Novel combretastatin–2–aminoimidazole analogues as potent tubulin assembly inhibitors: Exploration of unique pharmacophoric impact of bridging skeleton and aryl moiety

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Supplementary Information

Table of Content

Experimental

Chemistry:

Spectral Data of intermediates and compounds (1-15)

2-(3,4,5-Trimethoxyphenyl)imidazo[1,2-*a*]pyrimidine (Intermediate)

Light yellow solid; mp >200 °C; 157 mg, 55% yield; IR (neat) ν_{max} = 2945, 2912, 1634, 1585, 1124 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d6*): δ = 8.94 (dd, *J* = 6.7 Hz, 1.2, 1H), 8.52-8.51 (m, 1H), 8.41 (s, 1H), 7.32 (s, 2H), 7.06-7.03 (m, 1H), 3.88 (s, 6H), 3.70 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d6*): δ = 153.7, 150.7, 148.3, 145.8, 138.2, 135.3, 129.4, 109.3, 108.0, 103.5, 60.6, 56.4; HRMS (ESI-TOF) Calcd for $C_{15}H_{16}N_3O_3$: $[M + H]^+$ 286.1193 found m/z 286.1185.

3-Bromo-2-(3,4,5-trimethoxyphenyl)imidazo[1,2-*a*]pyrimidine (Intermediate)

Light yellow solid; mp >200 °C; 327 mg, 90% yield; IR (neat) ν_{max} = 2948, 2920, 1643, 1580, 1128 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.59 (dd, *J* = 4.1 Hz, 1.9, 1H), 8.47 (dd, *J* = 6.8 Hz, 1.9, 1H), 7.53 (s, 2H), 7.02 (dd, *J* = 4.1 Hz, 1.9, 1H), 3.98 (s, 6H), 3.92 (s, 3H); ^{13}C NMR (100

MHz, $CDCl_3$): δ = 153.3, 150.2, 148.0, 143.9, 138.8, 131.4, 127.6, 109.4, 105.3, 89.9, 60.9, 56.3; HRMS (ESI-TOF) Calcd for $C_{15}H_{15}{}^{81}BrN_3O_3$: $[M + H]^+$ 364.0299 found m/z 364.0299.

4,5-Diphenyl-1*H*-imidazol-2-amine (1)

Greenish yellow solid; mp 130-132 °C; 94 mg, 40% yield; IR (neat) ν_{max} = 3416, 3363, 2934, 1634, 1581, 1122 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.42 (d, *J* = 7.3 Hz, 4H), 7.28 (dd, *J* = 7.4 Hz, 7.7, 4H), 7.17 (dd, J = 7.4 Hz, 7.2, 2H), 5.36 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 150.1, 134.1, 128.2, 126.8, 125.9; HRMS (ESI-TOF) Calcd for $C_{15}H_{14}N_3$: $[M + H]^+$ 236.1189 found m/z 236.1159.

5-Phenyl-4-(3,4,5-trimethoxyphenyl)-1*H*-imidazol-2-amine (2)

Light yellow solid; mp 140-142 °C; 117 mg, 36% yield; IR (neat) ν_{max} = 3318, 1634, 1590, 1508, 1124 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.42 (d, *J* = 7.4 Hz, 2H), 7.24 (dd, *J* = 7.2 Hz, 7.5, 2H), 7.17 (dd, J = 7.2 Hz, 7.1, 1H), 6.67 (s, 2H), 3.80 (s, 3H), 3.56 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.1, 153.1, 149.2, 136.5, 132.7, 128.8, 128.4, 127.7, 127.0, 126.5, 104.2, 60.9, 55.7; HRMS (ESI-TOF) Calcd for $C_{18}H_{20}N_3O_3$: $[M + H]^+$ 326.1506 found m/z 326.1496

4,5-Bis(3,4,5-trimethoxyphenyl)-1*H*-imidazol-2-amine (3)

Light yellow solid; mp >200 °C; 145 mg, 35% yield; IR (neat) ν_{max} = 3444, 3359, 1614, 1500, 1171, 1044 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ = 6.73 (s, 4H), 5.33 (s, 2H), 3.65 (s, 12H), 3.64 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 171.0, 168.3, 153.1, 148.7, 136.8, 128.7, 104.6, 60.9, 55.9; HRMS (ESI-TOF) Calcd for $C_{21}H_{26}N_3O_6$: $[M + H]^+$ 416.1823 found m/z 416.1822.

5-(4-Chlorophenyl)-4-(3,4,5-trimethoxyphenyl)-1*H*-imidazol-2-amine (4)

Light yellow solid; mp 170-172 °C; 115 mg, 32% yield; IR (neat) ν_{max} = 3327, 1624, 1587, 1126, 749 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.37 (d, J = 8.3 Hz, 2H), 7.20 (d, J = 8.4 Hz, 2H), 6.64 (s, 2H), 3.85 (s, 3H), 3.63 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.0, 153.2, 149.2, 136.8, 132.4, 131.6, 128.7, 128.5, 104.5, 60.9, 55.9; HRMS (ESI-TOF) Calcd for $C_{18}H_{19}ClN_3O_3$: $[M + H]^+$ 360.1117 found m/z 360.1112 and for $C_{18}H_{19}{}^{35}ClN_3O_3$ 362.1087 found m/z 362.1088.

5-(4-Fluorophenyl)-4-(3,4,5-trimethoxyphenyl)-1*H*-imidazol-2-amine (5)

Light yellow solid; mp 145-147 °C; 106 mg, 31% yield; IR (neat) ν_{max} = 3325, 2924, 1686, 1580, 1502, 1225, 1123, cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.42-7.38 (m, 2H), 6.96 (dd, J = 8.7 Hz, 8.7 Hz, 2H), 6.62 (s, 2H), 3.84 (s, 3H), 3.65 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 168.9, 161.8 ($^{1}J_{C–F}$ = 245 Hz), 153.1, 148.9, 136.7, 129.4 ($^{3}J_{C–F}$ = 8 Hz), 129.1 ($^{4}J_{C–F}$ = 1 Hz), 128.3, 126.9, 126.4, 115.3 ($^{2}J_{C–F}$ = 22 Hz), 104.2, 60.9, 55.9; HRMS (ESI-TOF) Calcd for $C_{18}H_{19}FN_3O_3$: $[M + H]^+$ 344.1412 found m/z 344.1414.

5-(4-Methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-1*H*-imidazol-2-amine (6)

Light yellow solid; mp 176-178 °C; 124 mg, 35% yield; IR (neat) ν_{max} = 3319, 1662, 1591, 1125 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.33 (d, J = 6.4 Hz, 2H), 6.77 (d, J = 6.7 Hz, 2H), 6.66 (s, 2H), 3.79 (s, 3H), 3.73 (s, 3H), 3.57 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.2, 168.9, 157.7, 152.0, 148.2, 135.3, 128.2, 124.4, 112.9, 103.0, 59.9, 54.8, 54.3; HRMS (ESI-TOF) Calcd for $C_{19}H_{22}N_3O_4$: $[M + H]^+$ 356.1612 found m/z 356.1611.

3-(2-Amino-4-(3,4,5-trimethoxyphenyl)-1*H*-imidazol-5-yl)phenol (7)

Pale yellow solid; mp 185-187 °C; 95 mg, 28% yield; IR (neat) ν_{max} = 3360, 2932, 1556, 1658, 1412, 1122 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$): δ = 7.42 (d, *J* = 7.2 Hz, 2H), 7.23 (dd, *J* = 7.1 Hz, 7.6 Hz, 2H), 7.16 (dd, *J* = 7.2 Hz, 7.3 Hz, 1H), 6.67 (s, 2H), 3.80 (s, 3H), 3.54 (s, 6H); ^{13}C NMR (100 MHz, CDCl$_3$): δ = 171.2, 152.9, 149.2, 136.3, 132.8, 128.9, 128.4, 127.9, 127.7, 126.9, 126.6, 104.1, 60.9, 55.7; HRMS (ESI-TOF) Calcd for $C_{18}H_{20}N_3O_4$: [M + H]$^+$ 342.1456 found m/z 342.1446.

5-(3-Methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-1*H*-imidazol-2-amine (8)

Light yellow solid; mp 180-182 °C; 114 mg, 32% yield; IR (neat) ν_{max} = 3315, 2936, 1664, 1581, 1231, 1123 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$): δ = 7.15 (dd, *J* = 8.1 Hz, 7.8 Hz, 1H), 7.00 (d, *J* = 7.2 Hz, 2H), 6.74 (d, *J* = 9.1 Hz, 2H), 6.67 (s, 2H), 3.82 (s, 3H), 3.67 (s, 3H), 3.63 (s, 6H); ^{13}C NMR (100 MHz, CDCl$_3$): δ = 180.1, 159.6, 153.1, 148.7, 137.0, 132.7, 129.5, 127.1, 126.3, 120.0, 113.2, 112.9, 104.4, 60.9, 55.9, 55.1; HRMS (ESI-TOF) Calcd for $C_{19}H_{22}N_3O_4$: [M + H]$^+$ 356.1612 found m/z 356.1610.

5-(3,4-Dimethoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-1*H*-imidazol-2-amine (9)

Yellow solid; mp >200 °C; 115 mg, 30% yield; IR (neat) ν_{max} = 3377, 2935, 1650, 1582, 1122 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$): δ = 7.00 (s, 2H), 6.76 (d, *J* = 8.6 Hz, 1H), 6.61 (s, 2H), 3.84 (s, 3H), 3.82 (s, 3H), 3.68 (s, 3H), 3.62 (s, 6H); ^{13}C NMR (100 MHz, CDCl$_3$): δ = 153.0, 148.7, 148.5, 148.1, 136.6, 128.8, 126.9, 125.5, 120.1, 111.1, 111.0, 104.3, 60.9, 55.9, 55.7; HRMS (ESI-TOF) Calcd for $C_{20}H_{24}N_3O_5$: [M + H]$^+$ 386.1718 found m/z 386.1699.

5-(2-Amino-4-(3,4,5-trimethoxyphenyl)-1*H*-imidazol-5-yl)pyridin-2-amine (10)

Yellow solid; mp 165-167 °C; 130 mg, 38% yield; IR (neat) ν_{max} = 3353, 2925, 1632, 1585, 1413, 1126 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, MeOD): δ = 8.01 (d, *J* = 2.2 Hz, 1H), 7.50 (dd, *J* = 2.3 Hz, 8.6 Hz, 1H), 6.63 (s, 2H), 6.47 (dd, *J* = 0.6 Hz, 8.6 Hz, 1H), 3.79 (s, 3H), 3.71 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$, MeOD): δ = 157.3, 153.0, 149.2, 145.8, 138.0, 136.3, 128.7, 119.3, 108.6, 105.7, 103.9, 60.8, 55.8; HRMS (ESI-TOF) Calcd for $C_{17}H_{20}N_5O_3$: $[M + H]^+$ 342.1568 found m/z 342.1562.

5-(Pyridin-3-yl)-4-(3,4,5-trimethoxyphenyl)-1*H*-imidazol-2-amine (11)

Yellow solid; mp 115-117 °C; 121 mg, 37% yield; IR (neat) ν_{max} = 3327, 2951, 1632, 1585, 1505, 1120 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.69 (s, 1H), 8.33 (d, *J* = 3.9 Hz, 1H), 7.72 (d, *J* = 7.9 Hz, 1H), 7.14-7.11 (m, 1H), 6.57 (s, 2H), 3.82 (s, 3H), 3.61 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 153.3, 149.6, 147.9, 147.2, 137.2, 134.7, 129.2, 127.7, 127.3, 123.2, 115.8, 104.5, 60.9, 55.9; HRMS (ESI-TOF) Calcd for $C_{17}H_{19}N_4O_3$: $[M + H]^+$ 327.1459 found m/z 327.1449.

5-(Quinolin-3-yl)-4-(3,4,5-trimethoxyphenyl)-1*H*-imidazol-2-amine (12)

Yellow solid; mp 120-122 °C; 150 mg, 40% yield; IR (neat) ν_{max} = 3338, 2935, 1636, 1590, 1508, 1125 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.02 (d, *J* = 2.1 Hz, 1H), 8.25 (d, *J* = 1.6 Hz, 1H), 7.99 (d, *J* = 8.4 Hz, 1H), 7.66 (d, *J* = 8.1 Hz, 1H), 7.62 (ddd, *J* = 1.3 Hz, 7.0 Hz, 8.3 Hz, 1H), 7.47 (ddd, *J* = 0.9 Hz, 7.9 Hz, 0.8 Hz, 1H), 7.41 (s, 3H), 6.67 (s, 2H), 3.84 (s, 3H), 3.62 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.1, 168.7, 153.4, 150.0, 149.9, 146.3, 137.2, 132.4 129.0, 128.8, 127.9, 127.6, 127.2, 126.9, 104.6, 60.9, 55.9; HRMS (ESI-TOF) Calcd for $C_{21}H_{21}N_4O_3$: $[M + H]^+$ 377.1615 found m/z 377.1606.

5-(1*H*-Indol-5-yl)-4-(3,4,5-trimethoxyphenyl)-1*H*-imidazol-2-amine (13)

Light yellow solid; mp 125-127 °C; 73 mg, 20% yield; IR (neat) ν_{max} = 3333, 2926, 1590, 1508, 1124, 905 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.42-7.39 (m, 3H), 7.26 (d, *J* = 5.8 Hz, 4H), 6.64 (s, 2H), 3.83 (s, 3H), 3.64 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 180.0, 153.3, 148.4, 137.6, 129.6, 128.8, 128.7, 128.3, 127.8, 124.9, 123.6, 122.9, 106.1, 104.3, 60.9, 55.9; HRMS (ESI-TOF) Calcd for $C_{20}H_{21}N_4O_3$: $[M + H]^+$ 365.1615 found m/z 365.1604.

