Electronic Supplementary Material (ESI) for Green Chemistry.

Supporting Information

Eco-friendly synthesis of aminoindamine and indoaniline dyes mediated by CotA-laccase

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1. Proposed pathway for the cross-coupling reactions mediated by laccases

The first step of the enzyme-mediated formation of the aminoindamine and indoaniline dyes (Figure S1), is the enzymatic oxidation of the primary intermediate (oxidation base or developer) under H-atom abstraction (i.e. $1H^+ + 1e^-$) promoting the formation of the benzoquinone-diimine intermediate (**A**). The nature of the specific reactive species involved in hair colouration with 1,4-PDA or other related oxidation bases is still under debate.[1] Nevertheless, the conjugated acid of the *p*-benzoquinonediimine has been proposed to this role, which is supported by the fact that the diiminium ion (**AH$^+$**) is more electrophilic than the correspondent diamine **A**).[2-6] The reactive species is further involved in a cross-coupling reaction, via electrophilic attack of the iminium group on the electron-rich coupler preferentially on the *p*-position to an amino or hydroxyl group, yielding the dinuclear leuco dyes (**B**) which result in the final indoaniline or aminoindamine chromophores (**C**) through a new oxidation step. The oxidation bases are expected to be less reactive than couplers and therefore no self-coupling in their presence occurs. When the *meta* couplers are blocked with a *para* substituent to one of the functional amino and/or hydroxyl groups (*m,p*-substituted couplers), the quinone-imine/diimine dimers (**C**) were found to be the final products.[1,7] The dyes formed from the *meta* difunctional couplers, having no substituents *para* to either of the functional groups, undergo further reaction yielding the trinuclear indo dyes (**D**). These dyes could be formed either by 1,4-addition of an unchanged diamine molecule to the aminoindamine intermediate or by electrophilic attack of the protonated diimine (**AH$^+$**) on the dinuclear leuco dye (**B**). Nevertheless, the latter suggestion is more consistent with the first step of the proposed pathway and the higher reactivity of the (**AH$^+$**) species towards the couplers than the neutral diimine (**A**).

The heterocoupling reactions with the naphthalene couplers (1-Nol and 1-NA), presumably follow a similar mechanistic pathway. The electrophilic attack of **AH$^+$** species on the 4-position of the naphthalene coupler leads to the naphthol leuco derivative (**E**), which suffers a new addition leading to the final product, the trimer (**F**).

For the heterocoupling reactions involving the 4-AP as the primary intermediate, a similar pathway can be proposed, considering the enzymatic formation of the benzoquinone-monoimine intermediate.

1 O.J.X. Morel, R.M. Christie, *Chem. Rev.*, 2011, **111**, 2537.
2 J.F. Corbett, *J. Soc. Cosmet. Chem.*,1973, **24**, 103.
3 K.C. Brown, J. F. Corbett, *J. Soc. Cosmet. Chem.*,1979, **30**, 191.
4 J.F. Corbett, *J. Chem. Soc. Perkin II,* 1972, 539.
5 J.F. Corbett, *J. Chem. Soc. (B),*1969, 818.
6 J.F. Corbett, *J. Chem. Soc. (B),*1969, 823.
7 J.F. Corbett, *Dyes & Pigments*, 1999, **41**, 127.

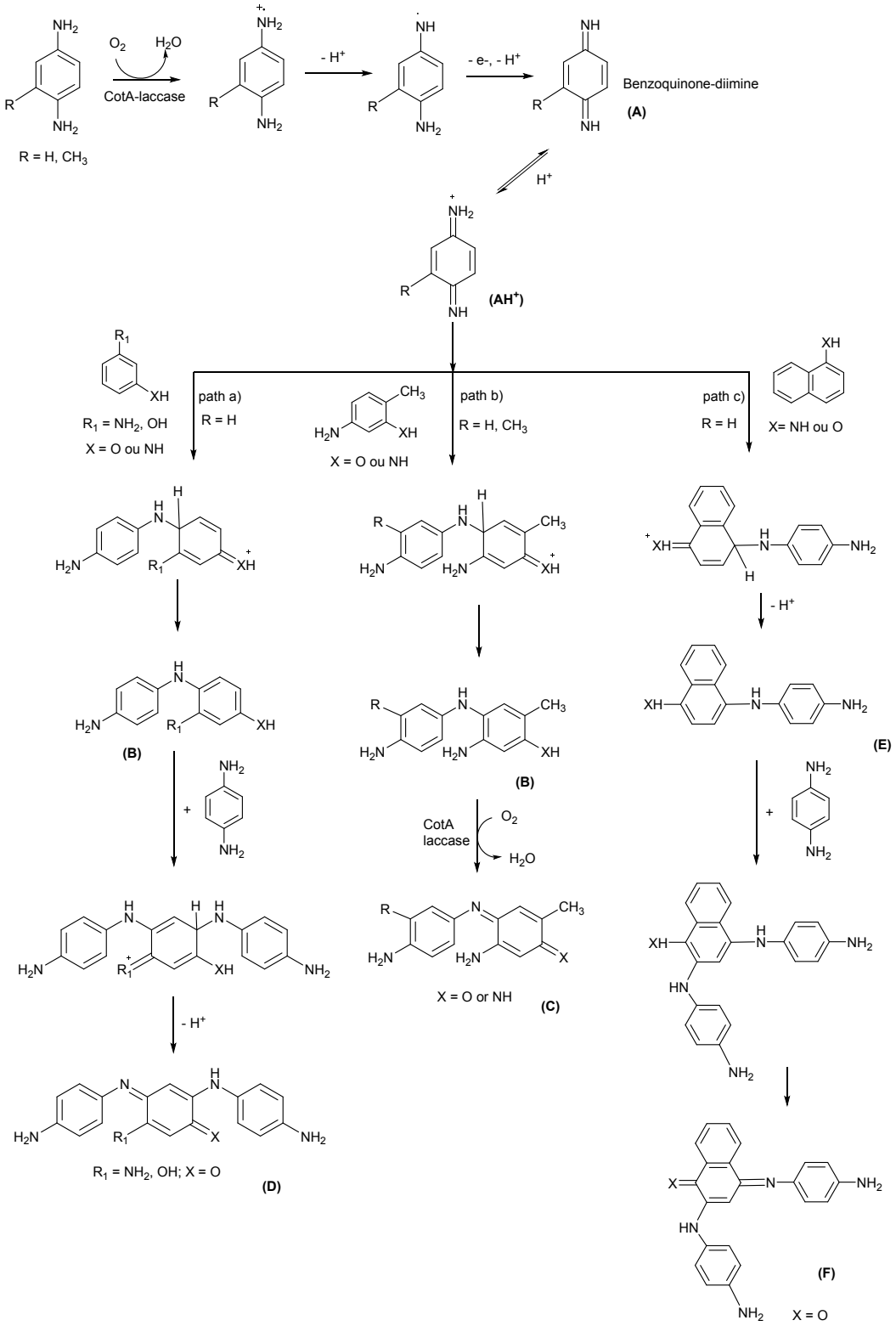


Fig. S1 Proposed pathway for the heterocoupling reactions involving the primary intermediates 1,4–PDA or 2,5-DAT and *meta*- or *meta,para*-substituted and naphthalene couplers, catalysed by CotA-laccase.

2. X-ray crystal structure data

Table S1. Crystal Data and structure refinement details for compound ***8***

Empirical formula	$C_{13}H_{13}N_3O$
Formula weight	227.26
T (K)	150(2)
Wavelength (Å)	0.71073
Crystal system	Monoclinic
Space group	P2(1)/n
a (Å)	13.5515(11)
b (Å)	3.9004(3)
c (Å)	20.9865(16)
β (º)	94.725(4)
V ($Å^3$)	1105.5(2)
Z	4
D_{calc} (Mg/m^3)	1.365
μ (Mo Ka) (mm^{-1})	0.090
Theta range for data collection (º)	3.18 to 28.40
Limiting indices	-18<=h<=16, -5<=k<=5, -28<=l<=28
Number of reflections collected	10590
Number of unique data	2710 [R(int) = 0.0295]
Completeness to theta = 28.40º	97.7 %
Data / restraints / parameters	2710 / 0 / 169
Final R_1[a], ωR_2[b] ($I \geq 2\delta$)	0.0400, 0.1017
Goodness-of-fit (GOF) on F^2	1.043
Largest diff. peak and hole ($eÅ^{-3}$)	0.322 and -0.237 e.-3

[a] $R_1 = \sum||F_o|-|F_c||/\sum|F_o|$.

[b] $\omega R_2=[\sum[\omega(F^2_o-F^2_c)^2]]/\sum[\omega(F^2_o)^2]]^{1/2}$

Table S2. Bond lengths [Å] and angles [deg] for compound ***8***

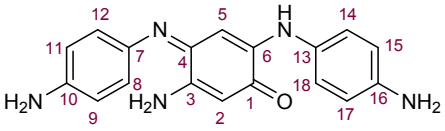
O(1)-C(10)	1.2531(13)
N(2)-C(7)	1.2948(14)
N(2)-C(4)	1.4003(14)
N(1)-C(1)	1.3929(14)
N(3)-C(8)	1.3363(15)
C(10)-C(9)	1.4199(17)
C(10)-C(11)	1.4890(16)
C(9)-C(8)	1.3684(16)
C(8)-C(7)	1.4902(15)
C(7)-C(12)	1.4603(16)
C(4)-C(5)	1.4041(16)
C(4)-C(3)	1.4046(15)
C(3)-C(2)	1.3778(15)
C(2)-C(1)	1.3985(16)
C(1)-C(6)	1.3958(15)
C(12)-C(11)	1.3438(15)
C(11)-C(13)	1.4928(16)
C(6)-C(5)	1.3780(15)
C(7)-N(2)-C(4)	123.94(10)
O(1)-C(10)-C(9)	122.10(10)
O(1)-C(10)-C(11)	119.20(11)
C(9)-C(10)-C(11)	118.68(10)
C(8)-C(9)-C(10)	122.53(10)
N(3)-C(8)-C(9)	123.93(10)
N(3)-C(8)-C(7)	116.73(10)
C(9)-C(8)-C(7)	119.30(10)
N(2)-C(7)-C(12)	127.12(10)
N(2)-C(7)-C(8)	115.58(10)
C(12)-C(7)-C(8)	117.26(10)
N(2)-C(4)-C(5)	123.94(10)
N(2)-C(4)-C(3)	117.69(10)
C(5)-C(4)-C(3)	117.97(10)
C(2)-C(3)-C(4)	120.63(11)
C(3)-C(2)-C(1)	121.05(10)
N(1)-C(1)-C(6)	120.36(11)
N(1)-C(1)-C(2)	121.22(10)
C(6)-C(1)-C(2)	118.40(10)
C(11)-C(12)-C(7)	122.66(10)
C(12)-C(11)-C(10)	119.41(11)
C(12)-C(11)-C(13)	123.19(10)
C(10)-C(11)-C(13)	117.40(10)
C(5)-C(6)-C(1)	120.74(11)
C(6)-C(5)-C(4)	121.07(10)

Table S3. Torsion angles [deg] for compound ***8***

O(1)-C(10)-C(9)-C(8)	-176.80(11)
C(11)-C(10)-C(9)-C(8)	1.90(18)
C(10)-C(9)-C(8)-N(3)	178.11(11)
C(10)-C(9)-C(8)-C(7)	-4.42(18)
C(4)-N(2)-C(7)-C(12)	-13.56(18)
C(4)-N(2)-C(7)-C(8)	168.73(10)
N(3)-C(8)-C(7)-N(2)	-1.03(15)
C(9)-C(8)-C(7)-N(2)	-178.68(10)
N(3)-C(8)-C(7)-C(12)	-178.97(10)
C(9)-C(8)-C(7)-C(12)	3.38(16)
C(7)-N(2)-C(4)-C(5)	-39.94(17)
C(7)-N(2)-C(4)-C(3)	147.49(11)
N(2)-C(4)-C(3)-C(2)	174.31(10)
C(5)-C(4)-C(3)-C(2)	1.29(17)
C(4)-C(3)-C(2)-C(1)	-4.08(18)
C(3)-C(2)-C(1)-N(1)	-177.13(11)
C(3)-C(2)-C(1)-C(6)	4.08(17)
N(2)-C(7)-C(12)-C(11)	-177.43(11)
C(8)-C(7)-C(12)-C(11)	0.23(17)
C(7)-C(12)-C(11)-C(10)	-2.73(17)
C(7)-C(12)-C(11)-C(13)	176.74(10)
O(1)-C(10)-C(11)-C(12)	-179.47(11)
C(9)-C(10)-C(11)-C(12)	1.79(17)
O(1)-C(10)-C(11)-C(13)	1.03(16)
C(9)-C(10)-C(11)-C(13)	-177.71(11)
N(1)-C(1)-C(6)-C(5)	179.83(11)
C(2)-C(1)-C(6)-C(5)	-1.37(17)
C(1)-C(6)-C(5)-C(4)	-1.36(17)
N(2)-C(4)-C(5)-C(6)	-171.14(10)
C(3)-C(4)-C(5)-C(6)	1.41(17)

3. 1D and 2D NMR and MS spectra

Compound 5:



NMR spectra in MeOD-d_4 (400MHz):

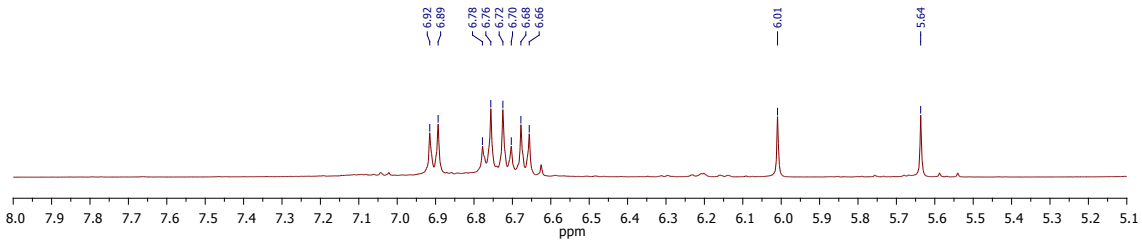


Figure S2 - ^{1}H-NMR spectrum of compound ***5*** in MeOD-d_4 (400MHz)

