Supplementary Data

Design, synthesis, biological evaluation of novel carbazole aminothiazoles as potential DNA-targeting antimicrobial 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 **4f** 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 indicated 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 **3a-j** and **4a-j** were evaluated for their antibacterial activities against *Methicillin-Resistant Staphylococcus aureus* N315, *Staphylococcus aureus* ATCC25923, *Bacillus subtilis* ATCC6633, *Micrococcus luteus* ATCC4698 as Gram-positive, *Escherichia coli* DH52, *Escherichia coli* JM109, *Shigella dysenteriae*, *Pseudomonas aeruginosa* ATCC27853, *Bacillus proteus* ATCC13315, *Eberthella typhosa ATCC14028* 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 Cytotoxicity investigation

2×10^6 cells per mL of Human epidermoid cancer cells (Hep-2) in Roswell Park Memorial Institute 1640 medium (RPMI 1640) supplemented with 10% fetal bovine serum were added to each well of a 96-well plate (100 μL per well). The cells were cultured first for 24 h in an incubator (37 ºC, 5% CO_2), and for another 24 h after the culture medium was replaced with 100 μL of RPMI 1640 containing 10 μL of the compound **4d** or **4f** at different doses (50, 100, 250, 500, 1000, 1250 μg/mL). Then, followed by removing the culture medium, 10 μL of Cell Counting Kit-8 (CCK-8) solution was added to every cell well, which were washed with PBS buffer twice and contained 90 μL RPMI 1640. The cells were further incubated for 1 h. The optical density (OD) of the mixture was measured at 450 nm with a Microplate Reader Model. The cell viability was estimated according to the following equation:

$$\text{Cell viability [\%]} = (\text{OD treated}/\text{OD control}) \times 100$$

OD control was obtained in the absence of target compound, and OD treated was obtained in the presence of target compound.

2. Interactions with calf thymus DNA

2.1 The plot of $A^0/(A–A^0)$ versus 1/[compound 4f]

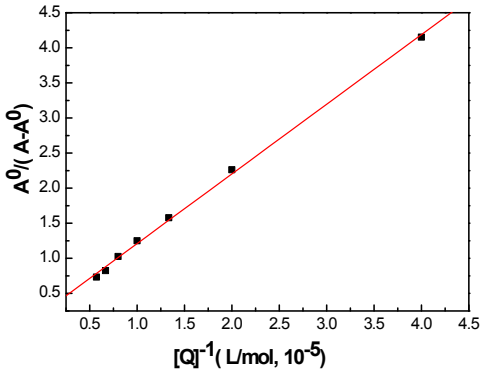


Figure S1. The plot of $A^0/(A-A^0)$ versus 1/[compound **4f**].

2.2 Fluorescence quenching spectra of compound 4f with DNA

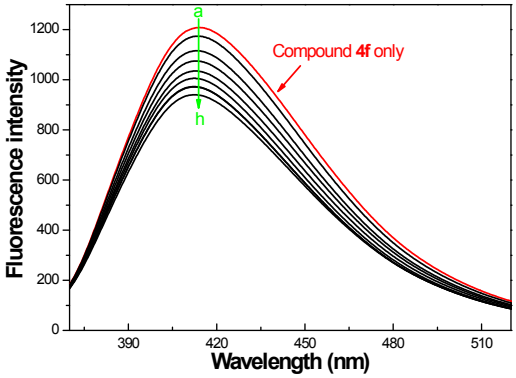


Figure S2. Fluorescence spectra of compound **4f** in the presence and absence of DNA at different concentrations (pH 7.4, T= 310 K). c(compound **4f**) = 5×10^{-5} mol/L, and c(DNA) = 0–1.392×10^{-6} mol/L for curves *a*–*h* at increment 1.74×10^{-7}. Red line shows the emission spectrum of compound **4f** only.

