Supporting Information

On the Sulfide Oxidation to Sulfoxides using Sodium Orthovanadate in Water

Yunhan Zhang[a†], Yiting Su[a†], Lan Liu[a,b], Bishuang Chen*[a,b]

a. School of Marine Sciences, Sun Yat-Sen University, Zhuhai 519080.

b. Southern Marine Science and Engineering Guangdong Laboratory (Zhuhai), Zhuhai 519080, China..

† The two authors contributed equally to this work.

Corresponding Author:

Bishuang Chen: chenbsh23@mail.sysu.edu.cn / (+86) 0756-3668775

Table of Contents

1. Materials and methods

All chemical raw materials used were purchased from commercial suppliers and were not subject to additional purification.

1.1 Experimental procedures for sulfoxidation

The sulfoxidation reaction was carried out according to the general procedure shown as below. The reaction was carried in 1 mL of PBS buffer containing substrate, H_2O_2 and Na_3VO_4. The specific concentration (substrate concentration of **1a-1r**: 5mM, substrate concentration of **2a-2g**: 1mM) of each substrate was used, sodium orthovanadate was added in the amount of 10 mM, and hydrogen peroxide was added in double the amount of substrate. The reaction was carried out at 35°C for 6 h. At the completion of the reaction, the mixture was extracted with an equal volume of ethyl acetate, dried with Na_2SO_4, and then the sample was analyzed by GC or HPLC. All the experiments were performed in triplicate.

Notably, most organic sulfides exhibit limited solubility in aqueous media such as phosphate buffer. To address this issue and ensure homogeneous reaction conditions, we employed a DMSO pre-dissolution strategy. Specifically, a stock solution of the sulfide (100 mM) was first prepared in DMSO, which ensured complete dissolution of the hydrophobic substrate. Subsequently, 50 μL of this DMSO solution was added to 950 μL of phosphate buffer containing the appropriate concentrations of Na_3VO_4 and H_2O_2, resulting in a final reaction mixture with: 5 mM sulfide, 5% v/v DMSO and PBS buffer (50 mM, pH 6.5).

1.2 Experimental procedure for investigation on effects of key parameters

The reaction set-up for the investigation on effects of key parameters was the same as used to perform the sulfoxidation reaction with temperature, pH, hydrogen peroxide concentration and substrate concentration changes within the given ranges.

1.3 Experimental procedure for isotope labelling

The oxygen origin in the reaction was investigated using ^{18}O-labeled H_2O_2. Specifically, 1 mL reaction system containing 10 mM Na_3VO_4, 5 mM methyl phenyl sulfide, and 10 mM ^{18}O-H_2O_2 was incubated in a shaker (35 °C, 700 rpm) for 6 h. After the reaction, the mixture was extracted with an equal volume of ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4 and subsequently analyzed by gas chromatography-mass spectrometry (GC-MS).

1.4 The enzymatic sulfoxidation by *Ci*VCPO

1.4.1 The synthesized encoding gene of *Ci*VCPO

ATGGGCAGCGTGACCCCCATCCCCCTGCCCAAGATCGACGAGCCCGAGGAGTACAACACC
AACTACATCCTGTTCTGGAACCACGTGGGCCTGGAGCTGAACAGGGTGACCCACACCGTG
GGCGGCCCCCTGACCGGCCCCCCCCTGAGCGCCAGGGCCCTGGGCATGCTGCACCTGGCC
ATCCACGACGCCTACTTCAGCATCTGCCCCCCCACCGACTTCACCACCTTCCTGAGCCCC
GACACCGAGAACGCCGCCTACAGGCTGCCCAGCCCCAACGGCGCCAACGACGCCAGGCAG
GCCGTGGCCGGCGCCGCCCTGAAGATGCTGAGCAGCCTGTACATGAAGCCCGTGGAGCAG
CCCAACCCCAACCCCGGCGCCAACATCAGCGACAACGCCTACGCCCAGCTGGGCCTGGTG
CTGGACAGGAGCGTGCTGGAGGCCCCCGGCGGCGTGGACAGGGAGAGCGCCAGCTTCATG
TTCGGCGAGGACGTGGCCGACGTGTTCTTCGCCCTGCTGAACGACCCCAGGGGCGCCAGC
CAGGAGGGCTACCACCCCACCCCCGGCAGGTACAAGTTCGACGACGAGCCCACCCACCCC
GTGGTGCTGATCCCCGTGGACCCCAACAACCCCAACGGCCCCAAGATGCCCTTCAGGCAG
TACCACGCCCCCTTCTACGGCAAGACCACCAAGAGGTTCGCCACCCAGAGCGAGCACTTC
CTGGCCGACCCCCCCGGCCTGAGGAGCAACGCCGACGAGACCGCCGAGTACGACGACGCC
GTGAGGGTGGCCATCGCCATGGGCGGCGCCCAGGCCCTGAACAGCACCAAGAGGAGCCCC
TGGCAGACCGCCCAGGGCCTGTACTGGGCCTACGACGGCAGCAACCTGATCGGCACCCCC
CCCAGGTTCTACAACCAGATCGTGAGGAGGATCGCCGTGACCTACAAGAAGGAGGAGGAC
CTGGCCAACAGCGAGGTGAACAACGCCGACTTCGCCAGGCTGTTCGCCCTGGTGGACGTG
GCCTGCACCGACGCCGGCATCTTCAGCTGGAAGGAGAAGTGGGAGTTCGAGTTCTGGAGG
CCCCTGAGCGGCGTGAGGGACGACGGCAGGCCCGACCACGGCGACCCCTTCTGGCTGACC
CTGGGCGCCCCCGCCACCAACACCAACGACATCCCCTTCAAGCCCCCCTTCCCCGCCTAC

CCCAGCGGCCACGCCACCTTCGGCGGCGCCGTGTTCCAGATGGTGAGGAGGTACTACAAC
GGCAGGGTGGGCACCTGGAAGGACGACGAGCCCGACAACATCGCCATCGACATGATGATC
AGCGAGGAGCTGAACGGCGTGAACAGGGACCTGAGGCAGCCCTACGACCCCACCGCCCCC
ATCGAGGACCAGCCCGGCATCGTGAGGACCAGGATCGTGAGGCACTTCGACAGCGCCTGG
GAGCTGATGTTCGAGAACGCCATCAGCAGGATCTTCCTGGGCGTGCACTGGAGGTTCGAC
GCCGCCGCCGCCAGGGACATCCTGATCCCCACCACCACCAAGGACGTGTACGCCGTGGAC
AACAACGGCGCCACCGTGTTCCAGAACGTGGAGGACATCAGGTACACCACCAGGGGCACC
AGGGAGGACCCCGAGGGCCTGTTCCCCATCGGCGGCGTGCCCCTGGGCATCGAGATCGCC
GACGAGATCTTCAACAACGGCCTGAAGCCCACCCCCCCCGAGATCCAGCCCATGCCCCAG
GAGACCCCCGTGCAGAAGCCCGTGGGCCAGCAGCCCGTGAAGGGCATGTGGGAGGAGGAG
CAGGCCCCCGTGGTGAAGGAGGCCCCC

1.4.2 Gene synthesis and sub cloning

The *Ci*VCPO-recombinant plasmids were constructed according to a previous procedure. The genes were codon-optimized for *E. coli*, synthesized, and synthesized and cloned in frame with the N-terminal His-tag of the expression vector pET28a(+) between the NdeI and HindIII restriction sites by Tsingke Biotechnology Co., Ltd. (Beijing, China).