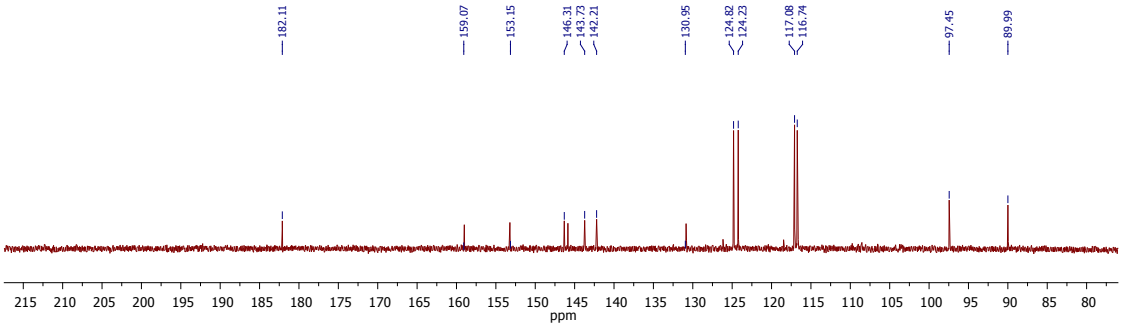


Figure S3 – ^{13}C -NMR spectrum of compound ***5*** in MeOD-d_4 (400MHz)

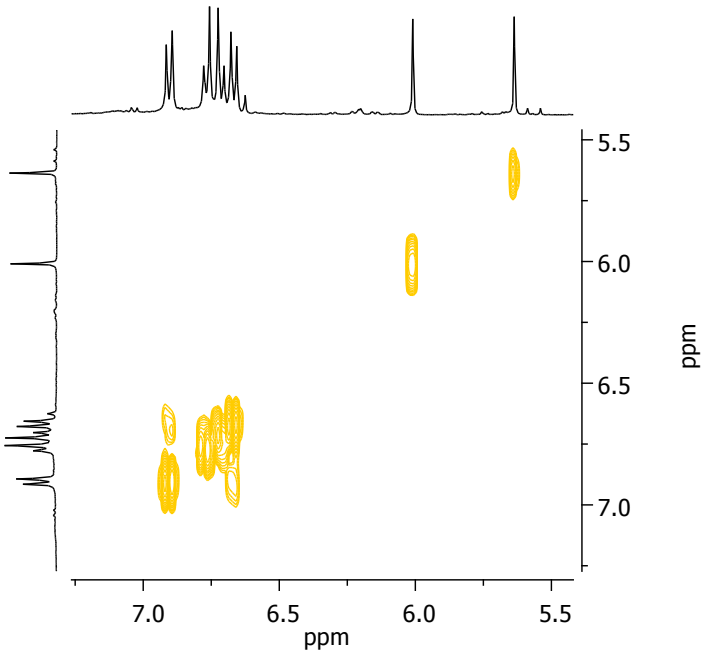


Figure S4 – COSY-NMR spectrum of compound ***5*** in MeOD-d_4 (400MHz)

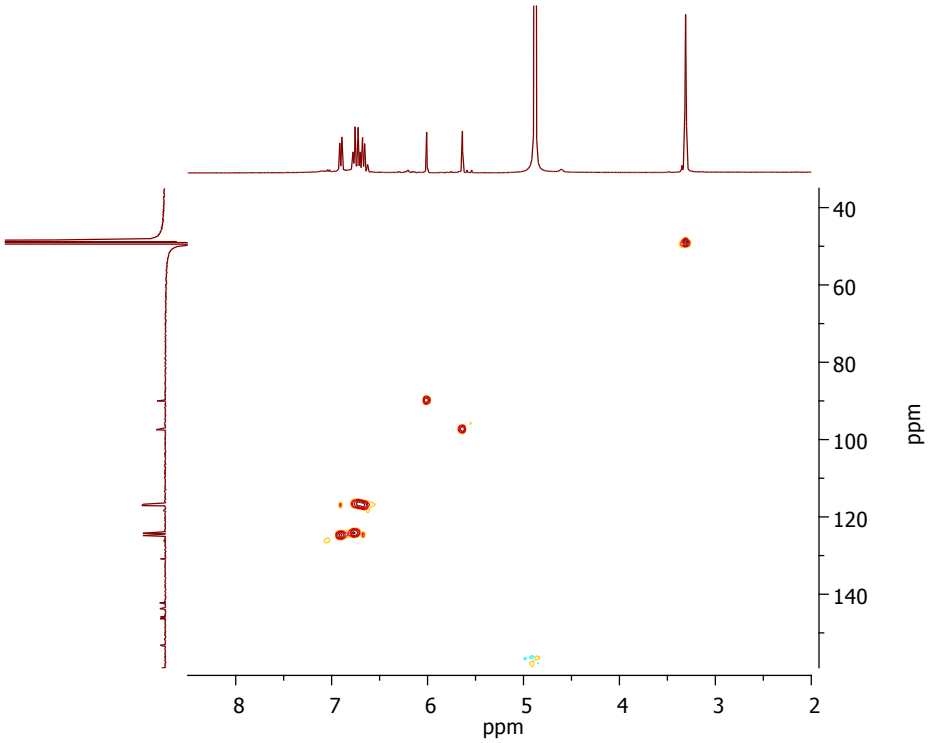


Figure S5 – HSQC-NMR spectrum of compound ***5*** in MeOD-d_4 (400MHz)

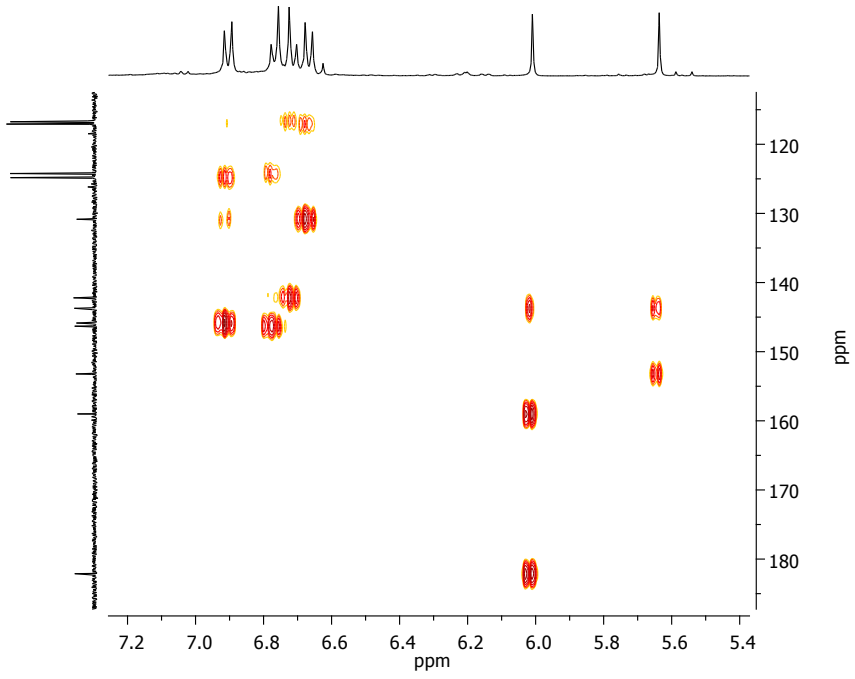


Figure S6 – HMBC-NMR spectrum of compound ***5*** in MeOD-d_4 (400MHz)

ESI(+)/MS spectrum of compound ***5*** ($C_{18}H_{17}N_5O$) MW= 319.36 g/mol

Positive mode *m/z* = 320 $[M+H]^+$

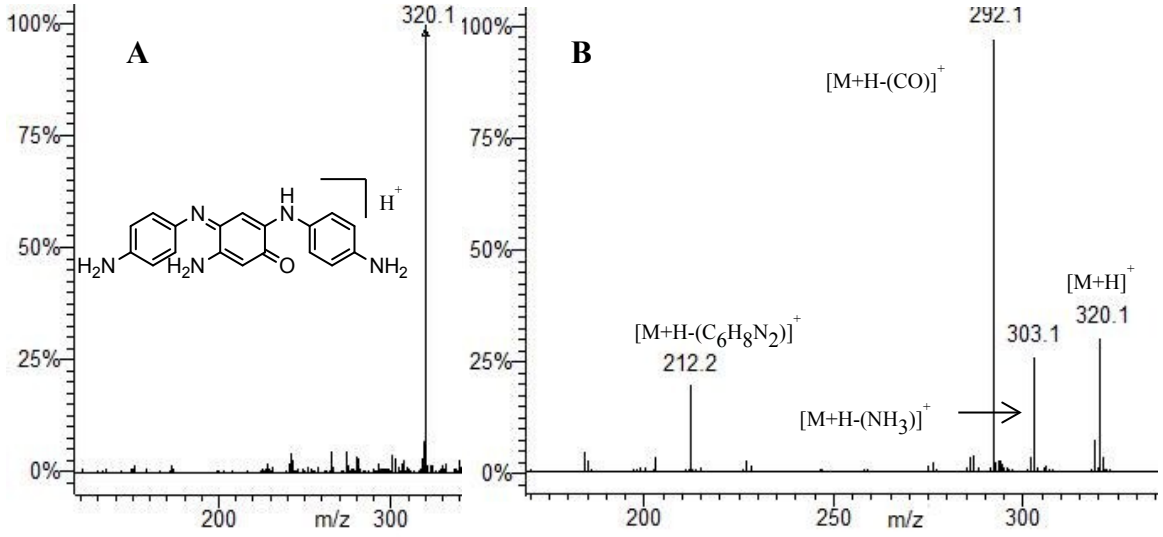
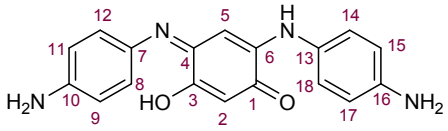


Figure S7 – A) ESI(+)/MS spectrum of compound ***5*** **B)** MS/MS spectrum of *m/z* 320 of compound ***5***

Compound 6:



NMR spectra in MeOD-d_4 (400MHz):

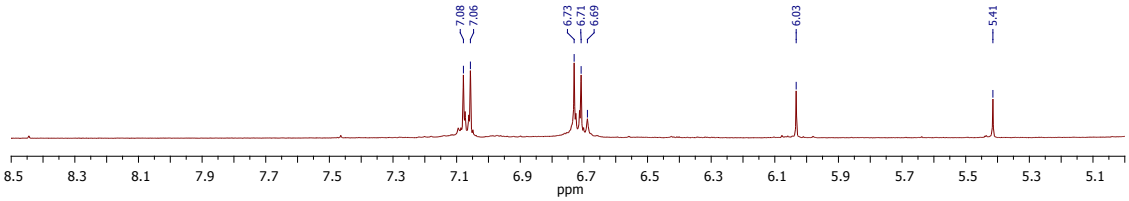


Figure S8 - ^{1}H-NMR spectrum of compound ***6*** in MeOD-d_4 (400MHz)

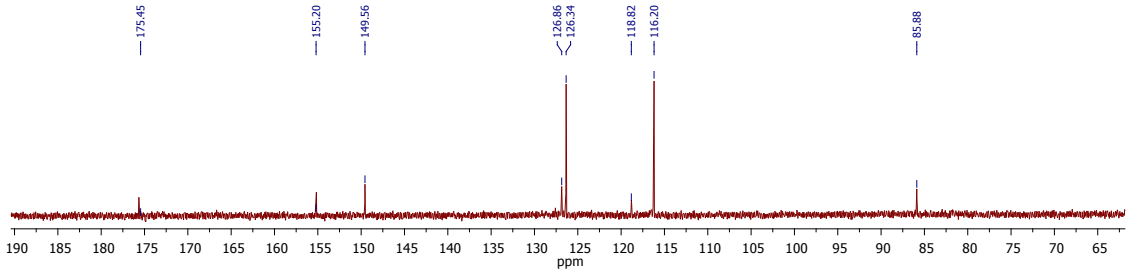


Figure S9 – ^{13}C-NMR spectrum of compound ***6*** in MeOD-d_4 (400MHz)

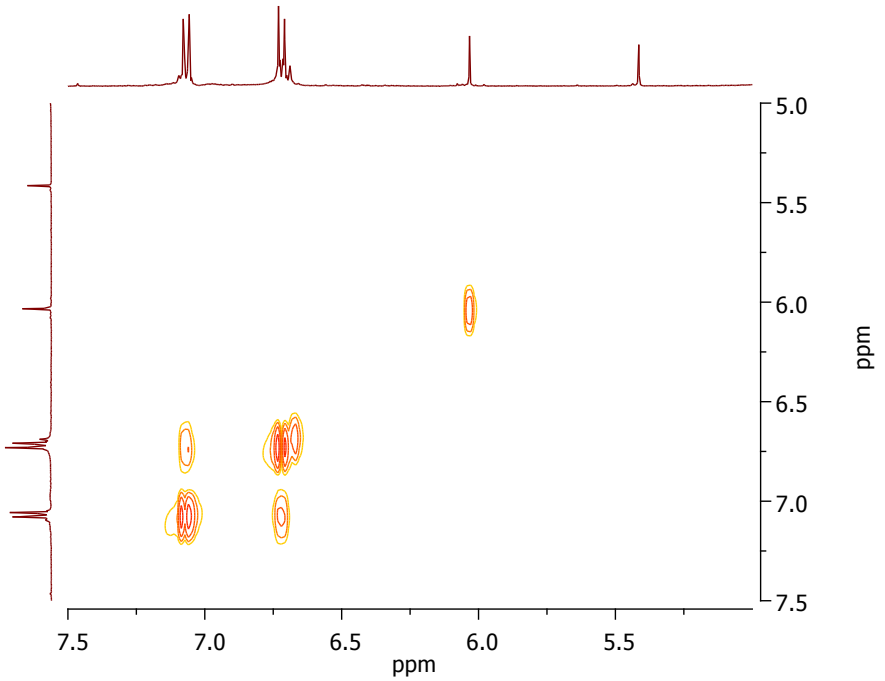


Figure S10 - COSY-NMR spectrum of compound ***6*** in MeOD-d_4 (400MHz)

