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Synthesis, characterization and anti-inflammatory activity of some novel pyrimidin-2-amines on Carrageenan–induced paw edema in *balb/c* mice

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ABSTRACT

We report here the synthesis of some new pyrimidin-2-amines and their anti-inflammatory activity against Balb/c mice with locally induced edema. Spectral characterization of the compounds is explained. The better drug candidate is optimized among the tested compounds. Of the synthesized compounds, 4-(9H-Fluoren-2-yl)-6-phenylpyrimidin-2-amine and 4-[4-(Diphenylamino) phenyl]pyrimidine-2-amine are found to have more efficient anti-inflammatory activity than that for 4,6-Bis-(9H-fluoren-2-yl)pyrimidin-2-amine and 3-[3-(9H-Fluoren-2-yl)-3-oxoprop-1en-1-yl]-4H-chromen-4-one.

Keywords: Anti-inflammatory activity, Pyrimidin-2-amines, Paw edema

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INTRODUCTION

Search for easy synthetic procedures for preparing biologically active building blocks like aminopyrimidines is extensive [1–4]. Pyrimidine derivatives are a class of heterocycles which possess remarkable biological activity and have been used in medicinal applications. Much of the earlier years work on pyrimidine-2-amine and a similar compound were on their derivatization and structural conformation of the products [5-8]. Since scaffolds containing pyrimidine moiety are of interest in medicinal chemistry and chemical biology, newer methodologies to prepare them are growing in the literature. The importance of pyrimidine–fused heterocycles is felt as they appear as the core of biologically active compounds [9, 10]. Recent reports reveal the anti-inflammatory activities of pyrimidine and thienopyrimidine derivatives [11, 12].

Pyrimidine and aminopyrimidine derivatives are having interesting structures as these structures occur in nature as components of nucleic acids. Aryl-substituted pyrimidine-2-amines and pyridine-2-amines are useful drugs for the treatment of diseases modulated by the adenosine receptor [13]. Pyrimidine-2-amines are often encountered in biological systems and they are capable of forming either supramolecular homosynthons or supramolecular heterosynthons [14]. Crystal engineering using the hydrogen bonding ability of $–NH_2$ group in amino pyrimidines has attracted a chemist which has aided them designing a number of supramolecular nano-architectures, layers, ribbons, rosettes, rods, tapes, tubes, sheets, and spheres [15, 16]. Apart from being the underlying constituent of nucleobases and a large number of natural products such as vitamin B1, and components of supramolecular structures, pyrimidin-2-amines are present in agrochemicals [17] pharmaceutical agents [18, 19], and materials [20]. The rigidity of fused heterocycles prepared from pyrimidin-2-amines helps tremendously in overcoming the entropic helps tremendously in overcoming the entropic barriers associated with receptor-ligand interactions. In addition, the basicity of the majority helping address at an early stages any unforeseen solubility [21].

Inflammation is the local response of living mammalian tissue to injury due to any agent. It is a body defense reaction in order to eliminate or the spread of injurious agent as well as to remove the necrosed cells inflammatory diseases like arthritis, allergy, multiple sclerosis, asthma, etc need considerable attention as these are quite common. Organic synthesis has a focus on deriving safer anti–inflammatory drugs. To reduce human health problems due to inflammatory diseases, there is an urgent need to develop safer and newer anti–inflammatory drugs. Newer drugs need to be non–steroidal anti–inflammatory agents. The search for better candidates in terms of higher efficacy and selectivity with lesser side effects continues. As inflammation is caused as a complex reaction to injurious agents including microbes and involving vascular responses such as activation and migration of leukocytes and systemic reactions, several newer anti–inflammatory drugs can be tested [22]. Due to the fast development of microbial resistance towards the existing drugs, new chemical entities are synthesized with better efficacy and wider medicinal properties. Both the modern aspects of structure activity relationship and classical therapies with non–steroidal anti–inflammatory drugs (NSAIDS) and opiates are in use. Putting all the points discussed so far, we based our present study with the following considerations: (i) synthesizing pyrimidin-2-amines of new structures as NSAIDs, (ii) testing them against animals with locally induced edema for effective anti-inflammatory activity, and (iii) optimizing the better drug candidate of the tested compounds.

EXPERIMENTAL SECTION

2.1 Chemicals

Fluorene-2-carboxaldehyde, 4-diphenylaminobenzaldehyde, 2–acetylfluorene, guanidine nitrate and 3-formylchromone were purchased from Aldrich, Bangalore, India. Benzaldehyde and sodium hydroxide were received from Qualigens and S.D. Fine chemicals respectively. All the above- mentioned chemicals were of Analar grade, purity 99%. The solvents for Column chromatography and TLC, viz., Petroleum ether, Dichloromethane, Hexane, Chloroform and Ethyl acetate were purchased from Merck Chemicals. Ethanol (99%) was used as solvent in synthesis as purchased. Gum acacia was purchased from Hi-Media, Carrageenan from Hi-Pure Fine Chemical Industries (Chennai, India), All chemicals used were analytical or reagent grade.

2.2 Instruments

Melting points of the synthesized compounds were recorded using a Labtronics (India) melting point apparatus with capillary tubes. FT-IR Spectra were recorded using a Brucker-α FT-IR spectrometer using KBr pellets. NMR spectra were recorded in a Brucker-AMX 400 MHz spectrometer with $CDCl_3$ as the solvent.

2.3 Synthesis of compounds

The compounds 1,3-diarylprop-2-en-1-ones and pyrimidin-2-amines were synthesized as per the reported general procedure [23].

General procedure for the preparation of 1,3-diarylprop-2-en-1-ones

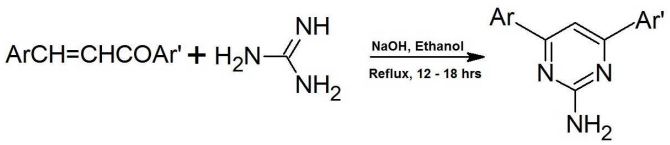
A 0.01 mol alcoholic solution of benzaldehyde (or fluorene-2-carboxaldehyde or 4-diphenylaminobenzaldehyde or 3-formylchromone) and 0.01 mol of 2-acetylfluorene in ethanol (50 ml) containing aqueous sodium hydroxide (0.5 mol in 5 ml of water) was heated over a water bath for half an hour. Heating was stopped and crystals were formed when cooled. The product thus obtained was filtered, washed with distilled water, and dried. Recrystallization from ethanol was carried out. The range of yield was 80-85%. The reaction for the preparation of 1,3-diarylprop-2-en-1-ones is given in Scheme 1.

$$ArCHO + CH_3COAr' \xrightarrow[Ethanol]{NaOH} ArCH{=}CHCOAr'$$

Scheme 1. General reaction for the preparation of 1,3-diaryl prop-2-en-1-ones

General procedure for the preparation of pyrimidin-2-amines

A mixture of 1,3-diarylprop-2-en-1-one (0.01 mol), guanidine nitrate (0.01 mol) in ethanol (50 ml) was heated while a solution of sodium hydroxide (0.5 mol in 5 ml of water) was added in portion for two hours. Refluxing was continued for further 12-18 h and the mixture was concentrated. The product was then filtered and dried. The products were separated from the solvent mentioned in Table 1. The scheme of preparation of pyrimidin-2-amines is given in Scheme 2.