1.4.3 Expression and purification of *CiVCPO*

Single colonies of the recombinant strains were picked and then incubated in 5 mL of LB medium containing 50 μg/mL kanamycin at 37 ◦C and 200 rpm for 6 h. The seed broth was transferred to 250 mL of LB medium containing 50 μg/mL of kanamycin with a 2% inoculation dose and shaken at 37 °C and 200 rpm for 3 h. An amount of 0.5 mM of Isopropyl-β-D-thiogalactopyranoside (IPTG) was added to induce the protein expression. After induction at 18 °C for 16 h, the cells were harvested by centrifugation at 4 °C at 12,000× *g* for 20 min.

The harvested cells expressing enzymes were resuspended in binding buffer (20 mM sodium phosphate, a pH of 7.4, 500 mM of NaCl, 100 μM of sodium

orthovanadate, and 20 mM of imidazole), disrupted by ultrasonication in an ice bath, and followed by centrifugation at 12,000× *g* for 20 min to remove the cell debris. The resulting supernatant was loaded onto a Ni-NTA column (5 mL, Sangon Biotech, Shanghai, China) that was pre-equilibrated with buffer A (20 mM of sodium phosphate, a pH of 7.4, 500 mM of NaCl, and 20 mM of imidazole). After washing with washing buffer (20 mM of sodium phosphate, a pH of 7.4, 500 mM of NaCl, and 50 mM of imidazole), the target protein was eluted with elution buffer (w0 mM of sodium phosphate, a pH of 7.4, 500 mM of NaCl, and 250 mM of imidazole). The crude extract was concentrated by an ultrafiltration centrifuge tube (Merck Millipore, 30 kDa) and then stored at −20 °C for subsequent analysis.

1.4.4 Sulfoxidation catalyzed by *Ci*VCPO, thermally inactivated *Ci*VCPO, and Na_3VO_4

In a 1 mL reaction system, the substrate sulfide concentration was 5 mM and double the amount of hydrogen peroxide added to the substrate, 1 μM *Ci*VCPO was added, and the reaction was carried out in PBS (50 mM, pH 6.0) buffer for 6 h. At the end of the reaction, the mixture was extracted with an equal volume of ethyl acetate, and the mixture was dried with Na_2SO_4, and the samples were analyzed using a gas chromatograph. The reaction system for the thermally inactivate *Ci*VCPO is the sameas above, with the addition of 1 μM *Ci*VCPO inactivated at 99 °C for an hour as the biocatalyst. The reaction system for Na_3NO_4 is the same as above, with no enzyme adding to the reaction system but the addition of 10 mM sodium orthovanadate. All the reactions were incubated in a shaker (35 °C, 700 rpm) for 6 h. After the reaction, the mixture was extracted with an equal volume of ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4 and subsequently analyzed by gas chromatography-mass spectrometry (GC-MS).

1.5 Time-course of substrate conversion and product formation

In 1 mL reaction volumes, the substrate methyl phenyl sulfide was added at a concentration of 5 mM, 10 mM Na_3VO_4 was added, and hydrogen peroxide was added in double the amount of the substrate. The reaction was carried out at 35°C for 8 h. Samples were taken at hourly intervals, the mixture was extracted with an equal volume of ethyl acetate, dried with Na_2SO_4 and the samples were analyzed by gas chromatography.

1.6 GC analysis

1.6.1 GC method

(1) The yield of sulfoxides (**1a-1f, 1n**) generated from the corresponding sulfides were analyzed using gas chromatography with a Scion GC 456 system equipped with an Agilent J&W DB-1 GC column (60 m × 0.53 mm × 2.5 μm) and nitrogen as the carrier gas. The specific method was as follows (Table S1).

Table S1.GC Temperature Program

Heating Rate (°C/min)	Column Temperature (°C）	Hold Time (min)
initial	110	1.2
25	150	2
30	200	0.5
30	300	0.5

(2) The yield of sulfoxides (**1g**, **1i**, **1m**, **1r**) generated from the corresponding sulfides were analyzed using gas chromatography with a Scion GC 456 system equipped with an Agilent J&W DB-1 GC column (60 m x 0.53 mm × 2.5 μm) and nitrogen as the carrier gas. The specific method was as follows (Table S2).

Table S2.GC Temperature Program

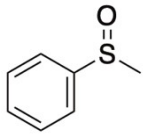
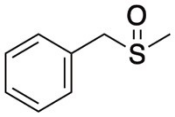
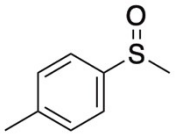
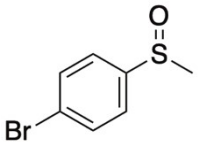
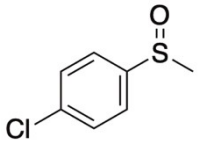
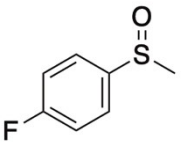
Heating Rate (°C/min)	Column Temperature (°C）	Hold Time (min)

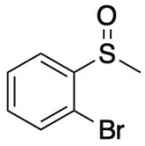
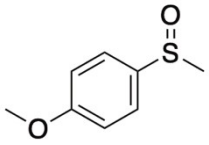
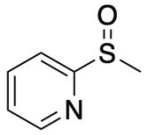
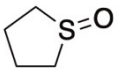
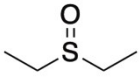
initial	35	1.2
10	150	2
15	230	0.5
20	280	0.5

1.6.2 The retention times of each compound

The GC retention times were shown in Table S3.

Table S3. GC retention times for selected compounds

entry	compound	retention time (min)
1a		7.71
1b		8.90
1c		8.77
1d		9.92
1e		9.18
1f		7.45

1g		20.32
1i		20.80
1m		16.63
1n		5.58
1r		10.72

1.6.3 The product yield determination

Product linear regression equation and correlation coefficient were shown in Table S4.

Table S4. Product linear regression equation and correlation coefficient

entry	product regression equation	correlation coefficient
1a	y = 508.48x - 144.93	0.9977
1b	y = 265.74x - 398.57	0.9975
1c	y = 578.96x - 167.24	0.9972
1d	y = 714.15x - 169.39	0.9989
1e	y = 629.88x - 293.37	0.9974
1f	y = 462.75x - 110.07	0.9995
1g	y = 923.49x - 356.62	0.9975
1i	y = 434.8x - 199.7	0.9976
1m	y = 232.08x - 73.299	0.9995
1n	y = 153.88x - 13.815	0.9983

1r	y = 246.8x - 82.217	0.9982

yield(%) = (Ca/C0) × 100%

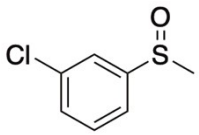
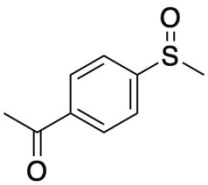
In the formula, Ca represents the molar amount of sulfoxide produced by the reaction for a certain period of time; C0 represents the molar amount of substrate added initially.

