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Article

# Synthesis and Antimicrobial Properties of 1,3,4-Oxadiazole Analogs Containing Dibenzosuberane Moiety

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Uma série de dez novos análogos de 1,3,4-oxadiazóis contendo uma parte dibenzosuberano foram sintetizados usando tanto uma aproximação de síntese linear, quanto convergente. Todos os compostos foram caracterizados por espectrometria de massas, espectroscopias no infravermelho (IR), de ressonância magnética nuclear de $^{1}$H ($^{1}$H NMR ) e $^{13}$C ($^{13}$C NMR) e análise elementar. As atividades bactericidas e antifúngicas destes compostos foram avaliadas, determinando-se que entre estes, quatro derivados, especificamente **8a, 8d**, **8e** e **8j**, foram altamente bactericidas e antifúngicos.

A series of ten novel 1,3,4-oxadiazole analogs containing dibenzosuberane moiety were synthesized using linear as well as convergent synthesis approach. All the compounds were characterized by mass spectrometry, infrared (IR), $^{1}$H and $^{13}$C nuclear magnetic resonance ($^{1}$H NMR and $^{13}$C NMR) spectroscopies and elemental analysis. These compounds were evaluated for antibacterial and antifungal activities. Among ten analogs, four compounds, namely, **8a, 8d**, **8e** and **8j** were found to be highly active antibacterial and antifungal agents.

**Keywords:** dibenzosuberane, 1,3,4-oxadiazole, antibacterial, antifungal

## Introduction

The rigid, tricyclic framework of dibenzosuberane (10,11-dihydro-5*H*-dibenzo[a,d]cycloheptene, (A), Figure 1) constitutes an integral part of the structure of molecules that are known to be effective for the treatment of depressive disorders.[1] Analogs of dibenzosuberane such as amitriptyline[2] ((B), Figure 1) and nortriptyline[3] ((C), Figure 1) are well known tricyclic antidepressants which are used as first line medicines in the treatment of migraines, tension headaches, anxiety, psychosis, aggression and violent behavior. Derivative of dibenzosuberane, 1,1a,6,10b-tetrahydrodibenzo[a,e]cyclopropa[c]cycloheptene ((D), Figure 1), obtained on annellation of a cyclopropyl ring to the dibenzosuberane framework, has been incorporated in the structure of potent multiple drug resistance modulators.[4] Easy access and close structural resemblance to popular pharmaceutical intermediate, dibenzazepine, makes dibenzosuberane an attractive drug scaffold.

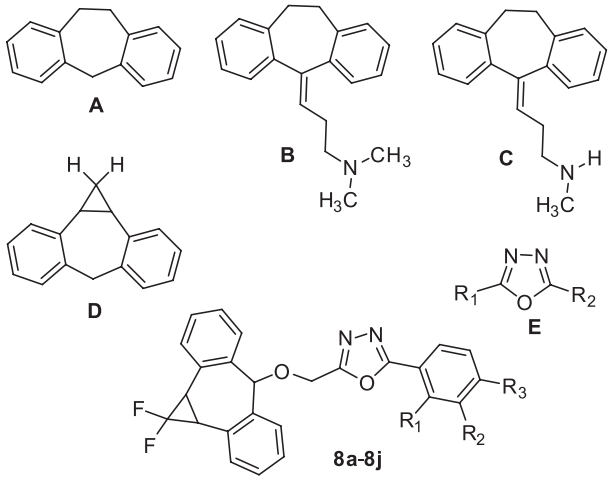


**Figure 1.** Structures of dibenzosuberane (A), amitriptyline (B), nortriptyline (C), 1,1a,6,10b-tetrahydrodibenzo[a,e]cyclopropa[c] cycloheptene (D), 2,5-disubstituted-1,3,4-oxadiazole (E) and 1,3,4-oxadiazole analogs containing dibenzosuberane unit (**8a**-**8j**).

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2,5-Disubstituted-1,3,4-oxadiazoles ((E), Figure 1) are known to exhibit a broad range of potent biological activities. A number of 1,3,4-oxadiazoles having appropriate substituents at 2 and 5 position are widely used as antimicrobial,[5] anti-HIV,[6] analgesic,[7] antiinflammatory,[8,9] anticonvulsant,[10,11] and antitumor[12,13] agents. We envisaged that attachment of dibenzosuberane framework to versatile 1,3,4-oxadiazole ring would produce synergistic effect and lead to novel hybrid molecules that may possess interesting biological activities. To validate this hypothesis, a series of ten novel hybrid molecules (**8a**-**8j**, Figure 1) in which a derivative of dibenzosuberane, 1,1-difluoro-1a,10b-dihydrodibenzo[a,e]cyclopropa[c]cycloheptane, was linked to 2-methyl-5-(substituted-phenyl)-1,3,4-oxadiazole ring were synthesized. All ten novel compounds were characterized by mass spectrometry, IR and NMR spectroscopy and elemental analysis and evaluated for antimicrobial properties. The study revealed that a number of novel dibenzosuberane containing 1,3,4-oxadiazole analogs possessed excellent antibacterial as well as antifungal activity.

## Results and Discussion

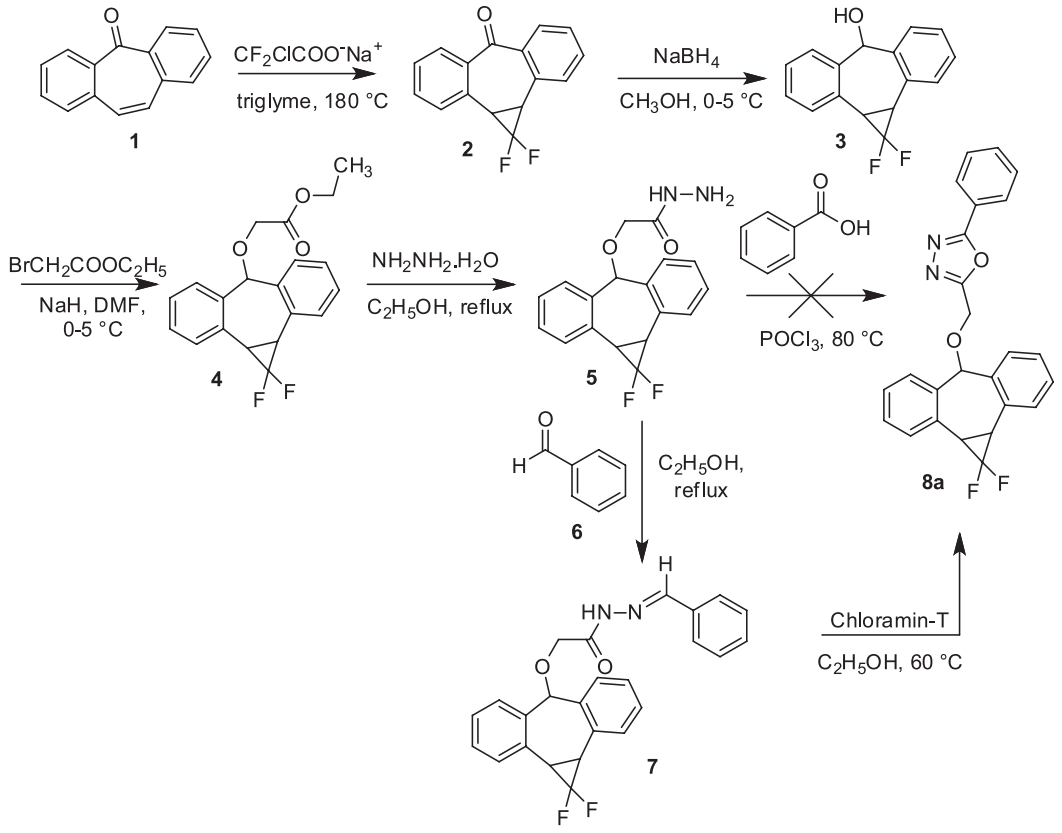
### Synthesis of novel 1,3,4-oxadiazole analogs containing dibenzosuberane moiety (**8a**-**8j**)

In order to synthesize novel 1,3,4-oxadiazole analogs containing dibenzosuberane unit (**8a**-**8j**), first, we adopted linear strategy as disclosed in Scheme 1. Dibenzosuberenone **1** was heated with sodium chlorodifluoroacetate in triglyme at 180-190 °C to obtain 1,1-difluoro-1a,10b-dihydrodibenzo[a,e]cyclopropa[c] cyclohepten-6-one **2**. Compound **2** on reduction with sodium borohydride in methanol at 0-5 °C afforded corresponding alcohol **3**[14] which on treatment with ethyl bromoacetate in presence of sodium hydride in DMF gave compound **4**. The reaction of compound **4** with hydrazine hydrate in ethanol under reflux yielded hydrazide **5**. Interestingly, cyclization reaction of hydrazide **5** with benzoic acid in phosphorous oxychloride to construct 1,3,4-oxadiazole framework did not give corresponding dibenzosuberane-1,3,4-oxadiazole analog **8a**. Alternatively, hydrazide **5** was treated with benzaldehyde (**6**) in ethanol under reflux to obtain corresponding *N*'-benzylidenehydrazide (**7**) which was cyclized using chloramine-T in ethanol at 60 °C to afford desired analog (**8a**). Although, the compound was isolated in good purity, yield of the final cyclization reaction did not exceed 15%.

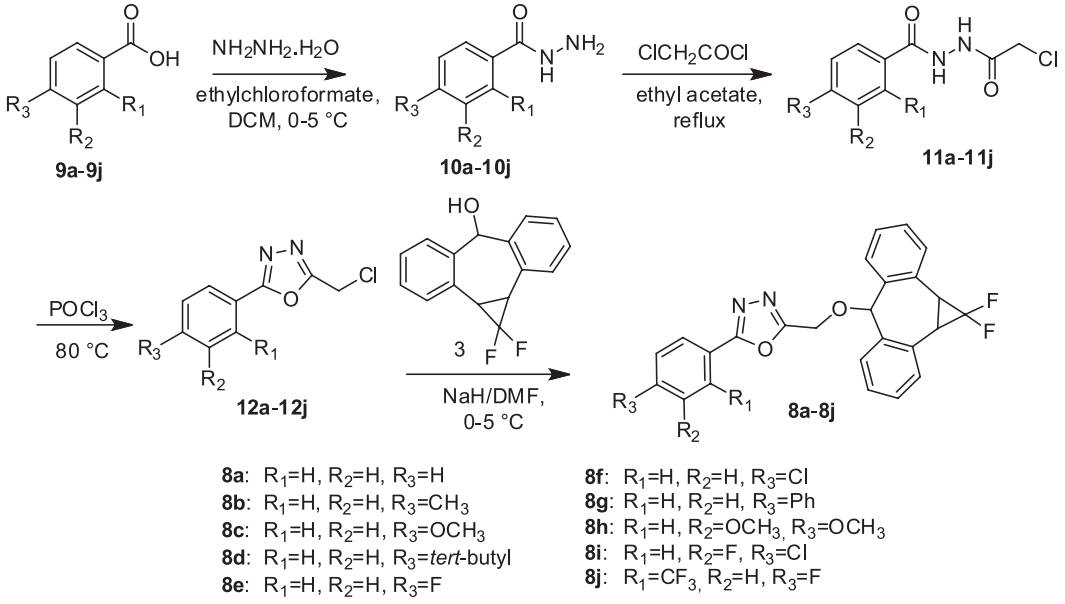
Lower yield in the linear synthesis strategy (Scheme 1) prompted us to explore alternate convergent synthesis approach as depicted in Scheme 2. 2-Chloromethyl-5-(substituted-phenyl)-1,3,4-oxadiazoles[15] (**12a**-**12j**) needed for the convergent synthesis route were prepared by reacting appropriately substituted benzoic acids (**9a**-**9j**) with hydrazine hydrate in the presence of ethyl chloroformate in DCM to obtain corresponding hydrazides (**10a**-**10j**) which were further reacted with chloroacetyl chloride in ethyl acetate under reflux to afford corresponding *N*'-(2-chloroacetyl)-substituted-phenyl hydrazides (**11a**-**11j**). Compounds (**11a**-**11j**) thus obtained were cyclized in phosphorus oxychloride at 80 °C to yield corresponding 2-chloromethyl-5-(substituted-phenyl)-1,3,4-oxadiazoles (**12a**-**12j**). Coupling of compound **3**[14] with suitable 2-chloromethyl-5-(substituted-phenyl)-1,3,4-oxadiazole (**12a**-**12j**) in DMF in the presence of sodium hydride gave desired dibenzosuberane containing analogs of 1,3,4-oxadiazole (**8a**-**8j**) in good to very good (60-77%) yield. All the compounds were purified by column chromatography using silica gel and gradient (0-50%) ethyl acetate in hexane as the eluent and characterized by $^{1}$H NMR, $^{13}$C NMR, IR spectroscopy, mass spectrometry and elemental analysis.

### Antibacterial and antifungal activity of 1,3,4-oxadiazole analogs containing dibenzosuberane unit (**8a-8j**)

With a range of target molecules at hand, their antibacterial activity against four bacterial strains namely, *Staphylococcus aureus, Escherichia coli, Pseudomonas aeroginosa* and *Klebsiella pneumonia* was determined using disc diffusion method[16,17] and compared with well-known antibacterial drug, nitrofurazone. The minimum inhibitory concentration (MIC, μg mL$^{-1}$) was determined for each compound in triplicate experiments, the values were averaged and are presented in Table 1. Among ten analogs, four analogs **8a**, **8d**, **8e** and **8j** showed very good activity against all the bacterial stains. Except three compounds **8g**, **8h** and **8i** remaining all compounds were found to be active against *Staphylococcus aureus*. Out of ten analogs, two analogs **8b** and **8h** were found to be inactive against *Escherichia coli*. Eight analogs namely, **8a**, **8b**, **8d**, **8e**, **8f**, **8g**, **8h**, and **8j** showed very good activity against *Pseudomonas aeroginosa*. Two analogs **8f** and **8i** were found to be inactive against *Klebsiella pneumonia*. The values presented in Table 1 reveal that type of substituent on the phenyl ring directly attached to 1,3,4-oxadiazole ring has a significant impact on the antibacterial activity of these novel analogs. In particular, electron donating methoxy substituent decreases the activity to a greater extent. Halogen substituent, especially fluorine, at the *para* position on




**Scheme 1.** Linear synthesis pathway of 1,3,4-oxadiazole analogs containing dibenzosuberane unit.




**8a**: $R_1$=H, $R_2$=H, $R_3$=H
**8b**: $R_1$=H, $R_2$=H, $R_3$=$CH_3$
**8c**: $R_1$=H, $R_2$=H, $R_3$=$OCH_3$
**8d**: $R_1$=H, $R_2$=H, $R_3$=*tert*-butyl
**8e**: $R_1$=H, $R_2$=H, $R_3$=F
**8f**: $R_1$=H, $R_2$=H, $R_3$=Cl
**8g**: $R_1$=H, $R_2$=H, $R_3$=Ph
**8h**: $R_1$=H, $R_2$=$OCH_3$, $R_3$=$OCH_3$
**8i**: $R_1$=H, $R_2$=F, $R_3$=Cl
**8j**: $R_1$=$CF_3$, $R_2$=H, $R_3$=F

**Scheme 2.** Convergent synthesis pathway of 1,3,4-oxadiazole analogs containing dibenzosuberane unit.

phenyl ring result in higher antibacterial activity (e.g., compounds **8e**, **8f** and **8j**). However, fluorine atom at *meta* position in combination with chlorine at *para* position of the phenyl ring diminishes antibacterial activity to a great extent (compound **8i**). Interestingly, compound **8a**, devoid of any substitution on the phenyl ring, showed remarkable activity against all four bacterial strains. Compounds **8a**, **8d**, **8e**, and **8j** showed uniform activity against gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli, Pseudomonas aeroginosa* and *Klebsiella pneumoniae*) bacterial strains. Compound **8g** was found to be inactive against gram-positive bacteria and moderately active

**Table 1.** Antibacterial activity of analogs (**8a**-**8j**)

| Compound | Antibacterial activity Minimum Inhibitory Concentration (MIC) and zone of inhibition[a] | | | |
|---|---|---|---|---|
| | *Staphylococcus aureus* | *Escherichia coli* | *Pseudomonas aeroginosa* | *Klebsiella pneumoniae* |
| **8a** | 6.25 (18) | 12.5 (21) | 6.25 (23) | 6.25 (16) |
| **8b** | 12.5 (17) | 50 (14) | 12.5 (11) | 12.5 (18) |
| **8c** | 6.25 (20) | 12.5 (17) | 100 (16) | 12.5 (16) |
| **8d** | 12.5 (18) | 12.5 (32) | 12.5 (21) | 12.5 (18) |
| **8e** | 6.25 (18) | 6.25 (20) | 6.25 (14) | 12.5 (19) |
| **8f** | 6.25 (23) | 12.5 (18) | 12.5 (16) | 50 (11) |
| **8g** | 100 (26) | 12.5 (24) | 12.25 (15) | 12.5 (13) |
| **8h** | 50 (11) | 100 (09) | 6.25 (12) | 12.5 (17) |
| **8i** | 50 (15) | 12.5 (21) | 100 (16) | 100 (13) |
| **8j** | 12.5 (26) | 6.25 (22) | 6.25 (20) | 6.25 (25) |
| Nitrofurazone | < 6.25 (32) | < 6.25 (30) | < 6.25 (36) | < 6.25 (32) |
| DMSO (1%) Solvent control | 00 | 00 | 00 | 00 |

[a]Zone of inhibition in mm is given in parenthesis; MIC is expressed in μg $mL^{-1}$ (MIC for the standard drug, nitrofurazone, is reported earlier[19]); standard drug used: nitrofurazone; solvent control: 1% DMSO.

against gram-negative bacteria. In general, all compounds showed broad spectrum of activity against both gram-positive and gram-negative bacterial strains.