Scheme 2. General reaction for the preparation of pyrimidin-2-amines

Table1 Melting point and solvents for separation of the compounds

Compound	Melting point, ºC	Solvent
FPP	132 – 134	Hexane: Ethyl acetate (80:20)
BFP	138 – 140	Hexane: Ethyl acetate (80:20)
DPAPP	182 – 184	Hexane: Ethyl acetate (80:20)
FOPC	124 – 126	Hexane : Ethyl acetate (75:25)

2.4 Anti-inflammatory activity

Toxicity studies

Acute *in vivo* toxicity studies with different concentrations of drug was carried out to determine the LD_{50} value by the Miller and Tainter method.[24] No deaths or adverse effects were detected during the 24 hour observation period in mice treated with up to 200mg/kg.bw of FPP, BFP, DPAPP, and FOPC drugs (data not shown). Based on the results, the dose at the concentration of 10mg/kg.bw was chosen for the experiments.

Preparation of the drug

A 10 mg of the each drug was dissolved separately in 1ml of methanol and added with phosphate buffer solution (PBS, pH 7.4) containing 1% gum acacia. The resultant mixture was subjected to vortex and the solvent was removed by evaporation.

Animals

Balb/c mice were bought from the KMCH College of Pharmacy, Coimbatore. The animals were fed with standard pellet diet (Sai Durga feeds, Bangalore, India) and water ad libitum. They were maintained in controlled environment (12:12 h light/dark cycle) and temperature (30 ± 2 ºC). All the animal experiments were performed according to the rules and regulations of the Animal Ethical Committee, Government of India.

Experimental Design

Edema was induced in rats according to the reported method [25]. *Balb/c* mice were divided into six groups (n = 6/group). Group 1: Carrageenan control; Group 2: Carrageenan + Drug 6-(9H-fluoren-2-yl)-4-phenyl pyrimidin-2-amine (FPP); Group 3: Carrageenan + Drug 4,6-Bis–(9H-fluoren-2-yl) pyrimidin-2-amine (BFP); Group 4: Carrageenan + Drug 4-[4-(diphenylaminophenyl]-6-(9H-fluoren-2-yl)-pyrimidine-2-amine (DPAPP); Group 5: Carrageenan + Drug 3-[3-(9H-fluoren-2-yl)-3-oxoprop-1-en-1-yl]-4H-chromen-4-one (FOPC). Group 6: Carrageenan + Diclofenac standard drug, separately re-suspended in 1% gum acacia that was injected IP (10 mg/kg) on 10 consecutive days; the last dose was provided 60 min before induction of inflammation. All mice received a subcutaneous injection of 0.1 ml of a 1% (w/v) carrageenan solution in the plantar region of their right hind paw to induce edema. The paw volume was measured initially and then at 30 min intervals for up to 8 hr and 24th hour alone after the injection using a Vernier caliper. All data were expressed as mean (± SD).

RESULTS AND DISCUSSION

The molecular structure of the synthesized compounds FPP, BFP, DPAPP and FOPC are shown in Fig. 1. Compounds were characterized by IR, ^{1}H, ^{13}C NMR and Mass spectroscopic techniques and the spectral data are given in Table 2.

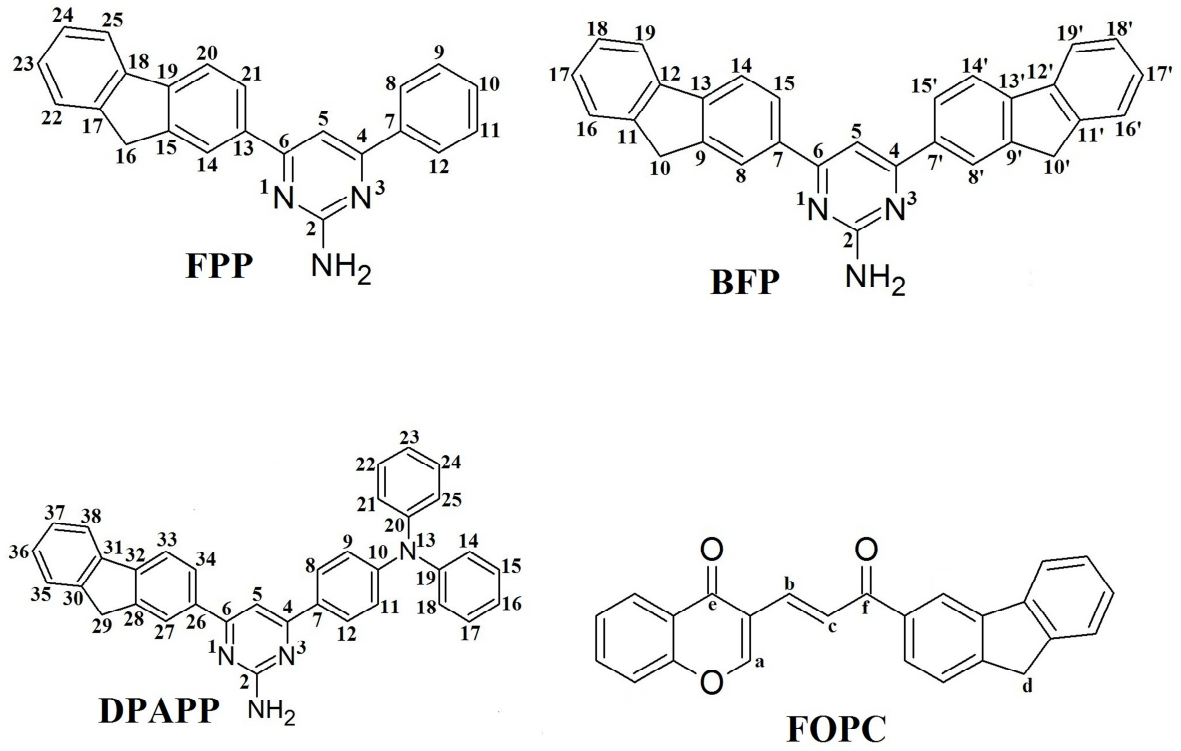
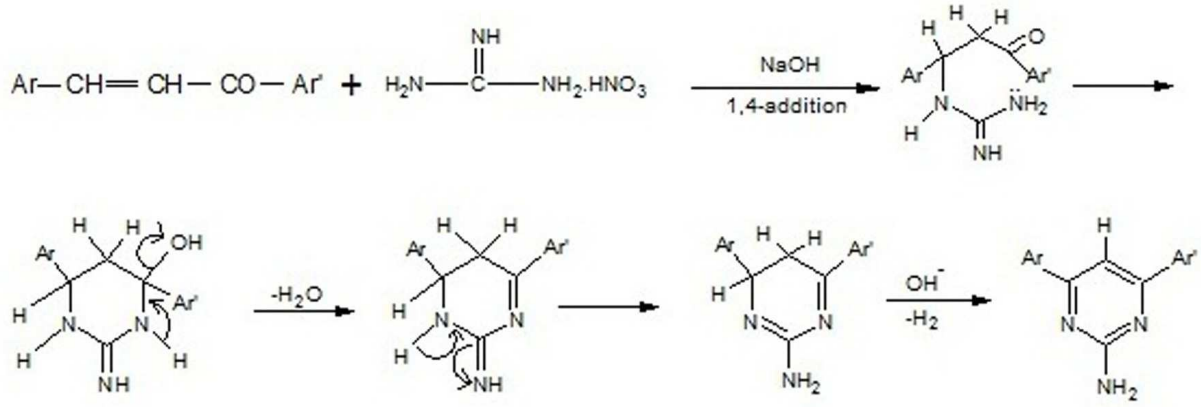