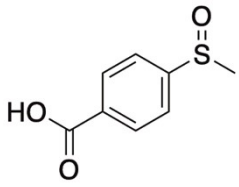
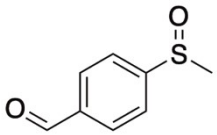
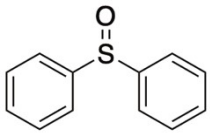
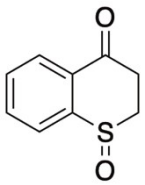
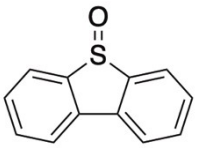
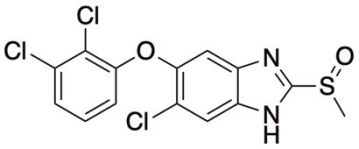
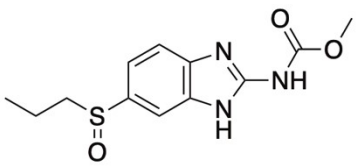
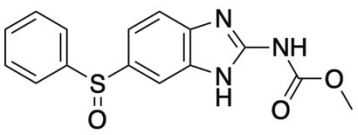
1.7 HPLC analysis

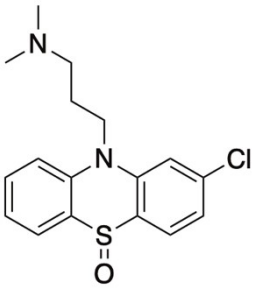
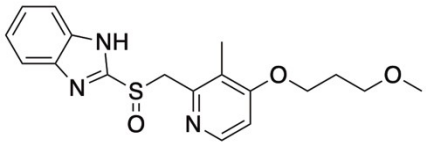
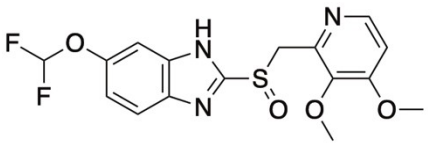
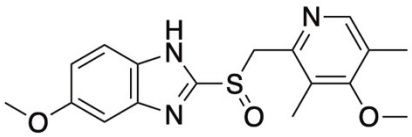
1.7.1 The HPLC method

The yield of sulfoxides (**1h**, **1j-1l**,**1o-1q**, **2a-2g**) generated from the corresponding sulfides were analyzed using HPLC. Detection conditions were given as follows: The Ultimate Cellu-D (Welch) HPLC column (250×4.6 mm, Chiral Technologies), temperature: 30 °C, flow rate: 1 mL/min, loading volume: 5 μl, mobile phase buffer A: hexane, buffer B: iso-propyl alcohol (IPA). The detailed analytical conditions and the retention times of each compound were shown in Table S5. (This column is a chiral stationary phase that can separate the enantiomers of some compounds, resulting in two distinct peaks on the chromatogram).

Table S5. HPLC methods for analysis

entry	compound	program	retention time
1h		Hexane 70%, IPA 30% 254nm	7.883
1j		Hexane 30%, IPA 70% 254nm	9.709

1k		Hexane 30%, IPA 70% 210nm	 9.944
1l		Hexane 70%, IPA 30% 310nm	 9.981
1o		Hexane 10%, IPA 90% 254nm	10.045
1p		Hexane 50%, IPA 50% 280nm	20.283 21.007
1q		Hexane 50%, IPA 50% 254nm	24.421
2a		Hexane 10%, IPA 90% 310nm	12.613
2b		Hexane 10%, IPA 90% 310nm	8.942
2c		Hexane 70%, IPA 30% 254nm	11.772 12.892

2d		Hexane 10%, IPA 90% 310nm	13.614
2e		Hexane 30%, IPA 70% 280nm	12.114
2f		Hexane 50%, IPA 50% 310nm	16.109
2g		Hexane 70%, IPA 30% 254nm	24.421

1.7.2 The determination of the product yield by HPLC

Product linear regression equation and correlation coefficient were shown in Table S6.

Table S6. Product linear regression equation and correlation coefficient

entry	product regression equation	correlation coefficient
1h	y = 844.16x - 874.66	0.9939
1j	y = 2729.6x - 112.23	0.9996
1k	y = 431.43x - 52.46	0.9988
1l	y = 51x - 2.9333	0.9979
1o	y = 4373.8x + 1542.	0.9994
1p	y = 1034.6x - 68.681	0.9990
1q	y = 4540.2x - 4600.7	0.9979

2a	y = 3175.9x - 2479.2	0.9995
2b	y = 5570.8x - 676.76	0.9967
2c	y = 3602.3x - 1133.3	0.9982
2d	y = 2231.9x - 347.8	0.9941
2e	y = 3048.1x - 51.8	0.9999
2f	y = 788.68x + 344	0.9995
2g	y = 1803.2x + 501.98	0.9944

yield(%) = (Ca/C0) × 100%

In the formula, Ca represents the molar amount of sulfoxide produced by the reaction for a certain period of time; C0 represents the molar amount of substrate added initially.

2. Supplementary Figures

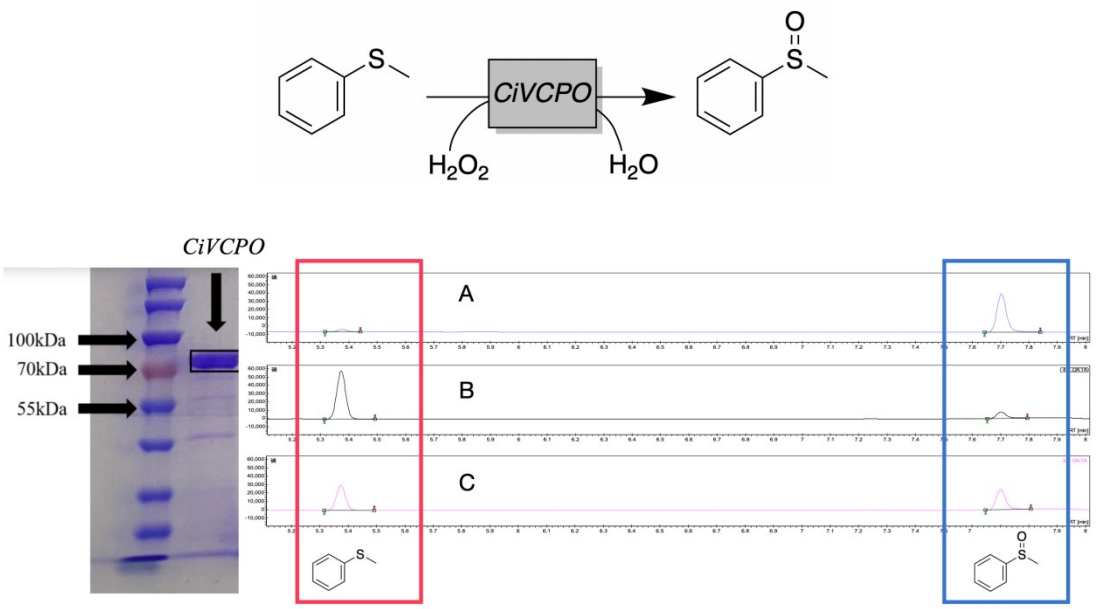


Figure S1. sulfoxidation catalyzed by *Ci*VCPO, thermally inactivated *Ci*VCPO, and Na_3VO_4. Left: SDS-PAGE of *Ci*VCPO. Right: The GC chromatogram of sulfoxidation catalyzed by *Ci*VCPO (A), thermally inactivated *Ci*VCPO (B) and Na_3VO_4 (C).

