methylmorpholine *N*-oxide (71 mg, 0.61 mmol) in CH_2Cl_2 (2.0 mL) was added TPAP (9.0 mg, 0.03 mmol) and the reaction stirred for 1 h at 0 °C under a nitrogen atmosphere. The reaction mixture was submitted to silica gel column chromatography (AcOEt/hexane = 1:4, v/v) to give 1-{6-[(2-chloro-4-fluorobenzyl)sulfanyl]cyclohex-1-en-1-yl}propan-1-one (43 mg, 54%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 1.09 (3H, t, J = 7.2 Hz), 1.61–1.72 (2H, m), 1.83–1.96 (2H, m), 2.15–2.41 (2H, m), 2.45–2.81 (2H, m), 3.94 (3H, br s), 6.86 (1H, t, J = 3.2 Hz), 6.96 (1H, td, J = 8.4, 2.6 Hz), 7.11 (1H, dd, J = 8.4, 2.6 Hz), 7.47 (1H, dd, J = 8.4, 6.0 Hz).

(2) To a solution of the above product (30 mg, 0.10 mmol) in AcOEt (1.0 mL), *m*CPBA (52 mg, 0.21 mmol) was added and the mixture was stirred for 1 h, then worked up (AcOEt; water, brine). The residue was crystallized from *i*-Pr_2O–hexane to afford **15h** (23 mg, 70%) as white crystals. IR (KBr): 2940, 1671, 1493, 1310, 1127, 912 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 1.66 (3H, t, J = 7.5 Hz), 1.55–2.56 (4H, m), 2.47–2.58 (2H, m), 2.72–2.86 (2H, m), 4.52, 4.57 (2H, ABq, J = 13.8 Hz), 4.60 (1H, br s), 7.02 (1H, td, J = 8.1, 2.7 Hz), 7.20 (1H, dd, J = 8.1, 2.7 Hz), 7.27–7.30 (1H, m), 7.59 (1H, dd, J = 8.7, 6.0 Hz). Anal. Calcd for $C_{16}H_{18}ClFO_3S$: C, 55.73; H, 5.26. Found: C, 55.50; H, 5.29.

5.13.2. 1-{6-[(2-Chloro-4-fluorobenzyl)sulfonyl]cyclohex-1-en-1-yl}butan-1-one (15i). Compound **15i** was prepared by a similar procedure to that described for the synthesis of **15h**.

(1) 1-{6-[(2-Chloro-4-fluorobenzyl)sulfanyl]cyclohex-1-en-1-yl}butan-1-one: Yield 65%. Colorless oil. ^{1}H NMR ($CDCl_3$) δ: 0.92 (3H, t, J = 7.2 Hz), 1.59–1.68 (4H, m), 1.85–1.99 (2H, m), 2.10–2.38 (2H, m), 2.46–2.67 (2H, m), 3.93 (3H, s), 6.85 (1H, t, J = 4.8 Hz), 6.96 (1H, td, J = 8.4, 2.7 Hz), 7.11 (1H, dd, J = 8.4, 2.7 Hz), 7.47 (1H, dd, J = 8.4, 6.0 Hz).

(2) **15i**: Yield 29%. IR (KBr): 2963, 1669, 1493, 1312, 1127, 914 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 0.98 (3H, t, J = 7.5 Hz), 1.54–1.80 (4H, m), 1.94–2.58 (4H, m), 2.73

(3H, t, $J = 7.5$ Hz), 4.52, 4.56 (2H, ABq, $J = 13.8$ Hz), 4.61 (1H, br s), 6.99–7.06 (1H, m), 7.20 (1H, dd, $J = 8.4$, 2.7 Hz), 7.28–7.31 (1H, m), 7.59 (1H, dd, $J = 8.7$, 6.0 Hz).

5.14. Optical resolution with chiral HPLC (Table 6): ethyl 6-[(+)- and (−)-][*N*-(2-chloro-4-fluorobenzyl)sulfonyl]cyclohex-1-ene-1-carboxylate [(+)- and (−)-10a]

The racemate **10a** was resolved into its enantiomers by HPLC (column; CHIRALPAK AD,[15] 20 mm ID × 250 mm, mobile phase; hexane/EtOH = 8:2, v/v, flow rate; 8.0 mL/min, temperature; 30 °C, detection; UV 260 nm), followed by crystallization from hexane to afford both enantiomers: (+)-**10a** (49 mg, retention time: 19.5 min) and (−)-**10a** (50 mg, retention time: 14.2 min), as white powder, respectively.

Compounds **10e** and **22a** were resolved by chiral HPLC in the same method as above. The optical purity of each enantiomer obtained and HPLC conditions are summarized in Table 6.

5.15. Biology

5.15.1. Assay for inhibitory activity against NO production. RAW264.7 cells were plated at 1×10^5 cells/well in 96-well culture plates (Nunc, Rochester, NY) and incubated overnight. After removing cell culture supernatants, cells were stimulated with 10 ng/mL LPS in the presence of various concentrations of test compounds for 20 h in stimulation medium (phenol red-free RPMI1640 containing 1% heat-inactivated FBS and 10 μg/mL kanamycin) at 37 °C in a humidified atmosphere of 5% CO_2 in air. The test compounds were prepared as 10 mM solutions in DMF, diluted with an RPMI-1640 medium to the appropriate concentrations, and added to the culture.

Production of NO was estimated by measuring the amount of nitrite, a stable metabolite of NO, according to a fluorometric method[16] using 2,3-diaminonapthalene (DAN, Dojindo Laboratories, Kumamoto, Japan). Briefly, 25 μL of 20 μg/mL DAN was added to 50 μL of the culture supernatant and incubated at room temperature for 10 min. After adding 25 μL of 0.5 N NaOH, fluorescence at 460 nm (excitation wavelength: 355 nm) was measured.

5.15.2. Endotoxin shock model. Mice were intraperitoneally injected with LPS at a lethal dose of 7 mg/kg. Survival of mice was recorded for 7 days following LPS challenge. Compounds (*R*)-(+)-**10a** and (6*R*, 1*S*)-(+)-**22a** were dissolved in 10% (w/v) Glucuronylglucosyl-beta-cyclodextrin sodium salt (Wako, Osaka, Japan) and administered intravenously 1 h before the LPS injection.

Acknowledgments

We thank Drs. A. Miyake, Y. Iizawa and S. Hashiguchi for their encouragement and helpful advice. We thank Drs. T. Yamano and T. Ishikawa for synthesis, and Mr. S. Yamamoto and Mr. M. Tanaka for opitical resolution. We thank Ms. K. Higashikawa for X-ray crystallographic analysis. We thank Dr. T. Sha, Mr. K. Oda, Ms. M. Uekata and Ms. N. Matsunaga for in vitro and in vivo assays.

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