Supporting Information

Genetically encoded fluorescent screening probe for MgrA, a global regulator in *Staphylococcus aureus*

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Supporting Figures

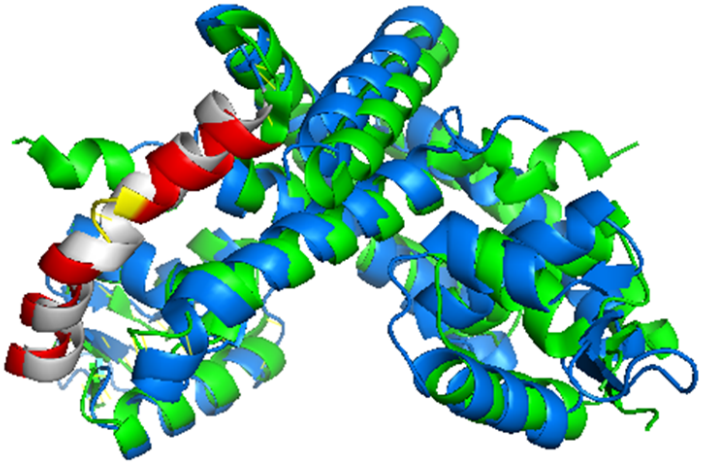


Figure S1. Alignment of MgrA (PDB entry: 2BV6) and OhrR (PDB entry: 2PEX). The red helix bundle is OhrR's alpha 5 moiety and the silver helix bundle is MgrA's alpha 5 and alpha 6.The yellow piece in the red bundle is Ohser's fluorescent protein insertion site.[1] Superpositions were analyzed by Pymol.

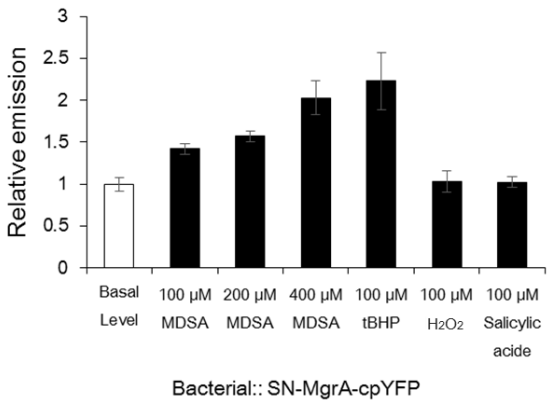


Figure S2. *E. coli* cells expressing SN-cpYFP-MgrA fusion protein were treated with MDSA, *t*BHP, H_2O_2 and salicylic acid. (1×10^5 cells/mL in 20mM HEPES, pH 7.5, 37°C, 30min).

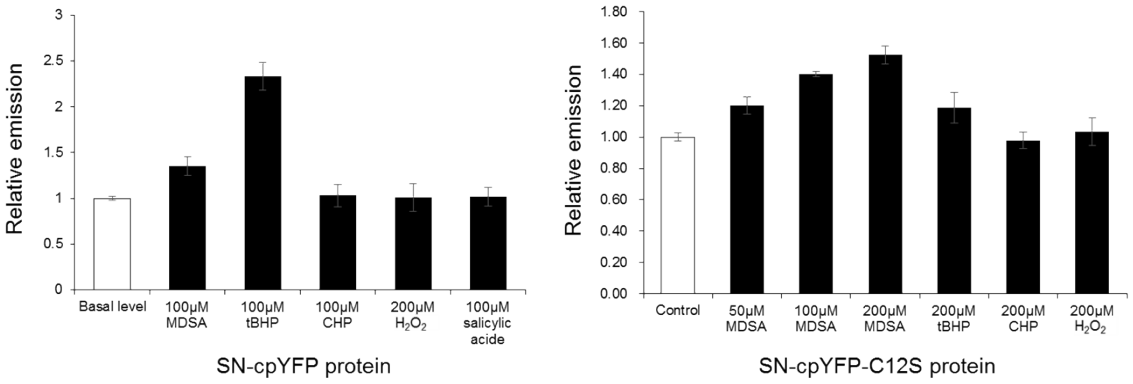


Figure S3. Fluorescence response of SN-MgrA-cpYFP/SN-MgrA-cpYFP-C12S proteins to MDSA, *t*BHP, CHP, H_2O_2, and SN-MgrA-cpYFP-C12S proteins to reduction reagent NADH, GSH, DTT, $NaNO_3$. (1×10^5 cells/mL in 20mM HEPES, pH 7.5, 37°C, 30min) .

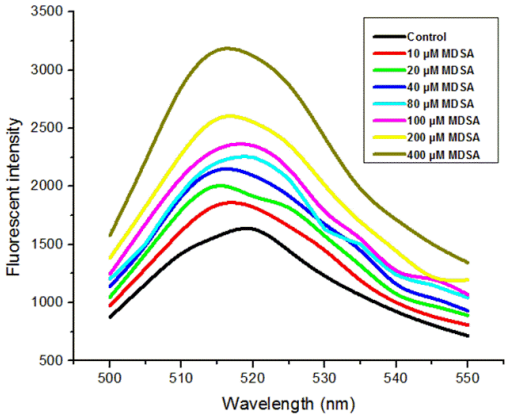


Figure S4. Sensitivity test of *E. coli* cells expressing SN-MgrA-cpYFP-C12S. The fluorescent intensities of *E. coli* cells expressing SN-MgrA-cpYFP-C12S in the presence of various amounts of MDSA with excitation set at 470 nm. (1×10^5 cells/mL in 20mM HEPES, pH 7.5, 37°C, 30min).

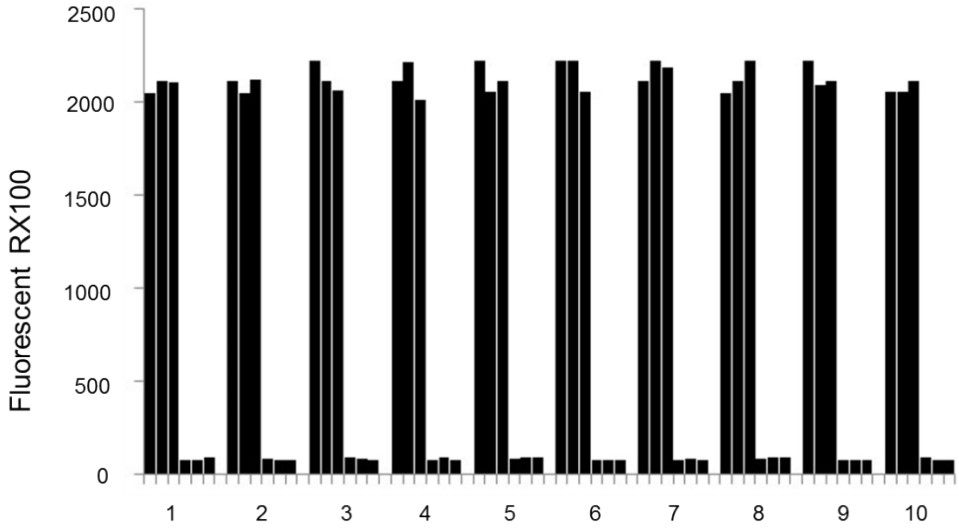


Figure S5. Measurement and calculation of Z' factor. Z' factor was measured to be 0.78 by using *E. coli* cells expressing SN-C12S fusion protein as the negative controls while the expressed cells treated with 400 μM MDSA as the positive controls. (1×10^5 cells/mL in 20mM HEPES, pH 7.5, 37°C, 30min).

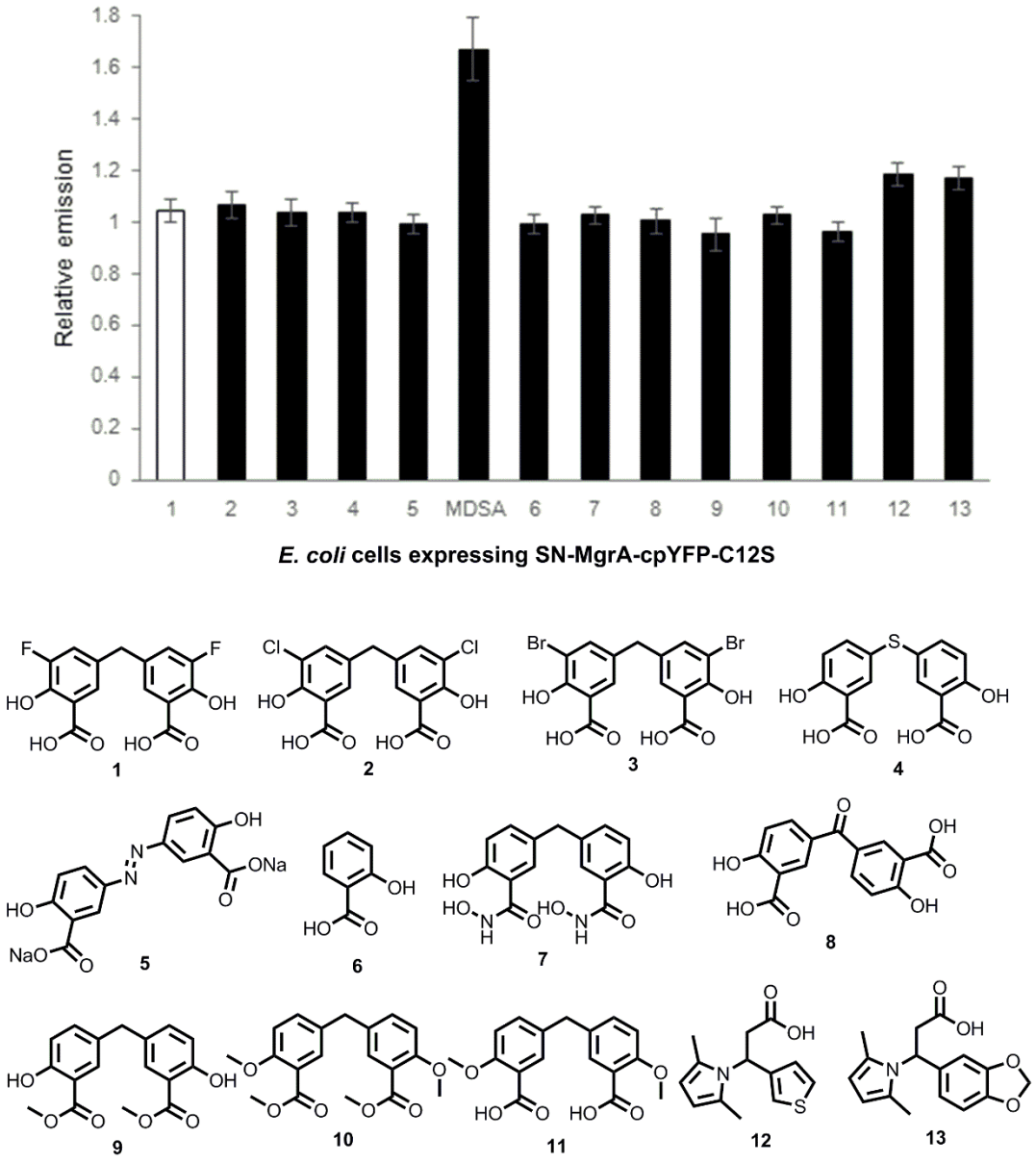


Figure S6. *E. coli* cells expressing SN-MgrA-cpYFP-C12S fusion protein were treated with 200 μM MDSA, its derivatives **1**-**11** and compound **12**, **13**. In our former study, compound **12** and **13** showed 32 μM and 30 μM inhibitory activity respectively. (1×10^5 cells/mL in 20mM HEPES, pH 7.5, 37°C, 30min)

