Supplementary Data

Synthesis and biological evaluation of Schiff base-linked imidazolyl naphthalimides as novel potential anti-MRSA agents

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1. Experimental Protocols

1.1 General Methods

Melting points were recorded on X-6 melting point apparatus and uncorrected. TLC analysis was done using pre-coated silica gel plates. FT-IR spectra were carried out on Bruker RFS100/S spectrophotometer (Bio-Rad, Cambridge, MA, USA) using KBr pellets in the 400–4000 cm^{-1} range. NMR spectra were recorded on a Bruker AV 300 and 600 spectrometer using TMS as an internal standard. The chemical shifts were reported in parts per million (ppm), the coupling constants (*J*) were expressed in hertz (Hz) and signals were described as singlet (s), doublet (d), triplet (t), as well as multiplet (m). The mass spectra were recorded on LCMS-2010A and the high-resolution mass spectra (HRMS) were recorded on an IonSpec FT-ICR mass spectrometer with ESI resource. All fluorescence spectra were recorded on F-7000 Spectrofluorimeter (Hitachi, Tokyo, Japan) equipped with 1.0 cm quartz cells, the widths of both the excitation and emission slit were set as 2.5 nm, and the excitation wavelength was 295 nm. Fluorescence spectra were recorded at room temperature in the range of 250–800 nm. The UV spectrum was recorded at room temperature on a TU-2450 spectrophotometer (Puxi Analytic Instrument Ltd. of Beijing, China) equipped with 1.0 cm quartz cells. All other chemicals and solvents were commercially available, and were used without further purification.

The stock solution of compound **9i** was prepared in DMSO. *Calf thymus* DNA (Sigma Chemical Co., St. Louis, MO) was used without further purification, and its stock solution was prepared by dissolving an appropriate amount of DNA in doubly distilled water. The solution was allowed to stand overnight and store at 4 ^{o}C in the dark for about a week. The concentration of DNA in stock solution was determined by UV absorption at 260 nm using a molar absorption coefficient ξ_{260} = 6600 L mol^{-1} cm^{-1} (expressed as molarity of phosphate

groups) by Bouguer-Lambert-Beer law. The purity of DNA was checked by monitoring the ratio of the absorbance at 260 nm to that at 280 nm. The solution gave a ratio of > 1.8 at A260/A280, which indicates that DNA was sufficiently free from protein. NR stock solution (2.0×10^{-3} mol/L) was prepared by dissolving its solid (Sigma Chemical Co.) in doubly distilled water and was kept in a cool and dark place. All the solutions were adjusted with Tris-HCl buffer solution (pH = 7.4), which was prepared by mixing and diluting Tris solution with HCl solution. All chemicals were of analytical reagent grade, and doubly distilled water was used throughout.

1.2 Biological Assay Procedures

Minimal inhibitory concentration (MIC, μg/mL) is defined as the lowest concentration of the new compounds that completely inhibit the growth of bacteria, by means of standard two folds serial dilution method in 96-well microtest plates according to the National Committee for Clinical Laboratory Standards (NCCLS). The tested microorganism strains were provided by the School of Pharmaceutical Sciences, Southwest University and the College of Pharmacy, Third Military Medical University. Chloromycin, Norfloxacin and Fluconazole were used as control drugs. To ensure that the solvent had no effect on bacterial growth, a control test was performed with test medium supplemented with DMSO at the same dilutions as used in the experiment. All the bacteria and fungi growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimal inhibitory concentration (MIC).

1.2.1. Antibacterial Assays

The prepared compounds **4a-c**, **5**, **8a-e** and **9a-k** were evaluated for their antibacterial activities against *Methicillin-Resistant Staphylococcus aureus* N315, *Staphylococcus aureus* ATCC25923, *Bacillus subtilis* ATCC6633, *Micrococcus luteus* ATCC4698 as Gram-positive bacteria, *Escherichia coli* DH52, *Escherichia coli* JM109, *Shigella dysenteriae*, *Pseudomonas aeruginosa* ATCC27853, *Bacillus proteus* ATCC13315, *Bacillus typhi* as Gram-negative bacteria. The bacterial suspension was adjusted with sterile saline to a concentration of 1×10^5 CFU/mL. The compounds were dissolved in DMSO to prepare the stock solutions. The compounds and reference drugs were prepared in Mueller–Hinton broth (Guangdong huaikai microbial sci. & tech co., Ltd, Guangzhou, Guangdong, China) by twofold serial dilution to obtain the required concentrations. These dilutions were inoculated and incubated at 37 ºC for 24 h. To ensure that the solvent had no effect on bacterial growth, a control test was performed with test medium supplemented with DMSO at the same dilutions as used in the experiment.

