Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Isolation, Structure Elucidation, and KDD study of (-)-Celosine, a new Skeleton with Potent Anti-Atherosclerosis Activity

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1. Extraction and Isolation

The dried seeds of *Celosia cristata* L. (5 kg) was pulverized and consecutively extracted with 70% EtOH (5L/Kg) for 3 times under reflux. The liquid extract was concentrated under vacuum and suspended in appropriate amount of water, which was extracted with equal volume of petroleum ether, ethyl acetate and butanol successively (each 4 times). The organic phases after liquid-liquid extractions were evaporated under vacuum, yielding 62 g of petroleum ether extract, 8 g of ethyl acetate extract and 40 g of butanol extract.

The petroleum ether extract (62 g) was chromatographed on a silica gel (200-300 mesh) column with petroleum ether/EtOAc (100:1, 50:1, 20:1, 5:1, 0:1, v/v) to yield five fractions (Fr.1 to Fr.5). The fraction Fr.4 (5 g) was subjected to MCI (75-150 μm) column and eluted with EtOH/H_2O (3:7, 5:5, and 7:3, 9:1) to obtain 20 fractions (Fr.4-1 to Fr.4-20). Fr.4-16 (200 mg) was subjected to anRP-18(40-60 μm) column, eluted with 65% ACN-H2O) to give the crude target compound(-)-Celosine (20 mg), which was then subjected to semi-preparative HPLC on an RP-18 column (250 mm × 20 mm, wavelength 254 nm) with MeCN/H_2O (63:37, v/v) to give pure target compounds (5.6 mg).

2. Structure Elucidation of Natural Source (-)-Celosine

2.1. NMR spectra of Natural Source (-)-Celosine

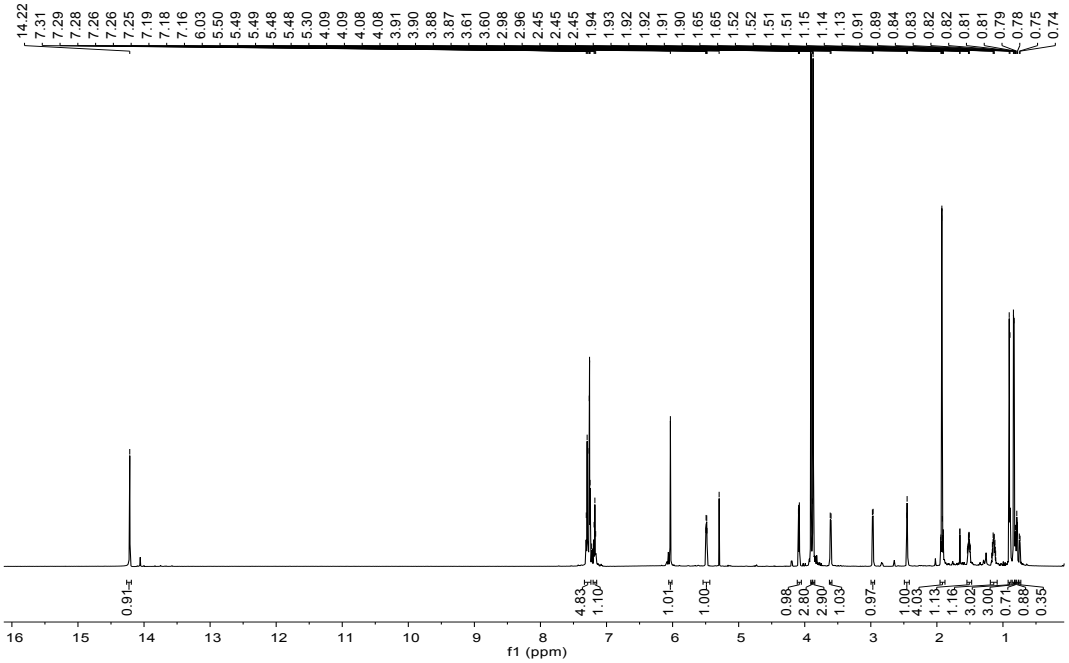


Figure S1. ^{1}H-NMR of (-)-Celosine (600MHz, *$CDCl_3$*)

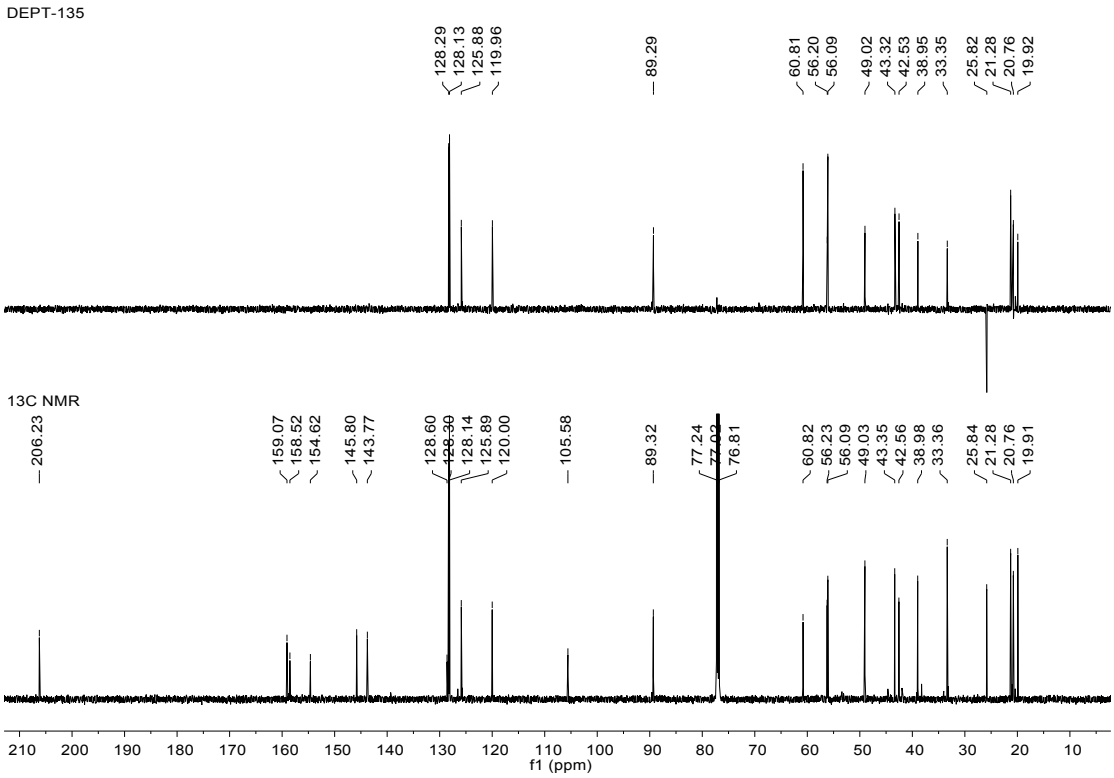


Figure S2.[13]C-NMR and 135 DEPT Spectra of (-)-Celosine (600MHz, $CDCl_3$)

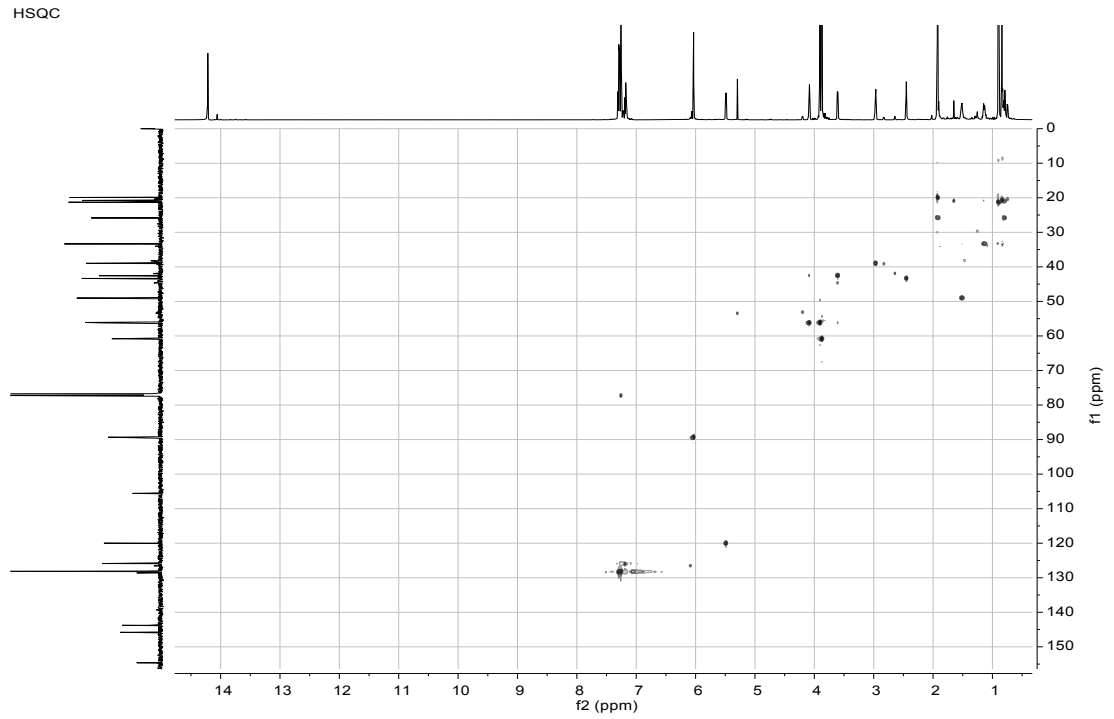


Figure S3. HSQC of (-)-Celosine (600MHz, $CDCl_3$)