3. Molecular Docking

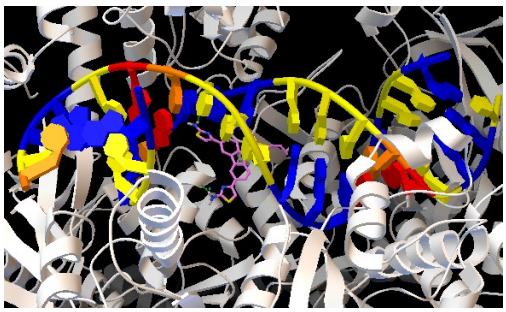


Figure S3. Three-dimensional conformation of compound **4f** docked in DNA.

4. General Procedure and Spectral data for the Prepared Compounds

*4.1 General procedures for the preparation of intermediates (**2a–j**)*

The intermediates **2a**–**j** were prepared according to the previously reported methods.

*4.2 Synthesis of 1,1'-(9-pentyl-9H-carbazole-3,6-diyl)bis(2-chloroethanone) (**3d**)*

To anhydrous aluminium chloride (7.73 g, 58.1 mmol) in round bottom flask, chloroacetyl chloride (3.94 g, 34.88 mmol) dissolved in dry CH_2Cl_2 (10 mL) was added drop-wise with vigorous stirring maintaining the temperature below 5 ºC. Subsequently, a solution of *N*-pentylcarbazole **2d** (2.76 g, 11.62 mmol) in dry CH_2Cl_2 (20 mL) was also added to the mixture under 0−5 ºC. When the addition was complete, the reaction mixture was stirred at RT for 24 h. After completion of reaction (by TLC), the reaction mixture was then poured into cooled water and extracted with CH_2Cl_2. The combined organic layer was washed with 5% aqueous $NaHCO_3$ (3×30 mL) and subsequently with water (3 × 30 mL) and dried over Na_2SO_4. After CH_2Cl_2 was removed in vacuo, the crude product was purified by silica gel column chromatography to give 1.58 g of the brown solid. Yield: 35.4%, m.p: 119–121 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.77 (d, J = 1.4 Hz, 2H, Cab 4,5-*H*), 8.20–8.13 (m, 2H, Cab 2,7-*H*), 7.48 (d, J = 6.5 Hz, 2H, Cab 1,8-*H*), 4.87 (s, 4H, COC*H*$_2$Cl), 4.39 (t, J = 7.3Hz, 2H, C*H*$_2$), 1.9–1.87 (m, 2H, C*H*$_2$), 1.39–1.34 (m, 4H, C*H*$_2$C*H*$_2$), 0.87 (t, J = 6.5 Hz, 3H, C*H*$_3$) ppm.

*4.3 Synthesis of 1,1'-(9-heptyl-9H-carbazole-3,6-diyl)bis(2-chloroethanone) (**3f**)*

To anhydrous aluminium chloride (7.73 g, 58.1 mmol) in round bottom flask, chloroacetyl chloride (3.94 g, 34.88 mmol) dissolved in dry CH_2Cl_2 (10 mL) was added drop-wise with vigorous stirring maintaining the temperature below 5 ºC. Subsequently, a solution of *N*-heptyl carbazole **2f** (3.08 g, 11.62 mmol) in dry CH_2Cl_2 (20 mL) was also added to the mixture under 0−5 ºC. When the addition was completed, the reaction mixture was stirred at RT for 24 h. After completion of reaction (by TLC), the reaction mixture was then poured into cooled water and extracted with CH_2Cl_2. The combined organic layer was washed with 5% aqueous $NaHCO_3$ (3 × 30 mL) and subsequently with water (3 × 30 mL) and dried over Na_2SO_4. After CH_2Cl_2 was removed in vacuo, the crude product was purified by silica gel column chromatography to give 1.46 g of the brown solid. Yield: 30.7%, m.p: 124–126°C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.79 (s, 2H, Cab 4,5-*H*), 8.18 (d, J = 8.6 Hz, 2H, Cab 2,7-*H*), 7.50 (d, J = 8.7 Hz, 2H, Cab 1,8-*H*), 4.83 (s, 4H, COC*H*$_2$Cl), 4.36 (t, J = 6.9 Hz, 2H, C*H*$_2$), 1.92–1.88 (m, 2H, C*H*$_2$), 1.35–1.32 (m, 4H, C*H*$_2$C*H*$_2$), 1.28–1.24 (m, 4H, C*H*$_2$C*H*$_2$), 0.86 (t, J = 6.6 Hz, 3H, C*H*$_3$) ppm.