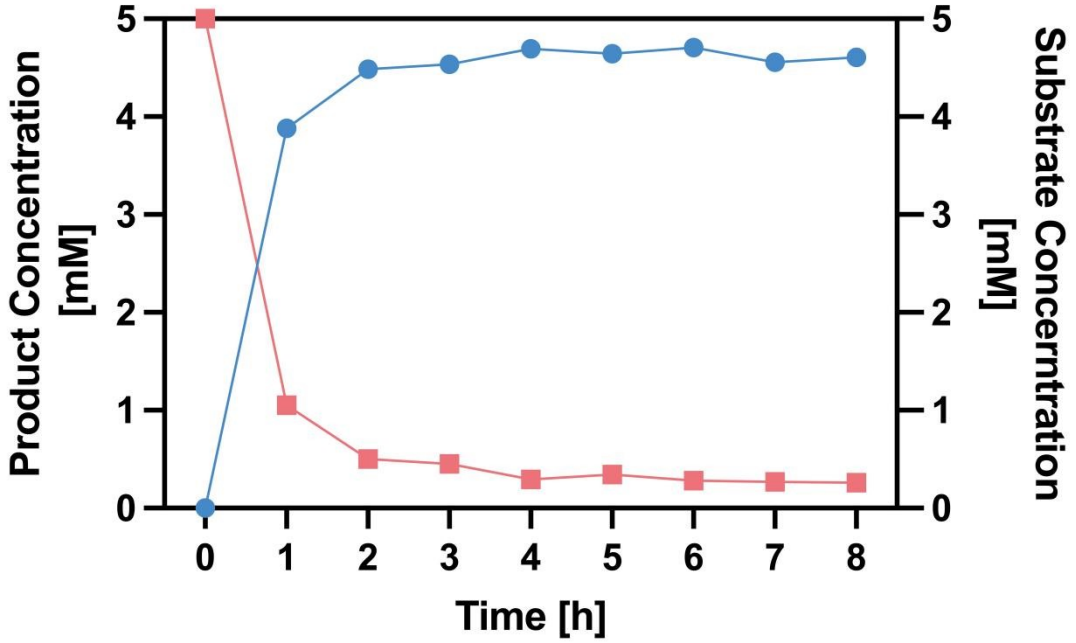


Figure S2. Time-course of substrate conversion and product formation.

3. GC Chromatography

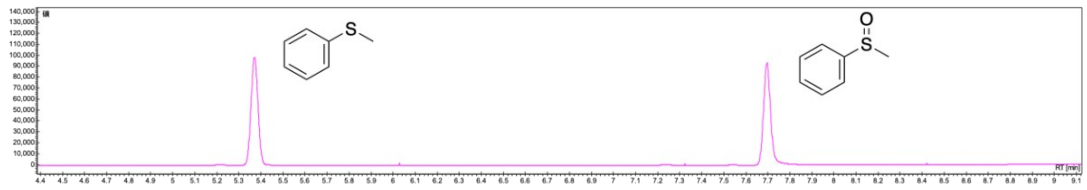


Figure S3. Representative GC chromatogram of **1a** and the corresponding substrate.

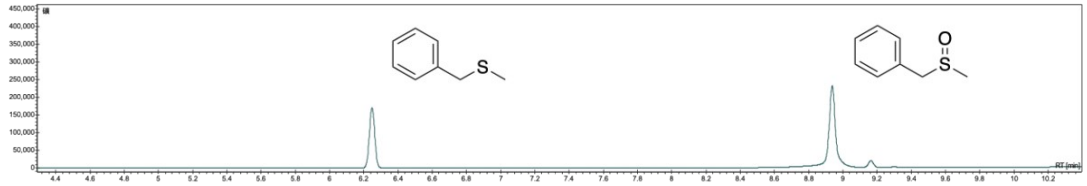


Figure S4. Representative GC chromatogram of **1b** and the corresponding substrate.

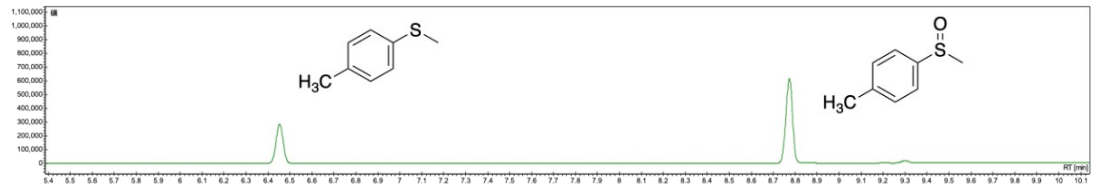


Figure S5. Representative GC chromatogram of **1c** and the corresponding substrate.

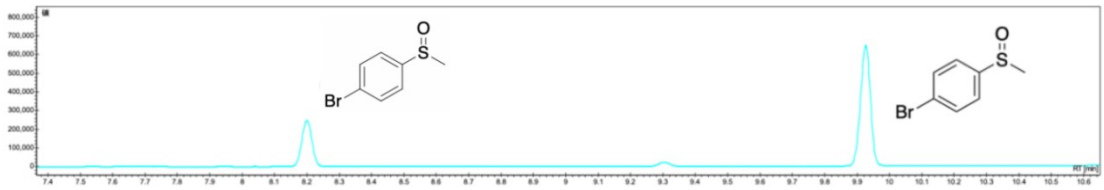


Figure S6. Representative GC chromatogram of **1d** and the corresponding substrate.

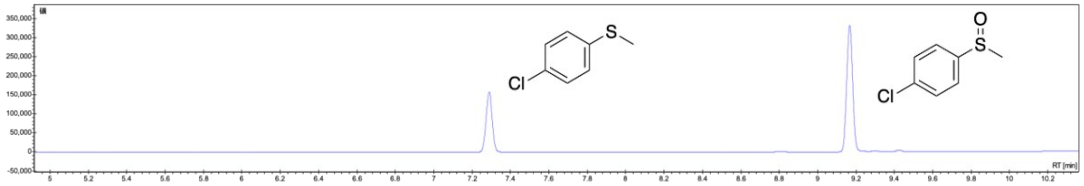


Figure S7. Representative GC chromatogram of **1e** and the corresponding substrate.

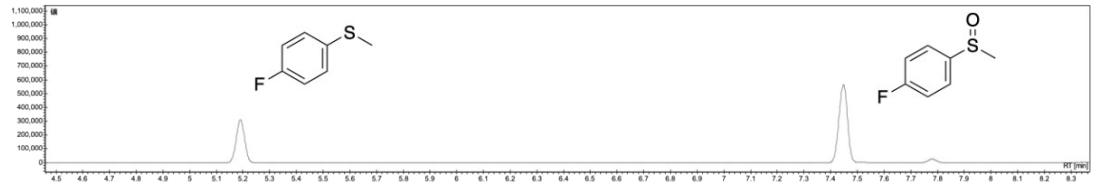


Figure S8. Representative GC chromatogram of 1f and the corresponding substrate.

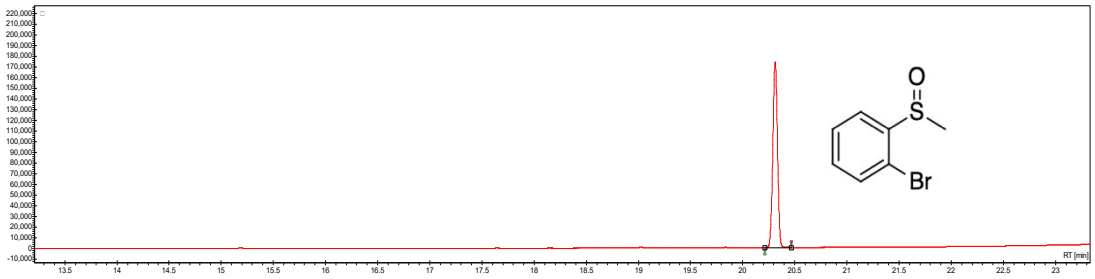


Figure S9. Representative GC chromatogram of **1g**.

