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Synthesis of chiral ND-322, ND-364 and ND-364 derivatives as selective inhibitors of human gelatinase

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ABSTRACT

Compounds **10** (**ND-322**) and **15** (**ND-364**) are potent selective inhibitors for gelatinases, matrix metalloproteinase 2 (MMP2) and matrix metalloproteinase 9 (MMP9). However, both of them are racemates. Herein we report facile synthesis of optically active (*R*)- and (*S*)-enantiomers of compounds **10** and **15**. And the sulfonyl of **15** was transformed to sulfinyl to obtain four epimeric mixtures. All synthesized thiirane-based compounds were evaluated in MMP2 and MMP9 inhibitory assays. Our results indicated that the configuration of thiirane moiety had little effects on gelatinase inhibition, but the substitution of sulfinyl for sulfonyl was detrimental to gelatinase inhibition. Besides, all target compounds exhibited no inhibition against other two Zn^{2+} dependant metalloproteases, aminopeptidase N (APN) and histone deacetylases (HDACs), which confirmed the unique Zn^{2+} chelation mechanism of thiirane moiety against gelatinases.

1. Introduction

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases that regulate functions of the extracellular matrix (ECM),[1] which are implicated in cancer growth,[2–4] tumor metastasis and angiogenesis,[5–7] arthritis,[8,9] connective tissue diseases,[10,11] inflammation,[12] cardiovascular,[13,14] neurological,[15,16] and autoimmune diseases.[17,18] To date, MMPs constitute more than 26 subfamily members and can be grouped into five classes: collagenases (MMP1, MMP8 and MMP13), gelatinases (MMP2 and MMP9), stromelysins (MMP3, MMP7, MMP10, MMP11 and MMP12), membrane-type MMPs (MT1-MMP, MT2-MMP, MT3-MMP and MT4-MMP), and other enzymes.[19,20] Of these enzymes, gelatinases A (MMP2) and B (MMP9) are implicated in many diseases, and selective inhibitors for gelatinases are highly sought.

Recently, compounds with thiirane moiety for human gelatinases are discovered. These compounds are selective against gelatinases due to their unique mechanism of action: enzyme inhibition occurs by Glu404-dependent deprotonation at the methylene adjacent to the sulfone, initiating ring opening of the thiirane and formation of a stable zinc–thiolate complex[21] (Fig. 1A). Among these inhibitors **ND-322**[22] (Fig. 1B) with modifiable 4-substituted phenoxy-aniline is a potent inhibitor in many scientific investigations,[22–24] and its *N*-acetylated metabolite **ND-364** (Fig. 1C) was even more potent.[24] It should be mentioned that both **ND-322** and **ND-364** are racemates. Herein, in order to investigate the effects of configuration of thiirane moiety on gelatinases inhibition, the optically active (*R*)-and (*S*)-enantiomers of compounds **ND-322** and **ND-364** were synthesized and evaluated. Moreover, sulfonyl group of **ND-364** was transformed to sulfinyl to find novel gelatinases inhibitors.

Abbreviations: MMP2, matrix metalloproteinase 2; MMP9, matrix metalloproteinase 9; APN, aminopeptidase N; HDACs, histone deacetylases; MMPs, matrix metalloproteinases; ECM, extracellular matrix; CEs, Cotton effects; PTSC, para-toluenesulfonyl chloride; DCM, dichloromethane; TEA, triethylamine; DMF, dimethyl formamide; THF, tetrahydrofuran; Zn, zinc powder; AcOH, acetic acid; MeOH, methanol; $(Boc)_2O$, di-*tert*-butyl dicarbonate; $Ti(O\text{-}i\text{-}Pr)_4$, tetra isopropyl titanate; (CHPO, 80%), 80% cumene hydroperoxide; EtOAc, ethyl acetate; $MgSO_4$, anhydrous magnesium sulfate; D-DET, (−)-diethyl D-tartrate; L-DET, diethyl-L-tartrate.

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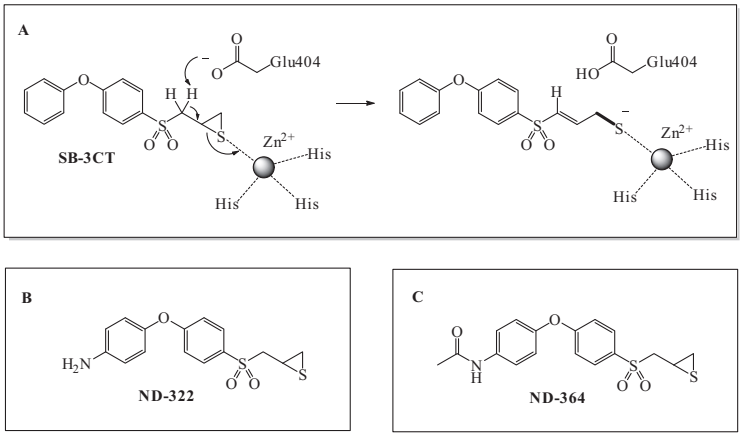


Figure 1. (A) The unique mechanism: enzyme inhibition occurs by Glu404-dependent deprotonation at the methylene adjacent to the sulfone, initiating ring opening of the thiirane and formation of a stable zinc–thiolate complex. (B) The structure of **ND-322**. (C) The structure of **ND-364**.

2. Chemistry

In Scheme 1, synthesis commences with chlorosulfonation of (*R*)-oxiran-2-ylmethanol (98.0% ee) (**1**) to get (*S*)-oxiran-2-ylmethyl 4-methylbenzenesulfonate (**2**), which was then treated with 4-mercaptophenol to generate compound **3**. The nucleophilic attack of the thioalcohol occurred exclusively at the C-3 of compound **2** and the stereocenter was not scrambled during this step.[25] Formation of the epoxide ring and reacting with 1-fluoro-4-nitrobenzene took place in one step in the presence of Cs_2CO_2. The nitro was reduced by zinc powder, then filtered by kieselguhr with the filtrate without further purification being added $(Boc)_2O$ for protecting amino group. Followed by three steps (sulfide oxidation, thiirane ring formation and N-Boc deprotection), the title compound **8** was prepared. The penultimate step, conversion of the oxirane to the thiirane ring, involved inversion of stereochemistry.[25]

Compounds (*R*)-4-(4-((thiiran-2-ylmethyl)sulfonyl)phenoxy) aniline hydrochloride (**9**) and 4-(4-((thiiran-2-ylmethyl)sulfonyl) phenoxy)aniline hydrochloride (**10**) were synthesized according to the same procedure as compound **8**, using (*S*)-oxiran-2-ylmethanol and oxiran-2-ylmethanol as the starting materials, respectively. The optical purity of **8** and **9** was comparative (Table 1). Compound **10**, namely **ND-322**, was synthesized as control drug.