1.2.2 Antifungal Assays

The synthesized compounds were also evaluated for their antifungal activities against *Candida albicans* ATCC76615, *Candida mycoderma* ATCC9888, *Candida utilis* ATCC9950, *Aspergillus flavus* ATCC204304 and *Beer yeast* ATCC9763. A spore suspension in sterile distilled water was prepared from 1-day old culture of the fungi growing on Sabouraud agar (SA) media. The final spore concentration was $1–5\times10^3$ spore/mL. From the stock solutions of the tested compounds and reference antifungal Fluconazole, dilutions in sterile RPMI 1640 medium (Neuronbc Laboraton Technology CO., Ltd, Beijing, China) were made resulting in eleven wanted concentrations of each tested compounds. These dilutions were inoculated and incubated at 35 ºC for 24 h. The drug's MIC was defined as the first well with an approximate 80% reduction in growth compared to the growth of the drug-free well.

1.2.3 Bacterial Membrane Permeabilization

The 6 h grown culture (mid log phase) of *B. proteus* and MRSA was harvested (3500 rpm, 5 min), washed, and resuspended in 5 mM glucose and 5 mM HEPES buffer (pH 7.2) in 1:1 ratio. Then an amount of 10 μL of tested compound **9i** (12 × MIC) was added to a cuvette containing 2 mL of bacterial suspension and 10 μM propidium iodide (PI). Fluorescence was monitored at excitation wavelength of 535 nm (slit width of 10 nm) and emission wavelength of 617 nm (slit width of 5 nm). As a measure of inner membrane permeabilization, the uptake of PI was monitored by the increase in fluorescence for 2 h.

1.2.4 Development of Resistance to Compound 9i

Considering the high-level resistance of Norfloxacin to MRSA strains, we selected the representative compound **9i** to investigate the developing rate of bacterial resistance according to the paper "Konai, M. M.; Ghosh, C.; Yarlagadda, V.; Samaddar, S.; Haldar, J. *J. Med. Chem.* **2014**, *57*, 9409". We exposed a standard strain of MRSA towards increasing concentrations of compound **9i** from sub-MIC for sustained passages and determined the MIC values of compound **9i** for each passage of MRSA. The initial MIC value of compound **9i** and Norfloxacin was determined against MRSA as aforementioned antimicrobial assay. For the next MIC experiment, the bacterial dilution was made by using the bacteria from sub-MIC concentration of the compound (0.5 × MIC). After a 12 h incubation period, again bacterial dilution was prepared by using the bacterial suspension from sub-MIC concentration of the compound (0.5 × MIC) and assayed for the next MIC experiment. The process was repeated for 13 passages. The MIC for compound **9i** exhibited no obvious change after 10 passages, whereas that of Norfloxacin was started to dramatically increase after 6 passages and could reach to 100-fold after 10 passages. This assay indicated that bacteria MRSA do not develop resistance against compound **9i** as easily as they do against Norfloxacin.

1.2.5 Time−kill Kinetic of Compound 9i

The 6 h grown culture (mid log phase) of MRSA was harvested (3500 rpm, 5 min), washed, and resuspended in yeast−dextrose broth. Compound **9i** was added to the bacterial solution (MRSA of approximately 1.8×10^6 CFU/mL) with the working concentration of 8 μg/mL (4 × MIC) and the mixture was incubated at 37 °C with shaking. Aliquots (20 μL) were removed from the cultures at 0, 60, 90, 120, 150, 180, 210, 240, 270, 300 min from that solution and were serially diluted 10-fold in 0.9% sodium chloride. The 20 μL of these obtained dilutions were then plated onto sterile yeast−dextrose agar medium. Plates were then incubated at 37 °C for 24 h, CFU was counted and the total bacterial log CFU/mL was determined.