Fig. 1. Molecular structure of 6-(9H-Fluoren-2-yl)-4-phenylpyrimidin-2-amine (FPP), 4,6-Bis-(9H-fluoren-2-yl)pyrimidin-2-amine (BFP), 4-[4-(Diphenylamino)phenyl]-6-(9H-Fluoren-2-yl)-pyrimidin-2-amine (DPAPP), and 3-[3-(9H-Fluoren-2-yl)-3-oxoprop-1-en-1-yl]-4H-chromen-4-one (FOPC)

Mechanism of the formation of pyrimidin-2-amines

The general mechanism of the formation of pyrimidine-2-amines is given in Scheme 3. Shortly, it can be explained by 1,4-addition of the guanidine to chalcones and the cyclization of the intermediates. These intermediates undergo proton shift and aromatization to yield pyrimidin-2-amines. The mechanism (given in Scheme 3) is similar to the one reported by El-Rayyes [26].



Scheme 3. Mechanism of formation of pyrimidin-2-amines

Table2 Spectral data for the synthesized compounds

Compounds	Spectral Data[#]
FPP	IR ῡ/cm^{-1} (KBr): 3328.59 and 3178.55 (N–H asymmetric and symmetric str.), 2922.73 (aromatic C-H str.), 1573.36 (N-H in-plane bending), 1229.38 (C-N str.). ^{1}H NMR δ (ppm), 400 MHz ($CDCl_3$): 6.82 (s, NH_2), 7.88 (s, H-5), 3.81 (s, 2 × H-15), 7.23 – 8.34 (m, Aromatic protons) ^{13}C NMR δ (ppm), 400 MHz ($CDCl_3$): 114.97 (C5), 162.49 (C2), 163.58 (C4), 163.15 (C6), 37.24 (C16), 120.27 – 143.63 (Aromatic carbons) Mass (m/z): 411.19
BFP	IR ῡ/cm^{-1} (KBr): 3406.04 cm^{-1} and 3367.00 (N–H asymmetric and symmetric str.), 3053.76 (aromatic C-H str.), 2852.10 (Methylene C-H str.), 1535.73 (N-H in-plane bending), 1223.08 (C-N str.). ^{1}H NMR δ (ppm), 400 MHz ($CDCl_3$): 6.91 (s, NH_2), 7.43 (s, H-5), 3.83 (s, 4 × H-10 & 10′), 7.16 – 8.27 (m, Aromatic protons) ^{13}C NMR δ (ppm), 400 MHz ($CDCl_3$): 114.31 (C5), 162.83 (C2), 163.51 (C4), 164.02 (C6), 37.97 (C10 & 10′), 120.04 – 143.41 (Aromatic carbons) Mass (m/z): 714.30
DPAPP	IR ῡ/cm^{-1} (KBr): 3444.16 cm^{-1} and 3351.34 (N–H asymmetric and symmetric str.), 3059.99 (aromatic C-H str.), 2852.37 (Methylene C-H str.), 1653.65 and 1585.49 (N-H in-plane bending), 1229.38 (C-N str.), 1332.65 and 1272.76 (C-N str.) ^{1}H NMR δ (ppm), 400 MHz ($CDCl_3$): 6.28 (s, NH_2), 6.61 (s, H-5), 3.85 (s, 2 × H-29), 6.83 – 8.27 (m, Aromatic protons) ^{13}C NMR δ (ppm), 400 MHz ($CDCl_3$): 114.84 (C5), 162.13 (C2), 162.99 (C4), 163.64 (C6), 37.86 (C29), 147.96, 148.06, and 148.97 (C10, C19 and C20), 116.12 – 147.57 (Aromatic carbons) Mass (m/z): 817.27
FOPC	^{1}H NMR δ (ppm), 400 MHz ($CDCl_3$): 8.26 (s, Chromone proton – ‘a’), 7.41 (d, CH – ‘b’), 6.79 (d, CH – ‘c’), 3.92 (s, Methylene proton – ‘d’), 7.32 – 8.35 (Aromaticprotons) ^{13}C NMR δ (ppm), 400 MHz ($CDCl_3$): 36.68 (C – ‘d’), 176.02 (C – ‘e’), 189.13 (C – ‘f’), 118.75 (Quaternary Carbon, Chromone attached to the propen-3-one), 133.53 (Quaternary Carbon, Fluorene attached to the propen-3-one), 117.56 - 146.04 (Aomatic Carbons) Mass (m/z): 531.07

[#] *For atom labeling see Fig.1*

Anti-inflammatory activity of FPP, BFP, DPAPP and FOPC against acute inflammation (Carrageenan induced paw edema)

The effect of synthesized drugs FPP, DPAPP, BFP and FOPC against carrageenan-induced paw edema is shown in Fig. 2. All the Drugs at 10 mg/kg.bw for 10 consecutive days exerted an anti-inflammatory property as illustrated by a significant reduction in paw size during at 2160th min post-carrageenan injection FPP (0.28 [± 0.01] mm), DPAPP (0.26 [± 0.01] mm), BFP (0.37 [± 0.01] mm) and FOPC (0.32 [± 0.04] mm) relative to that seen in carrageenan control group maximum up to (0.32 [± 0.01] mm). Among the four drugs, the drug DPAPP acts as most potent anti-inflammatory agent and this activity might be due to presence of aminopyrimidine ring substituted with diphenylaminophenyl ring. The inhibition of inflammatory response exerted by the drug DPAPP was comparable to the standard drug diclofenac at concentration of 10 mg/kg.bw (0.25 [± 0.01] mm) on the same time. The discovery of cyclooxygenase-2 (COX-2) enzyme led to the hypothesis that selective inhibitors of this isoform would exhibit effective clinical efficacy but reduced ulcerogenicity than traditional nonsteroidal antiinflammatory drugs (NSAIDs). A series of pyrimidine derivatives have been reported as COX-2 inhibitors.[27] The aminopyrimidine moiety is a common structural subunit in a large number of both natural products and synthetic compounds. Aminopyrimidine and their derivatives have been extensively studied for their various biological and pharmacological activities include kinase inhibitor, analgesics and quite rarely reported as anti-inflammatory agents.[28] In conclusion, the synthesized drug DPAPP in which aminopyrimidine ring substituted with diphenylaminophenyl ring might be an adequate combination of substituents to inhibit COX-2 expression with selectivity and efficacy.