In Scheme 2, reduction of intermediate **4** followed by N-acetylation led to compound **11**, which was transformed to compound **13** in two steps: sulfide oxidation and thiirane ring formation. The conversion of the oxirane to thiirane ring also involved inversion of stereochemistry.[25]

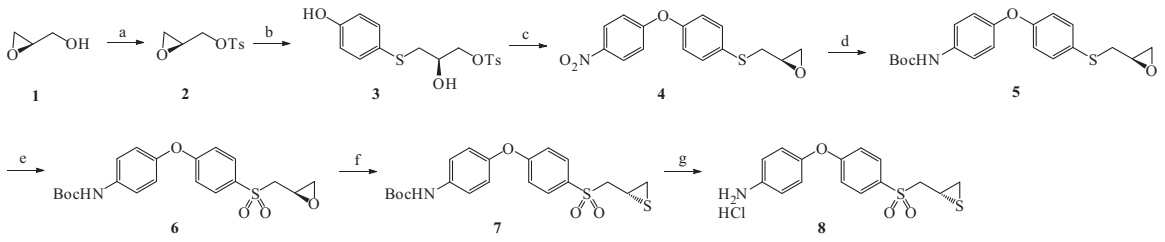
Compounds (*R*)-*N*-(4-(4-((thiiran-2-ylmethyl)sulfonyl)phenoxy)phenyl)acetamide (**14**) and *N*-(4-(4-((thiiran-2-ylmethyl)sulfonyl)phenoxy)phenyl)acetamide (**15**) were synthesized according to the same procedure as compound **13**, using (*S*)-oxiran-2-ylmethanol and oxiran-2-ylmethanol as the starting materials, respectively. The optical purity of **13** and **14** was comparative (Table 1). Compound **15**, namely **ND-364**, was synthesized as control drug.

In Scheme 3, compound **16** was synthesized with catalyst (Ti(O-*i*-Pr)$_4$), ligand (D-DET) and oxidant (CHPO, 80%). When the reaction was over, the mixture was immediately purified by flash column chromatography with ethyl acetate to quench reaction. Then, thiirane ring formation with thiourea involving inversion of stereochemistry gave compound **18**.[25]

Compound **19** was synthesized according to the same procedure as compound **18**, with the sole substitution of diethyl-L-tartrate as the ligand of choice. Compounds **20** and **21** were synthesized according to the same procedure as compound **18** and **19**, respectively, with the sole substitution of (*S*)-oxiran-2-ylmethanol as the reagent of choice.

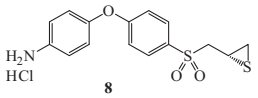
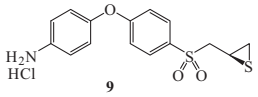
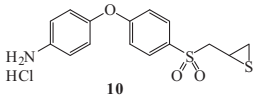
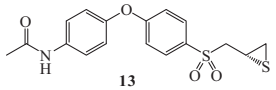
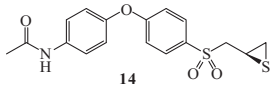
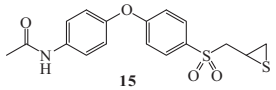
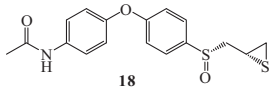
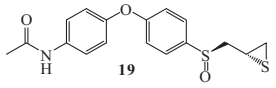
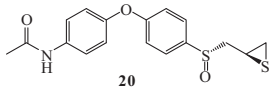
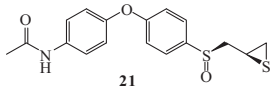
3. Results and discussion

To determine the stereochemistry of chiral sulfoxide sulfur of **18**, **19**, **20** and **21**, the Cotton effects of products **18**, **19**, **20** and **21** with the same concentration (200 μg/mL) in acetonitrile were measured by Chirascan CD Spectrometer (Fig. 2A and B). Because of the lack of previously investigated CD data of similar samples, there was no possibility for the interpretation of the CD spectra of such phenyl methyl sulfoxide—thiirane hybrids directly by an empirical comparison. Considering that the products **18**, **19**, **20** and **21** are a conjugate of phenyl methyl sulfoxide moiety with thiirane moiety, and there were only two stereoelements in the structures, the addition of the CD spectra of the two molecular moieties possibly resulted in the overall CD spectra of **18**, **19**, **20** and **21**.

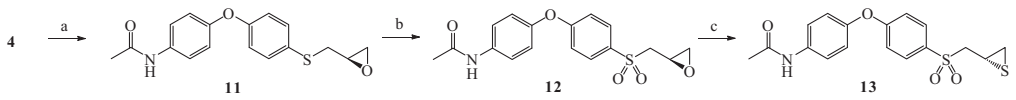


Scheme 1. Reagents and conditions: (a) PTSC, TEA, DCM, 0 °C, 85.0%; (b) 4-mercaptophenol, TEA, DCM, 0 °C, 81.5%; (c) 1-fluoro-4-nitrobenzene, Cs_2CO_2, DMF, rt, 83.0%; (d) Zn, AcOH, THF, 0 °C, $(Boc)_2O$, TEA, MeOH, 0 °C–rt, 81.1%; (e) Ti(O-*i*-Pr)$_4$, CHPO (80%), DCM, rt, 87.3%; (f) thiourea, DCM, MeOH, rt, 84.5%; (g) HCl, anhydrous EtOAc, rt, 89.0%, 96.2% ee.

Table 1
The inhibitory activities of the target compounds against MMP2, MMP9, APN and HDACs

Compounds	Stereo	ee [a] or dr[a]	MMP2 (IC_{50})[c] (nM)	MMP9 (IC_{50})[c] (nM)	MMP2 % inhib.[d]	MMP9 % inhib.[d]	APN % inhib.[d]	HDACs % inhib.[d]
 **8**	(*S*)	96.2%	235.2	709.7	97.7	98.3	n.i.	n.i.
 **9**	(*R*)	96.2%	144.6	833.0	100	97.4	n.i.	n.i.
 **10**	(±)		63.6	611.0	100	96.7	n.i.	n.i.
 **13**	(*S*)	92.9%	256.2	677.2	92.6	91.1	n.i.	n.i.
 **14**	(*R*)	93.6%	299.2	428.6	100	96.2	n.i.	n.i.
 **15**	(±)		170.0	529.1	100	97.2	n.i.	n.i.
 **18**	(*S*, *S*)	23:76:1[b]	ND	ND	30.7	23.7	n.i.	n.i.
 **19**	(*R*, *S*)	60:38:2[b]	ND	ND	7.9	3.4	n.i.	n.i.
 **20**	(*S*, *R*)	1:67:32[b]	ND	ND	12.9	34.4	n.i.	n.i.
 **21**	(*R*, *R*)	2:27:71[b]	ND	ND	14.1	24.8	n.i.	n.i.
NNGH			2.0	5.9	100	100	ND	ND
Bestatin			ND	ND	ND	ND	6.9[e]	ND
SAHA			ND	ND	ND	ND	ND	176.6[f]

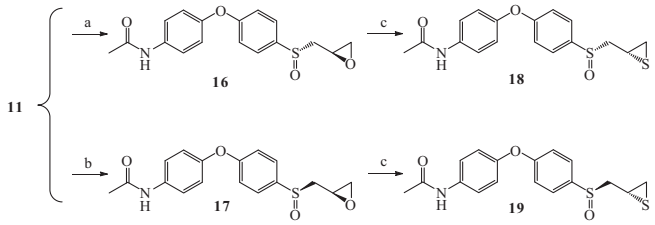
ND: not determined.
n.i.: no inhibition.
[a] Assayed by chiral HPLC.
[b] Corresponding compound is the main component.
[c] IC_{50} values are mean of three experiments, the standard derivations are <20% of the mean.
[d] The inhibitory potency was determined at 10 μM concn of the compounds. % inhibition values are reported as mean of three experiments.
[e] The IC_{50} of Bestatin, unit is μm, the standard derivations are <20% of the mean.
[f] The IC_{50} of SAHA, unit is nM, the standard derivations are <20% of the mean.



Scheme 2. Reagents and conditions: (a) Zn, AcOH, THF, 0 °C, acetyl chloride, TEA, MeOH, 0 °C–rt, 78.1%; (b) Ti(O-*i*-Pr)$_4$, CHPO (80%), DCM, rt, 91.0%; (c) thiourea, THF, MeOH, rt, 92.5%, 92.9% ee.