*4.4 Synthesis of 4,4'-(9-pentyl-9H-carbazole-3,6-diyl)bis(thiazol-2-amine) (**4d**)*

The mixture of **3d** (0.091 g, 0.232 mmol) and thiourea (0.045 g, 0.58 mmol) in EtOH (10 mL) was heated to

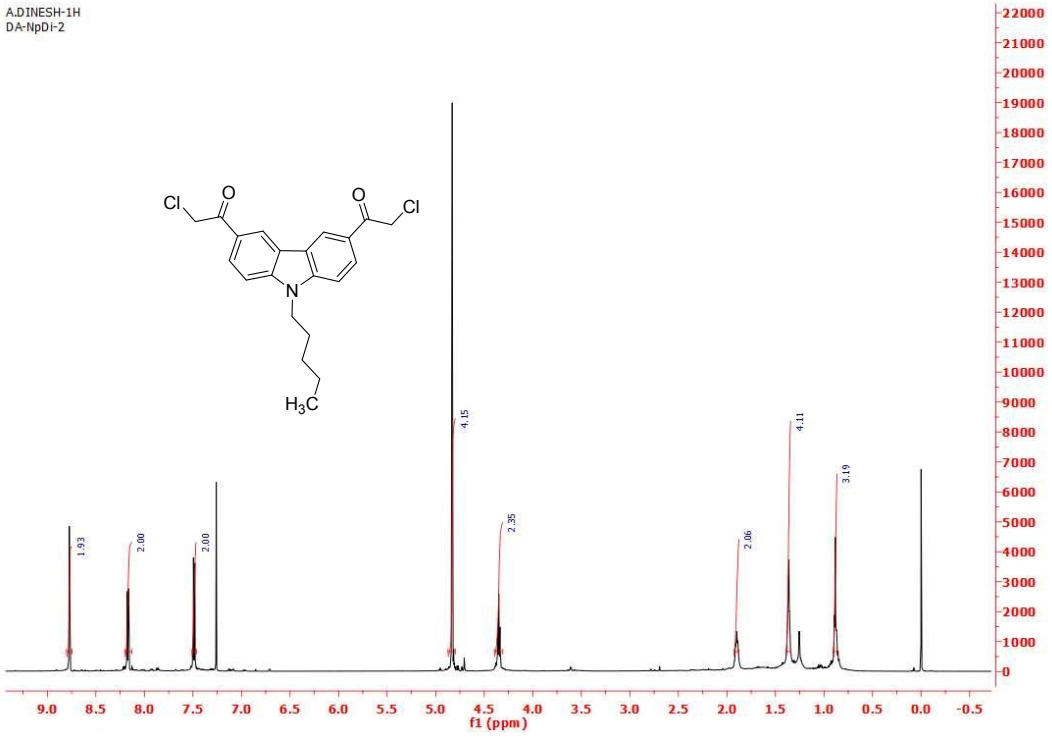
reflux for 1 h. After that, the solvent was removed in vacuo and the saturated aqueous $NaHCO_3$ was added to make the mixture basic (pH = 8–9). The resulting mixture was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic phases were dried with anhydrous Na_2SO_4. After removal of all the solvent, the residue was purified by silica gel chromatography to give 95.6 mg of the light yellow solid. Yield: 95 %, m.p: 235–237 ºC. IR (KBr, cm^{-1}) ν: 3455, 3363 (NH_2), 3112 (Ar-H), 2954, 2926, 2856 (CH_2, CH_3) cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6) δ 8.57 (s, 2H, Cab 4,5-*H*), 7.92 (d, J = 8.5 Hz, 2H, Cab 2,7-*H*), 7.56 (d, J = 8.6 Hz, 2H, Cab 1,8-*H*), 7.01 (s, 4H, NH_2), 6.97 (s, 2H, Thi-*H*), 4.38 (t, J = 6.8 Hz, 2H, CH_2), 1.79–1.77 (m, 2H, CH_2), 1.30–1.26 (m, 4H, CH_2CH_2), 0.82 (t, J = 6.5 Hz, 3H, CH_3) ppm. ^{13}C NMR (151 MHz, DMSO-d_6) δ 168.46, 151.26, 140.34, 127.03, 124.44, 122.82, 117.84, 109.74, 99.47, 42.92, 29.14, 28.78, 22.37, 14.32 ppm. ESI-MS (m/z): 434 $[M+H]^+$, HRMS (TOF) calcd for $C_{23}H_{24}N_5S_2$ $[M+H]^+$, 434.1395; found, 434.1470.