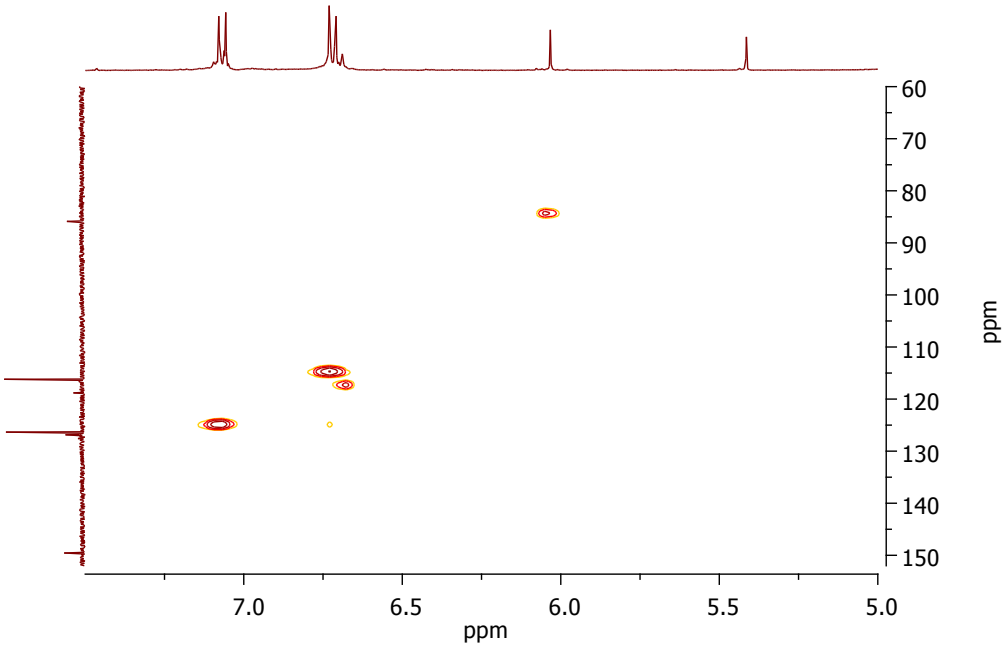


Figure S11 - HSQC-NMR spectrum of compound ***6*** in MeOD-d_4 (400MHz)

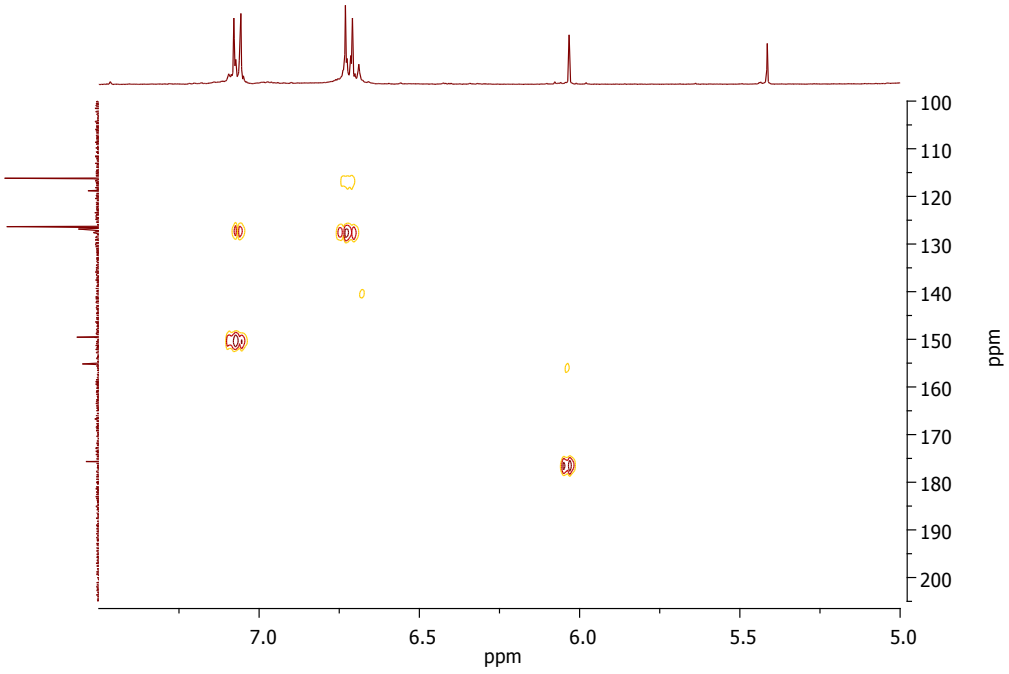


Figure S12 - HMBC-NMR spectrum of compound ***6*** in MeOD-d_4 (400MHz)

ESI(+)/MS spectrum of compound ***6*** ($C_{18}H_{16}N_4O_2$) MW= 320.13 g/mol

Positive mode *m/z* = 321 $[M+H]^+$

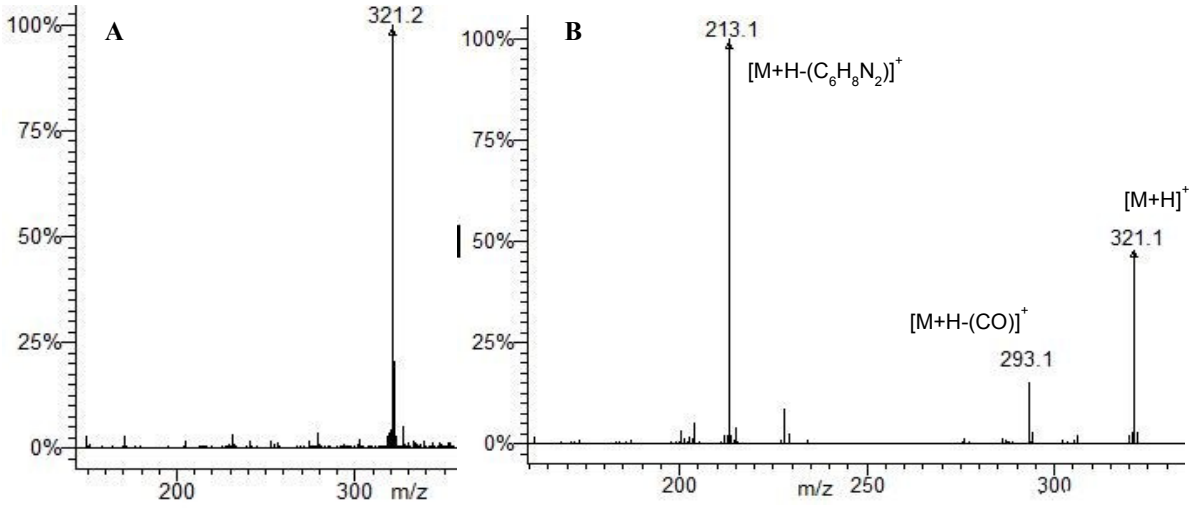
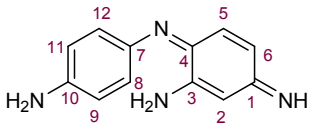


Figure S13 – A) ESI(+)/MS spectrum of compound ***6***; **B)** MS/MS spectrum of *m/z* 321 of compound ***6***

Compound 7:



NMR spectra in MeOD-d_4 (400MHz):

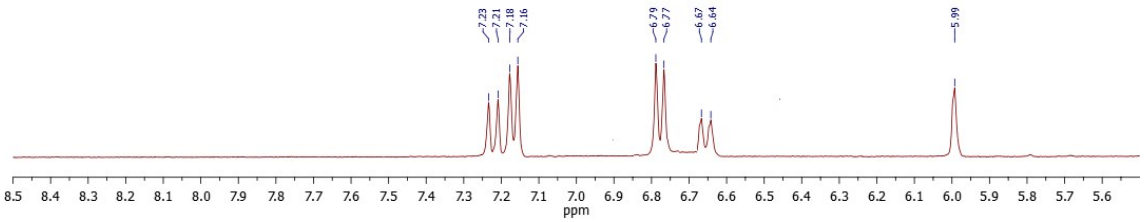


Figure S14 - ^{1}H-NMR spectrum of compound ***7*** in MeOD-d_4 (400MHz)

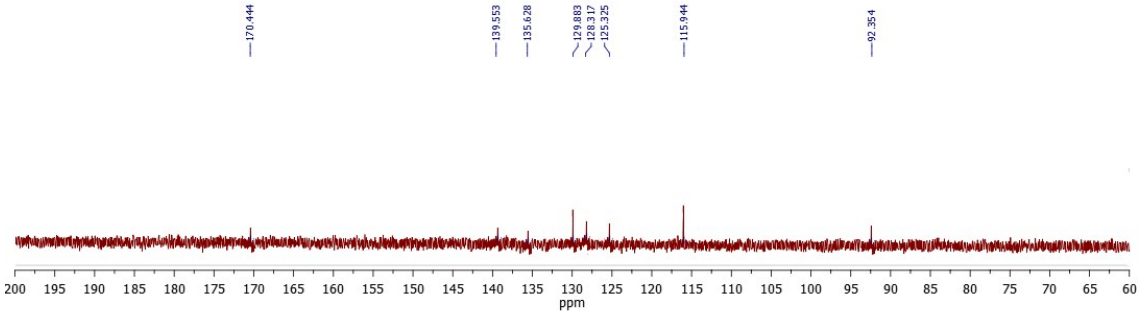


Figure S15 – ^{13}C-NMR spectrum of compound ***7*** in MeOD-d_4 (400MHz)

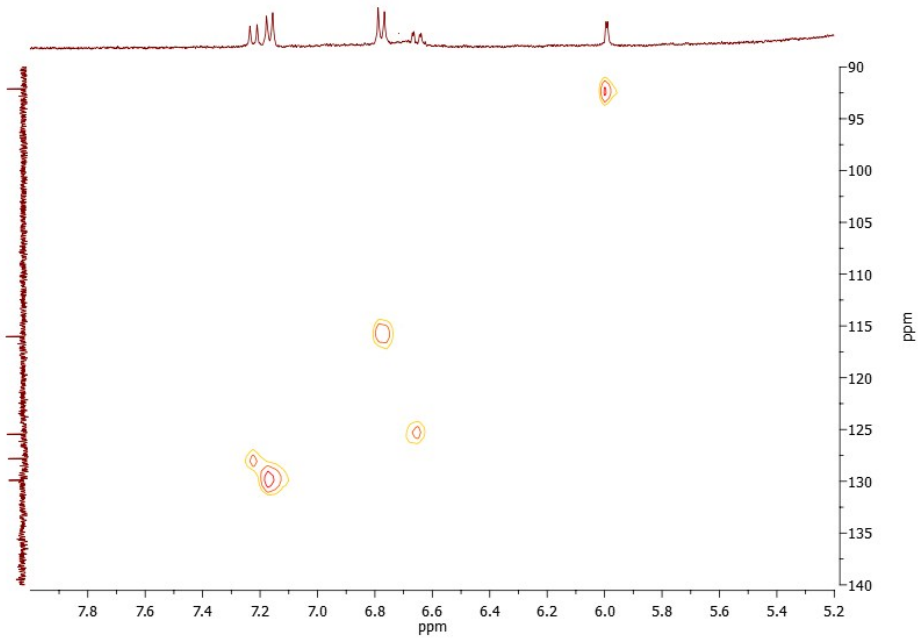


Figure S16 - HSQC-NMR spectrum of compound ***7*** in MeOD-d_4 (400MHz)

ESI(-)/MS spectrum of compound ***7*** ($C_{12}H_{12}N_4$) MW= 212.11 g/mol

positive mode *m/z* = 213 $[M+H]^+$

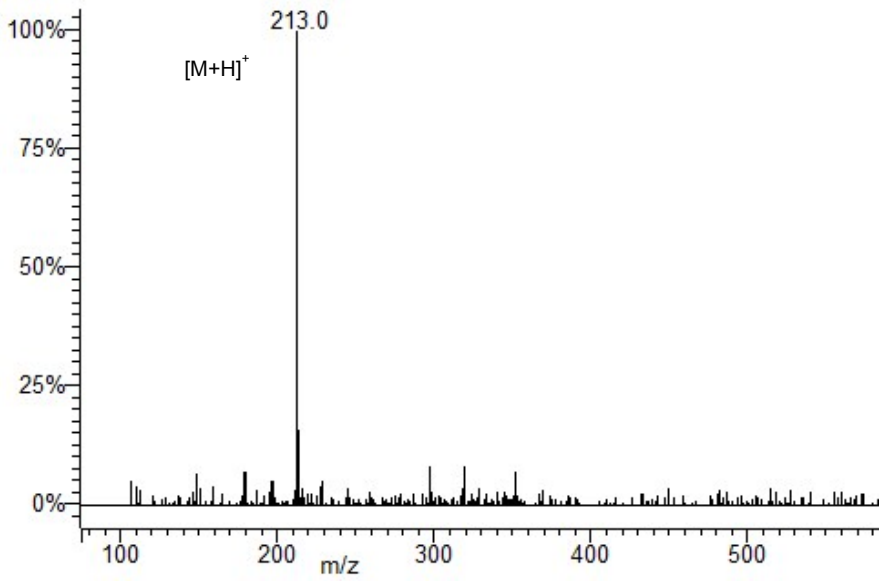
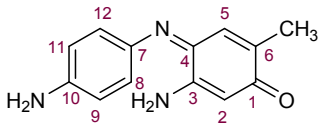


Figure S17 – ESI(+)/MS spectrum of compound ***7***

Compound 8:



NMR spectra in MeOD-d_4 (400MHz):