Thus, it seemed appropriate to compare the overall CD spectra of **18**, **19**, **20** and **21** with the experimental CD spectra of the corresponding moieties. It was reported that phenyl methyl sulfoxide showed two Cotton effects (CEs) with the λ_{max} based on CD was at 212 nm and 235 nm. The *R* isomer of phenyl methyl sulfoxide showed a negative Cotton effect around 212 nm and a positive Cotton effect around 235 nm; meanwhile, The *S* isomer of phenyl methyl sulfoxide showed a positive Cotton effect around 212 nm and a negative Cotton effect around 235 nm, respectively[26] (Fig. 2C).

In the case of **18**, **19**, **20** and **21**, it might be possible to eliminate the CD contribution originated from thiirane moiety to give the



Scheme 3. Reagents and conditions: (a) Ti(O-*i*-Pr)$_4$, D-DET, CHPO (80%), DCM, rt–0 °C, 75.2%; (b) Ti(O-*i*-Pr)$_4$, L-DET, CHPO, DCM, rt–0 °C, 76.8%; (c) thiourea, THF, MeOH, rt.

phenyl methyl sulfoxide moiety contributions, by subtracting one CD spectrum from that of the other stereoisomer with the same configuration at thiirane moiety. As both **18** and **19** had *S*-configuration from thiirane moiety, subtracting the CD spectrum of **19** from that of **18** could calculatorily eliminated the effect of *S*-thiirane moiety, the resulting differential curve indicated the CD contribution of *S*-sulfoxide. In the same way, if one subtracts the spectrum of **18** from that of **19**, the resulting curve would indicate the *R*-sulfoxide contribution. Following the same method, by subtracting the CD spectrum of **21** from that of **20**, the CD contribution of *R*-configuration from thiirane moiety should also be eliminated to obtain the same mirror imaged arithmetically 'isolated' CD curves for *S* and *R* sulfoxide. The arithmetically 'isolated' CD contribution of *S*-sulfoxide provided a positive Cotton effect nearby 222 nm and a negative Cotton effect nearby 258 nm. Accordingly, the 'isolated' CD curve of *R*-sulfoxide displayed a negative Cotton effect nearby 222 nm and a positive Cotton effect nearby 258 nm, respectively (Fig. 2D and E). The 'isolated' CD curves thus obtained showed good agreement with the reported CD spectra of phenyl methyl sulfoxide.[26]

Following the above discussion, the absolute configuration of stereoisomer **18** should be assigned as (*S*,*S*); stereoisomer **19** was consequently assigned as (*R*,*S*). Stereoisomers **20** and **21** were deduced as (*S*,*R*), and (*R*,*R*), respectively.

The inhibitory potency of our compounds against MMP2 and MMP9 was evaluated. For those compounds showing >50% inhibition activity at 10 μM, the IC_{50} values were determined. The activity toward the human gelatinases in Table 1 showed that enantiomers **8** (*S*) and **9** (*R*), and their racemate **10** exhibited comparative activities. The similar trend was also observed in enantiomers **13** (*S*) and **14** (*R*) and their racemate **15**. These results revealed the chirality configuration of the thiirane moiety had little impact on inhibitory activity. Unfortunately, the sulfoxide analogs were almost not active, regardless of the absolute configuration of sulfoxide. We presumed that the weaker electron withdrawing ability of sulfoxide relative to sulfone, which resulted in more difficult Glu404-dependent deprotonation at the methylene adjacent to the sulfoxide, might be the reason why sulfoxide analogs were not active.

Similar to MMP2 and MMP9, APN and HDACs are both zinc-dependant metalloproteinases. Thus the assay was performed on APN and HDACs so as to identify the compounds' selectivity.[27] Bestatin and SAHA were used as the positive controls for APN and HDACs, respectively. Our compounds tested at 10 μM showed no inhibition for APN and HDACs (Table 1), which confirmed the unique Zn^{2+} chelation mechanism of thiirane moiety against gelatinases (Fig. 1A).

The docking processes were performed using Surflex-dock in the SYBYL-X 2.0 software. The crystal structure of MMP2 (PDB entry: 1QIB) and MMP9 (PDB entry: 1L6J) were refined by removing water molecules and the crystallized metals ions in the protein structure, and assigning of Amber77 charge. The ligands used in the docking approach were sketched and minimized by SYBYL-X 2.0. The protomol was generated automatically, and other parameters were set as default. We chose **8** and **9**, which represented our compounds with thiirane moiety (*S*) and thiirane moiety (*R*) respectively, to be carried out docking studies. The computational study is supportive of the stereochemistry of the thiirane ring has no effects on biological activity that both **8** and **9** are able to bind to the active sites of MMP2 (Fig. 3a) and MMP9 (Fig. 3b).

4. Conclusion

All the target compounds with thiirane moiety were assayed for their activities against MMP2, MMP9, APN and HDACs. The results indicated that all the compounds exhibited highly selective inhibition against MMP2 and MMP9 compared with APN and HDACs. The optically active (*R*)- and (*S*)-enantiomers of compounds **10** and **15** were equally active in inhibition of human gelatinases. The results show that the configuration of thiirane moiety has little effect on inhibitory activity. It should be mentioned that the sulfoxide analogs of **15** were almost not active against gelatinases regardless of their stereochemistry. The reason may be the electron withdrawing ability of the sulfoxide is weaker than that of sulfone, which resulted in more difficult Glu404-dependent deprotonation at the methylene adjacent to the sulfoxide.

5. Experimental section

5.1. Chemistry

Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. The solvents of dichloromethane, methanol and tetrahydrofuran had been distilled before use. All reactions were monitored by TLC with 0.25 mm silica gel plates (60GF-254). UV light was used to visualize the spots. Silica gel (300–400 mesh HaiYang) was used for column chromatographic purification with Combiflash Rf 200 (Teledyne Isco) instruments. Melting points were determined uncorrected on an electrothermal melting point apparatus. The IR spectra were recorded by means of KBr plate on a Nicolet 6700 FT-IR Spectrometer. ^{1}H NMR was recorded on Bruker DRX spectrometers at 400 MHz. Chemical shifts are given in parts per million (ppm) with tetramethylsilane as an internal standard. Abbreviations are used as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublets of doublet, br = broad. Coupling constants (*J* values) are given in hertz (Hz). High-resolution mass spectra were determined on an Agilent Q-TOF-6250 spectrometer in Shandong Analysis and Test Center in Ji'nan, China. ESI-MS spectra were recorded on an API 4000 spectrometer. Analytical chiral HPLC performed on a Shimadzu 20A HPLC instrument using a CHIRALPAK IA column. Optical rotations were determined on a MCP 200 polarimeter.