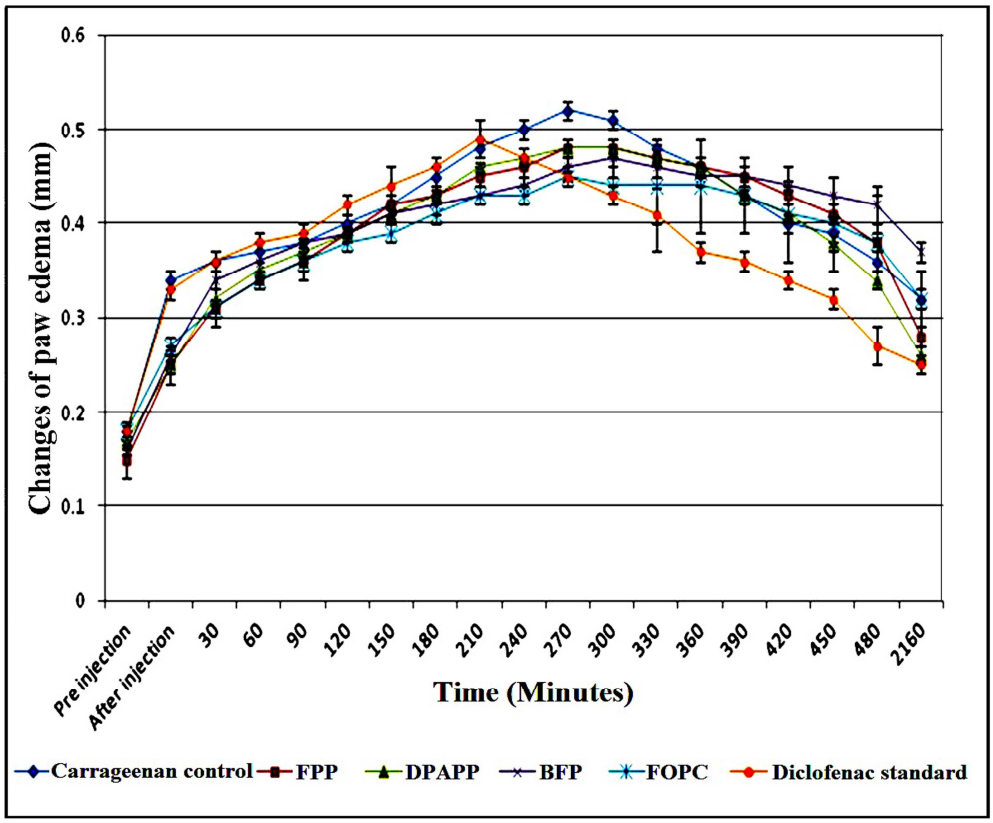


Fig. 2. Effect of FPP, DPAPP, BFP and FOPC against acute inflammation

CONCLUSION

The compounds 4-(9H-fluoren-2-yl)-6-phenylpyrimidin-2-amine (FPP), 4,6-bis-(9H-fluoren-2-yl)pyrimidin-2-amine (BFP), 4-[4-(diphenylamino)phenyl]pyrimidine-2-amine (DPAPP), 3-[3-(9H-fluoren-2-yl)-3-oxoprop-1en-1-yl]-4H-chromen-4-one (FOPC) were synthesized. The synthesized compounds were characterized by IR, ^{1}H, ^{13}C NMR and mass spectroscopic techniques. The synthesis demonstrated easy and facile method of preparation of novel pyrimidin-2-amines. The compounds were tested for their anti-inflammatory activity with *Balb/c* mice inducing local acute paw edema with carrageenan as control. The anti-inflammatory response for FPP and DPAPP were found higher than that for BFP and FOPC.

Acknowledgement
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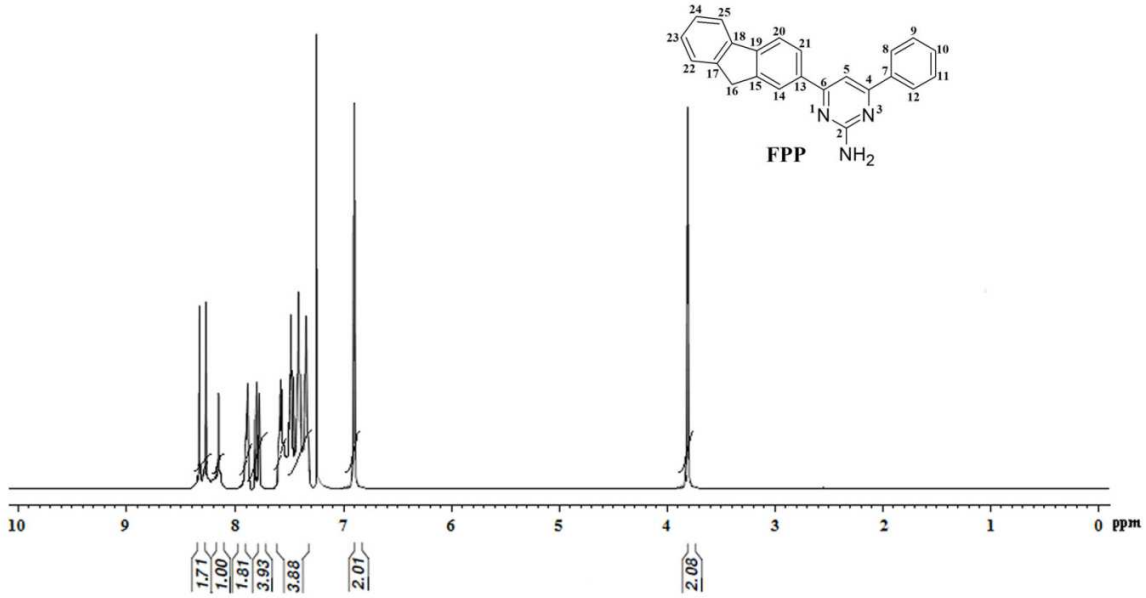
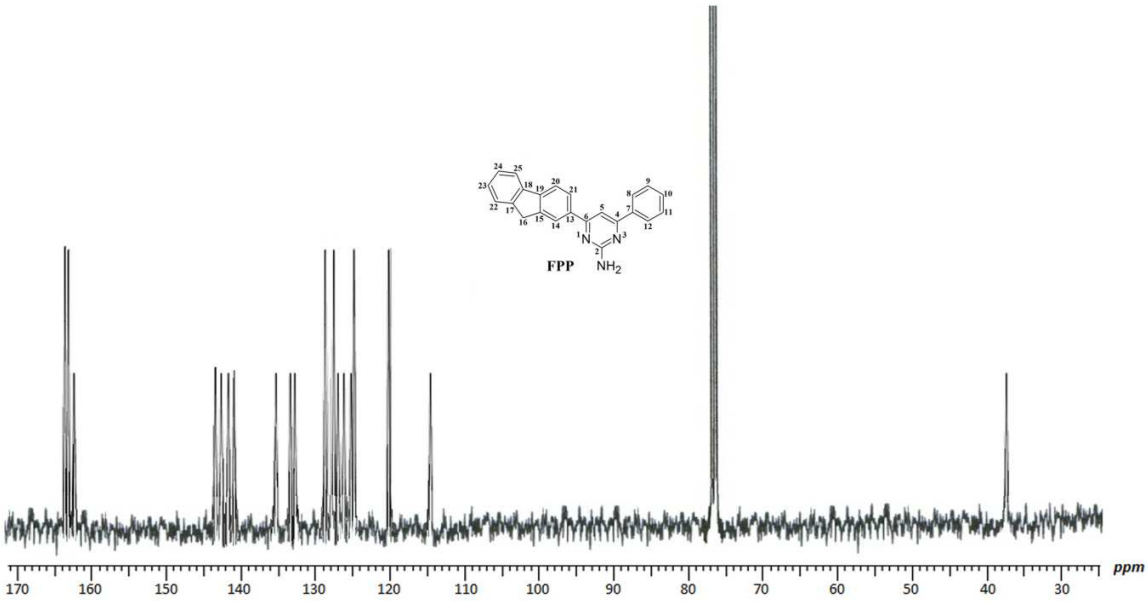