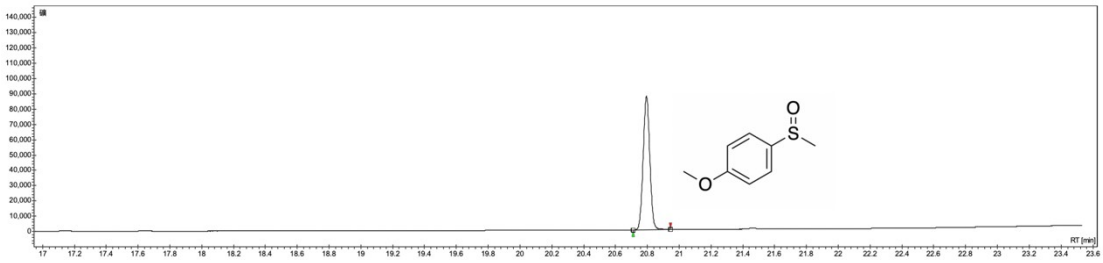


Figure S10. Representative GC chromatogram of **1i**.

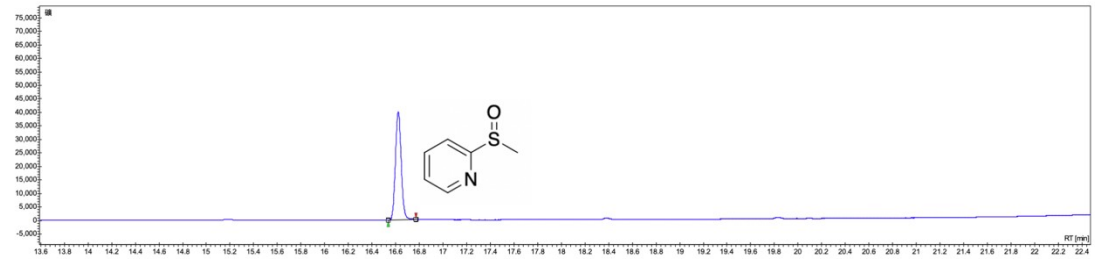


Figure S11. Representative GC chromatogram of **1m**.

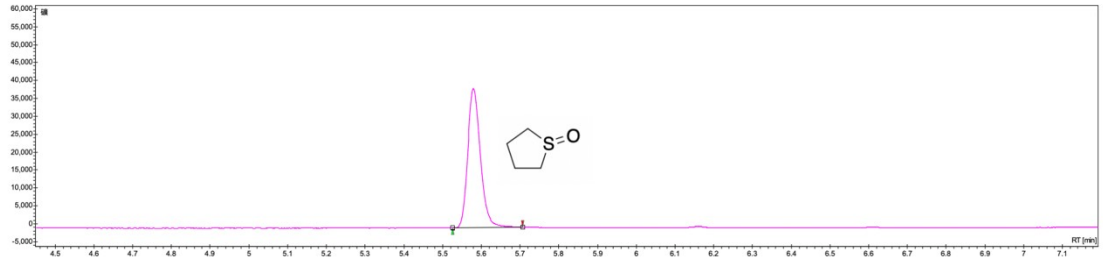


Figure S12. Representative GC chromatogram of **1n**.

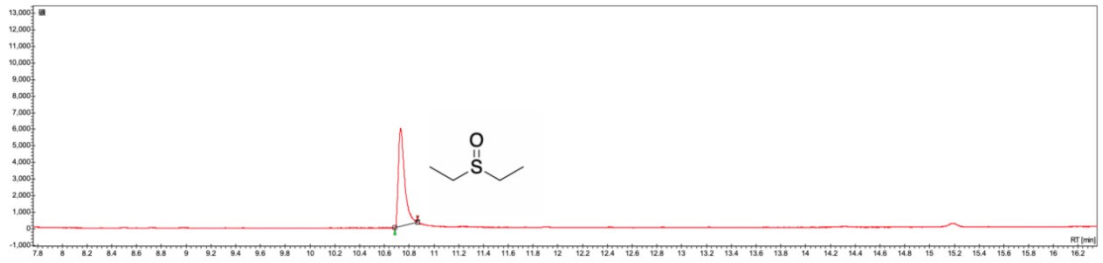


Figure S13. Representative GC chromatogram of **1r**.

4. HPLC Chromatography

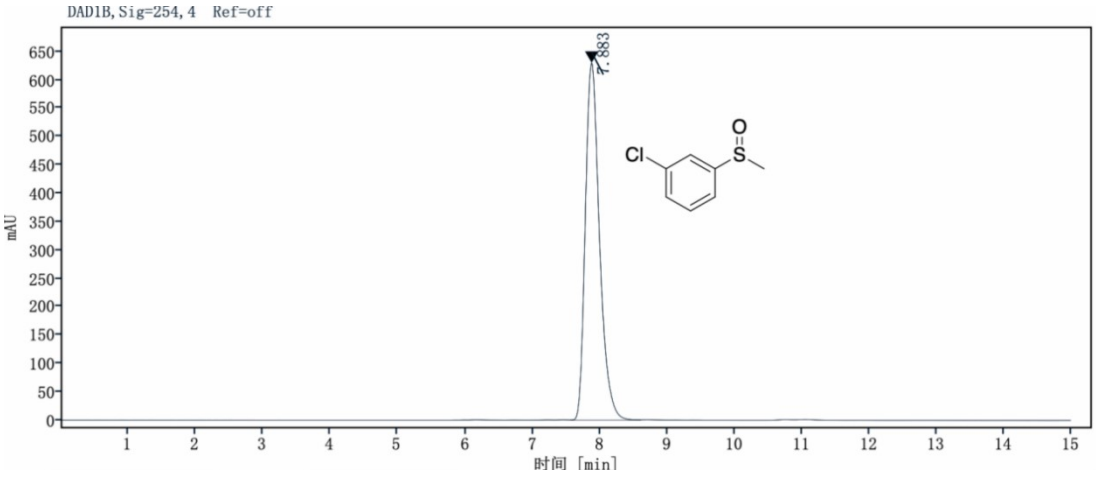


Figure S14. Representative chiral HPLC chromatogram of **1h**.

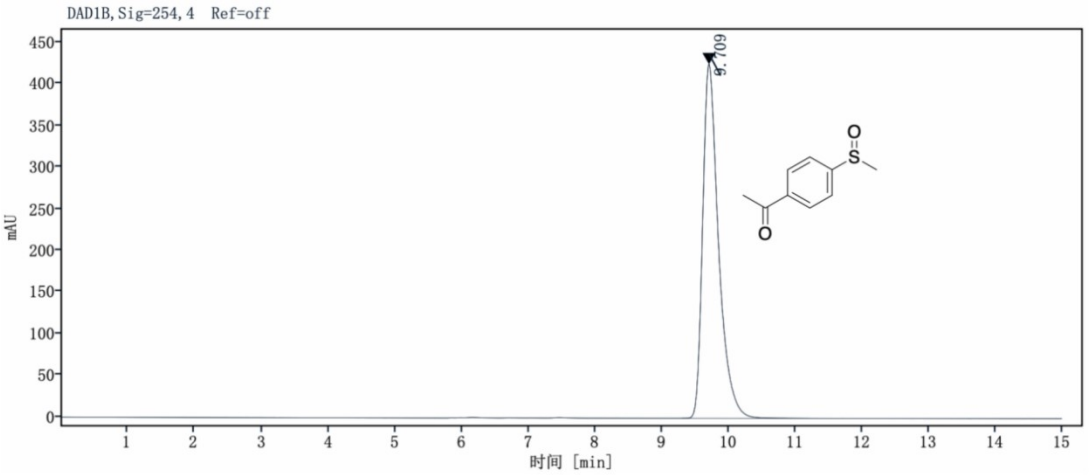


Figure S15. Representative chiral HPLC chromatogram of **1j**.