5.1.1. (*S*)-Oxiran-2-ylmethyl 4-methylbenzenesulfonate (2)

Paratoluenesulfonyl chloride (PTSC, 25.0 g) and (*R*)-oxiran-2-ylmethanol (6.0 mL) were dissolved in anhydrous dichloromethane (DCM, 250 mL), then, triethylamine (TEA, 36.0 mL) was dropwise added in the mixture at 0 °C. After reacting for 3 h at 0 °C, the reaction liquid was concentrated. At last, the residue was purified by flash column chromatography with ethyl acetate/petroleum ether to obtain the title compound **2** (17.6 g). White solid; yield: 85.0%; mp: 44–46 °C; $[\alpha]_D^{25}$ = +18.3 (*c* 1.7, acetonitrile). IR (KBr) 1598, 1363, 1177, 969, 666, 556. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.83 (d, *J* = 8.1 Hz, 2H), 7.52–7.46 (m, 2H), 4.43 (d, *J* = 11.6 Hz,

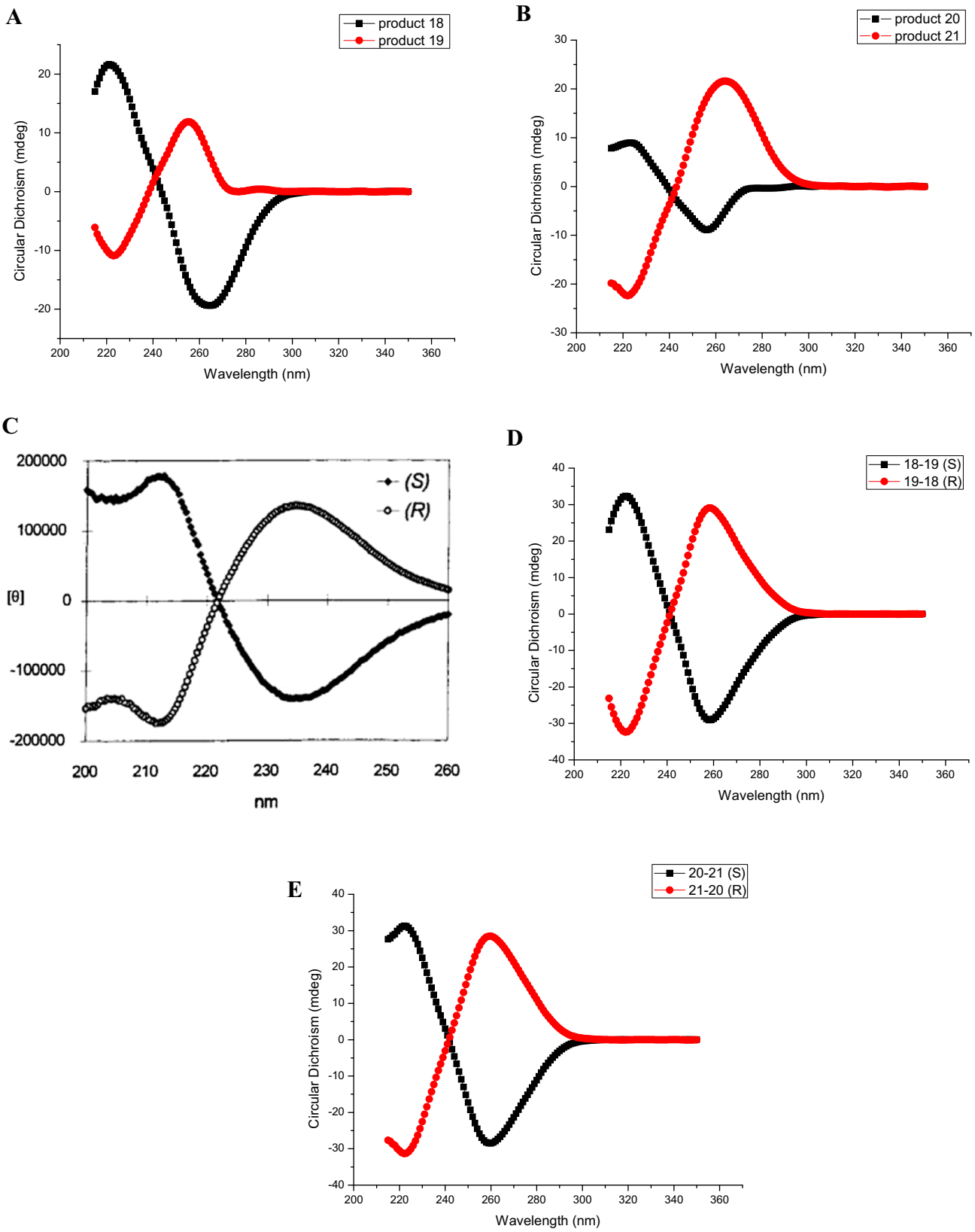


Figure 2. (A) shows Cotton effects in CD spectra of products **18** and **19** in acetonitrile. The Cotton effects of products **18** and **19** with the same concentration (200 μg/mL) were measured by Chirascan CD Spectrometer at 25 °C. (B) Shows Cotton effects in CD spectra of products **20** and **21** in acetonitrile. The Cotton effects of products **20** and **21** with the same concentration (200 μg/mL) were measured by Chirascan CD Spectrometer at 25 °C. (C) Shows Cotton effects in CD spectra of phenyl methyl sulfoxide. (D) Shows Cotton effects in CD spectra of (**18**–**19**) and (**19**–**18**). (E) Shows Cotton effects in CD spectra of (**20**–**21**) and (**21**–**20**).

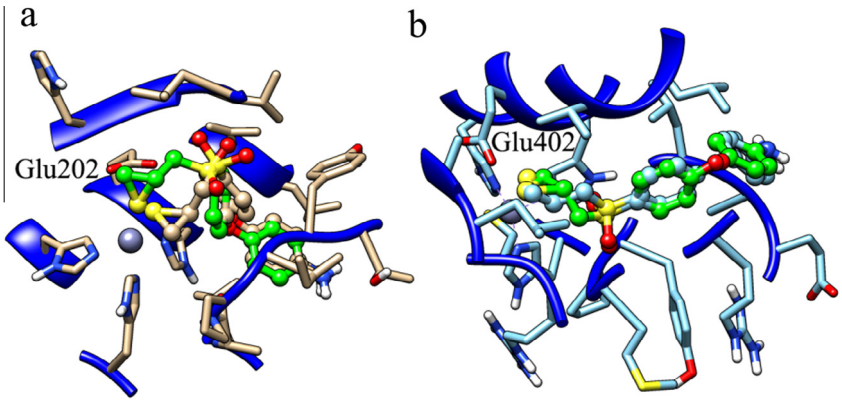


Figure 3. (a) Molecule **8** and **9** in the active site of MMP2, the carbon atoms of molecule **8** was colored green and the gray ball is zinc ion; (b) molecule **8** and **9** in the active site of MMP9, the carbon atoms of molecule **8** was colored green.

1H), 3.85 (dddd, J = 7.0, 5.7, 3.7, 1.9 Hz, 1H), 3.26–3.17 (m, 1H), 2.78 (t, J = 4.6 Hz, 1H), 2.66–2.58 (m, 1H), 2.42 (s, 3H). ESI-MS m/z: 251.2 $[M+Na]^+$.

5.1.2. (*R*)-2-Hydroxy-3-((4-hydroxyphenyl)thio)propyl 4-methylbenzenesulfonate (3)

(*S*)-Oxiran-2-ylmethyl 4-methylbenzenesulfonate (**2**) (9.0 g) and 4-mercaptophenol (5.0 g) were dissolved in anhydrous dichloromethane (150 mL). After TEA (11.1 mL) was dropwise added in the mixture at 0 °C, the ice bath was removed. The mixture was reacted at room temperature for 4–5 h. Then, it was concentrated and evaporated, with the residue being purified by flash column chromatography with ethyl acetate/petroleum ether to give desired compound **3** (5.9 g). White solid; yield: 81.5%; mp: 96–98 °C; $[\alpha]_D^{25}$ = +24.3 (c 0.6, acetonitrile). IR (KBr) 3372, 3146, 1597, 1582, 1495, 1365, 1257, 1190, 1177, 1097, 1074, 988, 905, 829, 815, 792, 665, 555. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.58 (s, 1H), 7.76 (d, J = 8.3 Hz, 2H), 7.48 (d, J = 8.1 Hz, 2H), 7.15 (d, J = 8.6 Hz, 2H), 6.71 (d, J = 8.6 Hz, 2H), 5.42 (d, J = 5.2 Hz, 1H), 4.06 (dd, J = 10.1, 3.3 Hz, 1H), 3.92 (dd, J = 10.1, 6.3 Hz, 1H), 3.69–3.59 (m, 1H), 2.77 (d, J = 6.5 Hz, 2H), 2.43 (s, 3H). ESI-MS m/z: 377.4 $[M+Na]^+$. ESI-MS m/z: 353.4 $[M-H]^-$.