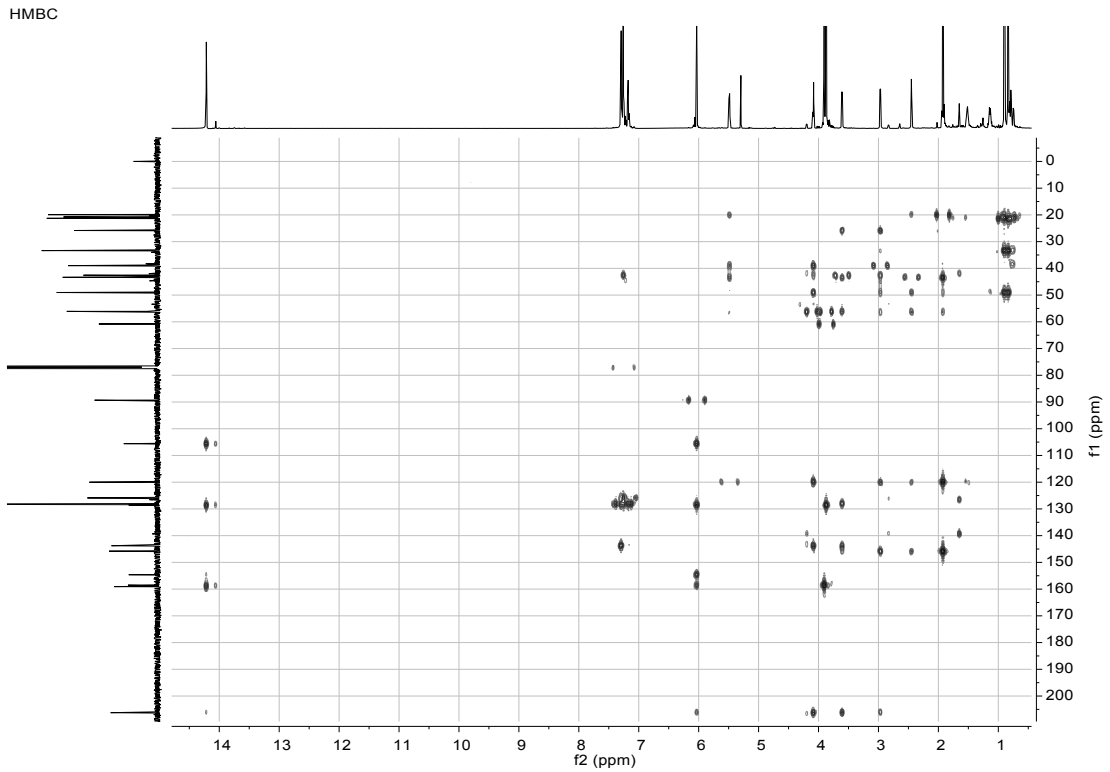


Figure S4. HMBC of (-)-Celosine (600MHz, $CDCl_3$)

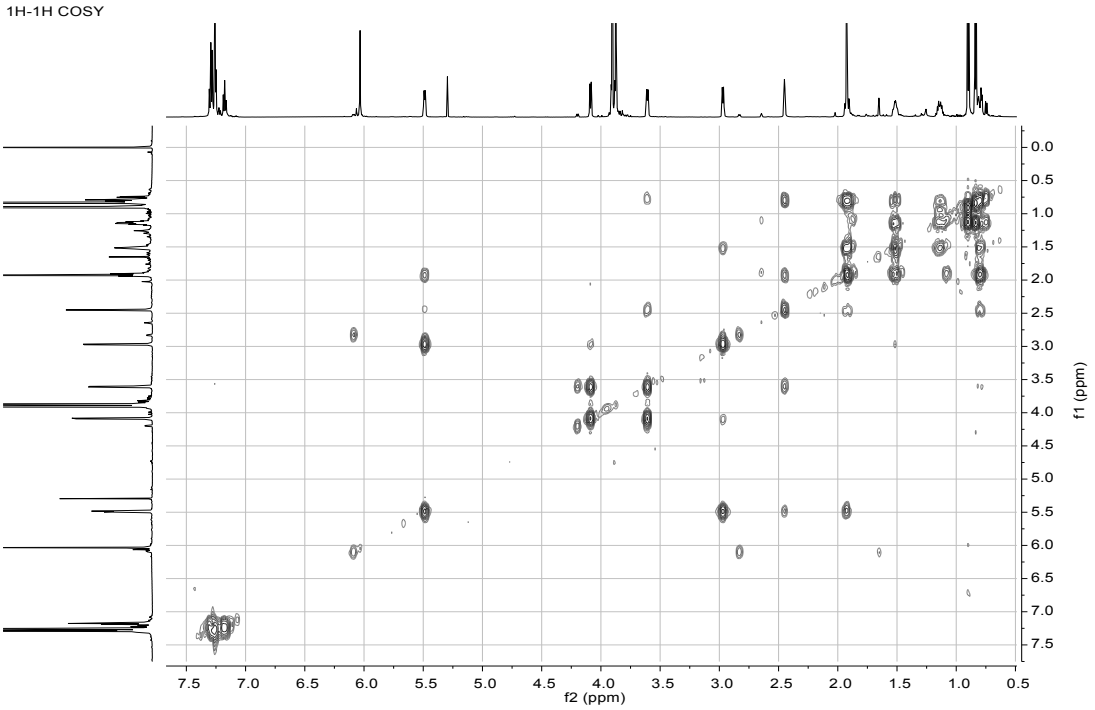


Figure S5. H-H COSY of (-)-Celosine (600MHz, $CDCl_3$)

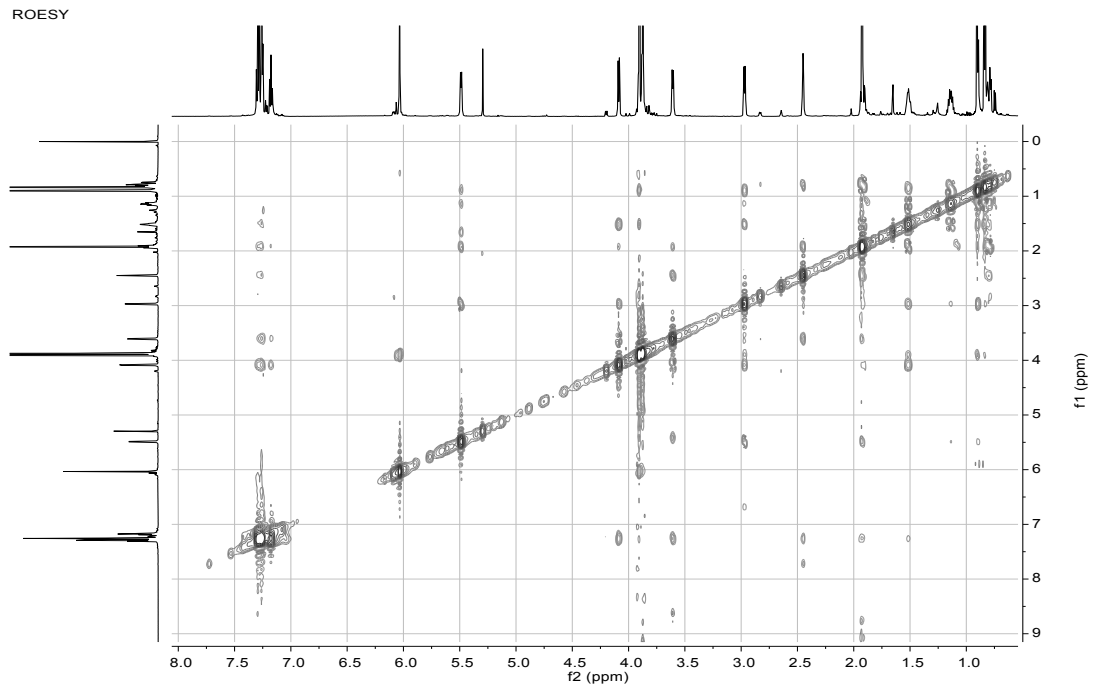


Figure S6. ROESY of (-)-Celosine (600MHz, $CDCl_3$)