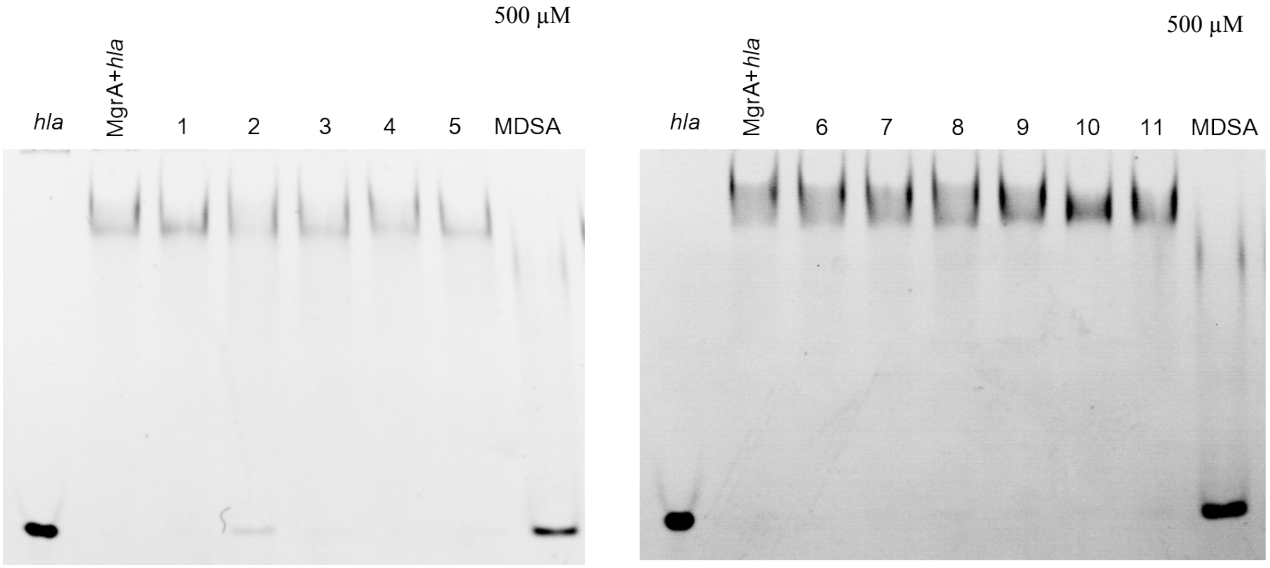


Figure S7. EMSA confirmed that all MDSA derivatives **1**-**11** could not induce dissociation of MgrA from its cognate DNA, with the exception of compound **2**. But the binding of Compound **2** to MgrA was very weak. The *hla* promoter region was used as DNA probe. MgrA protein (final concentration 2.5 μM) were incubated with *hla* promoter (final concentration 0.1 μM) at RT for 20 min in 20 μL of the binding buffer [10 mM Tris-HCl, pH 7.4, 50 mM KCl, 5 mM $MgCl_2$, 10% (wt/vol) glycerol]. It was then treated with several compounds (500 μM) for 30min and separated by 6% native polyacrylamide gel electrophoresis. The gel was visualized by SYBR Gold staining.

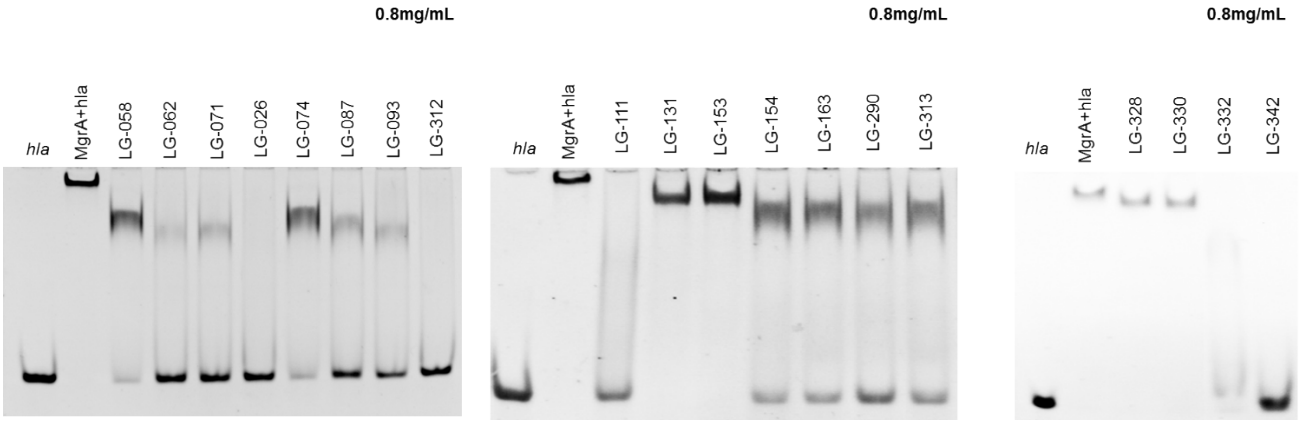


Figure S8. Screening of Chinese herbal extract hits with Electrophoretic Mobility Shift Assays (EMSA). EMSA confirms that most hits induce dissociation of MgrA from its cognate DNA except 4 hits.

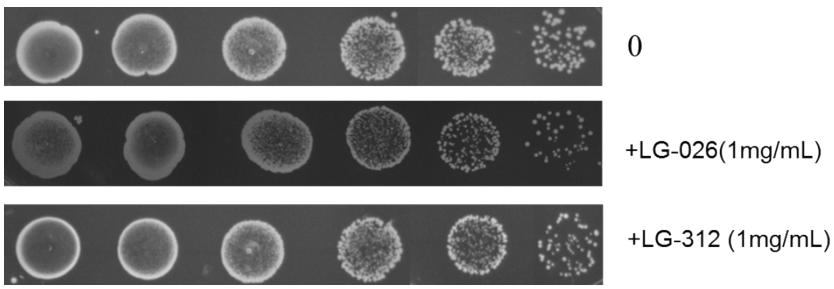


Figure S9. Neither LG-026 nor LG-312 is toxic to *S. aureus*. Mid-log culture (OD_{600}=0.4) was treated with LG-026 (1mg/mL) or LG-312 (1 mg/mL) for 1 hour at 37 °C. The resulting cultures were serially diluted with 9 volumes of PBS and plated on TSA.

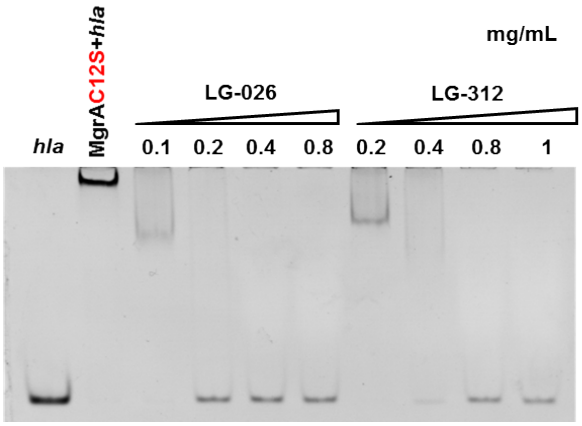


Figure S10. EMSAs Showing LG-026 and LG-312 could dissociate *hla* promoter from MgrA (C12S) protein.

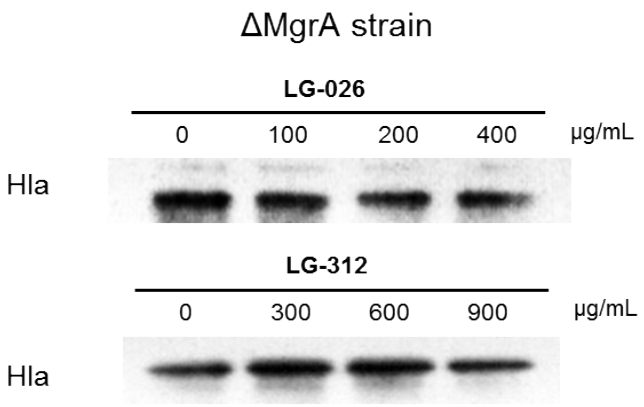


Figure S11. Western blot results showed that LG-026 and LG-312 showed no significant influence on expression of α-Hemolysin in *mgra-deleted* strain (ΔMgrA strain).

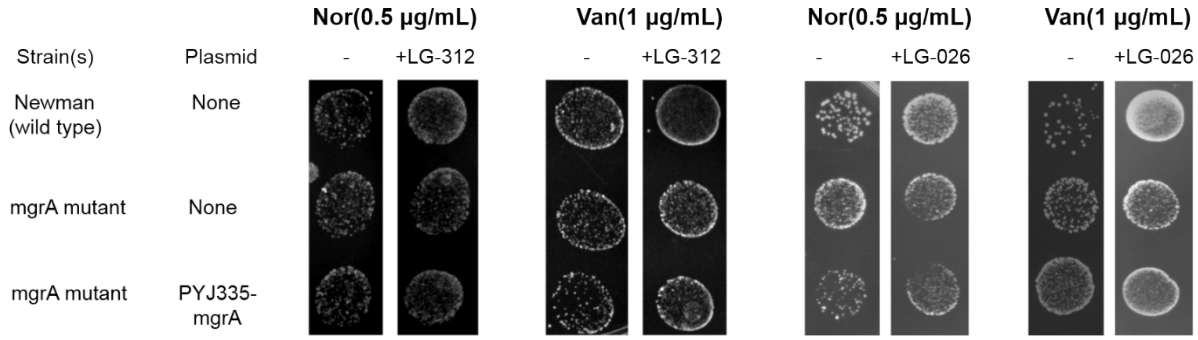


Figure S12. Antibiotic resistance plate assay of LG-026 and LG-312 towards Norfloxacin and Vancomycin. Mid-log culture (OD_{600}=0.4) was treated with LG-026 (1mg/mL) or LG-312 (1 mg/mL) for 1 hour at 37 °C. The resulting cultures were serially diluted with 9 volumes of PBS and plated on TSA.