SI 1. ^{1}H NMR spectrum of FPP



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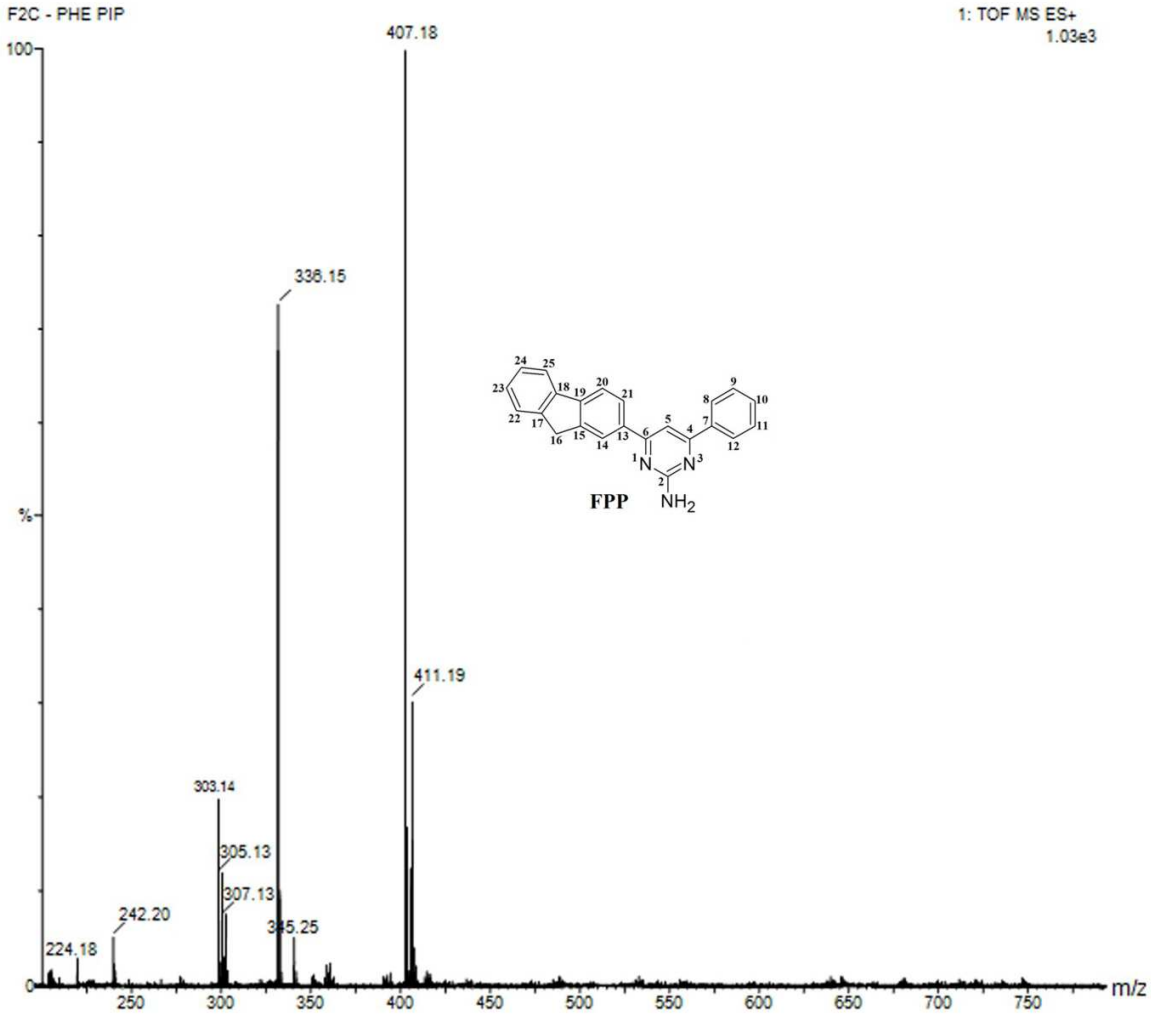
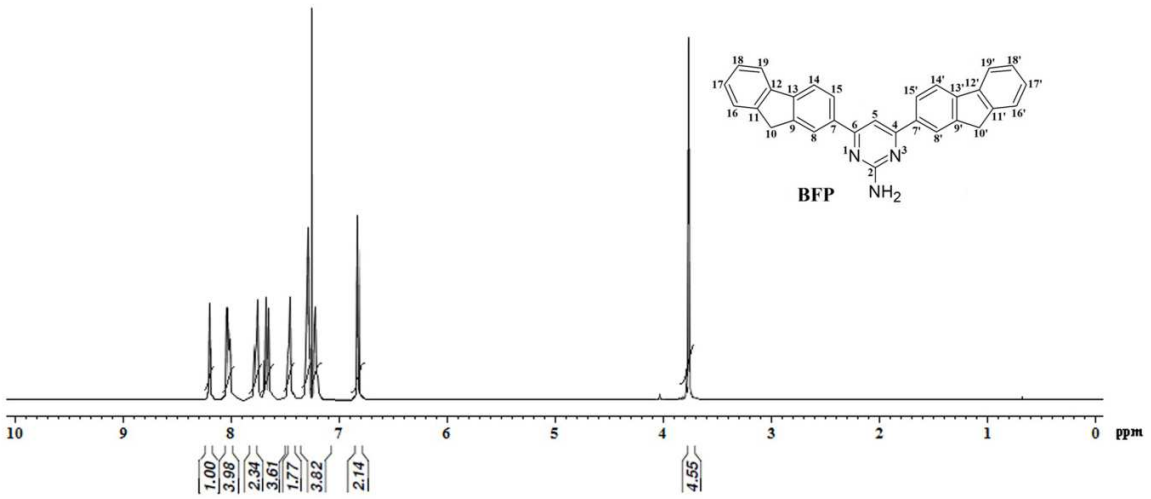
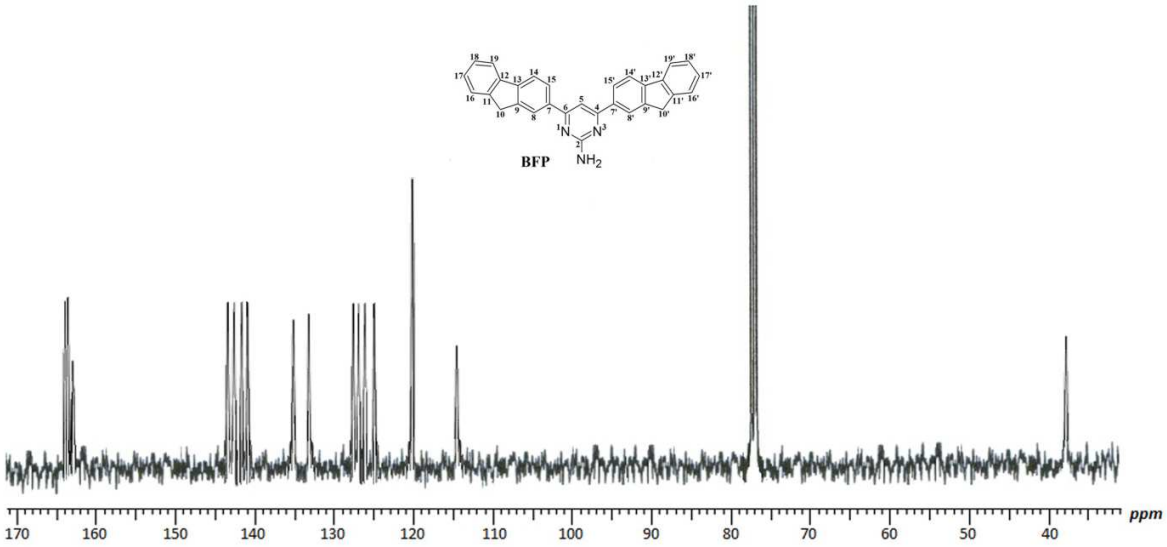