5.1.3. (*R*)-2-(((4-(4-Nitrophenoxy)phenyl)thio)methyl)oxirane (4)

At room temperature, (*R*)-2-hydroxy-3-((4-hydroxyphenyl) thio)propyl 4-methylbenzenesulfonate (**3**) (3.0 g) was added in anhydrous dimethyl formamide (DMF, 50 mL), followed by adding 1-fluoro-4-nitrobenzene (3.0 g) and Cs_2CO_2 (8.1 g). After 12 h, the reaction solutions was added some water, and then, extracted with ethyl acetate. The organic layer was washed with brine (30 mL × 3), dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by flash column chromatography with ethyl acetate/petroleum ether to obtain the title compound **4** (4.15 g). Pale yellow oil; yield: 83.0%; $[\alpha]_D^{25}$ = −3.5 (c 0.5, acetonitrile). IR (KBr) 1582, 1487, 1343, 1243, 877, 846, 751. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.29–8.23 (m, 2H), 7.57–7.51 (m, 2H), 7.19–7.12 (m, 4H), 3.24–3.11 (m, 3H), 2.78–2.74 (m, 1H), 2.59 (dd, J = 5.1, 2.2 Hz, 1H). ESI-MS m/z: 304.4 $[M+H]^+$. ESI-MS m/z: 348.4 $[M+HCOO]^-$.

5.1.4. *tert*-Butyl (*R*)-(4-(4-((oxiran-2-ylmethyl)thio)phenoxy) phenyl)carbamate (5)

At 0 °C, (*R*)-2-(((4-(4-nitrophenoxy)phenyl)thio)methyl)oxirane (**4**) (3.7 g) was added in anhydrous tetrahydrofuran (THF, 60 mL), followed by adding zinc powder (Zn, 32.0 g) and dropwise adding acetic acid (AcOH, 13.9 mL). After 15 min, the mixture was filtered. The filtrate was added absolute methanol (MeOH, 60 mL) and dropwise added TEA (37 mL). After 5 min, di-*tert*-butyl dicarbonate ($(Boc)_2O$, 12.8 g) was added. Then, the ice bath was removed. 24 h later, the reaction liquid was concentrated. And the residue was purified by flash column chromatography with ethyl acetate/petroleum ether to give the title compound **5** (3.7 g). Light yellow solid; yield: 81.1%; mp: 82–84 °C; $[\alpha]_D^{25}$ = −0.39 (c 1.0, acetonitrile). IR (KBr) 3345, 3298, 2978, 2918, 1718, 1488, 1229, 1155, 1053, 852, 826, 771, 738. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.37 (s, 1H), 7.50–7.40 (m, 4H), 7.00–6.87 (m, 4H), 3.13–3.02 (m, 3H), 2.75–2.70 (m, 1H), 2.53–2.51 (m, 1H), 1.48 (s, 9H). ESI-MS m/z: 374.4 $[M+H]^+$. ESI-MS m/z: 372.3 $[M-H]^-$.

5.1.5. *tert*-Butyl (*R*)-(4-(4-((oxiran-2-ylmethyl)sulfonyl) phenoxy)phenyl)carbamate (6)

At room temperature, *tert*-butyl (*R*)-(4-(4-((oxiran-2-ylmethyl) thio)phenoxy)phenyl)carbamate (**5**) (3.5 g) and tetra isopropyl titanate ($Ti(O\text{-}i\text{-}Pr)_4$, 3.8 mL) were added in anhydrous dichloromethane (30 mL). Then, 80% cumene hydroperoxide (CHPO, 80%, 2.2 mL) was dropwise added to the mixture. One hour later, the reaction liquid was concentrated. And the residue was purified by flash column chromatography with ethyl acetate/petroleum ether to give the title compound **6** (3.3 g). White solid; yield: 87.3%; mp: 118–120 °C; $[\alpha]_D^{25}$ = −14.1 (c 0.6, acetonitrile). IR (KBr) 3334, 2980, 1699, 1527, 1490, 1407, 1368, 1312, 1234, 1143, 850, 834, 767, 566. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.47 (s, 1H), 7.89–7.83 (m, 2H), 7.55 (d, J = 8.9 Hz, 2H), 7.13–7.06 (m, 4H), 3.68 (dd, J = 14.6, 4.3 Hz, 1H), 3.50 (dd, J = 14.6, 7.1 Hz, 1H), 3.158–3.113 (m, 1H), 2.72 (dd, J = 5.2, 4.1 Hz, 1H), 2.44 (dd, J = 5.3, 2.5 Hz, 1H), 1.48 (s, 9H). ESI-MS m/z: 428.4 $[M+Na]^+$. ESI-MS m/z: 404.6 $[M-H]^-$.

5.1.6. *tert*-Butyl (*S*)-(4-(4-((thiiran-2-ylmethyl)sulfonyl) phenoxy)phenyl)carbamate (7)

At room temperature, *tert*-butyl (*R*)-(4-(4-((oxiran-2-ylmethyl)-sulfonyl)phenoxy)phenyl)carbamate (**6**) (2.0 g) and thiourea (2.0 g) reacted in anhydrous dichloromethane (15 mL) and absolute methanol (30 mL) overnight. The reaction liquid was concentrated. And the residue was purified by flash column chromatography with ethyl acetate/petroleum ether to give the title compound **7** (1.76 g). White solid; yield: 84.5%; mp: 158–160 °C; $[\alpha]_D^{25}$ = −0.7 (c 0.14, acetonitrile). IR (KBr) 3325, 2979, 1698, 1530, 1310, 1239, 1140, 851, 834, 768, 565, 550, 515. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.47 (s, 1H), 7.92–7.84 (m, 2H), 7.54 (d, J = 8.9 Hz, 2H), 7.14–7.06 (m, 4H), 3.62 (d, J = 6.7 Hz, 2H), 2.98 (dd, J = 6.4, 5.5 Hz, 1H), 3.013–2.951 (m, 1H), 2.19 (dd, J = 5.3, 1.2 Hz, 1H), 1.48 (s, 9H). ESI-MS m/z: 444.5 $[M+Na]^+$. ESI-MS m/z: 420.3 $[M-H]^-$.

Compounds **9** and **10** were synthesized according to the same procedure as compound **8**.