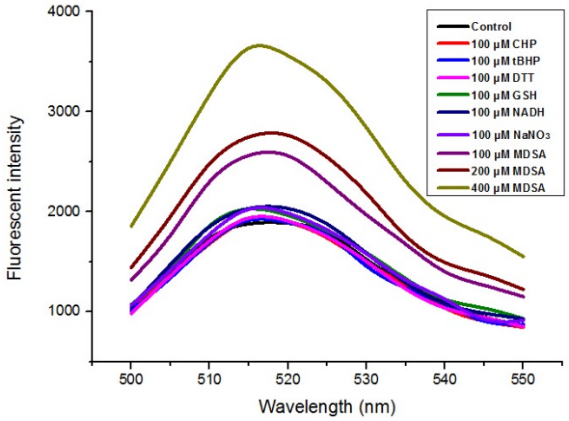


Figure S13. MgrA-cpYFP-C12S exhibited high selectivity towards various small molecules. The fluorescent intensities of *E. coli* cells expressing SN-MgrA-cpYFP-C12S towards different small molecules with the excitation at set 470 nm. (1×10^5 cells/mL in 20mM HEPES, pH 7.5, 37°C, 30min).

Supporting Tables

Table S1. Overview of selected Chinese herb extracts

Number	Latin names of Chinese herbs and corresponding fractions[a]	Fluorescent ratio	Gel shift (0.8 mg/mL)	Growth curve (1mg/mL)
LG-026	the barks of *Cinnamomum cassia* Presl (fraction IV)	1.9	√	√
LG-058	the leaves of *Garciniaoblongifolia* Champ. ex Benth(fraction III)	1.8	√	√
LG-062	the twigs of *Garciniaesculenta* Y. H. Li (fraction IIC)[b]	2.7	√	×
LG-071	the twigs of *Garciniaesculenta* Y. H. Li (fraction II)	3.1	√	√
LG-074	the twigs of *Garciniaesculenta* Y. H. Li (fraction III)	3.2	√	×
LG-087	the pericarps of *Garciniayunnanensis* Hu (fraction II20)[c]	2.0	√	√
LG-093	the barks of *Cinnamomum cassia* Presl (fraction III)	1.9	√	√
LG-111	the roots and rhizomes of *Salvia miltiorrhiza*Bunge (fraction IV)	2.3	√	√
LG-131	the pericarps of *Garciniayunnanensis* Hu (fraction II15)[d]	2.0	×	√
LG-153	the rhizomes of *Polygonatumsibiricum* Red. (fraction IV)	2.0	×	√
LG-154	the spikes of *Prunella vulgaris* L. (fraction III)	1.8	√	√
LG-163	the rhizomes of *Dryopteriscrassirhizoma* Nakai (fraction IV)	2.0	√	√
LG-290	the barks of *Eucommiaulmoides* Oliv. (fraction III)	2.1	√	√
LG-312	the whole plant of *Sargassumpallidum* (Turn) C. Ag (fraction II)	2.0	√	√
LG-313	the rhizomes of *Dryopteriscrassirhizoma* Nakai (fraction III)	1.9	√	√
LG-328	the roots of *Acacia catechu* (L.f.) Willd. (fraction II)	1.9	×	√
LG-330	the roots and rhizomes of *Notopterygiumincisum* Ting ex H. (fraction IV)	2.0	×	√
LG-332	the rhizomes of *Ligusticumchuanxiong* Hort. (fraction III)	1.8	√	√
LG-342	the young twigs with leaves of *Uncariagambier* Roxb.(fraction IV)	2.8	√	√

[a]. Detailed extract procedure see Experimental Section (Preparation of herbal extracts.)

[b]. Fraction II was subjected to column chromatography on MCI GEL CHP 20P, eluted with 30%, 60%, 90%, 100% EtOH, and EtOAc, successively, to obtain subfractions IIA−IIE, respectively.

[c,d]. Fraction II was evaporated in vacuum to give a residue (345 g), part of which (50 g) was subjected to silica gel column eluted with a gradient CH_2Cl_2/CH_3OH system (100:0 to 1:1, v/v) to yield 20 fractions (fractions II1-II20).

Table S2. Bacterial strains and plasmids used in this study

Strains/plasmids	Relevant Characteristics	Source
E.coli		
DH10B		Laboratory stock
BL21 (DE3)		Laboratory stock
BL21 star(DE3)		Laboratory stock
S.aureus		
Newman	Wild-type, human clinical isolate	[2]
NewmanΔmgrA	insertion in mgrA (= ΦNΞ-01594), Em^r	Laboratory stock
Plasmids		
PYJ335	Tet-inducible promoter; Ap^r in *E. coli*; Cam^r in *S. aureus*	Laboratory stock
PYJ335-*mgrA*	pYJ335 carrying *mgrA* gene	Laboratory stock
pET28a	T7 lac promoter-operator, N-terminal His tag, Kan^r	Novagen
pET28a::*mgrA*	pET28a derivative carrying *mgrA*	This study
pET28a::*vt-mgrA-cpYFP*	pET28a carrying *mgrA* gene fused with *cpYFP* between 59V and 60T	This study
pET28a::*sp-mgrA-cpYFP*	pET28a carrying *mgrA* gene fused with *cpYFP* between 71S and 72P	This study
pET28a::*sn-mgrA-cpYFP*	pET28a carrying *mgrA* gene fused with *cpYFP* between 110S and 111N	This study
pET28a::*dk-mgrA-cpYFP*	pET28a carrying *mgrA* gene fused with *cpYFP* between 114D and 115K	This study
pET28a::*sa-mgrA-cpYFP*	pET28a carrying *mgrA* gene fused with *cpYFP* between 118S and 119A	This study

Em^r: resistant to erythromycin; Cam^r, resistant to chloramphenicol.

Table S3. Primers used in this study

Primers	Sequence (5'-3')	Application
MgrA-NdeI-F	GTGTACACATATGATGTCTGATCAACATAATTTA	Protein cloning
MgrA-XhoI-R	GTAGGCACTCGAGTTATTTTTCCTTTGTTTCATC	Protein cloning
cpYFP-F	GCAGGTTCCGCCGGCTACAACAGCGACAA	Protein cloning
cpYFP-R	GACGGCACCGGTGCCGTTGTACTCCAGCTT	Protein cloning
VT-F	GGCACCGGTGCCGTCACTGAATTAGCACTCGAT	Protein cloning
VT-R	GCCGGCGGAACCTGCTGATACTGTACCAGTATCGAGT	Protein cloning
SP-F	GGCACCGGTGCCGTCCCATTATTAAAACGAATG	Protein cloning
SP-R	GCCGGCGGAACCTGCTGATACTGTACCAGTATCGAGT	Protein cloning
SN-F	GGCACCGGTGCCGTCAATGCATCTGACAAAGT	Protein cloning
SN-R	GCCGGCGGAACCTGCACTTAATTCTGGTCTA	Protein cloning
DK-F	GGCACCGGTGCCGTCAAAGTCGCTTCAGCTT	Protein cloning
DK-R	GCCGGCGGAACCTGCGTCAGATGCATTACTTA	Protein cloning
SA-F	GGCACCGGTGCCGTCTCAGCTTCTTCTTTATCTCAAG	Protein cloning
SA-R	GCCGGCGGAACCTGCAGCGACTTTGTCAGATGCATTA	Protein cloning
MgrAC12S –F	AATTTAAAAGAACAGCTAAGCTTTAGTTTG	Mutagenesis
MgrAC12S –R	TTAGCTGTTCTTTTAAATTATGTTGATCAGAC	Mutagenesis
hla-P-F	GTGTACAAACGAAAAAGTATC GTATGTATTTTTAATATAG	EMSA
hla-P-R	TCCGGTACCTACGAGTTTCATTAACGTCACA	EMSA
16s-rt-F	CCATAAAGTTGTTCTCAGTT	RT-PCR
16s-rt-R	CATGTCGATCTACGATTACT	RT-PCR
hla-rt-F	TATTAGAACGAAAGGTACCA	RT-PCR
hla-rt-R	ACTGTACCTTAAAGGCTGAA	RT-PCR

spa-rt-F	TATGCCTAACTTAAATGCTG	RT-PCR
spa-rt-R	TATGCCTAACTTAAATGCTG	RT-PCR

Experimental Section

Unless stated otherwise, all chemicals were purchased from Sigma-Aldrich, and the restriction enzymes were purchased from Takara, and T4 ligase was purchased from New England Biolabs. All Chinese herbal extracts were dissolved in DMSO for biochemical and biological assays. 5,5'-thioldisalicylic acid(**4**) was purchased from TCI Chemicals. Olsalazine sodium salt(**5**) was purchased from J&K Chemical.Co. 3-(2,5-dimethyl-1h-pyrrol-1-yl)-3-(3-thienyl) propanoic acid(**12**), 3-(1,3-benzodioxol-5-yl)-3-(2,5-dimethy-1h-pyrrol-1-yl) propanoic acid (**13**) was purchased from J&K Chemical. Co. **5**, 9, 10, 11 were synthesized as our previous study.[3] NMR spectra were recorded at room temperature with a Bruker Avance instrument operating at a frequency of 400 MHz and 300MHZ.

Bacterial strains, plasmids, and culture conditions. The bacterial strains and plasmids used in this study are listed in Table S2. All *S. aureus strains* were grown in tryptic soy broth (TSB) supplemented with 5 μg/mL nalidixic acid for Newman, 10μg/mL erythromycin for *mgrA* transposon mutants. *E. Coli* cells were grown in LB. *E. coli* strains DH10B, BL21 (DE3) and BL21 star (DE3) (Laboratory stock) were used for DNA manipulation and protein expression, respectively. Vector pET28a (Novagen) was used for cloning in *E. coli*.