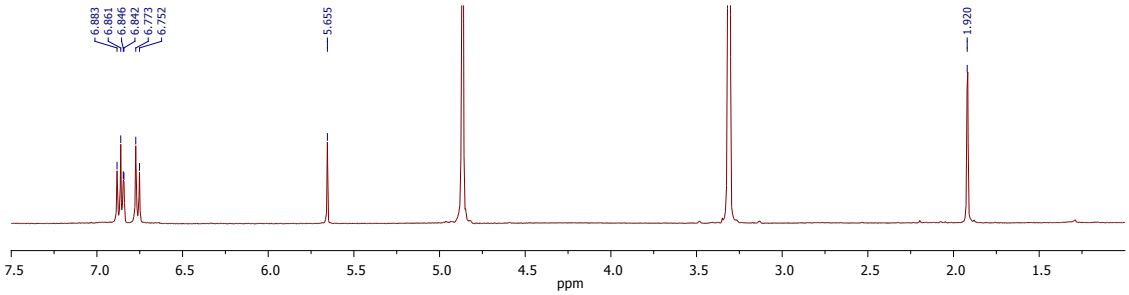


Figure S18 - ^{1}H-NMR spectrum of compound ***8*** in MeOD-d_4 (400MHz)

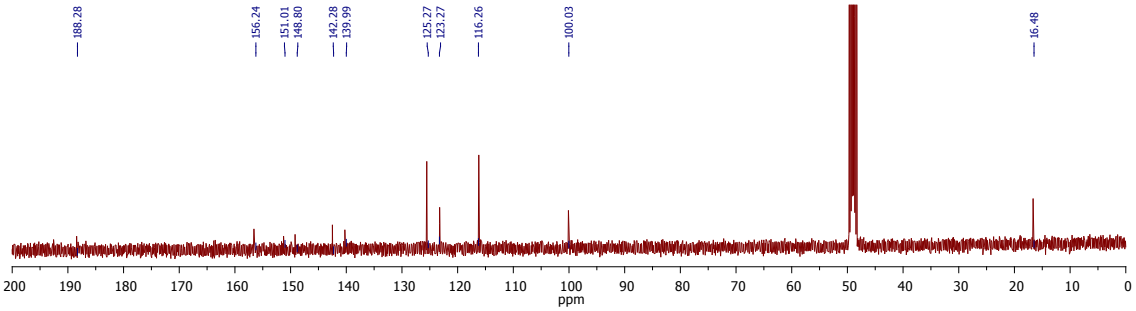


Figure S19 – ^{13}C-NMR spectrum of compound ***8*** in MeOD-d_4 (400MHz)

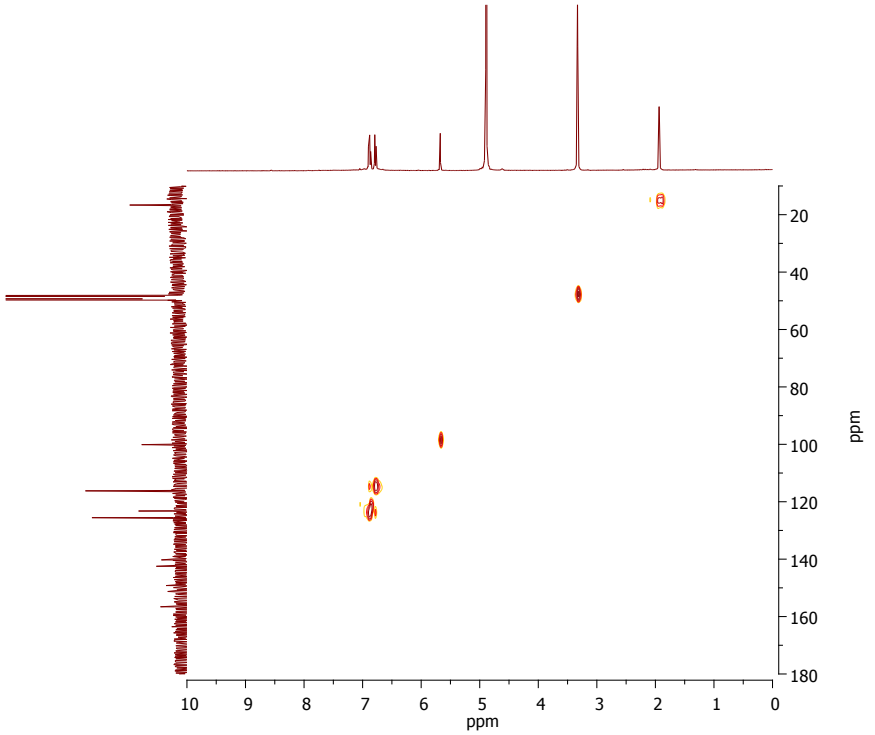


Figure S20 - HSQC-NMR spectrum of compound ***8*** in MeOD-d_4 (400MHz)

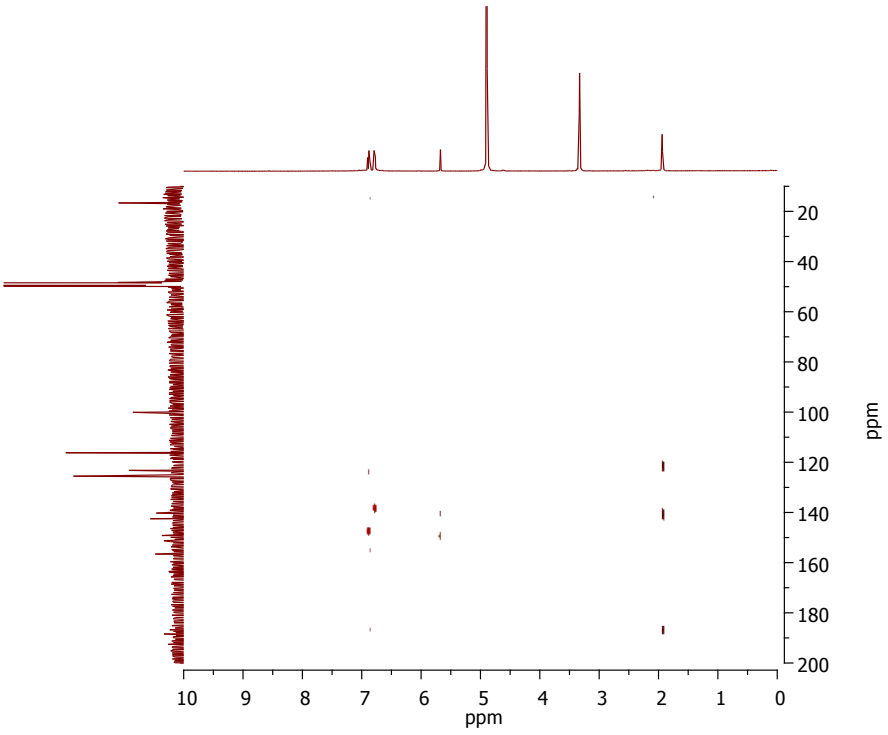


Figure S21 - HMBC-NMR spectrum of compound ***8*** in MeOD-d_4 (400MHz)

ESI(+)/MS spectrum of compound ***8*** ($C_{13}H_{13}N_3O$) MW= 227.26 g/mol

Positive mode *m/z* = 228 $[M+H]^+$, 250 $[M+Na]^+$

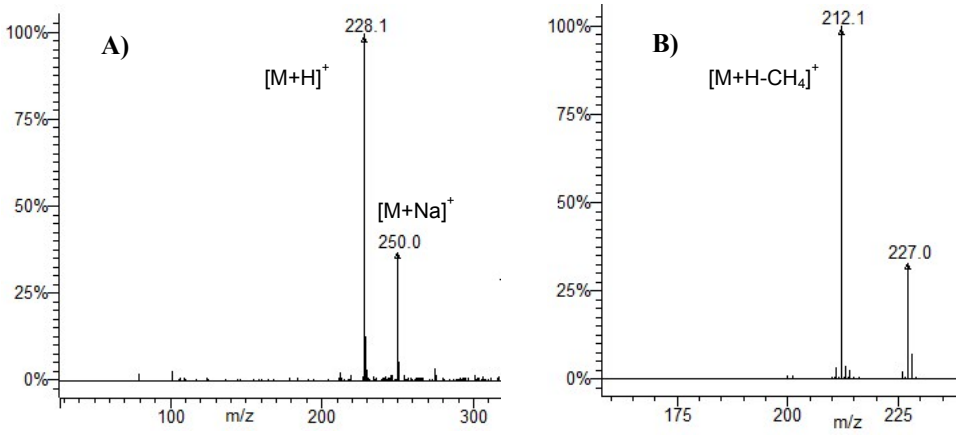
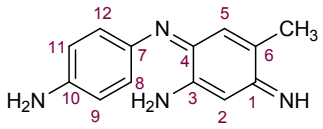


Figure S22 – A) ESI(+)/MS spectrum of compound ***8***; **B)** MS/MS spectrum of *m/z* 227 of compound ***8***

Compound 9:



NMR spectra in MeOD-d_4 (400MHz)

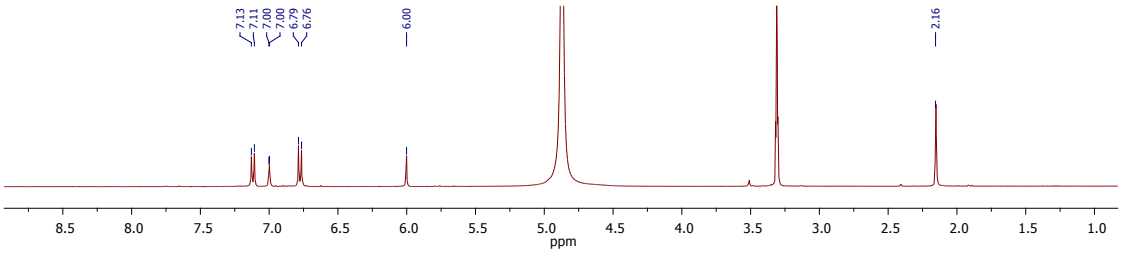


Figure S23 - ^{1}H-NMR spectrum of compound ***9*** in MeOD-d_4 (400MHz)

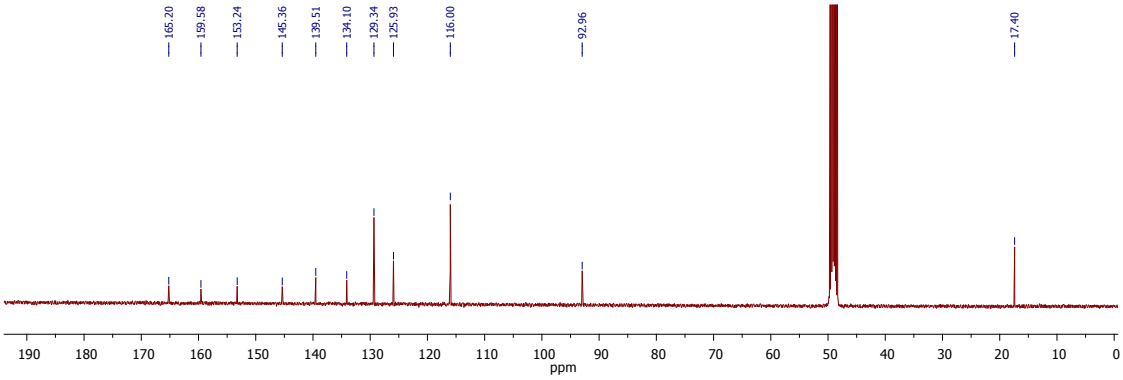


Figure S24 – ^{13}C-NMR spectrum of compound ***9*** in MeOD-d_4 (400MHz)

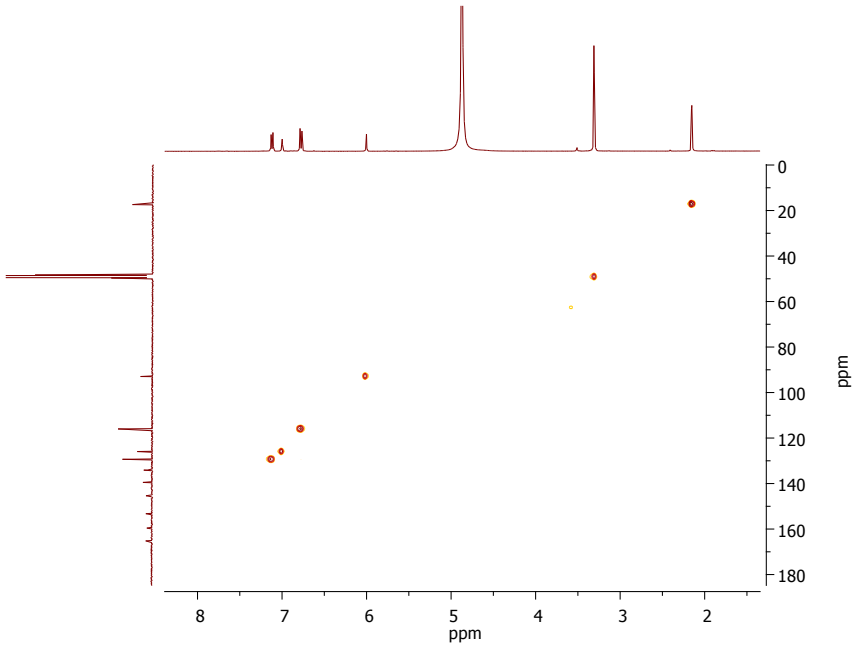


Figure S25 – HSQC-NMR spectrum of compound ***9*** in MeOD-d_4 (400MHz)