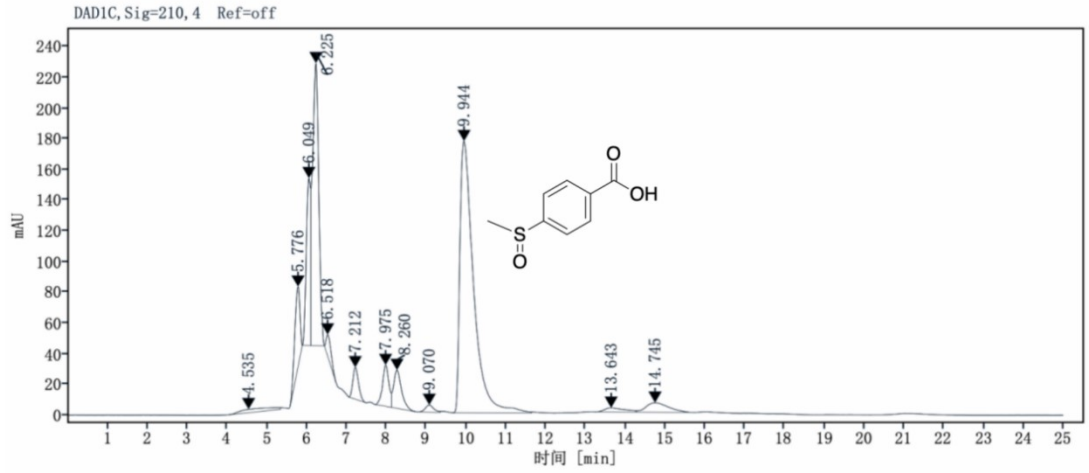


Figure S16. Representative chiral HPLC chromatogram of **1k.**

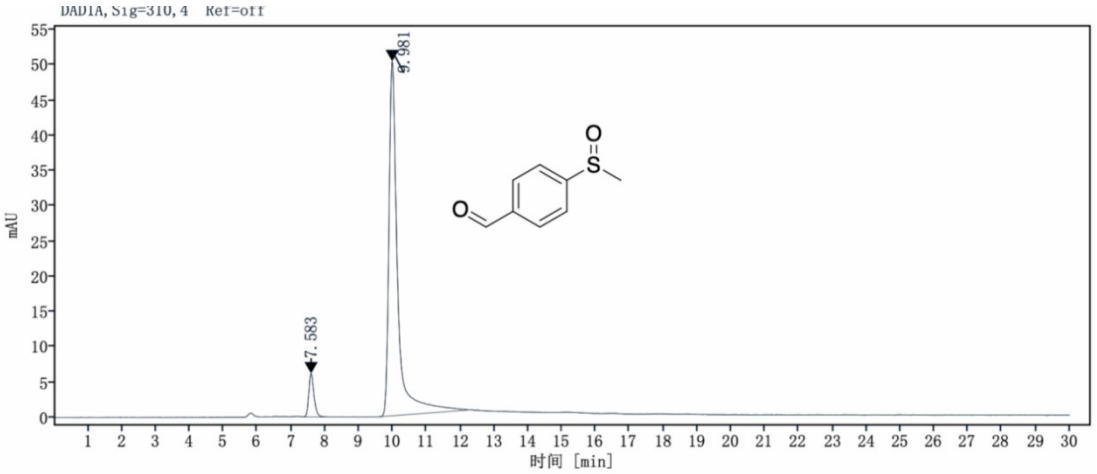


Figure S17. Representative chiral HPLC chromatogram of **1l**

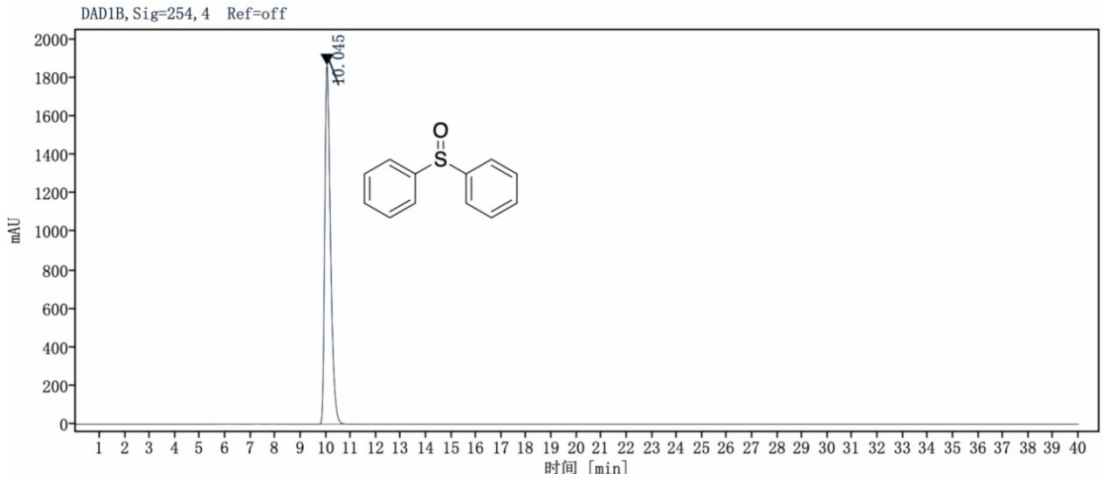


Figure S18. Representative chiral HPLC chromatogram of **1o.**

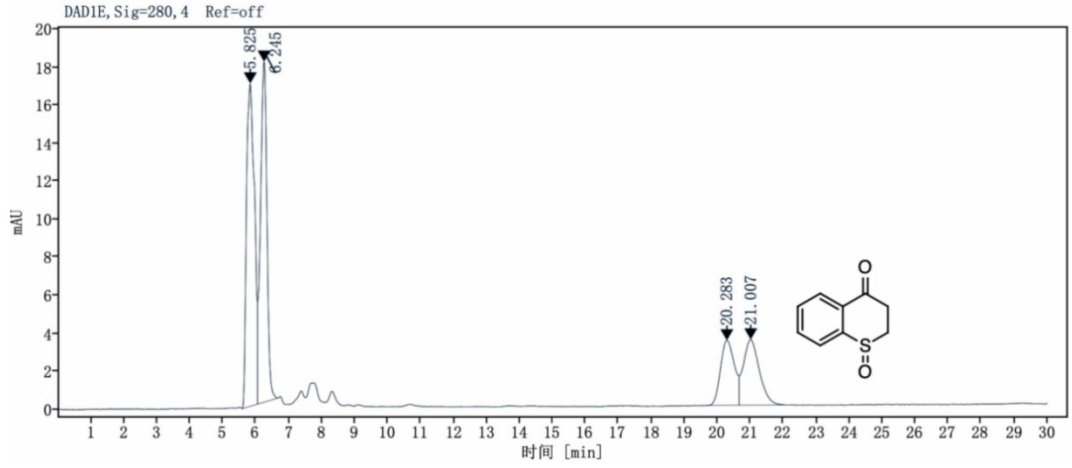


Figure S19. Representative chiral HPLC chromatogram of **1p.**