*4.5 Synthesis of 4,4'-(9-heptyl-9H-carbazole-3,6-diyl)bis(thiazol-2-amine) (**4f**)*

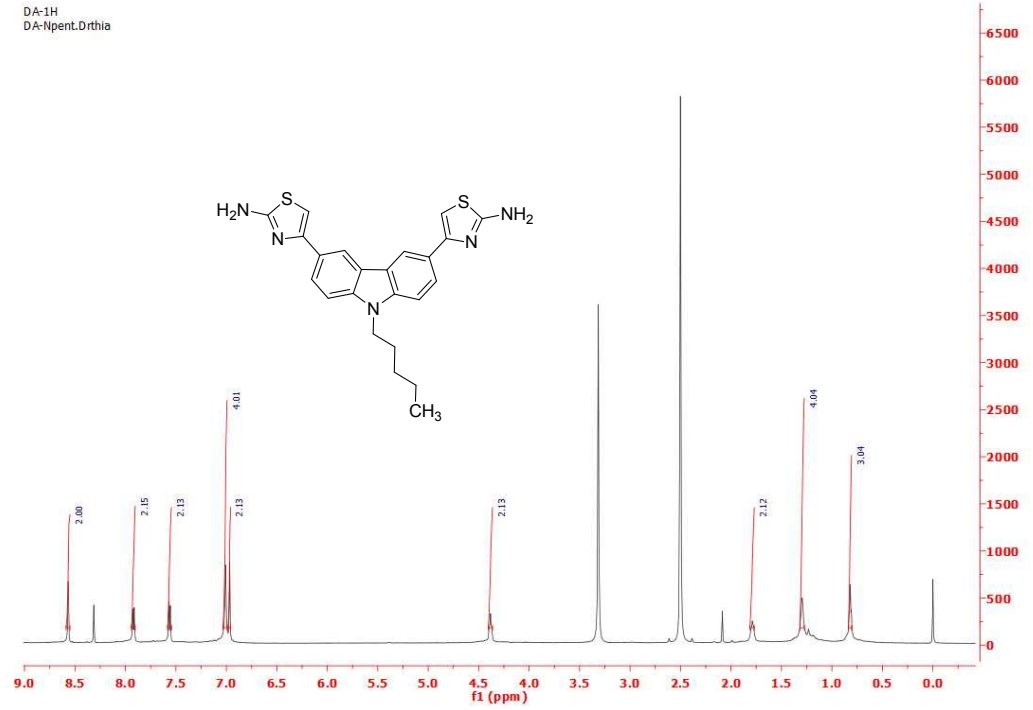
The mixture of **II**–**3f** (0.097 g, 0.232 mmol) and thiourea (0.045 g, 0.58 mmol) in EtOH (10 mL) was heated to reflux for 1 h. After that, the solvent was removed in vacuo and the saturated aqueous $NaHCO_3$ was added to make the mixture basic (pH = 8–9). The resulting mixture was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic phases were dried with anhydrous Na_2SO_4. After removal of all the solvent, the residue was purified by silica gel chromatography to give 101.7 mg of the light yellow solid. Yield: 95%, m.p: 239–241 ºC. IR (KBr, cm^{-1}) ν: 3460, 3363 (NH_2), 3112, (Ar-H), 2954, 2924, 2853 (CH_2, CH_3) cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6) δ 8.57 (s, 2H, Cab 4,5-*H*), 7.92 (d, J = 8.6 Hz, 2H, Cab 2,7-*H*), 7.55 (d, J = 8.6 Hz, 2H, Cab 1,8-*H*), 7.01 (s, 4H, NH_2), 6.96 (s, 2H, Thi-*H*), 4.37 (t, J = 6.8 Hz, 2H, CH_2), 1.80–1.75 (m, 2H, CH_2), 1.23–1.18 (m, 8H, CH_2CH_2CH_2CH_2), 0.81 (t, J = 7.0 Hz, 3H, CH_3) ppm. ^{13}C NMR (151 MHz, DMSO-d_6) δ 168.46, 151.28, 140.33, 127.04, 124.43, 122.82, 117.83, 109.74, 99.47, 42.93, 31.66, 29.08, 28.89, 26.93, 22.42, 14.32 ppm. ESI-MS (m/z): 462 $[M+H]^+$, HRMS (TOF) calcd for $C_{25}H_{28}N_5S_2$ $[M+H]^+$, 462.1708; found, 462.1785.