2. The plot of $A^0/(A-A^0)$ versus 1/[compound 9i].

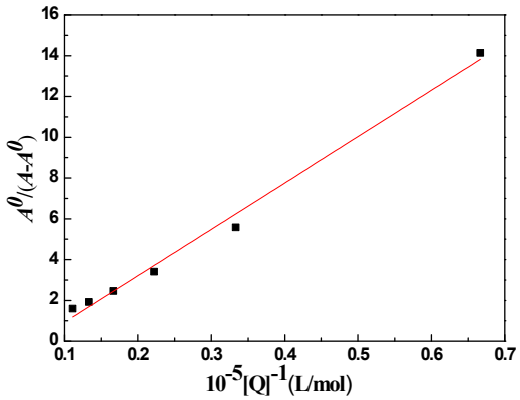


Figure S1. The plot of $A^0/(A-A^0)$ versus 1/[compound **9i**].

3. General Procedure and Spectral data for the Prepared Compounds.

*Synthesis of 2-((2-butyl-4-chloro-1-pentyl-1H-imidazol-5-yl)methyleneamino)-6-morpholino-1H-benzo[de]isoquinoline-1,3(2H)-dione (**8a**)*

Compound **3c** (200 mg, 0.67 mmol) was added to the suspension of compound **6a** (344 mg, 1.34 mmol) and P_2O_5 (2% mmol) in toluene. The mixture was stirred under reflux condition for 12 h under nitrogen atmosphere. After the reaction came to the end (monitored by TLC, eluent, dichloromethane), the solvent was evaporated under reduced pressure, and the residue was treated with water (30 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic phase was dried over anhydrous Na_2SO_4 and then evaporated under reduced pressure. The resulting residue was purified *via* silica gel column chromatography using CH_2Cl_2 as eluent and recrystallized from ethanol to give compound **8a** (121 mg) as yellow powder. Yield: 34%; mp: 159–161 °C; IR (KBr, cm^{-1}) *v*: 3059, 2955, 2849 (C–H), 1701, 1661 (C=O), 1594, 1465 (aromatic frame), 1389, 1348, 1272, 1198 (C–N), 1122,

1023 (C–O), 919, 893, 836, 785, 758, 725, 654, 603; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.64 (d, *J* = 6.2 Hz, 1H, naphthalimide-*H*), 8.61–8.53 (m, 1H, naphthalimide-*H*), 8.46 (d, *J* = 8.2 Hz, 1H, naphthalimide-*H*), 8.42 (s, 1H, N=C*H*), 7.74 (t, *J* = 7.3 Hz, 1H, naphthalimide-*H*), 7.26 (s, 1H, naphthalimide-*H*), 4.44 (s, 2H, imidazole 4-CH_2), 4.03 (s, 4H, morpholine-*H*), 3.29 (s, 4H, morpholine-*H*), 2.73 (t, *J* = 7.4 Hz, 2H, imidazole 2-CH_2), 1.93 (s, 2H, imidazole 4-CH_2CH_2), 1.83–1.75 (m, 2H, imidazole 2-CH_2CH_2), 1.46 (dd, *J* = 14.3, 7.1 Hz, 2H, imidazole 4-CH_2CH_2CH_2), 1.36 (s, 4H, imidazole 2-CH_2CH_2CH_2, imidazole 4-$CH_2CH_2CH_2$CH_2), 0.98 (t, *J* = 7.0 Hz, 3H, imidazole 4-$CH_2CH_2CH_2CH_2$CH_3), 0.90 (s, 3H, imidazole 2-$CH_2CH_2CH_2$CH_3) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ: 161.3, 160.9, 158.2, 156.0, 153.1, 138.8, 133.0, 131.6, 130.4, 129.3, 126.2, 125.9, 123.4, 119.7, 117.1, 115.1, 66.9, 53.5, 46.6, 29.9, 29.8, 28.7, 26.6, 22.5, 22.2, 13.9, 13.7 ppm; MS (m/z): 536 $[M+H]^+$. HR-MS (TOF) calcd for $C_{29}H_{34}ClN_5O_3$: $[M+Na]^+$, 558.2242; found, 558.2251.