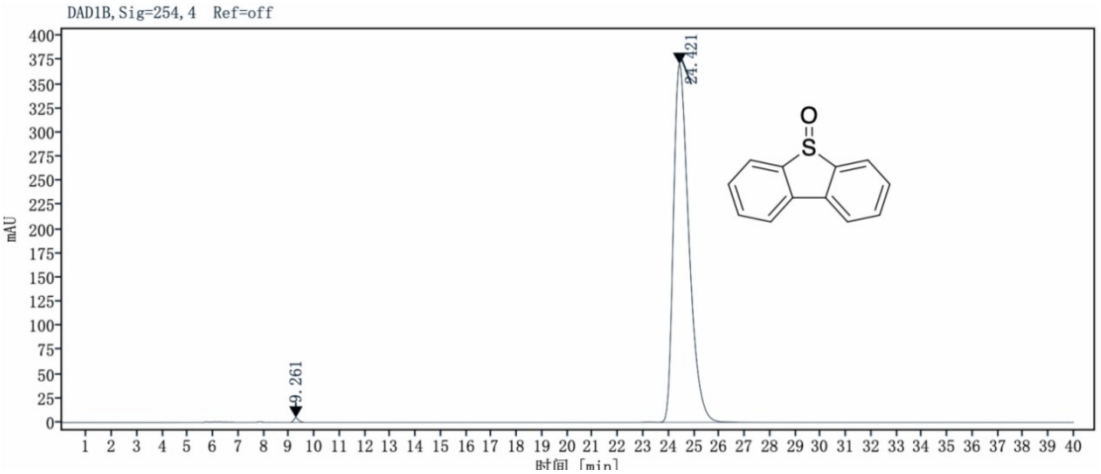


Figure S20. Representative chiral HPLC chromatogram of **1q.**

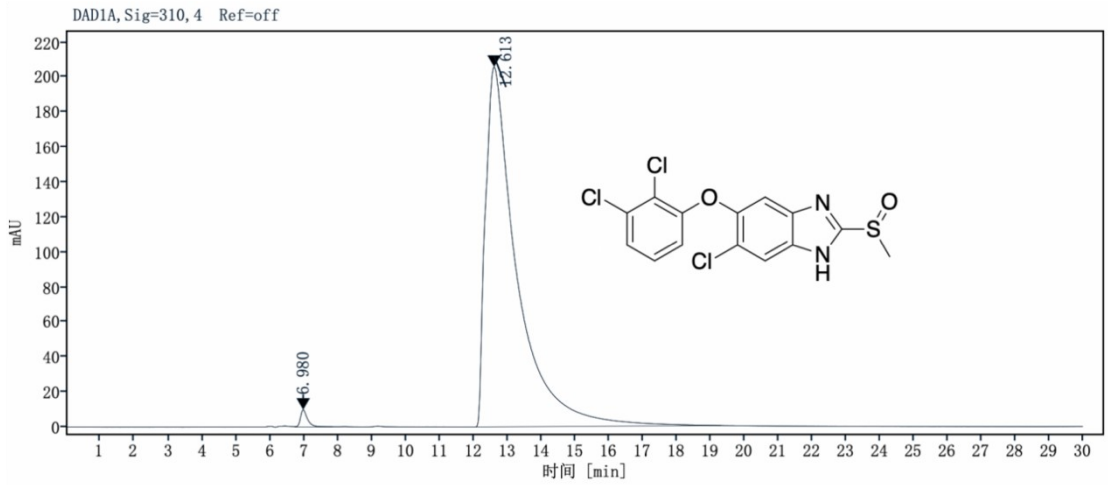


Figure S21. Representative chiral HPLC chromatogram of **2a**

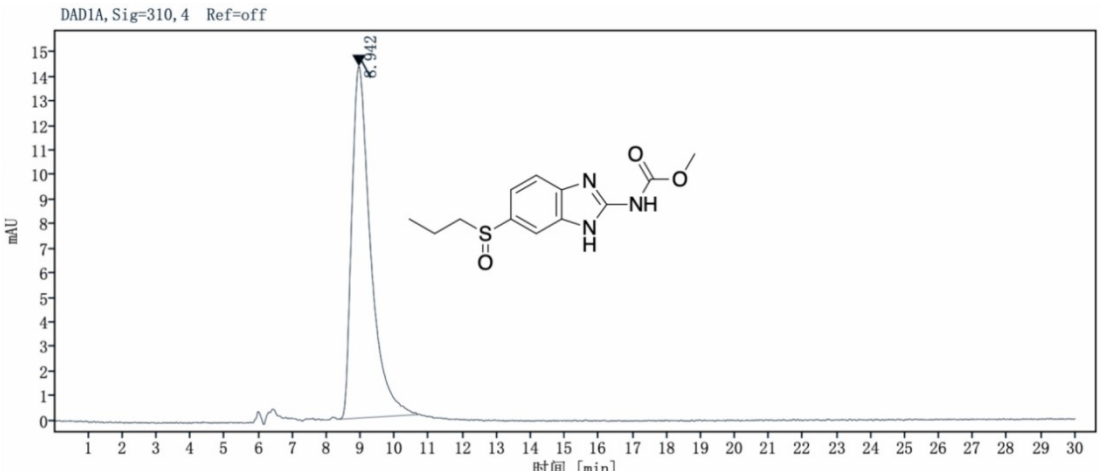


Figure S22. Representative chiral HPLC chromatogram of **2b.**

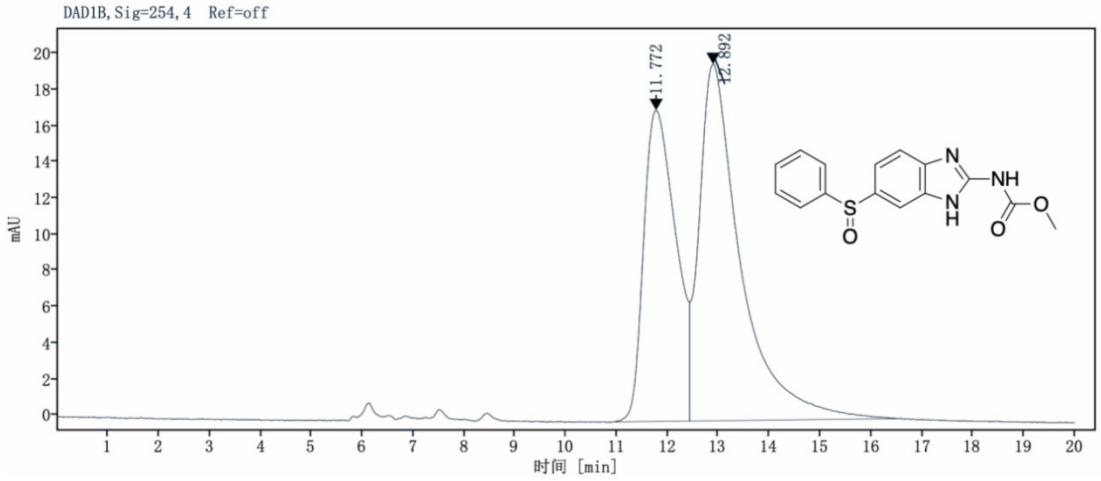


Figure S23. Representative chiral HPLC chromatogram of **2c.**