All compounds were also assessed for antifungal activity against *Penicillium marneffei* (recultured)*, Trichophyton mentagrophytes* (recultured)*, Aspergillus flovus* (NCIM No. 524) and *Aspergillus fumigatus* (NCIM No. 902). The compounds were dissolved in DMSO and antifungal activity was determined using serial dilution method.[18] For comparison, well-known antifungal drug Amphotericin B was used as a standard. The minimum inhibitory concentration (MIC, μg $mL^{-1}$) was determined for each compound in triplicate experiments; the values were averaged and are presented in Table 2. Six compounds **8a**, **8b**, **8d**, **8e**, **8h** and **8j** displayed very good activity against all four fungal strains. Compounds **8c** and **8i** showed activity against all strains except *Trichophyton mentagrophyte* and *Penicillium marneffei*, respectively. While compound **8f** was found to be active against two strains namely, *Penicillium marneffei* and *Trichophyton mentagrophyte*, compound **8g** showed activity against *Trichophyton mentagrophytes* and *Aspergillus fumigatus*. The values presented in the Table 2 reveal interesting trends in antifungal activity of compounds depending on substitution pattern on the phenyl ring attached to 1,3,4-oxadiazole ring. Alkyl substituents on the phenyl ring, as in the case of compounds **8b** and **8d** impart good antifungal activity against all four

**Table 2**. Antifungal activity of analogs (**8a**-**8j**)

| Compound | Antifungal activity Minimum Inhibitory Concentration (MIC) and zone of inhibition[a] | | | |
|---|---|---|---|---|
| | *Penicillium marneffei* | *Trichophyton mentagrophyte* | *Aspergillus flovus* | *Aspergillus fumigatus* |
| **8a** | 12.5 (10) | 12.5 (13) | 6.25 (11) | 6.25 (16) |
| **8b** | 12.5 (12) | 12.5 (11) | 12.5 (12) | 12.5 (18) |
| **8c** | 6.25 (11) | R | 12.5 (09) | 12.5 (11) |
| **8d** | 12.5 (16) | 12.5 (16) | 12.5 (12) | 12.5 (13) |
| **8e** | 6.25 (16) | 6.25 (12) | 6.25 (12) | 12.5 (14) |
| **8f** | 6.25 (14) | 12.5 (14) | R | R |
| **8g** | R | 12.5 (13) | R | 12.5 (14) |
| **8h** | 6.25 (12) | 12.5 (13) | 12.5 (12) | 12.5 (12) |
| **8i** | R | 6.25 (10) | 12.5 (14) | 6.25 (12) |
| **8j** | 12.5 (13) | 6.25 (12) | 6.25 (12) | 6.25 (13) |
| Amphotericin B | < 6.25 (33) | < 6.25 (38) | < 6.25 (29) | < 6.25 (24) |
| DMSO (1%) Solvent control | 00 | 00 | 00 | 00 |

[a]Zone of inhibition in mm is given in parenthesis; MIC is expressed in μg $mL^{-1}$ (MIC for the standard drug, amphotericin B, is reported earlier[19]); standard drug used: amphotericin B; solvent control: 1% DMSO; R: resistant.

strains. Electron withdrawing fluorine substituent at *para* position of the phenyl ring, as in the case of compounds **8e** and **8j**, resulted in remarkably high antifungal activity against all strains. Presence of chlorine substituent at the *para* position of the phenyl ring (compound **8f**) caused diminished activity against *Aspergillus flovus* and *Aspergillus fumigatus*. Interestingly, this trend was reversed by introduction of fluorine substituent at *meta* position next to chloro group (compound **8i**). Presence of electron donating methoxy group on the phenyl ring caused good to very good antifungal activity (compound **8c** and **8h**). However, compound **8c** having *para*-methoxy substituent showed resistance to *Trichophyton mentagrophyte* strain. The compound **8a** having no substitution on the phenyl ring attached to 1,3,4-oxadiazole ring showed good antifungal activity against *Penicillium marneffei* and *Trichophyton mentagrophyte* and very good activity against *Aspergillus flovus* and *Aspergillus fumigatus*. Phenyl substitution at *para* position of the phenyl ring (compound **8g**) caused resistance for *Penicillium marneffei* and *Aspergillus flovus* strains.

## Conclusions

A series of ten novel 1,3,4-oxadiazole analogs containing dibenzosuberane moiety (**8a**-**8j**) having various substituents on the phenyl ring attached to 1,3,4-oxadiazole ring were synthesized and well characterized. All the analogs were investigated for antibacterial and antifungal activities. In general, all compounds showed moderate-to-good activity against the bacterial and fungal strains used in this study. Four compounds **8a, 8d**, **8e** and **8j** were uniformly active against all bacterial as well as fungal strains used for the study. *para*-Fluoro substitution on the phenyl ring attached to 1,3,4-oxadiazole framework causes marked increase in both antibacterial as well as antifungal activities of the novel molecules.

## Experimental

### Material and methods

All chemicals used for the synthesis were of reagent grade and procured from Sigma-Aldrich, Bangalore, India. $^1$H and $^{13}$C NMR spectra were recorded on AS 400 MHz Varian NMR spectrometer using TMS as an internal standard. IR spectra were recorded by using Perkin-Elmer Spectrum 100 Series FT-IR spectrometer. Mass spectra were recorded on Agilent 1200 Series LC/MSD VL system. Melting points were determined by using Büchi melting point B-545 instrument and are uncorrected. All the reactions were monitored by thin layer chromatography (TLC) using precoated silica 60 F254, 0.25 mm aluminum plates (Merck). The crude compounds were purified by column chromatography using silica gel (100-200) and gradient (0-50%) ethyl acetate in hexane as the eluent system.

### Synthesis

#### General procedure for the preparation of 1,3,4-oxadiazole analogs containing dibenzosuberane moiety (**8a-8j**)

A solution of compound **3**[14] (200 mg, 0.77 mmol) in DMF (5.0 mL) was cooled to 0-5 °C. Sodium hydride (60%, 47.0 mg, 1.16 mmol) was added to it and stirred for 15 min. To the reaction mixture was added suitably substituted 2-chloromethyl-5-phenyl-1,3,4-oxadiazole[15] (**12a**-**12j**, Scheme 2) (0.85 mmol, 1.1 eq) at 0-5 °C and stirred for 1.0 h. Progress of the reaction was monitored by TLC. Upon completion, the reaction mixture was poured on crushed ice. The precipitated solid was filtered, washed with water and dried. The crude product was purified by column chromatography using silica gel (100-200) and gradient (0-50%) ethyl acetate in hexane as the eluent.

#### 1,1-Difluoro-1,1a,6,10b-tetrahydro-(1a,6,10b)-dibenzo[a,e] cyclopropa[c]cyclohepten-6-yloxymethyl-5-phenyl-[1,3,4]-oxadiazole (**8a**)

White solid; yield 77%; mp 183-185 °C; IR (KBr) $\nu_{max}$/cm$^{-1}$: 3011, 1456, 1169, 747; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.53 (d, 2H, *J* 13.6 Hz), 5.09 (s, 2H), 6.55 (s, 1H), 7.20-7.27 (m, 6H), 7.45 (d, 2H, *J* 7.2 Hz), 7.60-7.66 (m, 3H), 8.01-8.03 (m, 2H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 27.3, 61.7, 77.8, 122.5, 123.6, 126.8, 127.1, 127.8, 128.3, 129.9, 131.8, 132.6, 142.7, 163.7, 165.1; MS (ESI) *m/z*: 417.1 $[M + H]^+$; anal. calcd. for $C_{25}H_{18}F_2N_2O_5$: C, 72.11; H, 4.36; F, 9.12; N, 6.73; O, 7.68; found: C, 72.13; H, 4.31; F, 9.14; N, 6.68; O, 7.62.

#### 1,1-Difluoro-1,1a,6,10b-tetrahydro-(1a,6,10b)-dibenzo [a,e]cyclopropa[c]cyclohepten-6-yloxymethyl-5-(4-methyl-phenyl)-[1,3,4]-oxadiazole (**8b**)

Off white solid; yield 76%; mp 218-220 °C; IR (KBr) $\nu_{max}$/cm$^{-1}$: 3011, 1495, 1169, 747; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.4 (s, 3H), 3.52 (d, 2H, *J* 13.6 Hz), 5.07 (s, 2H), 6.54 (s, 1H), 7.23-7.26 (m, 6H), 7.41 (m, 4H), 7.88 (d, 2H, *J* 8.4 Hz); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 26.3, 32.2, 66.5, 82.5, 125.5, 127.3, 131.3, 131.8, 132.5, 133.1, 135.2, 136.6, 147.5, 147.6, 168.2, 169.9; MS (ESI) *m/z*: 431.1 $[M + H]^+$; anal. calcd. for $C_{26}H_{20}F_2N_2O_2$: C, 72.55; H, 4.68; F, 8.83; N, 6.51; O, 7.43; found: C, 72.51; H, 4.60; F, 8.85; N, 6.47; O, 7.41.

1,1-Difluoro-1,1a,6,10b-tetrahydro-(1a,6,10b)-dibenzo [a,e] cyclopropa[c]cyclohepten-6-yloxymethyl-5-(4-methoxyphenyl)-[1,3,4]-oxadiazole (**8c**)

White solid; yield 65%; mp 165-168 °C; IR (KBr) $\nu_{max}$/cm$^{-1}$: 3012, 1613, 1496, 1260, 746; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.53 (d, 2H, *J* 13.6 Hz), 3.86 (s, 3H), 5.06 (s, 2H), 6.54 (s, 1H), 7.15 (d, 2H, *J* 8.8 Hz), 7.23-7.27 (m, 6H), 7.44 (d, 2H, *J* 6.4 Hz), 7.93-7.97 (m, 2H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 27.3, 55.9, 61.7, 77.7, 115.4, 115.9, 122.5, 126.8, 127.8, 128.3, 128.9, 131.8,142.7, 162.6, 163.1, 165.1; MS (ESI) *m/z*: 447.1 [M + H]$^+$; anal. calcd. $C_{26}H_{20}F_2N_2O_3$: C, 69.12; H, 3.94; F, 13.12; N, 6.45; O, 7.37; found: C, 69.15; H, 3.91; F, 13.09; N, 6.41; O, 7.30.

1,1-Difluoro-1,1a,6,10b-tetrahydro-(1a,6,10b)-dibenzo[a,e] cyclopropa[c]cyclohepten-6-yloxymethyl-5-(4-*tert*-butylphenyl)-[1,3,4]-oxadiazole (**8d**)

White solid; yield 73%; mp 157-160 °C; IR (KBr) $\nu_{max}$/cm$^{-1}$: 2960, 1460, 1165, 749; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 1.32 (s, 9H), 3.53 (d, 2H, *J* 13.2 Hz), 5.08 (s, 2H), 6.54 (s, 1H), 7.21-7.27 (m, 6H), 7.44 (d, 2H, *J* 6.8 Hz), 7.63 (d, 2H, *J* 8.8 Hz), 7.92 (d, 2H, *J* 10.4 Hz); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 27.3, 31.2, 35.3, 61.7, 77.7, 120.8, 122.5, 126.7, 126.9, 127.8, 128.3, 131.9, 142.7, 155.6, 163.5, 165.1; MS (ESI) *m/z*: 473.2 [M + H]$^+$; anal. calcd. for $C_{29}H_{26}F_2N_2O_2$: C, 73.71; H, 5.55; F, 8.04; N, 5.93; O, 6.77; found: C, 73.75; H, 5.51; F, 8.10; N, 5.90; O, 6.77.

1,1-Difluoro-1,1a,6,10b-tetrahydro-(1a,6,10b)-dibenzo[a,e] cyclopropa[c]cyclohepten-6-yloxymethyl-5-(4-fluorophenyl)-[1,3,4]-oxadiazole (**8e**)

White solid; yield 62%; mp 220-223 °C; IR (KBr) $\nu_{max}$/cm$^{-1}$: 3011, 1607, 1496, 1168, 746; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.53 (d, 2H, *J* 13.2 Hz), 5.08 (s, 2H), 6.54 (s, 1H), 7.20-7.27 (m, 6H), 7.44-7.49 (m, 4H), 8.06-8.09 (m, 2H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 27.3, 61.7, 77.8, 117.1, 117.3, 120.3, 122.5, 126.8, 127.80, 128.3, 129.8, 129.9, 131.9,142.7, 163.7, 164.3; MS (ESI) *m/z*: 435.1 [M + H]$^+$; anal. calcd. for $C_{25}H_{17}F_3N_2O_2$: C 69.95; H, 4.52; F, 8.51; N, 6.27; O, 10.75; found: C, 69.93; H, 4.47; F, 8.49; N, 6.21; O, 10.72.

1,1-Difluoro-1,1a,6,10b-tetrahydro-(1a,6,10b)-dibenzo[a,e]cyclopropa[c]cyclohepten-6-yloxymethyl-5-(4-chlorophenyl)-[1,3,4]-oxadiazole (**8f**)

White solid; yield 70%; mp 212-214 °C; IR (KBr) $\nu_{max}$/cm$^{-1}$: 3006, 1607, 1458, 1166, 747; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.53 (d, 2H, *J* 13.2 Hz), 5.09 (s, 2H), 6.55 (s, 1H), 7.22-7.27 (m, 6H), 7.44 (d, 2H, *J* 6.4 Hz), 7.69 (d, 2H, *J* 8.4 Hz), 8.01 (d, 2H, *J* 8.8 Hz); $^{13}$C NMR (100 MHz, DMSO-d$_6$) $\delta$ 27.4, 61.7, 77.8, 122.5, 126.8, 127.8, 128.3, 128.9, 130.1, 131.8, 137.4, 142.7, 163.9, 164.3; MS (ESI) *m/z*: 451.0 [M + H]$^+$; anal. calcd. for $C_{25}H_{17}ClF_2N_2O_2$: C, 66.60; H, 3.80; Cl, 7.86; F, 8.43; N, 6.21; O, 7.10; found: C, 66.66; H, 3.77; Cl, 7.80; F, 8.47; N, 6.18; O, 7.06.

1,1-Difluoro-1,1a,6,10b-tetrahydro-(1a,6,10b)-dibenzo [a,e]cyclopropa[c]cyclohepten-6-yloxymethyl-5-(4-phenylphenyl)-[1,3,4]-oxadiazole (**8g**)

White solid; yield 70%; mp 241-244 °C; IR (KBr) $\nu_{max}$/cm$^{-1}$ 3011, 1455, 1169, 747; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.54 (d, 2H, *J* 13.6 Hz), 5.11 (s, 1H), 6.56 (s, 1H), 7.22-7.27 (m, 6H), 7.44-7.55 (m, 5H), 7.77 (d, 2H, *J* 7.2 Hz), 7.92 (d, 2H, *J* 8.4 Hz), 8.09 (d, 2H, *J* 8.4 Hz); MS (ESI) *m/z*: 493.0 [M + H]$^+$; anal. calcd. for $C_{31}H_{22}F_2N_2O_2$: C, 75.60; H, 4.50; F, 7.71; N, 5.69; O, 6.50; found: C, 75.65; H, 4.47; F, 7.76; N, 5.63; O, 6.55.

1,1-Difluoro-1,1a,6,10b-tetrahydro-(1a,6,10b)-dibenzo[a,e] cyclopropa[c]cyclohepten-6-yloxymethyl-5-(3,4-dimethoxyphenyl)-[1,3,4]-oxadiazole (**8h**)

White solid; yield 60%; mp 205-208 °C; IR (KBr) $\nu_{max}$/cm$^{-1}$: 3011, 1607, 1500, 1170, 748; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.53 (d, 2H, *J* 13.2 Hz), 3.85 (s, 6H), 5.07 (s, 2H), 6.54 (s, 1H ), 7.16-7.27 (m, 7H), 7.44-7.48 (m, 3H), 7.57 (dd, 1H, *J* 2.0, 8.0 Hz, ); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 27.4, 56.1, 61.7, 77.7, 109.5, 112.5, 115.8, 120.6, 122.5, 126.7, 127.8, 128.3, 131.9, 142.7, 149.5, 152.4, 163.2, 165.1; MS (ESI) *m/z*: 477.1 [M + H]$^+$; anal. calcd. $C_{27}H_{22}F_2N_2O_4$: C, 68.06; H, 4.65; F, 7.97; N, 5.88; O, 13.43; found: C, 68.02; H, 4.60; F, 7.92; N, 5.72; O, 13.41.

1,1-Difluoro-1,1a,6,10b-tetrahydro-(1a,6,10b)-dibenzo[a,e] cyclopropa[c]cyclohepten-6-yloxymethyl-5-(4-chloro-3-fluorophenyl)-[1,3,4]-oxadiazole (**8i**)

Off white solid; yield 60%; mp 207-210 °C; IR (KBr) $\nu_{max}$/cm$^{-1}$: 3010, 1608, 1497, 1168, 746; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.52 (d, 2H, *J* 13.2 Hz), 5.09 (s, 2H), 6.54 (s, 1H), 7.23-7.27 (m, 6H), 7.43 (d, 2H, *J* 6.4 Hz), 7.86 (d, 2H, *J* 3.6 Hz), 8.01 (d, 1H, *J* 8.8 Hz); MS (ESI) *m/z*: 469.0 [M + H]$^+$; anal. calcd. for $C_{25}H_{16}ClF_3N_2O_2$: C, 64.04; H, 3.44; Cl, 7.56; F, 12.16; N, 5.97; O, 6.82; found: C, 64.07; H, 3.40; Cl, 7.51; F, 12.11; N, 5.99; O, 6.85.

1,1-Difluoro-1,1a,6,10b-tetrahydro-(1a,6,10b)-dibenzo[a,e] cyclopropa[c]cyclohepten-6-yloxymethyl-5-(4-fluoro-2-trifluoromethylphenyl)-[1,3,4]-oxadiazole (**8j**)

Off white solid; yield 60%; mp 153-155 °C; IR (KBr) $\nu_{max}$/cm$^{-1}$: 3011, 2932, 1479, 1168, 748; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.51 (d, 2H, *J* 13.2 Hz), 5.10 (s, 2H), 6.54 (s, 1H), 7.21-7.27 (m, 6H), 7.42-7.49 (m, 2H), 7.77-7.86 (m, 1H), 7.99 (dd, 1H, *J* 2.4, 8.8 Hz), 8.12-8.15

(m, 1H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 27.3, 61.5, 77.7, 115.9, 116.2, 118.4, 120.8, 121.1, 122.4, 126.8, 127.8, 128.2, 128.3, 131.9, 135.4, 135.5, 142.6, 162.5, 162.7, 164.7; MS (ESI) *m/z*: 503.1 $[M + H]^+$; anal. calcd. for $C_{26}H_{16}F_6N_2O_2$: C, 62.16; H, 3.21; F, 22.69; N, 5.58; O, 6.37; found: C, 62.10; H, 3.24; F, 22.63; N, 5.52; O, 6.30.

### Biological assays

The bacterial strains were collected from patients with different infectious status who had not been administered any antibacterial drugs for at least two weeks with the suggestions of an authorized physician and authenticated by a microbiologist at Kiran Diagnostic Health Center, Chitradurga, Karnataka (India). Fungal strains were taken from Dept. of Post Graduate Studies and Research in Microbiology, Tumkur University, Tumkur, Karnataka (India).