5-(3-Aminophenyl)-4-(3,4,5-trimethoxyphenyl)-1*H*-imidazol-2-amine (14)

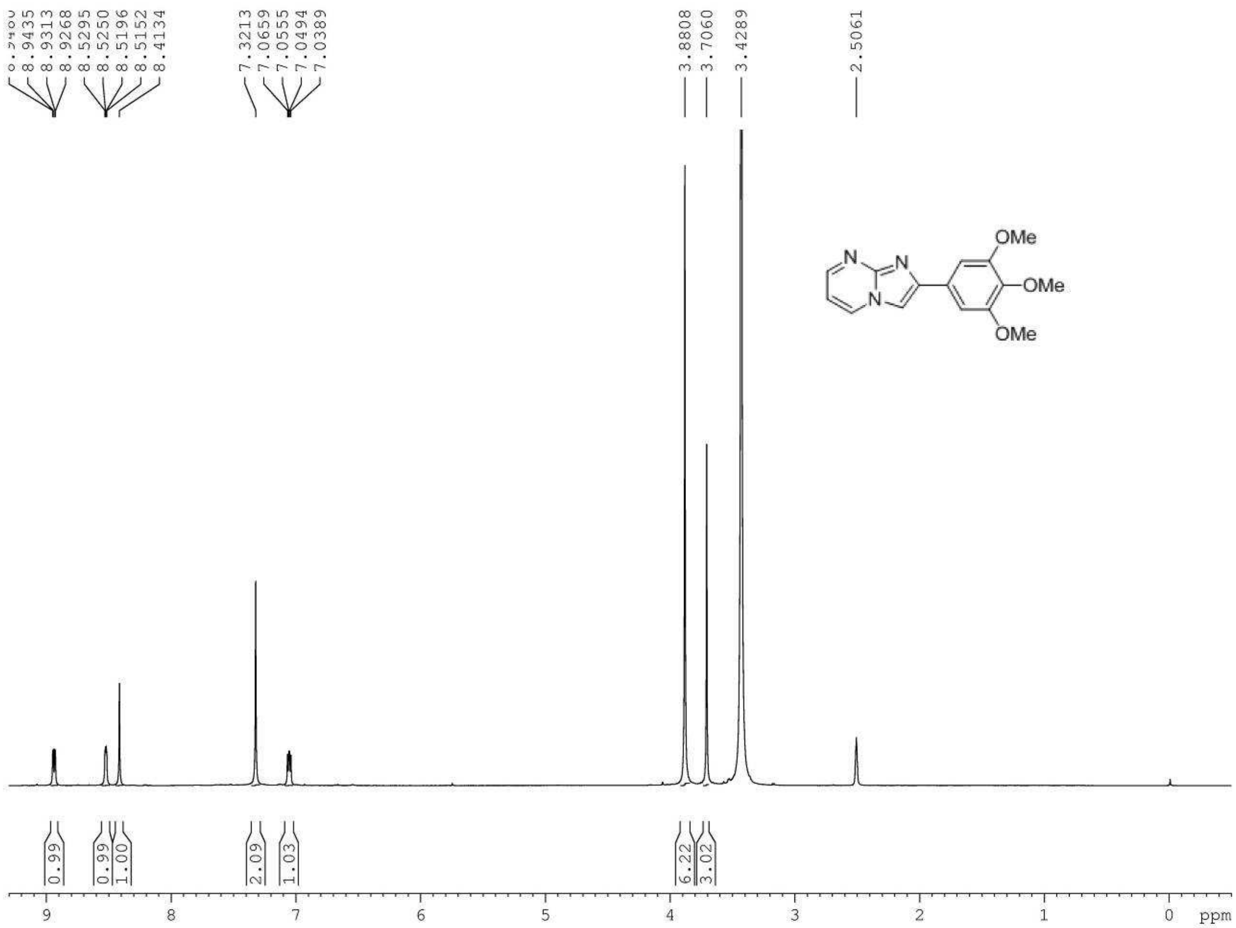
Pale yellow solid; mp 135-137 °C; 119 mg, 35% yield; IR (neat) ν_{max} = 3433, 3285, 3175, 1640, 1566, 1414, 1124 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ = 7.07 (d, *J* = 7.8 Hz, 7.8 Hz, 1H), 6.91 (s, 1H), 6.84 (d, *J* = 7.5 Hz, 1H), 6.77 (s, 2H), 6.58 (dd, J = 1.6 Hz, 8.0 Hz, 1H), 5.55 (s, 2H), 3.64 (s, 3H), 3.62 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6): δ =153.1, 148.3, 146.9, 137.0, 131.7, 129.4, 126.7, 125.5, 124.6, 118.0, 114.5, 114.1, 104.3, 60.9, 55.9; HRMS (ESI-TOF) Calcd for $C_{18}H_{21}N_4O_3$: $[M + H]^+$ 341.1615 found m/z 341.1608.

5-(2-Amino-4-(3,4,5-trimethoxyphenyl)-1*H*-imidazol-5-yl)-2-methoxyphenol (15)

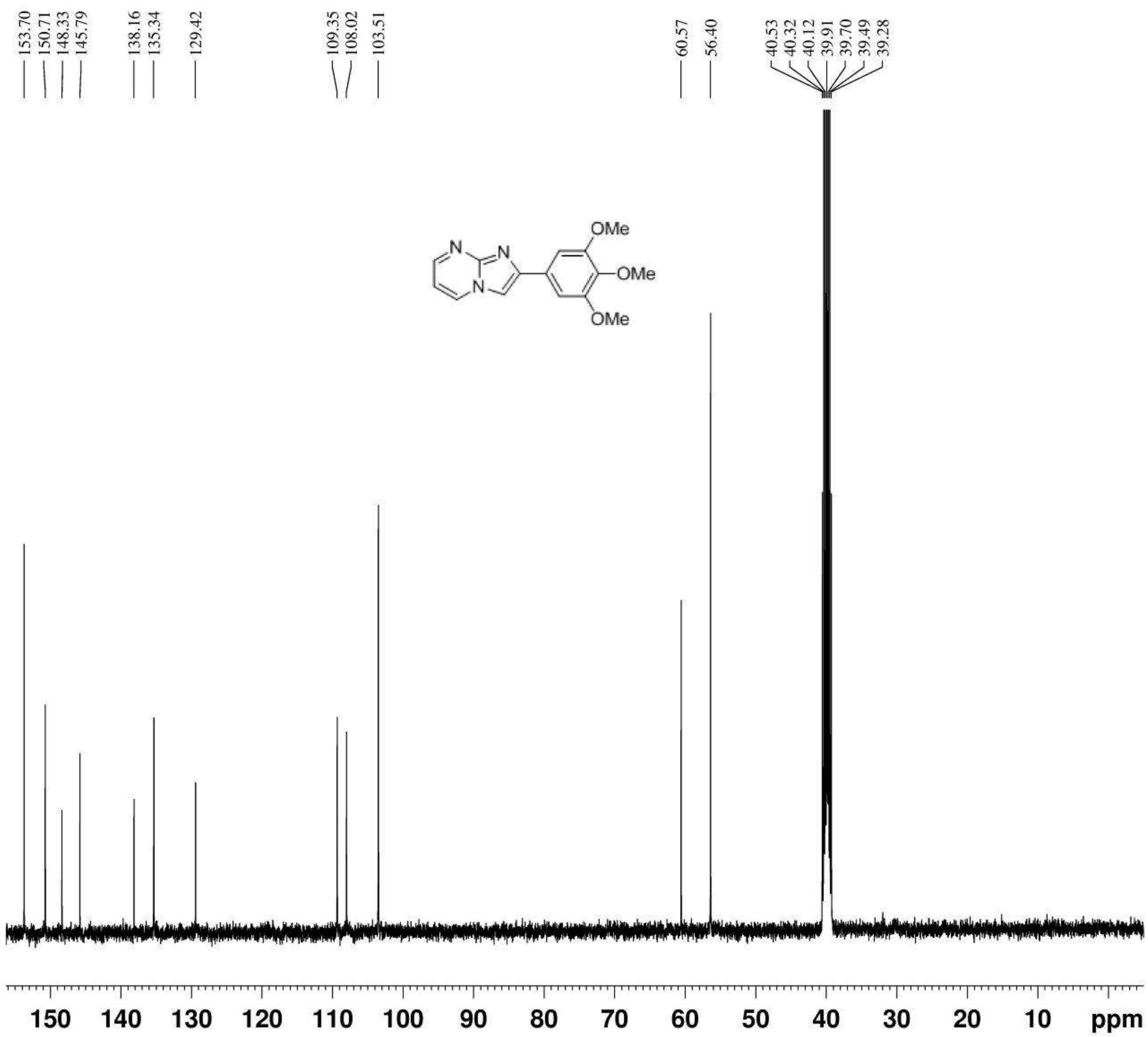
Pale yellow solid; mp 95-97 °C; 78 mg, 21% yield; IR (neat) ν_{max} = 3339, 2934, 1635, 1510, 1416, 1126 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 6.94 (s, 1H), 6.78 (s, 1H), 6.78 (s, 1H), 6.62 (s, 3H), 3.77 (s, 3H), 3.73 (s, 3H), 3.55 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 152.9, 147.8, 147.1, 146.2, 136.9, 126.7, 124.1, 123.5, 119.8, 114.6, 111.1, 104.2, 60.9, 55.9, 55.8; HRMS (ESI-TOF) Calcd for $C_{19}H_{22}N_3O_5$: $[M + H]^+$ 372.1561 found m/z 372.1565.

NMR spectra of intermediates and compounds 1-15 are given below.

2-(3,4,5-Trimethoxyphenyl)imidazo[1,2-*a*]pyrimidine : ^{1}H NMR



2-(3,4,5-Trimethoxyphenyl)imidazo[1,2-*a*]pyrimidine : ^{13}C NMR



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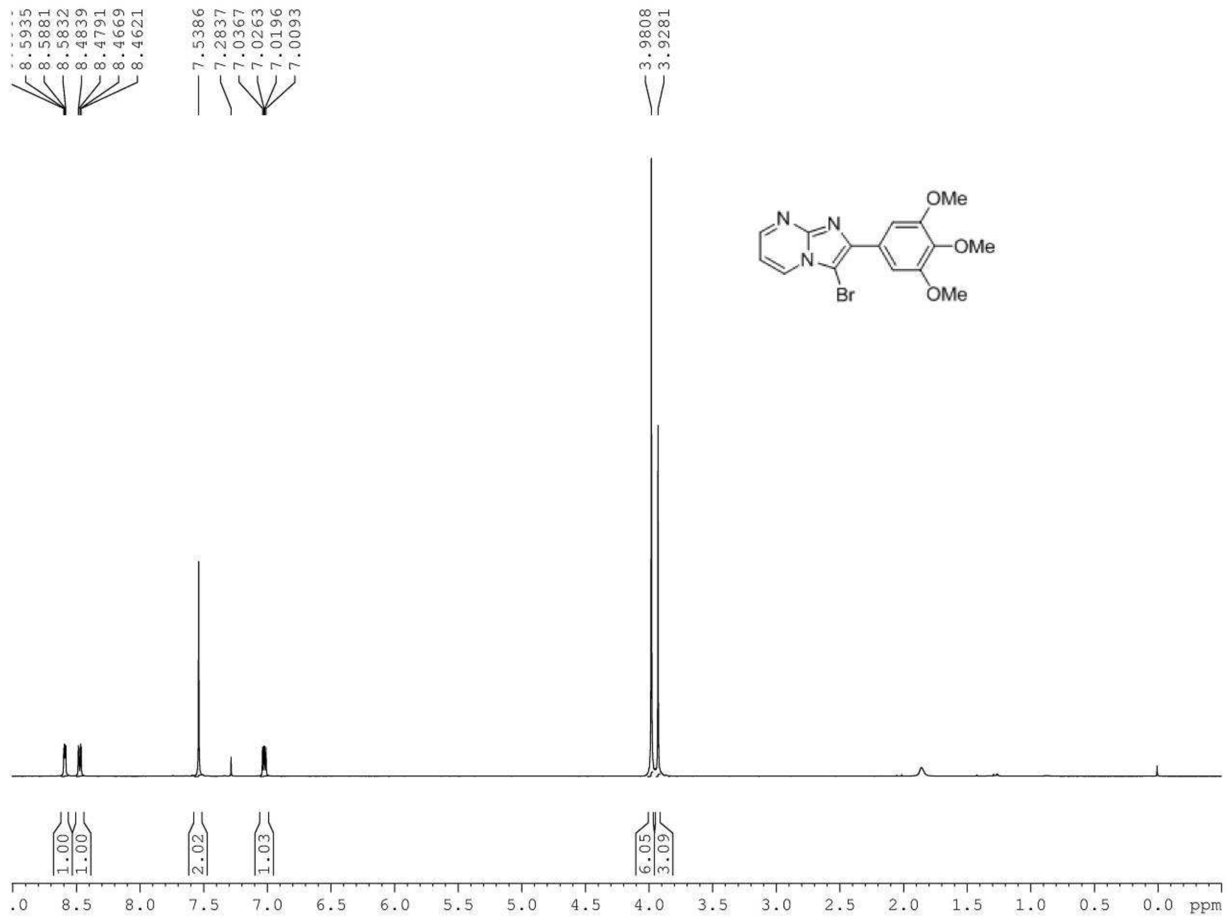

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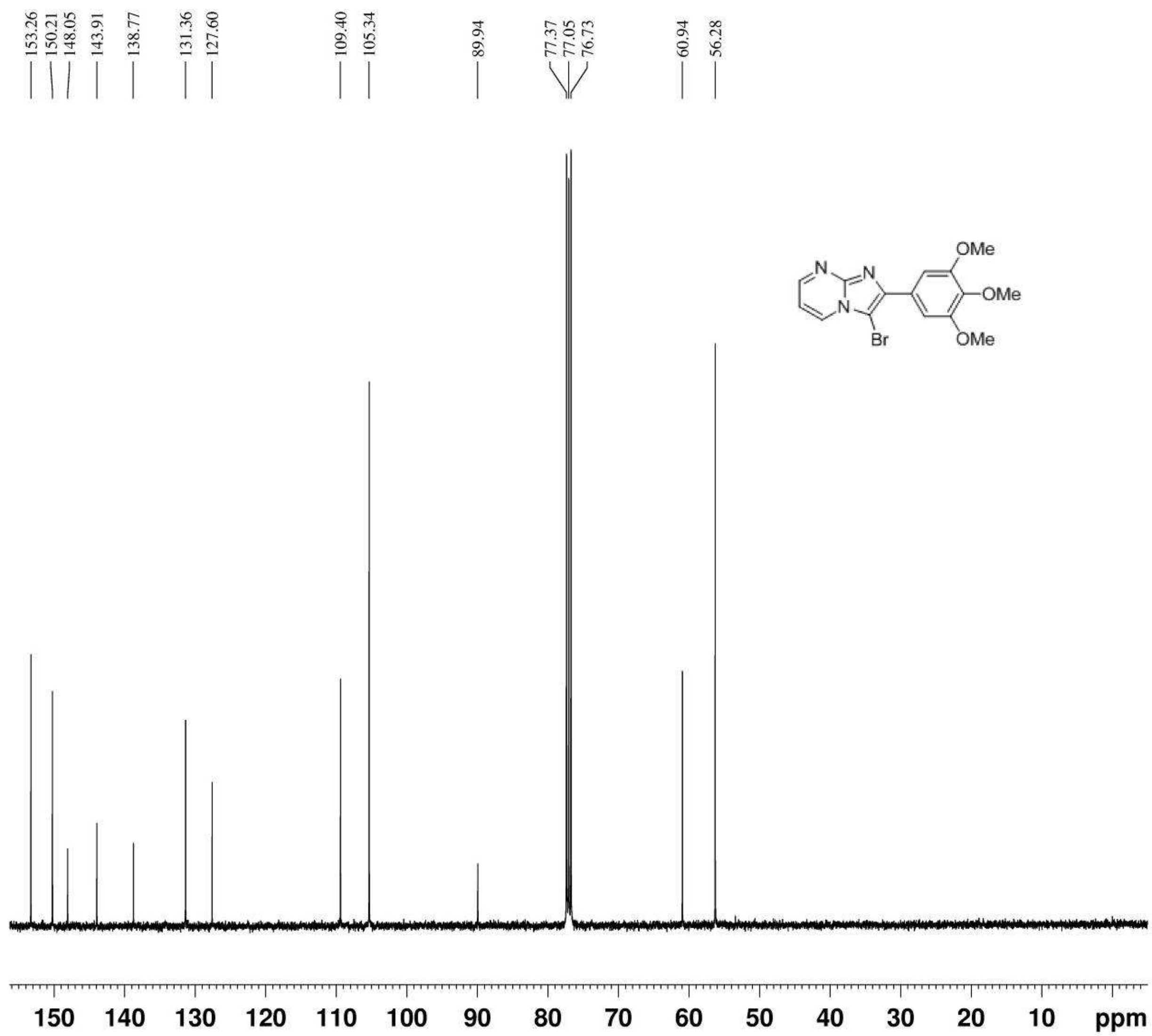

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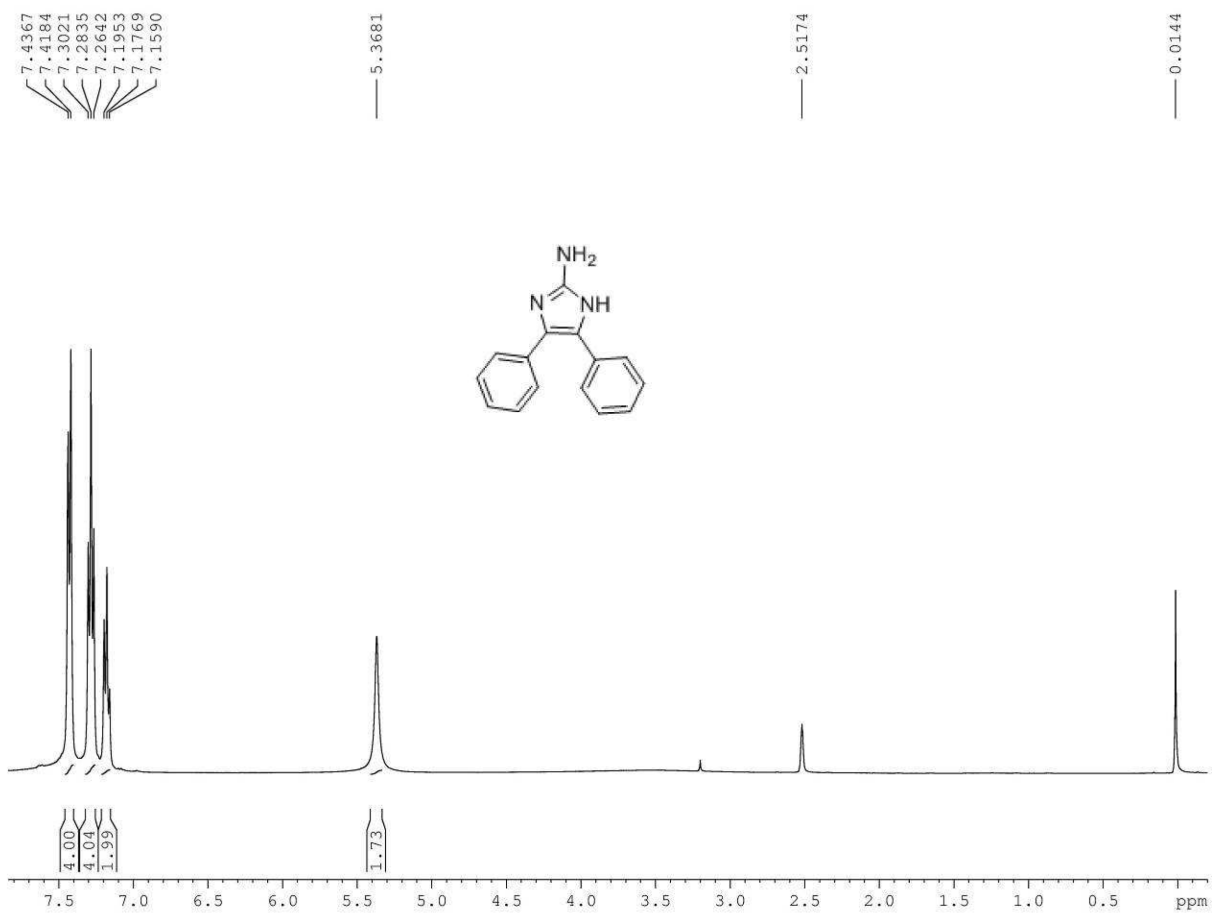

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NH
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6.0
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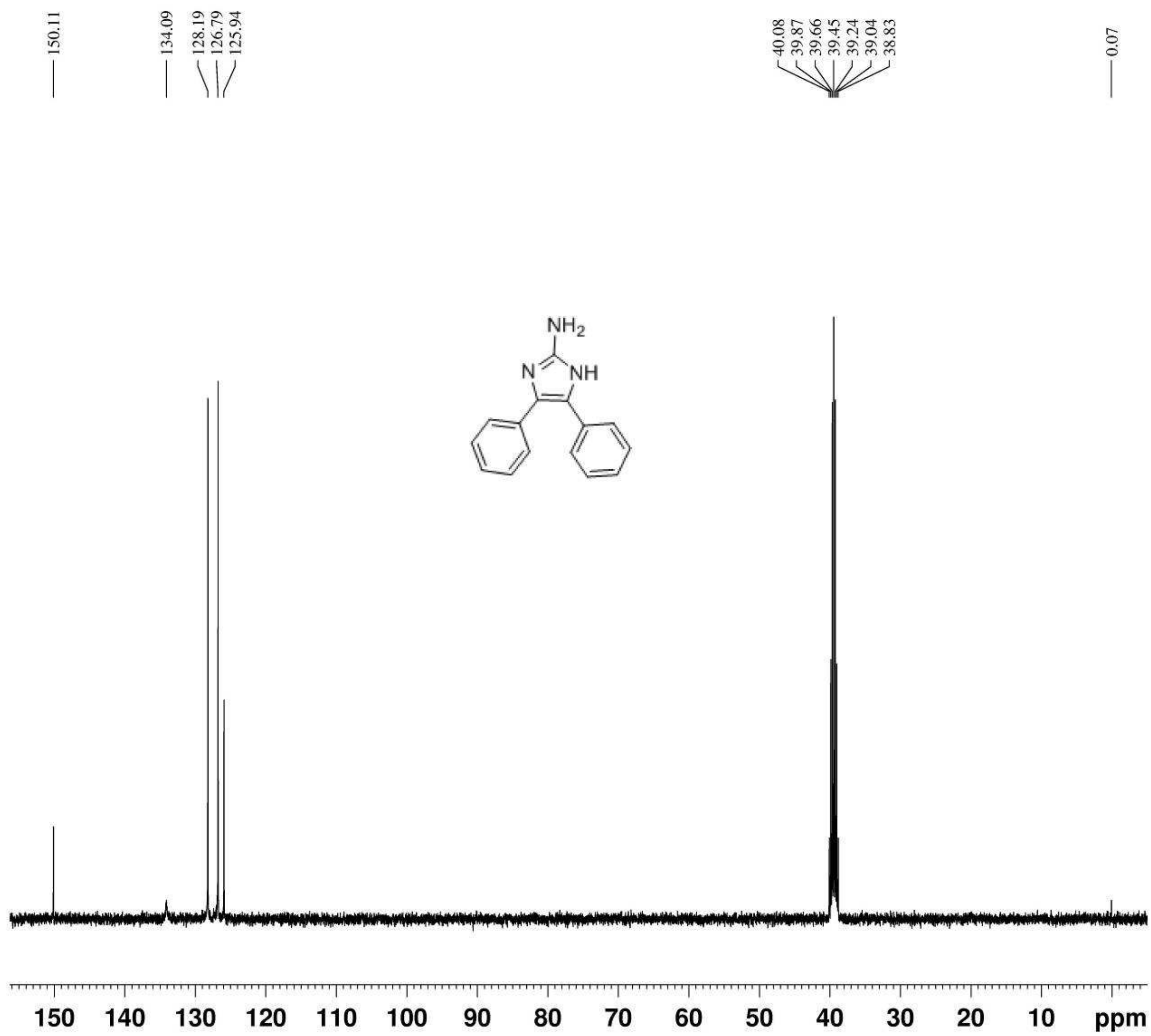
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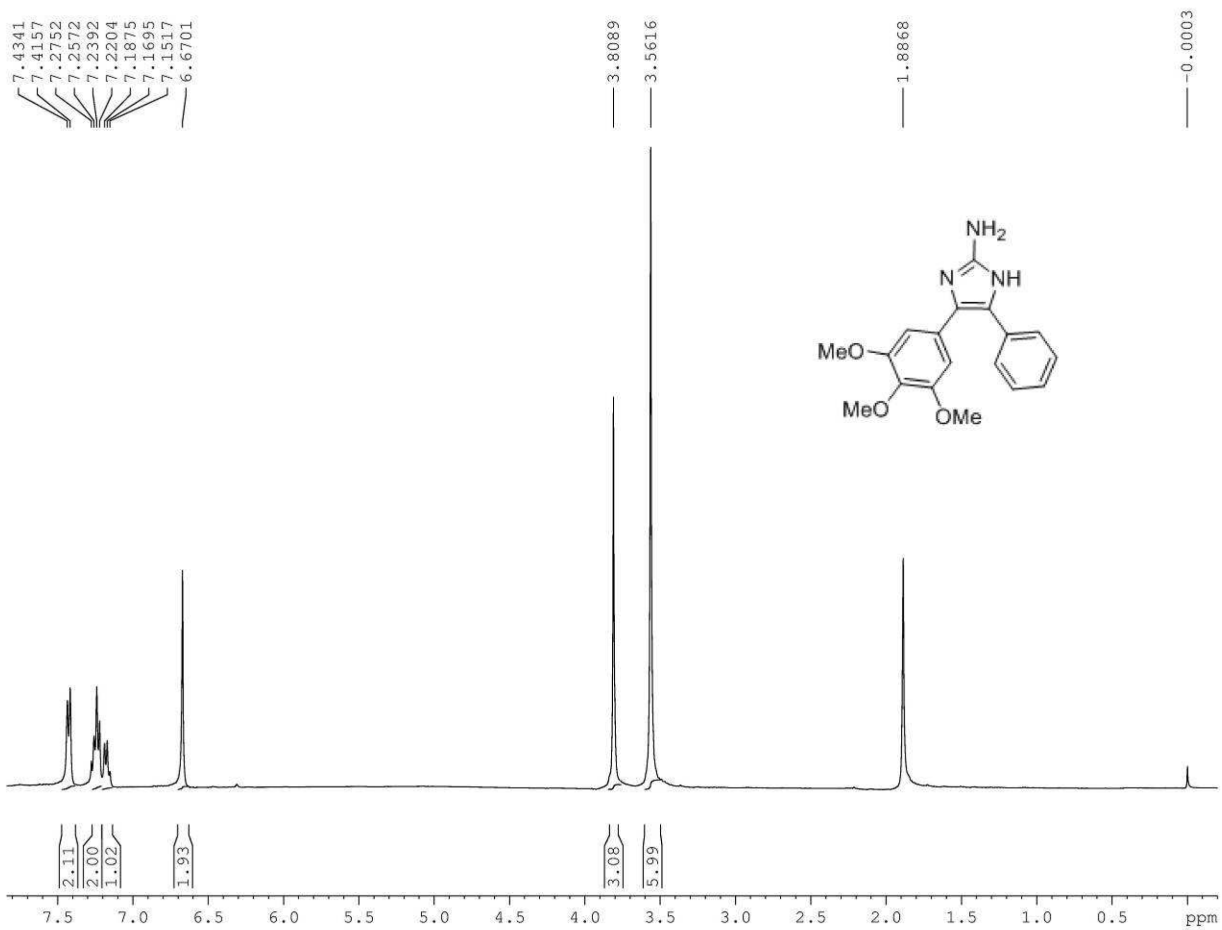
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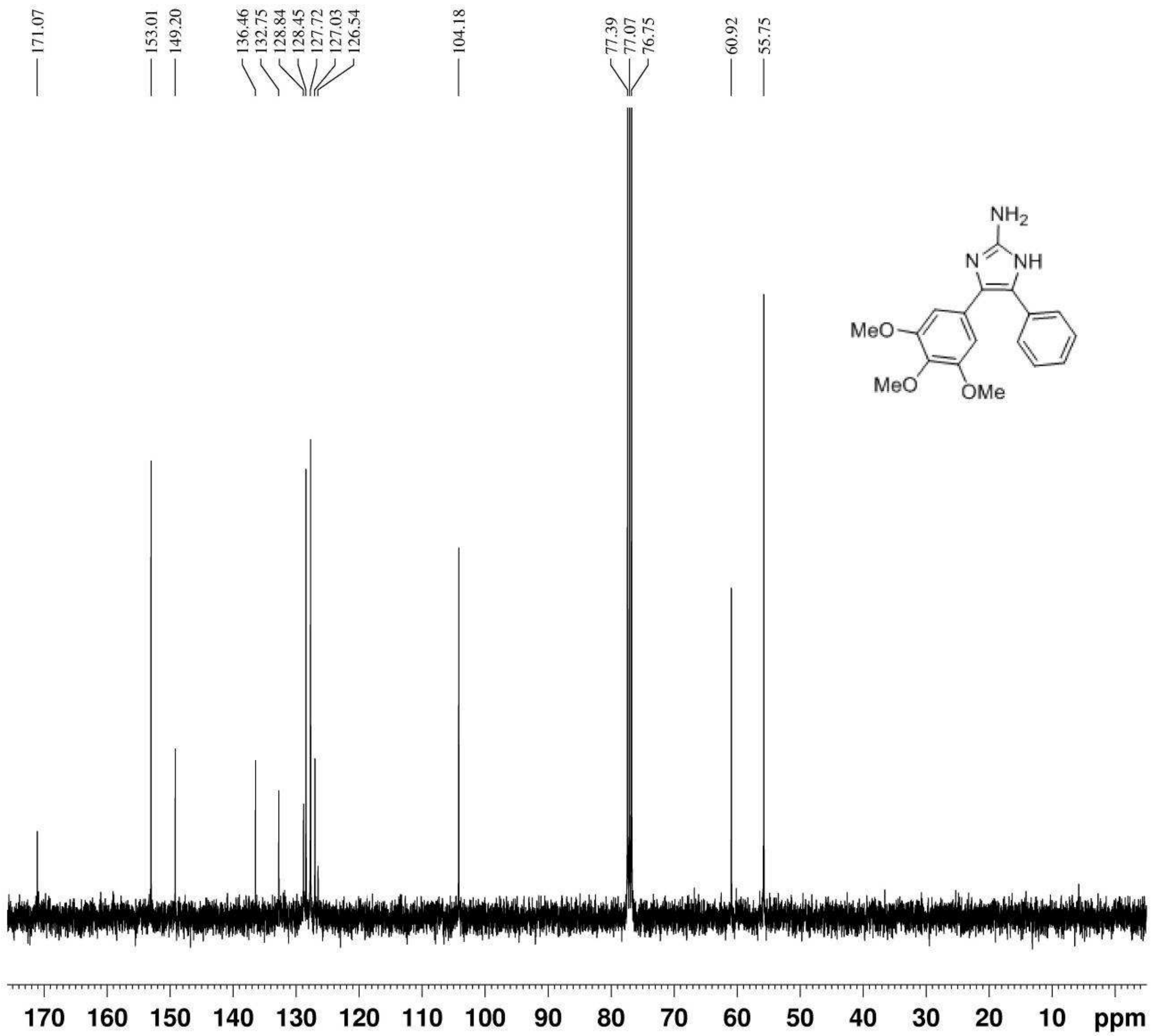
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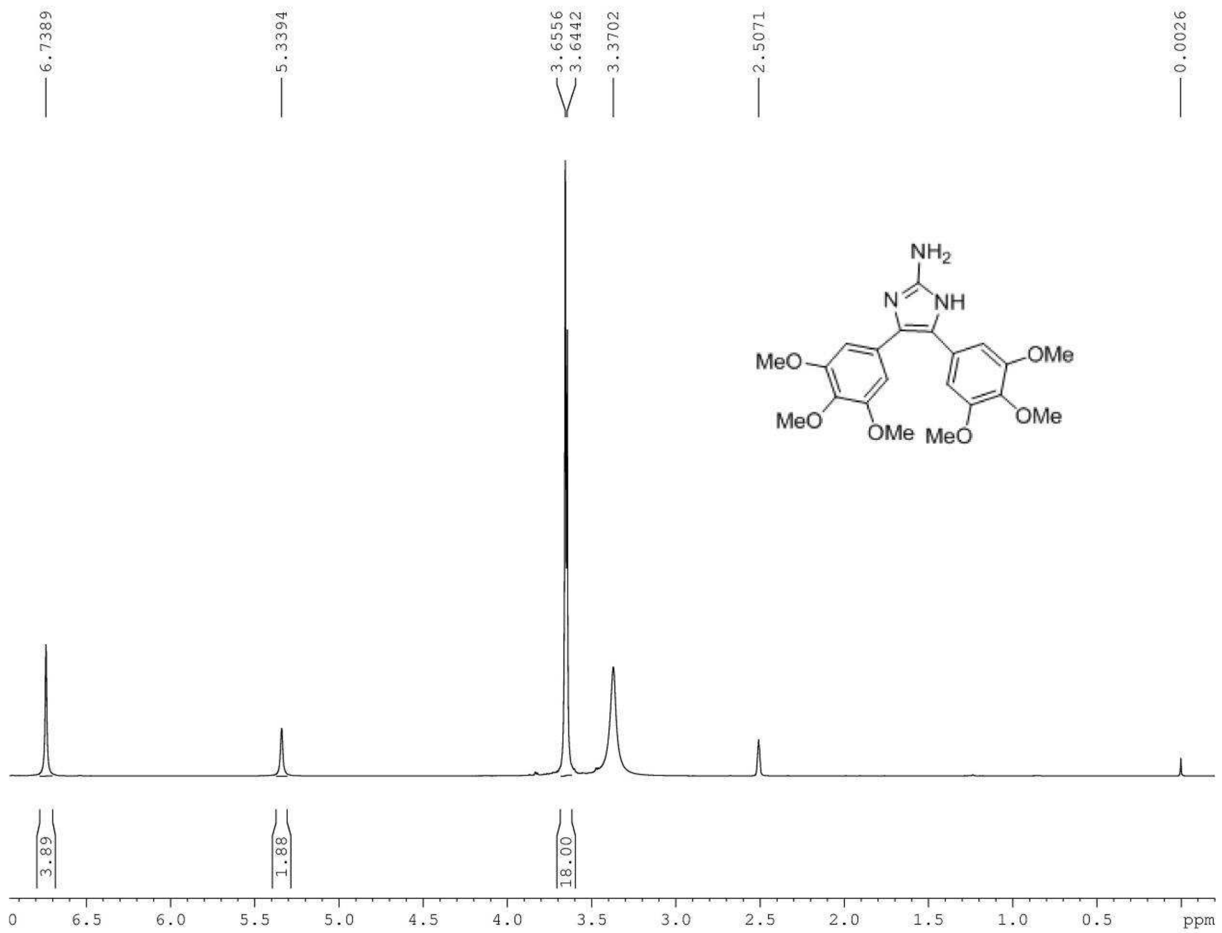
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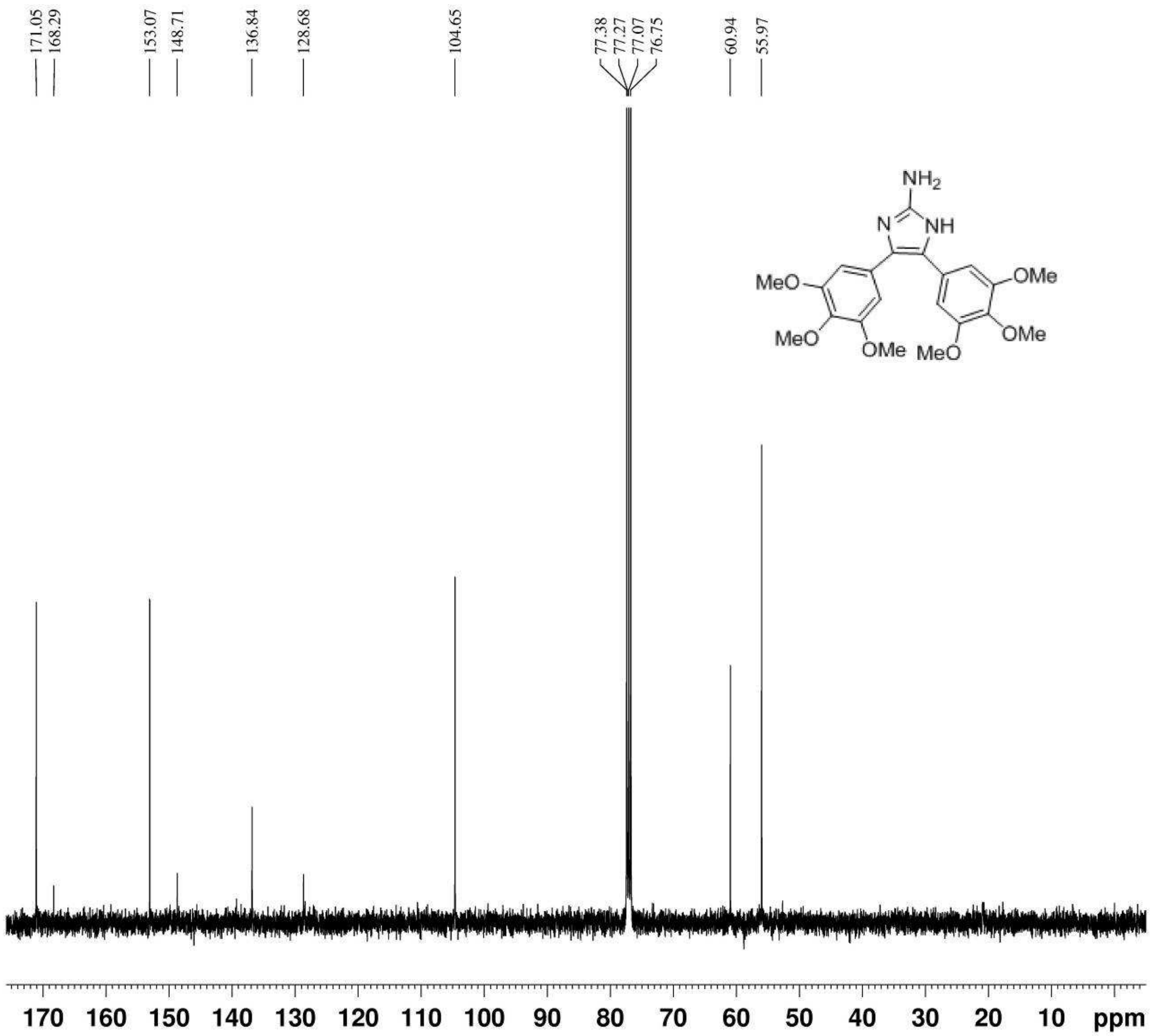