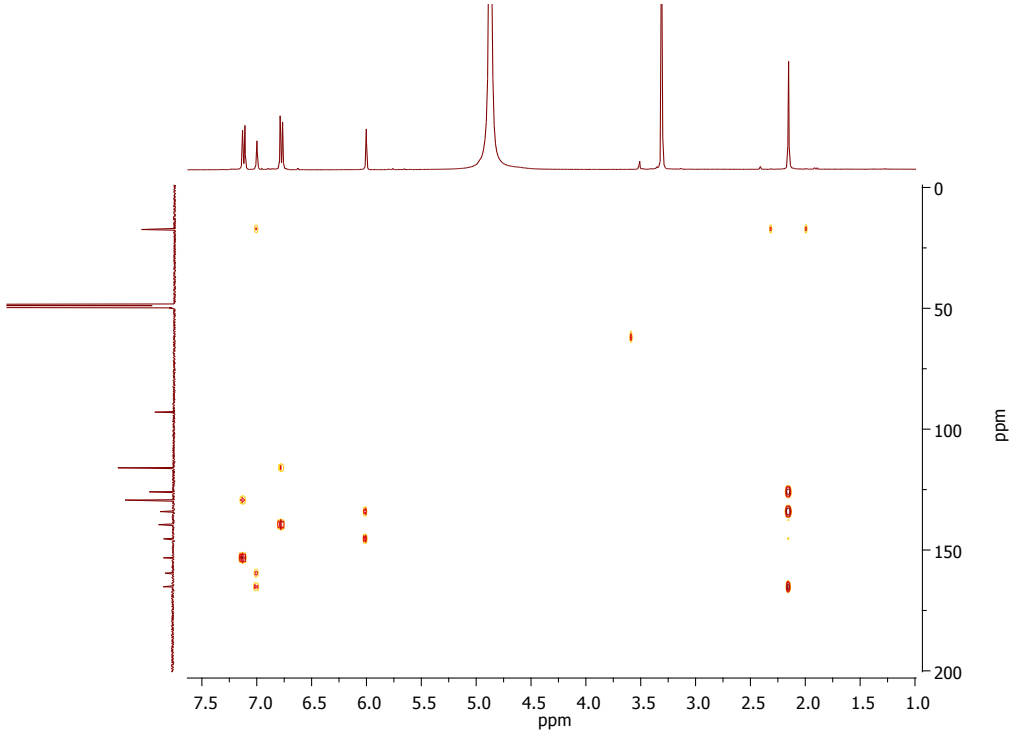


Figure S26 – HMBC-NMR spectrum of compound ***9*** in MeOD-d_4 (400MHz)

ESI(+)/MS spectrum of compound ***9*** ($C_{13}H_{14}N_4$) MW= 226.28 g/mol

Positive mode *m/z* = 227 $[M+H]^+$

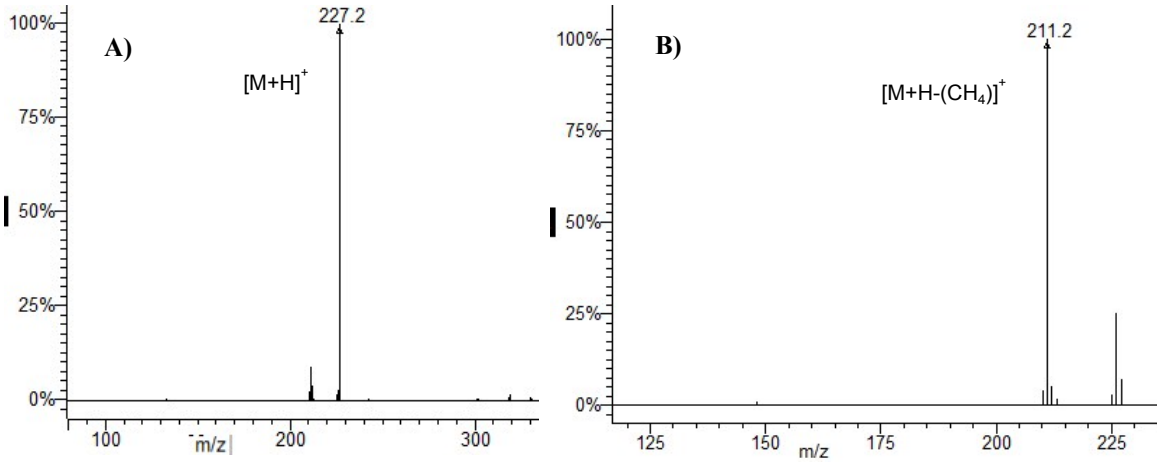
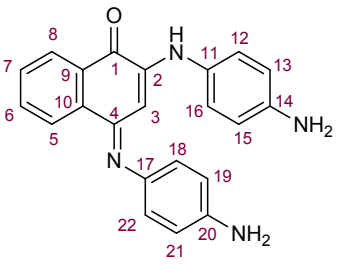


Figure S27 – A) ESI(+)/MS spectrum of compound ***9***; **B)** MS/MS spectrum of *m/z* 227 of compound ***9***

Compound 11:



NMR spectra in MeOD-d_4 (400MHz)

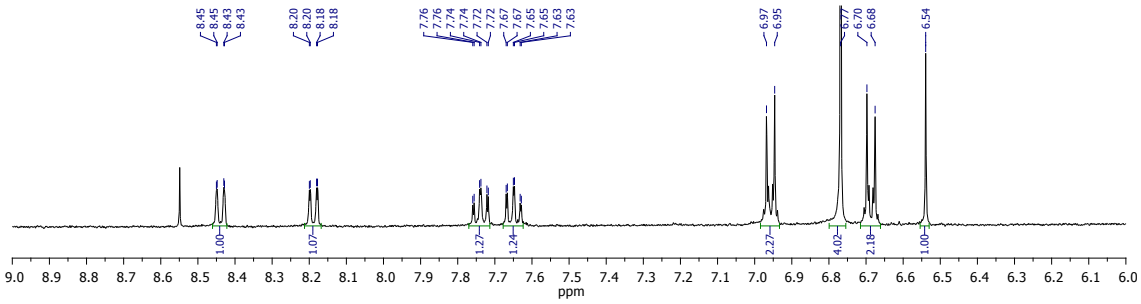


Figure S28 - ^{1}H-NMR spectrum of compound ***11*** in MeOD-d_4 (400MHz)

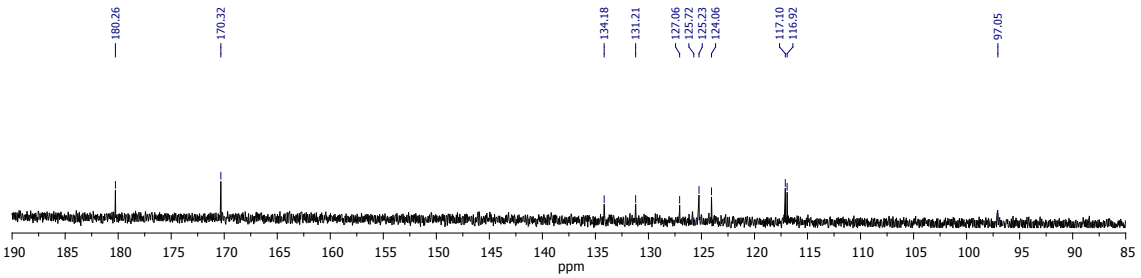


Figure S29 – ^{13}C-NMR spectrum of compound ***11*** in MeOD-d_4 (400MHz)

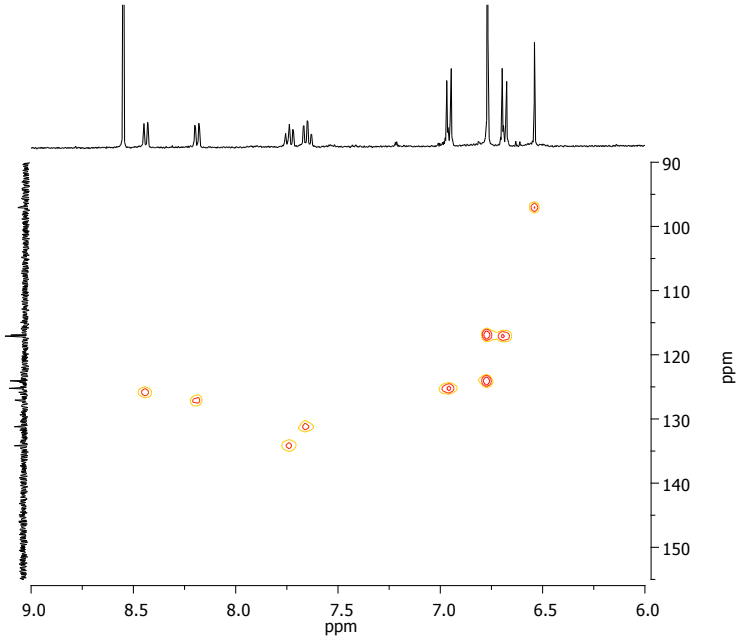
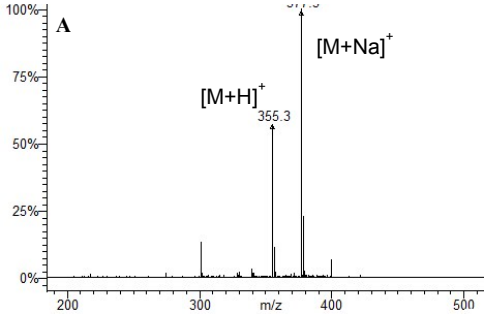


Figure S30 – HSQC-NMR spectrum of compound ***11*** in MeOD-d_4 (400MHz)

ESI(+)/MS spectrum of compound ***11*** ($C_{22}H_{18}N_4O$) MW= 354.40 g/mol

Positive mode *m/z* = 355 $[M+H]^+$, 377 $[M+Na]^+$



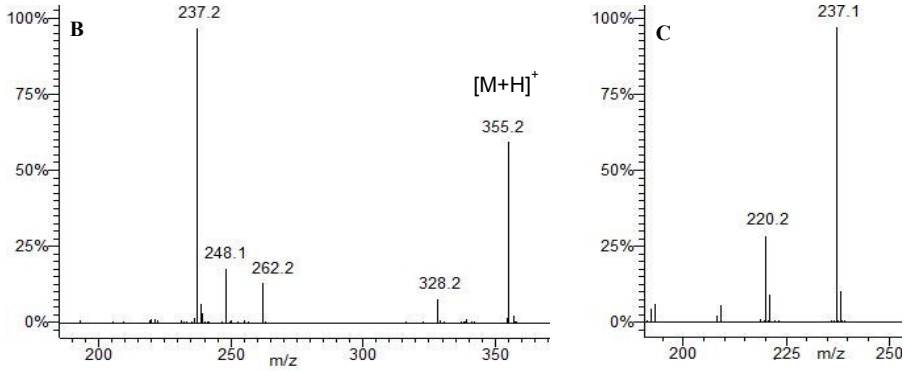
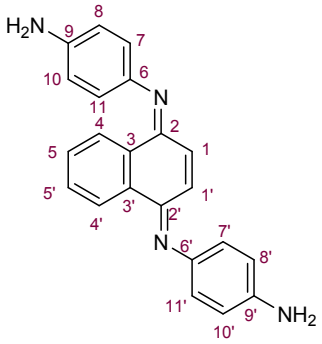


Figure S31 – A) ESI(+)/MS spectrum of compound ***11***; **B**) MS^2 spectrum of *m/z* 355 of compound ***11***; **C)** MS^3 spectrum of *m/z* 237.

Compound 12:



NMR spectra in MeOD-d_4 (400MHz)

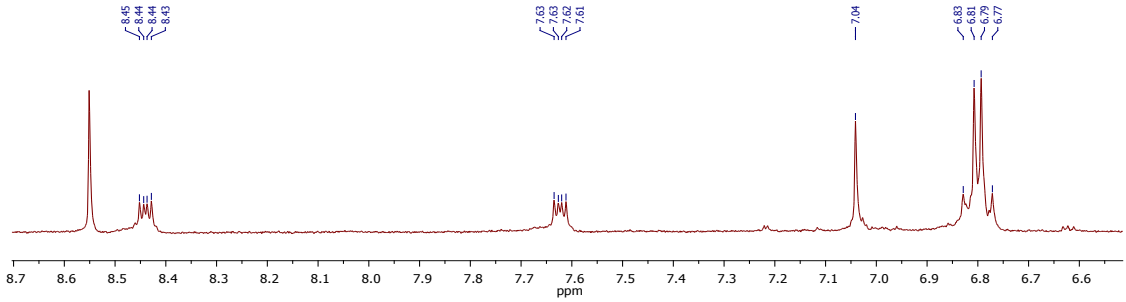


Figure S32 - ^{1}H-NMR spectrum of compound ***12*** in MeOD-d_4 (400MHz)

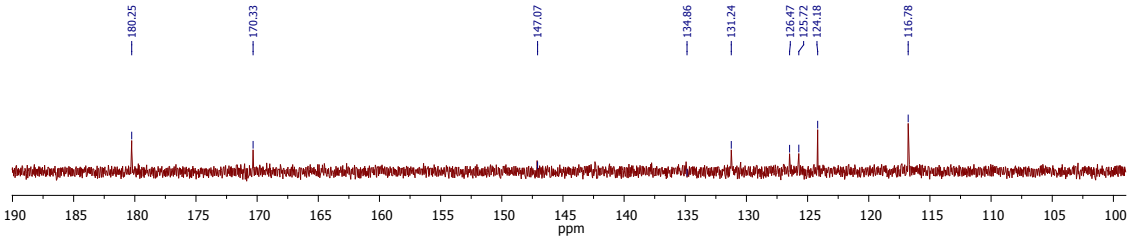


Figure S33 – ^{13}C-NMR spectrum of compound ***12*** in MeOD-d_4 (400MHz)