Construction, expression and purification of MgrA and MgrA (C12S) protein. The expression and purification of MgrA have been described previously.[4] Briefly, BL21 star (DE3) competent cells expressed MgrA were grown at 37 °C for 2.5 hr (OD_{600}, 0.6), then the cells were induced with 0.5 mM of isopropyl-β-D-thiogalactopyranoside (IPTG) for 4 hr at 30 °C. Cells were harvested and stored at -80 °C until use. The purification of MgrA and MgrA(C12S) used a Ni-NTA column as the user manual described. For the pET28a::*mgrAC12S* plasmid, pET28a::*mgrA* was used as the template for site-directed mutagenesis.

Construction of MgrA-cpYFP. The gene encoding MgrA was cloned into the pET28a vector (Novagen). To produce a fluorescent probe for screening, the cpYFP-coding sequence was inserted into the conformational sensitive region (between residues 58-59; 71-72; 110-111; 114-115; 117–118) of MgrA-coding sequence. The chimeric protein was obtained with the following composition: MgrA(N')-Ser-Ala-Gly–cpYFP–Gly-Thr-Gly–MgrA(C'). Ser-Ala-Gly and Gly-Thr-Gly are short amino acid linkers between the cpYFP and MgrA fragments. A modified version of the Polymerase Incomplete Primer Extension (PIPE) cloning method was used to construct the gene of MgrA-cpYFP.[5] The primers used were shown in Table S3. The cpYFP gene insert was amplified using cpYFP-F and cpYFP-R primer pair, which included extensions complementary to the desired vector cloning site. pET28a-MgrA plasmid was amplified using VT, SP, SN, DK and SA primer pair. The two distinct complementary regions between primers were where annealing occurs. Insert PCR reaction was treated as follows: Initial denaturation for 2 min at 95 °C, then 30 cycles of 94 °C for 30 s, 57 °C for 30 s, and 72 °C for 75 s followed by a cool down to 4 °C. MgrA plasmid PCR reaction was treated as follows: Initial denaturation for 4 min at 95 °C,

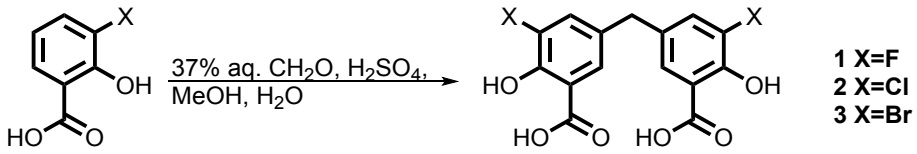
then 30 cycles of 94 °C for 30 s, 56 °C for 30 s, and 68 °C for 13 min followed by a cool down to 4 °C. Both products were purified and mixed 1:1 (v/v). Immediately after mixing, a 5 μL aliquot of the mixture products was incubated on ice for 30 min then were transformed into 50 μL DH10B competent cells (Invitrogen) for 30 min. Cells were transformed by heat shock at 42 °C for 90 s, cultured at 37 °C for 60 min, then plated on selective media. The transformants were sequenced. To improve affinity and specificity of the sensors, sited directed mutagenesis of SN-MgrA-cpYFP sensor required introduction of targeting residues involved in the redox switch (C12S) using the primer pairs P3 and P4. The sequences of all DNA oligonucleotide primers are listed in Table S3.

Preparation of herbal extracts. 351 herbal extracts used in this study were from a library prepared from Chinese herbs. The general operation procedure was as follows: Air-dried and powdered herbs (500 g) were extracted with petroleum ether (3 × 2.5 L, each 1 day). The combined extracts were evaporated to dryness under vacuum to produce the petroleum ether-soluble part (fraction I). The remaining materials were refluxed with 80% EtOH (v/v, 3 × 2.5 L). The combined extracts were evaporated to dryness under vacuum, and the residue was suspended in H_2O and extracted with EtOAc (the same volume as H_2O) to obtain fractions II (the EtOAc-soluble part) and III (the remaining H_2O portion), respectively. The remaining materials were refluxed with distilled water (3 × 2.5 L) to afford the H_2O-soluble part (fraction IV). Extracts were first filtered using Whatman No. 1 filter papers, filtrates were evaporated to dryness at RT in a steady air current. All dried crude extracts were made from one lot of each herb and were stored at − 20 °C until required for testing. The extracts were dissolved in 50% dimethyl sulfoxide (DMSO) before use. 50% DMSO served as negative controls.

Screen of MgrA inhibitors. Cells of *E. coli* (BL21star (DE3)) carrying the pET28a-MgrA-cpYFP expression plasmid were grown overnight and then diluted 1:100 in LB medium (50 μg/mL kanamycin) at 37 °C until the cultures reached about 0.6-0.8 OD_{600}. These probes were induced by addition of 0.1 mM IPTG and grew at 16 °C for overnight. Bacteria were then centrifuged at 4000 g for 5 min at 4 °C. The cell pellets were re-suspended with HEPES buffer (20 mM, pH 7.5) to 1×10^5 cells/mL. Each Chinese herbal extract was seed in the 96-well microplate then we added 100 μL SN-MgrA-cpYFP (C12S) expressing *E. coli* cells. The final concentration of each of the extracts was 500 μg/mL. After incubation for 30 min at 37 °C, the fluorescence data was recorded on a Perkin-Elmer plate reader (excitation: 485 nm, emission: 520 nm).

Fluorescent measurements. The fluorescent measurements were performed on Synergy H1 hybrid reader. The samples were excited at 470 nm and the emission spectrum was scanned from 500 nm to 550 nm with the emission slit width at 5 nm. The SN-MgrA-cpYFP (C12S) expressed by *E.coli* cells (1×10^5 cells/mL) were incubated with various compounds in the HEPES buffer (20 mM, pH 7.5) for 30 min at 37 °C before data collection.

Synthesis of compound 1~3, **7**, **8**.[6] Compound **1~3** were synthesized from 3-fluoro, 3-chloro- and 3-bromo- substituted salicylic acid.



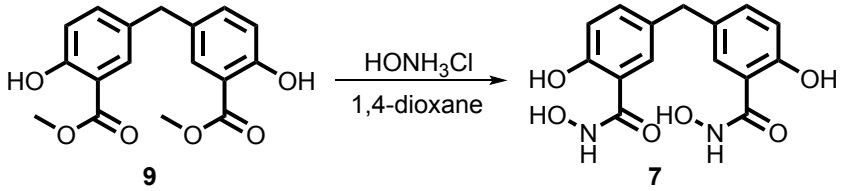
1 X=F
2 X=Cl
3 X=Br

3-Chlorosalicylic acid (97 mg, 0.56 mmol) was added in a 5mL round-bottomed flask. The solid was dissolved in methanol (0.5 mL), water (75 μL) was added, and the mixture was stirred with slow addition of sulfuric acid (1.0 mL) in ice bath. Then the reaction mixture was stirred in an ice bath for 1 h. An aqueous solution of 37% formaldehyde (220 μL) was added. The mixture was stirred in ice bath for 4 h and left overnight at room temperature. It was poured onto ice (5 g), and the precipitate was filtered, washed with water, and then dried to form a solid compound. Product **2** was recrystallized from chloroformmethanol (2:l). Produce **1** and **3** were synthesized with the same procedure.

1 1H NMR (300 MHz, $CDCl_3$) δ 7.64 (dd, J = 8.7, 2.1 Hz, 4H), 3.86 (s, 2H). 13C NMR (75 MHz, CDCl3) δ 176.74 (s), 160.42 (s), 140.82 (s), 137.60 (s), 134.00 (s). ESIMS m/z 341.0743 (MNa^+).

2 1H NMR (300 MHz, Acetone) δ 11.55 (s, 2H), 7.78 (d, J = 2.2 Hz, 2H), 7.62 (d, J = 2.2 Hz, 2H), 3.99 (s, 2H).

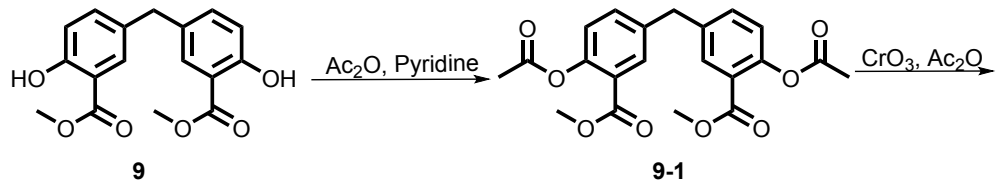
3 1H NMR (300 MHz, DMSO) δ 7.77 (d, J = 2.0 Hz, 2H), 7.70 (d, J = 2.1 Hz, 2H), 3.87 (s, 2H).



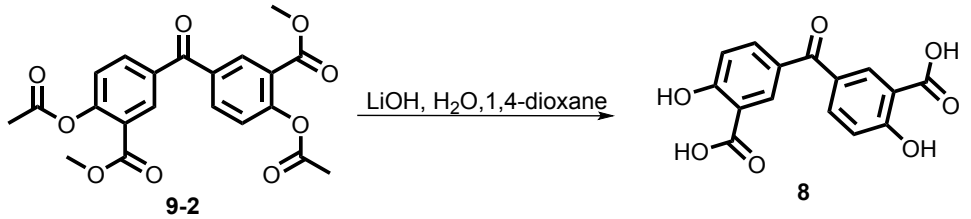
9 **7**

The MDSA methyl ester **9** was dissolved in 1,4-dioxane, then 5.0 equivalence of Hydroxylamine hydrochloride was added to the mixture in an ice bath. The mixture was stirred overnight at room temperature, The solvent was evaporated and the solid residue was purified to obtain the product **7** as white solid.

1H NMR (400 MHz, DMSO) δ 11.94 (s, 2H), 11.33 (s, 2H), 9.27 (s, 2H), 7.55 (s, 2H), 7.19 (dd, J = 8.4, 2.1 Hz, 2H), 6.81 (d, J = 8.4 Hz, 2H), 3.73 (s, 2H). 13C NMR (101 MHz, DMSO) δ 166.66 (s), 157.95 (s), 134.16 (s), 132.18 (s), 127.68 (s), 117.87 (s), 114.72 (s). ESIMS m/z 341.0743 (MNa^+).



9 **9-1**



9-2 **8**

MDSA methyl ester **9** was dissolved in pyridine. Then, 2.5 equivalence of acetic anhydride was added in drops. The mixture was stirred at room temperature for 5 hours. The mixture was diluted with excess of ethyl acetate and washed with brine and saturated sodium bicarbonate. The organic phase was collected and evaporated. The residue was purified with flash chromatography to obtain **9-1**.