SI 3. Mass spectrum of FPP



SI 4. ^{1}H NMR spectrum of BFP



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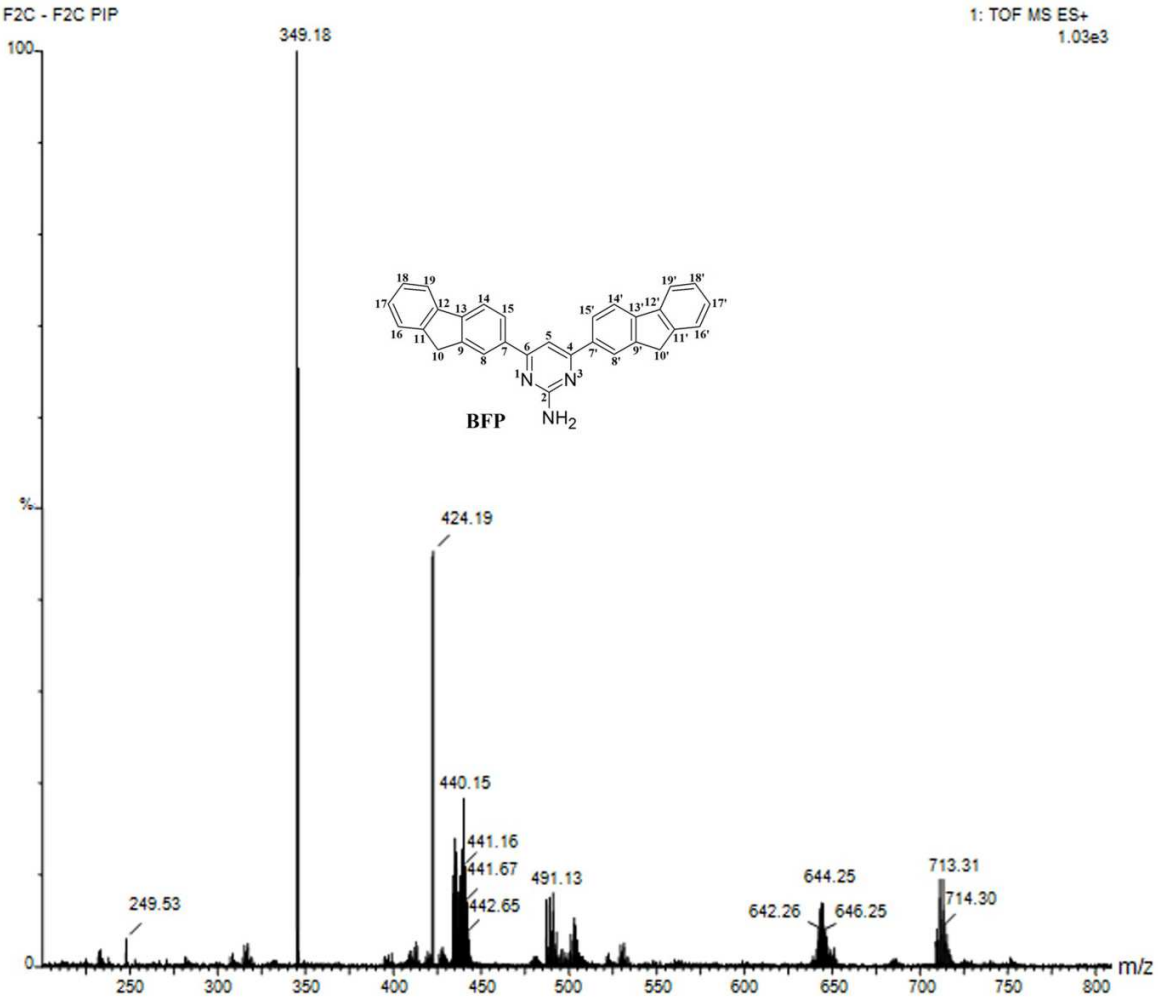
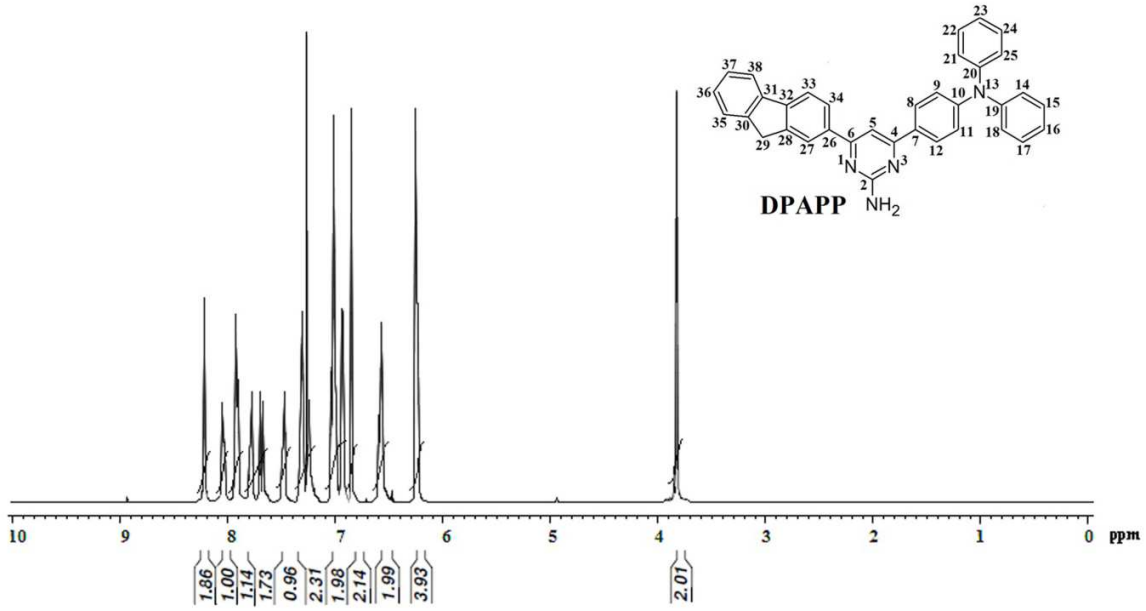
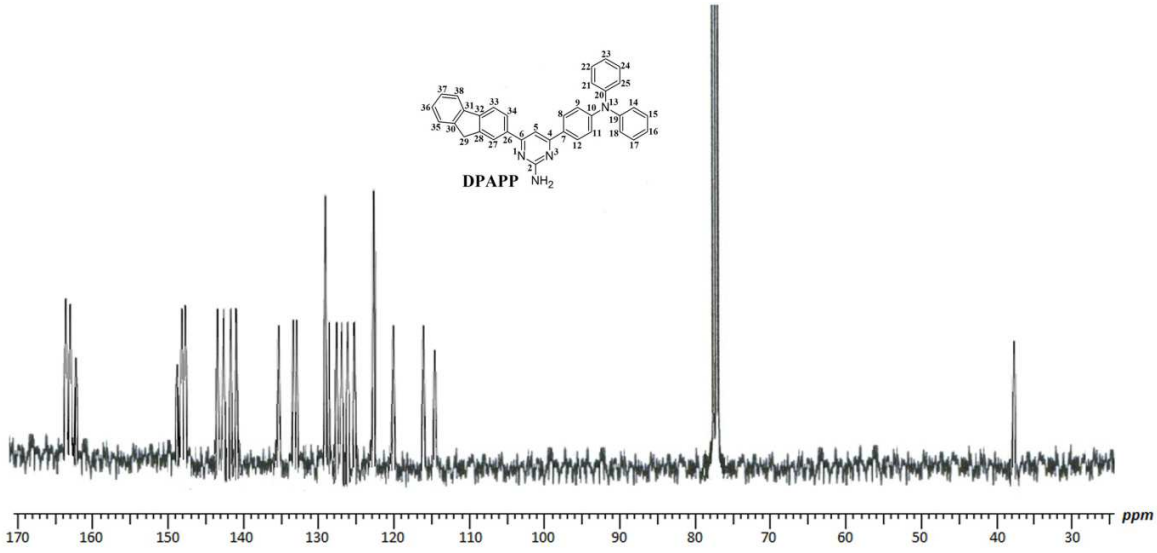