2.2. MS spectraof Natural Source (-)-Celosine

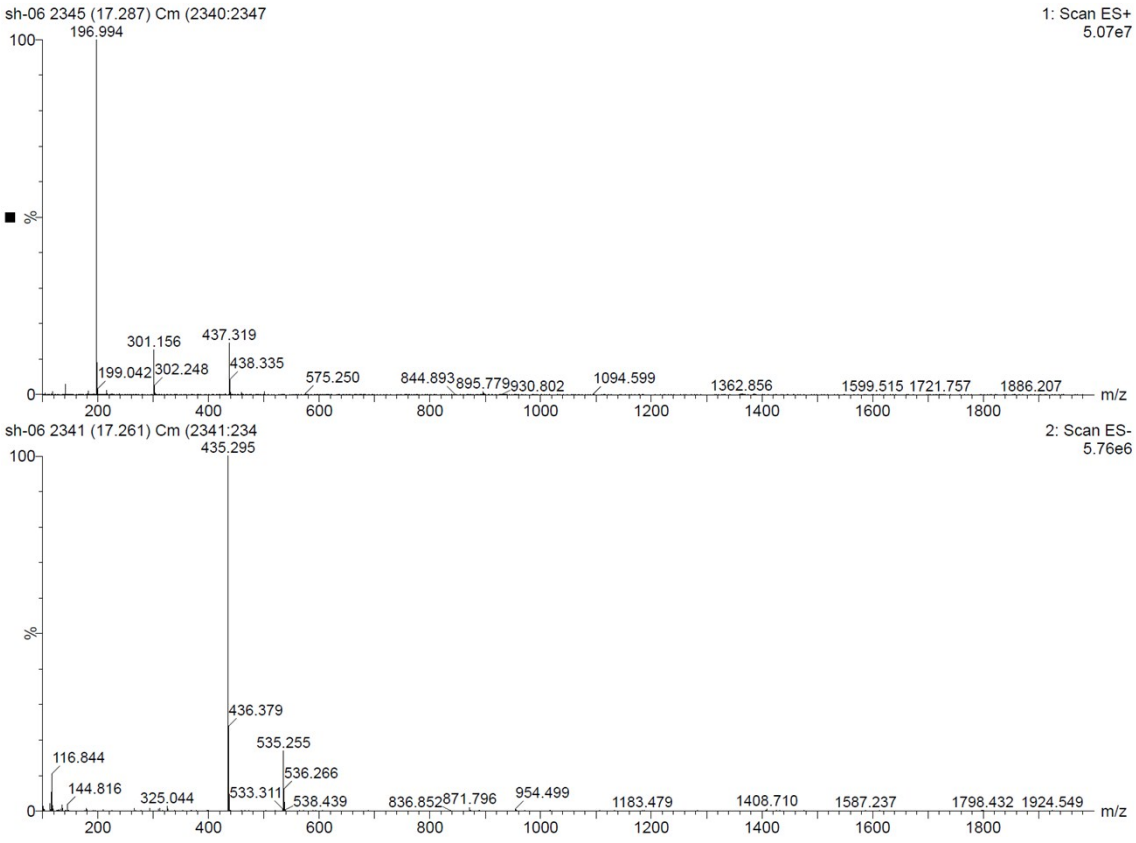


Figure S7. MS Spectra of (-)-Celosine

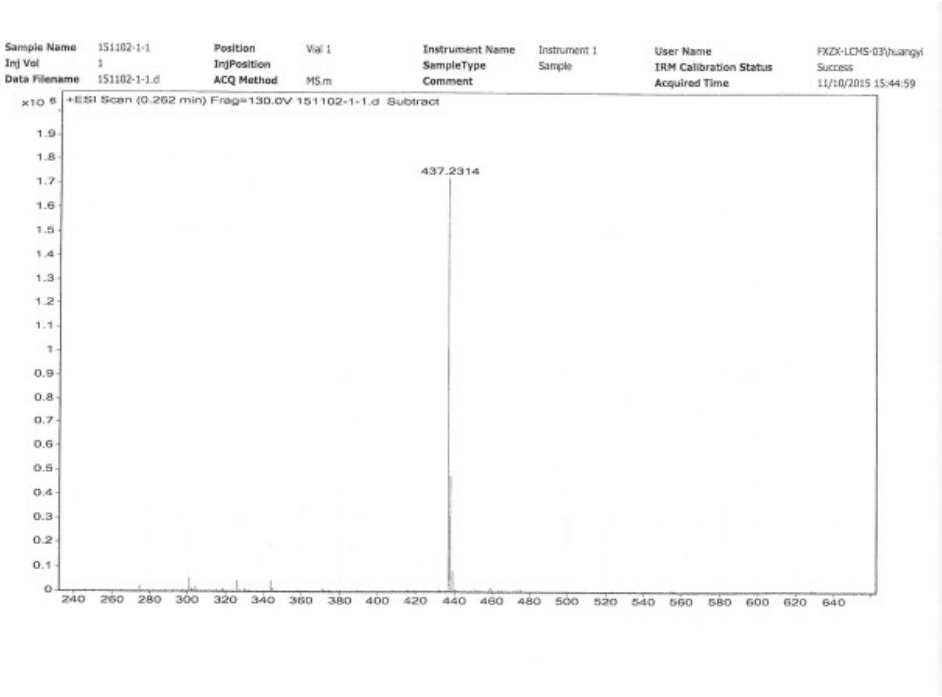
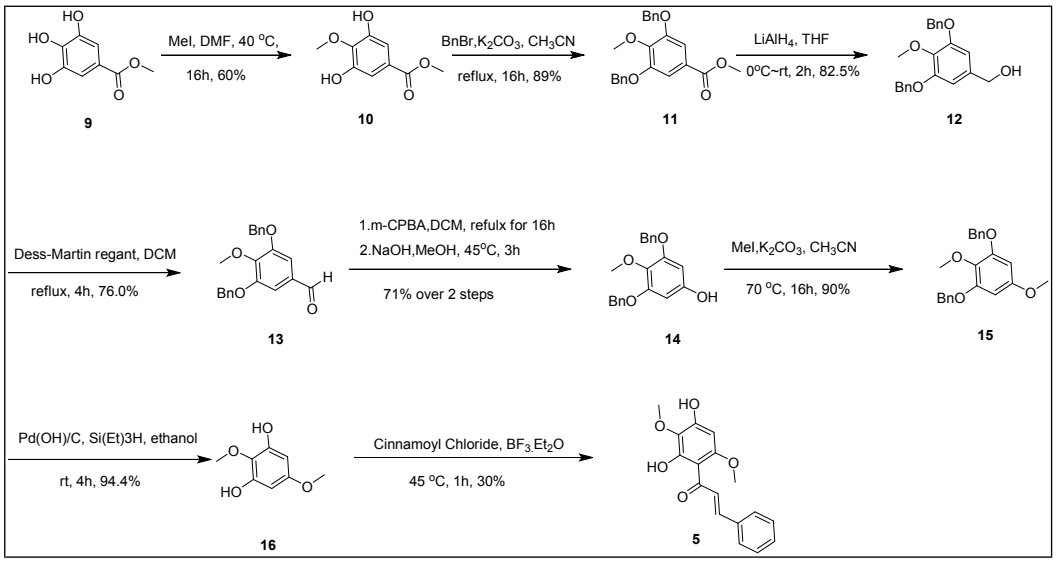


Figure S8. HR-EI-MS Spectra of (-)-Celosine

3. Total Synthesis of (+)-Celosine

3.1. Experimental Procedures and Characterization

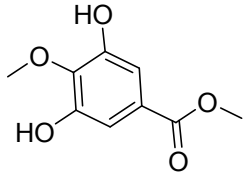


Scheme S1, Synthesis Route of intermediate compound 5

As shown in **Scheme S1**, the synthesis of compound 5 commenced with selective methylation of methyl gallate (compound 9) to give 3, 5-dihydroxy-4-methoxybenzoate (compound 10) in 60% yield. Compound 10 was protected with Benzyl group, and then reduced by LiAlH4 to afford alcohol 12, oxidized with Dess-Matin reagent to give substituted benzaldehyde 13 and followed by Baeyer-Villiger

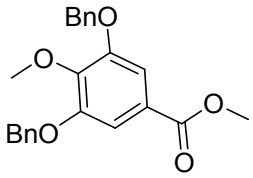
oxidation and hydrolysis reaction to afford 14 in high yield over five steps. Compound 16 was obtained in 85% yield over two steps by methylation of phenolic hydroxyl and deprotection of benzyl group. The key intermediate 5 was obtained after Friedel Crafts acylation reaction in 30% yield between compound 16 and cinnamoyl chloride.

Compound 10. methyl 3,5-dihydroxy-4-methoxybenzoate



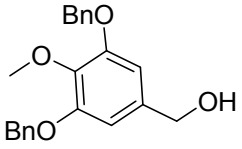
To a solution of methyl 3,4,5-trihydroxybenzoate (90.0g, 0.49 mol) in DMF (200 mL) was addedpotassium carbonate (87.0 g,0.63 mol) and MeI (69.0 g,0.49 mol), the reaction mixture was stirred at 40 ^{o}C for 16 h. Then water (150 mL) was added and the solution was adjusted to PH 4 with HCl (2N), and extracted with EA (4*100 mL), dried with sodium sulfate, concentrated, and purified by chromatography on silica gel (PE: EA=10:1) to get the desired product as a white solid. (58.0 g, Yield: 59.9%).
^{1}H-NMR (400Hz, DMSO)δ=9.47 (s, 1H), 6.95(s, 1H), 3.78(s,3H), 3.75 (s, 3H)

Compound 11.methyl 3,5-bis(benzyloxy)-4-methoxybenzoate



A mixture of methyl 3,5-dihydroxy-4-methoxybenzoate (50.0 g, 0.25 mol), benzyl bromide (107.7 g, 0.63 mol) and potassium carbonate (87.2 g,0.63 mol) in acetonitrile (150 mL) was stirred under flux for 16 h. Solvent was removed, water (150 mL) was added and extracted with DCM (3*100 mL), the organic layer was combined, dried with anhydroussodium sulfate, concentrated, and purified by chromatography on silica gel (PE: EA=10:1) to get the desired product as a light orange solid. (85.0g Yield: 88.9%).

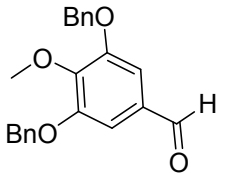
Compound 12.(3,5-bis(benzyloxy)-4-methoxyphenyl)methanol



To a solution of lithium aluminium hydride (17.0 g, 0.44mol) in THF (150 mL) was added methyl 3,5-bis(benzyloxy)-4-methoxybenzoate (85.0 g, 022 mol) in THF (150 mL) at 0^{o}C,Then the reaction was quenched with water, filtered and washed with DCM (4*50 mL), the filtrate was combined and dried with anhydroussodium sulfate, concentrated, and purified by chromatography on silica gel (PE: EA=10:1) to get the

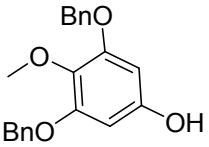
desired product as an off-white solid. (65.0 g, Yield: 82.6%).

Compound 13.3,5-bis(benzyloxy)-4-methoxybenzaldehyde



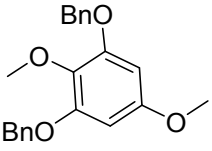
To a solution of (3,5-bis(benzyloxy)-4-methoxyphenyl)methanol (65.0 g, 0.19 mol) in DCM (200 mL) was added Dess-Martin regent (118.0 g, 0.28 mol), the reaction mixture was stirred at reflux for 4 h. The reaction was quenched with aqueous sodium thiosulfate solution and sodium bicarbonate solution, then extracted with DCM (4* 100 mL), the organic layer was combined, dried with anhydroussodium sulfate, concentrated, and purified by chromatography on silica gel (PE: EA=10:1) to get the desired product as a light orange solid. (49.0 g, Yield: 75.9%).

Compound 14. 3,5-bis(benzyloxy)-4-methoxyphenol



To a solution of 3,5-bis(benzyloxy)-4-methoxybenzaldehyde (89.0 g, 0.26 mol) in DCM (200 mL) was added m-CPBA (75.2 g,0.43 mol) in batches at 0^{o}C, the reaction mixture was stirred at reflux for 16 h. The reaction was quenched with aqueous sodium thiosulfate solution and sodium bicarbonate solution, then extracted with DCM (4* 100 mL), washed with brine (2*100 mL), the organic layer was combined, dried with anhydroussodium sulfate,concentrated to get the crude product as an orange oil (80.0 g), and was used in the next step without purification. The residue was dissolvedin methanol (100 mL), and 10% aqueous NaOH solution was added, the mixture was stirred at 45^{o}Cfor 3 h. Solvent was removed, aqueous HCl (2 N) was added to adjust PH value to 5.0, and extracted with EA (3* 100 mL), the organic layer was combined, dried with anhydroussodium sulfate, concentrated, and purified by chromatography on silica gel (PE: EA=10:1) to get the desired product as an orange solid. (61.0g Yield: 71.0%).

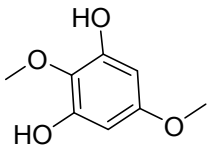
Compound 15.(2,5-dimethoxy-1,3-phenylene)bis(oxy)bis(methylene)dibenzene



A mixture of 3,5-bis(benzyloxy)-4-methoxyphenol (60.0 g, 0.18 mol), potassium carbonate (37.0 g, 0.27 mol), and methyl iodide (38.0 g, 0.27 mol) was dissolved in acetonitrile (150 mL), the reaction was stirred at 70^{o}Cfor 16 h. Solvent was removed and water (150 mL) was added, and extracted with EA (4* 100 mL), the organic layer

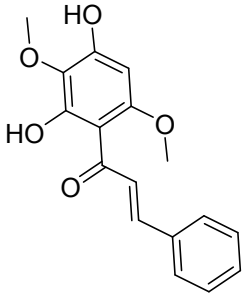
was combined, dried with anhydrous sodium sulfate, concentrated, and purified by chromatography on silica gel (PE:EA=10:1) to get the desired product as an orange solid. (55.7 g, Yield: 89.1%). EI-MS (m/z) = 351 $(M+H)^+$, ^{1}H-NMR(400Hz, $CDCl_3$)δ= 7.49-7.28 (m, 10 H), 6.22 (s, 2 H), 5.11 (s, 4 H), 3.88 (s, 3 H), 3.71 (s, 3 H).

Compound 16. 2,5-dimethoxybenzene-1, 3-diol



To a solution of (2,5-dimethoxy-1, 3-phenylene)bis(oxy)bis(methylene)dibenzene (37.0 g, 0.11 mol) and Pd(OH)/C (3.7 g) in anhydrous ethanol (150 mL) was added triethylsilane (61.3 g, 0.55 mol), the reaction mixture was at r.t. for 4 h. Solvent was removed, concentrated, and purified by chromatography on silica gel (PE:EA=5:1) to get the desired product as a light yellow solid. (17.0g Yield: 94.4%). EI-MS (m/z) = 171 $(M+H)^+$, ^{1}H-NMR(400Hz, *d6*-DMSO)δ= 9.00 (s, 2 H), 5.88 (s, 2 H), 3.59 (s, 6 H).

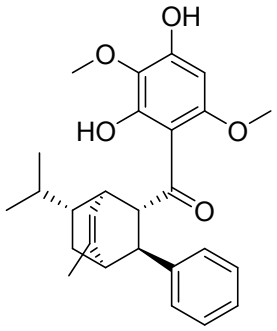
Compound 5. (E)-1-(2,4-dihydroxy-3, 6-dimethoxyphenyl)-3-phenylprop-2-en-1-one



A mixture of 2,5-dimethoxybenzene-1, 3-diol (2.0 g, 11.8 mmol), and cinnamoyl chloride (2.6 g, 17.7 mmol) in boron trifluoride diethyl etherate solution (20 mL) was stirred at 45 ^{o}C for 1 h. The reaction was quenched with water (10 mL), and extracted with EA (3* 50 mL), the organic layer was combined, dried with anhydroussodium sulfate, concentrated, and purified by reversed phase column chromatography on C18 silica gel (water:acetonitrile = 60 %) to get the desired product as an orange solid (1.0 g, Yield: 28.6%). EI-MS (m/z) = 301 $(M+H)^+$, ^{1}H-NMR(400Hz, *d6*-DMSO)δ= 13.83 (s, 1 H), 10.48 (s, 1 H), 7.87-7.67 (m, 4 H), 7.48-7.46 (m, 3 H), 6.10 (s, 1 H),

3.87 (s, 3H), 3.67 (s, 3 H).

(+)-Celosine: (2,4-dihydroxy-3, 6-dimethoxyphenyl)((1R,2S,3S,4R,7R)-7-isopropyl-5-methyl-3-phenylbicyclo[2.2.2]oct-5-en-2-yl)methanone



A mixture of (E)-1-(2,4-dihydroxy-3,6-dimethoxyphenyl)-3-phenylprop-2-en-1-one (320 mg, 1.07 mmol), (-)-alpha-Phellandrene (950 mg, 70.0 mmol), and silica-supported AgNP catalyst (1.0 g, containing 27 μg Ag, 0.25 mol%) in dichloroethane (15 mL) was stirred at 70^{o}C for 16 h. Then reaction was filtered, concentrated, and the residuewas purified by reversed phase column chromatography on C18 silica gel (water:acetonitrile = 60 %) to get the desired product as a light yellow solid (300 mg, Yield: 64.4 %). EI-MS (m/z) = 437 (M+H) +, ^{1}HNMR(400Hz, $CDCl_3$)δ= 14.23 (s, 1 H), 7.32-7.15 (m, 6 H), 6.29 (bs, 1 H), 6.04 (s, 1 H), 5.49 (d, *J*= 6.36 Hz,1H), 4.09 (dd, *J*= 6.9 Hz, 1.1 Hz, 1H), 3.91 (s, 3 H), 3.88 (s, 3H), 3.60 (d, *J*= 6.8 Hz, 1 H), 2.97 (d, *J*= 6.8 Hz, 1H), 2.45 (s, 1H), 2.00-1.89 (m, 4 H), 1.55-1.52 (m, 1 H), 1.17-1.11 (m, 1 H), 0.90 (d, *J*= 6.0 Hz, 3 H), 0.83(d, *J*= 6.5 Hz, 3 H).

3.2. NMR spectra and MS spectra of compound 15 ~ (-)-Celosine

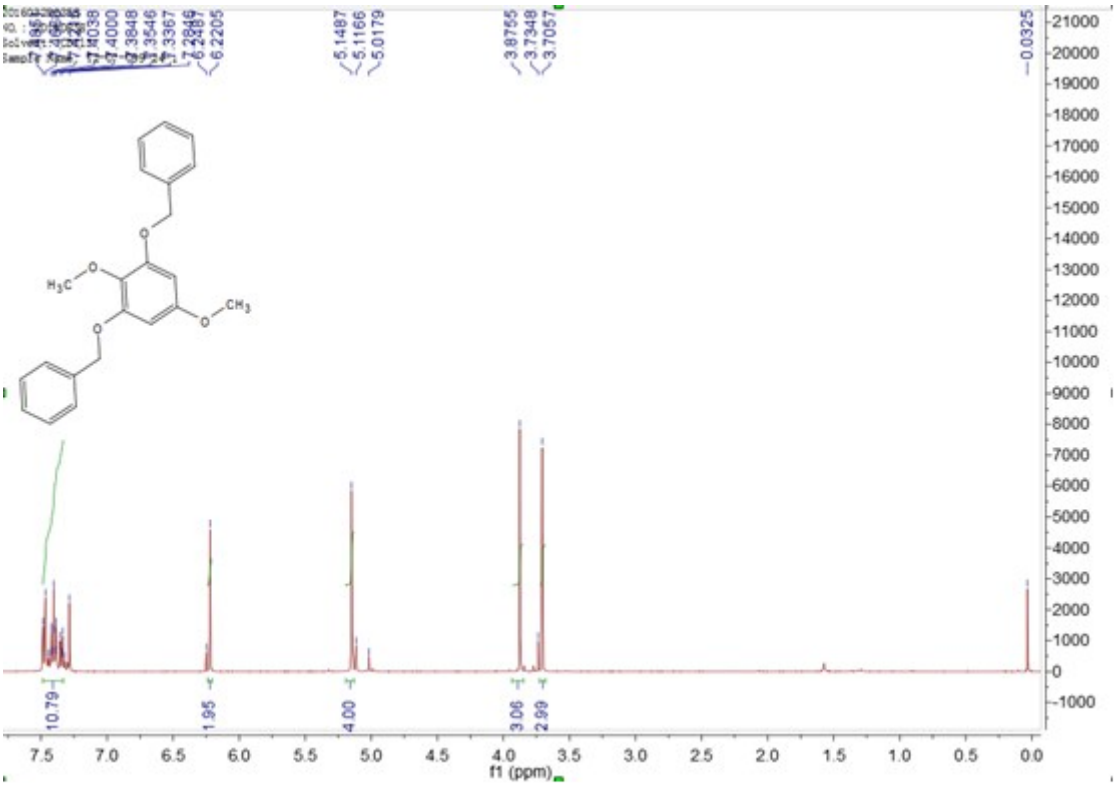


Figure S9. [1]H-NMR of compound 15 (400MHz, *d6-DMSO*)

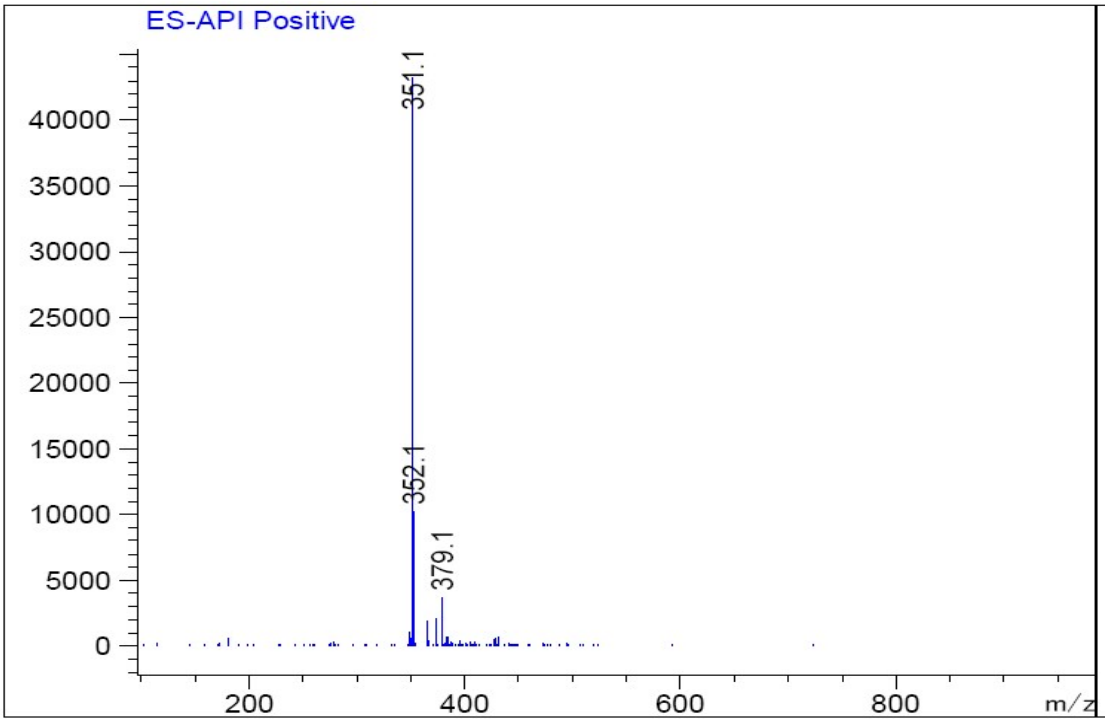


Figure S10. MS spectra of compound 15

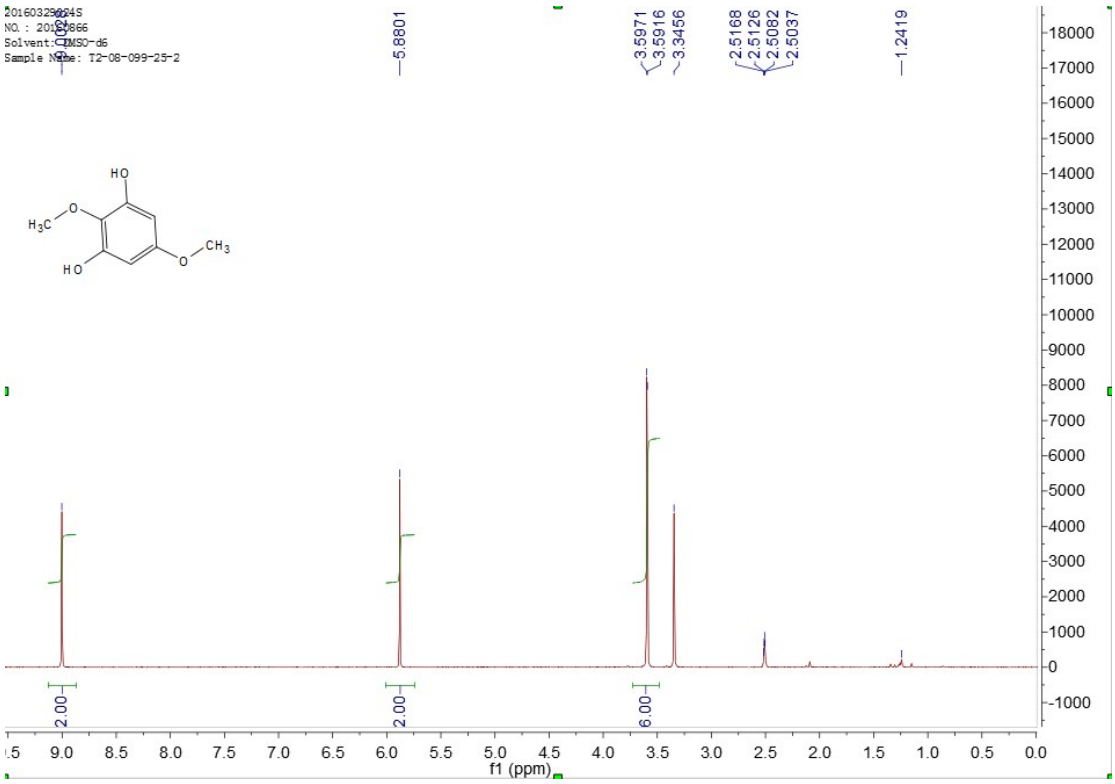
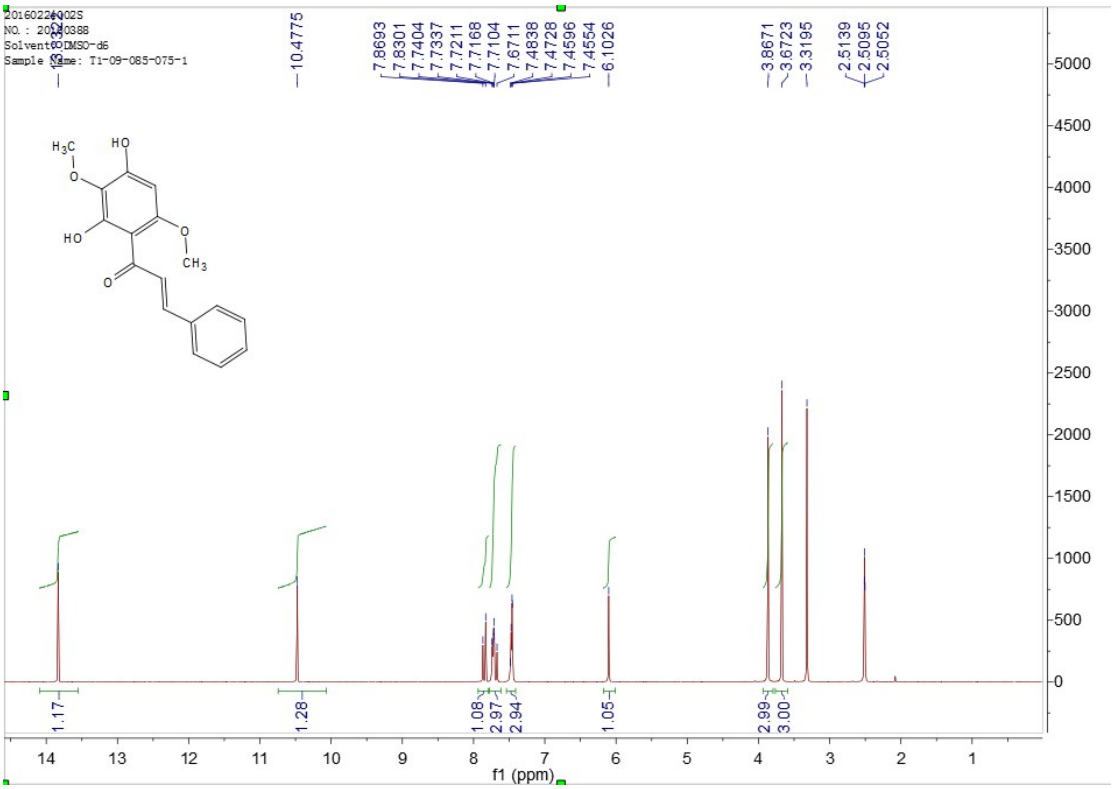


Figure S11. [1]H-NMR of compound 16 (400MHz, *d6-DMSO*)

Figure S12. [1]H-NMR of compound 5(400MHz, *d6-DMSO*)



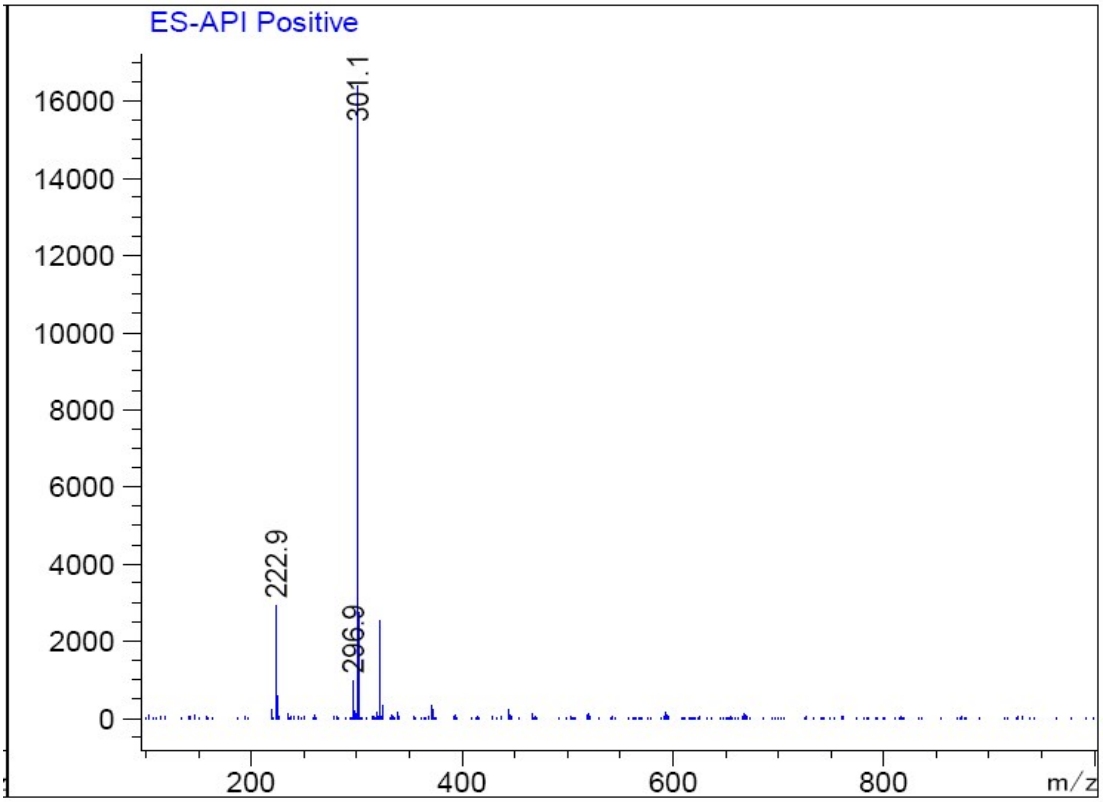


Figure S13. MS spectra of compound 5

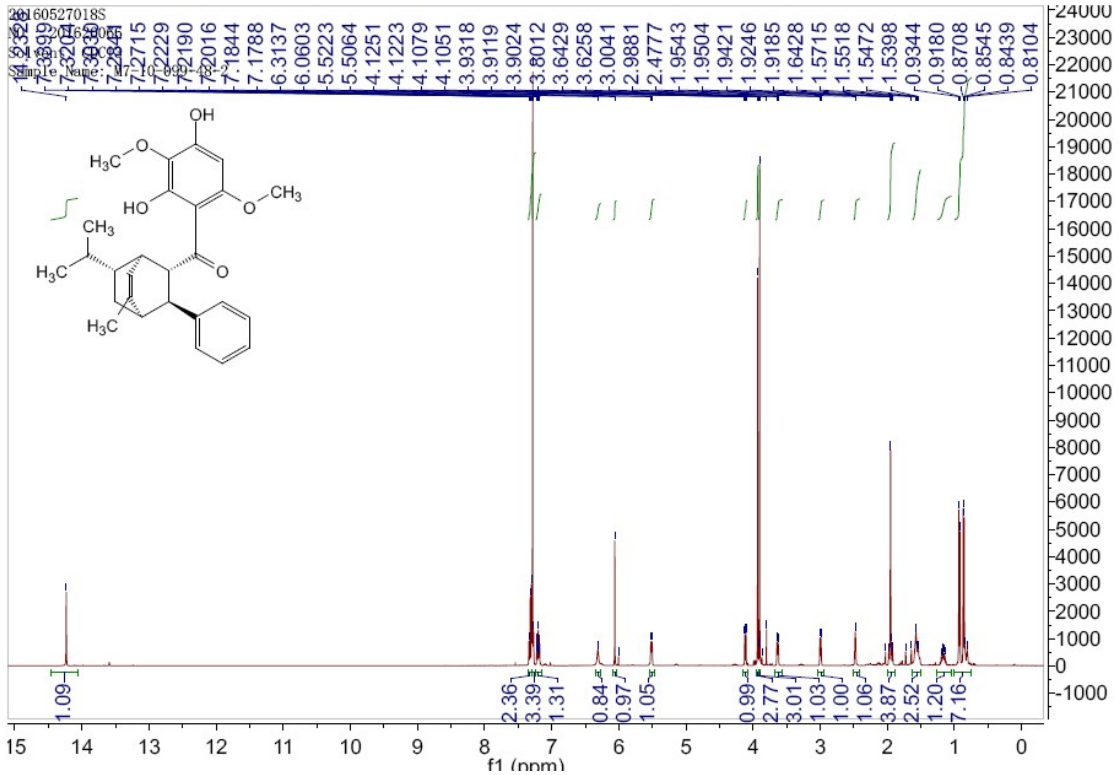


Figure S14. ^{1}H-NMR of (+)-Celosine (400MHz, *$CDCl_3$*)

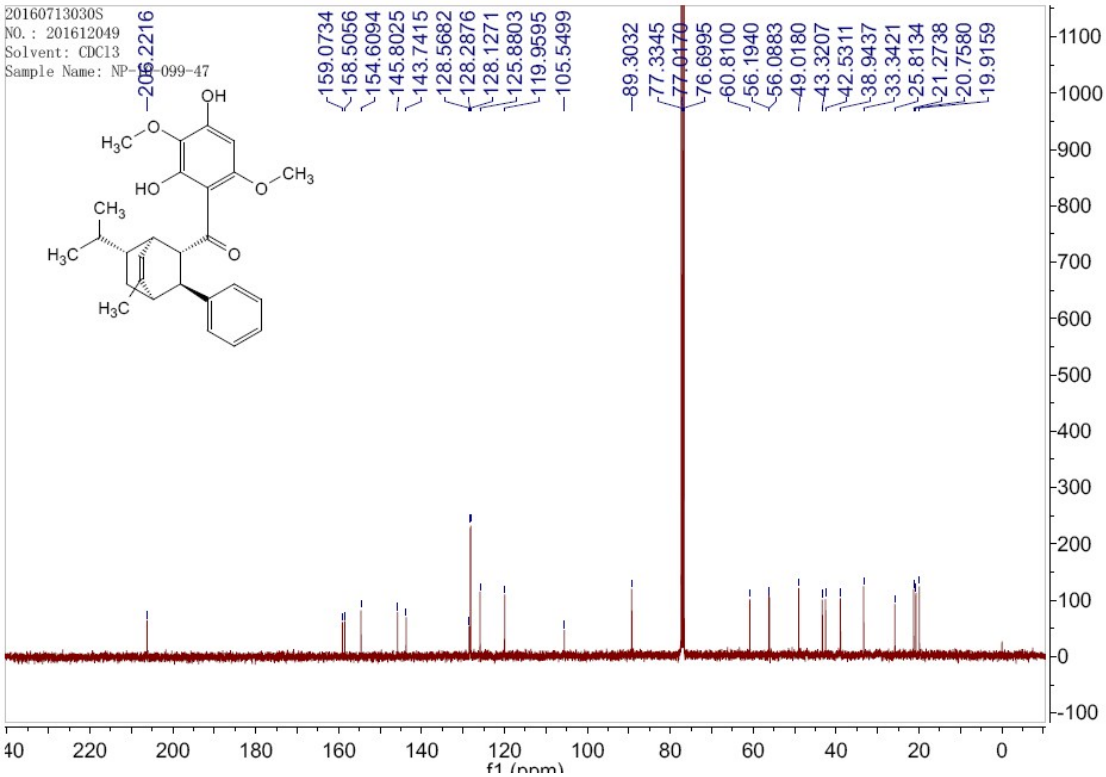


Figure S15. ^{13}C-NMR of (+)-Celosine (400MHz, *$CDCl_3$*)

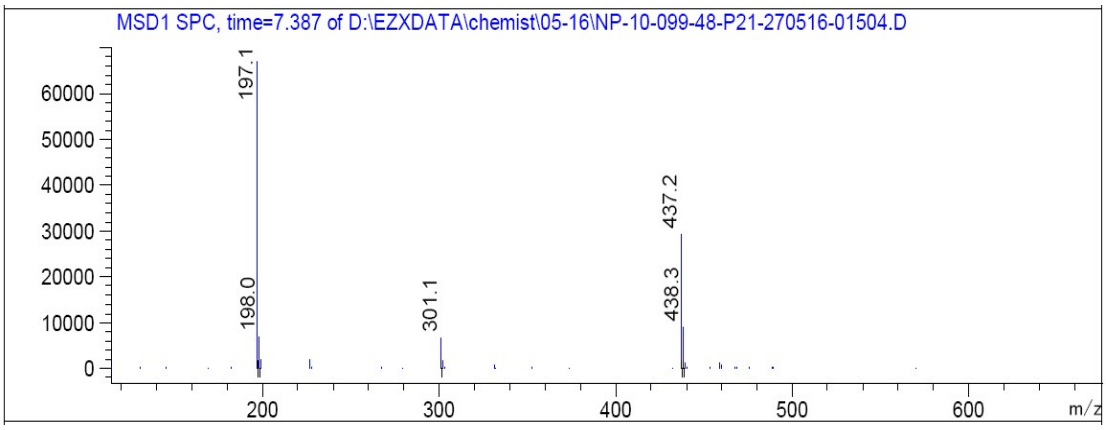


Figure S16. MS spectra of (+)-Celosine

Table S1. ^{1}H-NMR data of (-)-Celosine and (+)-Celosine

Natural Source Celosine*				Synthesized Celosine**			
No	δ_H (*J* in Hz)	δ_C	DEPT	No	δ_H (*J* in Hz)	δ_C	DEPT
1	2.97d (6.4)	39.0	CH	1	2.97d (6.4)	38.9	CH
2	4.09dd (6.8, 1.5)	56.2	CH	2	4.09dd (6.9, 1.1)	56.2	CH
3	3.61br. d (7.1)	42.6	CH	3	3.60br. d (6.8)	42.5	CH
4	2.45t (2.3)	43.4	CH	4	2.45s	43.3	CH
5α	1.92 (overlapped)	25.8	CH_2	5α	1.92dd (22.6, 2.3)	25.8	CH_2
5β	0.78-0.82 (overlapped)			5β	0.78-0.82 m		
6	1.52tdd (9.5, 4.7, 2.0)	49.0	CH	6	1.52tdd (9.5, 4.7, 2.0)	49.0	CH
7	5.49br. d (6.4)	120.0	CH	7	5.49br. d (6.4)	120.0	CH
8	-	145.8	C	8	-	145.8	C
9	1.93d (1.7)	19.9	CH_3	9	1.93d (1.7)	19.9	CH_3
10	1.14dp (9.4, 6.4)	33.4	CH	10	1.14dp (9.4, 6.4)	33.3	CH
11	0.90d (6)	21.3	CH_3	11	0.90d (6)	21.3	CH_3
12	0.84d (6.5)	20.8	CH_3	12	0.83d (6.5)	20.8	CH_3
13	-	206.2	C	13	-	206.2	C
14	-	105.6	C	14	-	105.5	C
15	-	159.1	C	15	-	159.1	C
16	-	128.6	C	16	-	128.6	C
17	-	154.6	C	17	-	154.6	C
18	6.03s	89.3	CH	18	6.03s	89.3	CH
19	-	158.5	C	19	-	158.5	C
20	-	143.8	C	20	-	143.8	C
21	7.26 (overlapped)	128.1	CH_2	21	7.26m	128.1	CH_2
22	7.29 (overlapped)	128.3	CH_2	22	7.29m	128.3	CH_2
23	7.18br. t (7.2)	125.9	CH_2	23	7.17br. t (7.2)	125.9	CH_2
24	7.29 (overlapped)	128.3	CH_2	24	7.31m	128.3	CH_2
25	7.26 (overlapped)	128.1	CH_2	25	7.25m	128.1	CH_2
15-OH	14.22s	-	-	15-OH	14.21s	-	-

16-OCH_3	3.87s	16.5	CH_3	16-OCH_3	3.88s	16.5	CH_3
19-OCH_3	3.90s	17.5	CH_3	19-OCH_3	3.91s	17.5	CH_3

*(600MHz in *$CDCl_3$*), **(400MHz in *$CDCl_3$*)

3.3. Crystal Structure of (+)-Celosine

Crystals suitable for single-crystal X-ray analysis were obtained from tetrahydrofuran/ethyl actate mixture (1:1) at -20 ^oC for 7 days. The data were collected at 173 K on a Burker APEX-II CCD diffractometer operating with graphite-monochrmatized Mo $K_\alpha$radiation. Frames of 0.8 ^{o} oscillation were exposed; deriving 8460 unique reflections (R_{int}= 0.035) in the θ range of 3.5 to 64 ^{o} with a completeness of ~97.8%. Structure solution and full least-squares refinement with anisotropic thermal parameters of all non-hydrogen atoms and rigid group refinement of the hydrogen were performed using SHELX. The final refinement results in R1= 0.0520 and the absolute configuration could be determined by anomalous-dispersion (Flack = -0.06(5)).

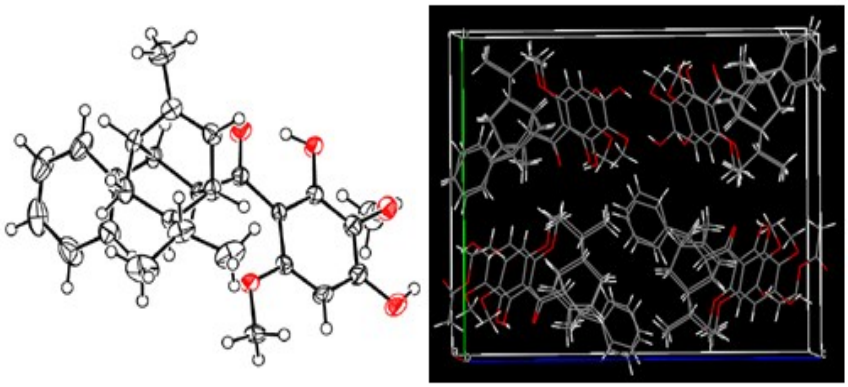


Figure S17. The Crystal structure and crystal packing view of (+)-Celosine

Table S2, Crystal data and structure refinement for (+)-Celosine

Bond precision:	C-C = 0.0048 A		Wavelength=1.54178
Cell:	a=16.1444(9)	b=16.5294(9)	c=19.7875(11)
	alpha=90	beta=90	gamma=90
Temperature:	173 K		
	Calculated		Reported
Volume	5280.4(5)		5280.4(5)
Space group	P 21 21 21		P 21 21 21
Hall group	P 2ac 2ab		P 2ac 2ab
Moiety formula	2(C27 H32 O5), C4 H8 O2		2(C27 H32 O5), C4 H8 O2
Sum formula	C58 H72 O12		C58 H72 O12
Mr	961.16		961.15
Dx,g cm-3	1.209		1.209

Z	4	4
Mu (mm-1)	0.675	0.675
F000	2064.0	2064.0
F000'	2070.34	
h,k,lmax	18,19,23	18,19,23
Nref	8751[4845]	8460
Tmin,Tmax	0.886,0.935	0.619,0.753
Tmin'	0.874	
Correction method= # Reported T Limits: Tmin=0.619 Tmax=0.753		
AbsCorr = MULTI-SCAN		
Data completeness= 1.75/0.97	Theta(max)= 63.998	
R(reflections)= 0.0520(8074)	Wr2(reflections)= 0.1392(8460)	
S = 1.069	Npar= 649	
CCDC No.	**1488037**	

4. Target Prediction

4.1. Docking Study of (-)-Celosine

Chapman & Hai's Dictionary of Natural Products (DNP6. 2) has collected and recorded about 200,000 natural products so far, and some of these natural products have be characterized in terms of their activities and related data such as ID50. MDL's MDDR3D has collected and recorded more than 100,000 synthetic compounds and listed the structures and activities of all these compounds.

Structure search (structural similarity=78%) showed that 18 natural and synthetic compounds were structurally similar to (-)-Celosine, and further search, comparison and analysis showed that all these compounds had anti atherosclerotic activities.

Crystal structure of MPO protein complex was obtained from the Protein Data Bank (PDB, Code 3f9p, and **FigureS18**). The primary ligand was extracted out from the binding site, and other ligands and water molecules had been deleted completely. The Biopolymer module in Sybil was used to do all the protein preparation, including adding related residues and hydrogen atoms. The compound (-)-Celosine was loaded with Gasteiger-Huckel charges and AMBER force filed. After the protein crystal docking zone was defined as Protomol, and Surflex-dock Geom (SFXC) was used as the accurate docking pattern, whereby a total of 20 docking results was obtained, which were sequenced one by one according to the toal acore (as listed in **Table S3**). The result of molecular docking (entry 1 in **Table S3**) is shown in **Figure S19**. As shown in Figure **S19-A** and **S19-B**, (-)-Celosine forms various forces with amino acid residues of the receptor protein, including hydrogen bond and hydrophobic bond. The residue HIE916 and ILE1060 form a alkyl-Pi hydrophobic bond with the benzene ring on the left side of (-)-Celosine, the length of which is 3.04 Å and 4.95 Å respectively; the residue bond ASN918, ARG934 and ASN996 form hydrogen bonds with the hydrogen and oxygen atoms on the left benzene ring, the length of which is 2.25 Å,

2.16 Å and 2.73 Å respectively. The alkyl moiety on the right side of the compound binds with the residue TYR776, PHE739 and ILE1060 to form the alkyl group and alkyl-Pi hydrophobic bond, the length of which is 5.24Å, 4.49Å and 4.98Å respectively. Based on the docking study, the residues HIE916, ASN918, ARG934, ASN996, TYR776, PHE739 and ILE1060 were illustrated of very importance for anti-atherosclerosis activity. The interactions between (-)-Celosine and the residues around the docking pocket were the main contribution to the anti-atherosclerosis activity of (-)-Celosine. The above docking analysis can provide some theoretical clues for the modification of such compounds, knowing that the benzene ring and some substituent groups on the left side can be decorated by adding an appropriate number of hydrophilic groups and the alkyl moiety on the right side can be used as the focus of decoration.

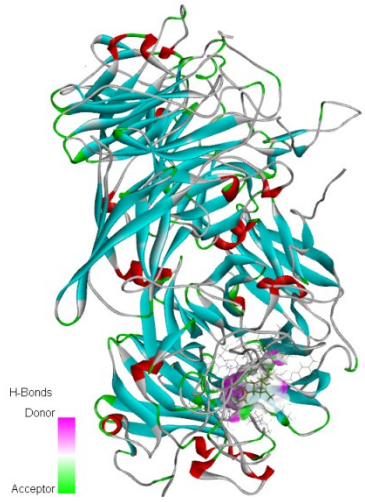


Figure S18. The conformation of MPO protein containing the compound

Table S3. 20 conformations produced from Celosine-MPO complex docking

Name	1: Total-Score	2: Crash	3: CSCORE
Celosine -1	6.97	-1.49	4
Celosine-2	6.64	-1.56	5
Celosine-3	6.55	-1.81	4
Celosine-4	6.53	-1.64	4
Celosine-5	6.36	-1.71	3
Celosine-6	6.33	-1.76	4
Celosine-7	6.27	-1.77	3
Celosine-8	6.19	-1.79	2
Celosine-9	6.16	-1.94	3
Celosine-10	6.12	-1.45	1
Celosine-11	6.10	-1.96	3
Celosine-12	6.08	-1.87	3

Celosine-13	6.07	-1.34	1
Celosine-14	6.05	-1.97	4
Celosine-15	5.98	-1.28	0
Celosine-16	5.97	-1.82	3
Celosine-17	5.96	-1.37	0
Celosine-18	5.95	-1.35	0
Celosine-19	5.95	-1.29	1
Celosine-20	5.93	-1.87	3

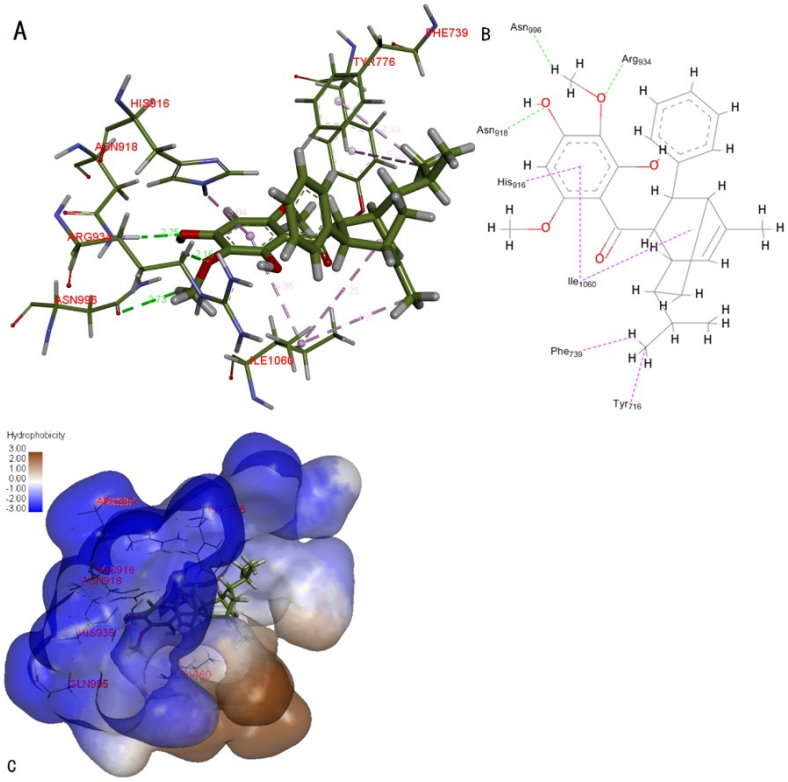


Figure S19. Docking of (-)-Celosine into the binding site of MPO. Hydrogen bonds are shown as green dotted line. Hydrophobic bonds are shown as pink dotted line.
(**A:** schematic perspective **B:** plan sketch **C:** MOLCAD map of hydrophobic interactions)

4.2. MD simulation study of (-)-Celosine

After molecular docking, the Amber12.0 package was used to perform a dynamics simulation to further verify the result of docking and get some further information about the state of amino acid binding around the binding pocket of (-)-Celosine and its receptor MPO. The docking result was used as the initial structure to perform the simulation. An ff99SB force field and a gaff force field were loaded onto the protein and the ligand respectively by using the antechamber in Amber12.0, and at

the same time bbc charge is loaded onto the small molecule to prepare parameters of the small molecule.

The whole system is pre-treated before initiation of the simulation. First, make sure that charge of the whole complex is zero, maintain electric neutrality of the system by adding Na+/Cl-, and finally add the neutralized complex to the 10Å regular-octahedral water box. Next, it is necessary to optimize energy of the system to eliminate the possibility of some high-energy collisions between molecules, knowing that they may be a hidden danger. The process of energy optimization is divided into two steps: first, make 1000-step optimization on the solvent environment under the promise of restraining the whole complex (500 steps for the steepest descent method and 500 steps for the conjugate gradient method), and second, make 1000-step optimization of the steepest descent method and 4000-step optimization of the conjugate gradient method without restraining the complex.