5.1.7. (*S*)-4-(4-((Thiiran-2-ylmethyl)sulfonyl)phenoxy)aniline hydrochloride (8)

tert-Butyl (*S*)-(4-(4-((thiiran-2-ylmethyl)sulfonyl)phenoxy) phenyl)carbamate (**7**) (1.7 g) was stirred in a solution of EtOAc (20 mL) saturated by dry HCl gas. The reaction solution was stirred at room temperature for overnight when the precipitation appeared. The suspension was filtered with the filter being washed with ether to give desired compound **8** (1.28 g). White powder; yield: 89.0%; mp: 192–194 °C; 96.2% ee [column, CHIRALPAK IA (4.6 mm I.D. × 250 mm 5 μm); mobile phase, *n*-hexane/ethanol = 50:50 (v/v); flow rate, 0.8 mL/min; detection, UV 244 nm; temperature, room temperature]; $[\alpha]_D^{25}$ = +5.2 (*c* 0.7, methanol). IR (KBr) 2850, 2595, 1586, 1501, 1488, 1314, 1254, 1142, 1085, 876, 860, 826, 780, 516. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.23 (br, 3H), 7.95–7.90 (m, 2H), 7.48–7.43 (m, 2H), 7.30–7.24 (m, 2H), 7.23–7.18 (m, 2H), 3.72–3.59 (m, 2H), 3.05–2.95 (m, 1H), 2.56 (dd, *J* = 6.3, 1.1 Hz, 1H), 2.20 (dd, *J* = 5.3, 1.2 Hz, 1H). ESI-MS *m*/*z*: 344.4 $[M+Na]^+$. ESI-MS *m*/*z*: 320.3 $[M-H]^-$. HRMS *m*/*z*: calcd for $C_{15}H_{15}NO_3S_2$ $[M+H]^+$ 322.0493, found: 322.0566.

5.1.8. (*R*)-4-(4-((Thiiran-2-ylmethyl)sulfonyl)phenoxy)aniline (9)

Synthesis of (*R*)-4-(4-((thiiran-2-ylmethyl)sulfonyl)phenoxy) aniline (**9**) was performed according to the same procedure as compound **8**, with the sole substitution of (*S*)-oxiran-2-ylmethanol (98.0% ee) as the reagent of choice. White powder; yield: 87.4%; mp: 152–154 °C; 96.2% ee [column, CHIRALPAK IA (4.6 mm I.D. × 250 mm 5 μm); mobile phase, *n*-hexane/ethanol = 50:50 (v/v); flow rate, 0.8 mL/min; detection, UV 244 nm; temperature, room temperature]; $[\alpha]_D^{25}$ = −14.5 (*c* 1.0, methanol). IR (KBr) 2853, 2579, 1590, 1508, 1489, 1313, 1259, 1143, 1087, 877, 853, 831, 549, 519. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.15 (br, 3H), 7.95–7.90 (m, 2H), 7.48–7.43 (m, 2H), 7.31–7.25 (m, 2H), 7.24–7.18 (m, 2H), 3.72–3.58 (m, 2H), 3.04–2.95 (m, 1H), 2.56 (dd, *J* = 6.3, 1.1 Hz, 1H), 2.20 (dd, *J* = 5.3, 1.2 Hz, 1H). ESI-MS *m*/*z*: 344.4 $[M+Na]^+$. ESI-MS *m*/*z*: 320.3 $[M-H]^-$. HRMS *m*/*z*: calcd for $C_{15}H_{15}NO_3S_2$ $[M+H]^+$ 322.0493, found: 322.0565.

5.1.9. 4-(4-((Thiiran-2-ylmethyl)sulfonyl)phenoxy)aniline (10, ND-322)

Synthesis of 4-(4-((thiiran-2-ylmethyl)sulfonyl)phenoxy)aniline (**10**, **ND-322**) was performed according to the same procedure as compound **8**, with the sole substitution of oxiran-2-ylmethanol as the reagent of choice. White powder; yield: 86.7%; mp: 176–178 °C. IR (KBr) 2850, 2591, 1586, 1502, 1313, 1255, 1142, 1086, 876, 860, 826, 780, 517. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.28 (br, 3H), 7.96–7.90 (m, 2H), 7.51–7.44 (m, 2H), 7.32–7.26 (m, 2H), 7.24–7.19 (m, 2H), 3.71–3.59 (m, 2H), 3.04–2.94 (m, 1H), 2.56 (dd, *J* = 6.3, 1.1 Hz, 1H), 2.20 (dd, *J* = 5.3, 1.2 Hz, 1H). ESI-MS *m*/*z*: 344.4 $[M+Na]^+$. ESI-MS *m*/*z*: 320.3 $[M-H]^-$. HRMS *m*/*z*: calcd for $C_{15}H_{15}NO_3S_2$ $[M+H]^+$ 322.0493, found: 322.0580.

5.1.10. (*R*)-*N*-(4-(4-((Oxiran-2-ylmethyl)thio)phenoxy)phenyl) acetamide (11)

At 0 °C, (*R*)-2-(((4-(4-nitrophenoxy)phenyl)thio)methyl)oxirane (**4**) (3.0 g) was added in anhydrous tetrahydrofuran (THF, 50 mL), followed by adding zinc powder (Zn, 26.0 g) and dropwise adding acetic acid (AcOH, 11.3 mL). After 15 min, the mixture was filtered. The filtrate was added absolute methanol (MeOH, 50 mL) and dropwise added TEA (30 mL). After 5 min, acetyl chloride (7.0 mL) was added. Then, the ice bath was removed. 15 min later, the reaction liquid was concentrated. And the residue was added some water, extracted with ethyl acetate (EtOAc) (30 mL × 3), washed with saturated brines (20 mL × 3), dried with anhydrous magnesium sulfate ($MgSO_4$) concentrated and purified by flash column chromatography with ethyl acetate/petroleum ether to give the title compound **11** (2.4 g). Light yellow solid; yield: 78.1%; mp: 96–98 °C; $[\alpha]_D^{25}$ = −4.8 (*c* 0.3, acetonitrile). IR (KBr) 3286, 1659, 1552, 1525, 1507, 1490, 1277, 1256, 819, 516. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.95 (s, 1H), 7.59 (d, *J* = 8.9 Hz, 2H), 7.43 (d, *J* = 8.7 Hz, 2H), 6.99 (d, *J* = 8.9 Hz, 2H), 6.92 (d, *J* = 8.7 Hz, 2H), 3.14–3.05 (m, 3H), 2.76–2.70 (m, 1H), 2.53 (d, *J* = 2.2 Hz, 1H), 2.03 (s, 3H). ESI-MS *m*/*z*: 316.4 $[M+H]^+$.

5.1.11. (*R*)-*N*-(4-(4-((Oxiran-2-ylmethyl)sulfonyl)phenoxy) phenyl)acetamide (12)

At room temperature, (*R*)-*N*-(4-(4-((oxiran-2-ylmethyl)thio) phenoxy)phenyl)acetamide (**11**) (0.5 g) and tetra isopropyl titanate ($Ti(O\text{-}i\text{-}Pr)_4$) (0.47 mL) were added in anhydrous dichloromethane (15 mL). Then, 80% cumene hydroperoxide (CHPO, 80%, 1.0 mL) was dropwise added to the mixture. One hour later, the reaction liquid was concentrated. And the residue was purified by flash column chromatography with ethyl acetate/petroleum ether to give the title compound **12** (0.5 g). Slight yellow oil; yield: 91.0%; $[\alpha]_D^{25}$ = −9.3 (*c* 0.4, acetonitrile). IR (KBr) 3301, 1665, 1531, 1506, 1488, 1314, 1296, 1245, 1137, 839, 788, 680, 595, 554, 526, 508. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.04 (s, 1H), 7.92–7.84 (m, 2H), 7.70–7.65 (m, 2H), 7.19–7.07 (m, 4H), 3.72–3.40 (m, 3H), 3.159–3.114 (m, 1H), 2.72 (dd, *J* = 5.2, 4.2 Hz, 1H), 2.44 (dd, *J* = 5.3, 2.5 Hz, 1H), 2.06 (s, 3H). ESI-MS *m*/*z*: 348.4 $[M+H]^+$.

Compounds **14** and **15** were synthesized according to the same procedure as compound **13**.