Compounds **8b–e** were prepared according to the procedure for the synthesis of **8a**

*2-((2-butyl-4-chloro-1-dodecyl-1H-imidazol-5-yl)methyleneamino)-6-morpholino-1H-benzo[de]isoquinoline-1,3(2H)-dione (**8e**)*

Yellow powder. Yield: 32%; mp: 127 °C; IR (KBr, cm^{-1}) *v*: 3065, 2993, 2944, 2879 (C–H), 1672, 1623 (C=O), 1604, 1495, 1442 (aromatic frame), 1371, 1289, 1252, 1212 (C–N), 1126, 1012 (C–O), 955, 907, 882, 827, 776, 740, 702; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.64 (d, *J* = 6.7 Hz, 1H, naphthalimide-*H*), 8.58 (d, *J* = 7.9 Hz, 1H, naphthalimide-*H*), 8.46 (d, *J* = 8.2 Hz, 1H, naphthalimide-*H*), 8.42 (s, 1H, N=C*H*), 7.74 (t, *J* = 7.7 Hz, 1H, naphthalimide-*H*), 7.25 (s, 1H, naphthalimide-*H*), 4.43 (t, *J* = 7.0 Hz, 2H, imidazole 4-CH_2), 4.03 (s, 4H, morpholine-*H*), 3.29 (s, 4H, morpholine-*H*), 2.73 (t, *J* = 7.5 Hz, 2H, imidazole 2-CH_2), 1.92 (s, 2H, imidazole 4-CH_2CH_2), 1.81–1.77 (m, 2H, imidazole 2-CH_2CH_2), 1.46 (dd, *J* = 14.5, 7.2 Hz, 2H, imidazole 4-CH_2CH_2CH_2), 1.26 (dd, *J* = 50.4, 34.2 Hz, 18H, imidazole 4-$CH_2CH_2CH_2$CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2, imidazole 2-CH_2CH_2CH_2), 0.98 (t, *J* = 7.2 Hz, 3H, imidazole 4-$CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_3$), 0.87 (t, *J* = 6.7 Hz, 3H, imidazole 2-$CH_2CH_2CH_2$CH_3) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ: 161.3, 160.9, 158.2, 156.0, 153.1, 138.8, 133.0, 131.6, 130.4, 129.4, 126.3, 125.9, 123.5, 119.7, 117.1, 115.1, 67.0, 53.5, 46.7, 31.9, 30.2, 29.8, 29.7, 29.6, 29.4, 29.3, 29.1, 26.7, 26.5, 22.7, 22.5, 14.1, 13.7 ppm; MS (m/z): 634 $[M+H]^+$. HR-MS (TOF) calcd for $C_{36}H_{48}ClN_5O_3$: $[M+H]^+$, 634.3518; found, 634.3474.

*Synthesis of 2-((2-Butyl-4-chloro-1-(2-chlorobenzyl)-1H-imidazol-5-yl)methyleneamino)-6-morpholino-1H-benzo[de]isoquinoline-1,3(2H)-dione (**9a**)*

Compound **3c** (297.31 mg, 1.0 mmol) was added to the suspension of compound **7a** (622.42 mg, 2.0 mmol) and P_2O_5 (2% mmol) in toluene. The mixture was stirred under reflux condition for 12 h under nitrogen atmosphere. After the reaction came to the end (monitored by TLC, eluent, dichloromethane), the solvent was evaporated under reduced pressure, and the residue was treated with water (30 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic phase was dried over anhydrous Na_2SO_4 and then evaporated under reduced pressure. The resulting residue was purified *via* silica gel column chromatography using CH_2Cl_2 as eluent and recrystallized from ethanol to give compound **9a** (210 mg) as yellow powder. Yield: 29%; mp: 175 ^oC; IR (KBr, cm^{-1}) *v*: 3062, 2989, 2880 (C–H), 1689, 1645 (C=O), 1529, 1479, 1450 (aromatic frame), 1379, 1290, 1249, 1197 (C–N), 1124, 1021 (C–O), 942, 779, 645; ^1H NMR (600 MHz, $CDCl_3$) δ: 8.57 (d, *J* = 7.2 Hz, 1H, naphthalimide-*H*), 8.53 (s, 1H, N=C*H*), 8.51 (d, *J* = 8.1 Hz, 1H, naphthalimide-*H*), 8.41 (d, *J* = 8.4 Hz, 1H, naphthalimide-*H*), 7.68 (t, *J* = 7.9 Hz, 1H, naphthalimide-*H*), 7.37 (d, *J* = 7.9 Hz, 1H, 2-Cl phenyl-*H*), 7.28 (d, *J* = 7.5 Hz, 1H, naphthalimide-*H*), 7.22 (dd, *J* = 12.5, 7.8 Hz, 2H, 2-Cl phenyl-*H*), 7.00 (d, *J* = 7.6 Hz, 1H, 2-Cl phenyl-*H*), 5.99 (s, 2H, 2-Cl phenyl-CH_2), 4.05–3.94 (m, 4H, morpholine-*H*), 3.31–3.20 (m, 4H, morpholine-*H*), 2.62–2.53 (m, 2H, imidazole 2-CH_2), 1.68–1.58 (m, 2H, imidazole 2-CH_2CH_2), 1.33 (dd, *J* = 15.0, 7.4 Hz, 2H, imidazole 2-CH_2CH_2CH_2), 0.87 (t, *J* = 7.4 Hz, 3H, imidazole 2-$CH_2CH_2CH_2$CH_3) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ: 161.1, 160.7, 158.1, 156.0, 154.4, 139.0, 133.9, 133.0, 132.1, 131.6, 130.4, 129.4, 128.8, 127.8, 127.5, 126.2, 125.9, 123.3, 120.4, 117.0, 115.0, 66.9, 53.5, 46.8, 29.4, 26.6, 22.3, 13.6 ppm; MS (m/z): 590 $[M+H]^+$; HR-MS (TOF) calcd for $C_{31}H_{29}Cl_2N_5O_3$: $[M+H]^+$, 590.1720; found, 590.1723.