#### Antibacterial activity

The newly synthesized compounds were screened for their antibacterial activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeroginosa* and *Klebsiella pneumoniae* strains by disc diffusion method.[16,17] The discs measuring 6.25 mm in diameter were punched from Whatman No. 1 filter paper. Batches of 100 discs were dispensed to each screw capped bottles and sterilized by dry heat at 140 °C for an hour. The test compounds were prepared with different concentrations using DMSO. One milliliter containing 100 times the amount of chemical required in each disc was added to each bottle which contained 100 discs. The discs of each concentration were placed in triplicate in nutrient agar medium seeded with fresh bacteria separately. The incubation was carried out at 37.8 °C for 24 h. The minimum inhibitory concentration (MIC) was noted. For comparison, nitrofurazone was used as a drug standard. Solvent and growth controls were kept. Antibacterial activity was determined by measuring the diameter of the inhibition zone. Zone of inhibition (inhibition halos) was calculated as average of three repeated experimental data. Compounds which inhibited bacteria growth (more than 3 mm zone of inhibition) were considered as active. MIC was determined for compounds which showed positive activity. The minimum inhibitory concentration (MIC) for the nitrofurazone in 1% DMSO was more than 1.0 mg mL$^{-1}$ against the tested species.[19] One percent DMSO was used as solvent control; the data is illustrated in Table 1.

#### Antifungal activity

Antifungal activity of the compounds was determined against *Penicillium marneffei* (recultured), *Trichophyton mentagrophytes* (recultured), *Aspergillus flovus* (NCIM No. 524) and *Aspergillus fumigatus* (NCIM No. 902) using serial dilution method.[18] Sabouraud's agar media was prepared by dissolving peptone (1.0 g), D-glucose (4.0 g) and agar (2.0 g) in distilled water (100 mL) and adjusting the pH to 5.7. Normal saline was used to make a suspension of spores of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3.0 mL saline to get a suspension of corresponding species. A 20.0 mL of agar media was poured into each of the Petri dishes. Excess of suspension was decanted and the plates were dried by placing in an incubator at 37 °C for 1.0 h. Using an agar punch wells were made on these seeded agar plates and 10 mg mL$^{-1}$ of the test compounds in 1% DMSO were added into each labeled well. A control was also prepared for the plates in the same way using 1% DMSO. The Petri dishes were prepared in triplicate and maintained at 37 °C for 3-4 days. Antifungal activity was determined by measuring the diameter of the inhibition zone. Zone of inhibition (inhibition halos) was calculated as average of three repeated experimental data. Compounds which inhibited fungal growth (more than 3 mm zone of inhibition) were considered as active. MIC was determined for compounds which showed positive activity. Activity of each compound was compared with amphotericin B as standard. The minimum inhibitory concentration (MIC) for the amphotericin B in 1% DMSO was more than 1.0 mg mL$^{-1}$ against the tested species.[19] One percent DMSO was used as solvent control; the data is illustrated in Table 2.

## Supplementary Information

$^1$H NMR, $^{13}$C NMR, IR and mass spectra of compounds are available free of charge at http://jbcs.sbq.org.br as a PDF file.

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## References

1. Gillman, P. K.; *Brit. J. Pharma.* **2007**, *151*, 737.
2. Mao, X.; Hou, T.; Cao, B.; Wang, W.; Li, Z.; Chen, S.; Fei, M.; Hurren, R.; Gronda, M.; Wu, D.; Trudel, S.; Schimmer, A. D.; *Mol. Pharm. Fast Forward* **2011**, *79*, 672.
3. Wilens, T. E.; Biederman, J.; Geist, D. E.; Steingard, R.; Spencer, T.; *J. Am. Acad. Child and Adolescent Psych.* **1993**, *32*, 343.

4. Pfister, J. R.; Makra, F.; Muehldorf, A. V.; Nelson, J. T.; Slate, D. L.; *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2473.
5. Oliveira, C. S.; Lira, B. F.; Barbosa-Filho, J. M.; Lorenzo, J. G.; Athayde-Filho, P. F.; *Molecules* **2012,** *17*, 5095.
6. Johns, B. A.; Weatherhead, J. G.; Allen, S. H.; Thompson, J. B.; Garvey, E. P.; Foster, S. A.; Jeffry, J. L.; Miller, W. H.; *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1807.
7. Amir, M.; Kumar, S.; *Acta Pharm.* **2007**, *57*, 31.
8. Manjunatha, K.; Poojary, B.; Lobo, P. L.; Fernandes, J.; Kumari, N. S.; *Eur. J. Med. Chem.* **2010**, *45*, 5225.
9. Palusa, S. K.; Udupi, R. H.; Himabindu, V.; Sridhara, A. M.; *Org. Commun.* **2011**, *4*, 82.
10. Kashaw, S. K.; Gupta, V.; Kashav, V; Mishra, P.; Stables, J. P.; Jain, N. K.; *Med. Chem. Res.* **2010**, *19*, 250.
11. Zarghi, A.; Tabatabai, S. A.; Faizi, M.; Ahadian, A.; Navabi, P.; Zanganeh, V.; Shafiee, A.; *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1863.
12. Savariz, F. C.; Formagio, A. S. N.; Barbosa, V. A.; Foglio, M. A.; Carvalho, J. E.; Duarte, M. C. T.; Dias Filho, B. P.; Sarragiotto, M. H.; *J. Braz. Chem. Soc.* **2010**, *21*, 288.
13. Tuma, M. C.; Malikzay, A.; Ouyang, X.; Surguladze, D.; Fleming, J.; Mitelman, S.; Camara, M.; Finnerty, B.; Doody, J.; Chekler, E. L.; Kussie, P.; Tonra, J. R.; *Trans. Oncology* **2010**, *3*, 318.
14. Astleford, B. A.; Barnett, C. J.; Kobierski, M. E.; Wilson, T. M.; *US pat. 6,570,016* **2003**.
15. Balsells, J.; DiMichele, L.; Liu, J.; Kubryk, M.; Hansen, K.; Armstrong, J. D.; *Org. Lett.* **2005**, *7*, 1039.
16. Collins, A. H.; *Microbiological Methods*, 2nd ed; Butterworth: London, 1976.
17. Arthington, B. A.; Motley, M.; Warnock, D. W.; Morrison, C. J.; *Clin. J. Microbiol.* **2000**, *38*, 2254.
18. Cruickshank, R.; Duguid, J. P.; Marmion, B. P.; Swain, R. H. A.; *Medical Microbiology*, *Vol. 2*, 12th ed.; Churchill Livingstone: London, 1975, p. 196.
19. Sarojini, B. K.; Krishna, B. G.; Darshanraj, C. G.; Bharath, B. R.; Majunatha, H.; *Eur. J. Med. Chem.* **2010**, *45*, 3490.

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# Supplementary Information

## Synthesis and Antimicrobial Properties of 1,3,4-Oxadiazole Analogs Containing Dibenzosuberane Moiety

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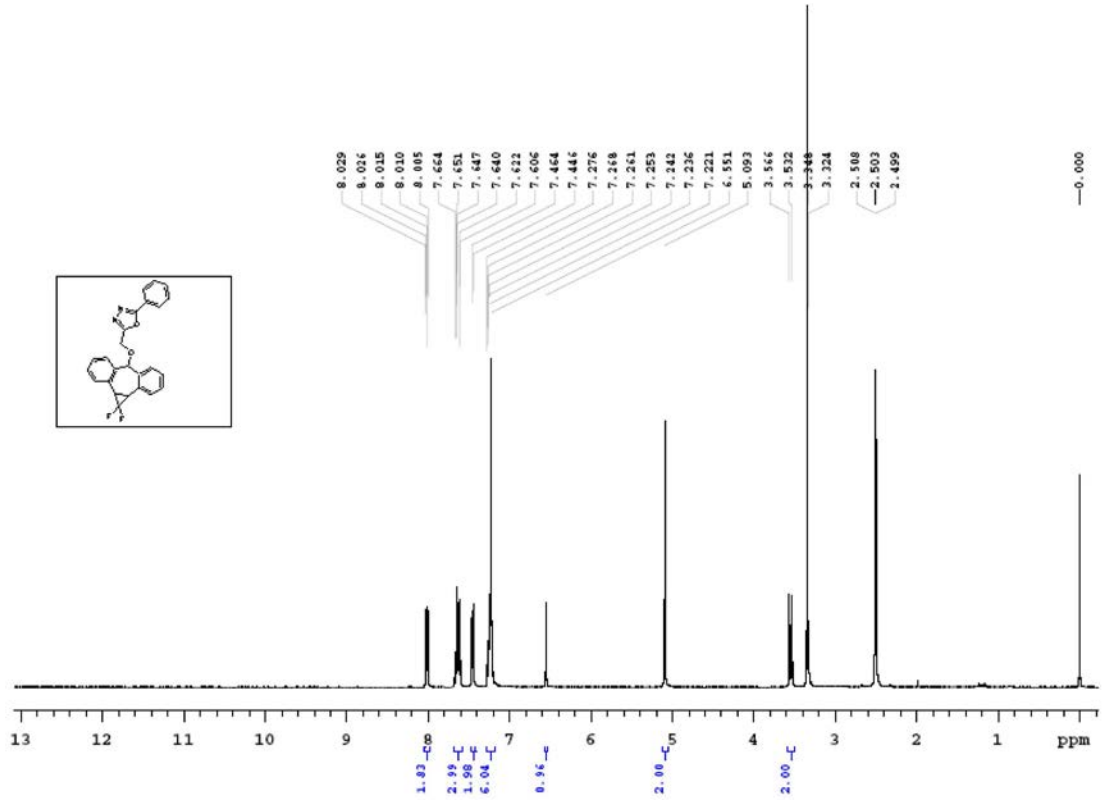


**Figure S1**. $^1$H NMR spectrum (400 MHz, DMSO-$d_6$) of compound **8a**.

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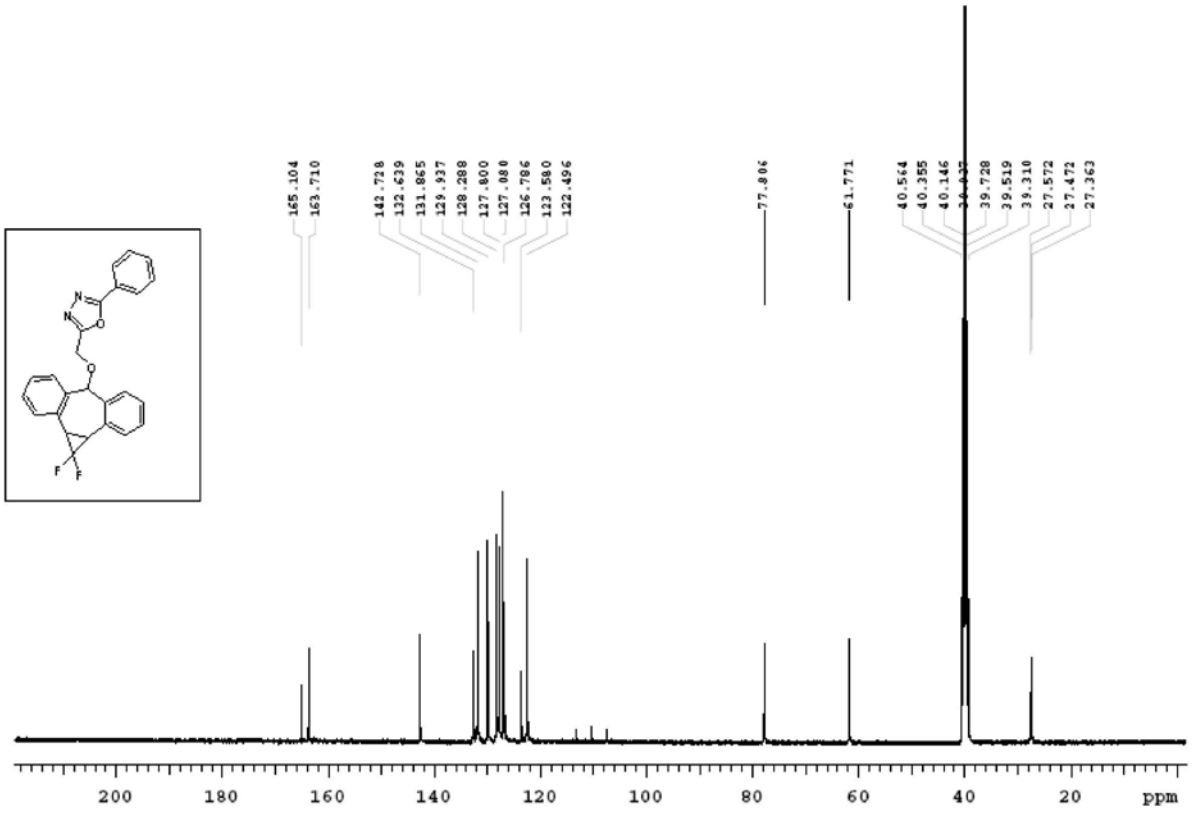


**Figure S2**. $^{13}C$ NMR spectrum (100 MHz, DMSO-$d_6$) of compound **8a**.

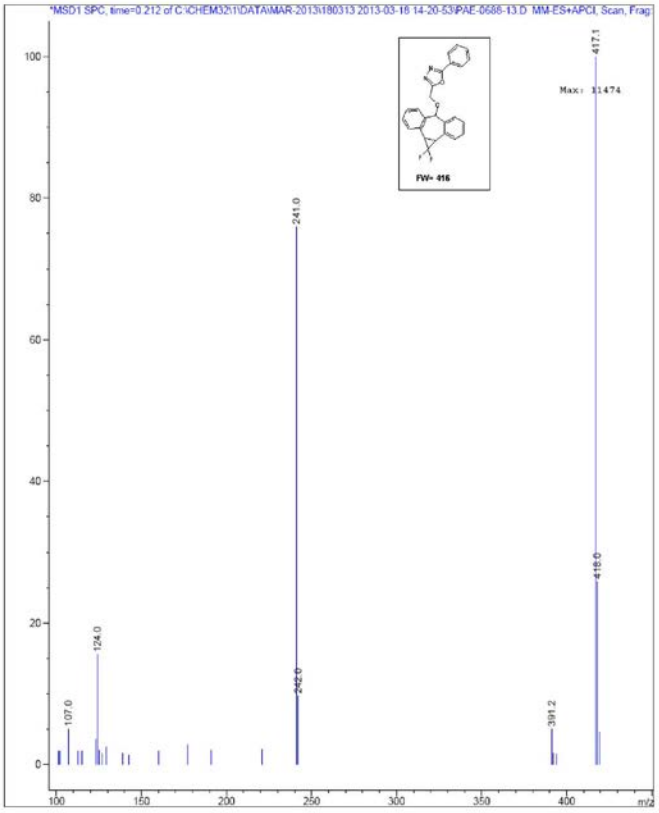


**Figure S3**. Mass spectrum of compound **8a**.

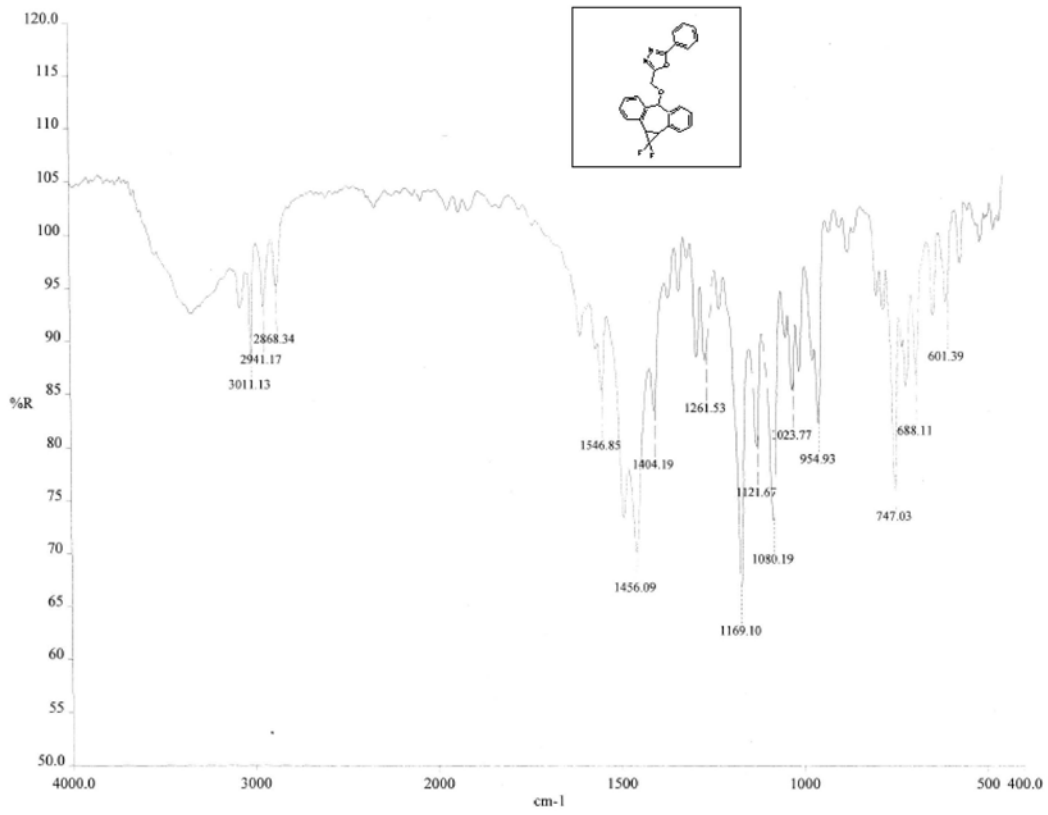


**Figure S4**. FT-IR (KBr) spectrum of compound **8a**.

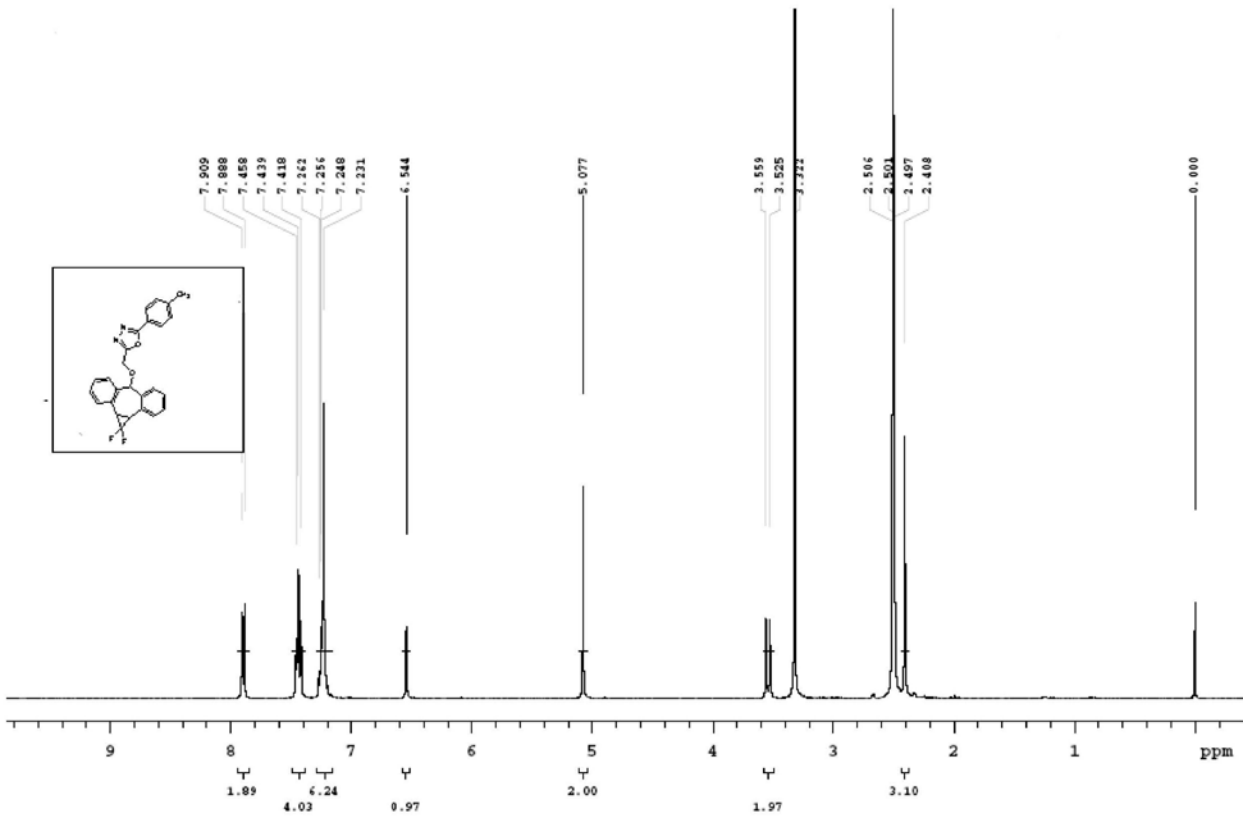


**Figure S5**. [1]H NMR spectrum (400 MHz, DMSO-$d_6$) of compound **8b**.

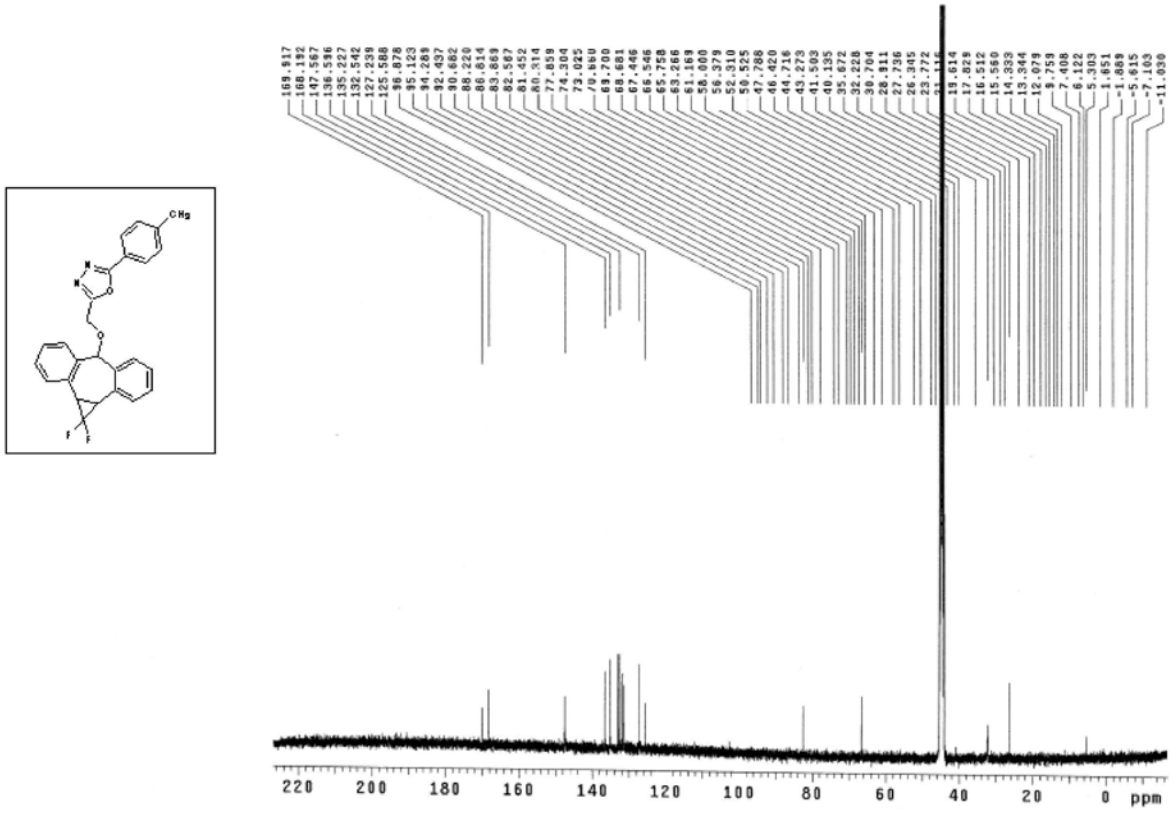


**Figure S6**. $^{13}C$ NMR spectrum (100 MHz, DMSO-$d_6$) of compound **8b**.

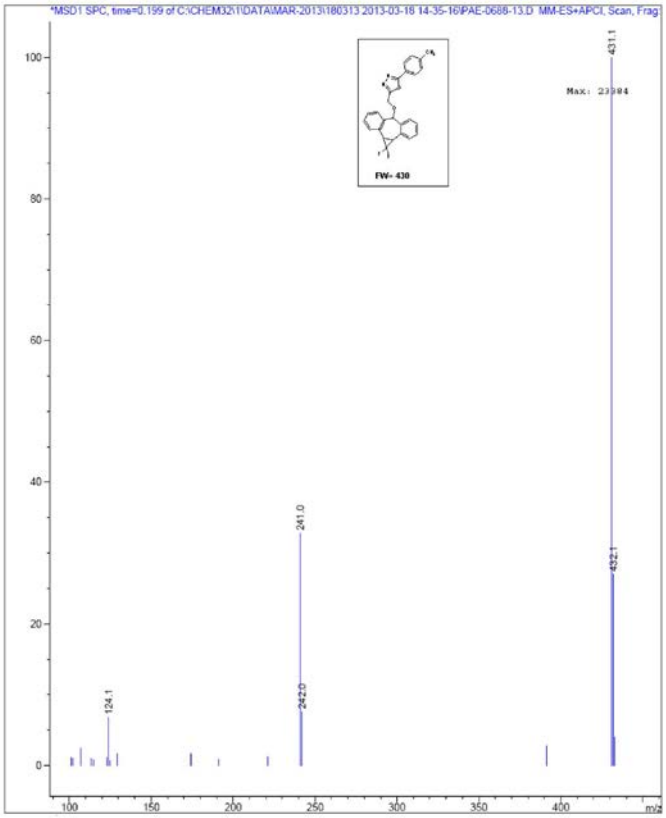


**Figure S7**. Mass spectrum of compound **8b**.

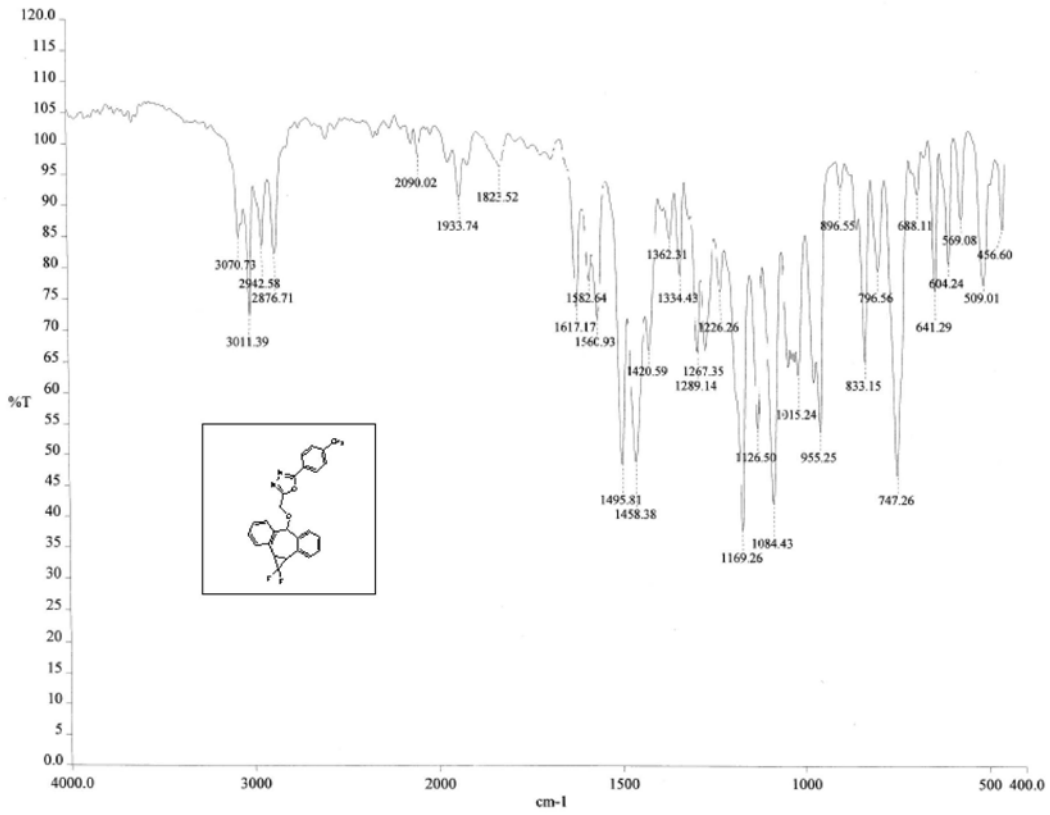


**Figure S8**. FT-IR (KBr) spectrum of compound **8b**.

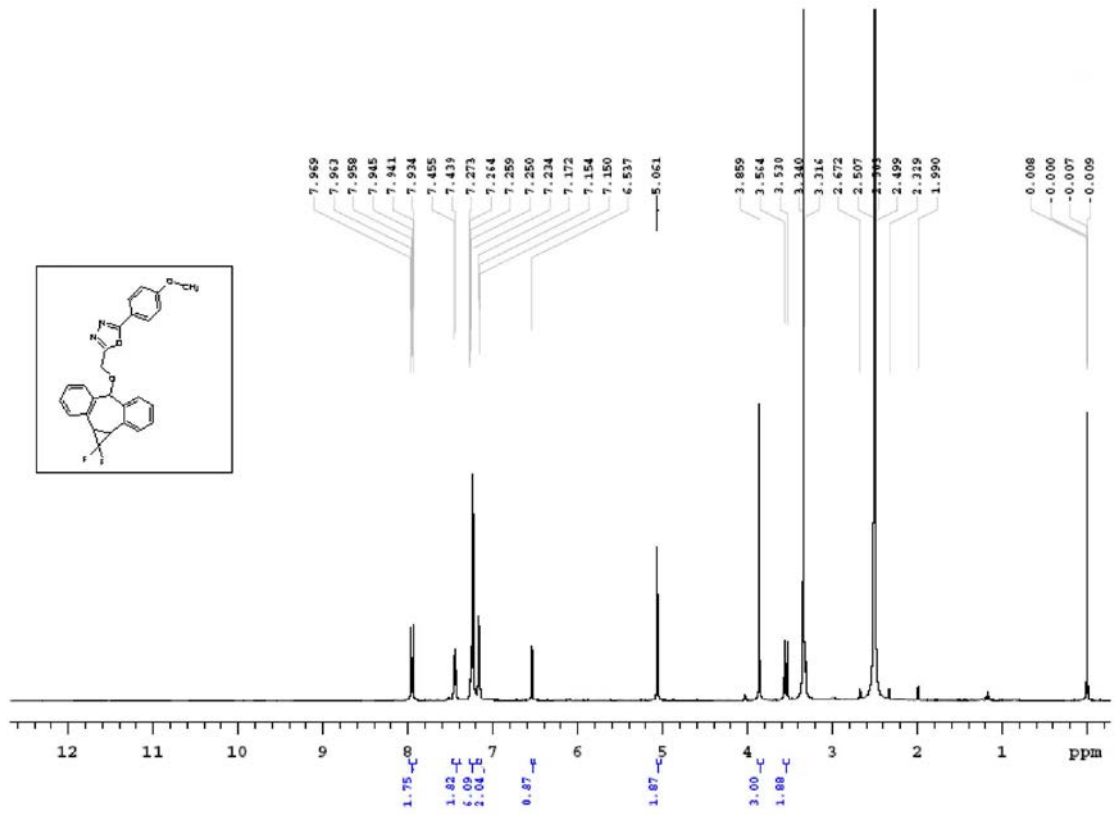


**Figure S9**. [1]H NMR spectrum (400 MHz, DMSO-$d_6$) of compound **8c**.

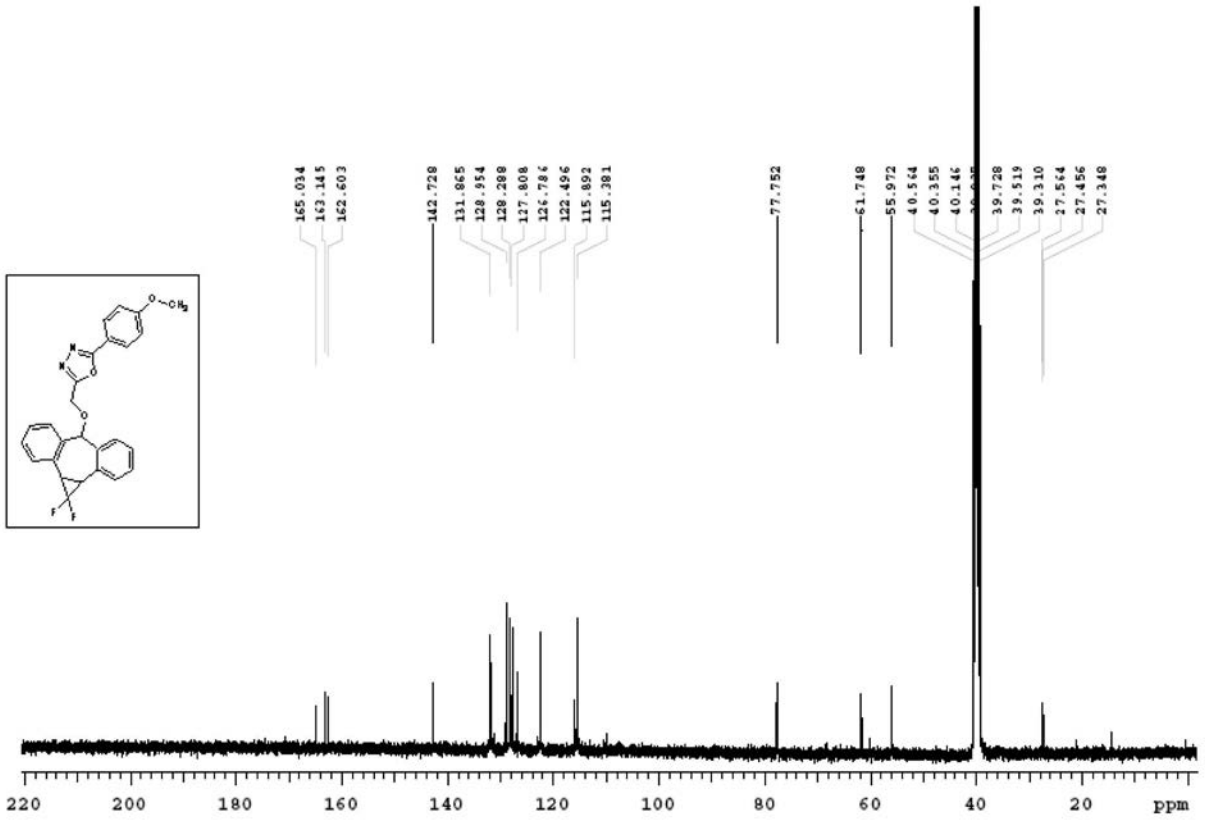


**Figure S10**. $^{13}C$ NMR spectrum (100 MHz, DMSO-$d_6$) of compound **8c**.

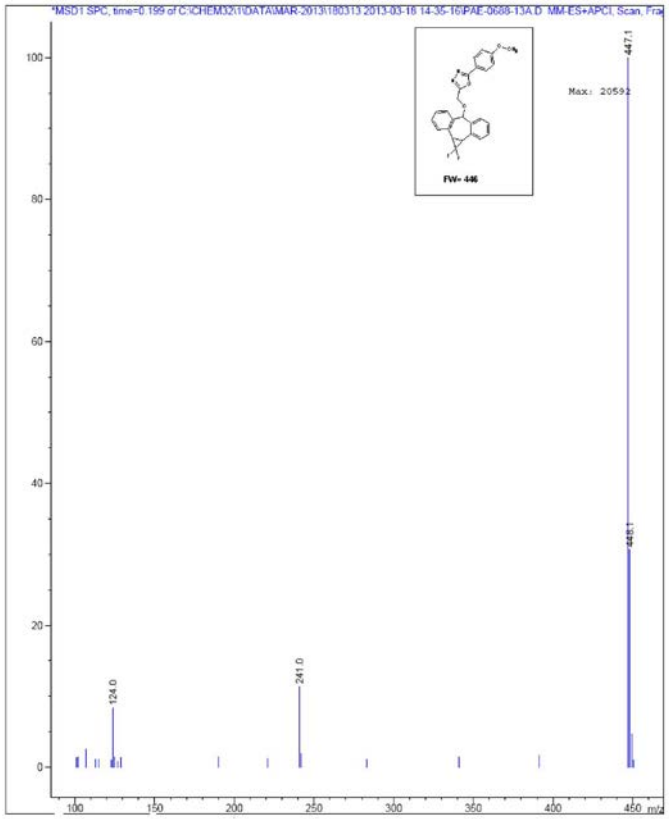


**Figure S11**. Mass spectrum of compound **8c**.

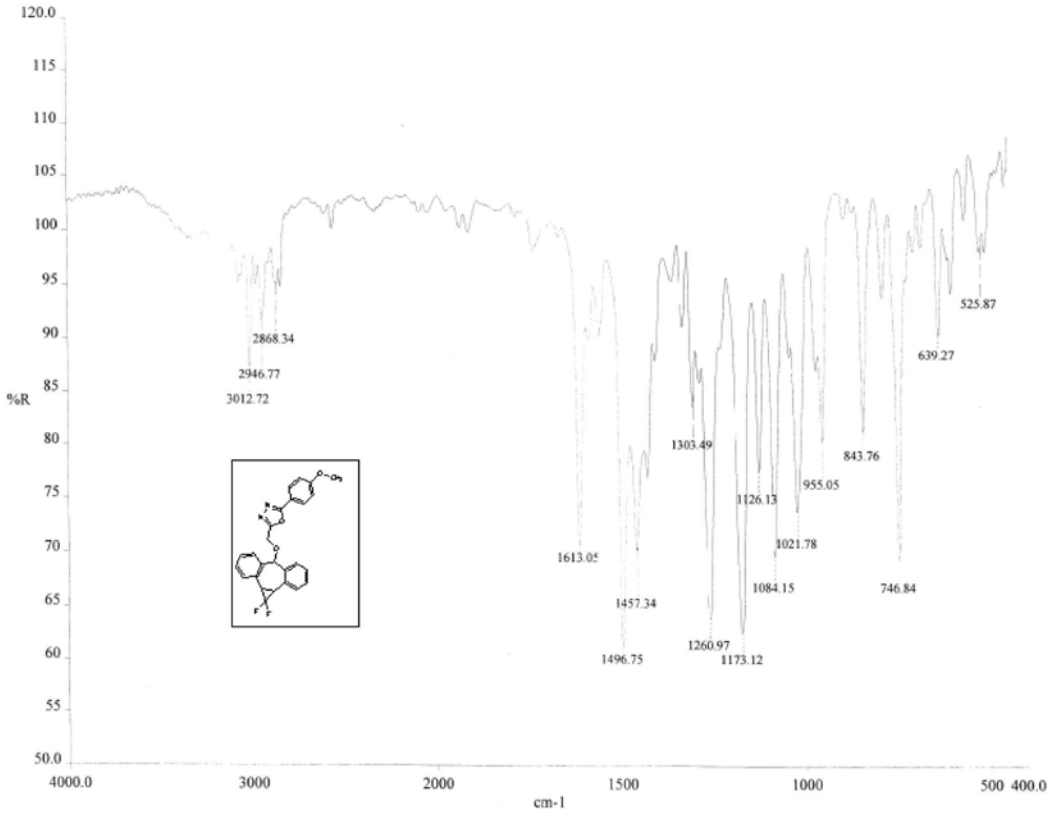


**Figure S12**. FT-IR (KBr) spectrum of compound **8c**.

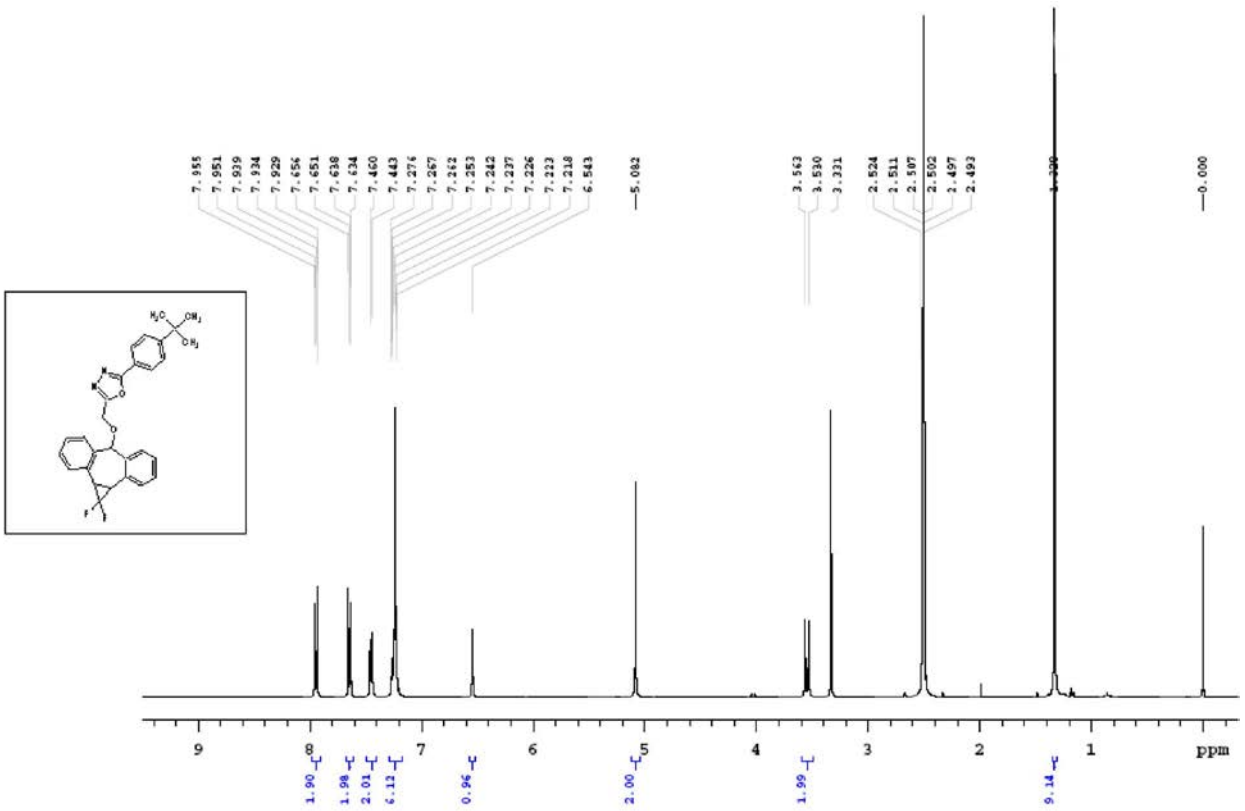


**Figure S13**. $^1$H NMR spectrum (400 MHz, DMSO-$d_6$) of compound **8d**.

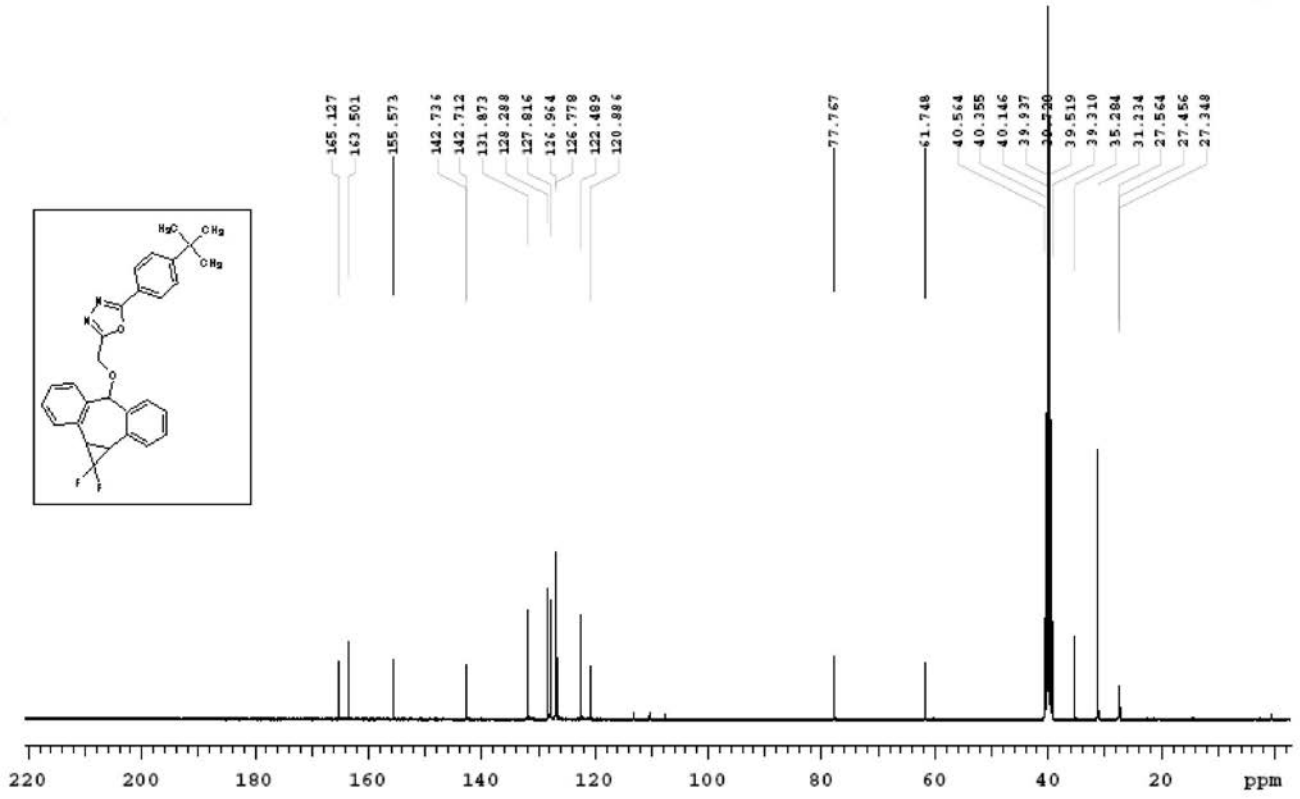


**Figure S14**. $^{13}C$ NMR spectrum (100 MHz, DMSO-$d_6$) of compound **8d**.

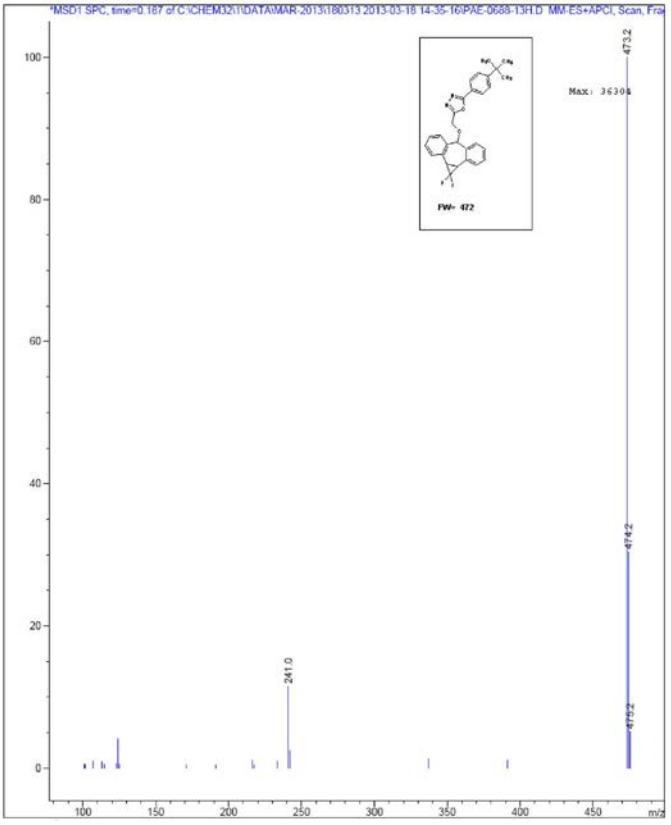


**Figure S15**. Mass spectrum of compound **8d**.

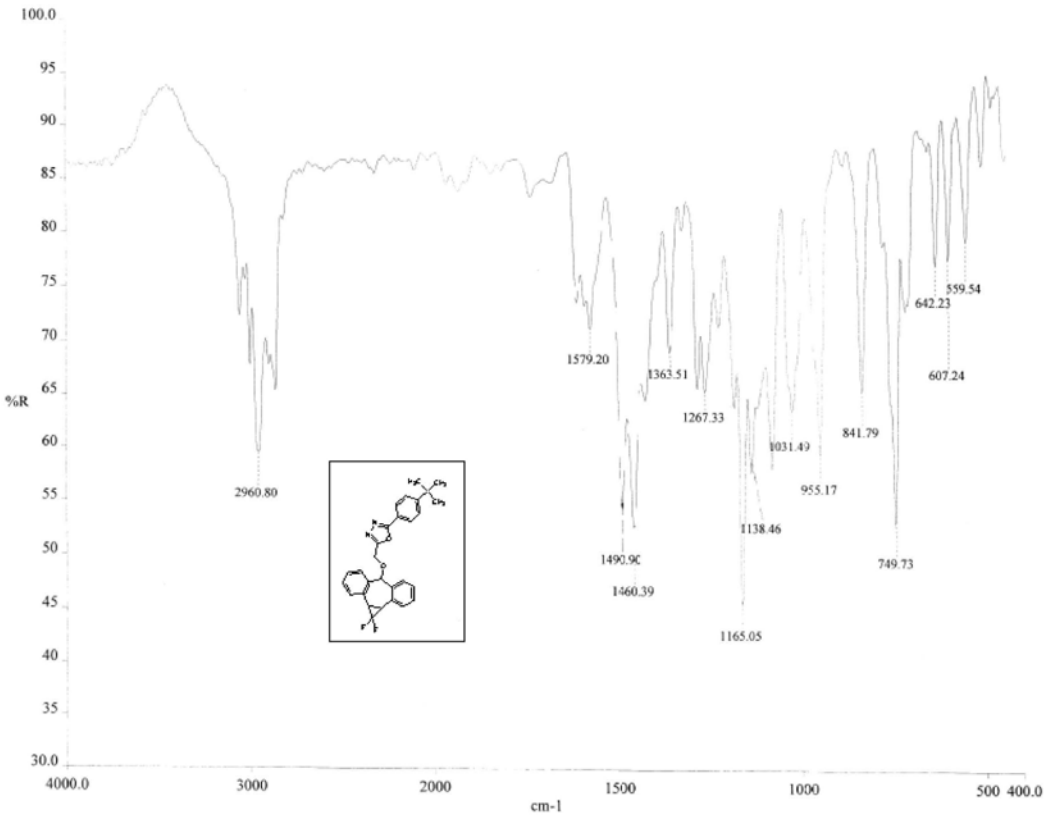


**Figure S16**. FT-IR (KBr) spectrum of compound **8d**.

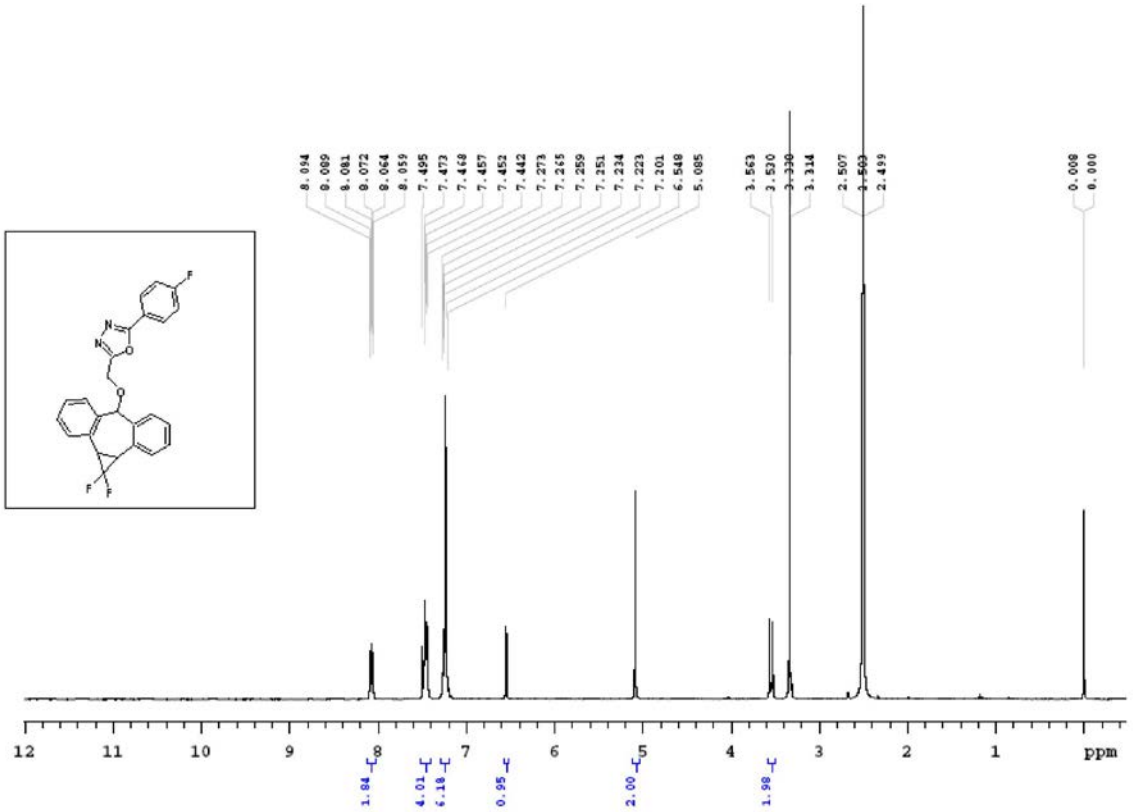