5.1.12. (*S*)-*N*-(4-(4-((Thiiran-2-ylmethyl)sulfonyl)phenoxy) phenyl)acetamide (13)

At room temperature, (*R*)-*N*-(4-(4-((oxiran-2-ylmethyl)sulfonyl)phenoxy)phenyl)acetamide (**12**) (0.4 g) and thiourea (0.4 g) reacted in anhydrous tetrahydrofuran (THF, 8.0 mL) and absolute methanol (4.0 mL) overnight. The reaction liquid was concentrated. And the residue was purified by flash column chromatography with ethyl acetate/petroleum ether to give the title compound **13** (0.39 g). White solid; yield: 92.5%; mp: 162–164 °C; 92.9% ee [column, CHIRALPAK IA (4.6 mm I.D. × 250 mm 5 μm); mobile phase, *n*-hexane/ethanol/tetrahydrofuran = 65:35:5 (v/v/v); flow rate, 1 mL/min; detection, UV 251 nm; temperature, room temperature]; $[\alpha]_D^{25}$ = +1.1 (*c* 0.4, acetonitrile). IR (KBr) 3298, 3258, 3197, 3138, 3093, 2927, 1663, 1615, 1584, 1557, 1505, 1489, 1244, 1225, 1145, 1087, 874, 858, 839, 755, 586, 542, 525, 511. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.05 (s, 1H), 7.88 (d, *J* = 8.9 Hz, 2H), 7.67 (d, *J* = 8.9 Hz, 2H), 7.17–7.09 (m, 4H), 3.63 (d, *J* = 6.7 Hz, 2H), 3.03–2.93 (m, 1H), 2.55 (d, *J* = 5.5 Hz, 1H), 2.19 (d, *J* = 5.2 Hz, 1H), 2.05 (s, 3H). HRMS *m*/*z*: calcd for $C_{17}H_{17}NO_4S_2$ $[M+H]^+$ 364.0599, found: 364.0674.

5.1.13. (*R*)-*N*-(4-(4-((Thiiran-2-ylmethyl)sulfonyl)phenoxy) phenyl)acetamide (14)

Synthesis of (*R*)-*N*-(4-(4-((thiiran-2-ylmethyl)sulfonyl)phenoxy)phenyl)acetamide (**14**) was performed according to the same procedure as compound **13**, with the sole substitution of (*S*)-oxiran-2-ylmethanol (98.0% ee) as the reagent of choice. White solid; yield: 91.4%; mp: 162–164 °C; 93.6% ee [column, CHIRALPAK IA (4.6 mm I.D. × 250 mm 5 μm); mobile phase, *n*-hexane/ethanol/tetrahydrofuran = 65:35:5 (v/v/v); flow rate, 1 mL/min; detection, UV 251 nm; temperature, room temperature]; $[\alpha]_D^{25}$ = −5.7 (*c* 0.5, acetonitrile). IR (KBr) 3298, 3258, 3198, 3138, 3093, 2927, 1663, 1615, 1557, 1505, 1489, 1244, 1145, 874, 859, 839, 755, 586, 542, 525, 511. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.05 (s, 1H), 7.88 (d, *J* = 8.9 Hz, 2H), 7.67 (d, *J* = 8.9 Hz, 2H), 7.17–7.08 (m, 4H), 3.63 (d, *J* = 6.7 Hz, 2H), 3.03–2.94 (m, 1H), 2.55 (d, *J* = 5.5 Hz, 1H), 2.19

(d, J = 5.3 Hz, 1H), 2.06 (s, 3H). HRMS m/z: calcd for $C_{17}H_{17}NO_4S_2$ $[M+H]^+$ 364.0599, found: 364.0683.

5.1.14. *N*-(4-(4-((Thiiran-2-ylmethyl)sulfonyl)phenoxy)phenyl) acetamide (15, ND-364)

Synthesis of *N*-(4-(4-((thiiran-2-ylmethyl)sulfonyl)phenoxy) phenyl)acetamide (**15**, **ND-364**) was performed according to the same procedure as compound **13**, with the sole substitution of oxiran-2-ylmethanol as the reagent of choice. White solid; yield: 89.6%; mp: 162–164 °C. IR (KBr) 3298, 3259, 3198, 3093, 2928, 1663, 1615, 1557, 1505, 1489, 1244, 1226, 1145, 874, 858, 839, 755, 586, 542, 525, 511. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.05 (s, 1H), 7.88 (d, J = 8.8 Hz, 2H), 7.67 (d, J = 8.9 Hz, 2H), 7.17–7.08 (m, 4H), 3.63 (d, J = 6.7 Hz, 2H), 3.03–2.94 (m, 1H), 2.58–2.53 (m, 1H), 2.19 (d, J = 5.3 Hz, 1H), 2.05 (s, 3H). HRMS m/z: calcd for $C_{17}H_{17}NO_4S_2$ $[M+H]^+$ 364.0599, found: 364.0662.

5.1.15. *N*-(4-(4-((*S*)-(((*R*)-Oxiran-2-yl)methyl)sulfinyl)phenoxy) phenyl)acetamide (16)

(*R*)-*N*-(4-(4-((Oxiran-2-ylmethyl)thio)phenoxy)phenyl)acetamide (**11**) (1.0 g), tetra isopropyl titanate ($Ti(O\text{-}i\text{-}Pr)_4$, 0.94 mL) and (−)-diethyl D-tartrate (D-DET, 2.2 mL) were added in anhydrous dichloromethane (25 mL). After 5 min, 80% cumene hydroperoxide (CHPO 80%, 0.64 mL) was dropwise added to the mixture at 0 °C, then, reacted for 24 h at the same temperature. The mixture was purified by flash column chromatography with ethyl acetate. The liquid was concentrated. And the residue was purified by flash column chromatography with ethyl acetate/petroleum ether to give the title compound **16** (0.79 g). Faint yellow solid; yield: 75.2%; mp: 86–88 °C; $[\alpha]_D^{25}$ = −43.8 (c 0.6, acetonitrile). IR (KBr) 3262, 3198, 3132, 3066, 2965, 2920, 1686, 1545, 1507, 1489, 1256, 1021, 852, 834, 523. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.01 (s, 1H), 7.71–7.60 (m, 4H), 7.11 (d, J = 8.7 Hz, 2H), 7.06 (d, J = 8.9 Hz, 2H), 3.26–3.17 (m, 1H), 3.14–2.99 (m, 2H), 2.84–2.73 (m, 1H), 2.61 (ddd, J = 20.0, 5.2, 2.4 Hz, 1H), 2.05 (s, 3H). ESI-MS m/z: 332.5 $[M+H]^+$.

Compounds **19**, **20 and 21** were synthesized according to the same procedure as compound **18**.

5.1.16. *N*-(4-(4-((*S*)-(((*S*)-Thiiran-2-yl)methyl)sulfinyl)phenoxy) phenyl)acetamide (18)

N-(4-(4-((*S*)-(((*R*)-Oxiran-2-yl)methyl)sulfinyl)phenoxy)phenyl)acetamide (**16**) (0.52 g) and thiourea (0.52 g) reacted in anhydrous tetrahydrofuran (THF, 10.4 mL) and absolute methanol (5.2 mL) overnight. The reaction liquid was concentrated. And the residue was purified by flash column chromatography with ethyl acetate/petroleum ether to give the title compound **18** (0.49 g). White solid; yield: 89.4%; mp: 134–136 °C; 23:76:1 dr [column, CHIRALPAK IA (4.6 mm I.D. × 250 mm 5 μm); mobile phase, *n*-hexane/ethanol/tetrahydrofuran = 74:25:1 (v/v/v); flow rate, 1 mL/min; detection, UV 255 nm; temperature, room temperature]; $[\alpha]_D^{25}$ = −36.3 (c 0.3, acetonitrile). IR (KBr) 3297, 1658, 1506, 1488, 1245, 1021, 834, 524. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.01 (s, 1H), 7.74–7.61 (m, 4H), 7.17–7.04 (m, 4H), 3.31–2.81 (m, 3H), 2.61 (d, J = 6.3 Hz, 1H), 2.35 (dd, J = 54.8, 5.2 Hz, 1H), 2.05 (s, 3H). HRMS m/z: calcd for $C_{17}H_{17}NO_3S_2$ $[M+H]^+$ 348.0650, found: 348.0719.