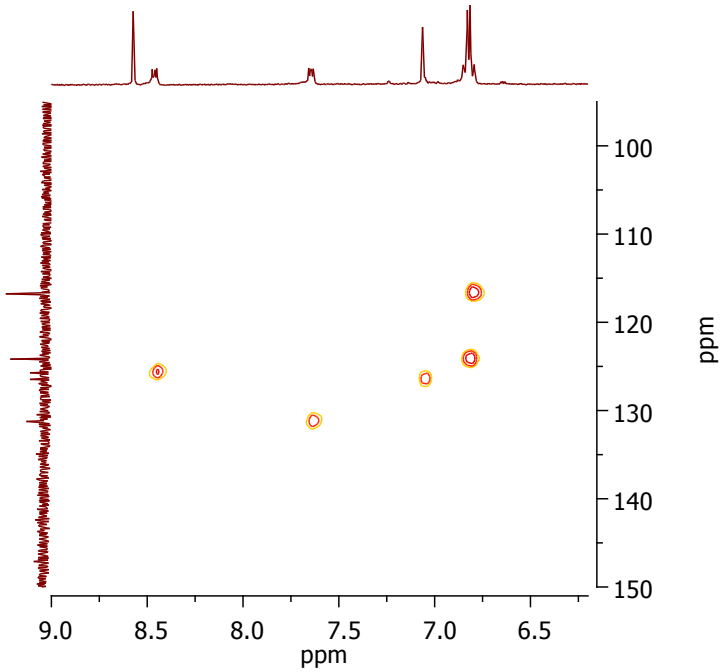


Figure S34 – HSQC-NMR spectrum of compound ***12*** in MeOD-d_4 (400MHz)

ESI(+)/MS spectrum of compound ***12*** ($C_{22}H_{18}N_4$) MW= 338.41 g/mol

Positive mode *m/z* = 339 $[M+H]^+$

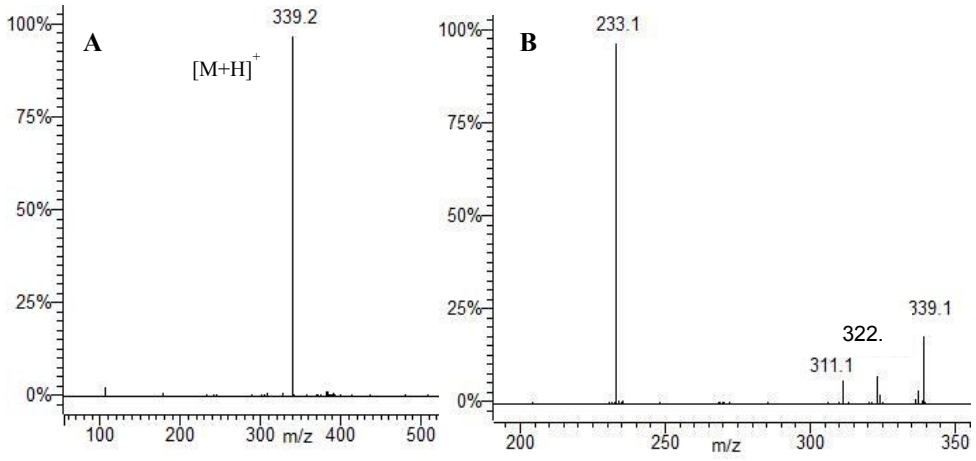
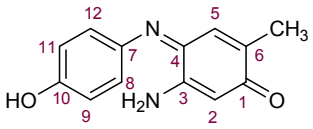


Figure S35 – A) ESI(+)/MS spectrum of compound ***12***; **B)** MS/MS spectrum of *m/z* 339 of compound ***12***.

Compound 13:



NMR spectra in MeOD-d_4 (400MHz)

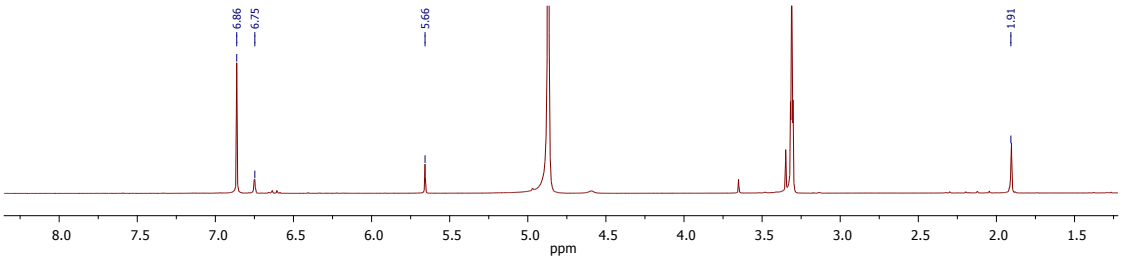


Figure S36 - ^{1}H-NMR spectrum of compound ***13*** in MeOD-d_4 (400MHz)

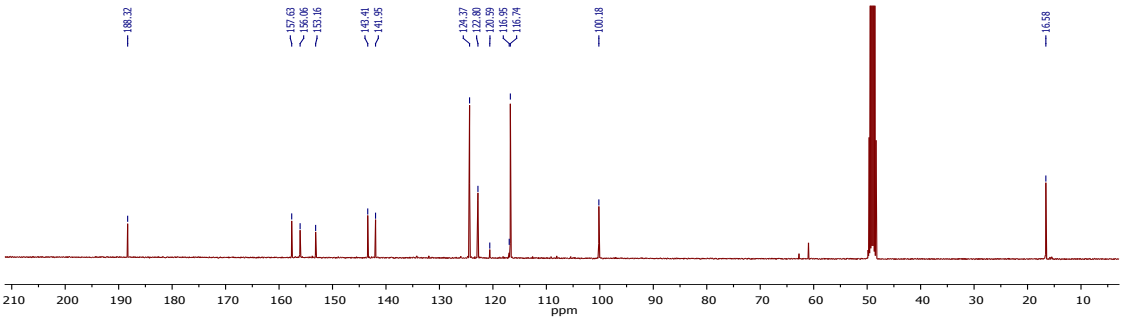


Figure S37 – ^{13}C-NMR spectrum of compound ***13*** in MeOD-d_4 (400MHz)

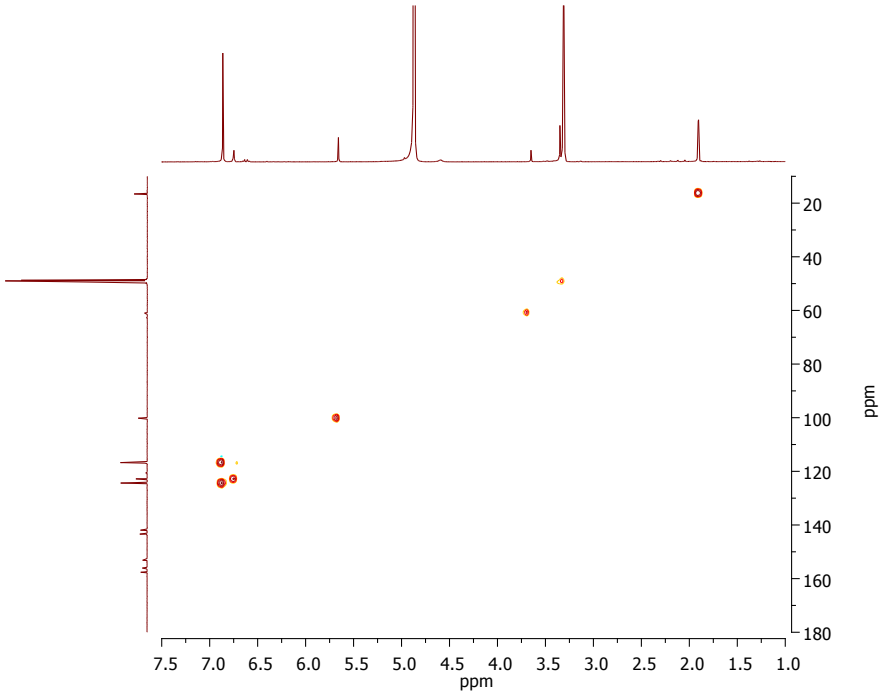


Figure S38 – HSQC-NMR spectrum of compound ***13*** in MeOD-d_4 (400MHz)

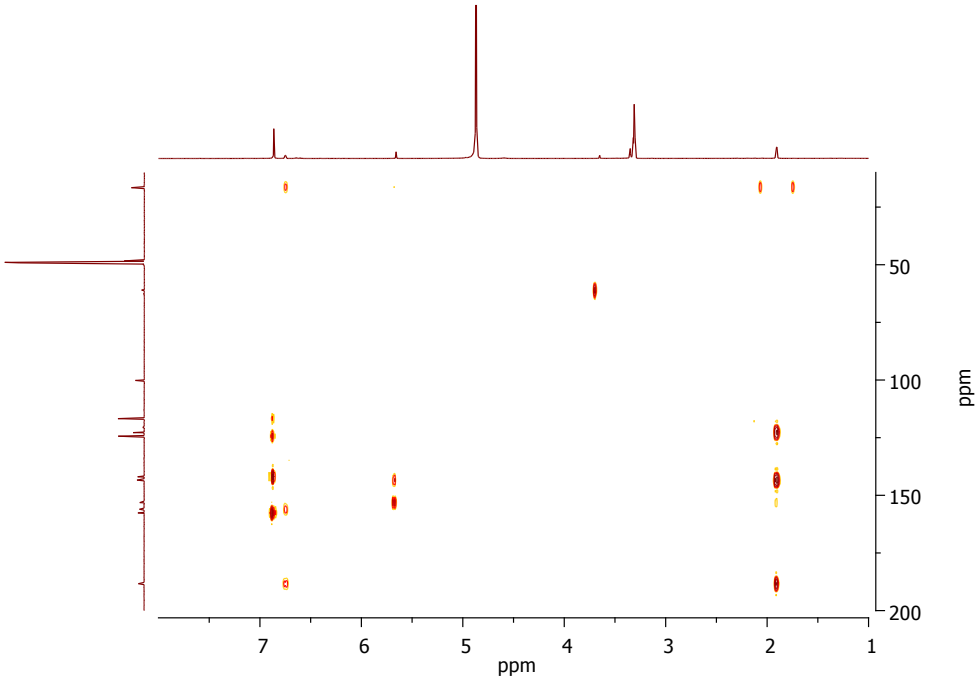


Figure S39 – HMBC-NMR spectrum of compound ***13*** in MeOD-d_4 (400MHz)

ESI(+)/MS of compound ***13*** ($C_{13}H_{12}N_2O_2$) MW=228.25 g/mol

Positive mode *m/z* = 229 $[M+H]^+$

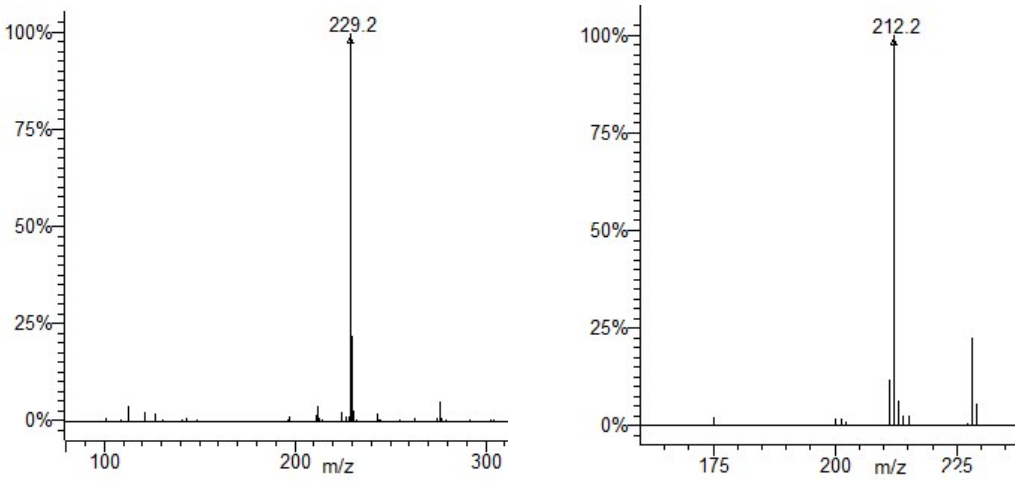
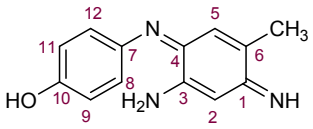


Figure S40 – A) ESI(+)/MS spectrum of compound ***13***; **B)** MS/MS spectrum of *m/z* 229 of compound ***13***

Compound 14:



NMR spectra in MeOD-d_4 (400MHz)

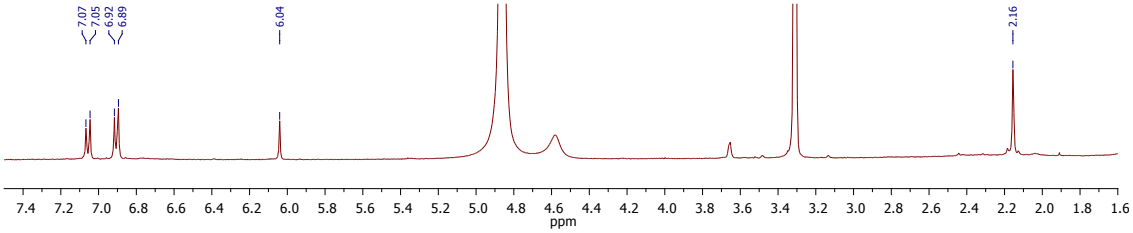


Figure S41 - ^{1}H-NMR spectrum of compound ***14*** in MeOD-d_4 (400MHz)

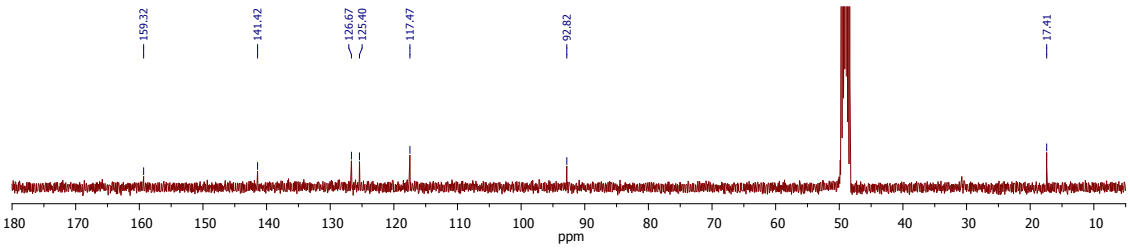


Figure S42 – ^{13}C-NMR spectrum of compound ***14*** in MeOD-d_4 (400MHz)