After optimization of the system, MD simulation is initiated in three steps. First step: elevate the temperature. Briefly, the system temperature is raised from OK to 300K under the NTV system, and at the same time the protein is restrained by a 2kca/mol $Å^{-2}$ constant force to 5restrain the H chemical bond by opening Shake. Second step: balance the system. Briefly, density balance of 100ps is performed after temperature elevation under constant temperature and pressure (300K and 1 atmosphere). Using the same way, the protein is restrained by a 2kca/mol $Å^{-2}$ constant force. Third step: dynamic sampling. Briefly, after temperature elevation and balance, dynamic sampling is performed by dynamically simulating 30ns under constant temperature and pressure (300K and 1 atmosphere). During the process of simulation, the cutoff value in calculating the non-covalent interaction is set at 10Å.

Molecular docking only reflects the static binding conformation. To understand the dynamic process between(-)-Celosine-protein binding for further analysis of the dynamic interaction pattern, we perform a (-)-Celosine-MPO dynamic simulation by using amber12.0 on the basis of the optimal conformation selected, and describe changes in conformation of the catalytic subunit relative to the initial structure in the simulated trajectory by using root mean square deviation (RMSD).

RMSD (t) $= \sqrt{\frac{1}{N}\sum_{i=1}^{N}\Delta r_i(t)^2}$, where $\Delta r_i(t) = r_i(t) - r_i(t_0)$ represents the deviation between the position a particular atom at t time point and the position of the corresponding atom in the initial structure, and N represents the total number of atoms. Using the initially optimized structure as the reference, RMJSD of the main skeleton atom of protein conformation in 30ns simulated trajectory is calculated (**Figure S20**) each conformation in the trajectory is overlapped with the reference structure by using the minimum variance fitting method without considering

translation, rotation and cycle effect, and with only conformational change of the protein itself. In the first 13ns, RMSD docking the complex undergoes a relatively large change, meaning that conformation of the receptor protein MPO and the position of Celosine in the active pocket have undergone a relatively change. However, Celosine dynamically travels to 13ns and RMSD of the subsequent fluctuates around 3.25 Å (a receptable deviation), meaning that no significant conformational change occurs when the initial and final conformations are in the same docking pocket. As shown in **Figure S20-B**, the left benzene ring maintains an important hydrogen-bonding force with THR994 and ILE1060 residues, while the right alkyl moiety maintains an important alkyl hydrophobic bond with PHE739 and ILE1060 residues. The total number of acting forces is reduced as compared with that before dynamics. However, the important acting forces (hydrogen bond and hydrophobic interaction) and the position thus formed do not undergo significant change. As shown in **Figure S21**, we overlap the docked protein-ligand complex before dynamic running with the ligand complex after 30ns dynamic running, where purple red represents the initial structure of the (-)-Celosine-MPO complex, and green represents the final structure of the complex. The figure shows that the initial and final conformations are in the same docking pocket without significant conformational change. A comprehensive analysis of **Figure S20** and **S21** demonstrates that both the docking pocket and ligand conformation are stable and the docking result is reliable.

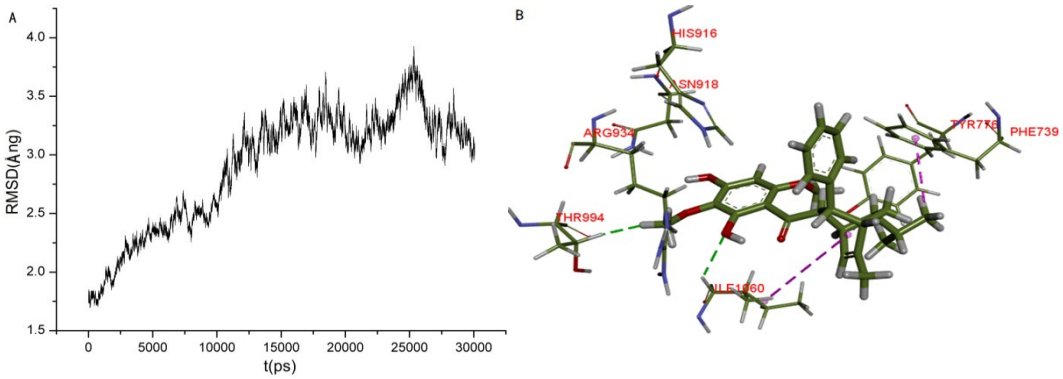


Figure S20.A: RMSD change diagram of the skeleton (C, Cα, N and O) atoms of Celosinet. B: Sketch map of the acting force of Celosine-MPO complex after 30ns MD simulation

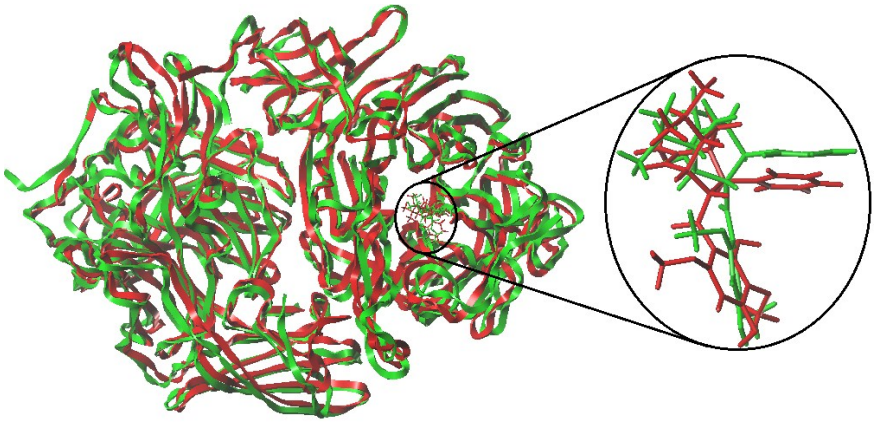


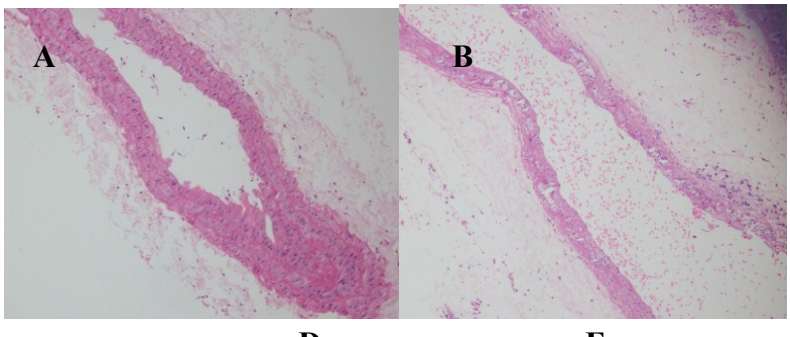
Figure S21. MD results of (-)-Celosine-MPO complex. Red represents the initial conformation and green represents the final conformation

5. Animal study protocol

After 4 weeks of high-fat diet, 30 male, 200-220g, Wistar rats were subjected to the right common carotid artery clamp and reperfusion under anaesthesia. Then animals except for the control group continued to receive high fat diet and 3 intraperitoneal injection of Vitamin D3 at the dose of 600,000 IU/kg. At teminal, after anesthesia with Isoflurane, blood samples were taken from abdominal aorta, serum was separated, and MPO, Lp-PLA2 and MMP-9 levels were determined by ELISA. Then, the common carotid artery was separated and removed, washed with normal saline, fixed with 10% neutral formaldehyde, dehydrated routinely and embedded in paraffin. MPO, Lp-PLA2 and MMP-9 levels were determined by HE staining and immunohistochemical staining.

6. Vascular intima change and expression of MMP-9

HE staining displayed that the vascular intima in normal group was smooth, holonomic and no lipid droplets formation. On the other hand, significant AS changes had been seen in the disease group, such as endothelial cell detachment, rupture, intimal thickening and inward bulge, and obvious plaque formation. The degree of intimal thickening and uplift was greatly improved after drug intervention.



C D E

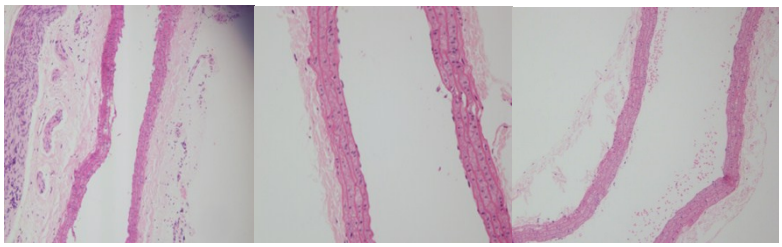


Figure S22. Vascular HE staining

(A. Normal group, B. Disease group, C. atorvastatin group, D and E. (-)-Celosine high/low dose group)

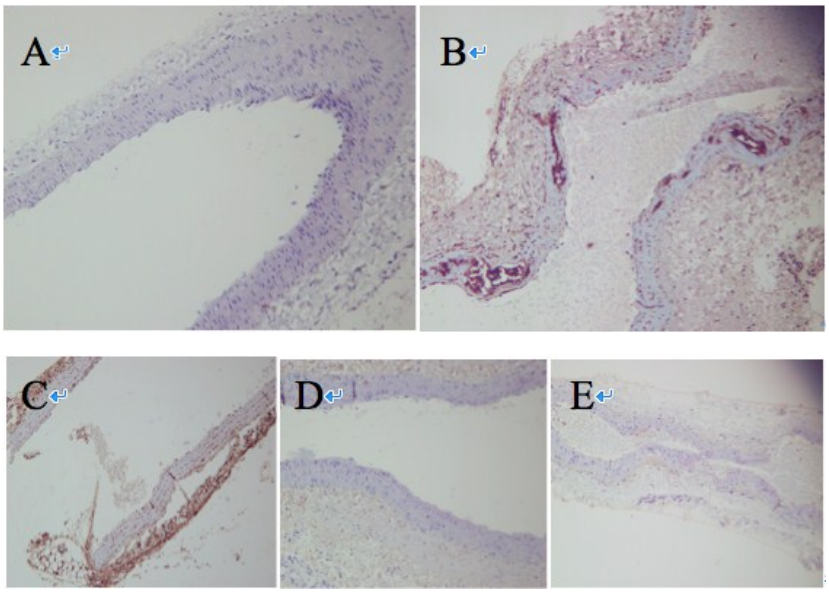


Figure S23. Immunohistochemical picture of vascular protein MMP-9

(A. Normal group, B. Disease group, C. atorvastatin group, D and E. (-)-Celosine high/low dose group)

Extremely high expression of MMP-9 was seen in disease group, and the expression was decreased significant in high and low dose treatment of (-)-Celosine, which showed a better treatment effect than the atorvastatin group.