5.1.17. *N*-(4-(4-((*R*)-(((*S*)-Thiiran-2-yl)methyl)sulfinyl)phenoxy) phenyl)acetamide (19)

Synthesis of *N*-(4-(4-((*R*)-(((*S*)-thiiran-2-yl)methyl)sulfinyl) phenoxy)phenyl)acetamide (**19**) was performed according to the same procedure as compound **18**, with the sole substitution of diethyl-L-tartrate (L-DET) as the ligand of choice. White solid; yield: 87.2%; mp: 114–116 °C; 60:38:2 dr [column, CHIRALPAK IA (4.6 mm I.D. × 250 mm 5 μm); mobile phase, *n*-hexane/ethanol/tetrahydrofuran = 74:25:1 (v/v/v); flow rate, 1 mL/min; detection, UV 255 nm; temperature, room temperature]; $[\alpha]_D^{25}$ = +24.4 (c 0.5, acetonitrile). IR (KBr) 3297, 1678, 1659, 1548, 1507, 1488, 1255, 1022, 833, 523. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.01 (s, 1H), 7.76–7.56 (m, 4H), 7.14–7.06 (m, 4H), 3.32–3.12 (m, 2H), 3.12–2.80 (m, 1H), 2.61 (d, J = 6.1 Hz, 1H), 2.35 (dd, J = 54.6, 5.2 Hz, 1H), 2.05 (s, 3H). HRMS m/z: calcd for $C_{17}H_{17}NO_3S_2$ $[M+H]^+$ 348.0650, found: 348.0720.

5.1.18. *N*-(4-(4-((*S*)-(((*R*)-Thiiran-2-yl)methyl)sulfinyl)phenoxy) phenyl)acetamide (20)

Synthesis of *N*-(4-(4-((*S*)-(((*R*)-thiiran-2-yl)methyl)sulfinyl) phenoxy)phenyl)acetamide (**20**) was performed according to the same procedure as compound **18**, with the sole substitution of (*S*)-oxiran-2-ylmethanol (98.0% ee) as the reagent of choice. White solid; yield: 88.9%; mp: 150–152 °C; 1:67:32 dr [column, CHIRALPAK IA (4.6 mm I.D. × 250 mm 5 μm); mobile phase, *n*-hexane/ethanol/tetrahydrofuran = 74:25:1 (v/v/v); flow rate, 1 mL/min; detection, UV 255 nm; temperature, room temperature]; $[\alpha]_D^{25}$ = −47.2 (c 0.4, acetonitrile). IR (KBr) 3296, 1662, 1617, 1557, 1505, 1488, 1242, 1222, 1035, 836, 520. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.01 (s, 1H), 7.72–7.61 (m, 4H), 7.14–7.05 (m, 4H), 3.31–3.12 (m, 2H), 3.12–2.82 (m, 1H), 2.61 (d, J = 6.0 Hz, 1H), 2.35 (dd, J = 54.7, 5.2 Hz, 1H), 2.05 (s, 3H). HRMS m/z: calcd for $C_{17}H_{17}NO_3S_2$ $[M+H]^+$ 348.0650, found: 348.0720.

5.1.19. *N*-(4-(4-((*R*)-(((*R*)-Thiiran-2-yl)methyl)sulfinyl)phenoxy) phenyl)acetamide (21)

N-(4-(4-((*R*)-(((*R*)-Thiiran-2-yl)methyl)sulfinyl)phenoxy)phenyl)acetamide (**21**) was performed according to the same procedure as compound **19**, with the sole substitution of (*S*)-oxiran-2-ylmethanol (98.0% ee) as the reagent of choice. White solid; yield: 86.4%; mp: 136–138 °C; 2:27:71 dr [column, CHIRALPAK IA (4.6 mm I.D. × 250 mm 5 μm); mobile phase, *n*-hexane/ethanol/tetrahydrofuran = 74:25:1 (v/v/v); flow rate, 1 mL/min; detection, UV 255 nm; temperature, room temperature]; $[\alpha]_D^{25}$ = +20.1 (c 0.4, acetonitrile). IR (KBr) 3297, 1656, 1552, 1507, 1258, 1021, 834, 524. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.01 (s, 1H), 7.75–7.57 (m, 4H), 7.17–7.03 (m, 4H), 3.32–3.12 (m, 2H), 3.12–2.81 (m, 1H), 2.61 (d, J = 6.3 Hz, 1H), 2.35 (dd, J = 54.7, 5.1 Hz, 1H), 2.05 (s, 3H). HRMS m/z: calcd for $C_{17}H_{17}NO_3S_2$ $[M+H]^+$ 348.0650, found: 348.0737.

5.2. In vitro MMP2 and MMP9 inhibition assay

Active human MMP2 full length protein and active human MMP9 full length protein were purchased from Abcam, The fluorogenic substrate Mca-Pro-Leu-Gly-Leu-Dap(Dnp)-Ala-Arg-NH_2 was purchased from AnaSpec. The inhibition potency of our compounds against MMP2 and MMP9 was evaluated though a fluorometric assay using 384-well plates with a plate reader (Varioskan, Thermo), at excitation and emission wavelengths of 328 and 393 nm. Substrate hydrolysis were monitored for 15 min in a buffer (50 mM HEPES, pH 7.5, 150 mM NaCl, 5 mM $CaCl_2$, 0.01% Brij-35 and 1% DMSO) contained 10 μM substrate. For those compounds showing >50% inhibition activity at 10 μm concn, the IC_{50} values were obtained by dose response measurements using inhibitor range of concentration (1 nM–10 μm) and enzyme concentration equal to 3 nM. The enzyme was preincubated with the inhibitor 2 h before assessment of activity. NNGH was a control drug. Data analysis was performed using Prism 5 software (GraphPad).

5.3. In vitro APN inhibition assay

Microsomal aminopeptidase from Porcine Kidney Microsomes as enzyme and L-Leu-P-nitroanilide as substrate were purchased from Sigma. The enzyme and inhibitors were dissolved in 50 mM PBS (pH 7.2) and incubated in 96-well microtiter plates for 2 h at 37 °C. Then the solution of substrate was added into the above mixture, which was incubated for another 30 min at 37 °C. The hydrolysis of the substrate was monitored by following the change in the absorbance measured at 405 nm with a plate reader (Varioskan, Thermo).

5.4. In vitro HDACs inhibition assay

In vitro HDACs inhibitory activity assay was determined by using Boc-Lys (acetyl)-AMC as substrate and Hela nuclear extract (mainly contains HDAC1 and HDAC2) as enzymes in 15 mM Tris–HCl (pH 8.0), at 37 °C. First, 10 μL of enzymes solution was added to tested compounds solutions (50 μL) and incubated for 2 h at 37 °C. Then 40 μL of substrate was added and the mixture continued to incubate for another 30 min in the same environment. Finally, 100 μL of developer which containing trypsin and TSA was putted into the mixture. Twenty minutes later, fluorescence intensity was measured at 390 nm excitation and 460 nm emission wavelengths with a microplate reader (Varioskan, Thermo).

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