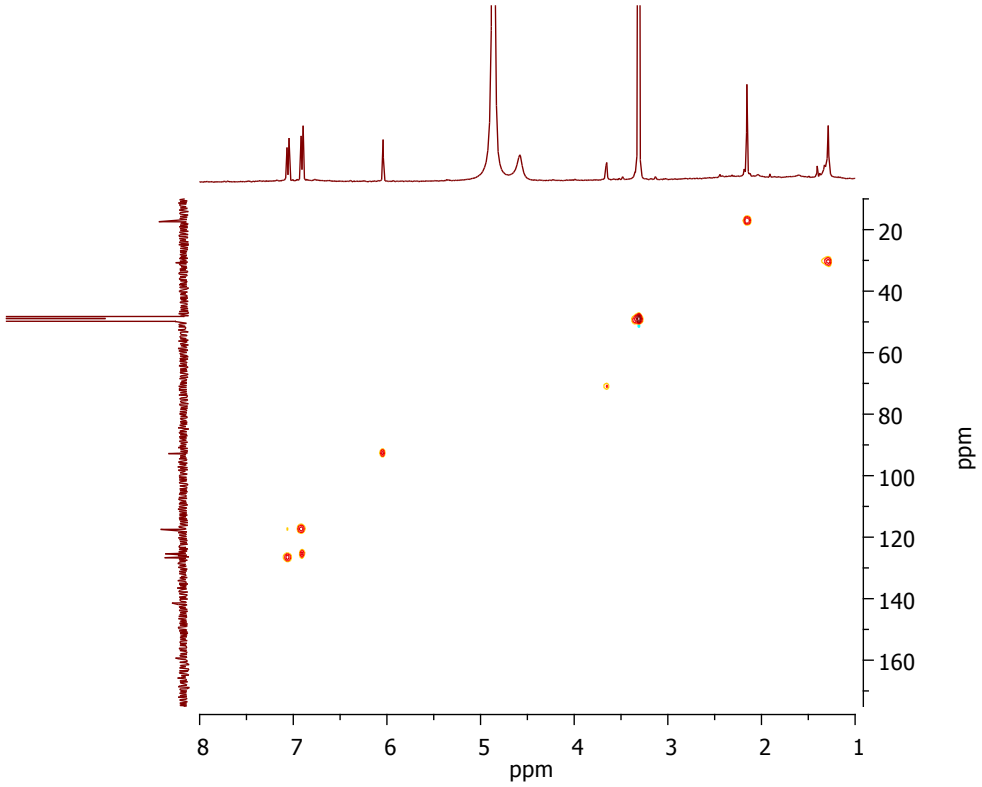


Figure S43 – HSQC-NMR spectrum of compound ***14*** in MeOD-d_4 (400MHz)

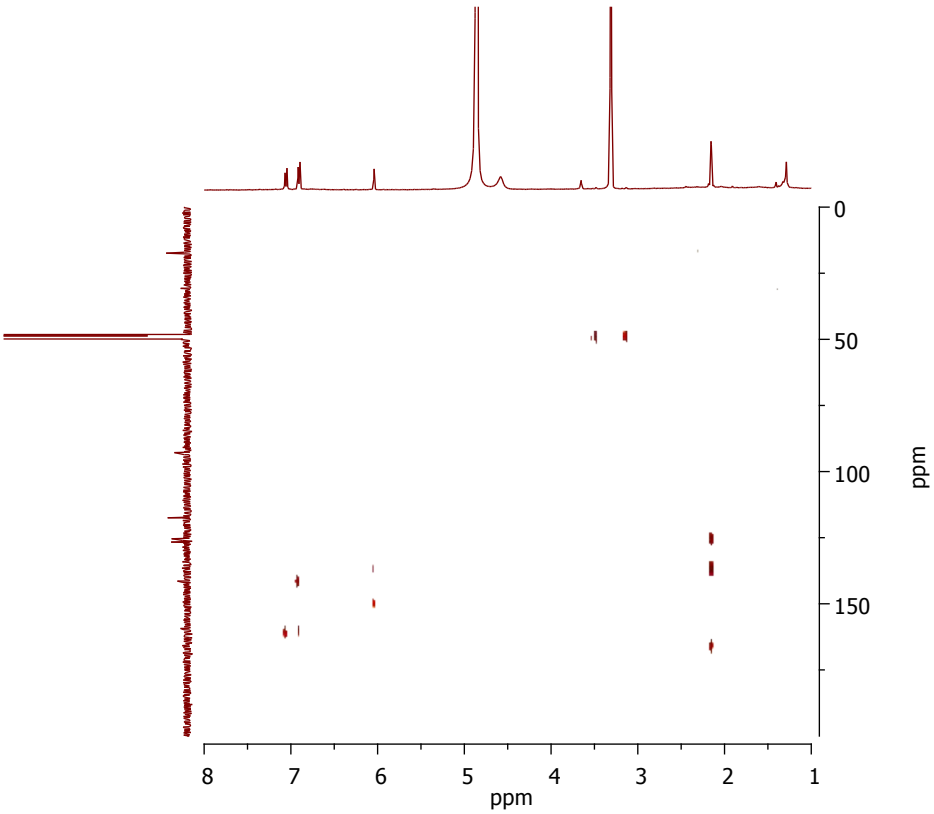


Figure S44 – HMBC-NMR spectrum of compound ***14*** in MeOD-d_4 (400MHz)

ESI(+)/MS of compound ***14*** ($C_{13}H_{13}N_3O$) MW=227.26 g/mol

Positive mode *m/z* = 228 $[M+H]^+$

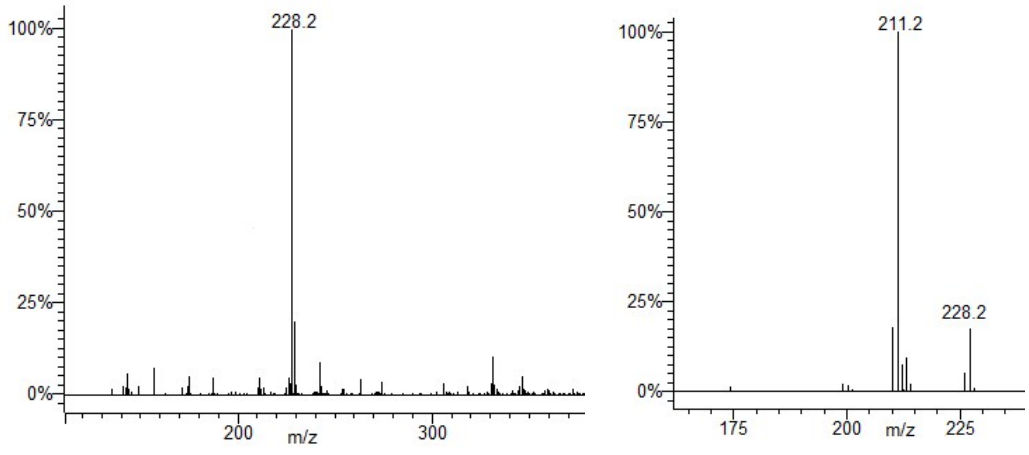
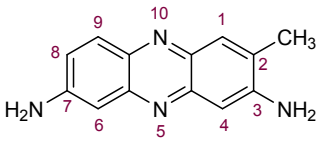


Figure S45 – A) ESI(+)/MS spectrum of compound ***14***; **B)** MS/MS spectrum of *m/z* 228 of compound ***14***.

Compound 15:



NMR spectra in MeOD-d_4 (400MHz)

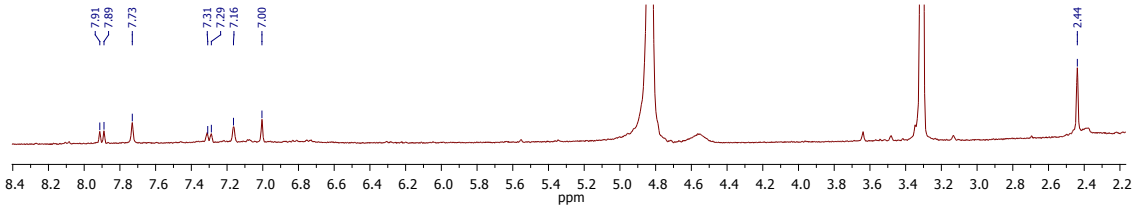


Figure S46 - ^{1}H-NMR spectrum of compound ***15*** in MeOD-d_4 (400MHz)

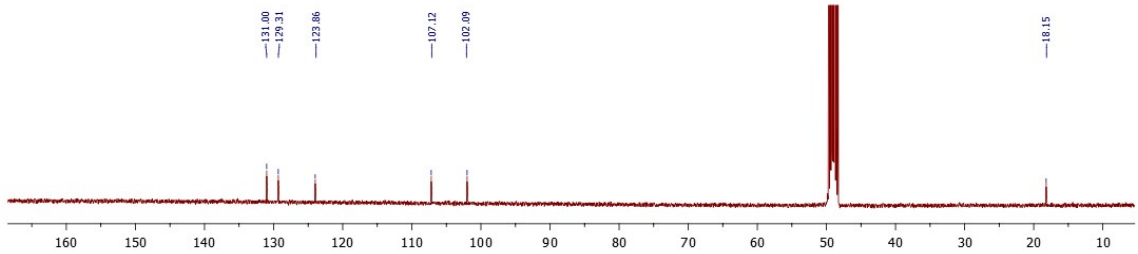


Figure S47 – ^{13}C-NMR spectrum of compound ***15*** in MeOD-d_4 (400MHz)

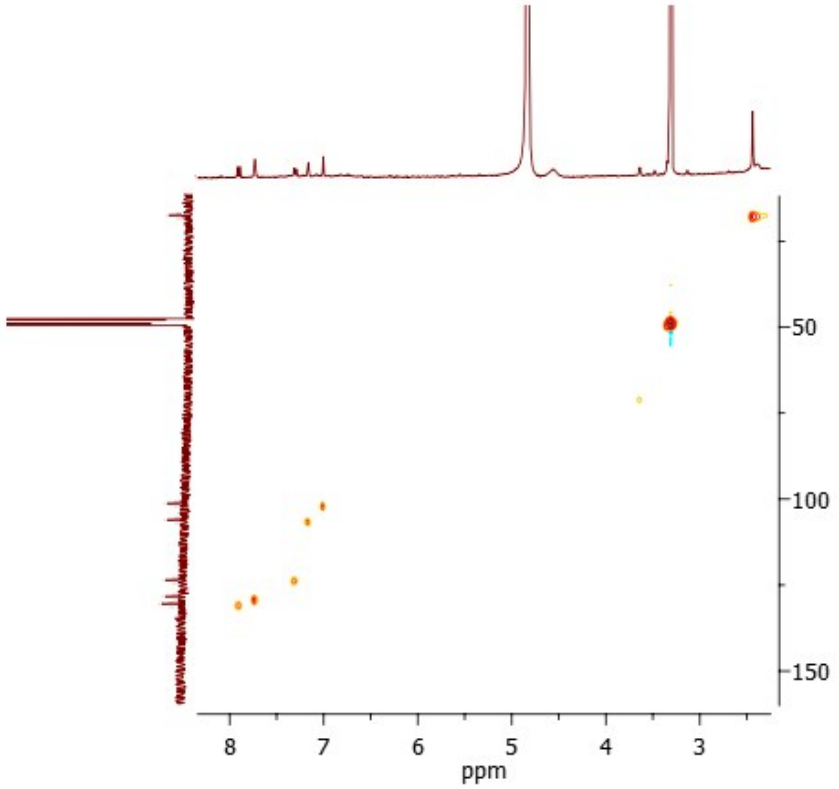


Figure S48 – HSQC-NMR spectrum of compound ***15*** in MeOD-d_4 (400MHz)

ESI(+)/MS spectrum of compound ***15*** ($C_{13}H_{11}N_3O$) MW = 225.25 g/mol

Positive mode *m/z* = 226 $[M+H]^+$

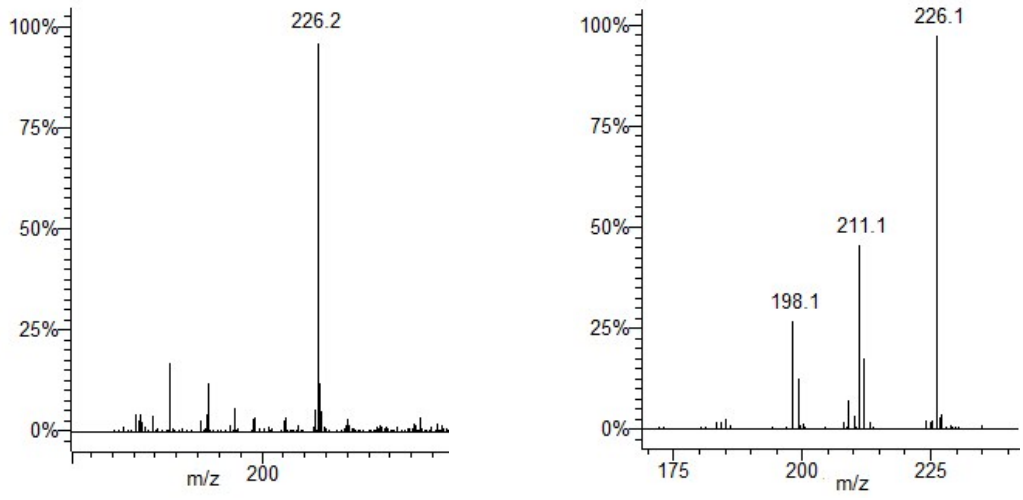
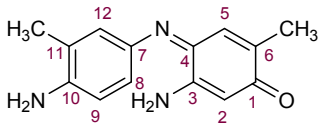