5. Some Representative Spectra

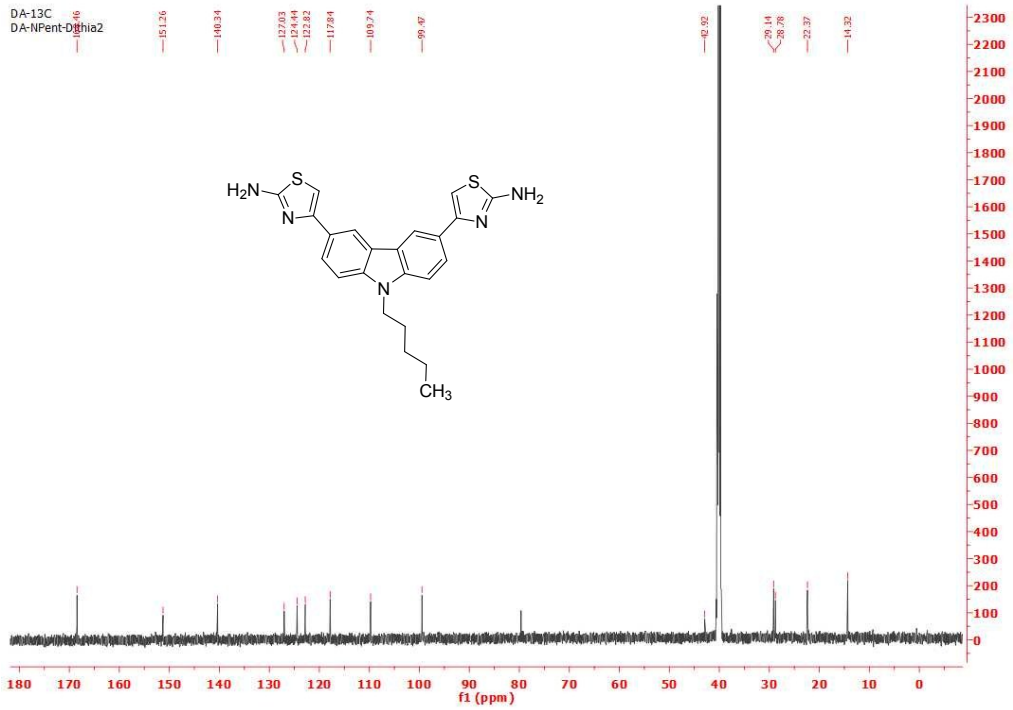
^{1}H NMR Spectrum of 3d:



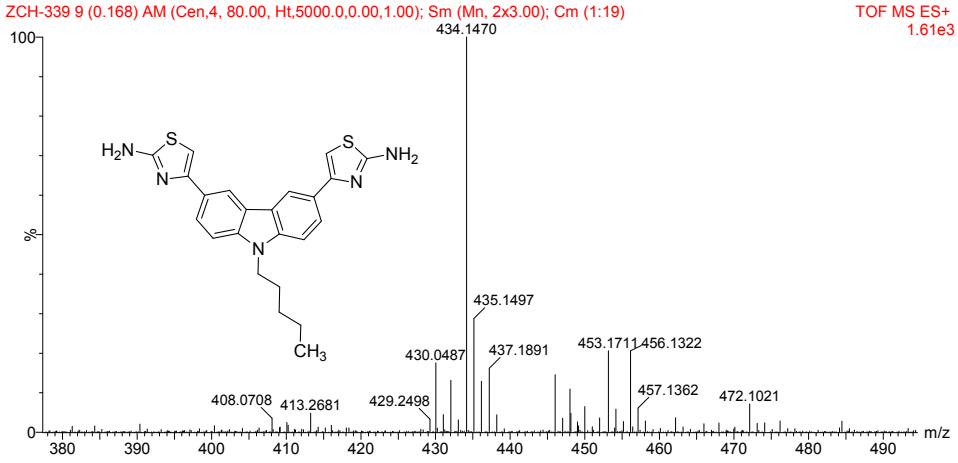
^{1}H NMR Spectrum of 4d:



^{13}C NMR Spectrum of 4d:

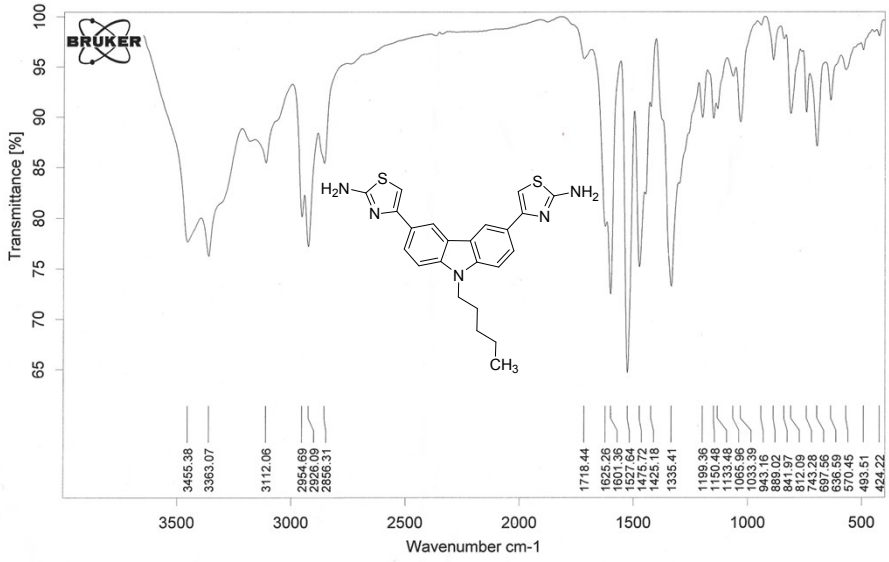


HRMS Spectrum of compound 4d:

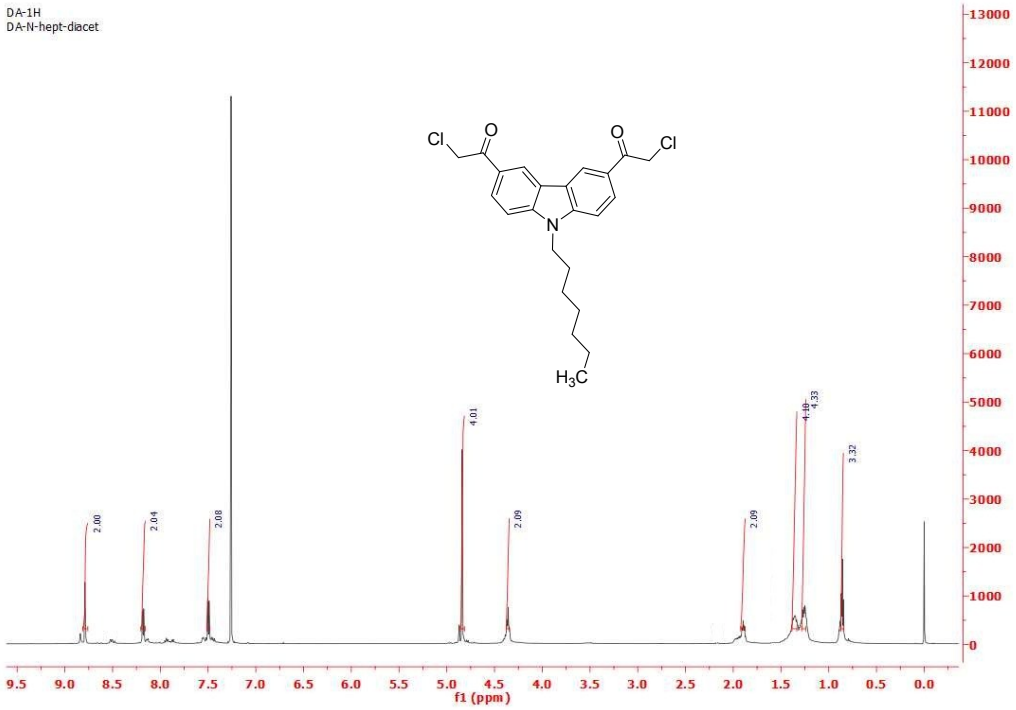


HRMS (TOF) calcd for $C_{23}H_{24}N_5S_2$ $[M+H]^+$, 434.1395; found, 434.1470.

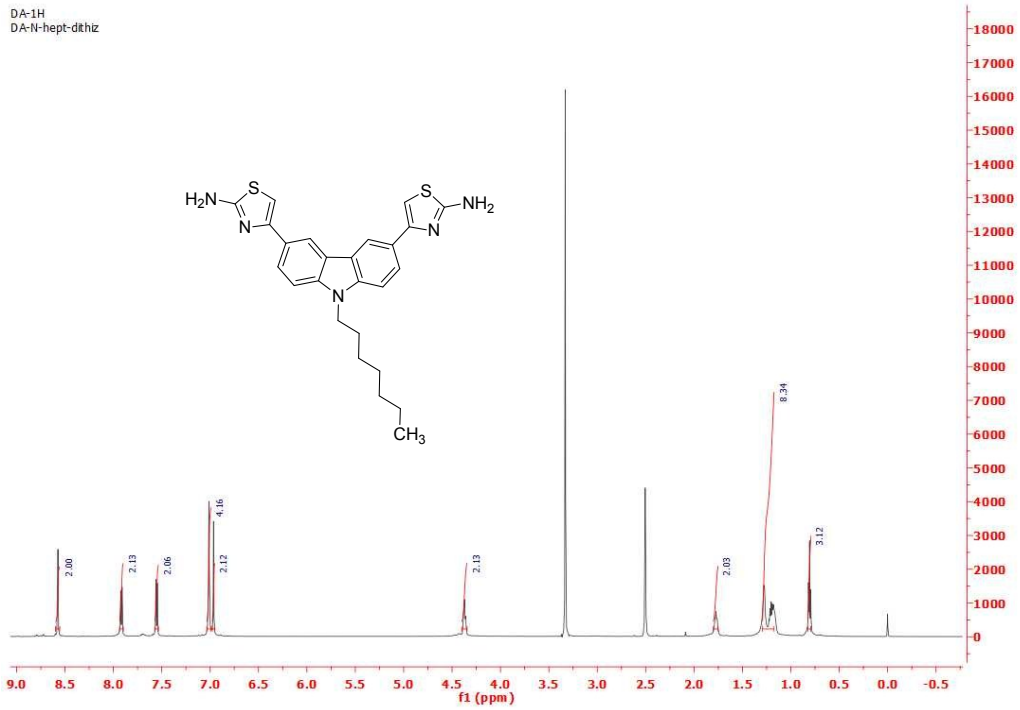
IR Spectrum of compound 4d:



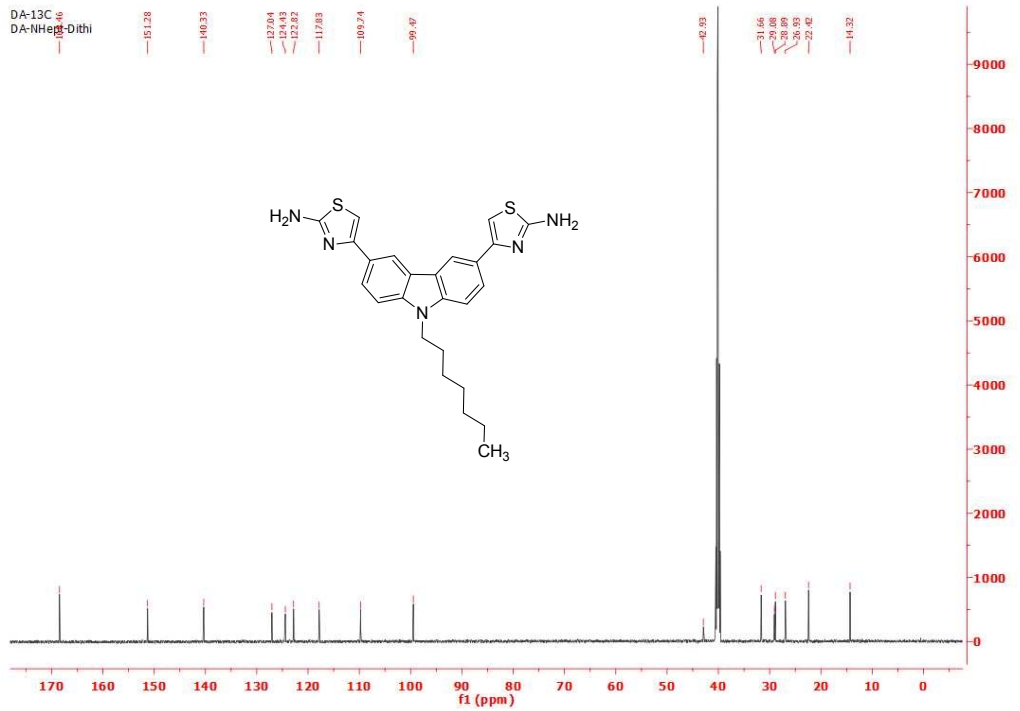
^{1}H NMR Spectrum of 3f:



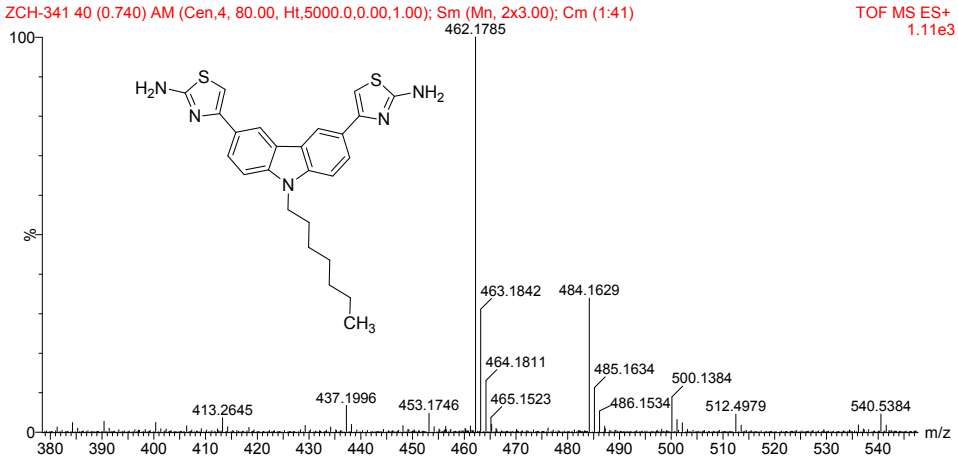
^{1}H NMR Spectrum of 4f:



^{13}C NMR Spectrum of 4f:



HRMS Spectrum of compound 4f:



HRMS (TOF) calcd for $C_{25}H_{28}N_5S_2$ $[M+H]^+$, 462.1708; found, 462.1785.

IR Spectrum of compound 4f: