Supporting Information

Alkyl chain substituted 1, 9- Pyrazoloanthrones exhibit prominent inhibitory effect on c-Jun N-terminal Kinase (JNK).

Karothu Durga Prasad[a], Jamma Trinath[b], Ansuman Biswas[c], Kanagaraj Sekar[d], Kithiganahalli N. Balaji*[#b] and Tayur N. Guru Row*[#a]

General Methods:

All chemicals (reagent grade) used were purchased from Sigma Aldrich. Separation and purification of the compounds was carried out with silica gel 60 (200-300 mesh) based column chromatography. Thin layer chromatography (TLC) was run on the silica gel coated aluminum sheets (silica gel 60 GF254, E. Merck, Germany) and visualized in ultraviolet (UV) light (254 nm). Melting points (uncorrected) were determined with Buchi melting point B-545 apparatus. ^{1}H and ^{13}C NMR spectra (400 MHz) were recorded on a ^{1}H-Bruker-400 spectrometer at 25°C, using tetramethylsilane (TMS) as the internal standard. HRMS was obtained using a micromass-QTOF spectrometer using electrospray ionization (ESI).

Synthesis of alkyl (CH_3-C_5H_{11}) derivatives of 1,9-pyrazoloanthrone:

These compounds were prepared according to the modified procedures.[1, 2] To the solution of 1equivalent of 1, 9-pyrazoloanthrone in 30 ml of dimethylformamide(DMF) was added 1.2 equivalents of K_2CO_3. After stirring for 30 min 1 equivalent of alkylhalide(R-X; R=CH_3-C_5H_{11}) added and continued stirring for overnight at reflux conditions. Reaction was monitored by TLC. The solvent was removed by vacuum after the completion of the reaction. The reaction mixture was extracted with EtOAc. The two isomers of each alkyl derivative were isolated by column chromatography by elucidating with different solvents like $CHCl_3$, EtOAc and hexane.

Preparation of SP : Compound SP was prepared according to the modified procedure. HRMS: m/z calcd for $C_{14}H_8N_2O$+Na calcd 243.0534; found 243.0538. HPLC: 99.20%, tR=4.97 min.

2-methyldibenzo[cd,g]indazol-6(2H)-one (SPM1): HPLC: 99.37%, tR=5.07 min. ^{1}H NMR (400 MHz, $CDCl_3$): δ8.45 (d, *J* = 7.88 Hz, 1H), 8.20 (d, *J* = 7.72 Hz, 1H), 8.05 (d, *J* = 6.72 Hz, 1H), 7.78-7.60 (m, 3H), 7.53 (t, *J* = 7.76 Hz, 1H), 4.25 (s, 3H).; ^{13}C NMR (100 MHz, $CDCl_3$): δ 184.2, 139.9, 139.2, 133.8(2C), 132.3, 129.7, 126.9, 124.1, 123.0, 121.2, 115.2, 36.9.; HRMS: m/z calcd for $C_{15}H_{10}N_2O$ +Na calcd 257.0793; found 257.0794.

1-methyldibenzo[cd,g]indazol-6(1H)-one (SPM2): HPLC: 99.10%, tR=5.11 min. ^{1}H NMR (400 MHz, $CDCl_3$): δ8.42 (d, *J* =7.76 Hz, 1H), 8.2 (d, *J* =7.51 Hz, 1H), 8.04 (d, *J* =6.7 Hz, 1H),

7.77- 7.58 (m, 3H), 7.53 (t, *J* =6.8 Hz, 3H), 4.24 (s, 3H).; ^{13}C NMR (100 MHz, $CDCl_3$): δ 184.18, 139.8, 139.7, 139.1, 133.7, 132.2, 129.6, 128.7, 126.7, 126.5, 124.0, 123.0, 121.0, 115.1, 36.8.; HRMS: m/z calcd for $C_{15}H_{10}N_2O$ +Na calcd 257.0793; found 257.0707.

2-ethyldibenzo[cd,g]indazol-6(2H)-one (SPE1): HPLC: 99.27%, tR=5.15 min. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.45 (d, *J* =7.96 Hz, 1H), 8.22 (d, *J* =7.68 Hz, 1H), 8.04 (d, *J* =6.96 Hz, 1H), 7.77-7.50 (m, 3H), 7.51 (t, *J* =7.48 Hz, 1H), 4.59 (q, *J* =14.56 Hz, 2H), 1.64 (t, *J* =7.28 Hz, 3H),; ^{13}C NMR (100 MHz, $CDCl_3$): δ 183.8, 138.5(2C), 133.2(2C), 131.9, 129.1(2C), 128.1, 126.4, 123.6, 122.5, 120.6, 114.8, 44.9, 15.5.;HRMS: m/z calcd for $C_{16}H_{12}N_2O$ +Na calcd 271.0847; found 271.0848.

1-ethyldibenzo[cd,g]indazol-6(1H)-one (SPE2): HPLC: 98.37%, tR=5.18 min. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.54 (d, *J* =7.96 Hz, 1H), 8.12 (d, *J* =6.96 Hz, 1H), 8.06 (d, *J* =8.44 Hz, 1H), 7.94-7.50 (m,3H), 7.52 (t, *J* =7.12 Hz, 1H), 4.95 (q, *J* =7.32 Hz, 2H), 1.75 (t, *J* =7.32 Hz, 3H).; ^{13}C NMR (100 MHz, $CDCl_3$): δ 183.6, 150.0, 146.4, 133.8, 133.3, 131.0, 128.6, 128.3(2C), 127.0, 124.6, 124.1, 122.1, 116.7, 49.3, 15.9.; HRMS: m/z calcd for $C_{16}H_{12}N_2O$ +Na calcd 271.0847; found 271.0844.

2-propyldibenzo[cd,g]indazol-6(2H)-one (SPP1): HPLC: 99.50%, tR=5.20 min. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.45 (d, *J* =7.52 Hz, 1H), 8.22 (d, *J* =7.64 Hz, 1H), 8.04 (d, *J* =6.84 Hz, 1H), 7.77-7.49 (m,3H), 7.51 (t, *J* =8.08 Hz, 1H), 4.49 (t, *J* =13.88 Hz, 2H), 2.15-1.99 (m,2H), 0.99 (t, *J* =14.72 Hz, 3H),; ^{13}C NMR (100 MHz, $CDCl_3$): δ184.3, 139.6, 139.0, 133.7(2C), 132.4, 129.7, 128.6(2C), 126.9, 123.9, 123.0, 121.1, 115.4, 52.6, 24.3, 11.9.; HRMS: m/z calcd for $C_{17}H_{14}N_2O$ +Na calcd 285.1004; found 285.1026.

1-propyldibenzo[cd,g]indazol-6(1H)-one (SPP2): HPLC: 98.67%, tR=5.28 min. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.53 (d, *J* =7.2 Hz, 1H), 8.11 (d, *J* =6.8 Hz, 1H), 8.05 (d, *J* =8.4 Hz, 1H), 7.9-7.54 (m,3H), 7.51 (t, *J* =7.2 Hz, 1H), 4.84 (t, *J* =7.2 Hz, 2H), 2.25-2.06 (m, 2H), 1.1 (t, *J* =7.2 Hz, 3H),; ^{13}C NMR (100 MHz, $CDCl_3$): δ 183.8, 146.4, 133.7, 133.3, 131.0, 128.8, 128.5, 128.2, 127.0, 124.9, 124.6, 124.0, 123.5, 122.5, 55.8, 11.7.; HRMS: m/z calcd for $C_{17}H_{14}N_2O$ +H calcd 263.1184; found 263.1174.

2-butyldibenzo[cd,g]indazol-6(2H)-one (SPB1): HPLC: 99.45%, tR=5.35 min. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.44 (d, *J* =7.88 Hz, 1H), 8.21 (d, *J* =7.72 Hz, 1H), 8.02 (d, *J* =6.96 Hz, 1H), 7.75-7.57 (m, 3H), 7.52 (t, *J* =8.36 Hz, 1H), 4.51 (t, *J* =7.12 Hz, 2H), 2.00 (p, *J* =5.48 Hz, 2H), 1.76-1.28 (m,2H), 0.97 (t, *J* =7.4 Hz, 3H).; ^{13}C NMR (100 MHz, $CDCl_3$): δ183.8, 139.1, 138.6, 133.2, 133.2, 132.0, 129.1(2C), 128.1, 126.4, 123.5, 122.6, 120.7, 114.9, 49.9, 32.4, 20.1, 13.7.; HRMS: m/z calcd for $C_{18}H_{16}N_2O$ +Na calcd 299.1160; found 299.1160.

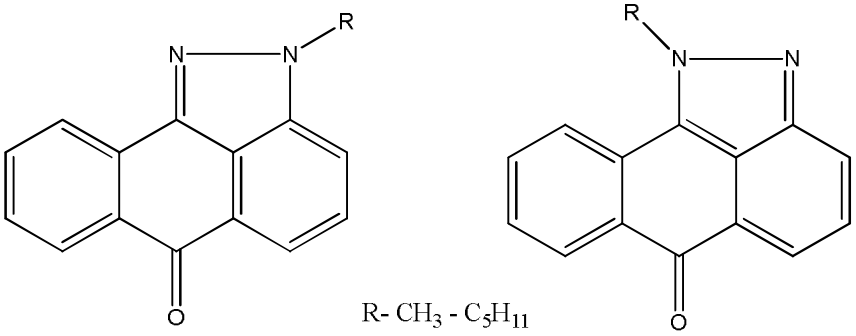
1-butyldibenzo[cd,g]indazol-6(1H)-one (SPB2): HPLC: 98.70%, tR=5.50 min. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.55 (d, *J* =8.8 Hz, 1H), 8.12 (d, *J* =7.2 Hz, 1H), 8.06 (d, *J* =8.8 Hz, 1H), 7.9-7.54(m, 3H), 7.53 (t, *J* =8.0 Hz, 1H), 4.88 (t, *J* =9.6 Hz, 2H), 2.11 (p, *J* =8.0 Hz, 2H), 1.62-1.46

(m,2H), 1.02 (d, *J* =7.2 Hz, 3H).; ^{13}C NMR (100 MHz, $CDCl_3$): δ 183.5, 146.4, 133.7, 133.3, 131.0, 128.8, 128.5, 128.4, 128.2, 127.0, 124.6, 124.0, 123.6, 122.6, 54.2, 32.9, 20.5, 14.2.; HRMS: m/z calcd for $C_{18}H_{16}N_2O$ +Na calcd 299.1160; found 299.1160.

2-pentyldibenzo[cd,g]indazol-6(2H)-one (SPEN1): HPLC: 99.10%, tR=5.60 min. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.43 (d, *J* =8.0 Hz, 1H), 8.20 (d, *J* =7.6 Hz, 1H), 8.01 (d, *J* =6.8 Hz, 1H), 7.75-7.58(m, 2H), 7.6(d, *J* =8.4 Hz, 1H), 7.51 (d, *J* =8.0 Hz, 1H), 4.49(t, *J* =14.4 Hz, 2H), 2.10-1.94 (m, 2H), 1.44-1.27 (m, 4H), 0.88 (t, *J* =13.6 Hz, 3H).; ^{13}C NMR (100 MHz, $CDCl_3$): δ 184.2, 139.4, 139.0, 133.8, 133.7, 132.3, 131.6, 129.5, 128.4, 126.8, 123.9, 123.0, 121.0, 115.2, 50.5, 30.5, 29.4, 22.7, 14.3.; HRMS: m/z calcd for $C_{19}H_{18}N_2O$ +Na calcd 313.1317; found 313.1319.

1-pentyldibenzo[cd,g]indazol-6(1H)-one (SPEN2): HPLC: 98.70%, tR=5.65 min. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.55 (d, *J* = 9.2 Hz, 1H), 8.12 (d, *J* = 6.8 Hz, 1H), 8.06 (d, *J* = 8.8 Hz, 1H), 7.86 (d, *J* = 8.0 Hz, 1H), 7.80-7.55 (m, 1H), 7.54 (t, *J* = 8.0 Hz, 1H), 4.87 (t, *J* = 7.6 Hz, 2H), 2.19-2.06 (m, 2H), 1.57-1.34 (m, 4H), 0.93 (t, *J* = 14.4 Hz, 3H), ; ^{13}C NMR (100 MHz, $CDCl_3$): δ 183.6, 146.4, 133.7, 133.3, 131.0, 128.8, 128.5, 128.2, 127.0, 124.6, 124.0, 123.5, 122.5, 116.7, 54.4, 30.6, 29.3, 22.7, 14.4.; HRMS: m/z calcd for $C_{19}H_{18}N_2O$ +Na calcd 313.1317; found 313.1317.

^{1}H and ^{13}C NMR spectras of both isomers.



R- CH_3 - C_5H_{11}

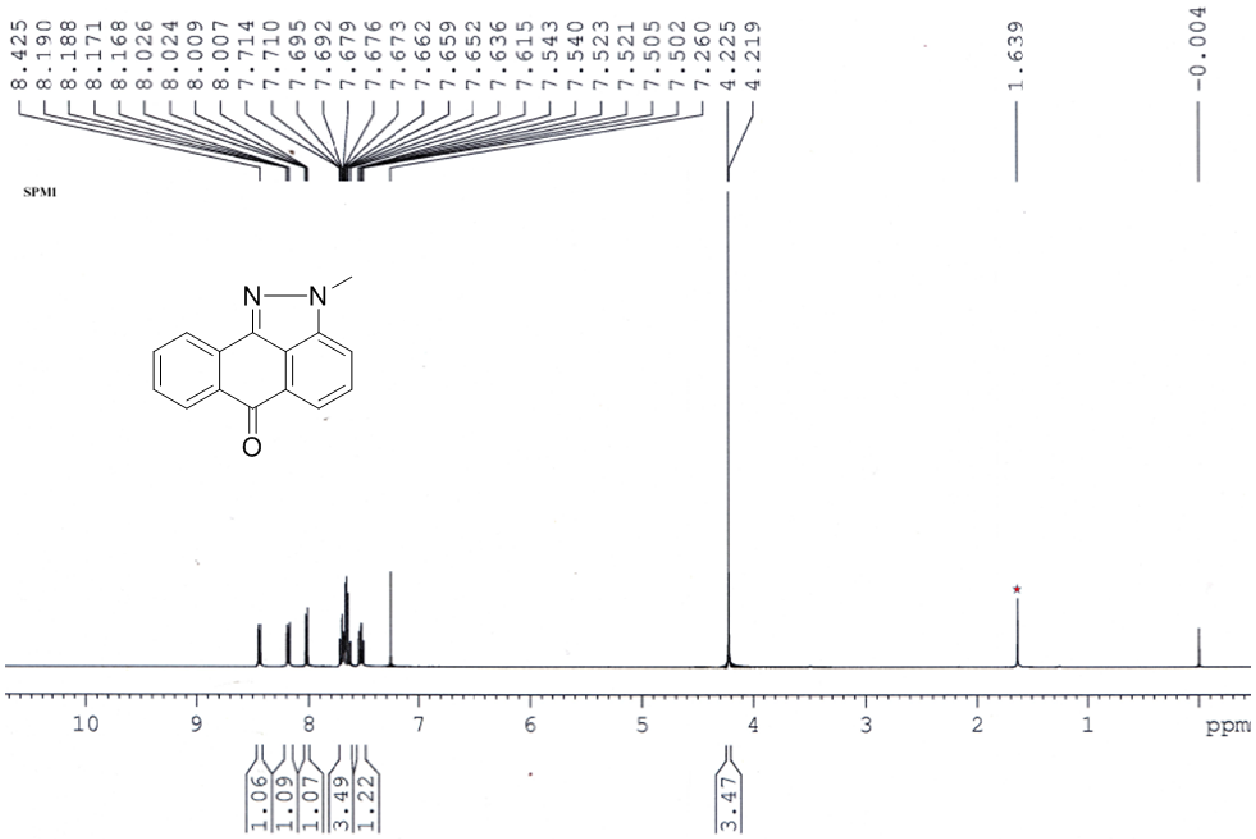


Fig. S1a ^{1}H NMR spectra of SPM1

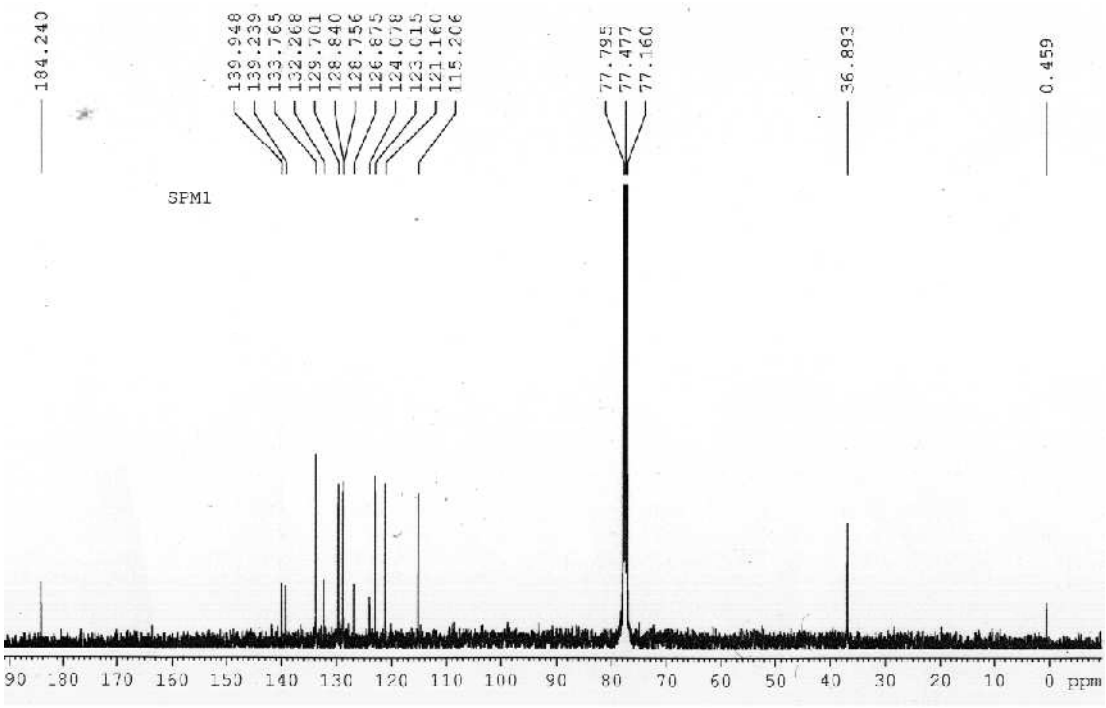


Fig. S1b ^{13}C NMR spectra of SPM1

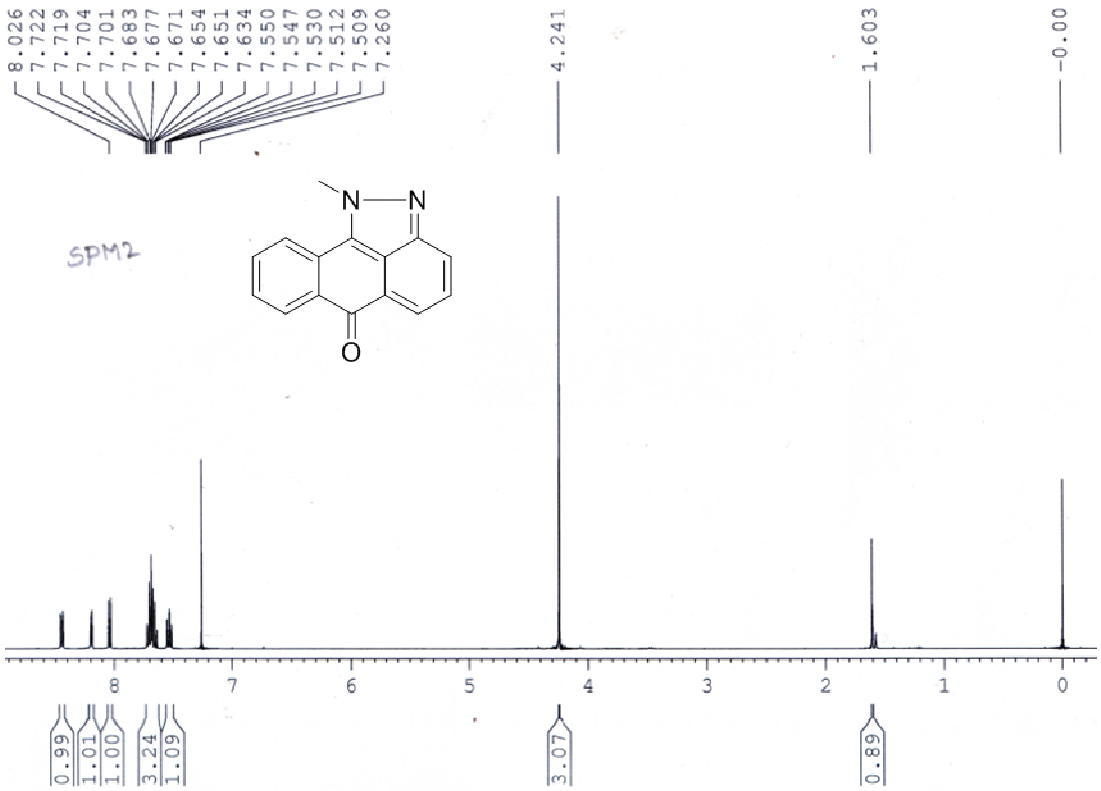


Fig. S2a ^{1}H NMR spectra of SPM2

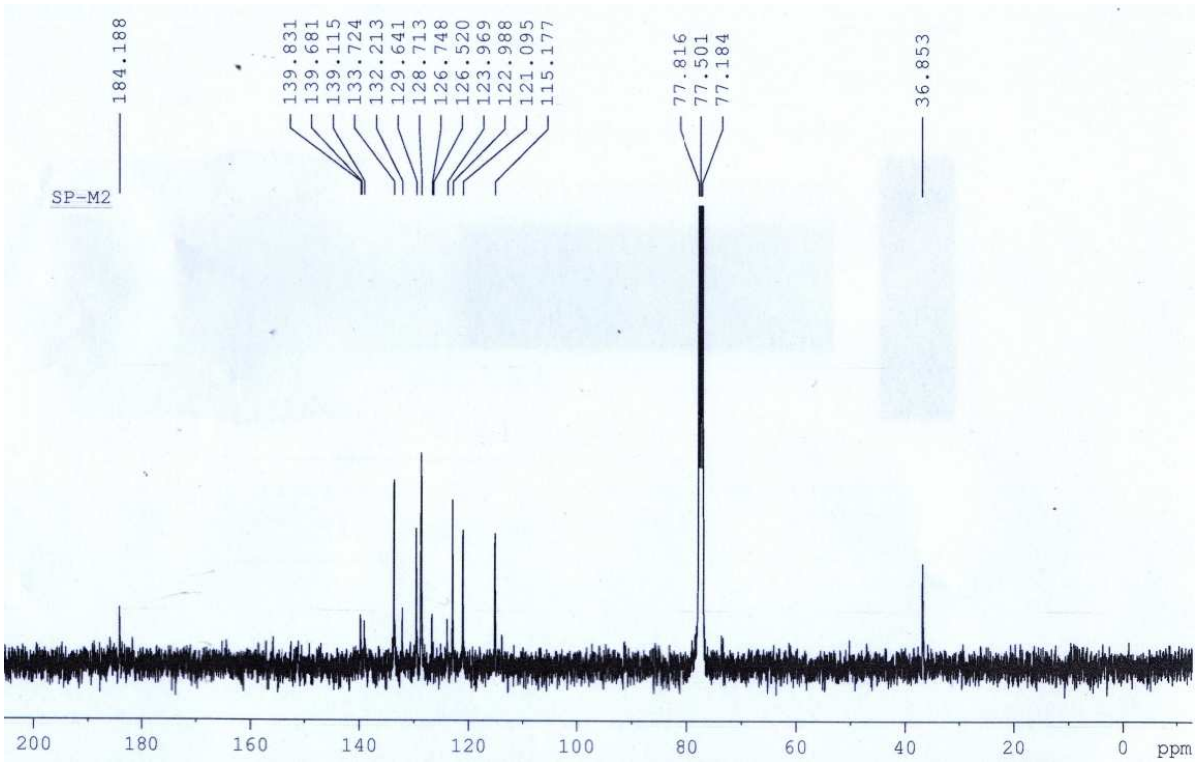


Fig. S2b ^{13}C NMR spectra of SPM2

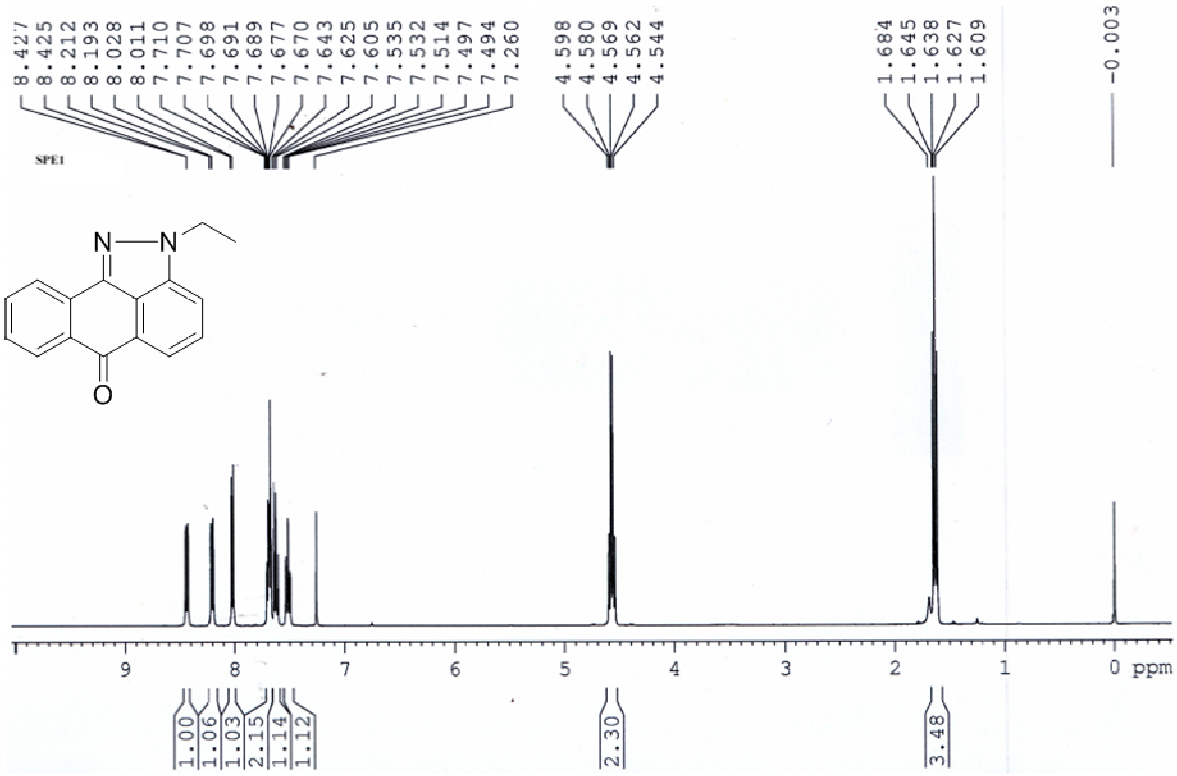


Fig. S3a ^{1}H NMR spectra of SPE1

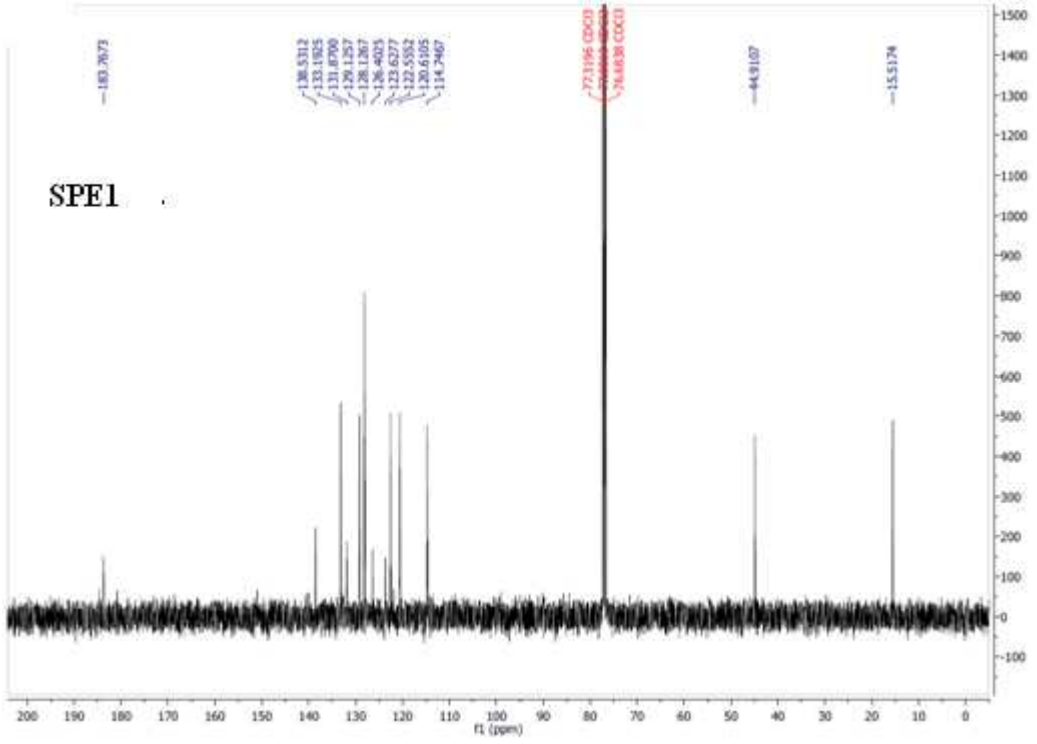


Fig. S3b ^{13}C NMR spectra of SPE1

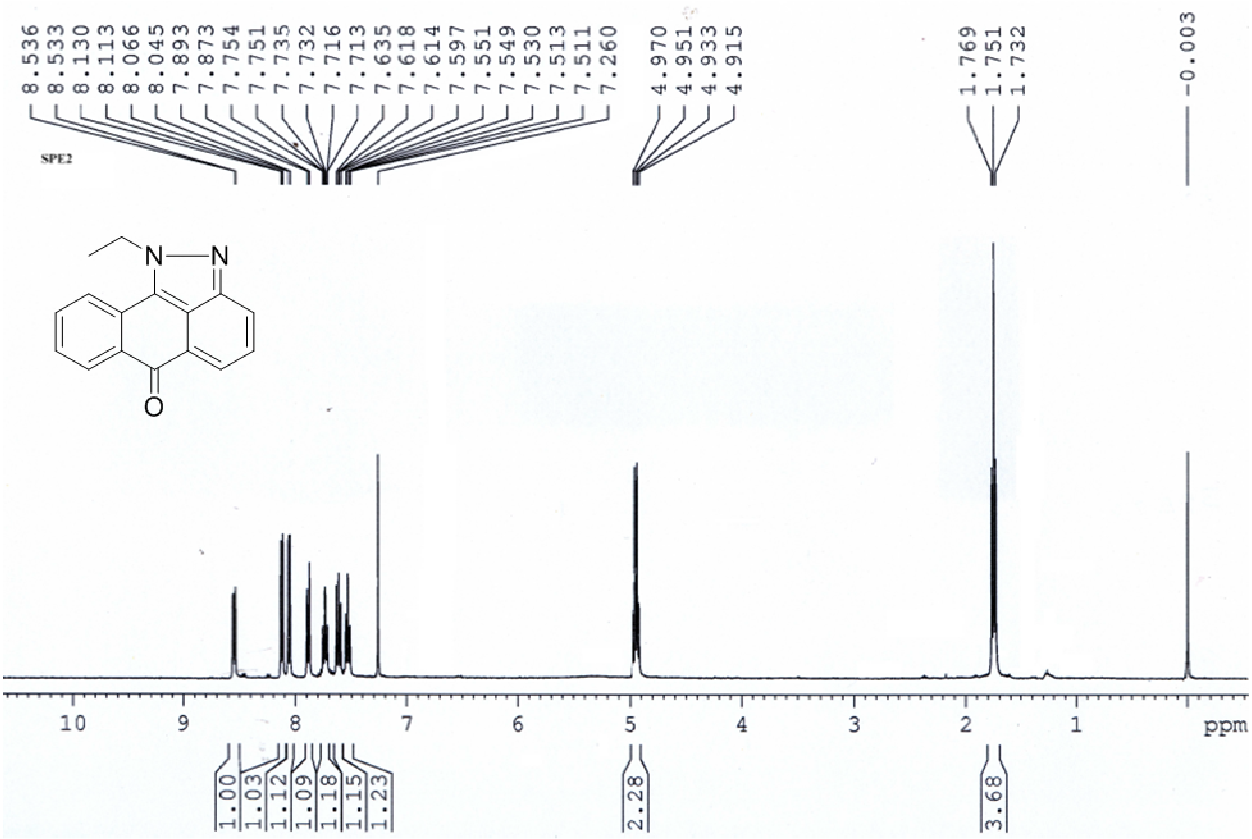


Fig. S4a ^{1}H NMR spectra of SPE2

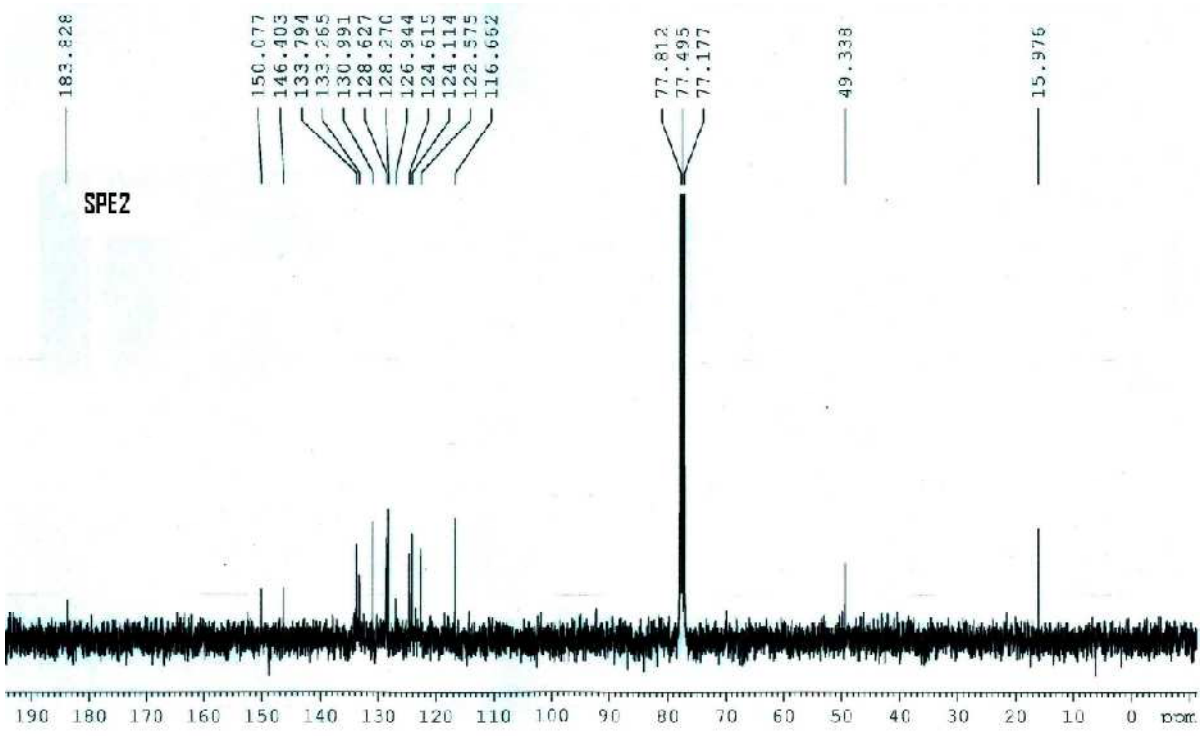


Fig. S4a ^{13}C NMR spectra of SPE2

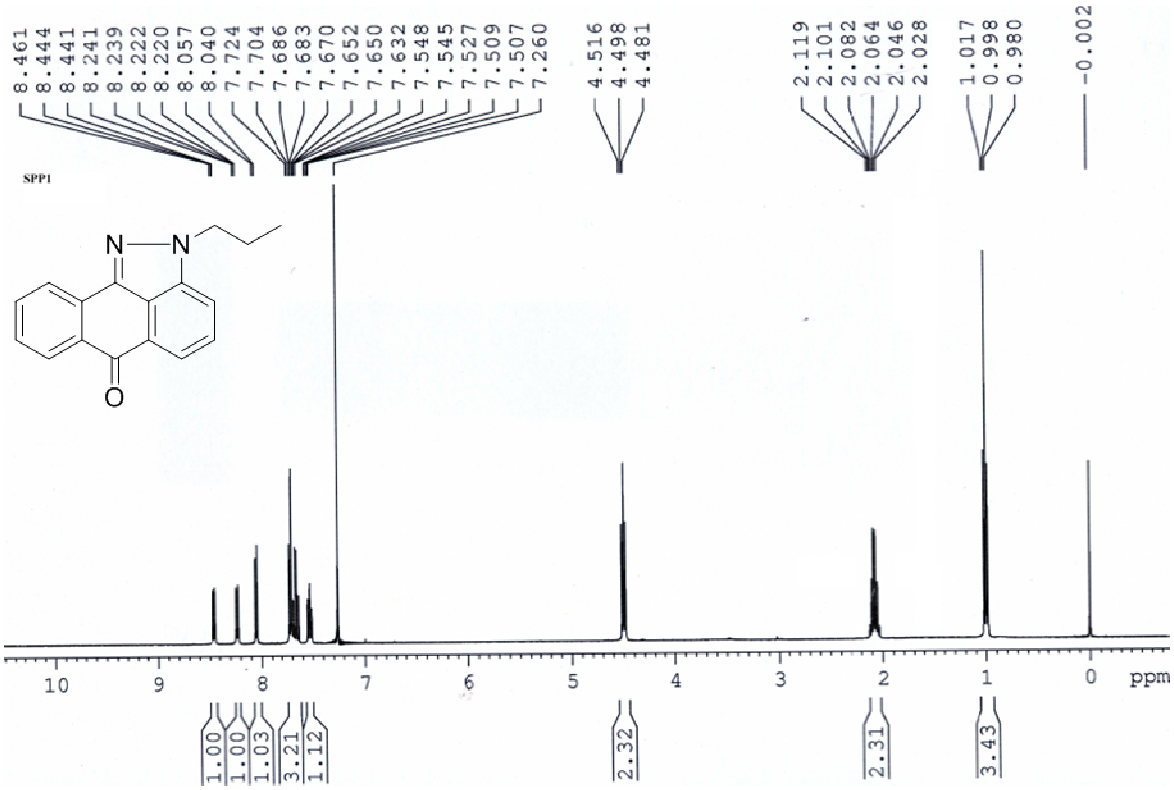


Fig. S5a ^{1}H NMR spectra of SPP1

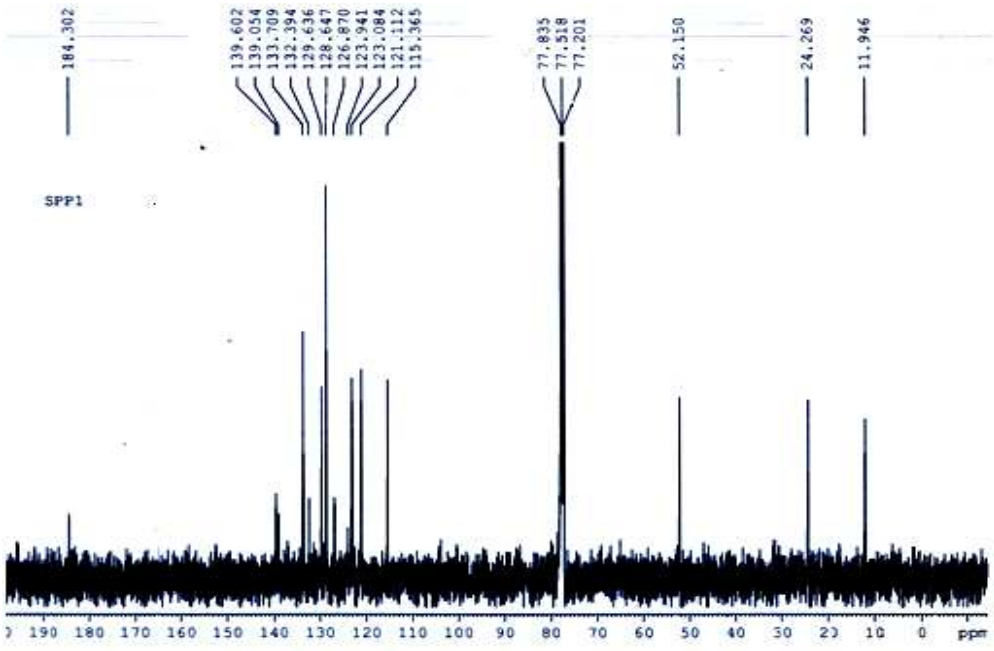


Fig. S5b ^{13}C NMR spectra of SPP1

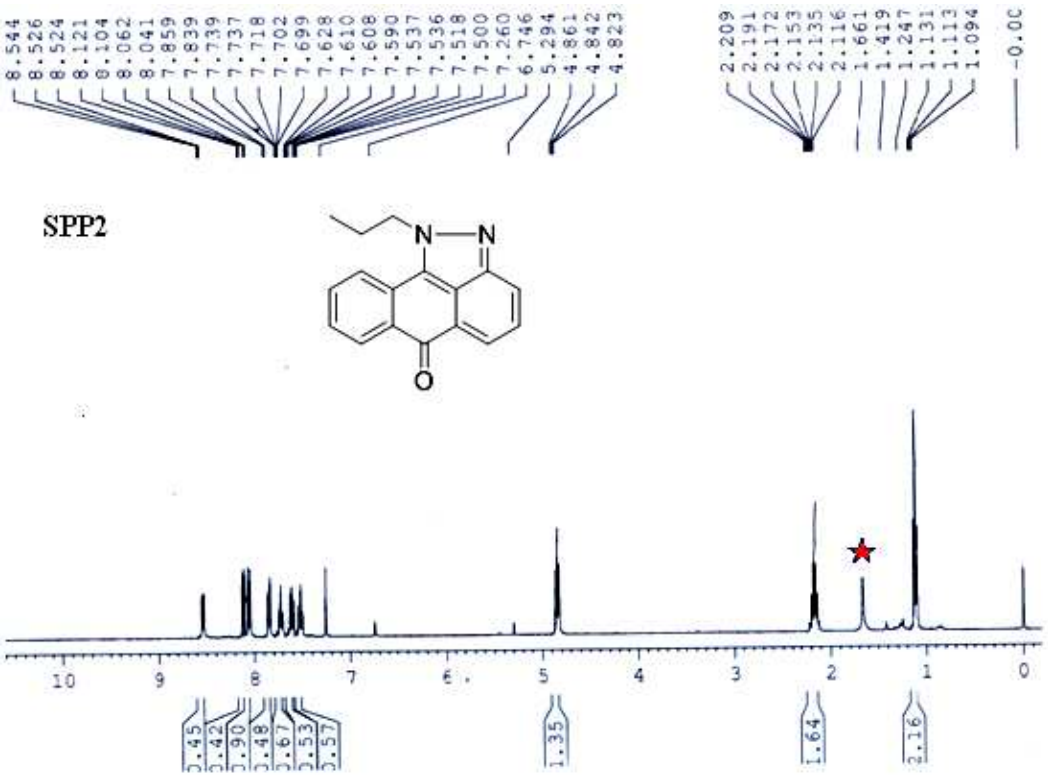


Fig. S6a ^{1}H NMR spectra of SPP2

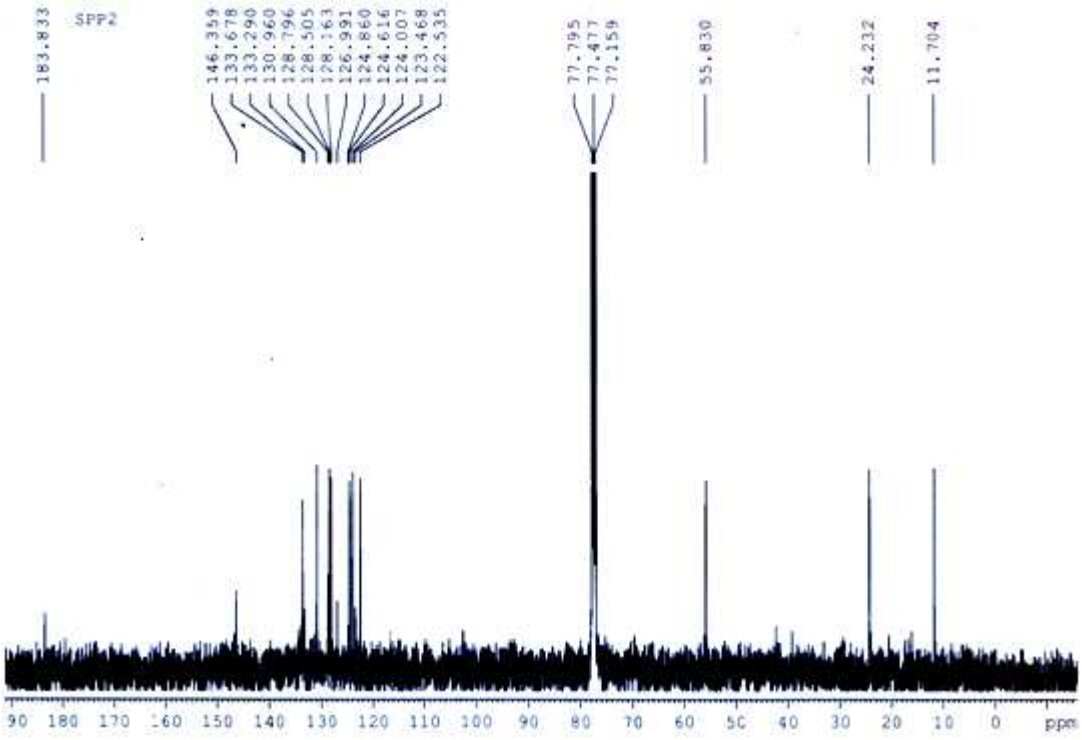


Fig. S6b ^{13}C NMR spectra of SPP2

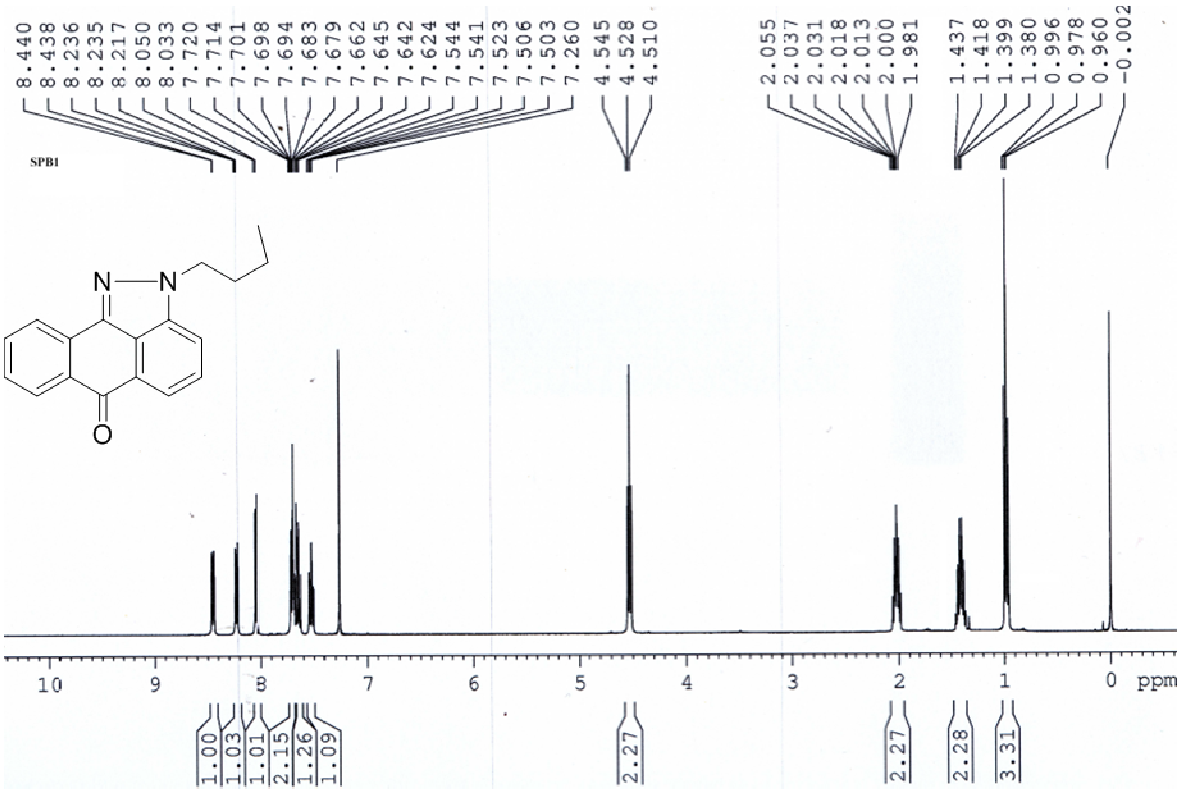


Fig. S7a ^{1}H NMR spectra of SPB1

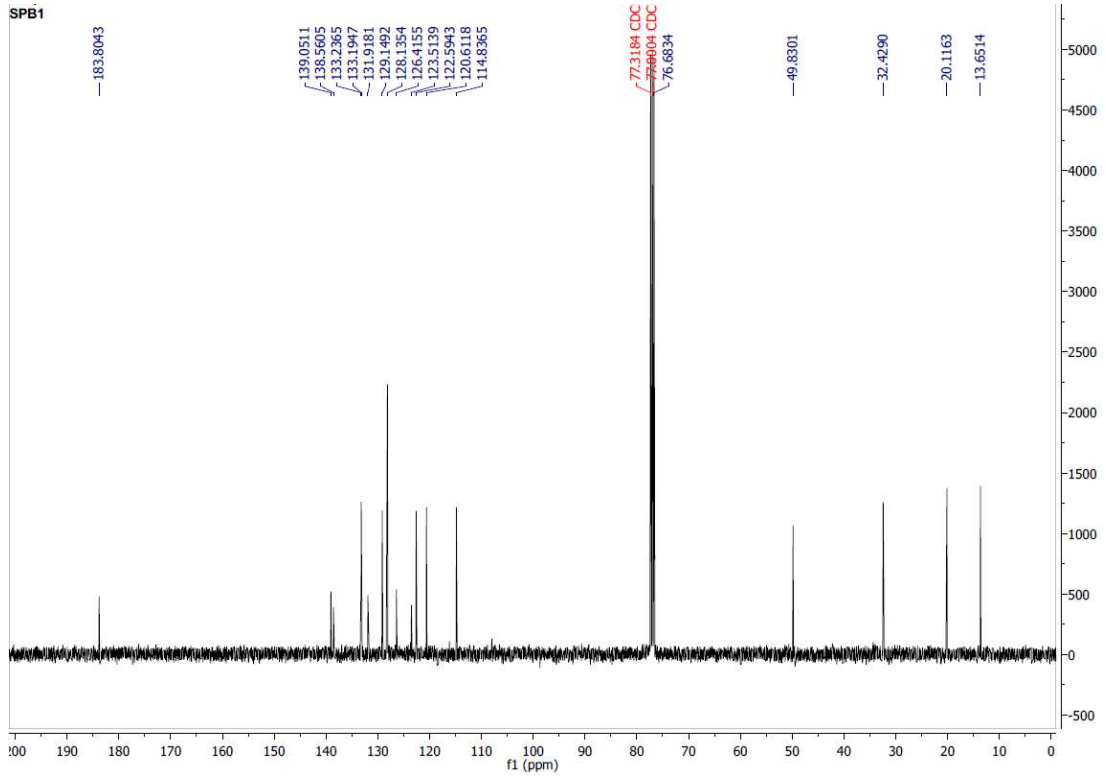


Fig. S7b ^{13}C NMR spectra of SPB1

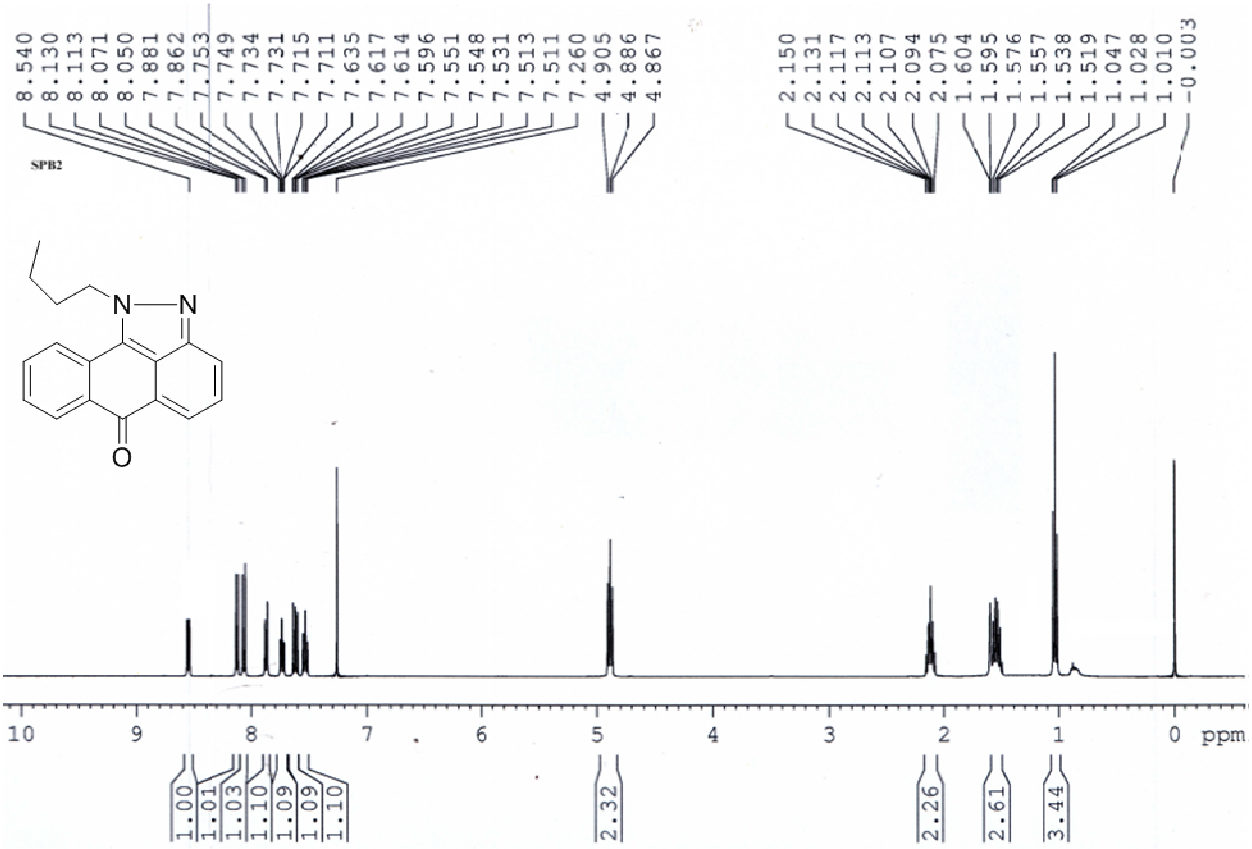


Fig. S8a ^{1}H NMR spectra of SPB2

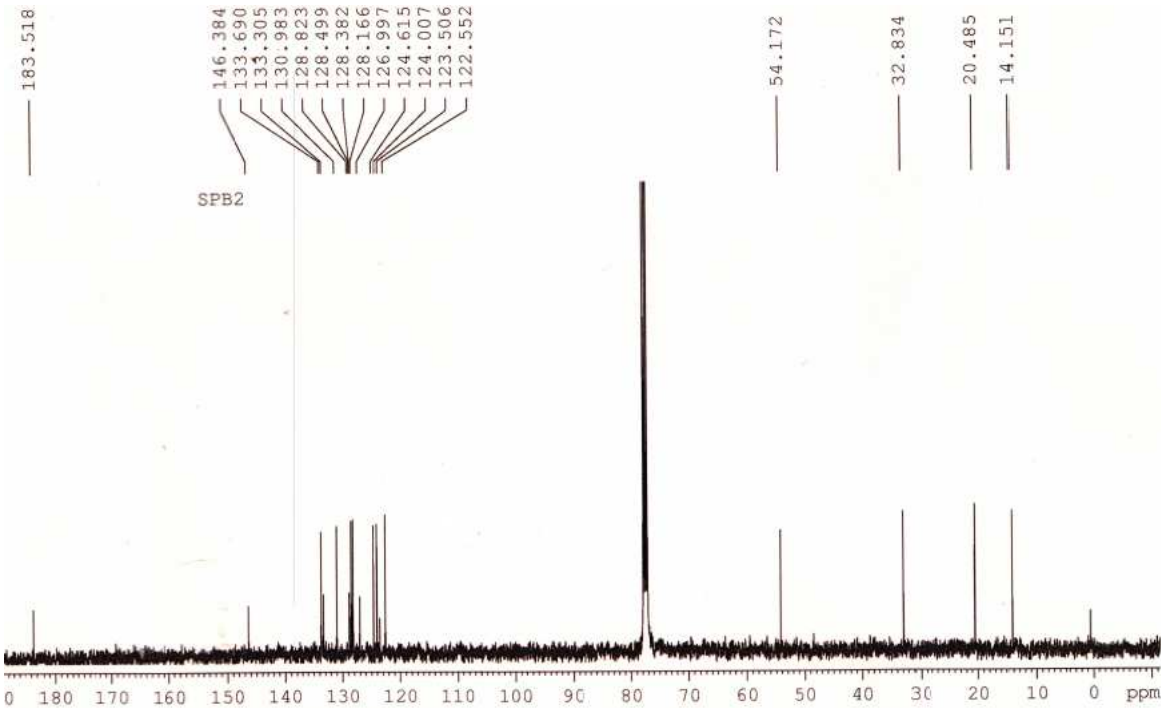


Fig. S8b ^{13}C NMR spectra of SPB2

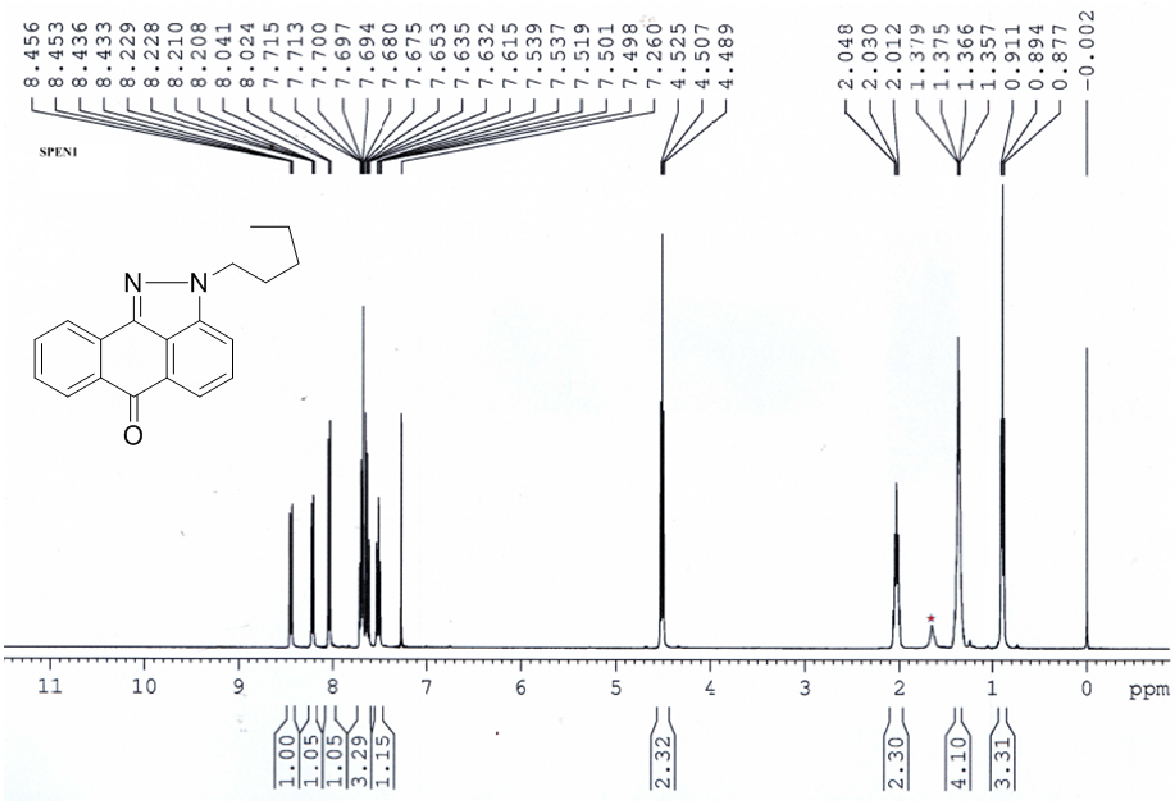


Fig. S9a ^{1}H NMR spectra of SPEN1

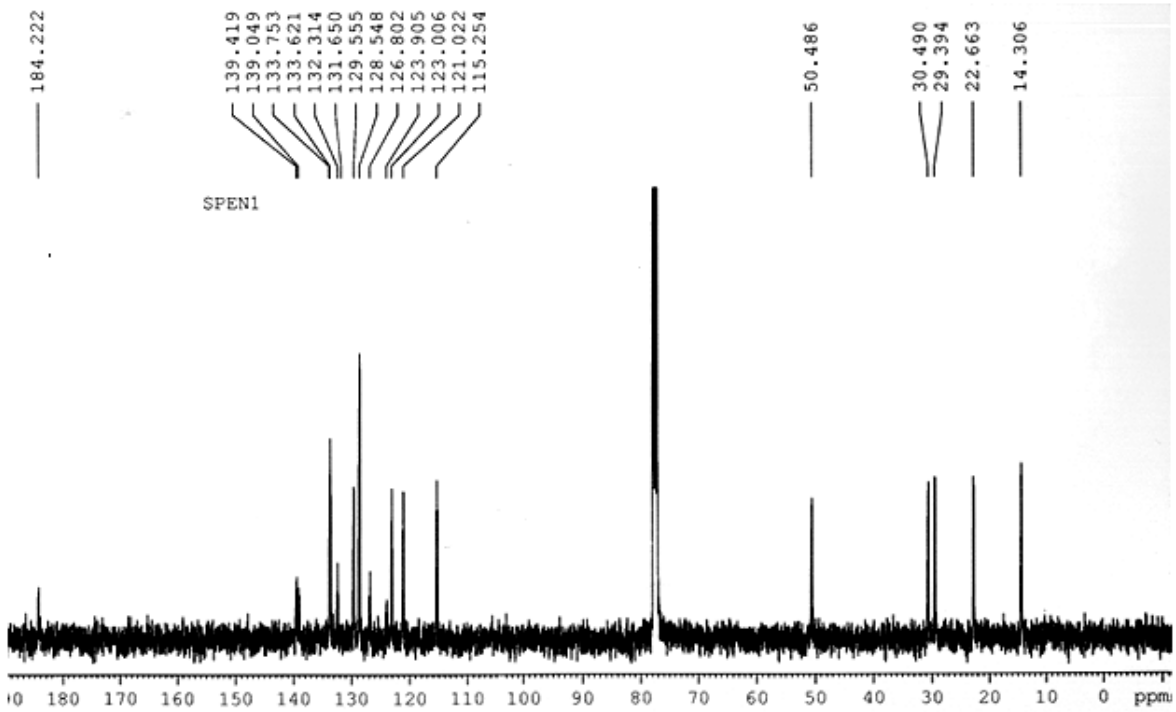


Fig. S9b ^{13}C NMR spectra of SPEN1

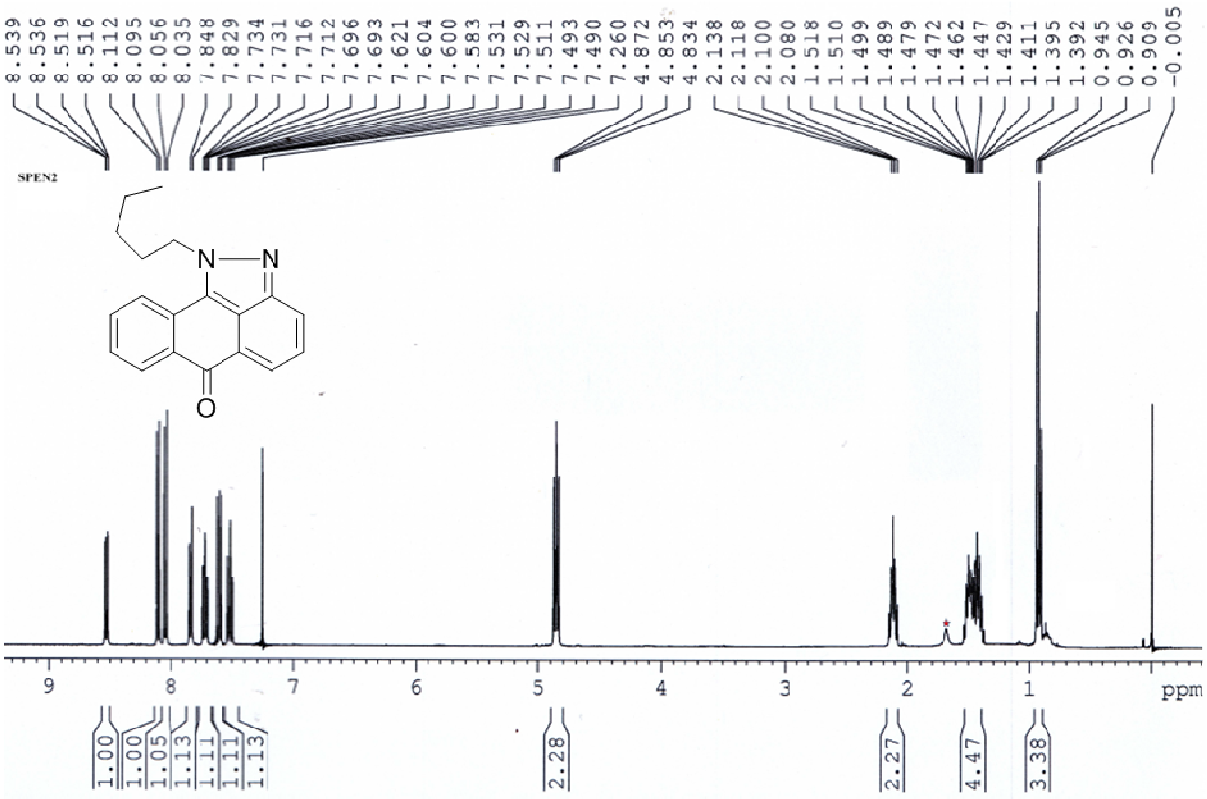


Fig. S10a ^{1}H NMR spectra of SPEN2

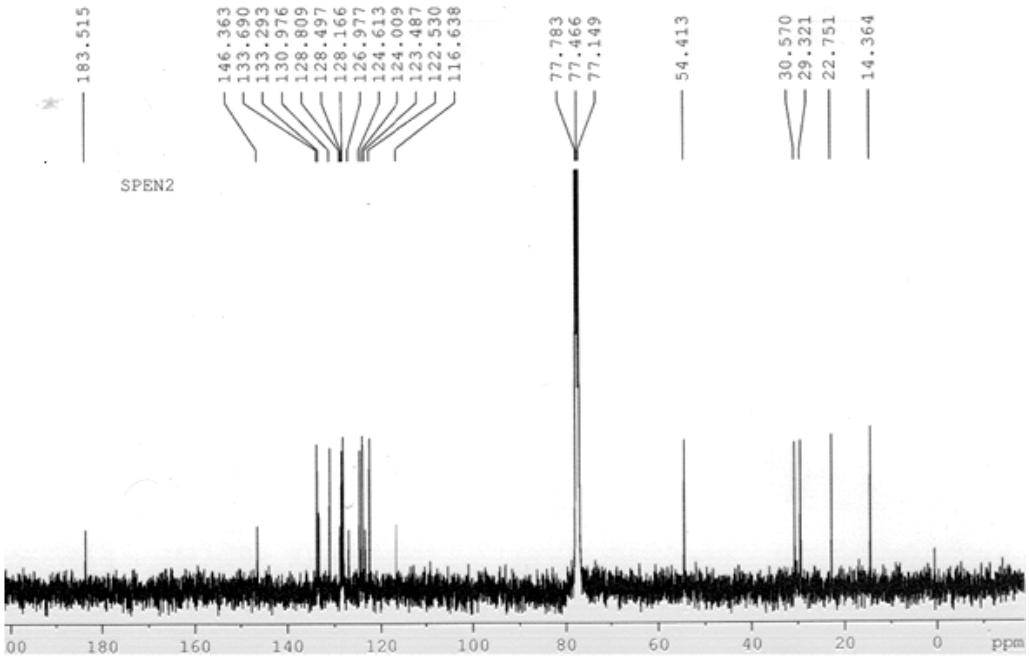


Fig. S10b ^{13}C NMR spectra of SPEN2

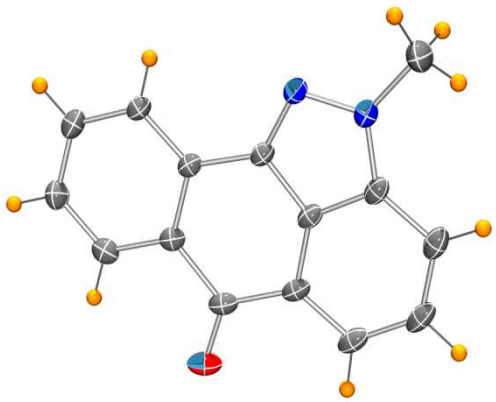
★ Indicates water.

Structural Determination: Single crystal X-ray diffraction data were collected on an Oxford Xcalibur (Mova) diffractometer equipped with an EOS CCD detector using MoKα radiation (λ= 0.71073 A). The temperature on crystal was maintained using the Oxford Instruments Cryojet-HT controller during data collection. All structures were solved by direct methods using SHELXS-97 and refined against F2 using SHELXL-97.[3] H-atoms were located geometrically and refined isotropically. The WinGX[4] package and OLEX2 (version 1.2)[5] was used for refinement and production of data tables and ORTEP-3[6] for structure visualization and making the molecular representations. Analysis of the H-bonded and π•••π interactions was carried out using PLATON[7] for all the structures. Packing diagrams were generated by using MERCURY.[8]

Structural determination of alkylated 1,9-pyrazolonthrones:

Methyl derivative of 1,9-pyrazoloanthrone: The two isomers were isolated by column chromatography by elucidating with 20 % of EtOAc/ hexane.

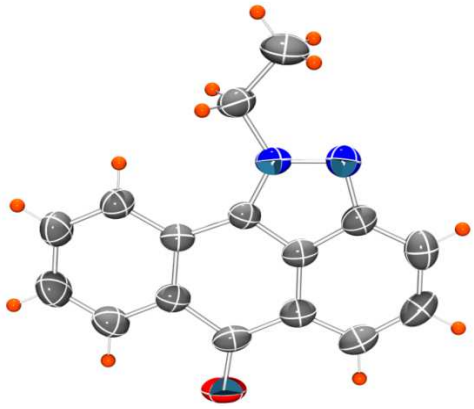
1-Methyl-(anthra[1,9-c,d]pyrazol)-6(1H)-one (SPM1): Single crystals of this isomer were efficaciously obtained by slow evaporation of $CHCl_3$/CH_3OH at ambient temperatures and solved in the monoclinic space group $P2_1/n$ with Z=4 where Z′=1



ORTEP of **SPM1** with displacement ellipsoids at 50% probability level at 120K

Ethyl derivative of 1,9-pyrazoloanthrone: The two isomers were isolated by column chromatography by elucidating with $CHCl_3$.

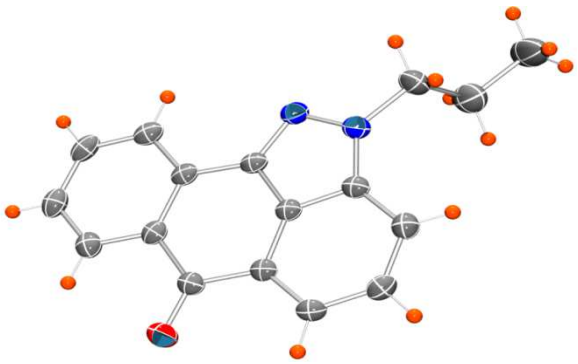
2-Ethyl-(anthra[1,9-c,d]pyrazol)-6(1H)-one (SPE2): Once again the single crystals were obtained from 20% EtOAc by slow evaporation and the reflection were collected and reduced in a monoclinic space group $P2_1$ with Z=2 and Z′ being 1. Synthesis of propyl derivative: The two isomers were isolated by column chromatography by elucidating with 1:1 mixture of $CHCl_3$ and EtOAc.



ORTEP of **SPE1** with displacement ellipsoids at 50% probability level at 290K

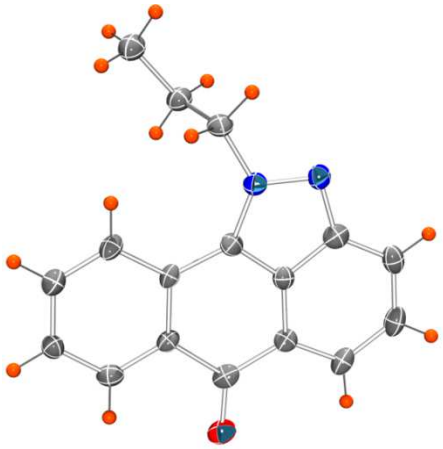
propyl derivative of 1,9-pyrazoloanthrone: These two isomers were isolated by column chromatography by elucidating with 1:1 mixture of $CHCl_3$ and EtOAc.

1-Propyl-(anthra[1,9-c,d]pyrazol)-6(1H)-one (SPP1): The single crystals of this isomer obtained from DCM/CH_3OH by slow evaporation and the compound crystallizes in a trigonal space group *R* -3 with Z=18 where Z′=1.



ORTEP of **SPP1** with displacement ellipsoids at 50% probability level at 120K

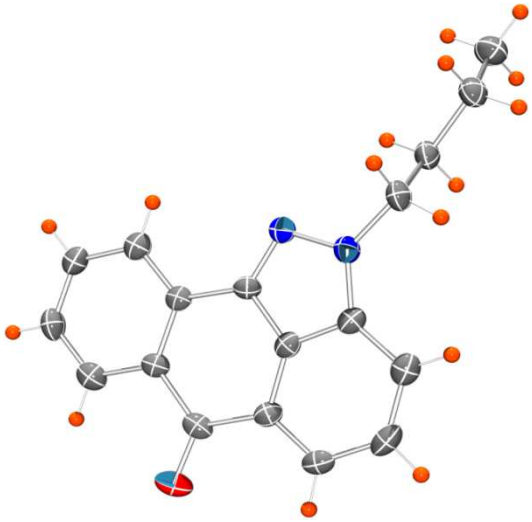
2-Propyl-(anthra[1,9-c,d]pyrazol)-6(1H)-one (SPP2): This isomer crystallized by slow evaporation of 30% EtOAc. It crystallizes in an Orthorhombic space group $P2_12_12_1$ with Z=4 where Z′=1.



ORTEP of **SPP2** with displacement ellipsoids at 50% probability level at 120K

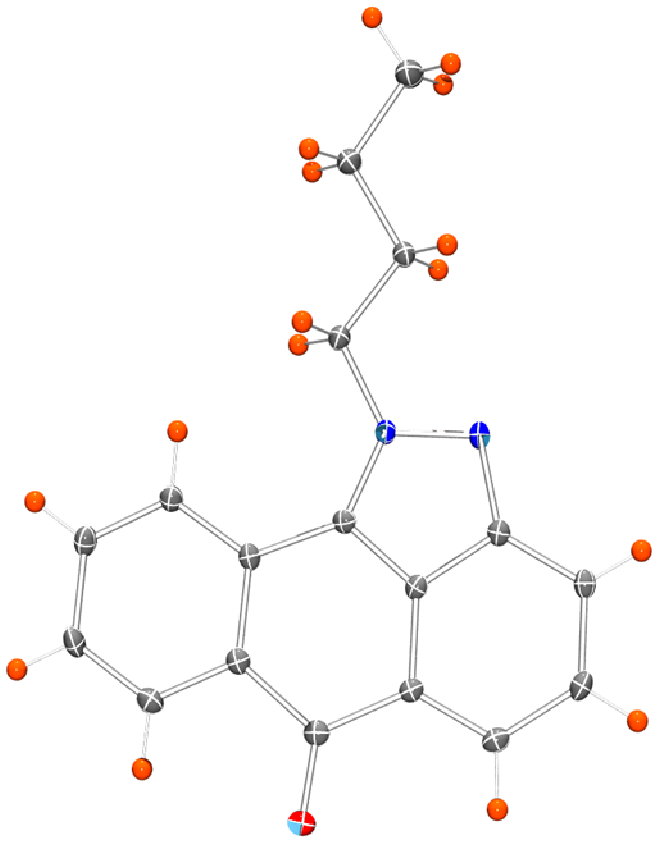
butyl derivative of 1,9-pyrazoloanthrone: These two isomers were isolated by column chromatography by elucidating with 1:1 mixture of $CHCl_3$ and EtOAc.

1-Butyl-(anthra[1,9-c,d]pyrazol)-6(1H)-one (SPB1): The single crystals were grown by slow evaporation of 30% EtOAc: hexane mixture at ambient temperatures. The compound crystallized in a Orthorhombic space group $P2_12_12_1$ with Z=4 where Z′=1.



ORTEP of **SPB1** with displacement ellipsoids at 50% probability level at 120K

2-Butyl-(anthra[1,9-c,d]pyrazol)-6(1H)-one (SPB2): Here again the compound crystallized from 30% EtOAc: hexane mixture at ambient temperatures. Reflections were collected and fitted for a monoclinic space group $P2_1/c$ with Z=4 where Z′=1.



ORTEP of **SPB2** with displacement ellipsoids at 50% probability level at 100K

Pentyl derivative of 1,9-pyrazoloanthrone : By using 1:1 mixture of $CHCl_3$ and EtOAc in column chromatography these two isomers were separated.

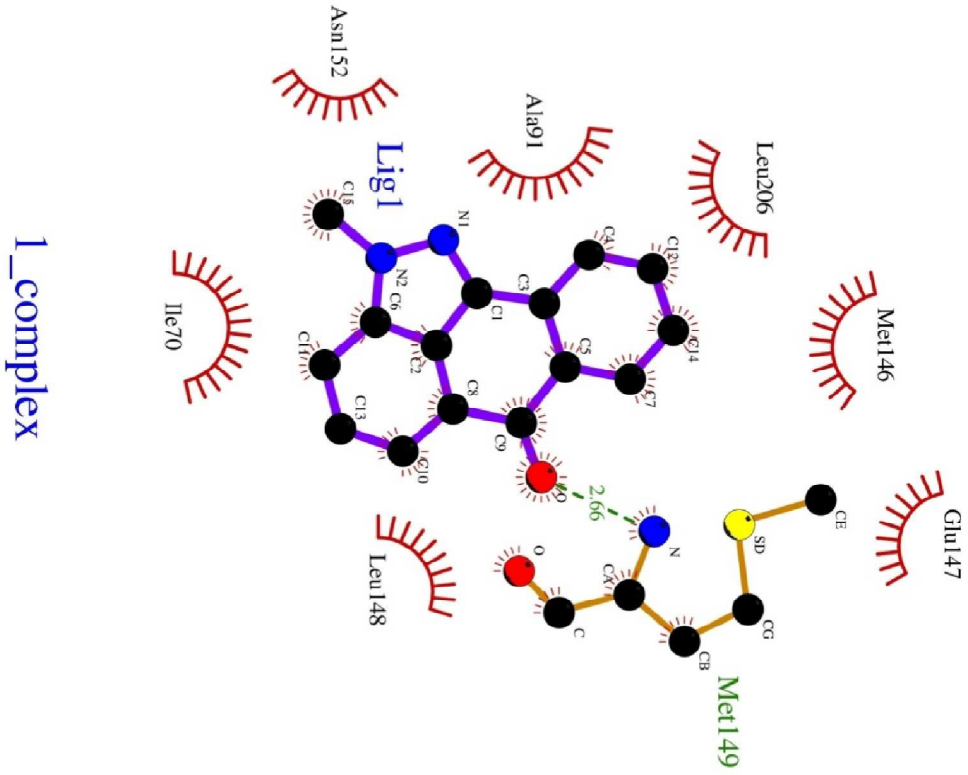


Fig. S11 Ligplot showing interactions in active site of JNK with SPM1

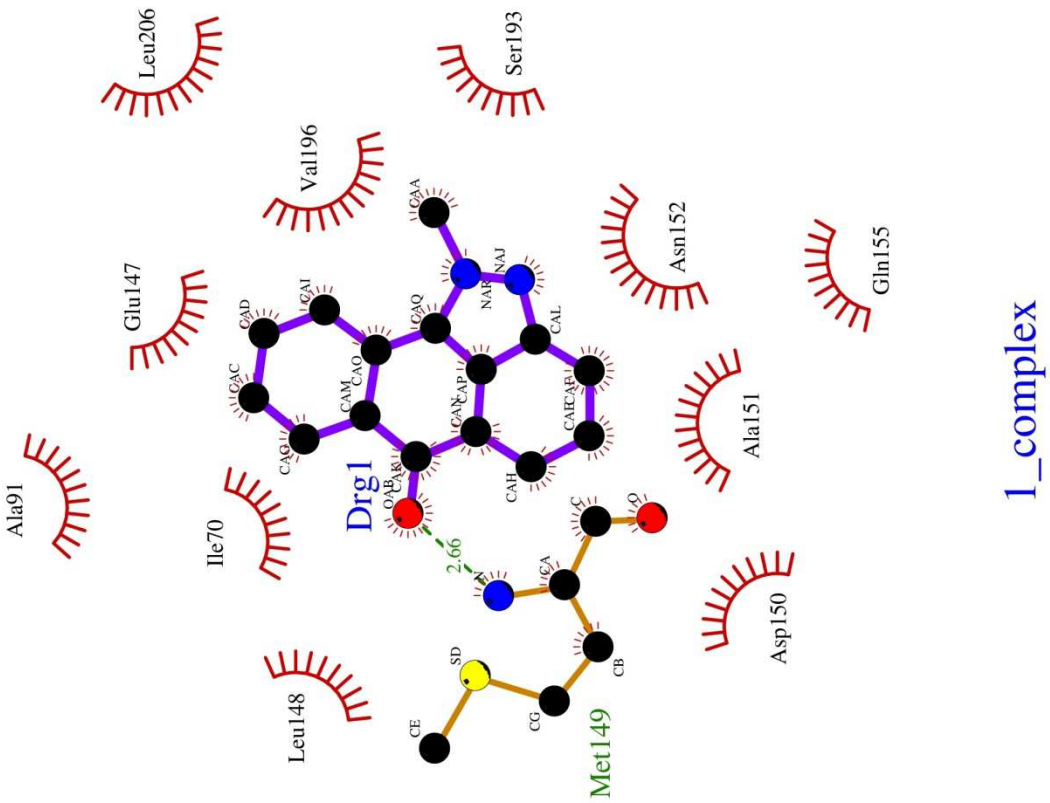


Fig. S12 Ligplot showing interactions in active site of JNK with SPM2

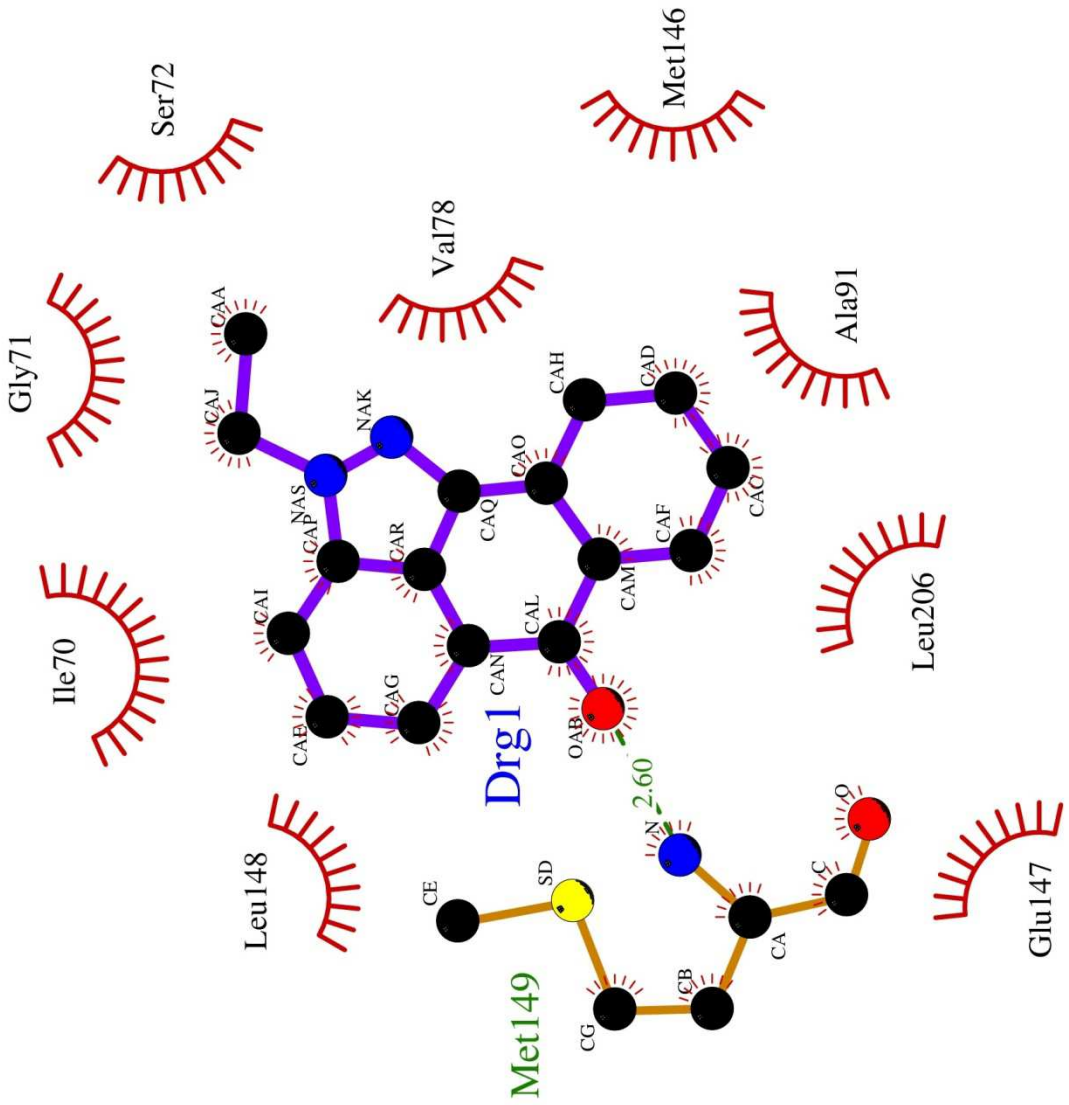


Fig. S13 Ligplot showing interactions in active site of JNK with SPE1

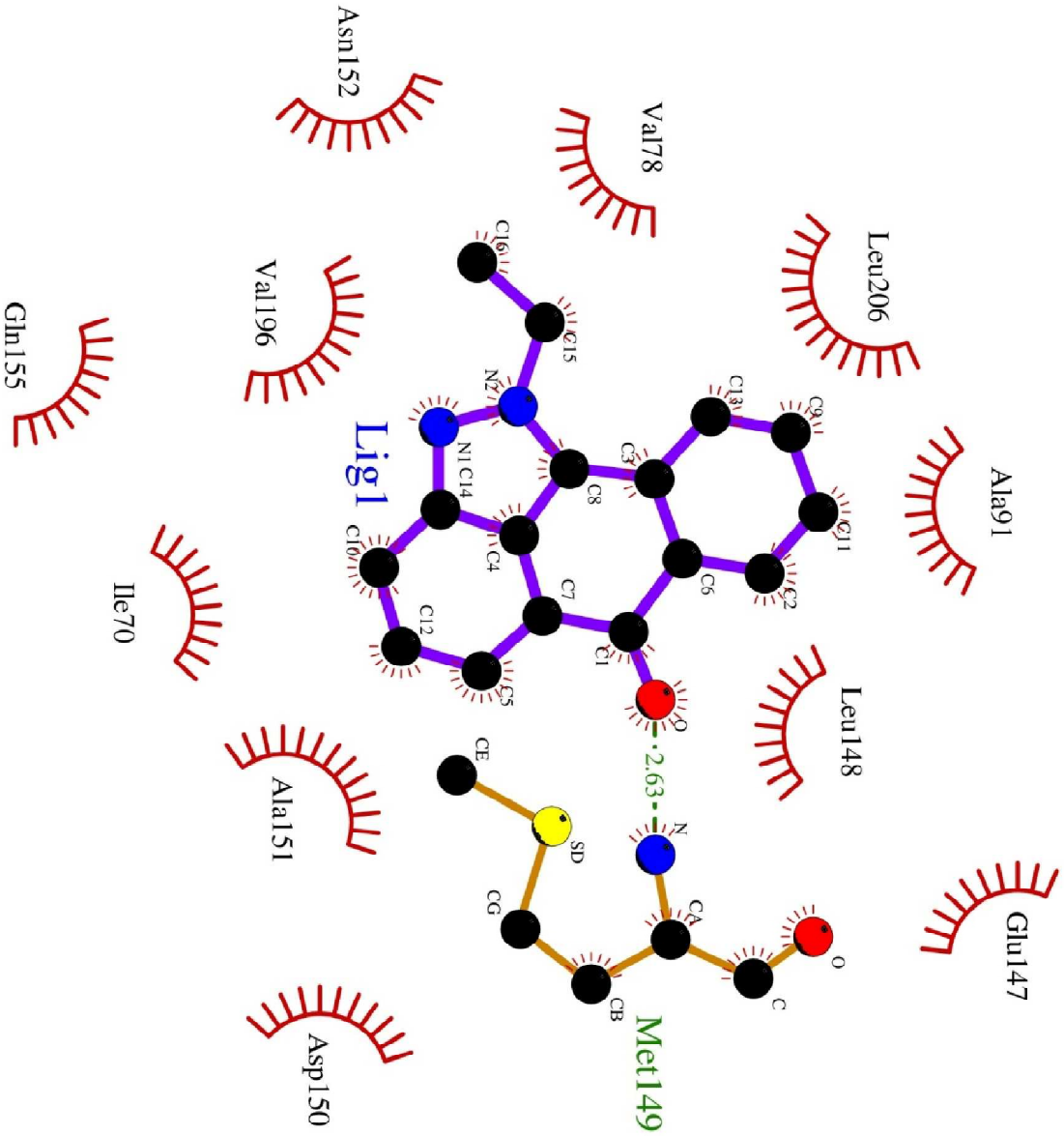


Fig. S14 Ligplot showing interactions in active site of JNK with SPE2

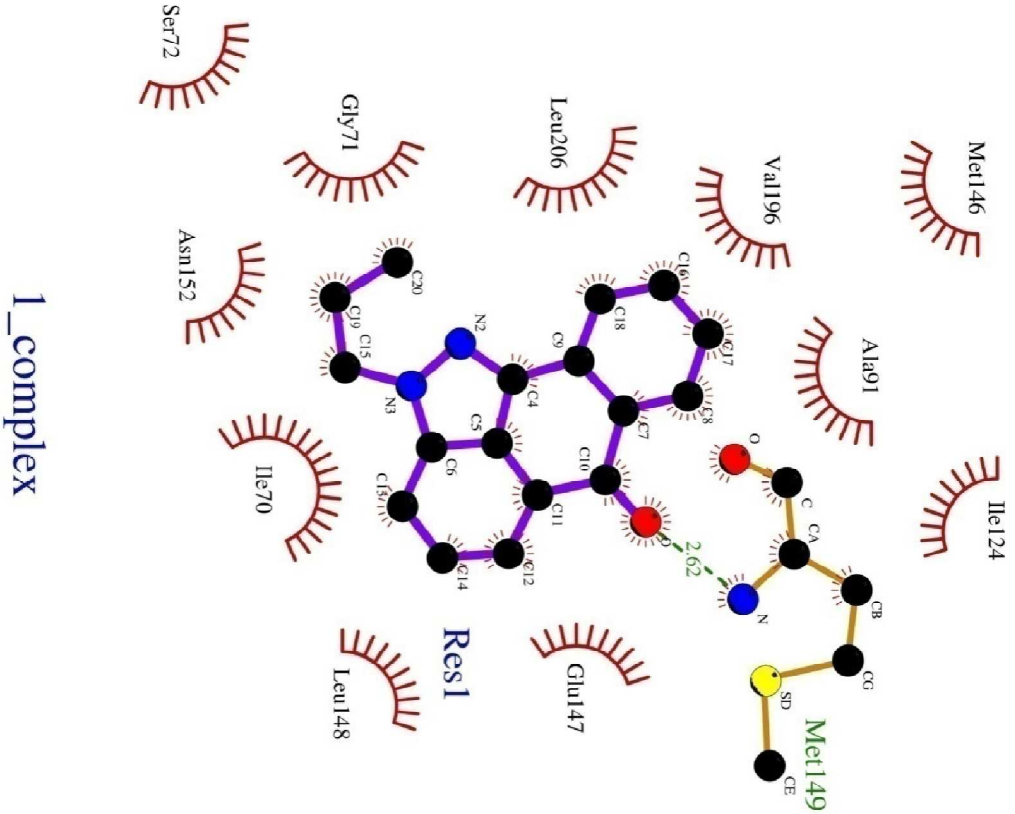


Fig. S15 Ligplot showing interactions in active site of JNK with SPP1

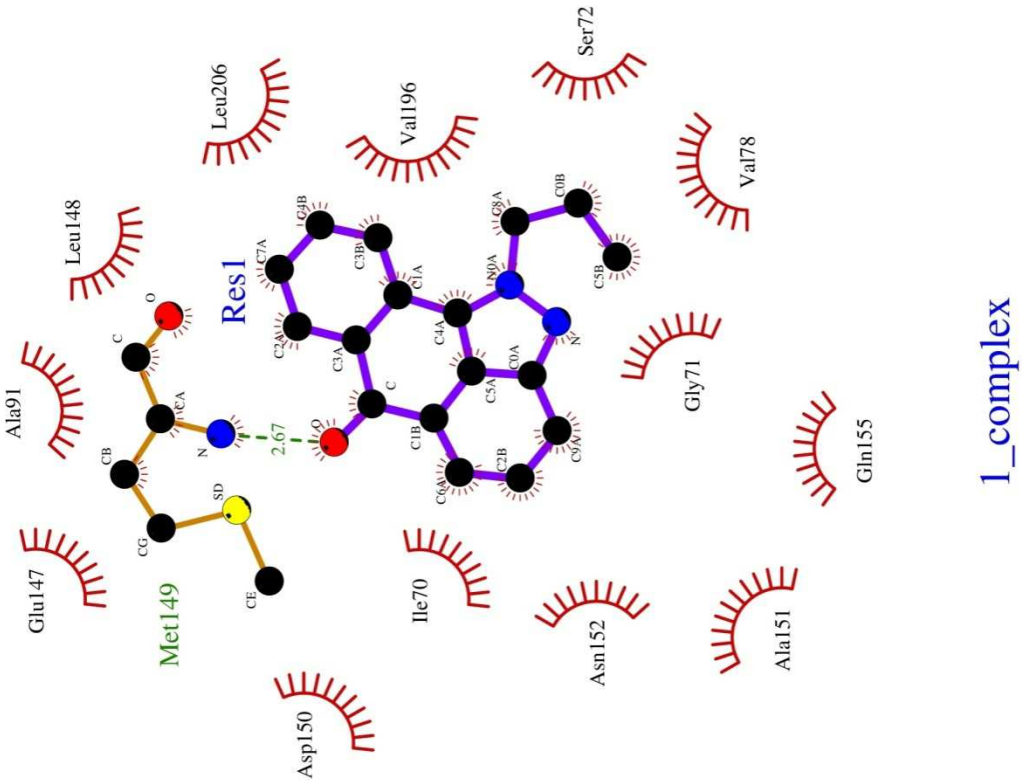


Fig. S16 Ligplot showing interactions in active site of JNK with SPP2

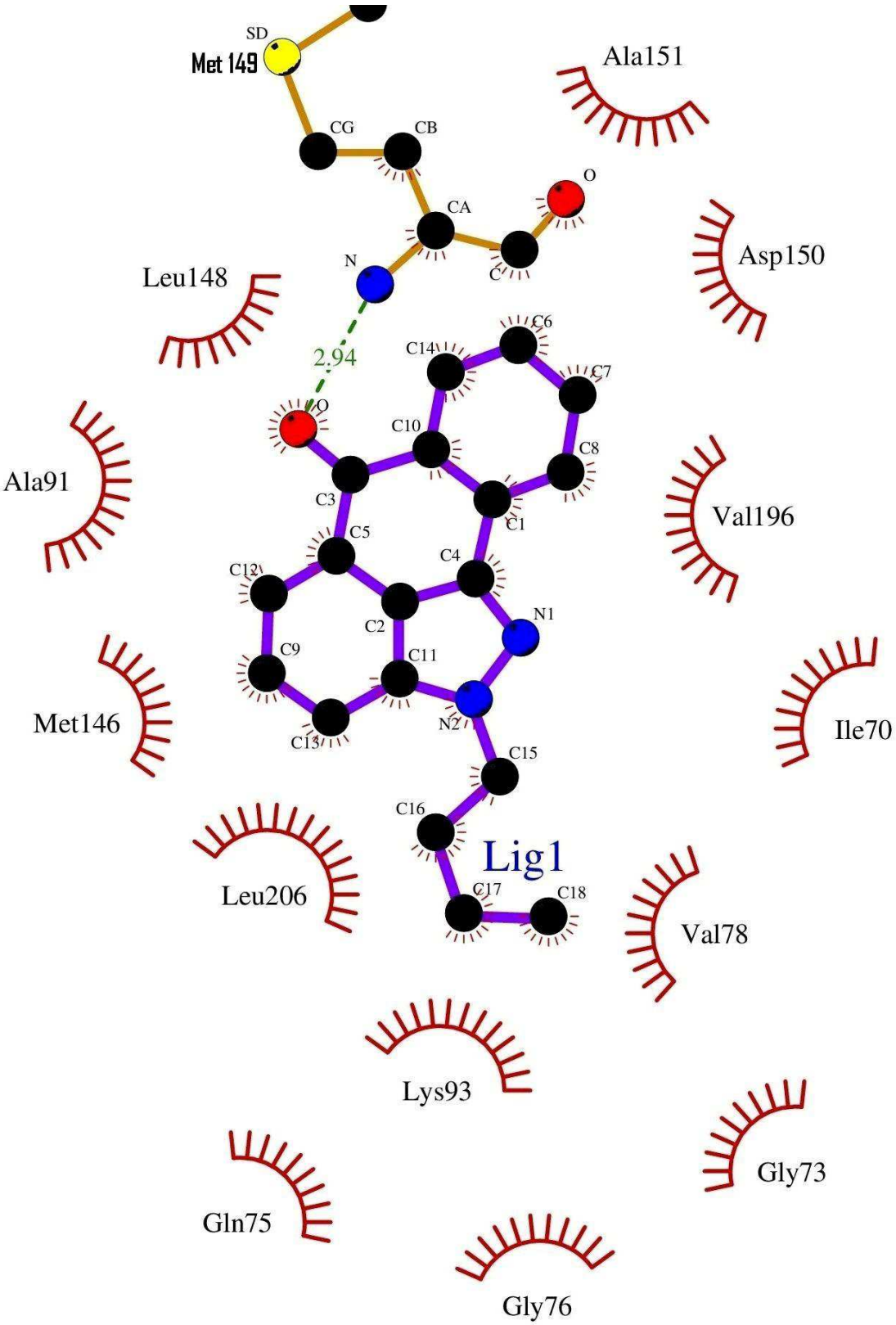


Fig. S17 Ligplot showing interactions in active site of JNK with SPB1

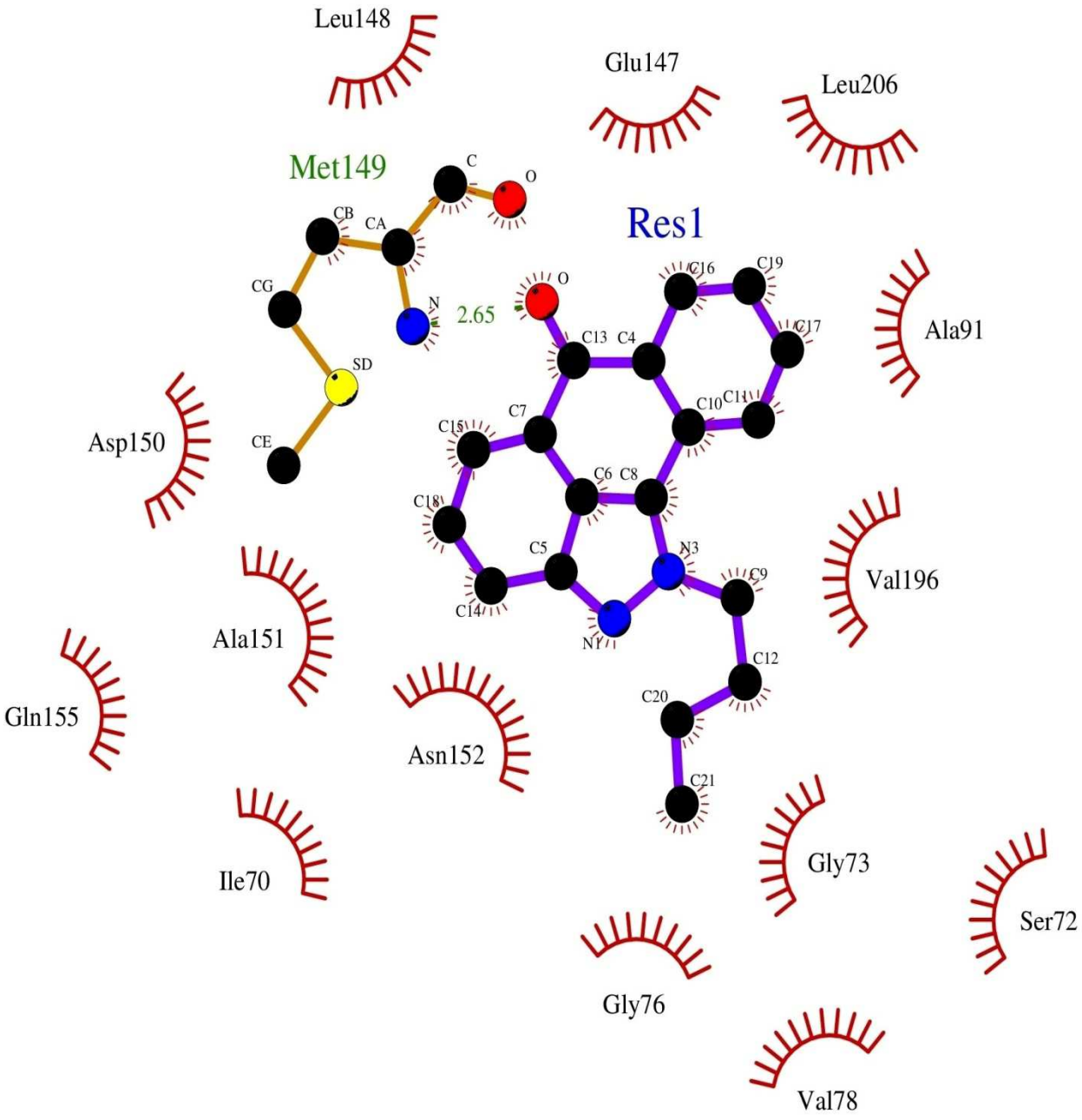


Fig. S18 Ligplot showing interactions in active site of JNK with SPB2

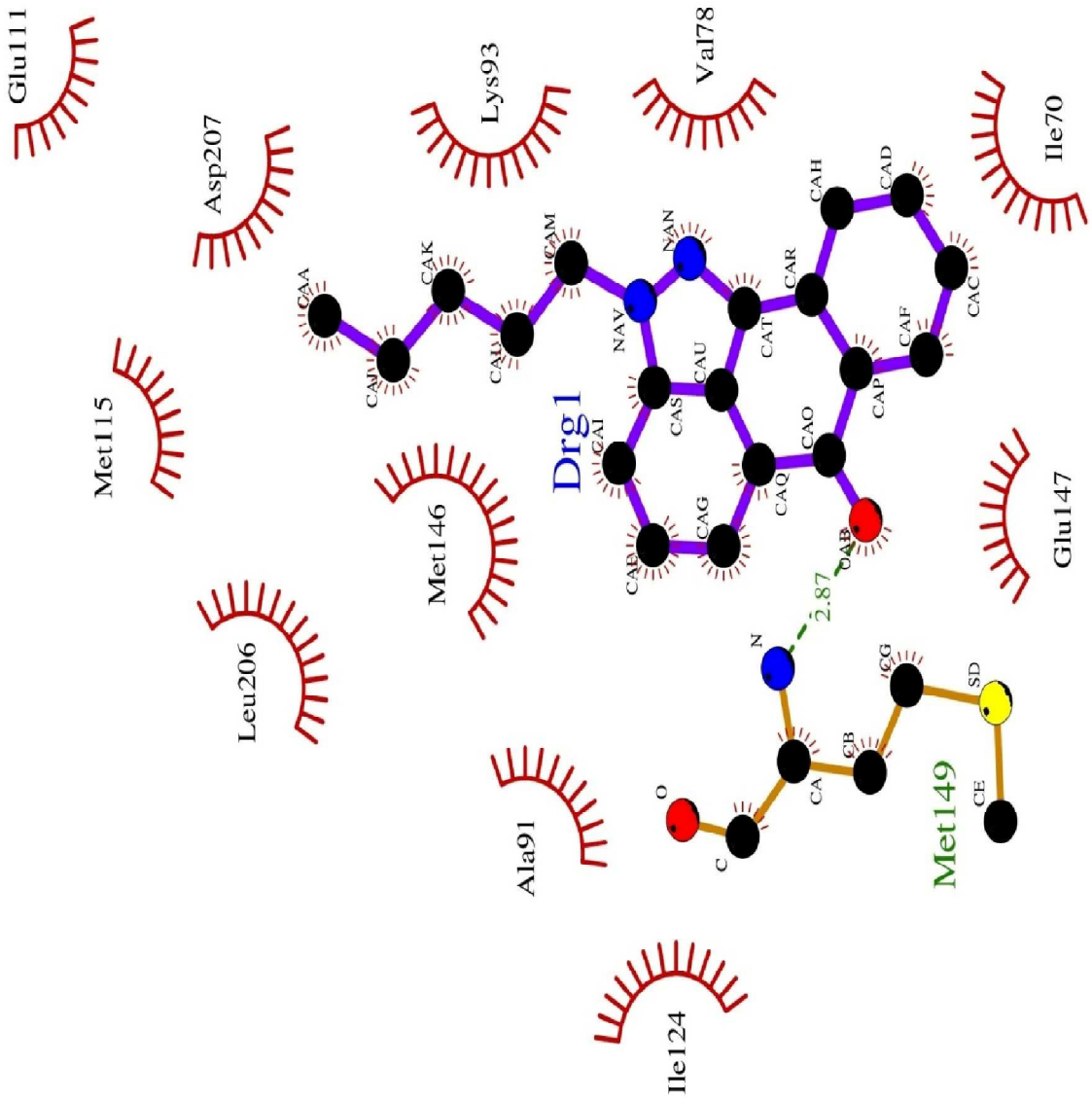


Fig. S19 Ligplot showing interactions in active site of JNK with SPEN1

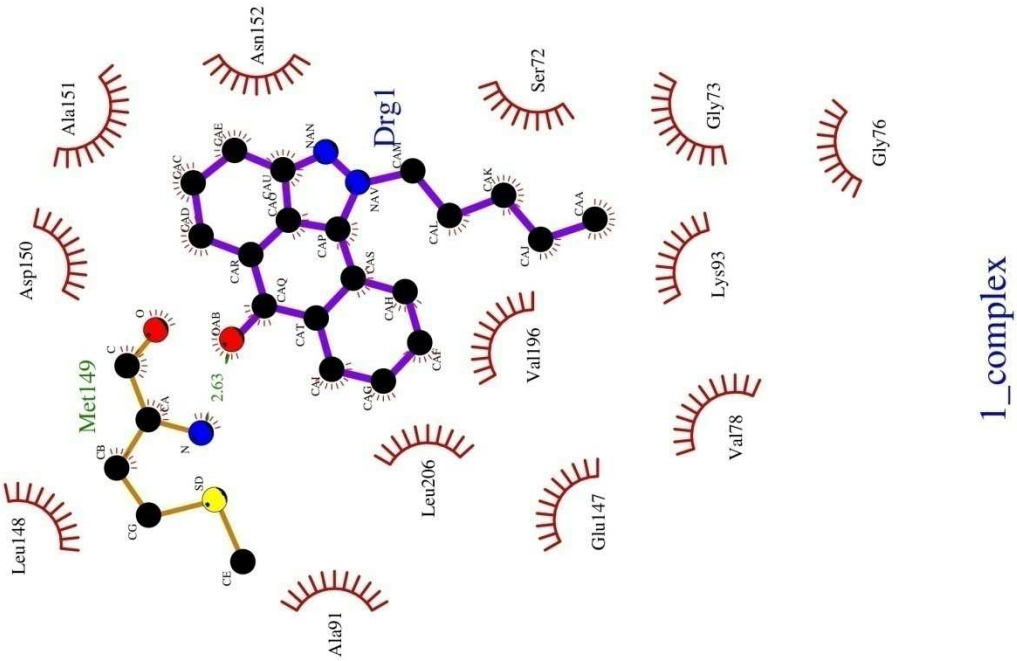


Fig. S20 Ligplot showing interactions in active site of JNK with SPEN2

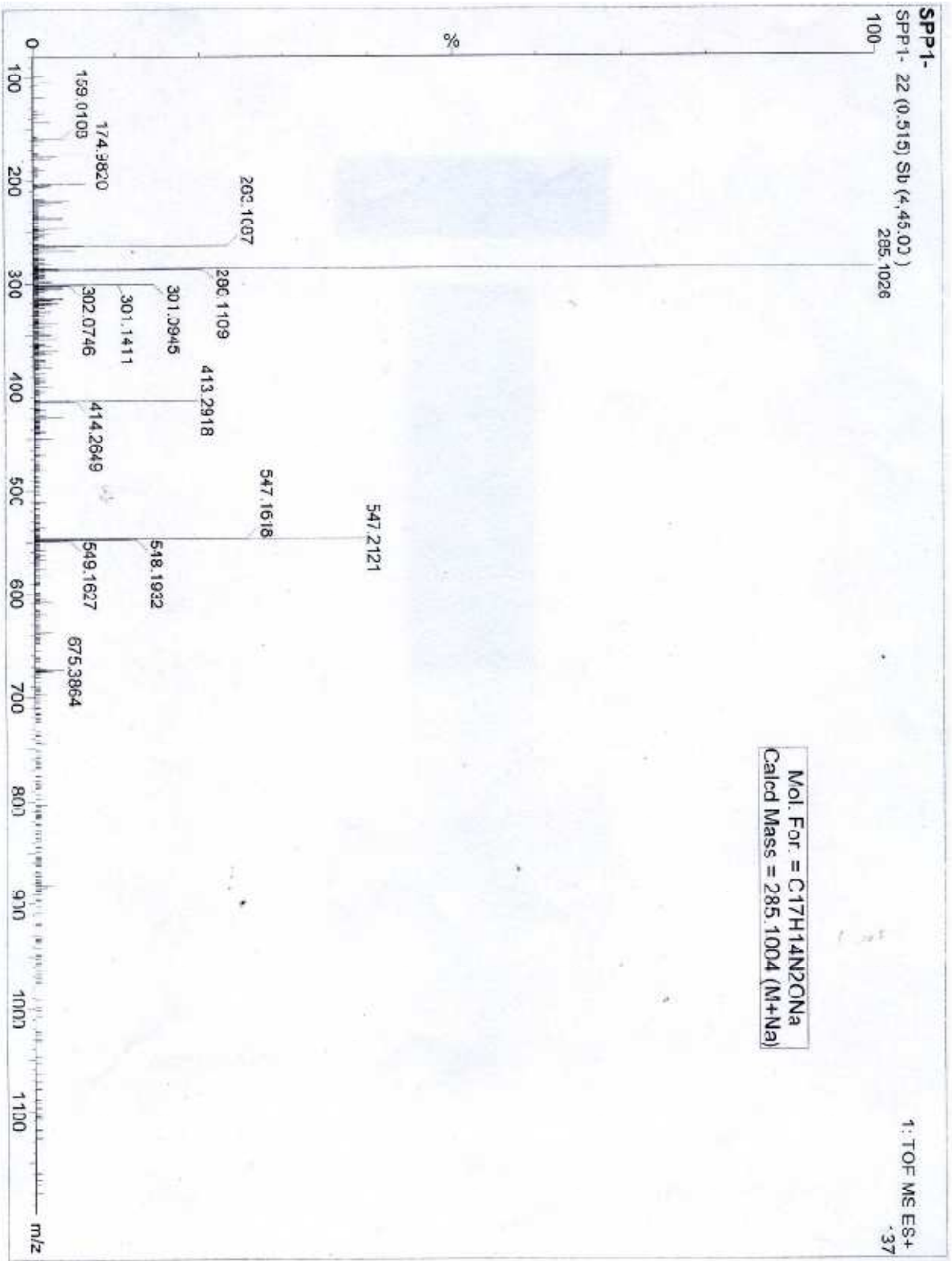


Fig. S21 HRMS of SPP1

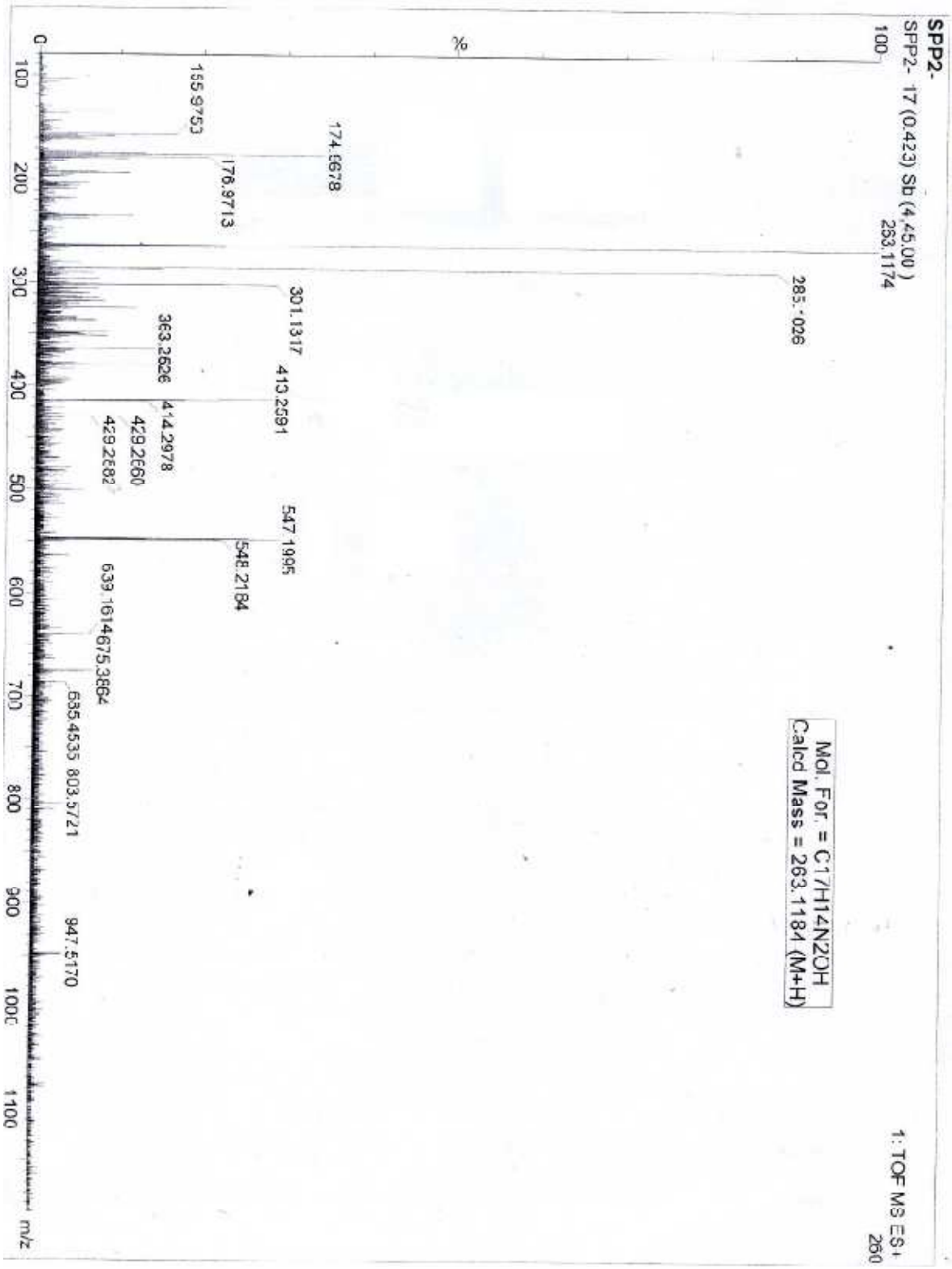


Fig. S22 HRMS of SPP2

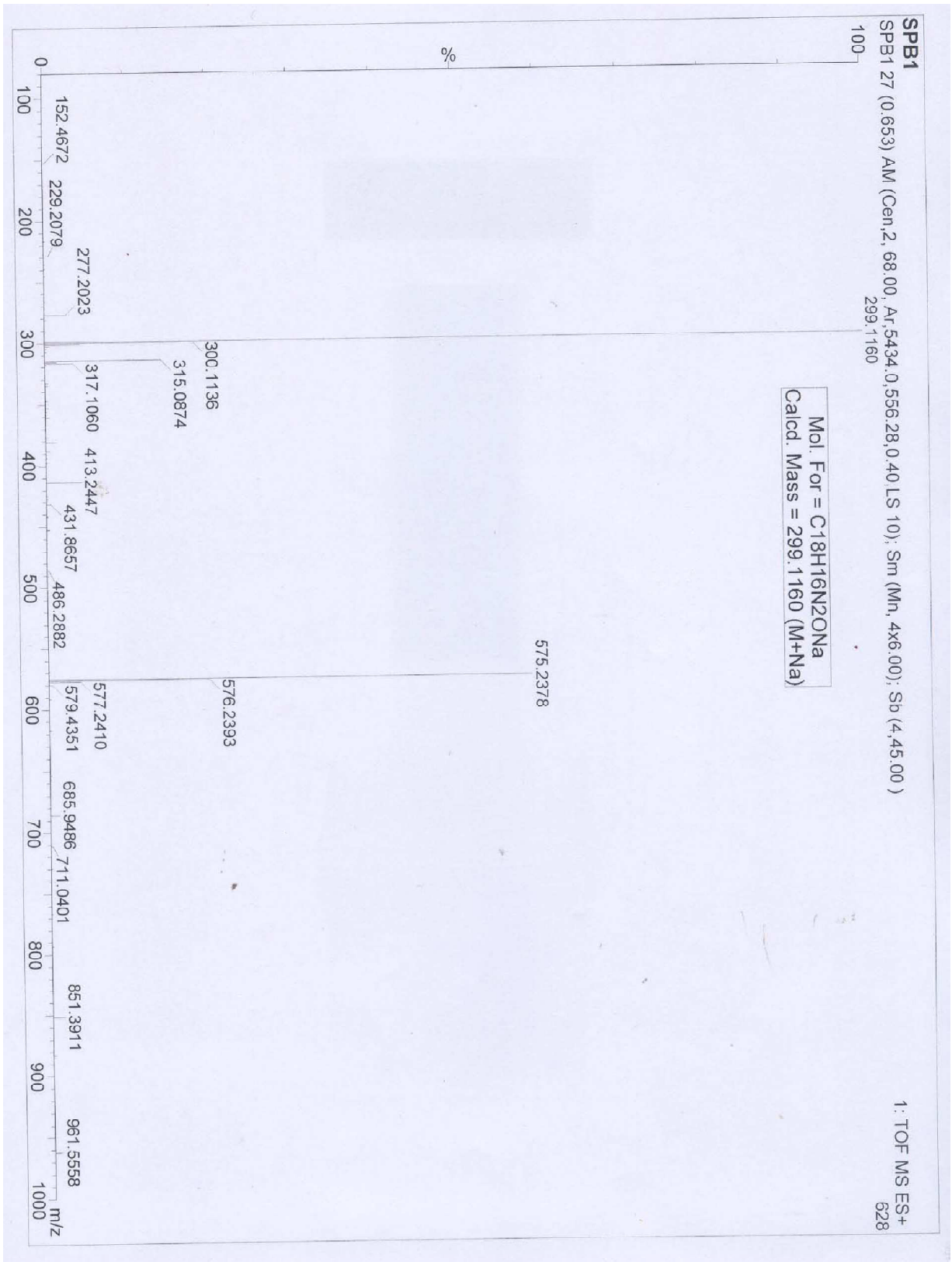


Fig. S23 HRMS of SPB1

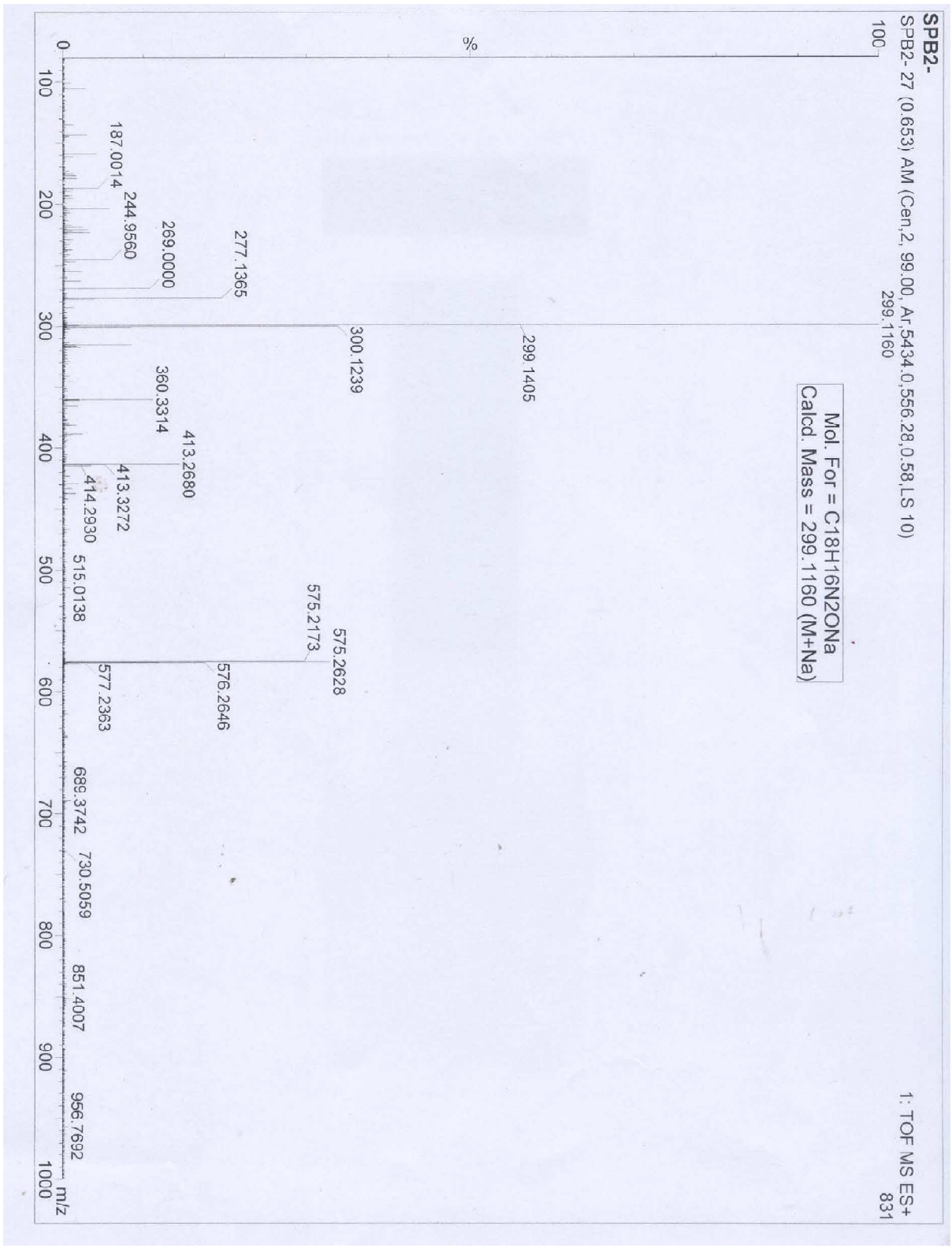


Fig. S24 HRMS of SPB2

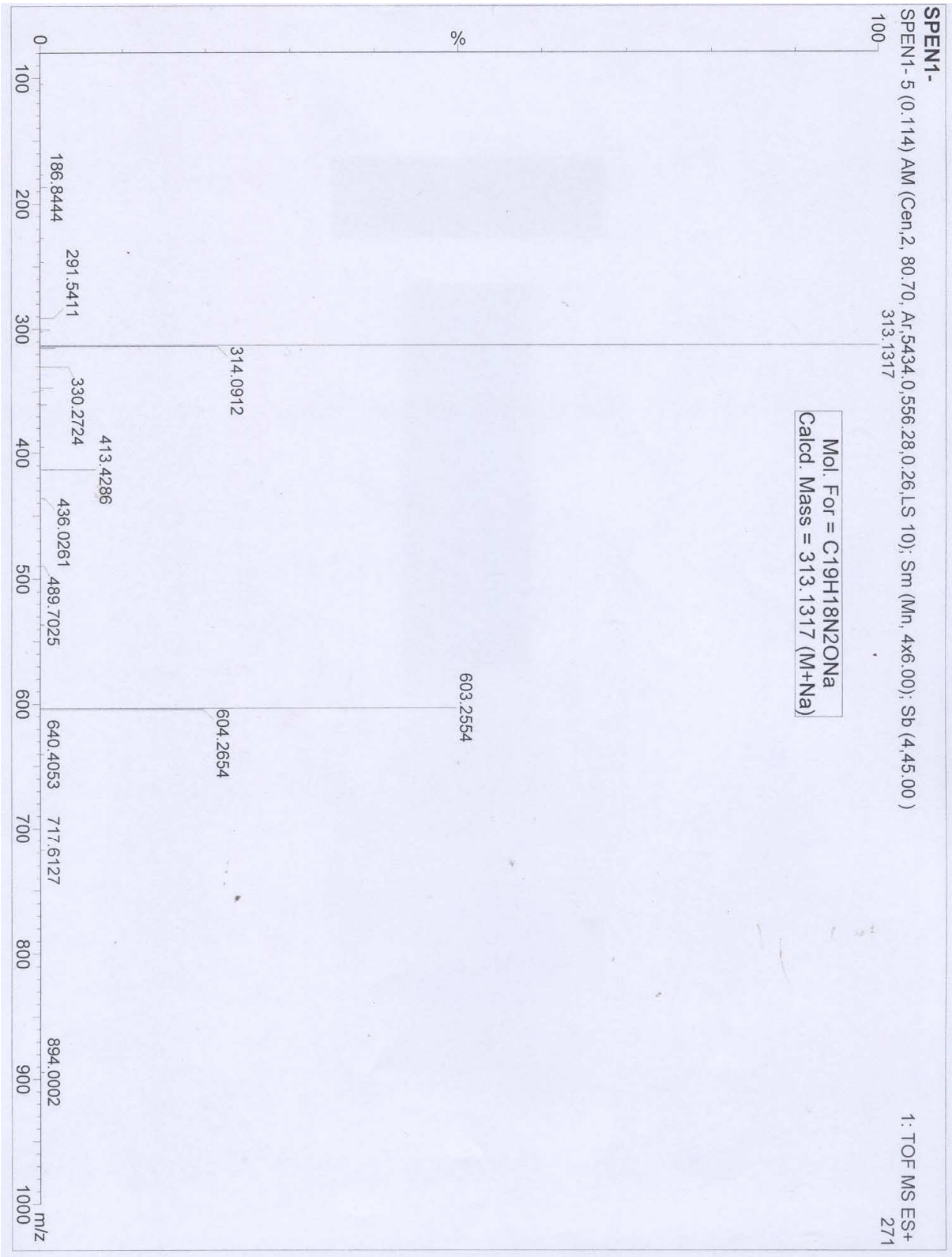


Fig. S25 HRMS of SPEN1

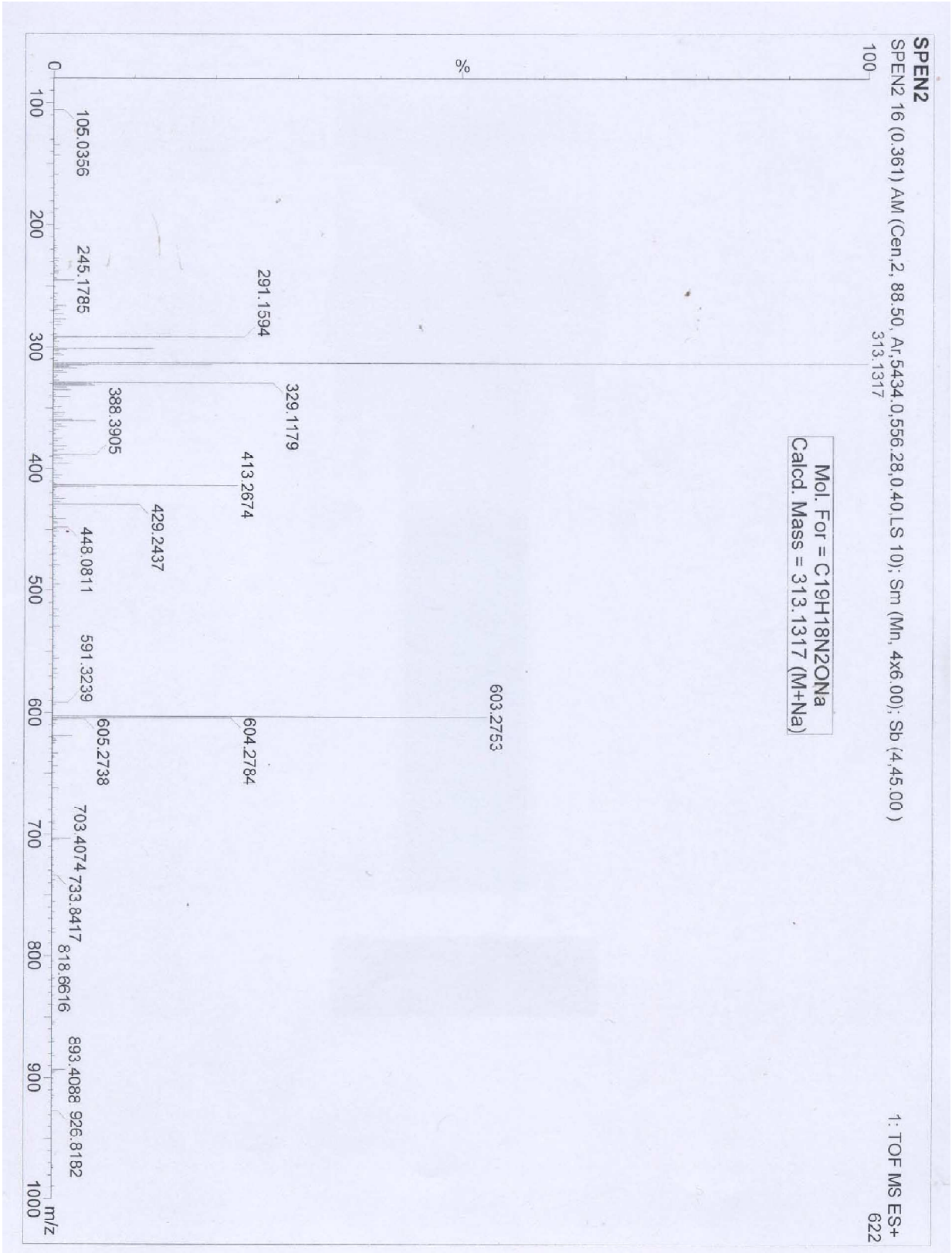


Fig. S26 HRMS of SPEN2

References:

1. (a) Showalter *et al., J. Med. Chem*., 1987, **30**, 121; (b) W. Bradley and K. W. Geddes, *J. Chem. Soc.* 1952, 1630; (c) M. Kim and D. F. Wiemer. *Tetrahedron Lett*. 2004, **45**, 4977.
2. Organic Syntheses, Coll. Vol. 6, p.75 (1988); Vol. 53, p.13 (1973).
3. G. M. Sheldrick, *Acta Crystallogr., Sect. A: Found. Crystallogr.,* 2008, **64**, 112.
4. L. J. Farrugia, *J. Appl. Crystallogr.*, 1999, **32**, 837.
5. O. V. Dolomanov, A. J. Blake, N. R. Champness and M. Schröder, *J. Appl. Crystallogr*., 2003, **36**, 1283.
6. Farrugia, L. *J. Appl. Crystallogr.*, 1997, **30**, 565.
7. A. Spek, *J. Appl. Crystallogr*., 2003, **36**, 7.
8. C. F. Macrae, P. R. Edgington, P. McCabe, E. Pidcock, G. P. Shields, R. Taylor, M. Towler and J. Streek, *J. Appl. Crystallogr.*, 2006, **39**, 453.