Figure S49 – A) ESI(+)/MS spectrum of compound ***15***; **B)** MS/MS spectrum of *m/z* 226 of compound ***15***

Compound 16:



NMR spectra in MeOD-d_4 (400MHz)

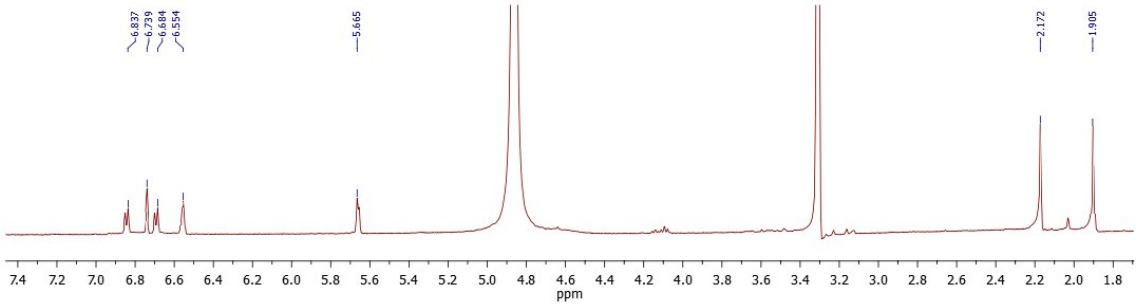


Figure S50 - ^{1}H-NMR spectrum of compound ***16*** in MeOD-d_4 (400MHz)

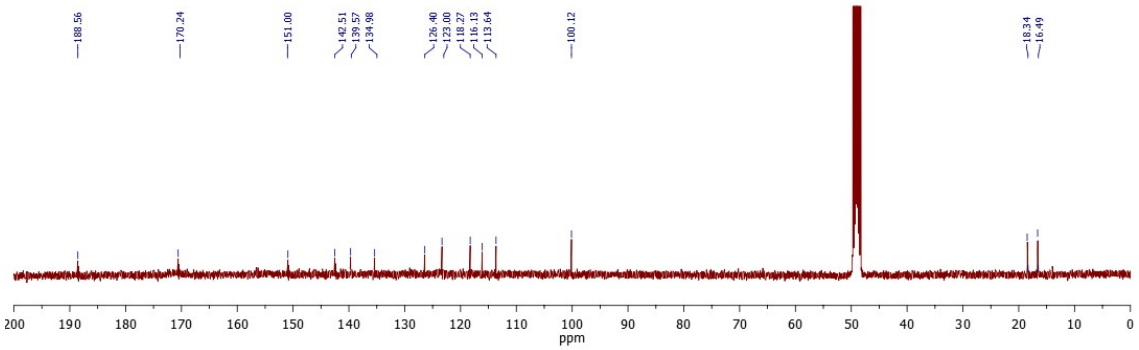
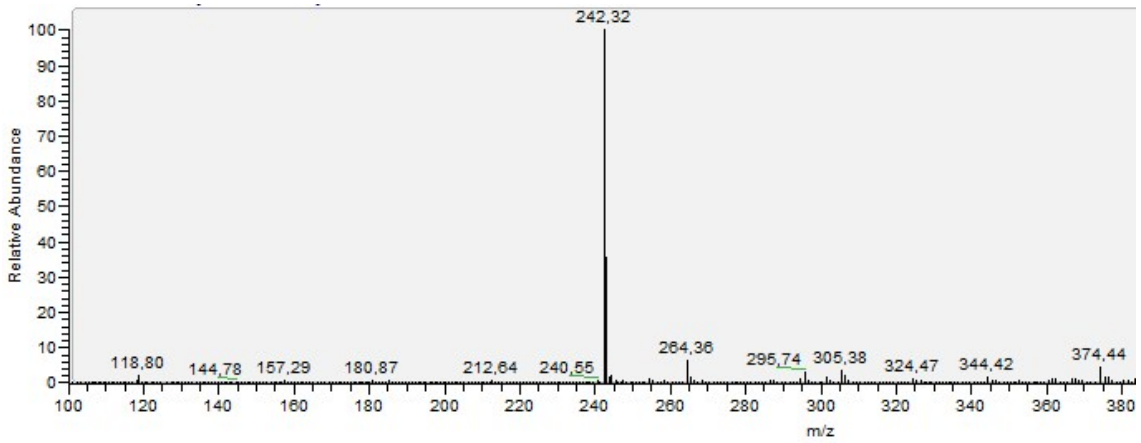


Figure S51 – ^{13}C-NMR spectrum of compound ***16*** in MeOD-d_4 (400MHz)

ESI(-)/MS spectrum of compound ***16*** ($C_{14}H_{15}N_3O$) MW = 241.29g/mol

Positive mode *m/z* = 242 $[M+H]^+$

A



B

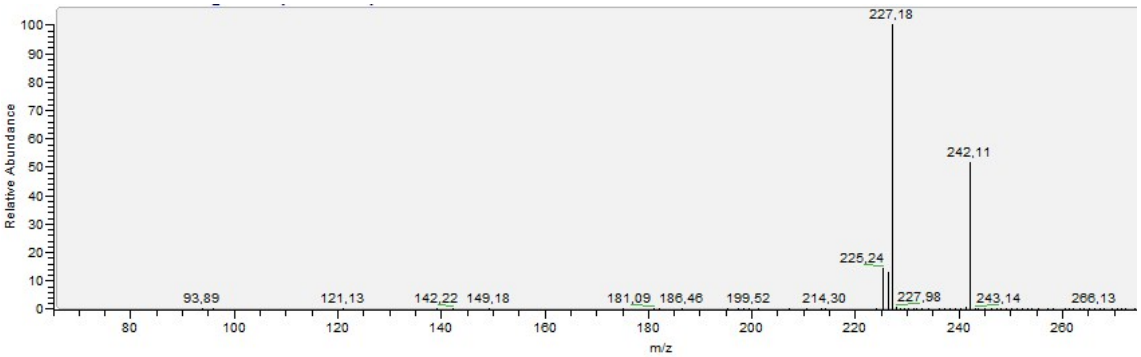
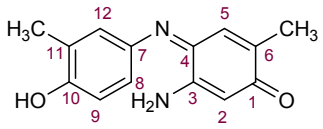


Figure S52 – A) ESI(+)/MS spectrum of compound ***16***; **B)** MS/MS spectrum of *m/z* 242 of compound ***16***.

Compound 17:



NMR spectra in MeOD-d_4 (400MHz)

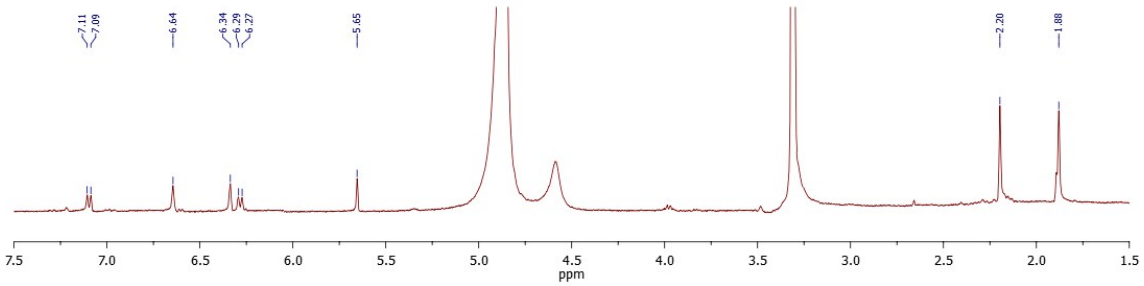


Figure S53 - ^{1}H-NMR spectrum of compound ***17*** in MeOD-d_4 (400MHz)

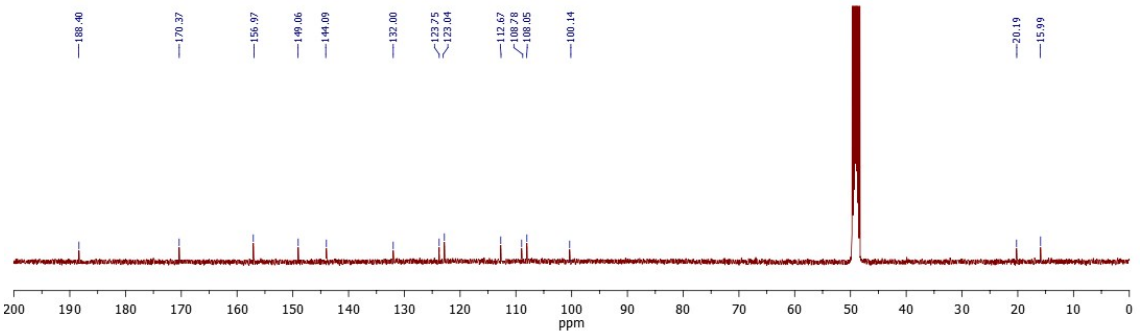


Figure S54 – ^{13}C-NMR spectrum of compound ***17*** in MeOD-d_4 (400MHz)

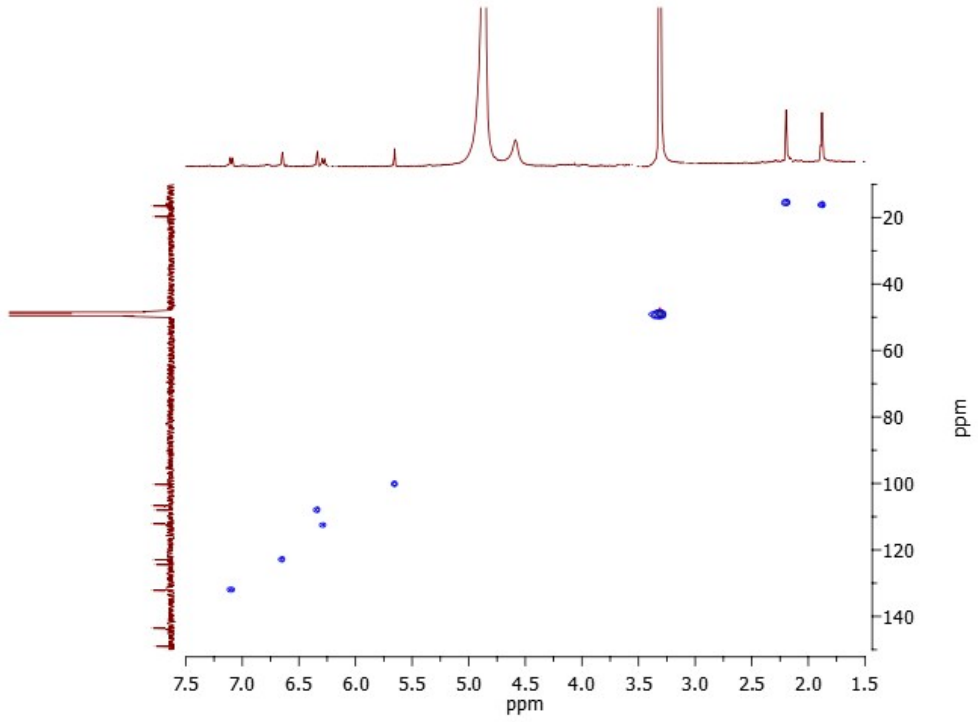
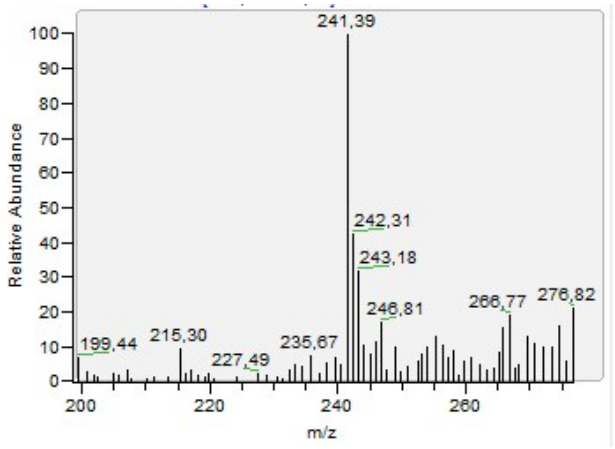


Figure S55 – HSQC-NMR spectrum of compound ***17*** in MeOD-d_4 (400MHz)

ESI(+)/MS spectrum of compound ***17*** ($C_{14}H_{14}N_2O_2$) MW = 242.20 g/mol

Negative mode *m/z* = 241 $[M-H]^-$

A)



B)

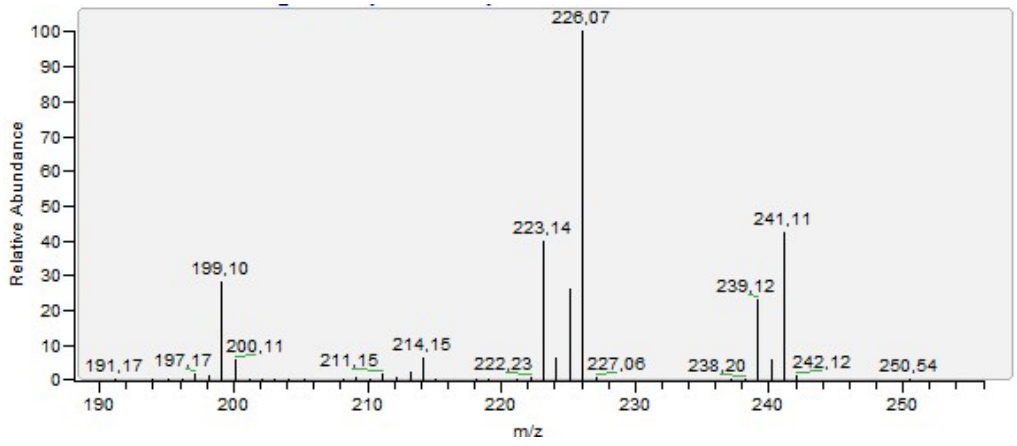


Figure S56 - A) ESI(-)/MS spectrum of compound ***17***; **B)** MS/MS spectrum of *m/z* 241 of compound ***17***