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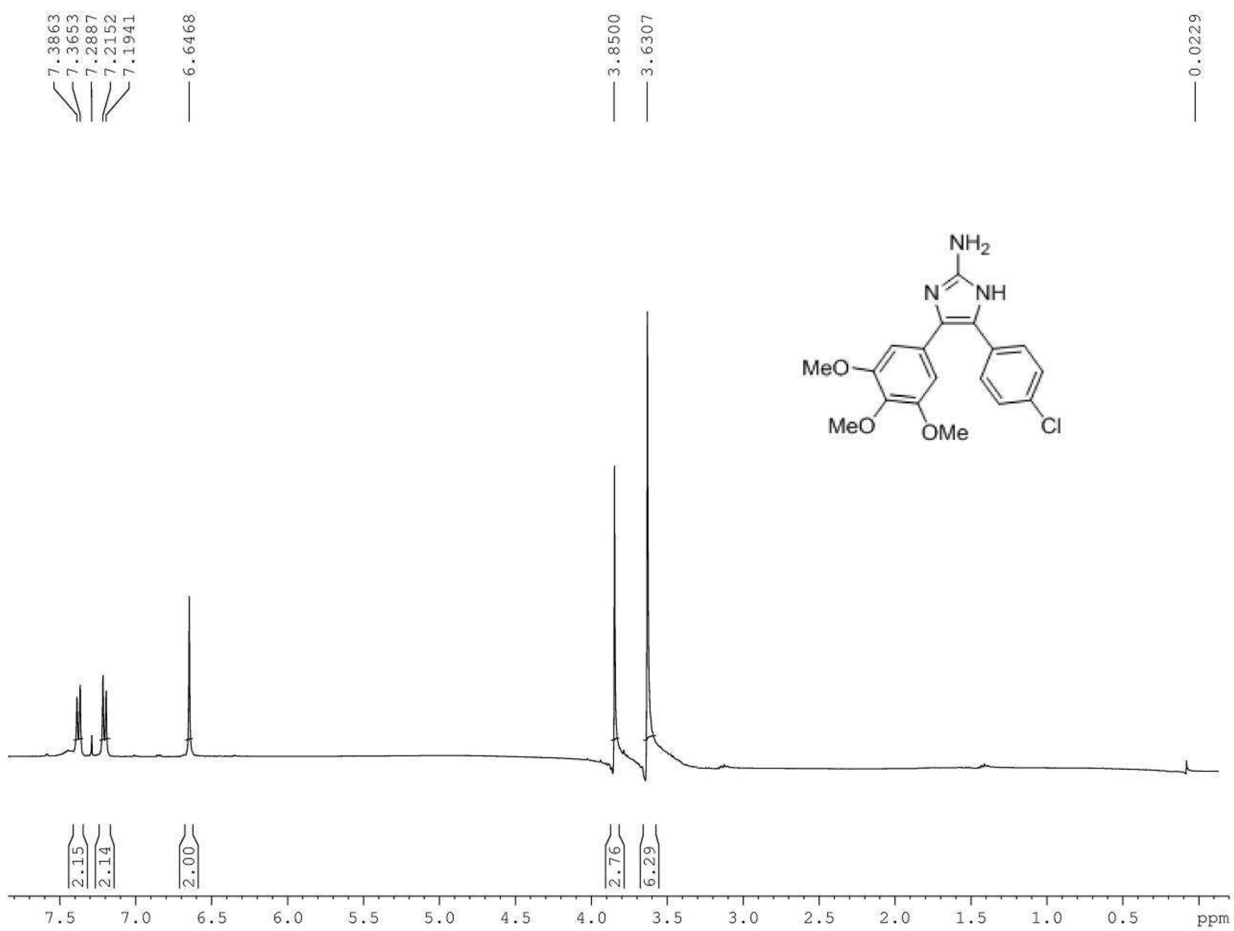
4: ^{1}H NMR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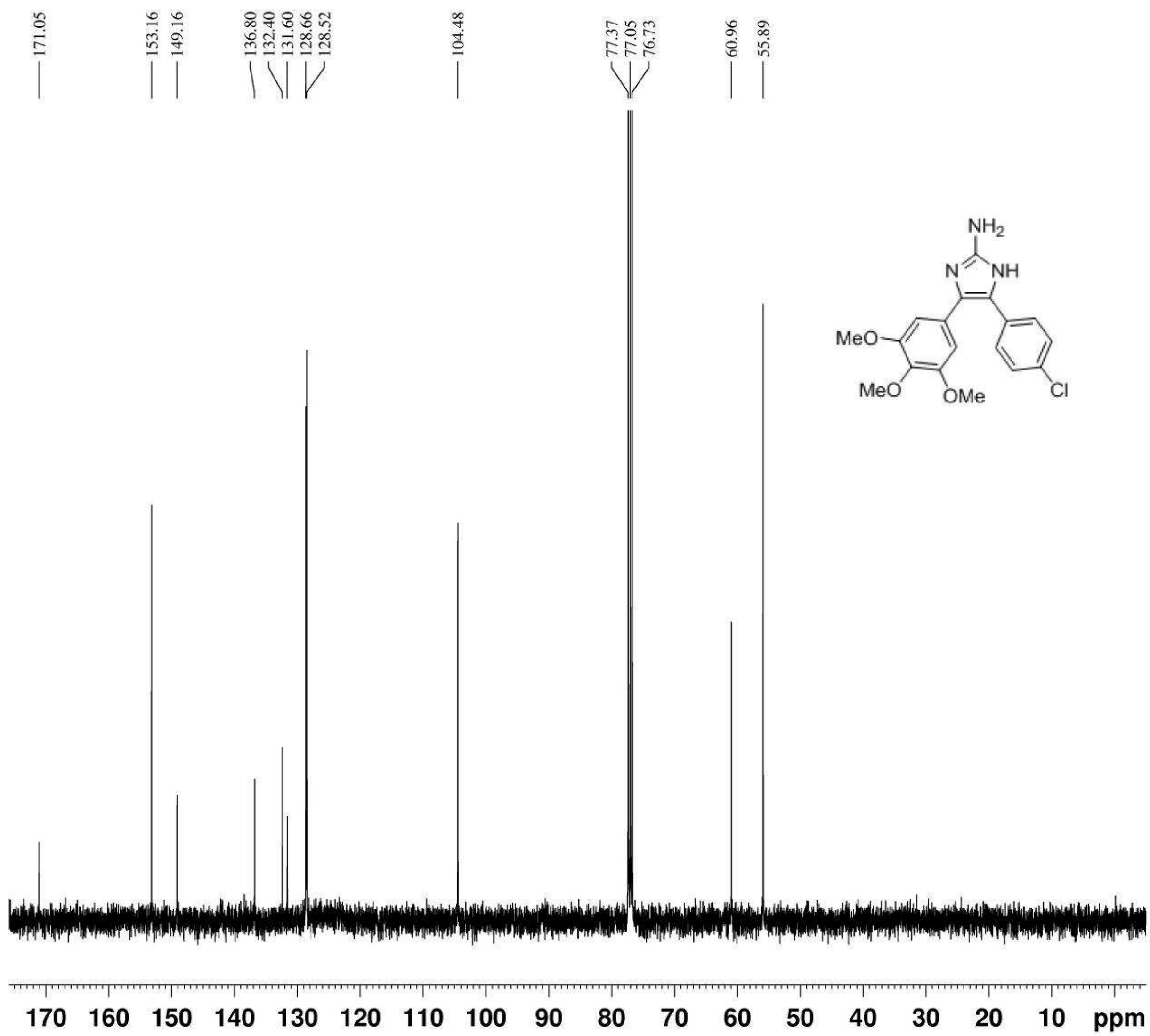