Compounds **9b–k** were prepared according to the procedure for the synthesis of **9a**

*2-((2-Butyl-4-chloro-1-(4-nitrobenzyl)-1H-imidazol-5-yl)methyleneamino)-6-morpholino-1H-benzo[de]isoquinoline-1,3(2H)-dione (**9i**)*

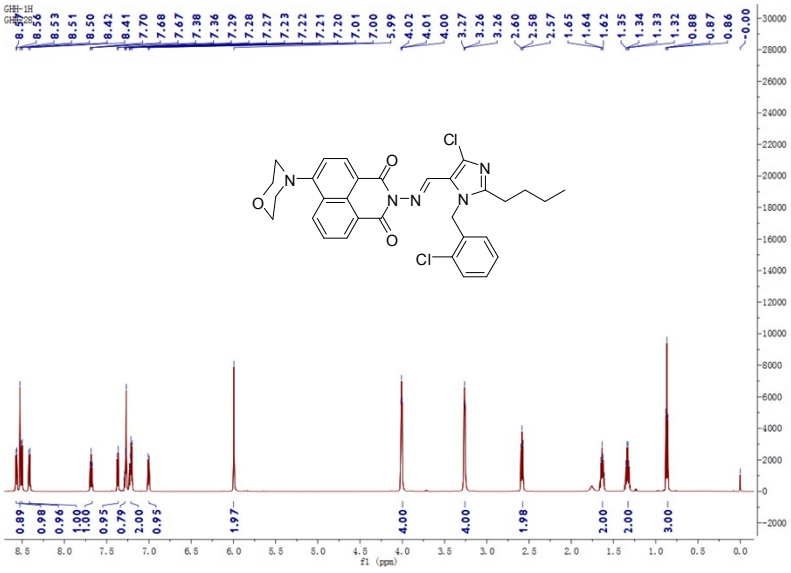
Yellow powder. Yield: 52%; mp: >250 ^oC; IR (KBr, cm^{-1}) *v*: 3069, 2961, 2857 (C–H), 1699, 1657 (C=O), 1590, 1516, 1459 (aromatic frame), 1351, 1264, 1234, 1189 (C–N), 1119, 1048, 1019 (C–O), 907, 835, 784, 752, 673; ^1H NMR (600 MHz, $CDCl_3$) δ: 8.59 (d, *J* = 7.2 Hz, 1H, naphthalimide-*H*), 8.57 (s, 1H, N=C*H*), 8.53 (d, *J* = 8.1 Hz, 1H, naphthalimide-*H*), 8.45 (d, *J* = 8.4 Hz, 1H, naphthalimide-*H*), 8.22 (d, *J* = 8.7 Hz, 2H, 4-NO_2 phenyl-*H*), 7.72 (t, *J* = 7.9 Hz, 1H, naphthalimide-*H*), 7.45 (d, *J* = 8.6 Hz, 2H, 4-NO_2 phenyl-*H*), 7.24 (d, *J* = 8.1 Hz, 1H, naphthalimide-*H*), 5.95 (s, 2H, 4-NO_2 phenyl-CH_2), 4.07–3.96 (m, 4H, morpholine-*H*), 3.34–3.22 (m, 4H, morpholine-*H*), 2.69–2.63 (m, 2H, imidazole 2-CH_2), 1.70 (dd, *J* = 15.3, 7.7 Hz, 2H, imidazole 2-CH_2CH_2), 1.37 (dd, *J* = 15.0, 7.5 Hz, 2H, imidazole 2-CH_2CH_2CH_2), 0.91 (t, *J* = 7.3 Hz, 3H, imidazole 2-$CH_2CH_2CH_2$CH_3) ppm;