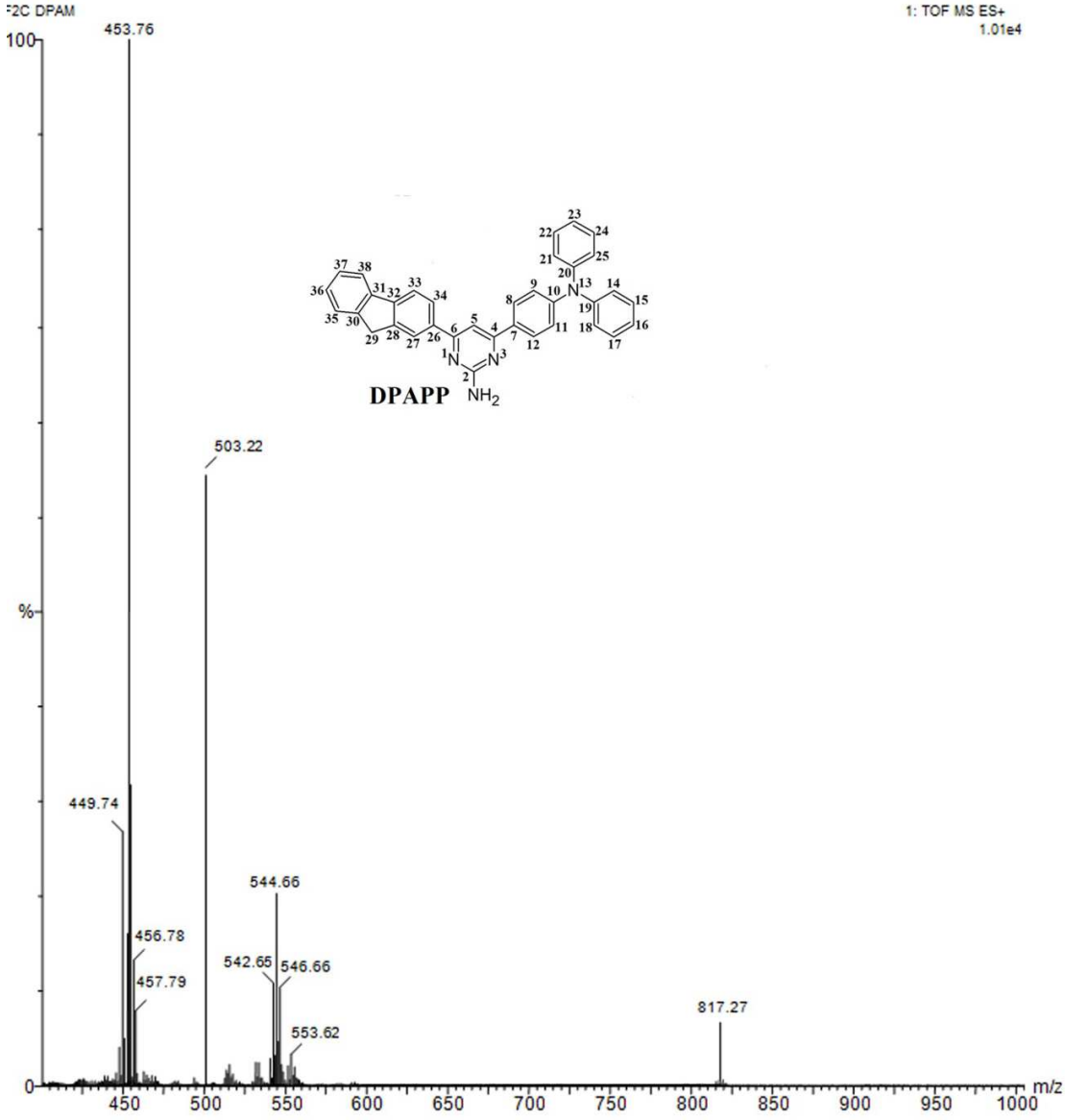
SI 6. Mass spectrum of BFP



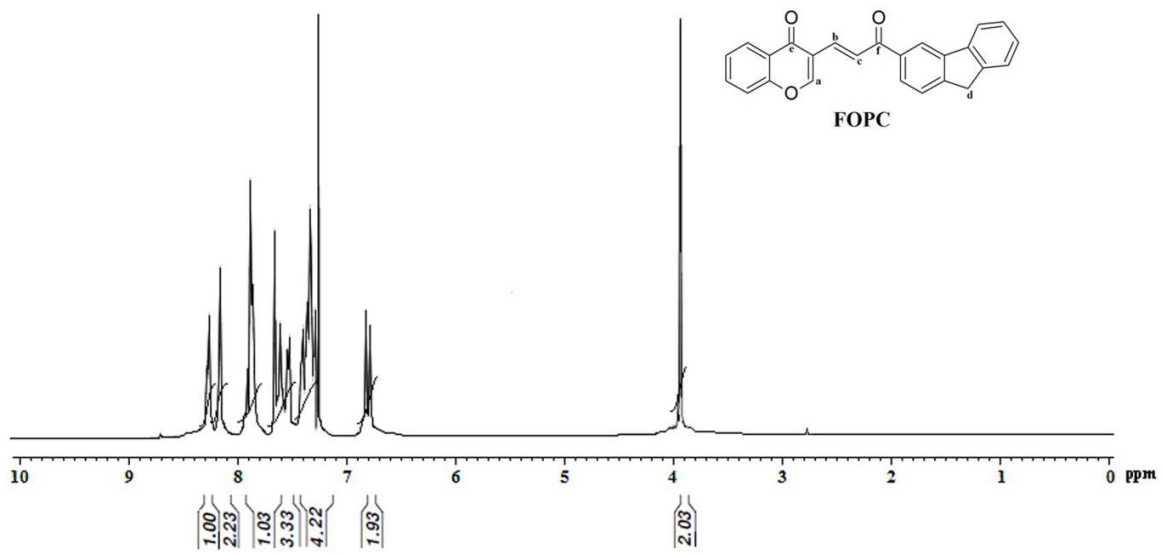
SI 7. ^{1}H NMR spectrum of DPAPP



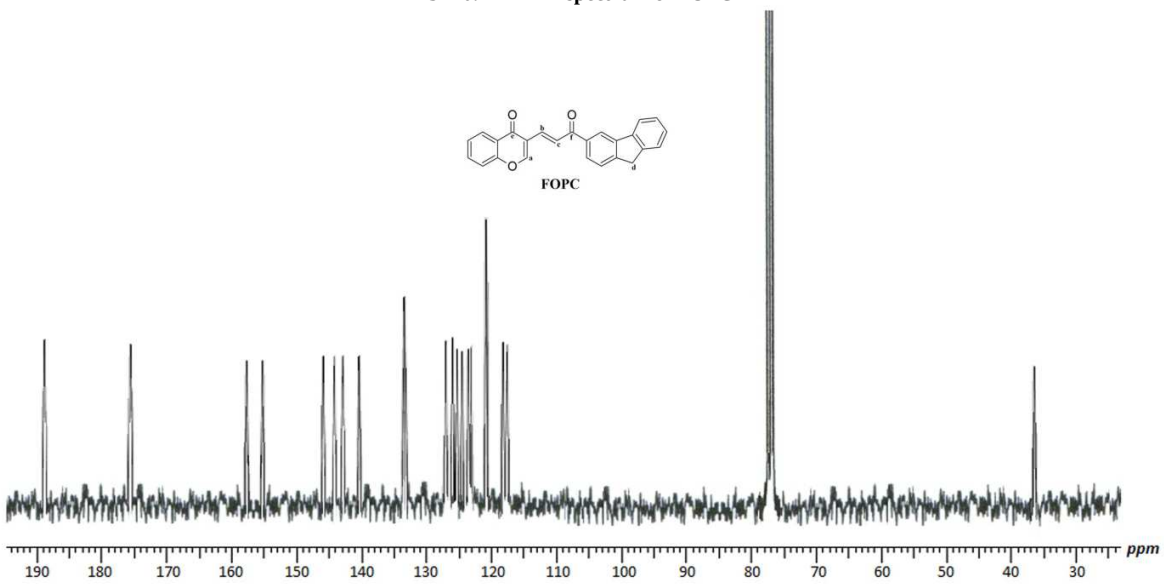