9-1 was dissolved in acetic anhydride with stirring. 4 equivalence of chromic acid was added one portion at a time and the mixture was stirred continuously for 12 hours. The reaction mixture was filtered through a pad of celite, then wash the celite with ethyl acetate. The filtrate and washes was combined, and the solvent was evaporated. The crude product was purified with flash chromatography to obtain **9-2** as white solid.

9-2 was dissolved in water/1,4-dioxne. 4 equivalence of LiOH was added with the mixture in an ice bath. The mixture was stirred for 12 hours at room temperature and the organic solvent was removed by a rotary evaporator. The pH was adjusted to 2 using 1N HCl. White solid was precipitated and washed with water to obtain **8**.

9-1 1H NMR (400 MHz, CDCl3) δ 7.85 (d, J = 2.2 Hz, 2H), 7.35 (dd, J = 8.3, 2.3 Hz, 2H), 7.04 (d, J = 8.3 Hz, 2H), 4.03 (s, 2H), 3.86 (d, J = 2.3 Hz, 6H), 2.34 (d, J = 2.2 Hz, 6H). 13C NMR (101 MHz, CDCl3) δ 169.98 (s), 164.98 (s), 149.38 (s), 138.18 (s), 134.43 (s), 132.18 (s), 124.22 (s), 123.30 (s), 52.40 (s), 40.46 (s), 21.13 (s). ESIMS m/z 423.1050 (MNa^+).

9-2 1H NMR (400 MHz, CDCl3) δ 8.45 (d, J = 2.2 Hz, 2H), 8.00 (dd, J = 8.4, 2.2 Hz, 2H), 7.28 – 7.24 (m, 2H), 3.89 (s, 6H), 2.39 (s, 6H). 13C NMR (101 MHz, CDCl3) δ 192.78 (s), 169.43 (s), 164.14 (s), 154.17 (s), 135.25 (s), 134.85 (s), 133.61 (s), 124.58 (s), 123.75 (s), 52.67 (s), 21.14 (s). ESIMS m/z 437.0843 (MNa^+).

8 1H NMR (400 MHz, DMSO) δ 8.15 (d, J = 1.9 Hz, 2H), 7.90 (dd, J = 8.6, 1.9 Hz, 2H), 7.11 (d, J = 8.6 Hz, 2H). 13C NMR (101 MHz, DMSO) δ 192.38 (s), 171.71 (s), 164.88 (s), 137.13 (s), 133.28 (s), 128.84 (s), 118.21 (s), 113.75 (s). ESIMS m/z 325.0358 (MNa^+).

Electrophoretic Mobility Shift Assays (EMSA). A 50 bp *hla* promoter fragment (sequence in Table S3) was used for the MgrA binding assays. Proteins (final concentration 2.5 μM) were incubated with DNA fragments (final concentration 0.1 μM) and various amounts of Chinese herbal extracts at RT for 20 min in 20 μL of the binding buffer [10 mM Tris-HCl, pH 7.4, 50 mM KCl, 5 mM $MgCl_2$, 10% (wt/vol) glycerol]. The samples were subjected to 6% native polyacrylamide gel electrophoresis for separation (120 V for 40 min pre-run, then 150 V for 40 min). The gels were stained with GelRed (Biotium) and imaged with Gel imager (Tanon).

Western blot analysis. For the western blot assay, *S. aureus* Newman strains were grown at 37 °C overnight in TSB containing 5 μg/mL nalidixic acid, then diluted by 100-fold in fresh 5 mL TSB, and incubated at 37 °C with shaking at 250 rpm to OD_{600} = 0.6. Then various amount of LG-026 (0, 100, 200, 400 μg/mL) and LG-312 (300, 600, 900 μg/mL) were added and shaken at 250 rpm for 3 hr. Then added 4 × SDS-PAGE loading buffer. Samples were separated by electrophoresis on 12% SDS-PAGE gel. Proteins were then transferred to a PVDF membrane at 350 mA for 60 min. The membrane was washed with TBST buffer [10 mM Tris-HCl, pH 7.5, 150 mM NaCl, and 0.1 % (wt/vol) Tween 20] followed by the

blocking step [20 mL of 5% (wt/vol) skim milk in TBST buffer] at RT for 2 hr. The membranes were briefly rinsed with 20 mL TBST buffer and then incubated with the antibody solution [1/10,000 dilution of antibody (anti-hemolysin, Sigma) in TBST buffer containing 5% (wt/vol) skim milk] at 4 °C overnight. The membranes were then washed 3 times at RT for 15 min in TBST buffer. Membranes were then incubated with the secondary antibody (Peroxidase-Conjugated AffiniPure Goat Anti-Rabbit IgG from ZSGB-BIO) at RT for 2 hr and washed with TBST for 3 times. The chemiluminescent detection was performed and monitored by ChemiDoc XRS System (Bio-Rad).

Plate Assays for Hemolytic Activity. Hemolytic activity was analyzed on 5% sheep blood agar plates. Overnight Newman strain culture was diluted by 100-fold in fresh 200 μL fresh TSB containing LG-026(0, 1 mg/mL) and LG-312 (1mg/mL), then incubated at 37 °C with shaking at 250 rpm for 3 hr (OD_{600} = 0.8). Then 3 μL of culture was spotted on blood agar plate and grown at 37 °C for 24 hr, then 4 °C for 48 hr. The zones of clearance surrounding bacterial colonies indicated the hemolysis.

Plate sensitivity assay for antibiotic resistance: Various amounts of Chinese herb extracts C (0.2 mg/mL), F (0.2 mg/mL) and antibiotics were used in this assy. Newman strains were grown overnight, then diluted 100 fold in fresh TSB with 1 mg/mL C and F, respectively. After 3 h at 37 °C (OD600, 0.8), the culture was diluted 1000 fold in PBS. Aliquots (10 μL) of the diluted culture were spotted onto TSA plates and grownat 37°C for 24 h.

Growth Curve assay. Wild-type Newman strain and *mgra-deleted* strain were grown overnight in TSB medium and then diluted in 50 ml of TSB medium(1:100). Cultures grown until mid-log phase during 2 hr (OD_{600}, 0.4) were divided and either untreated or treated with Chinese herbal extracts (1mg/mL). Aliquots of all cultures were collected at several time intervals, and the growth of strains was evaluated by measurement of optical density. The data presented are the mean of quadruplicate measurements.

RNA isolation and real-time RT-PCR. Newman strain was cultured in TSB at 37 °C overnight, then diluted 100-fold in fresh 3 mL TSB containing various amount of LG-026 (100, 200, 400 μg/mL) and LG-312 (0, 300, 600, 900 μg/mL) in 15 mL tube and incubated at 37 °C with rotary shaking (250 rpm) for 3 hr (OD_{600} = 0.8). Cells were harvested and the RNA was isolated as previously described.[7] Cells were re-suspended in 800 μL lysis buffer (sodium acetate 2.7 g, SDS 5 g, EDTA 0.34 g per liter, pH 5.5), glass beads (Sigma) were added to break the cell wall through vortex (2000 rpm, 3 min). After centrifugation, the supernatants were collected and mixed with saturated phenol (pH 5.5) and incubated at 68 °C for 5 min. Samples were then centrifuged at 10,000 g for 3 min. The aqueous layer was mixed with chloroform. After centrifugation, the aqueous layer was collected and precipitated with ethanol and sodium acetate. RNA was reverse transcribed into cDNA using the Promega GoScript reverse transcription system as described by the manufacturer. cDNA was stored at -20 °C until needed. The primer used for real time RT-PCR are listed in Table 3. PCR was performed with SYBR Premix Ex TaqTM (TakaRa) in ABI 7500 Real-time PCR system. Each reaction was performed in triplicate in 25 μL reaction volume and the 16S rRNA gene served as an endogenous control.