^{13}C NMR (151 MHz, $CDCl_3$) δ: 177.9, 157.6, 153.9, 143.3, 133.1, 132.8, 131.7, 131.4, 130.6, 130.5, 127.8, 127.1, 126.2, 125.9, 125.9, 124.2, 124.1, 115.1, 66.9, 53.5, 47.7, 29.4, 26.8, 22.4, 13.6 ppm; MS (m/z): 601 $[M+H]^+$; HR-MS (TOF) calcd for $C_{31}H_{29}ClN_6O_5$: $[M+H]^+$, 601.1961; found, 601.1968.

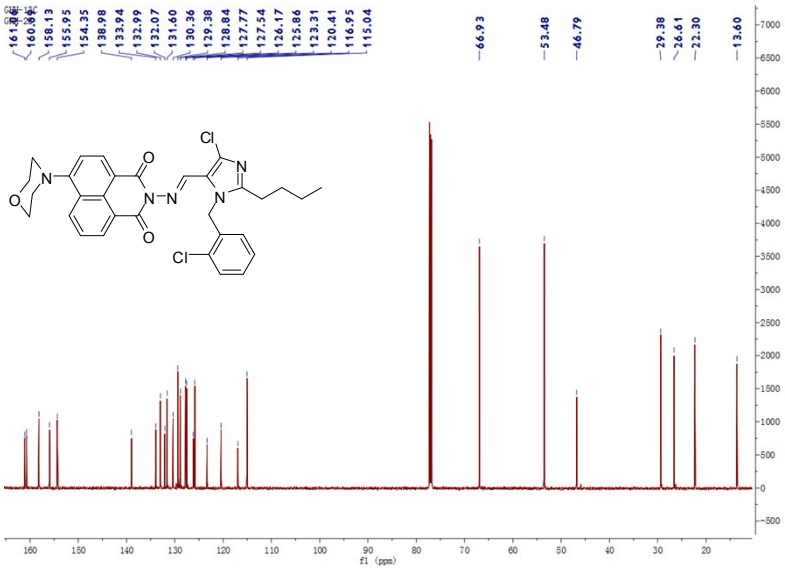
4. Some Representative Spectra

4.1 Spectra of Compound 9a

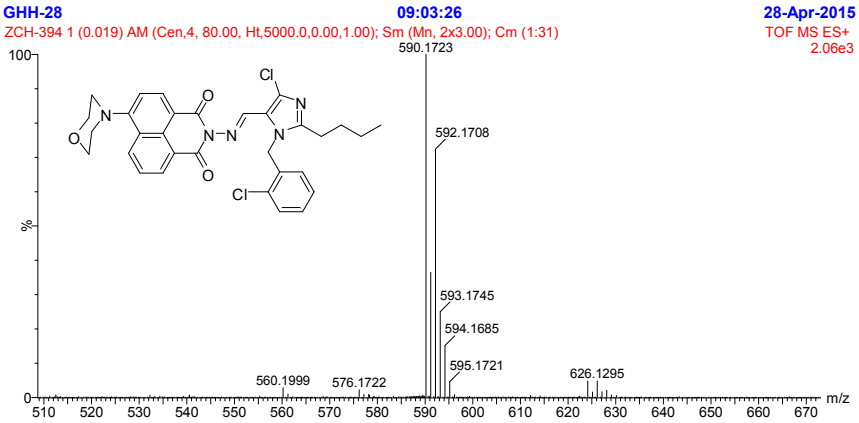
^{1}H NMR Spectrum



^{13}C NMR Spectrum



HRMS Spectrum



HR-MS (TOF) calcd for $C_{31}H_{29}Cl_2N_5O_3$: $[M+H]^+$, 590.1720; found, 590.1723.