SI 8. ^{13}C NMR spectrum of DPAPP



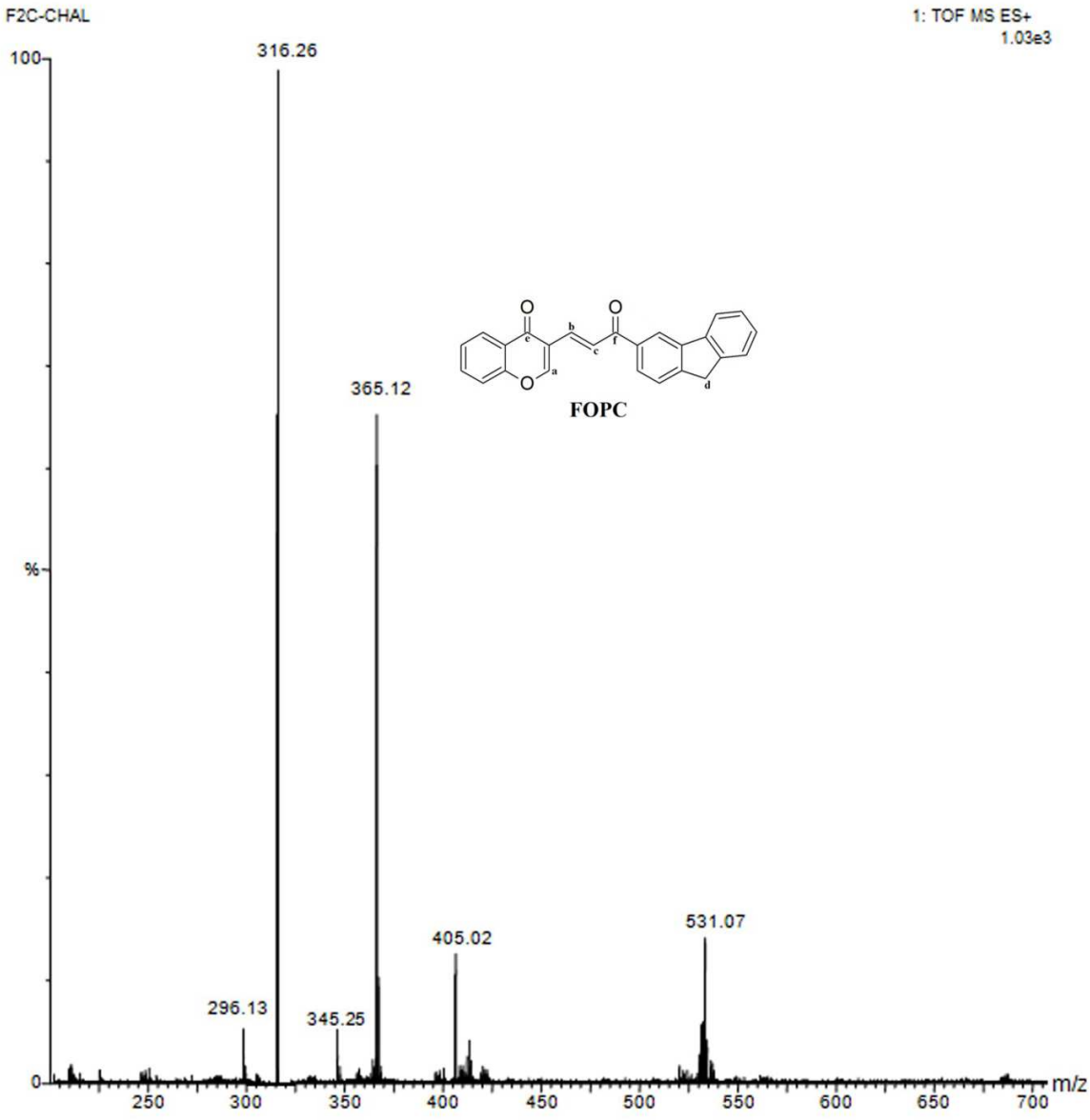
SI 9. Mass spectrum of DPAPP



SI 10. ^{1}H NMR spectrum of FOPC



SI 11. ^{13}C NMR spectrum of FOPC



SI 12. Mass spectrum of FOPC