References

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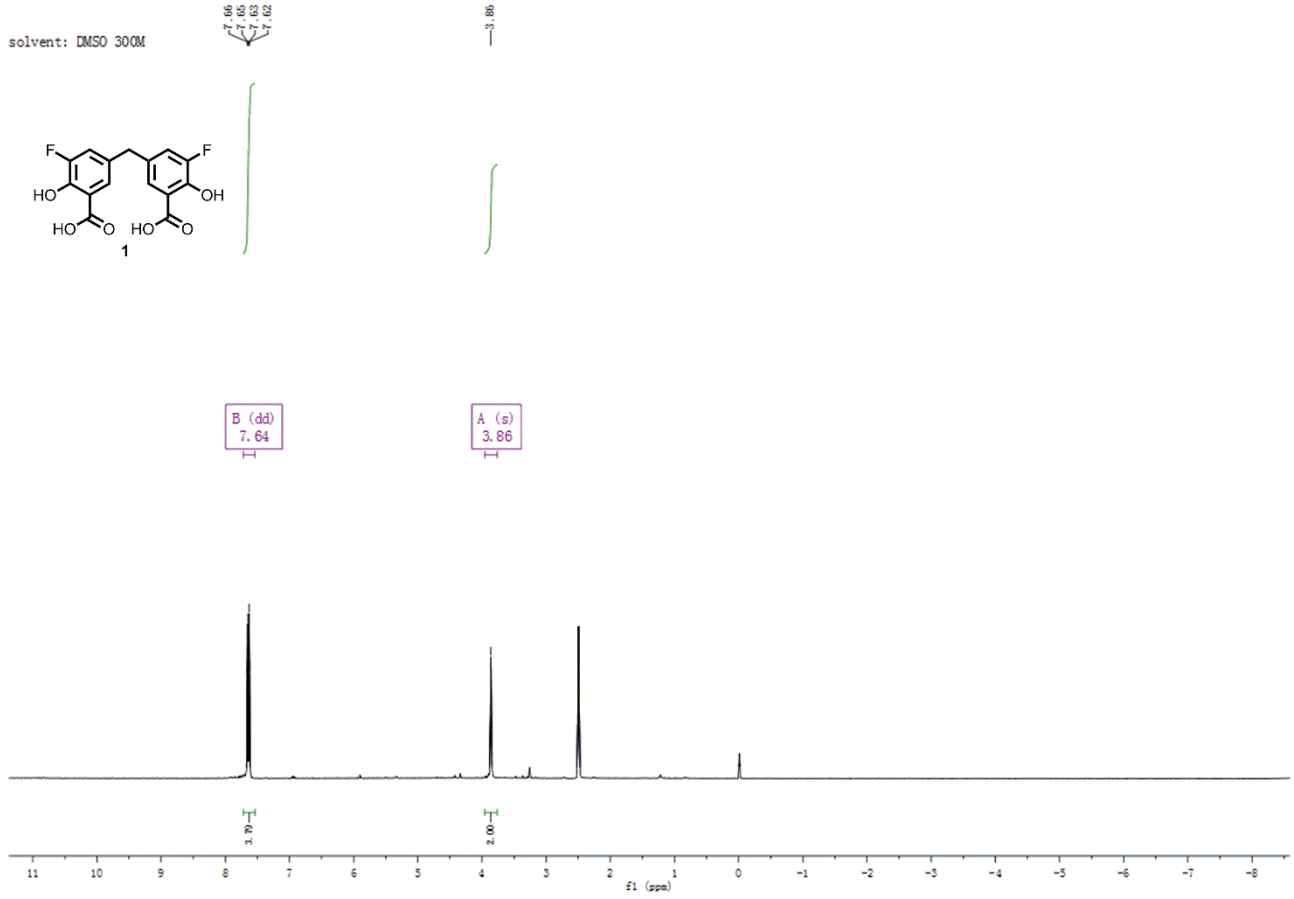
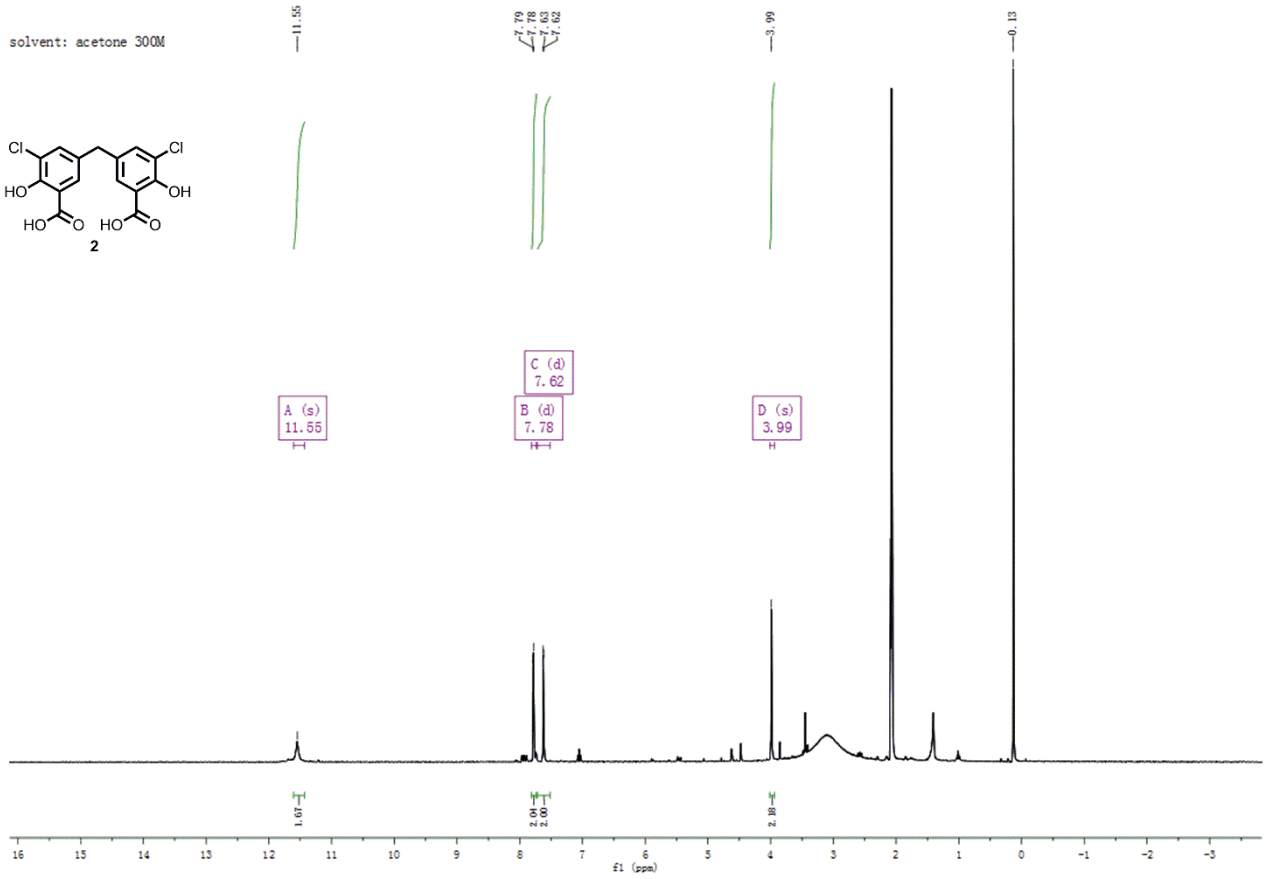
NMR Spectrum

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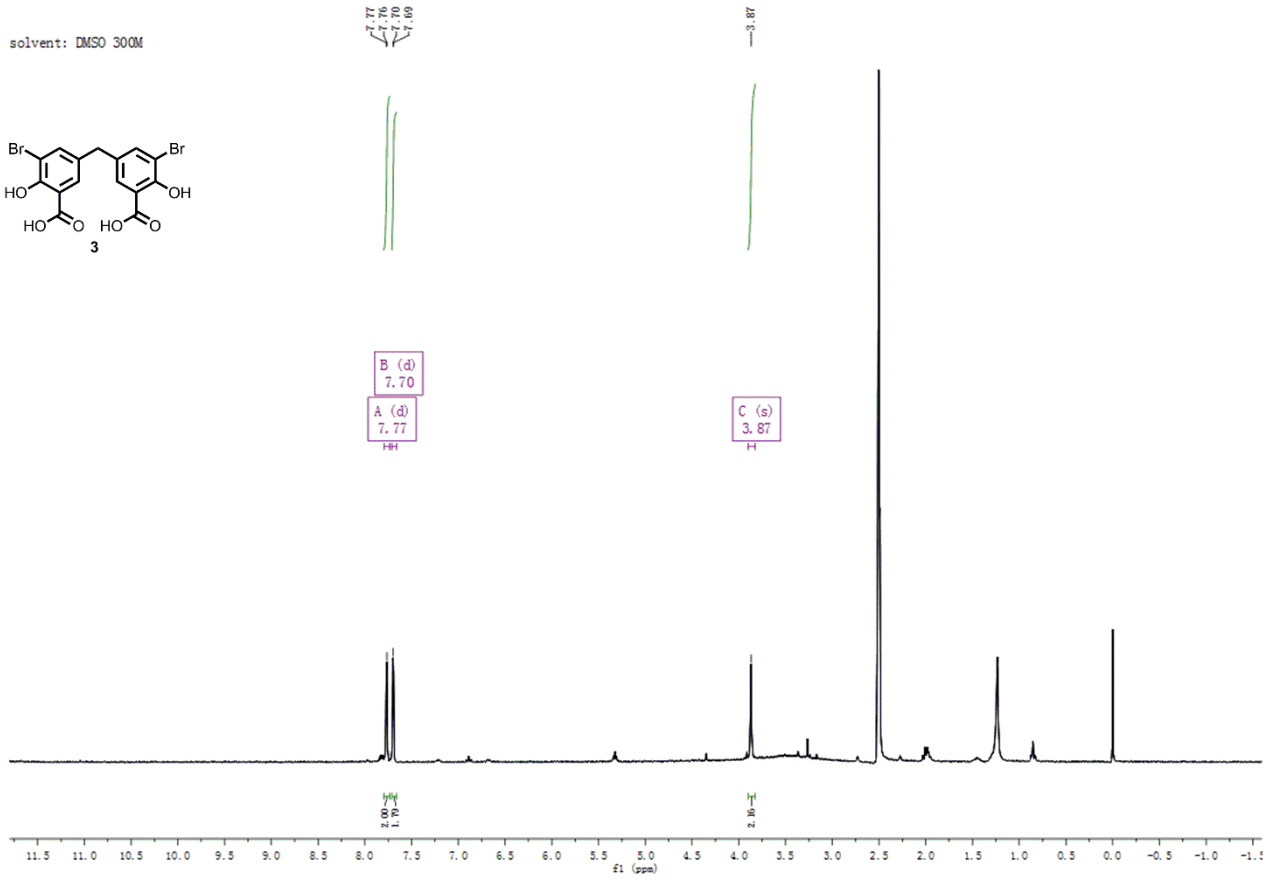
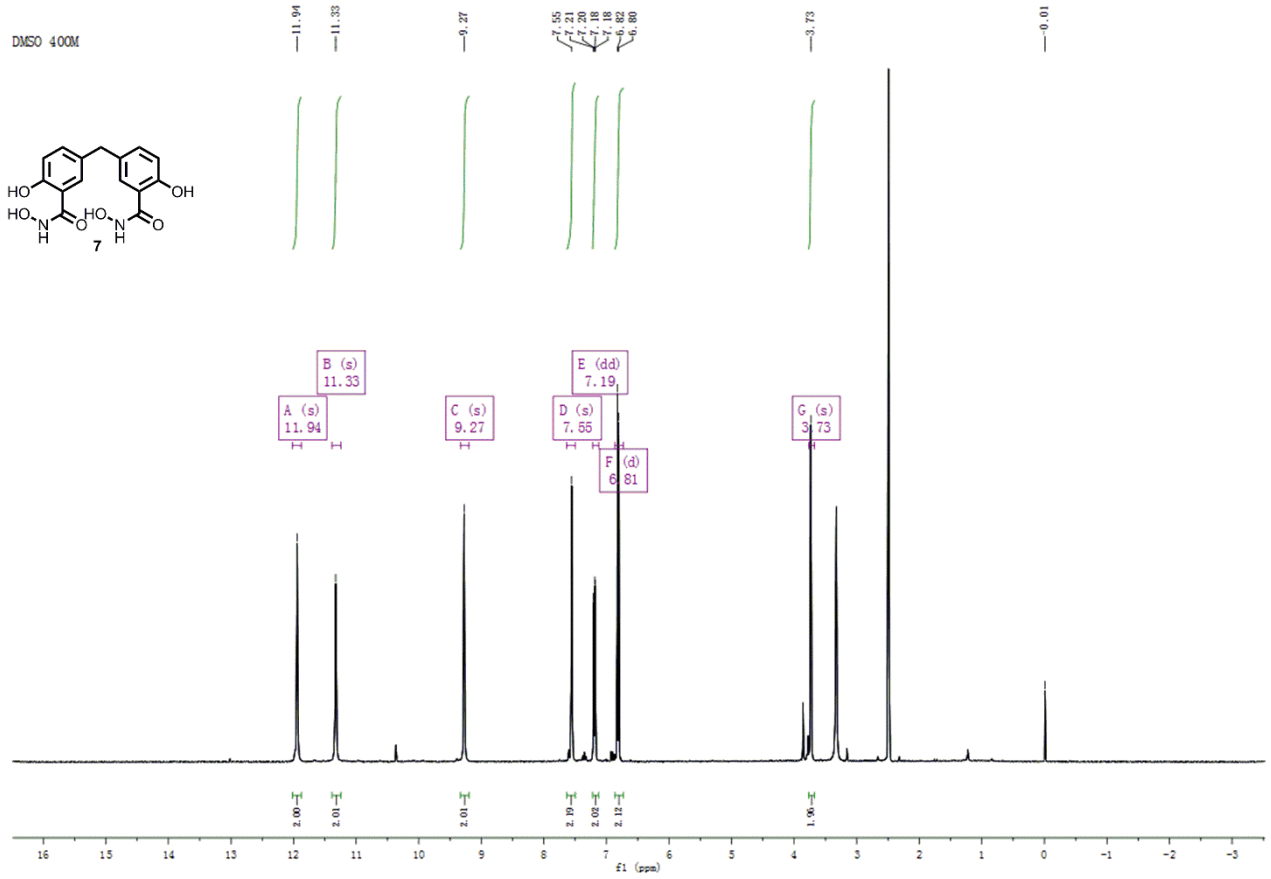
11.55
7.79
7.78
7.63
7.62
3.99
0.13
Cl
Cl
HO
OH
HO
O
HO
O
2
A (s)
11.55
C (d)
7.62
B (d)
7.78
D (s)
3.99
1.67
2.04
2.00
2.88
f1 (ppm)