**Figure S17**. $^1$H NMR spectrum (400 MHz, DMSO-$d_6$) of compound **8e**.

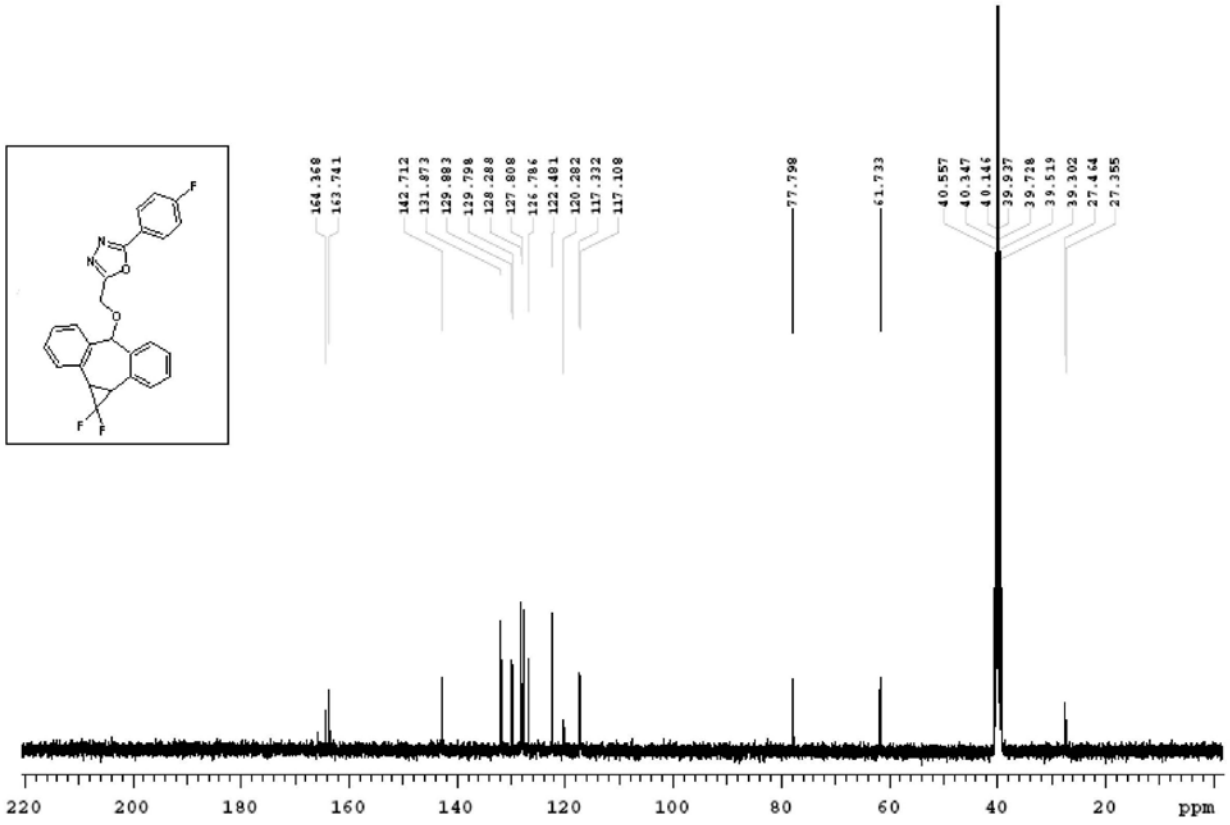


**Figure S18**. $^{13}C$ NMR spectrum (100 MHz, DMSO-$d_6$) of compound **8e**.

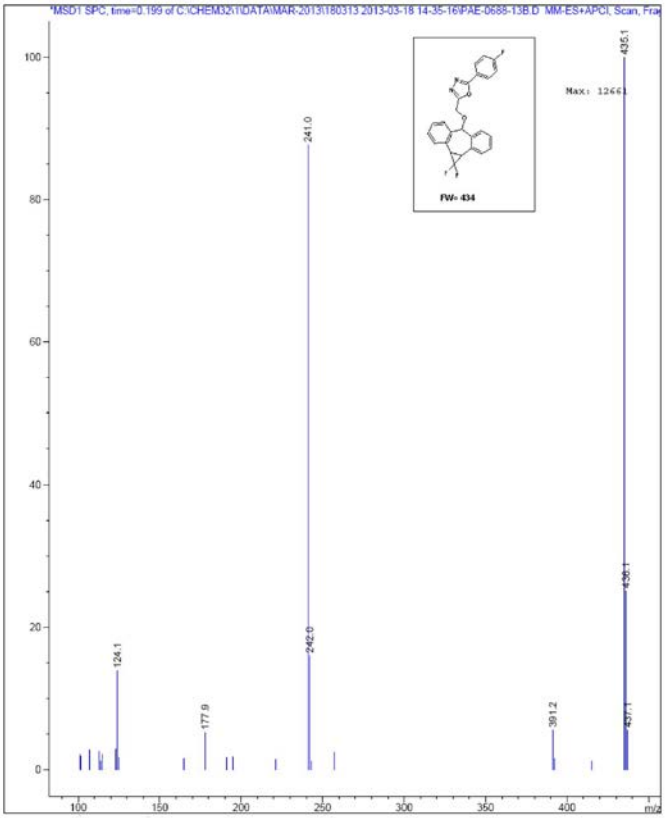


**Figure S19**. Mass spectrum of compound **8e**.

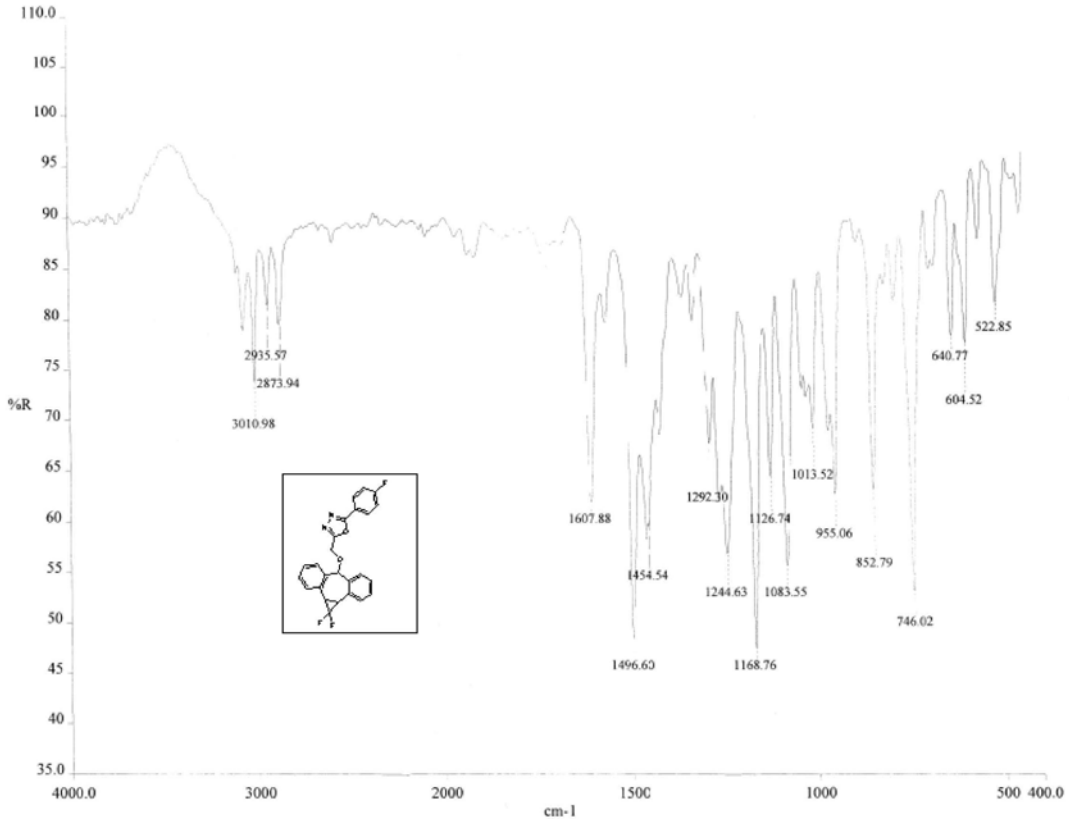


**Figure S20**. FT-IR (KBr) spectrum of compound **8e**.

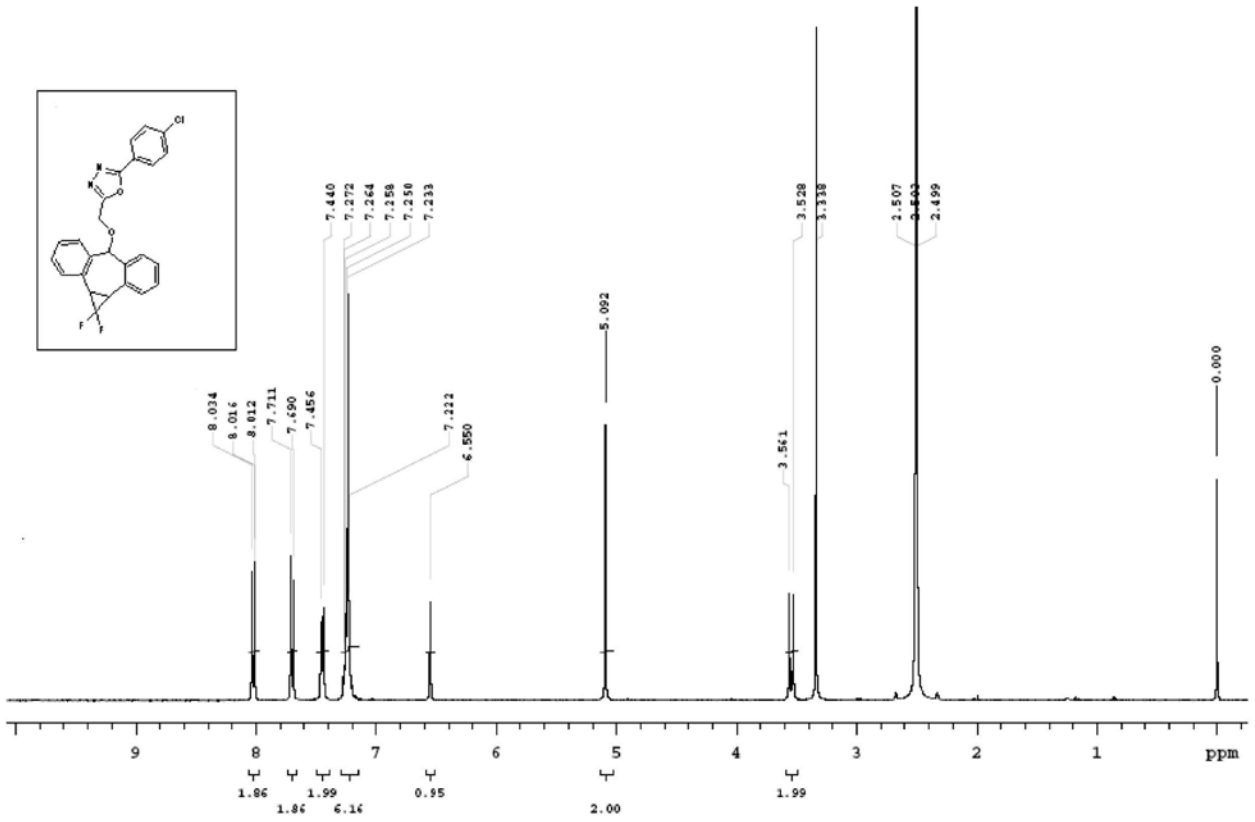


**Figure S21**. $^1$H NMR spectrum (400 MHz, DMSO-$d_6$) of compound **8f**.

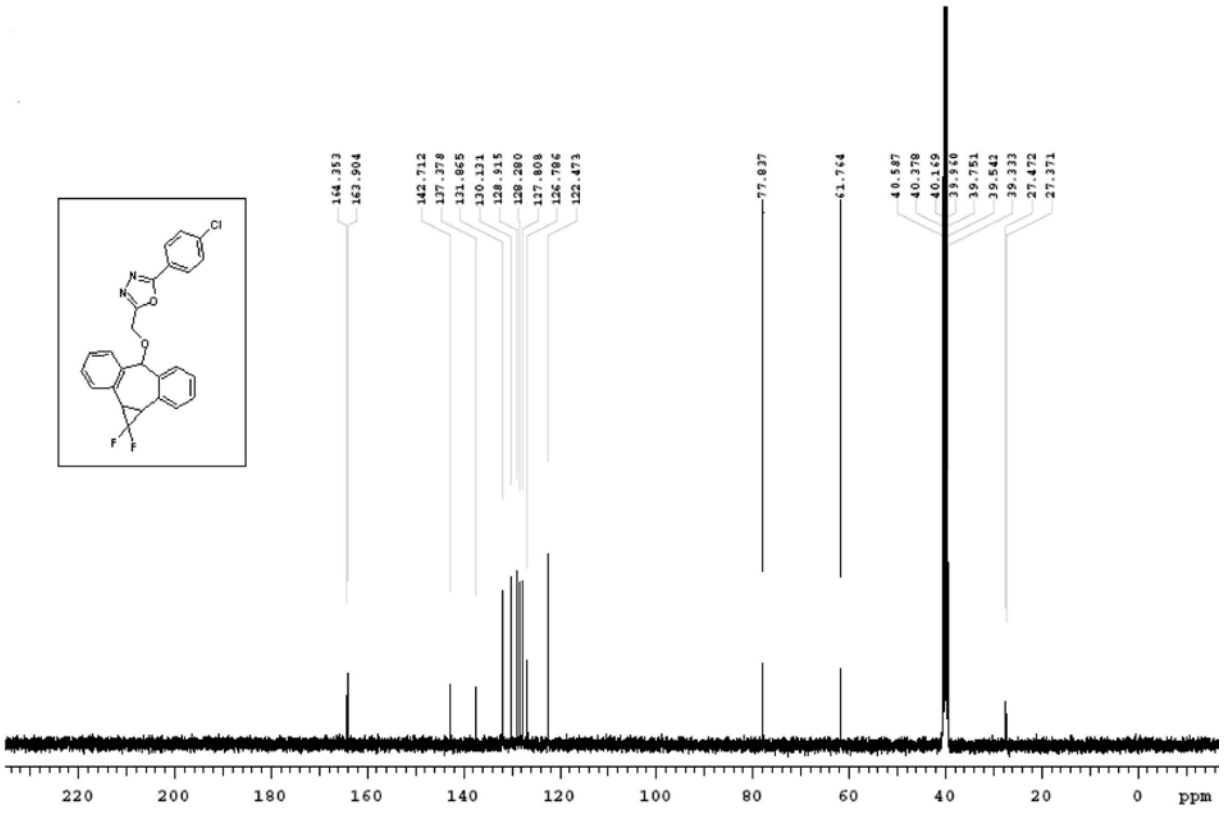


**Figure S22**. $^{13}C$ NMR spectrum (100 MHz, DMSO-$d_6$) of compound **8f**.

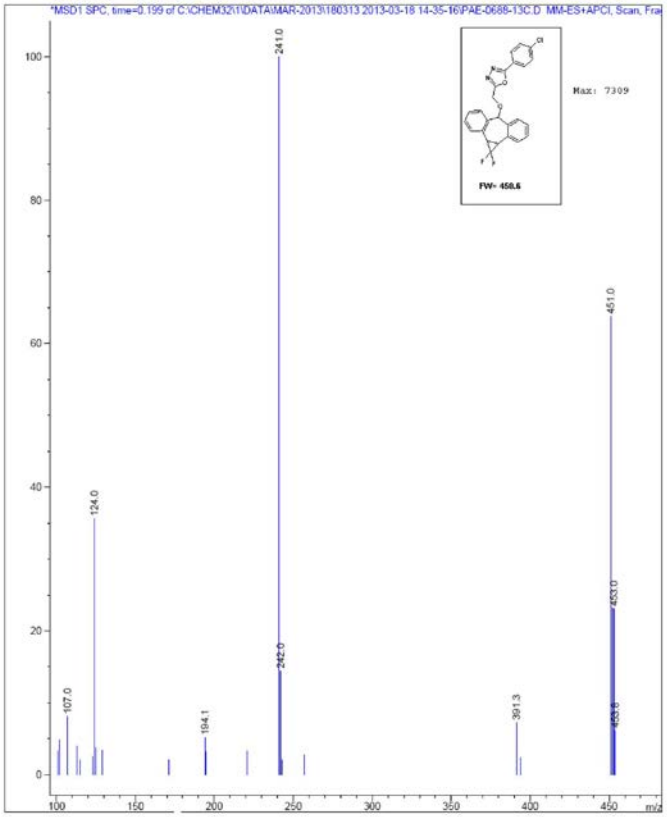


**Figure S23**. Mass spectrum of compound **8f**.

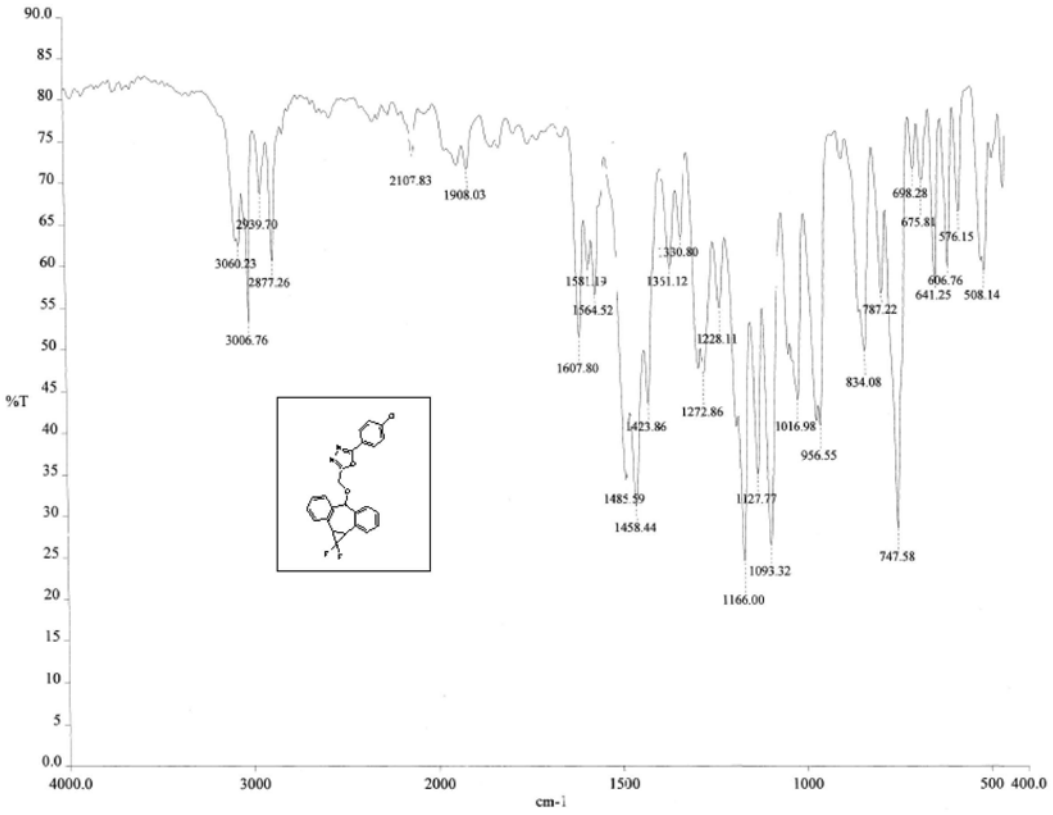


**Figure S24**. FT-IR (KBr) spectrum of compound **8f**.

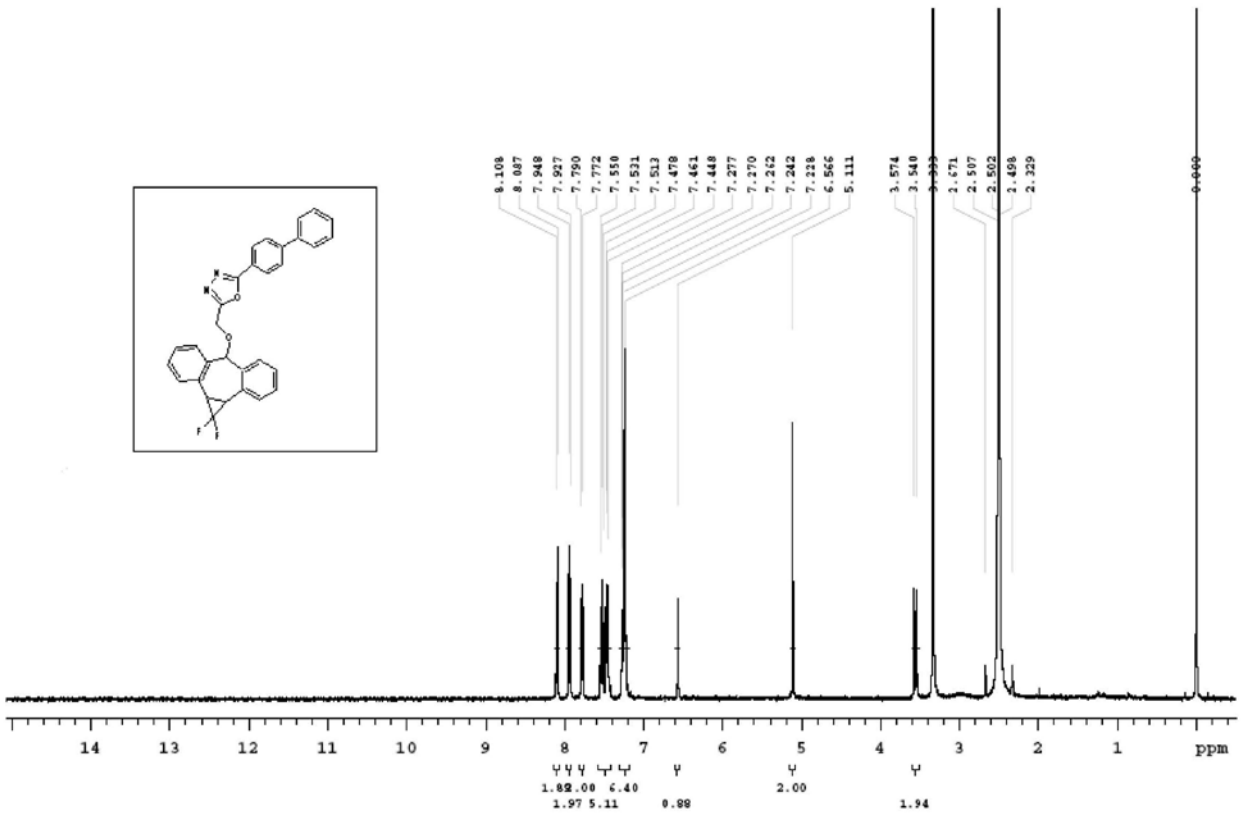


**Figure S25**. $^1$H NMR spectrum (400 MHz, DMSO-$d_6$) of compound **8g**.

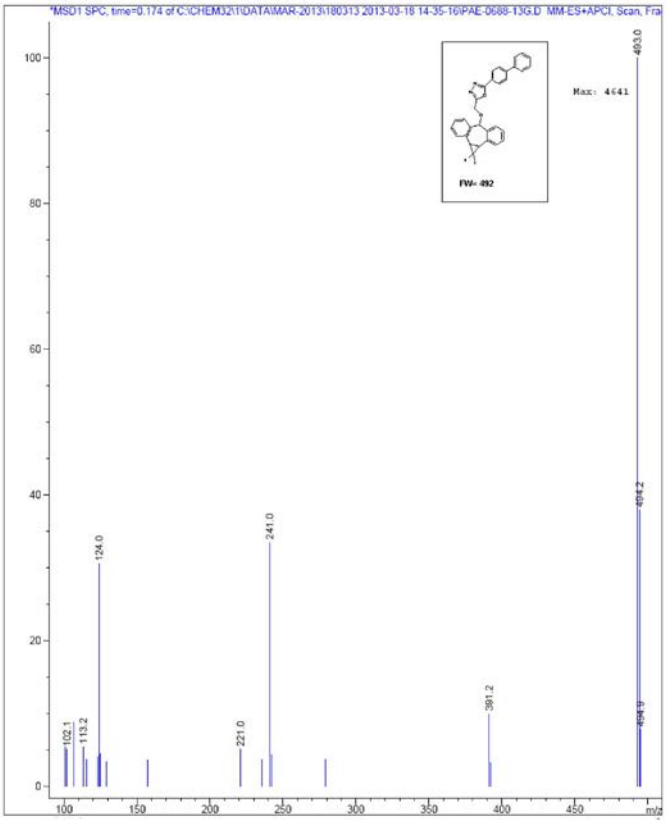


**Figure S26**. Mass spectrum of compound **8g**.

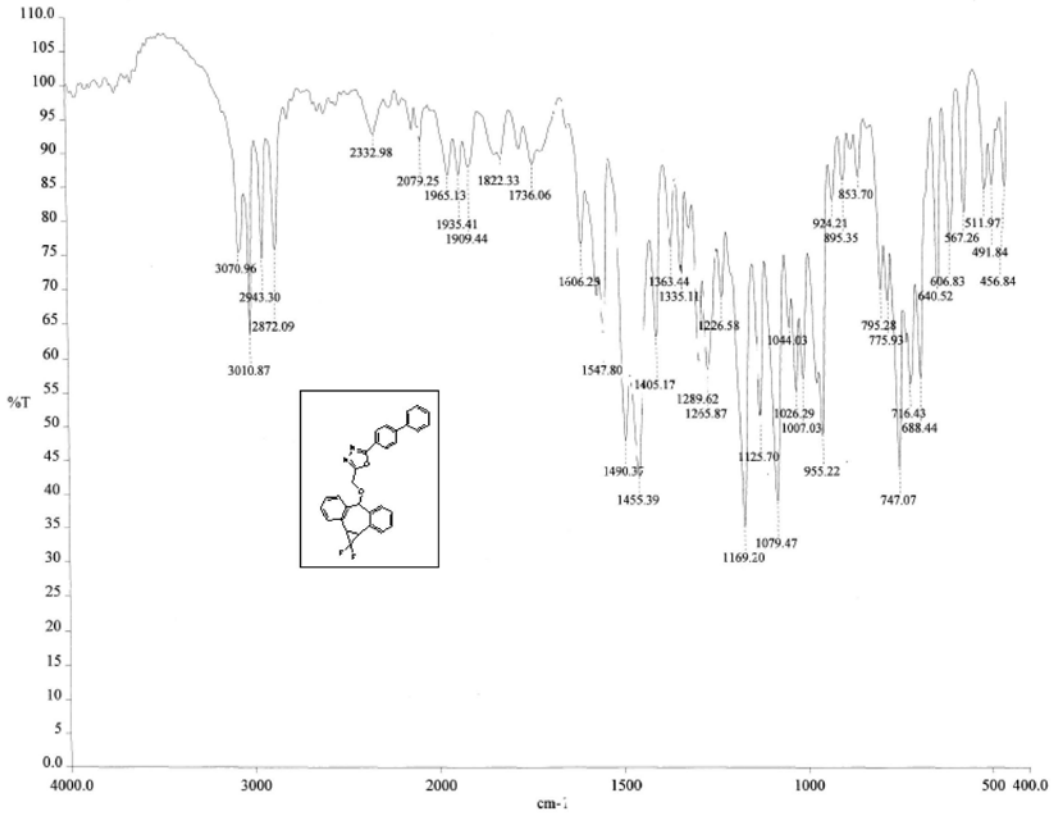


**Figure S27**. FT-IR (KBr) spectrum of compound **8g**.

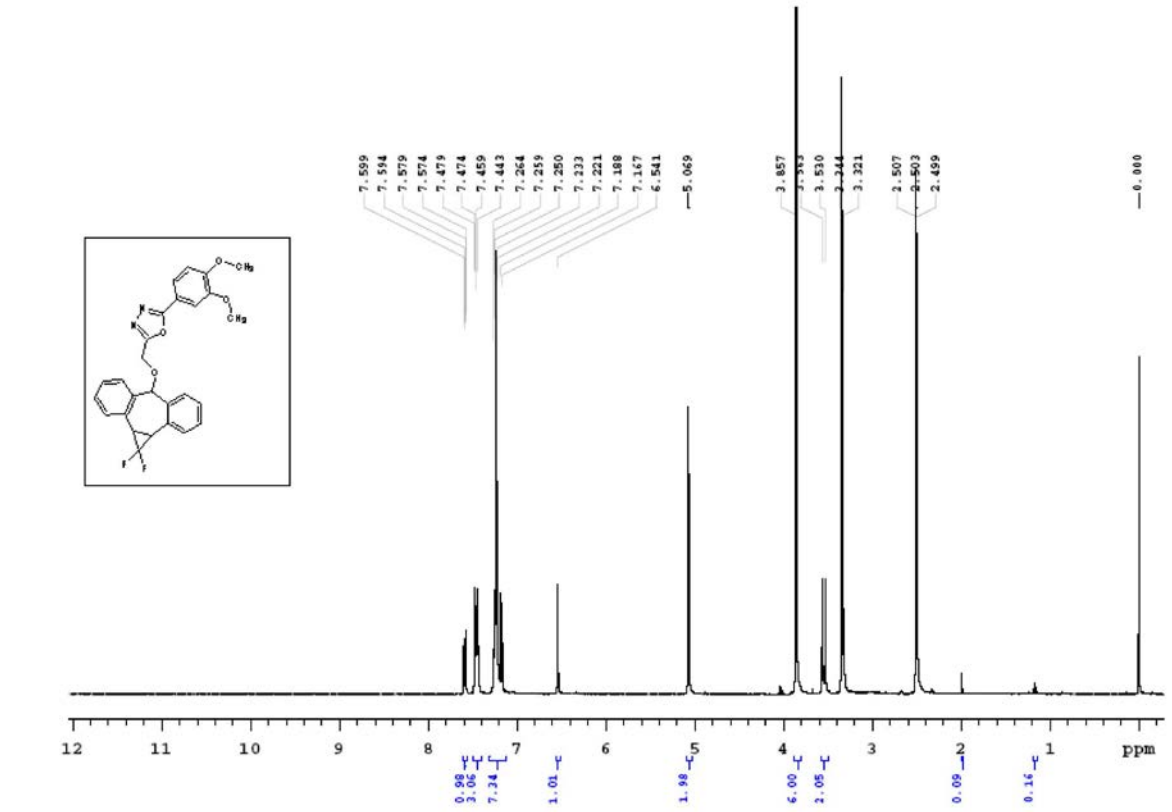


**Figure S28**. [1]H NMR spectrum (400 MHz, DMSO-$d_6$) of compound **8h**.

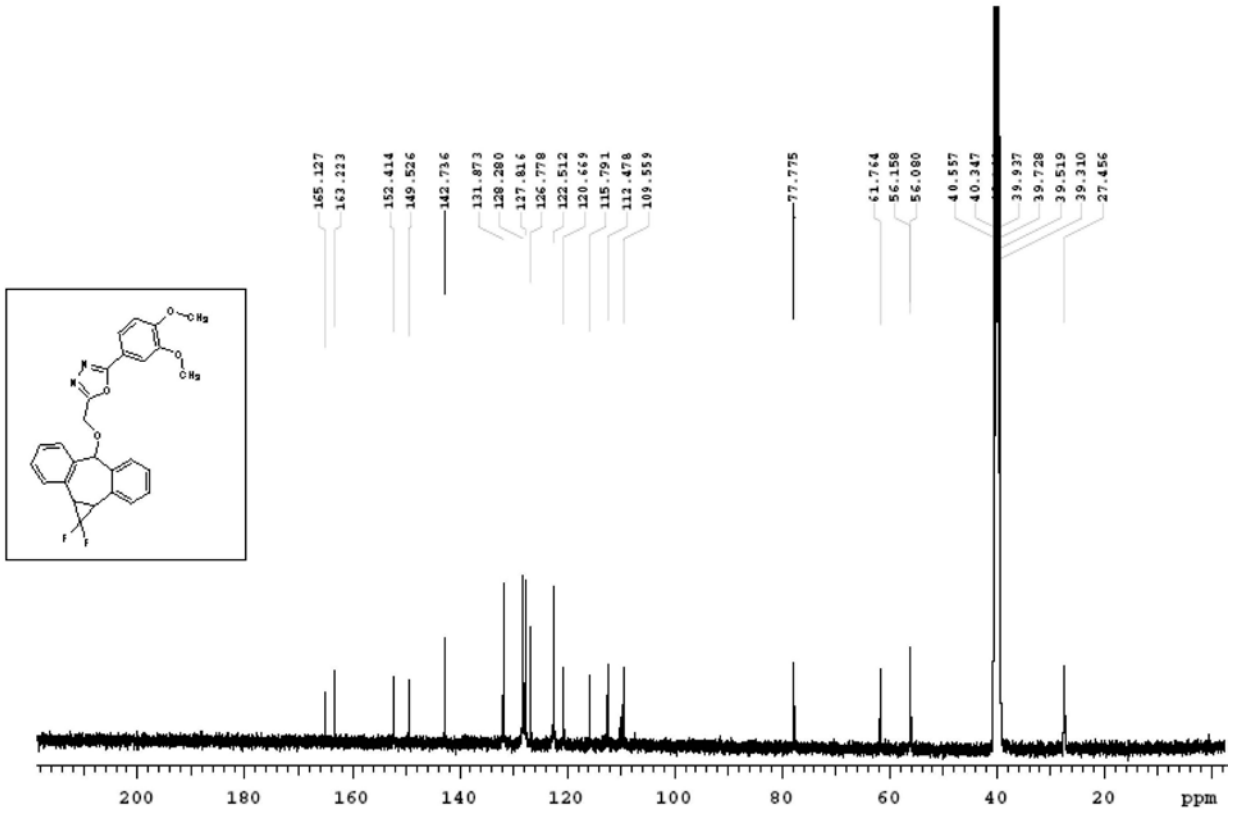


**Figure S29**. [13]C NMR spectrum (100 MHz, DMSO-$d_6$) of compound **8h**.

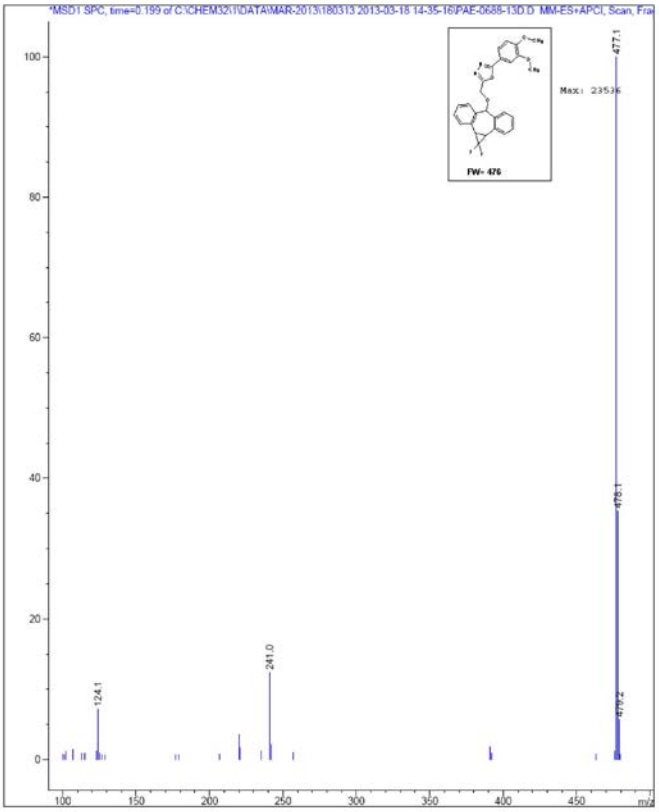


**Figure S30**. Mass spectrum of compound **8h**.

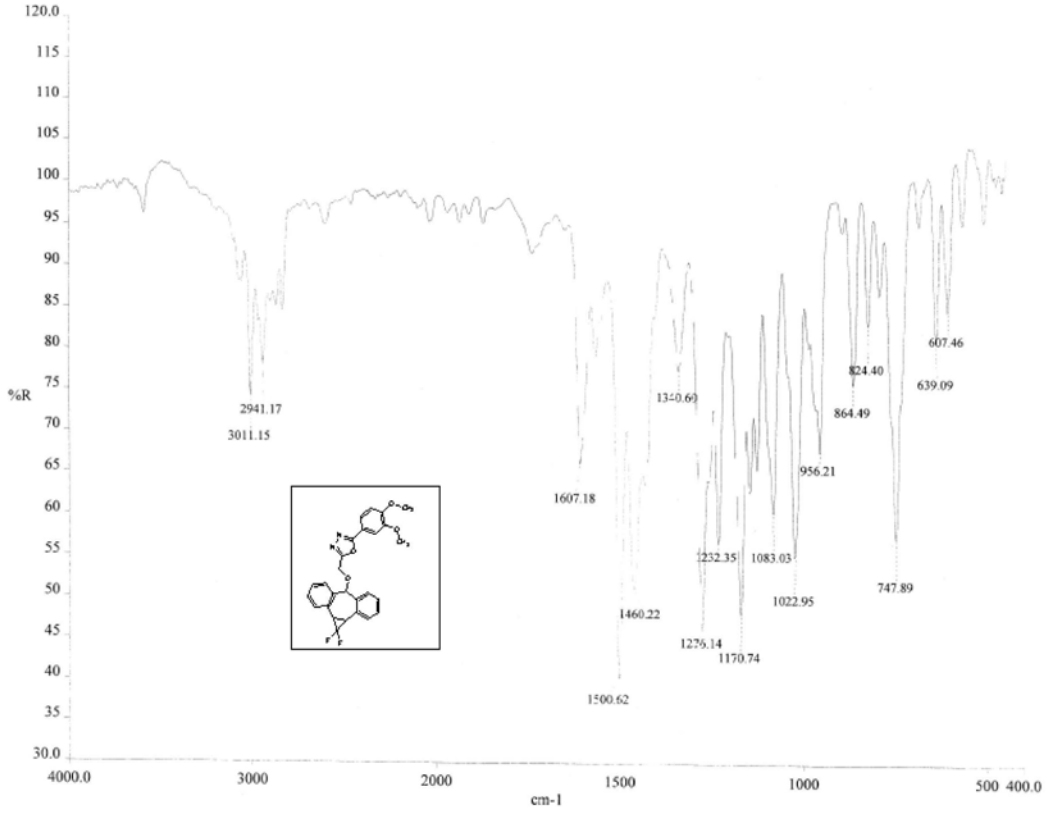


**Figure S31**. FT-IR (KBr) spectrum of compound **8h**.

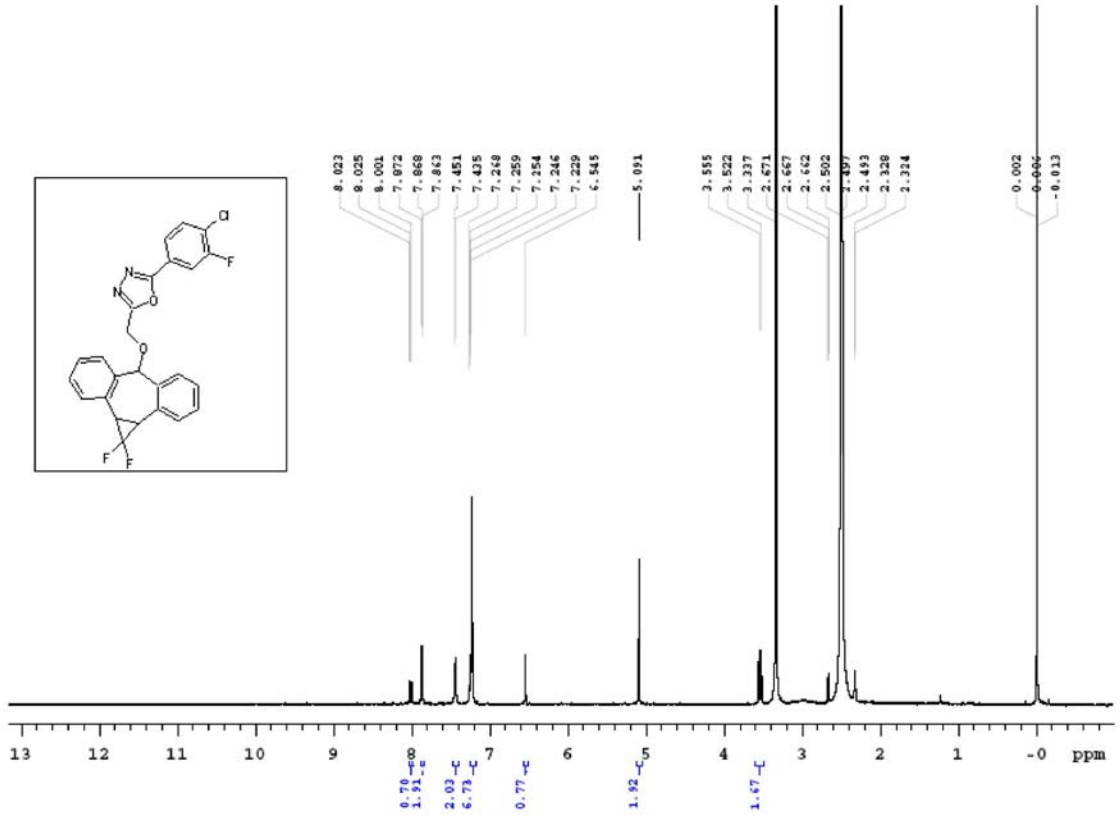


**Figure S32**. $^1$H NMR spectrum (400 MHz, DMSO-$d_6$) of compound **8i**.

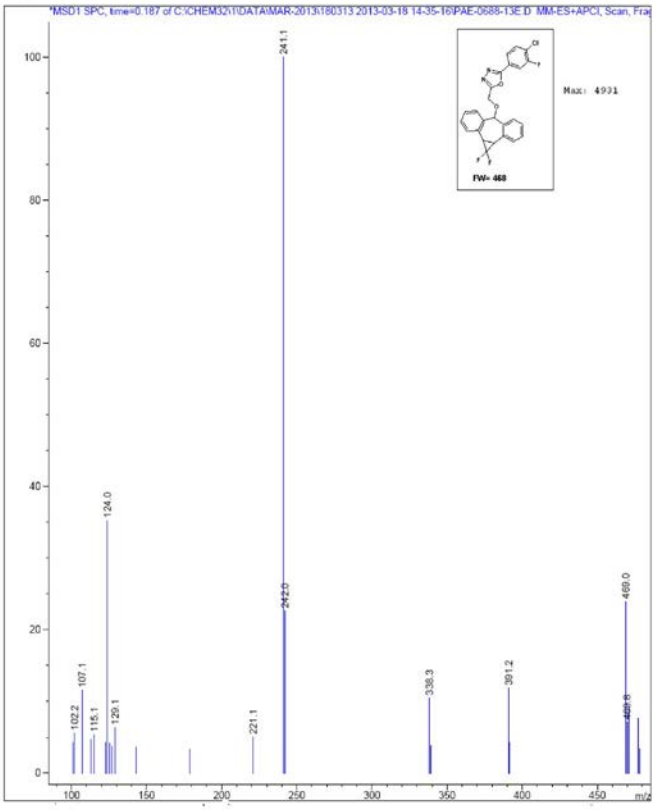


**Figure S33**. Mass spectrum of compound **8i**.

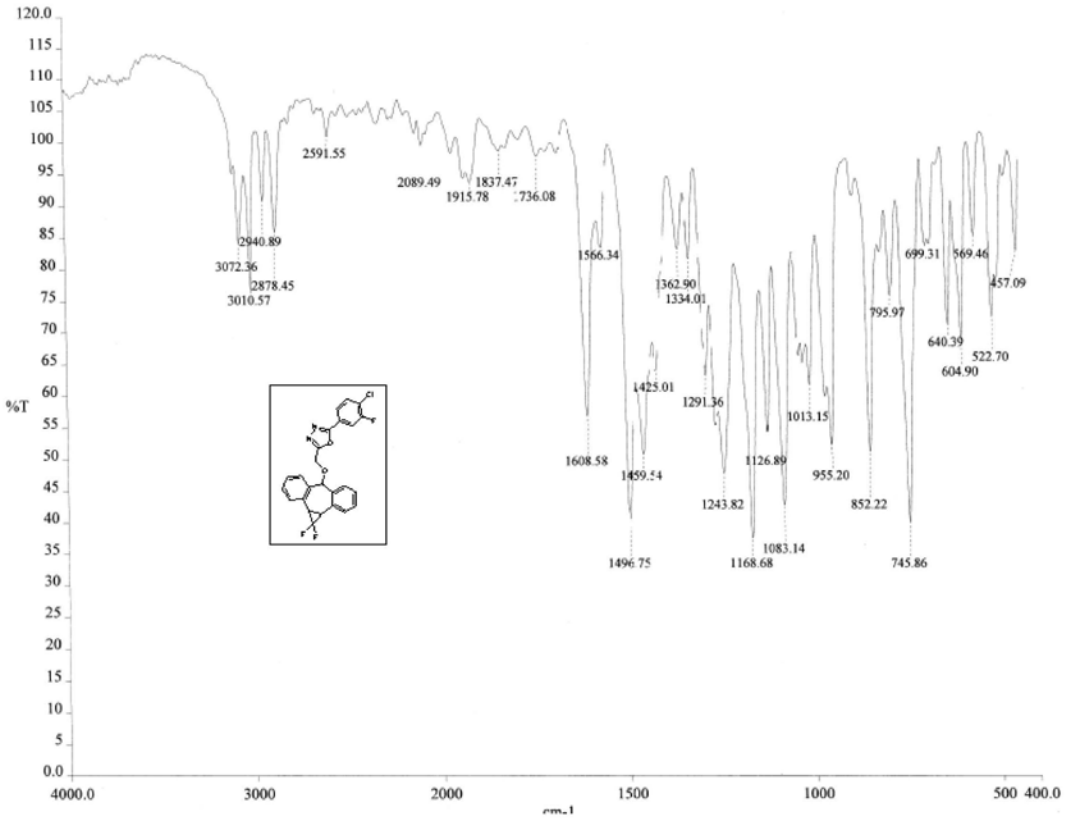


**Figure S34**. FT-IR (KBr) spectrum of compound **8i**.

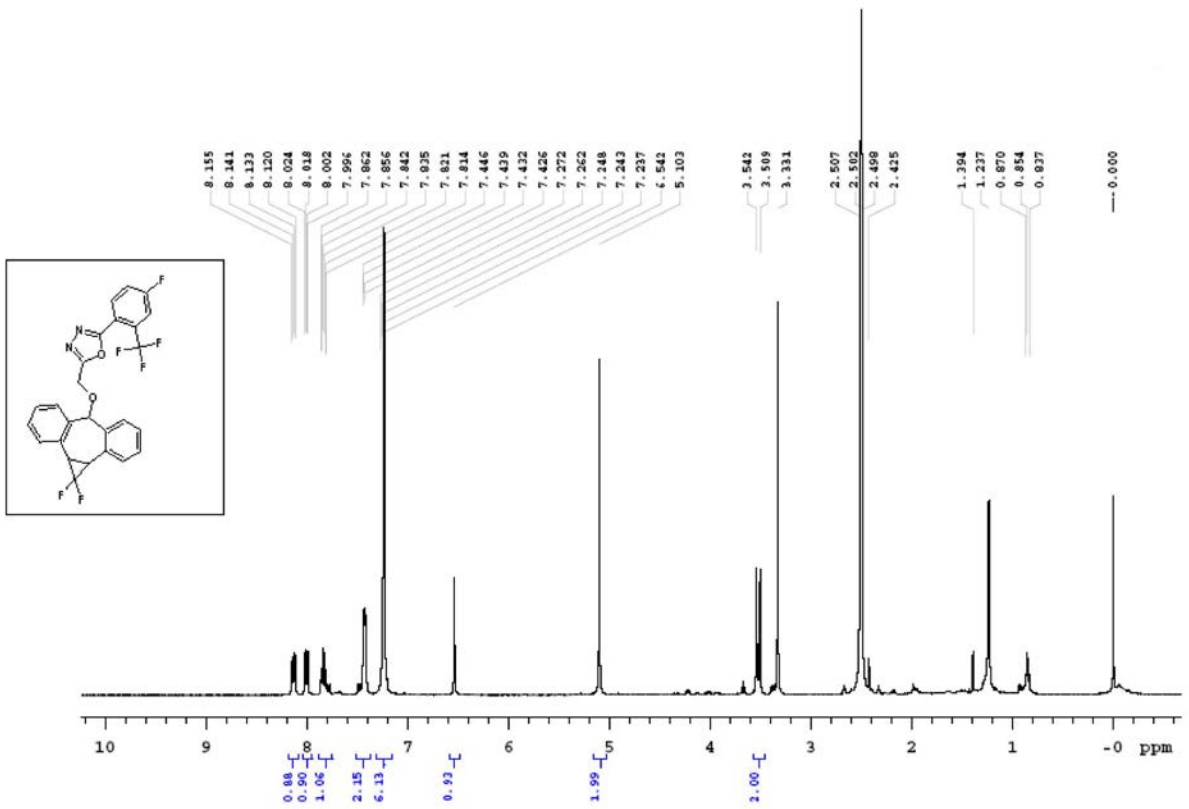


**Figure S35**. $^1$H NMR spectrum (400 MHz, DMSO-$d_6$) of compound **8j**.

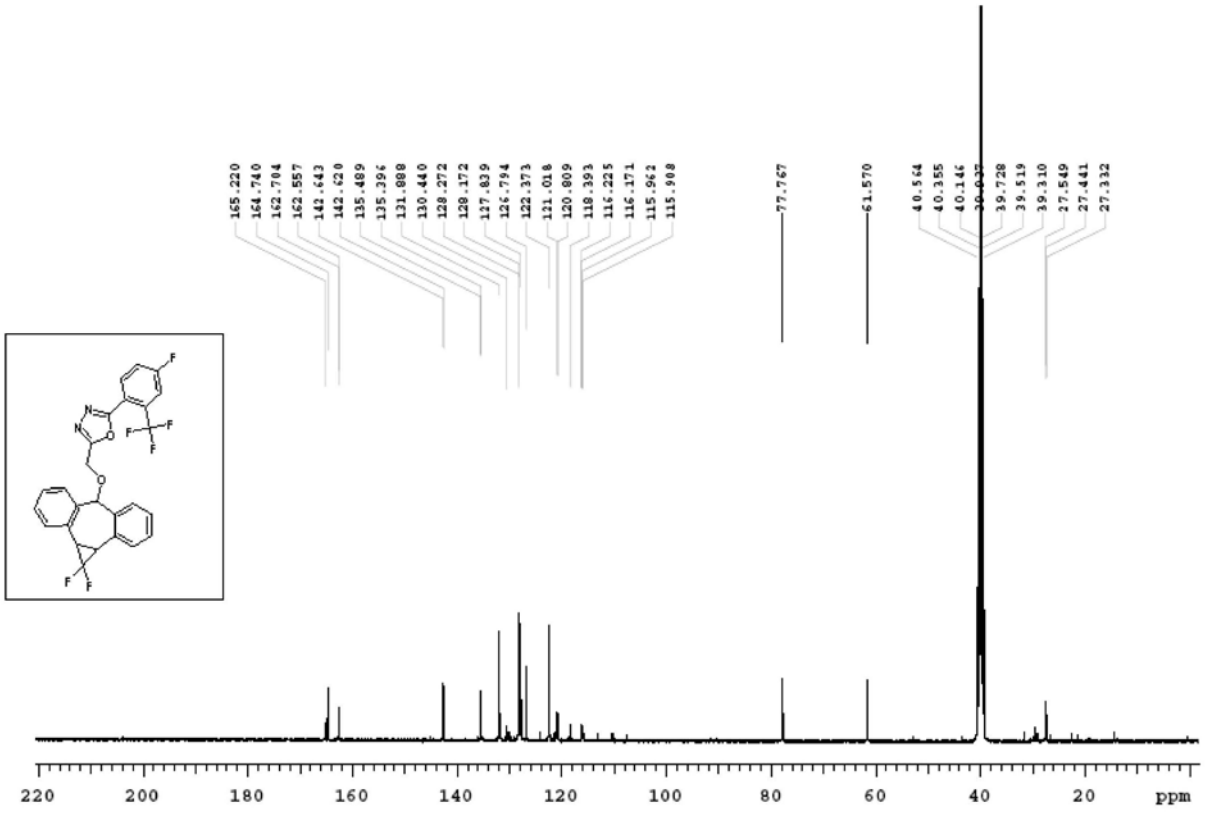


**Figure S36**. $^{13}$C NMR spectrum (100 MHz, DMSO-$d_6$) of compound **8j**.

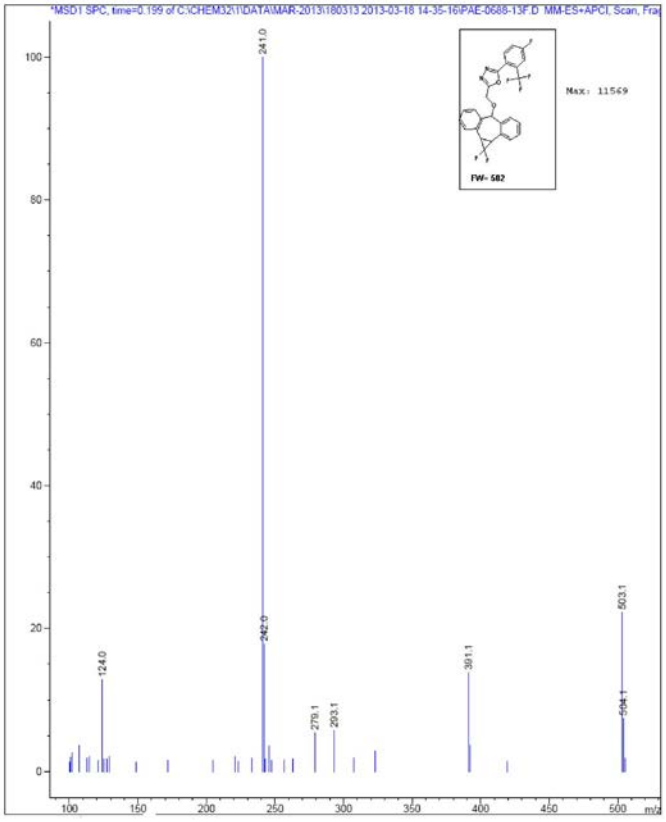


**Figure S37**. Mass spectrum of compound **8j**.

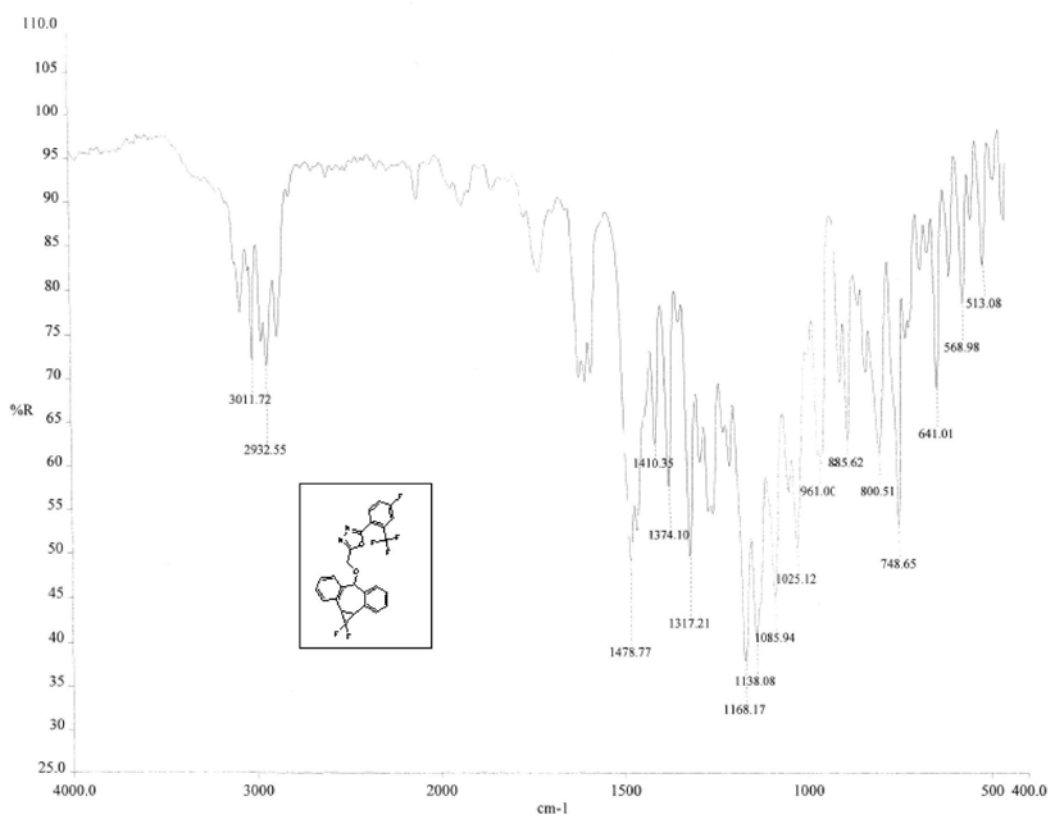


**Figure S38**. FT-IR (KBr) spectrum of compound **8j**.