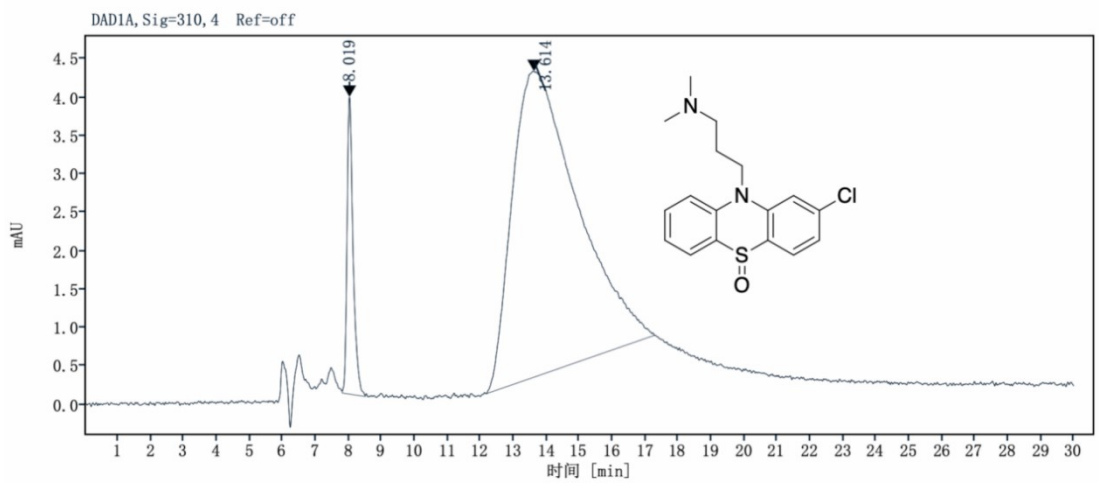


Figure S24. Representative chiral HPLC chromatogram of **2d.**

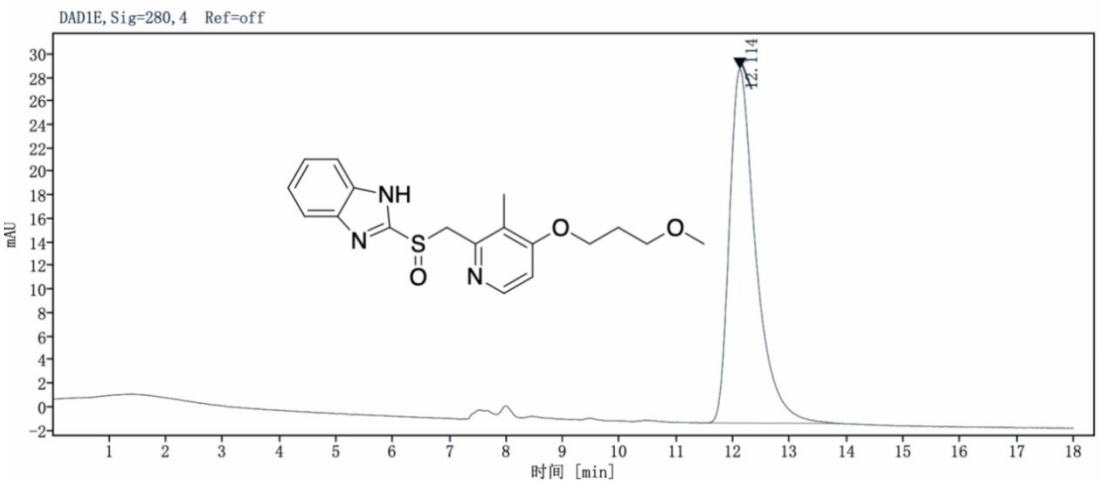


Figure S25. Representative chiral HPLC chromatogram of **2e.**

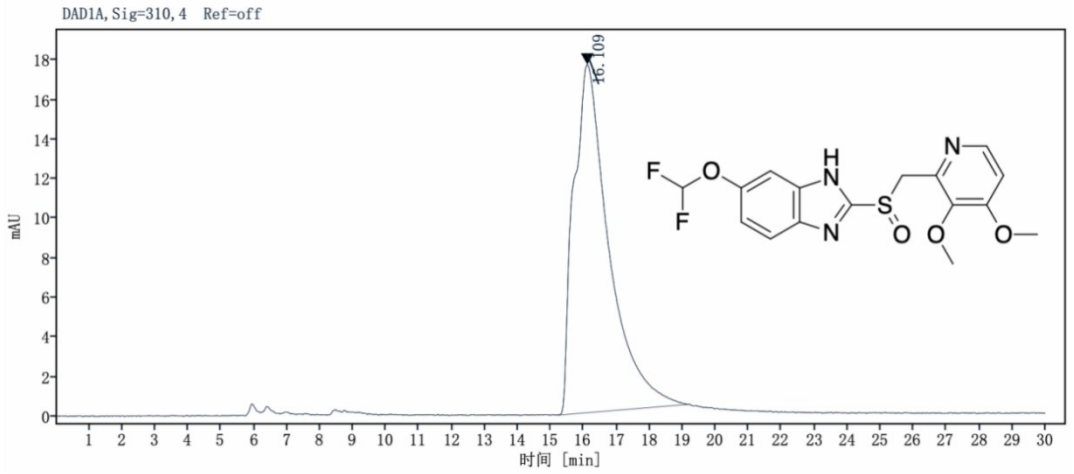


Figure S26. Representative chiral HPLC chromatogram of **2f.**

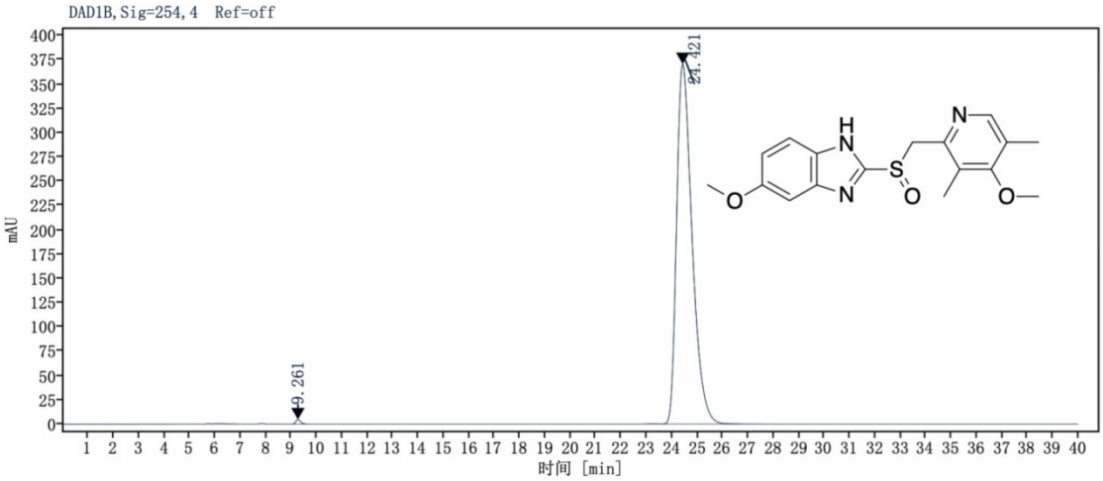


Figure S27. Representative chiral HPLC chromatogram of **2g.**