solvent: DMSO 300M
7.77
7.76
7.70
7.69
3.87
Br
Br
HO
OH
HO
O
HO
O
3
B (d)
7.70
A (d)
7.77
C (s)
3.87
2.00
1.79
2.16
f1 (ppm)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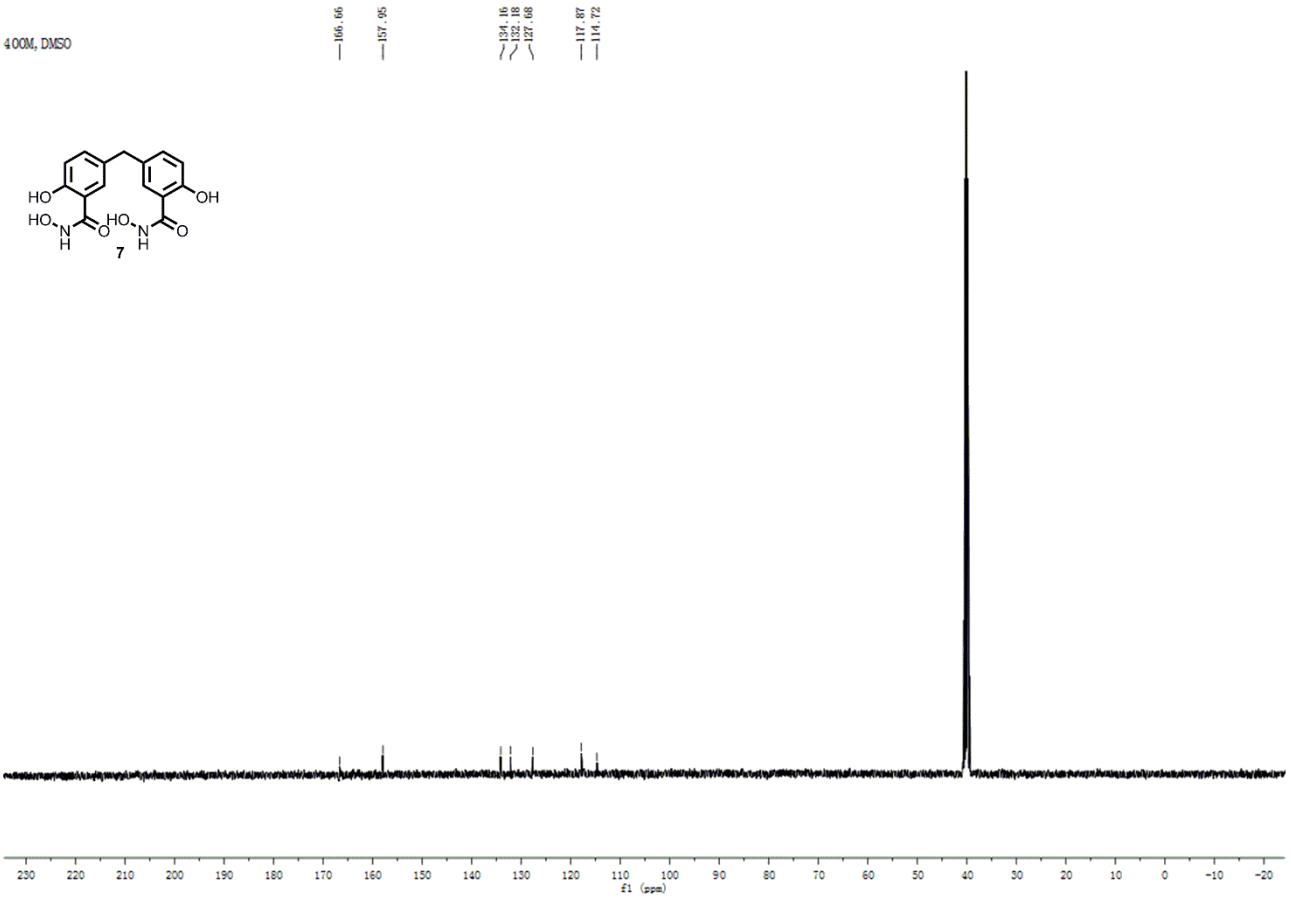
11.33
9.27
7.55
7.21
7.20
7.18
6.82
6.80
3.73
0.01
HO
HO
N
H
O
HO
N
H
O
OH
7
A (s) 11.94
B (s) 11.33
C (s) 9.27
D (s) 7.55
E (dd) 7.19
F (d) 6.81
G (s) 3.73
2.00
2.01
2.01
2.19
2.08
2.12
1.90
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2 −3
f1 (ppm)


400M, DMSO
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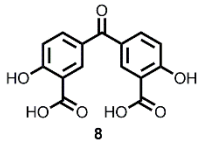
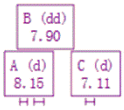
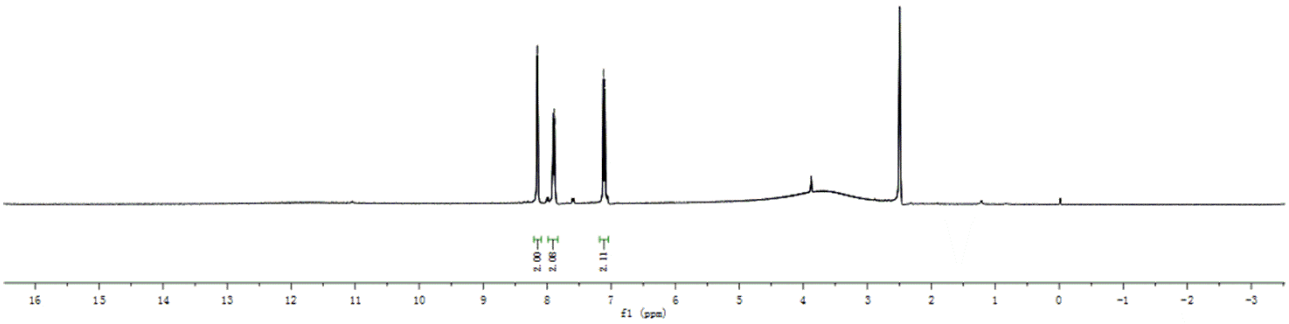
157.95
134.16
132.18
127.68
117.87
114.72
HO
HO
N
H
O
HO
N
H
O
OH
7
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10 −20
f1 (ppm)

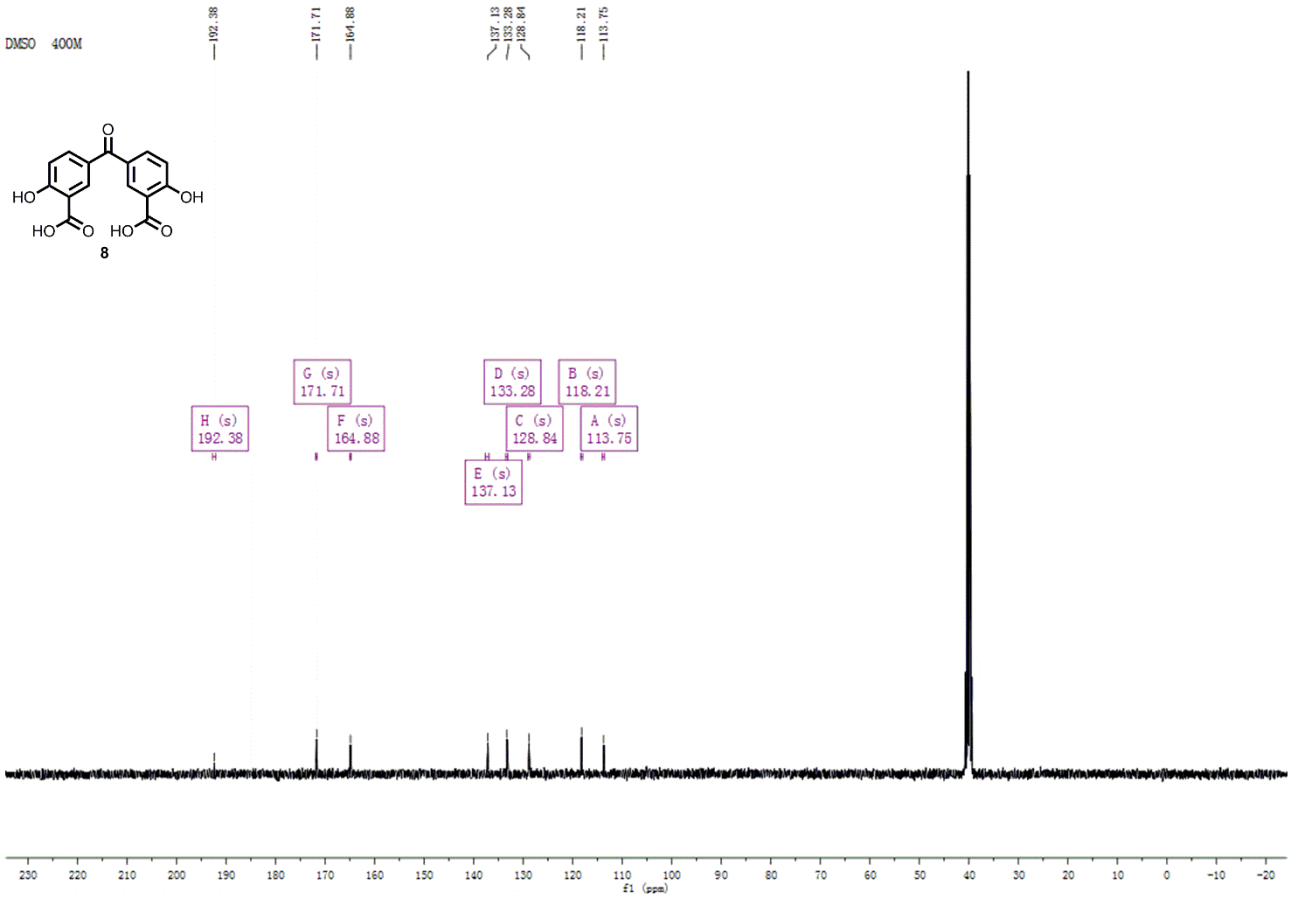
MDSA CO-4 final
MDSA CO-4 final
CDCl3 400M