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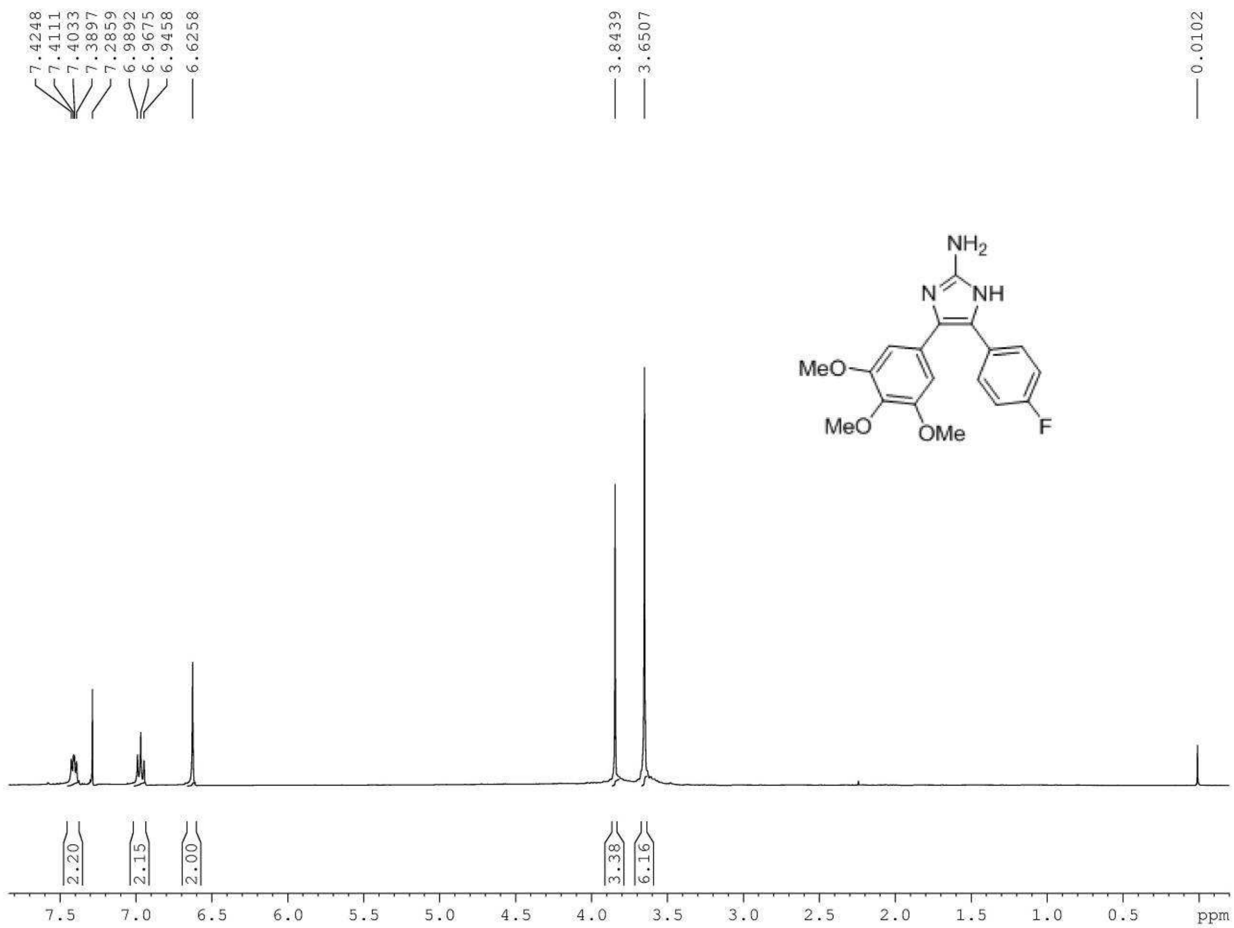
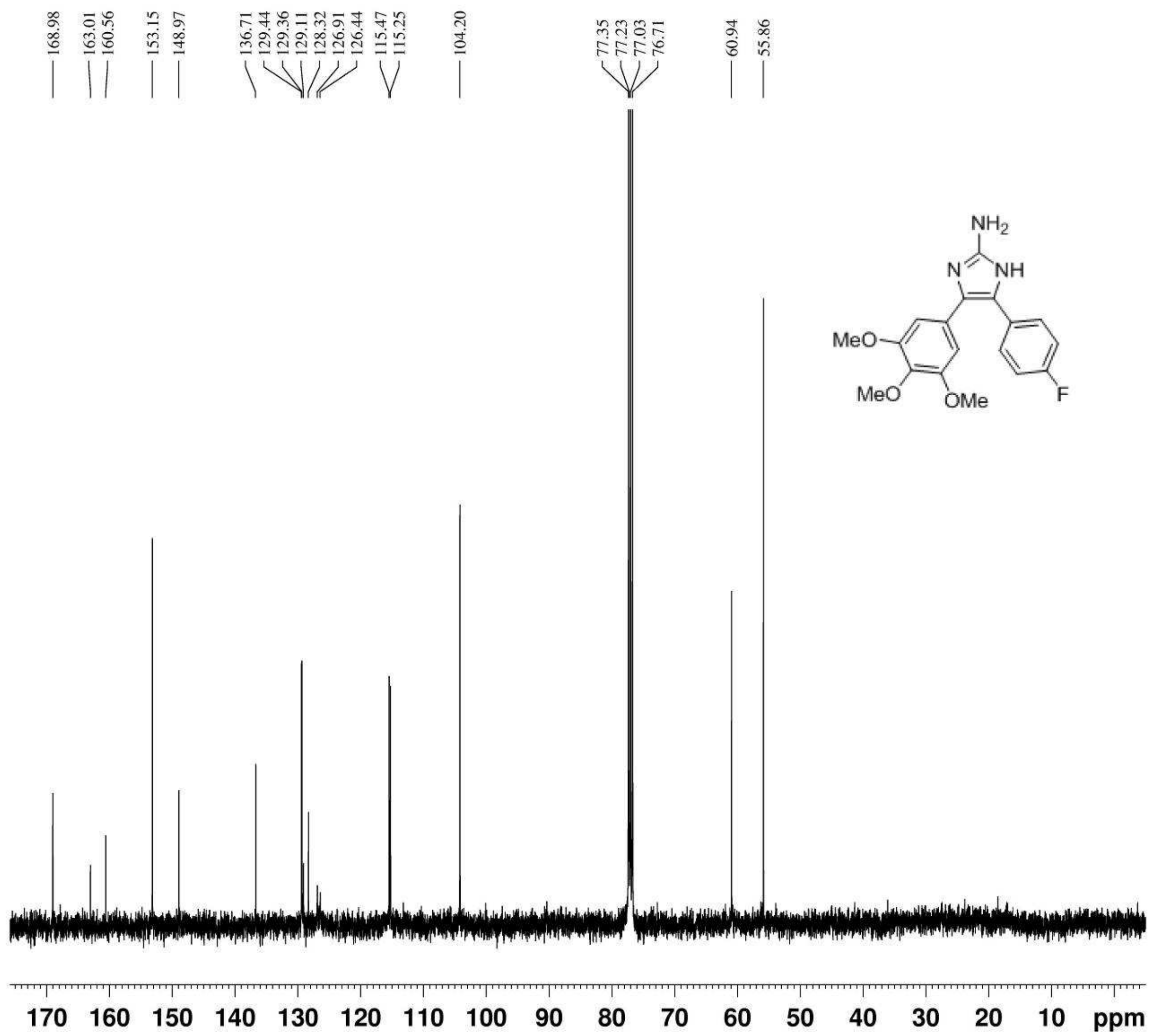
128.66
128.52
104.48
77.37
77.05
76.73
60.96
55.89
NH2
N
NH
MeO
MeO
OMe
Cl
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

5: ¹H NMR



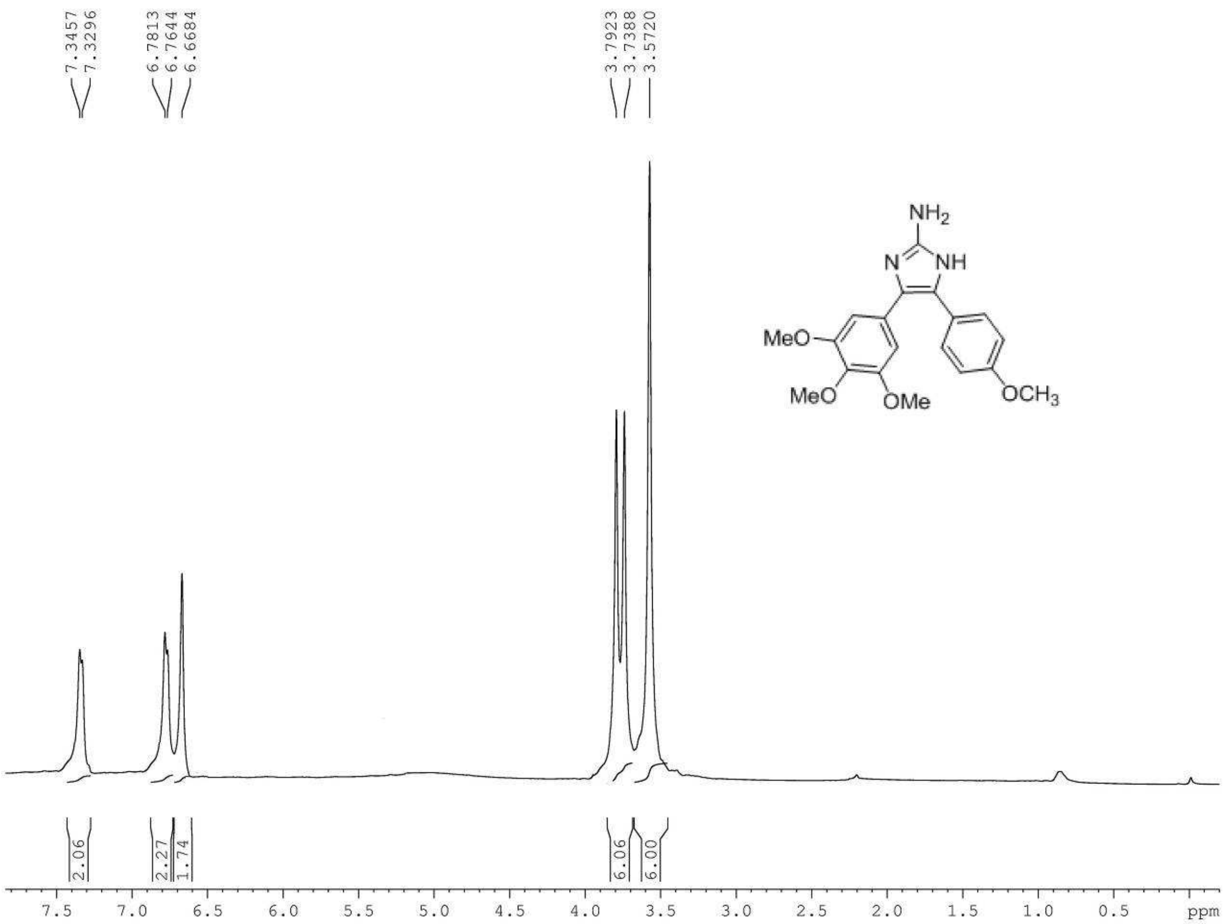
7.4248
7.4111
7.4033
7.3897
7.2859
6.9892
6.9675
6.9458
6.6258
3.8439
3.6507
0.0102
NH2
N
NH
MeO
MeO
OMe
F
2.20
2.15
2.00
3.38
6.16
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm

5: ^{13}C NMR



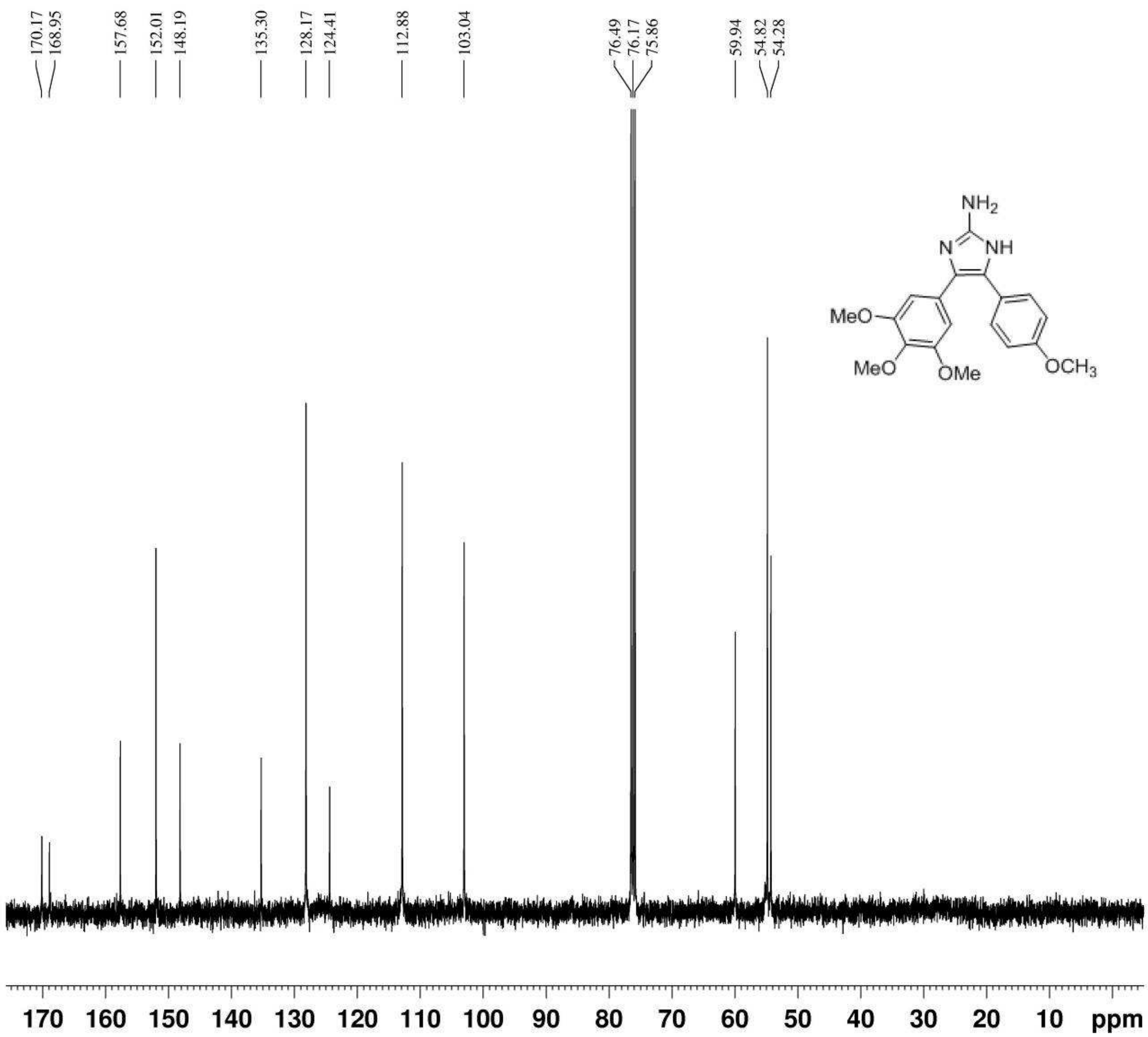
168.98
163.01
160.56
153.15
148.97
136.71
129.44
129.36
129.11
128.32
126.91
126.44
115.47
115.25
104.20
77.35
77.23
77.03
76.71
60.94
55.86
NH_2
N
NH
MeO
MeO
OMe
F
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

6: ^{1}H NMR

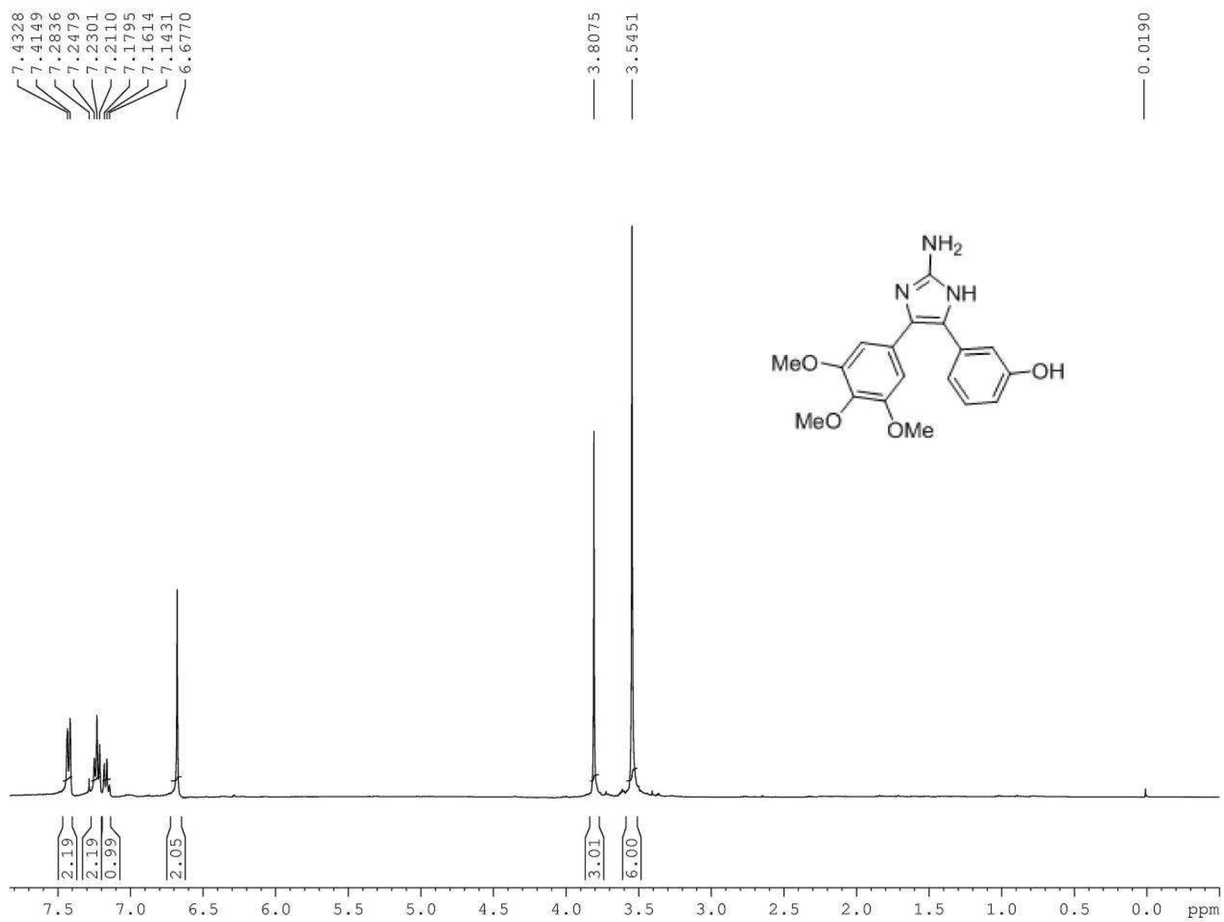


7.3457
7.3296
6.7813
6.7644
6.6684
3.7923
3.7388
3.5720
NH2
N
NH
MeO
MeO
OMe
OCH3
2.06
2.27
1.74
6.06
6.00
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm

6: ^{13}C NMR

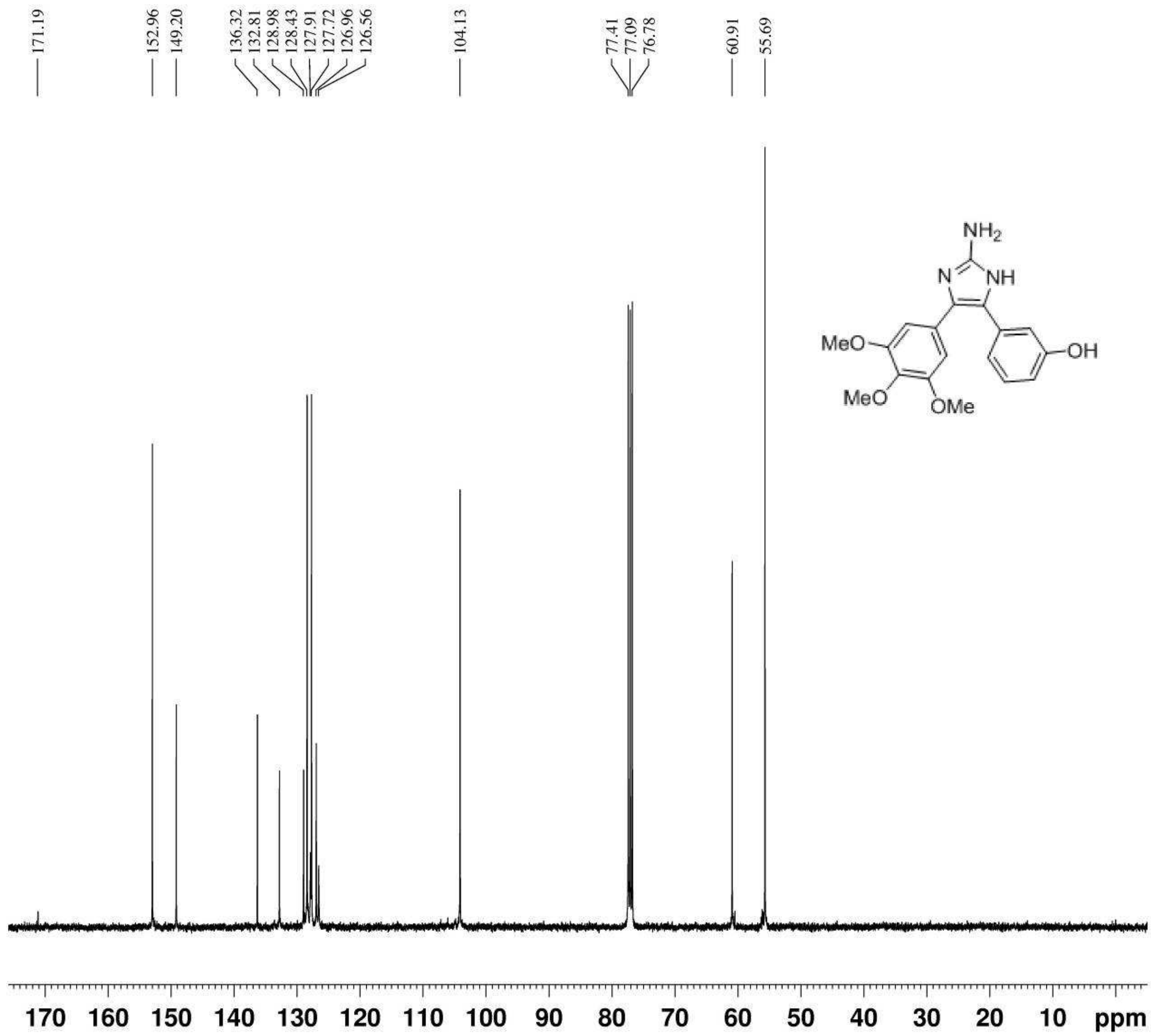

170.17
168.95
157.68
152.01
148.19
135.30
128.17
124.41
112.88
103.04
76.49
76.17
75.86
59.94
54.82
54.28
NH_2
N
NH
MeO
MeO
OMe
OCH_3
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

7: ^{1}H NMR

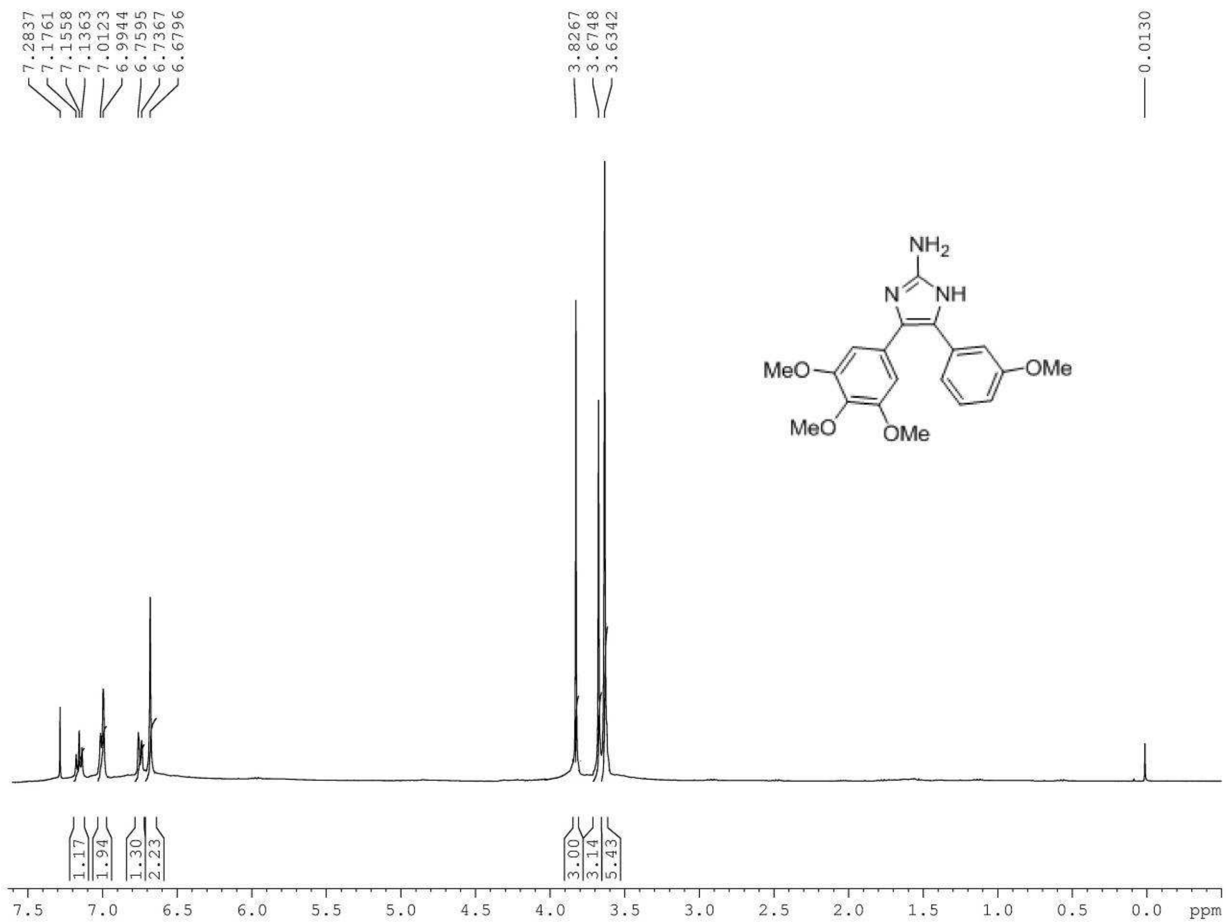


7.4328
7.4149
7.2836
7.2479
7.2301
7.2110
7.1795
7.1614
7.1431
6.6770
3.8075
3.5451
0.0190
NH2
N
NH
MeO
OH
MeO
OMe
2.19
2.19
0.99
2.05
3.01
6.00
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm

7: ^{13}C NMR

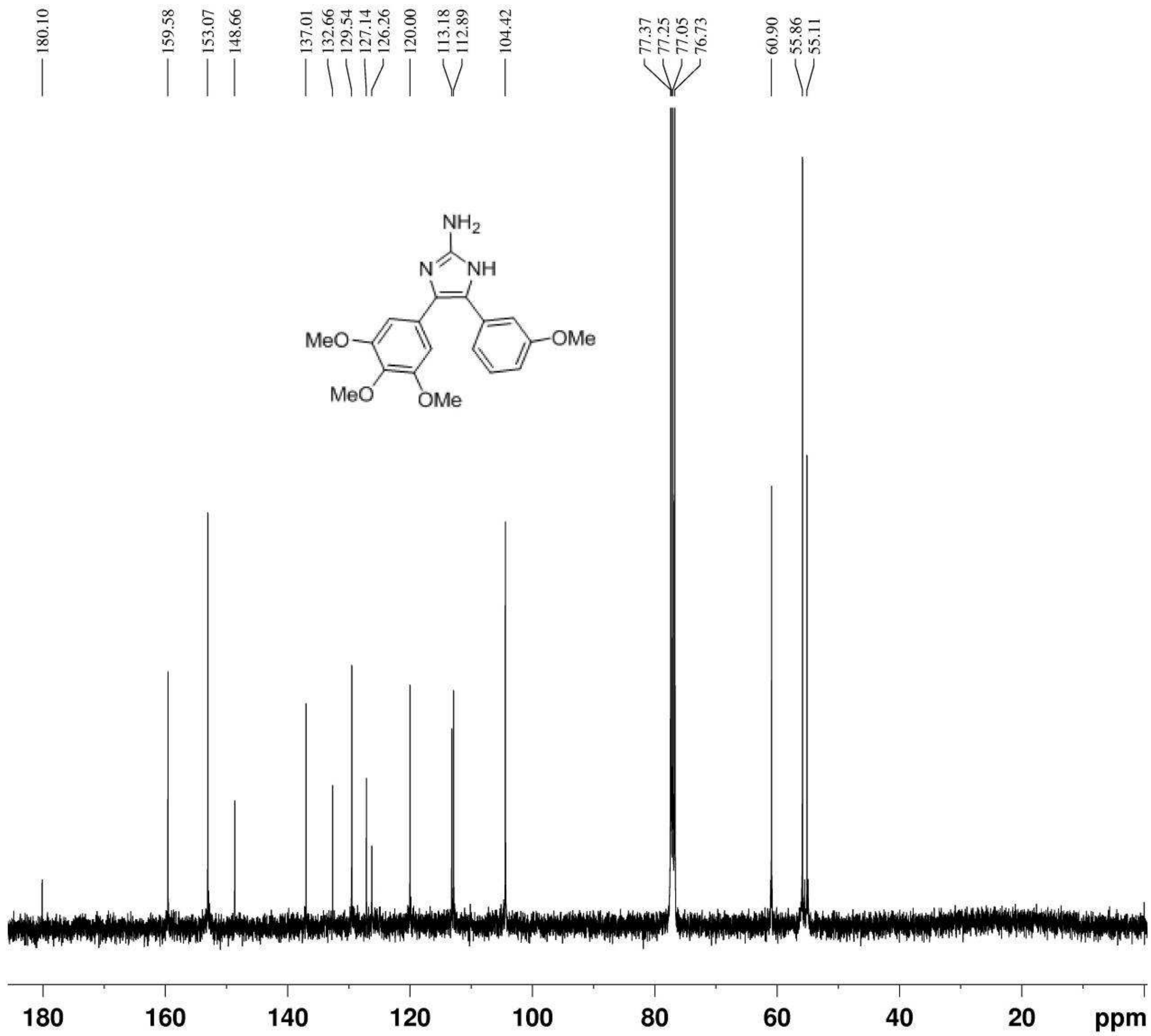

171.19
152.96
149.20
136.32
132.81
128.98
128.43
127.91
127.72
126.96
126.56
104.13
77.41
77.09
76.78
60.91
55.69
NH_2
N
NH
MeO
MeO
OMe
OH
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