8



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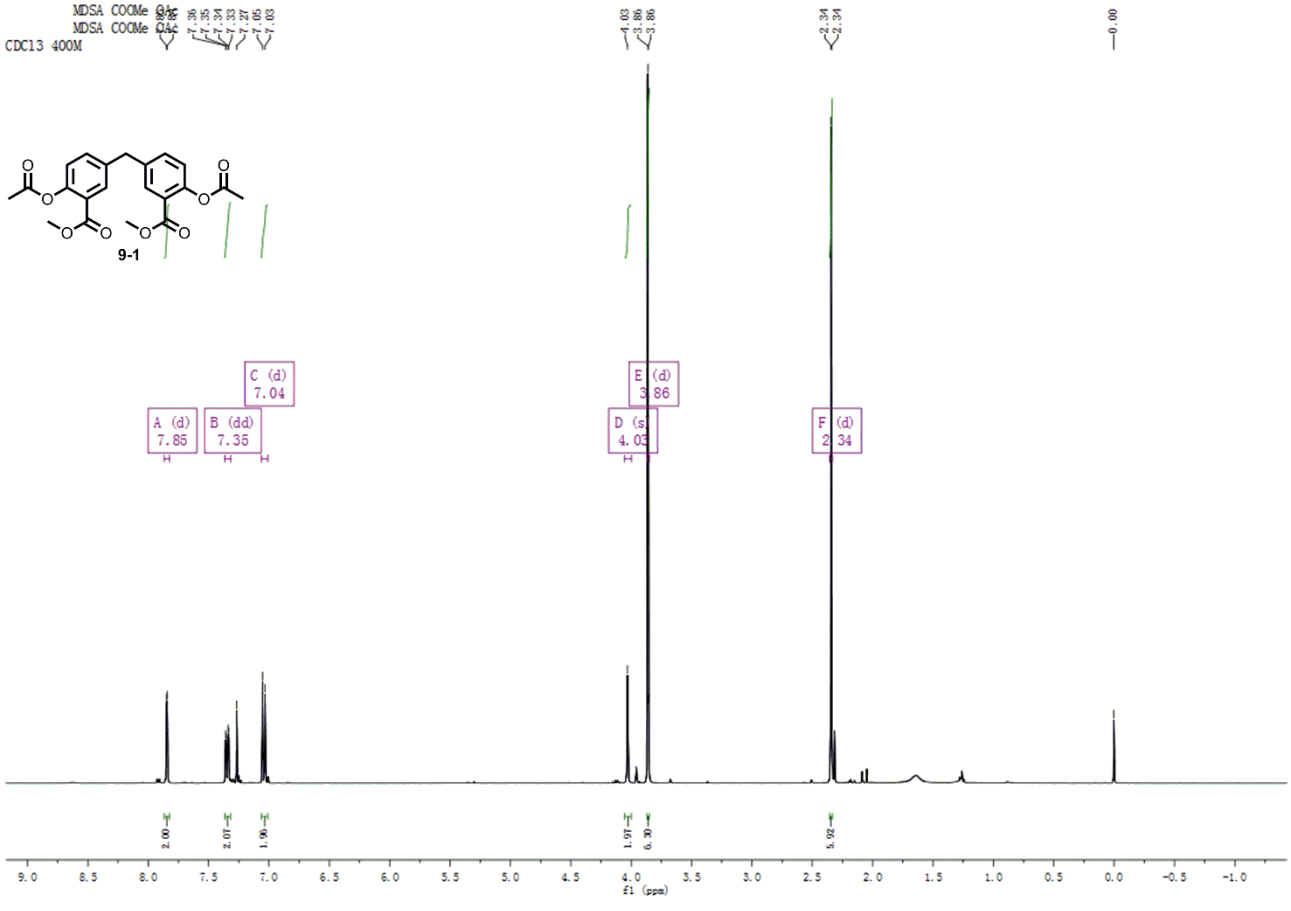
171.71
164.88
137.13
133.28
128.84
118.21
113.75
HO
OH
HO
HO
O
8
G (s)
171.71
D (s)
133.28
B (s)
118.21
H (s)
192.38
F (s)
164.88
C (s)
128.84
A (s)
113.75
E (s)
137.13
f1 (ppm)


CDCl3 400M
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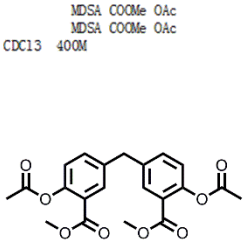
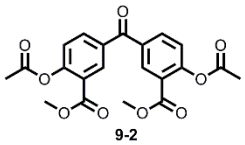
7.36
7.35
7.34
7.33
7.27
7.05
7.03
4.03
3.86
3.86
2.34
2.34
0.00
9-1
C (d)
7.04
A (d)
7.85
B (dd)
7.35
E (d)
3.86
D (s)
4.03
F (d)
2.34
2.00
2.07
1.90
1.97
6.30
5.92
f1 (ppm)

MDSA COOMe OAc
MDSA COOMe OAc
CDCl3 400M

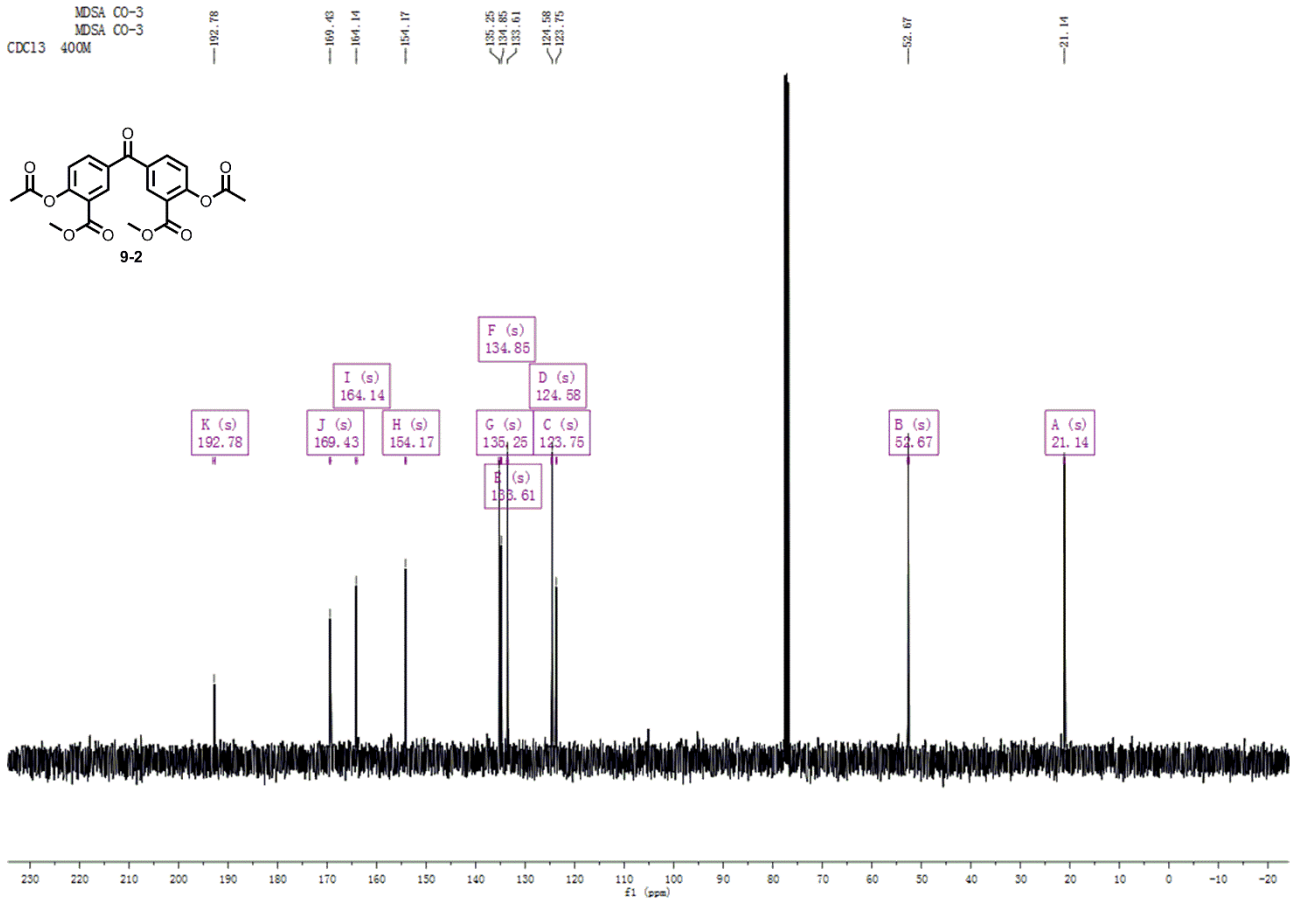


9-1

MDSA CO-3
MDSA CO-3
CDCl3 400M



9-2


MDSA CO-3
MDSA CO-3
CDC13 400M
192.78
169.43
164.14
154.17
135.25
134.85
133.61
124.58
123.75
52.67
21.14
9-2
F (s)
134.85
I (s)
164.14
D (s)
124.58
K (s)
192.78
J (s)
169.43
H (s)
154.17
G (s)
135.25
C (s)
123.75
E (s)
133.61
B (s)
52.67
A (s)
21.14
f1 (ppm)