8: ^{1}H NMR

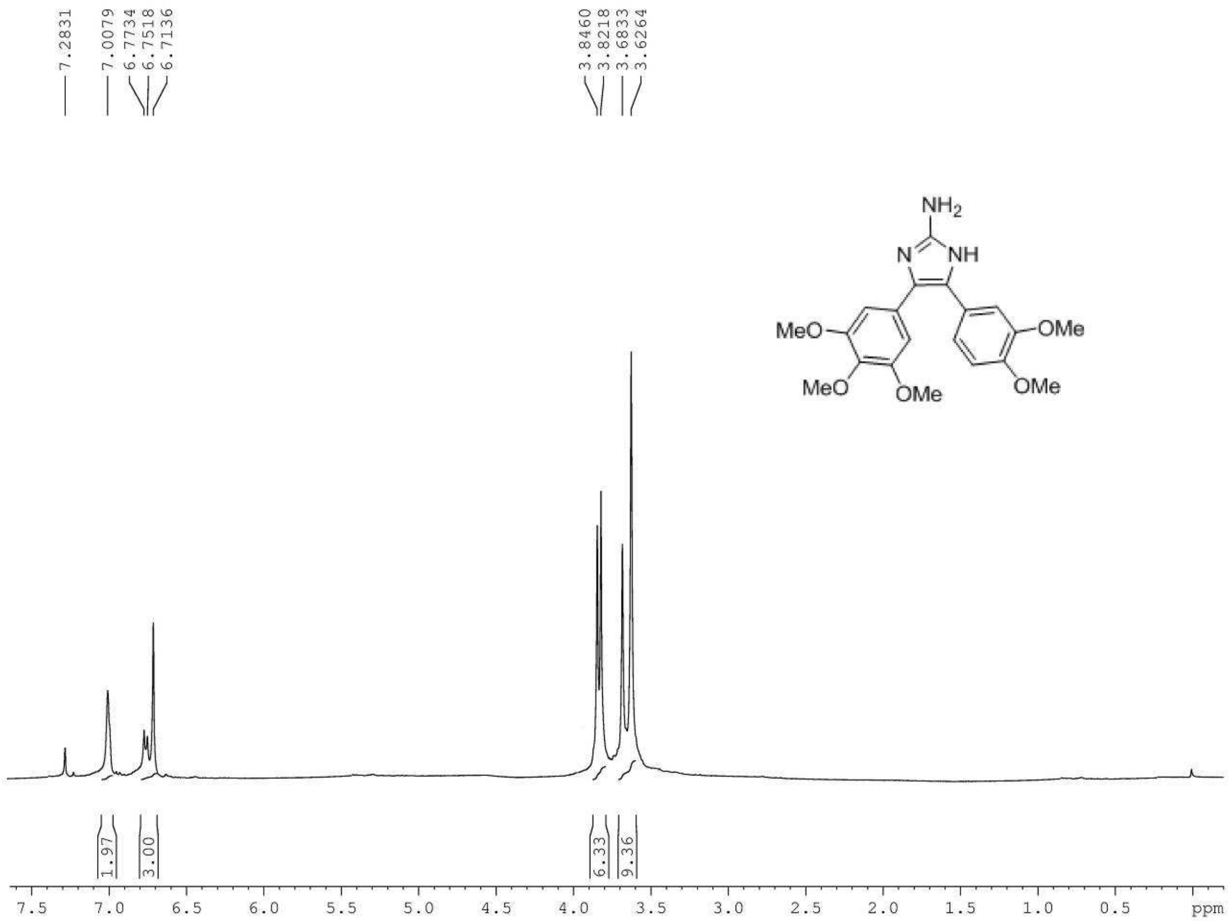


7.2837
7.1761
7.1558
7.1363
7.0123
6.9944
6.7595
6.7367
6.6796
3.8267
3.6748
3.6342
0.0130
NH2
N
NH
MeO
OMe
MeO
OMe
1.17
1.94
1.30
2.23
3.00
3.14
5.43
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm

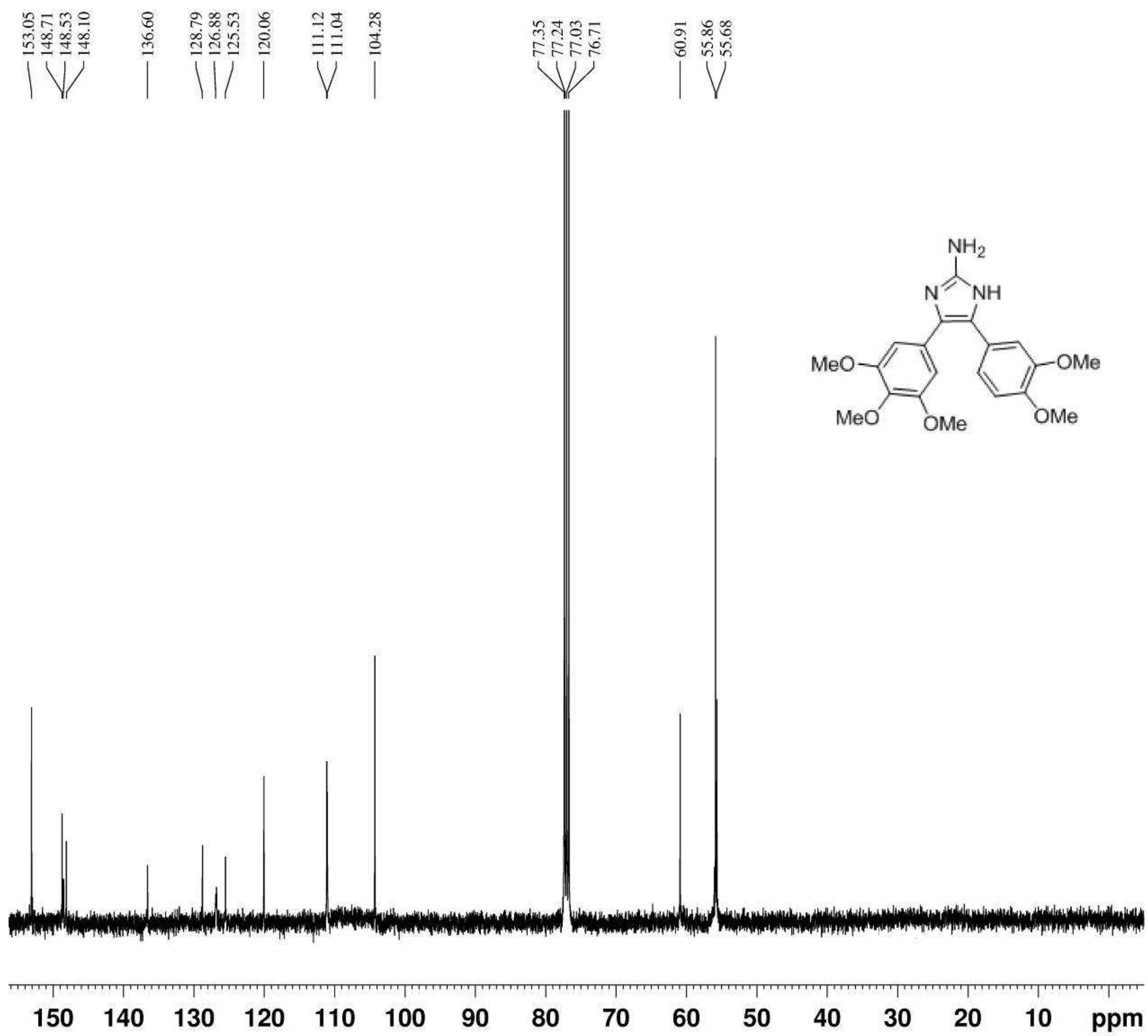
8: ^{13}C NMR


180.10
159.58
153.07
148.66
137.01
132.66
129.54
127.14
126.26
120.00
113.18
112.89
104.42
77.37
77.25
77.05
76.73
60.90
55.86
55.11
NH2
N
NH
MeO
MeO
OMe
OMe
180
160
140
120
100
80
60
40
20
ppm

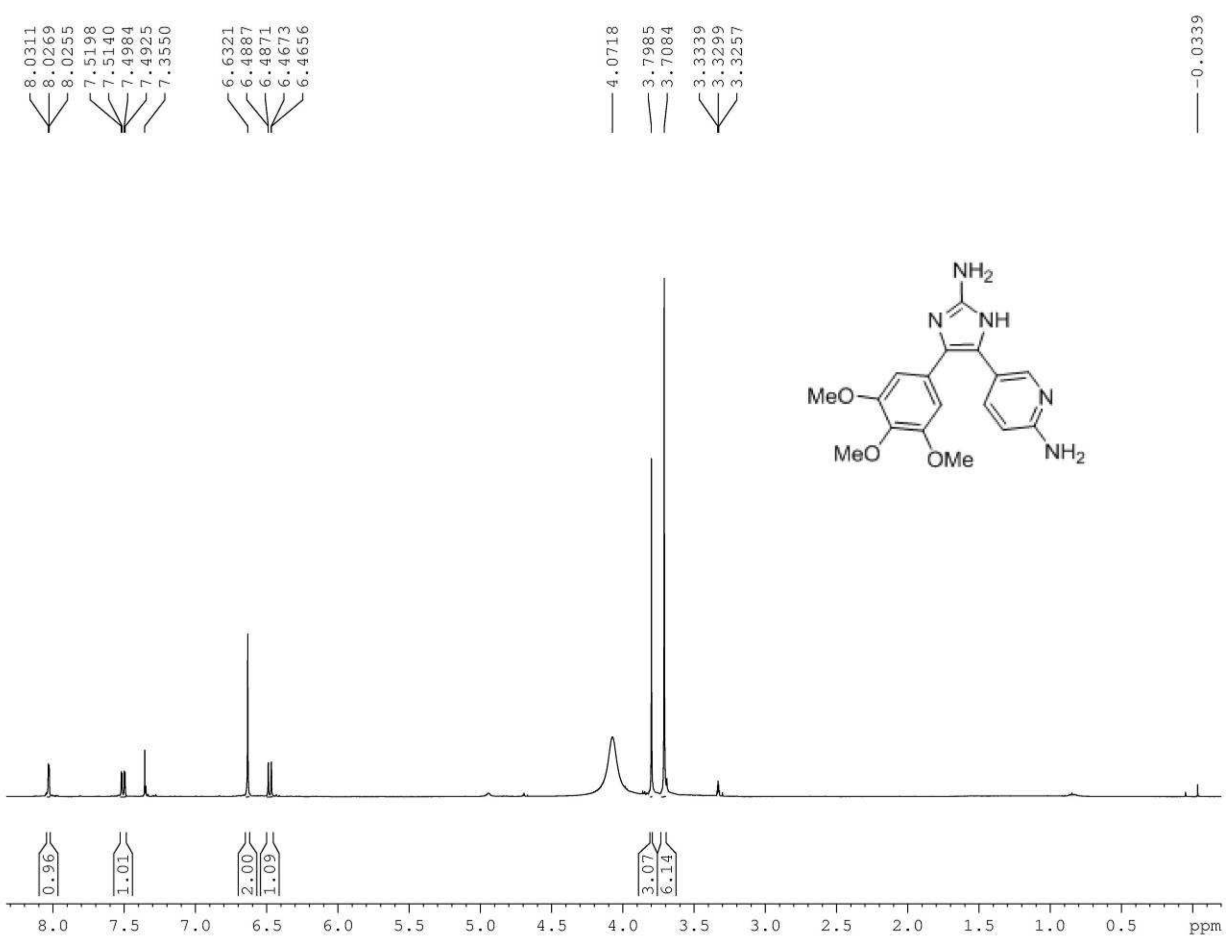
9: ^{1}H NMR


7.2831
7.0079
6.7734
6.7518
6.7136
3.8460
3.8218
3.6833
3.6264
NH2
N
NH
MeO
MeO
OMe
OMe
OMe
1.97
3.00
6.33
9.36
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm

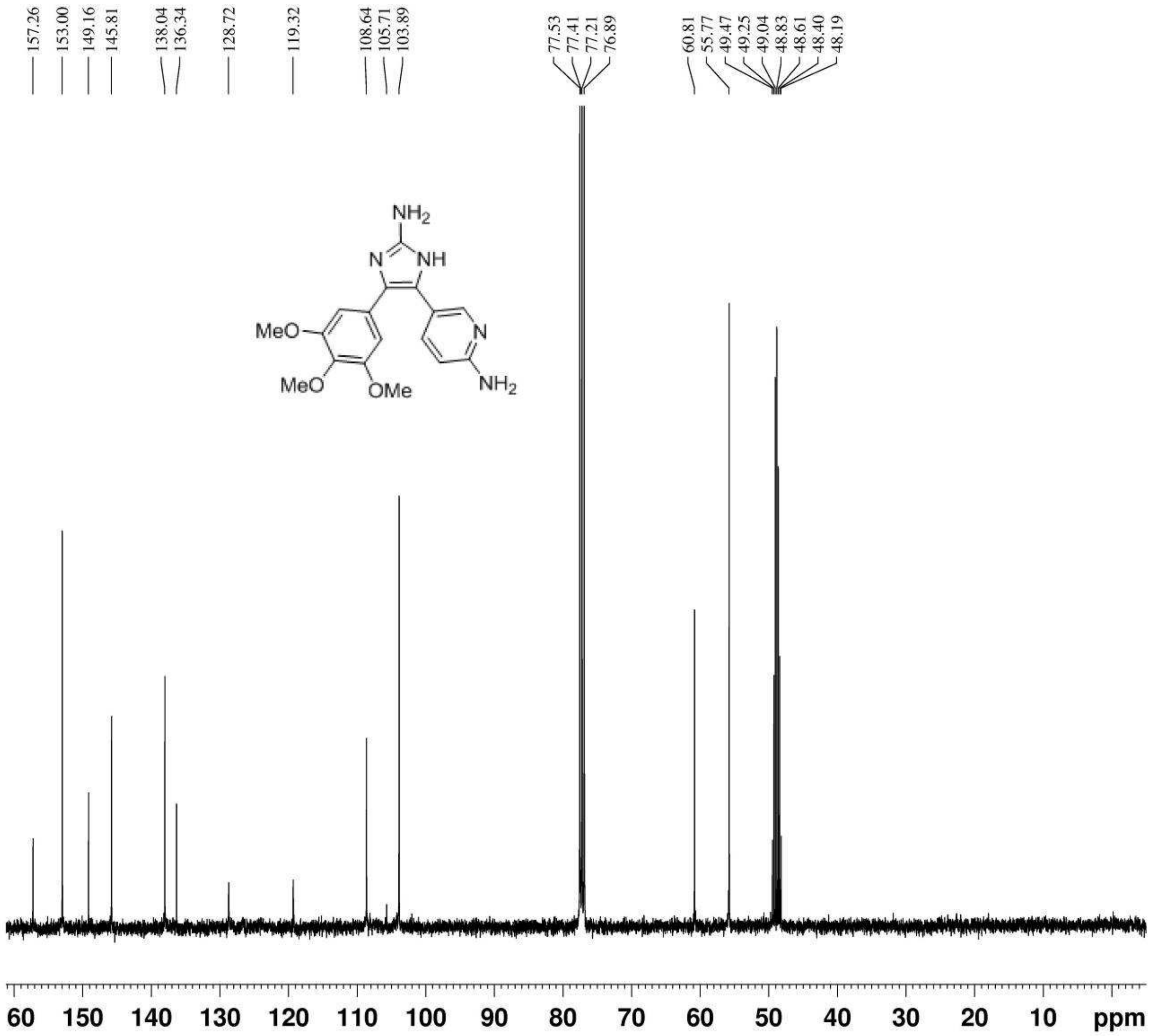
9: ^{13}C NMR


153.05
148.71
148.53
148.10
136.60
128.79
126.88
125.53
120.06
111.12
111.04
104.28
77.35
77.24
77.03
76.71
60.91
55.86
55.68
NH2
N
NH
MeO
OMe
MeO
OMe
OMe
150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

10: ^{1}H NMR

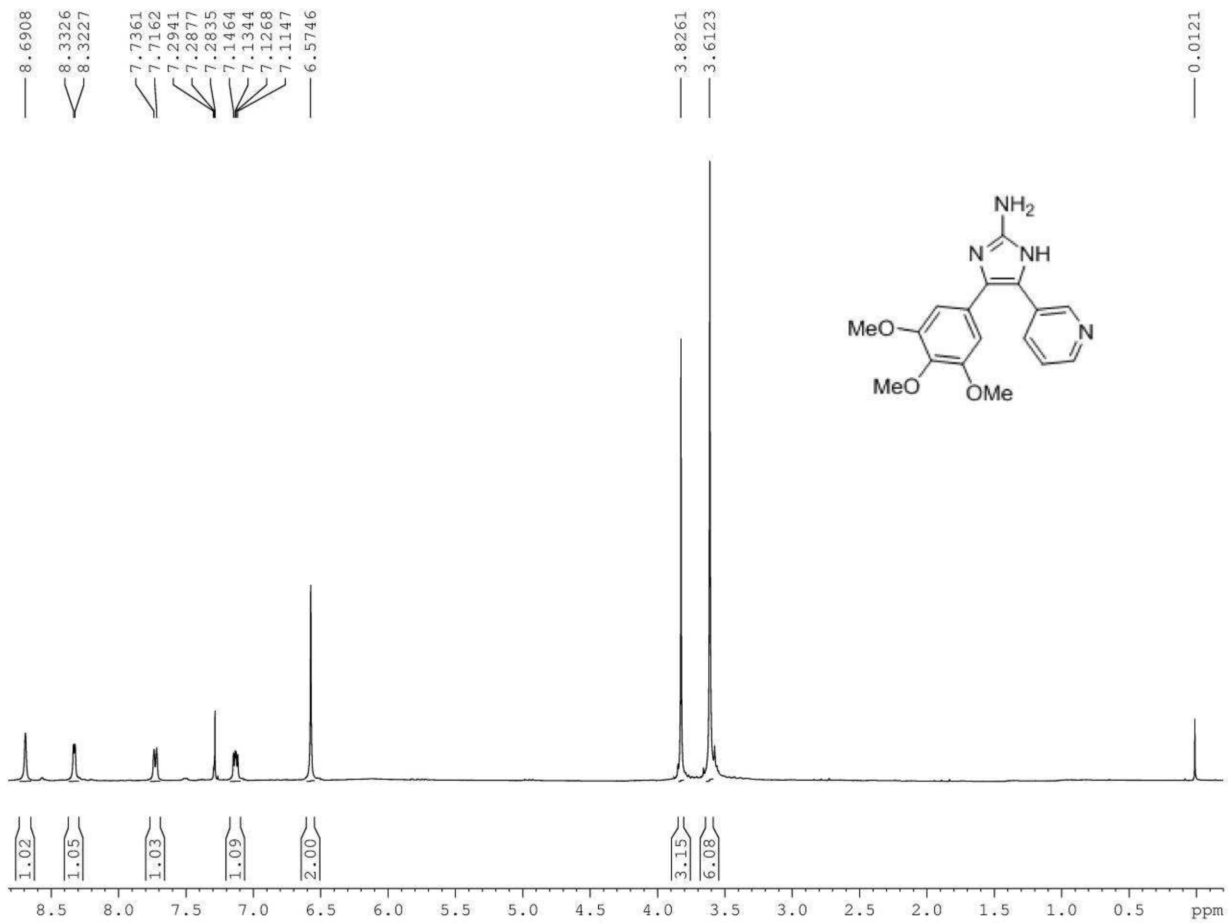

8.0311
8.0269
8.0255
7.5198
7.5140
7.4984
7.4925
7.3550
6.6321
6.4887
6.4871
6.4673
6.4656
4.0718
3.7985
3.7084
3.3339
3.3299
3.3257
-0.0339
NH2
N
NH
MeO
MeO
OMe
N
NH2
0.96
1.01
2.00
1.09
3.07
6.14
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm

10: ^{13}C NMR

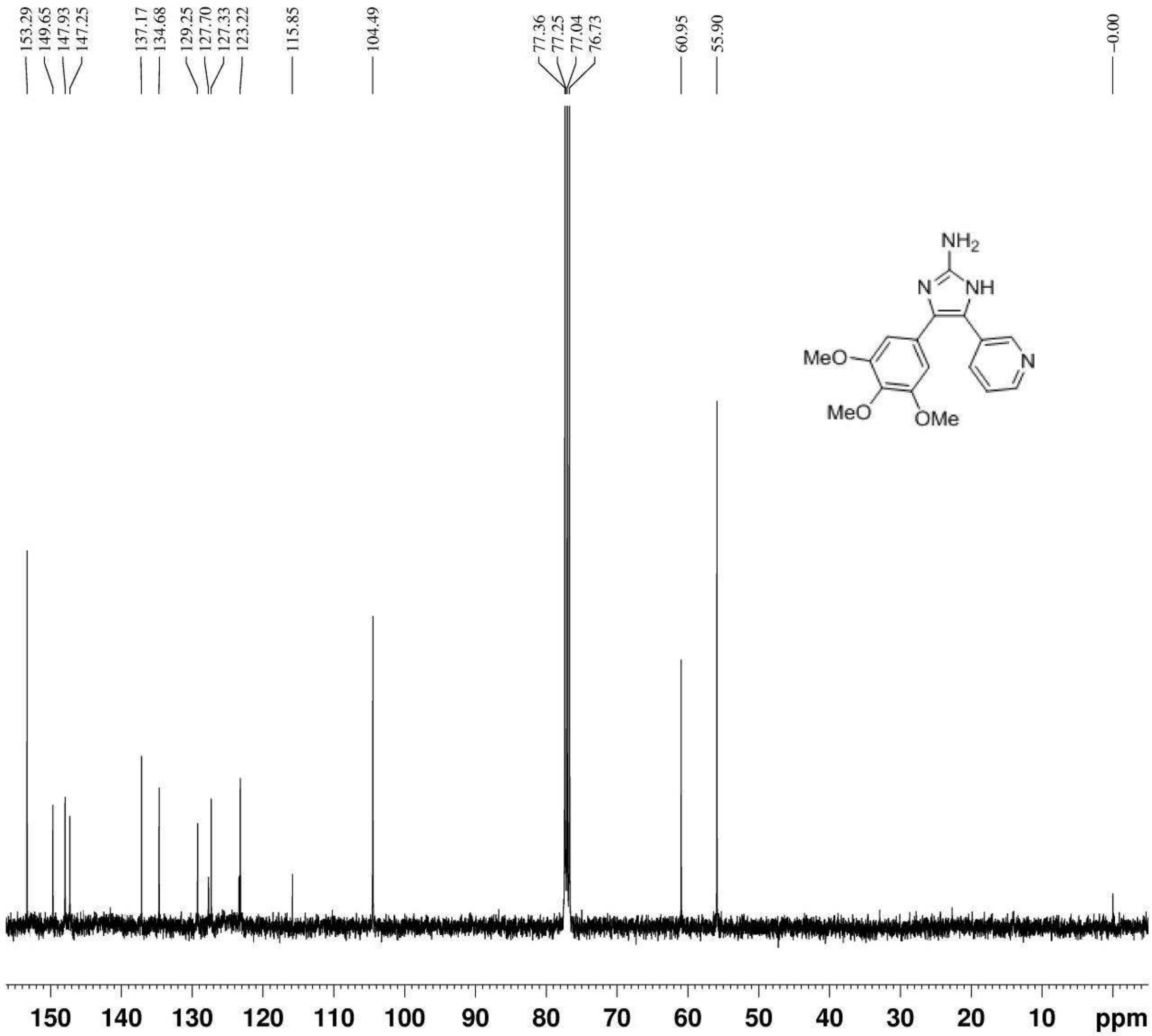


157.26
153.00
149.16
145.81
138.04
136.34
128.72
119.32
108.64
105.71
103.89
77.53
77.41
77.21
76.89
60.81
55.77
49.47
49.25
49.04
48.83
48.61
48.40
48.19
NH2
N
NH
MeO
MeO
OMe
N
NH2
160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

11: ^{1}H NMR

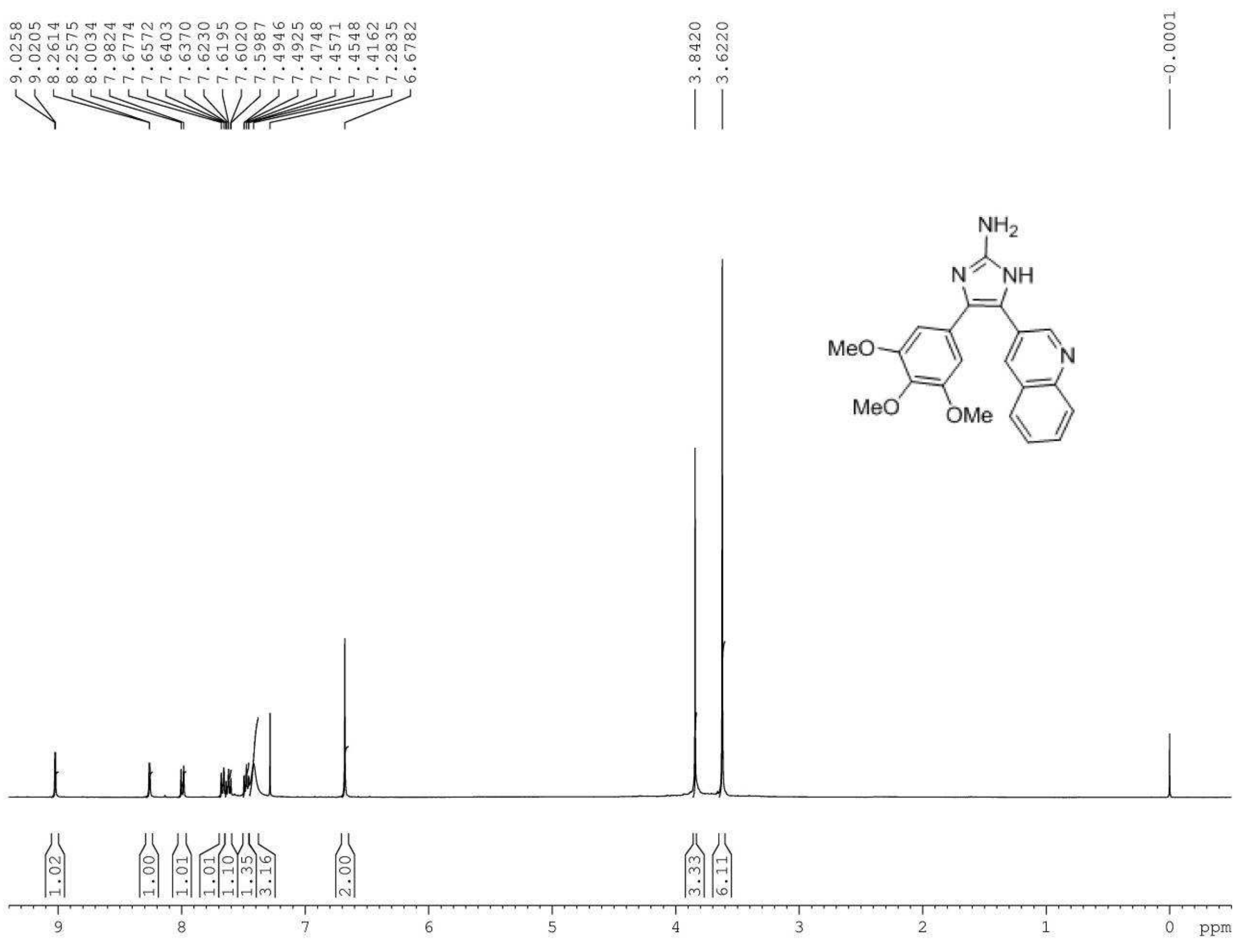

8.6908
8.3326
8.3227
7.7361
7.7162
7.2941
7.2877
7.2835
7.1464
7.1344
7.1268
7.1147
6.5746
3.8261
3.6123
0.0121
NH2
N
NH
N
MeO
MeO
OMe
1.02
1.05
1.03
1.09
2.00
3.15
6.08
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm

11: ^{13}C NMR

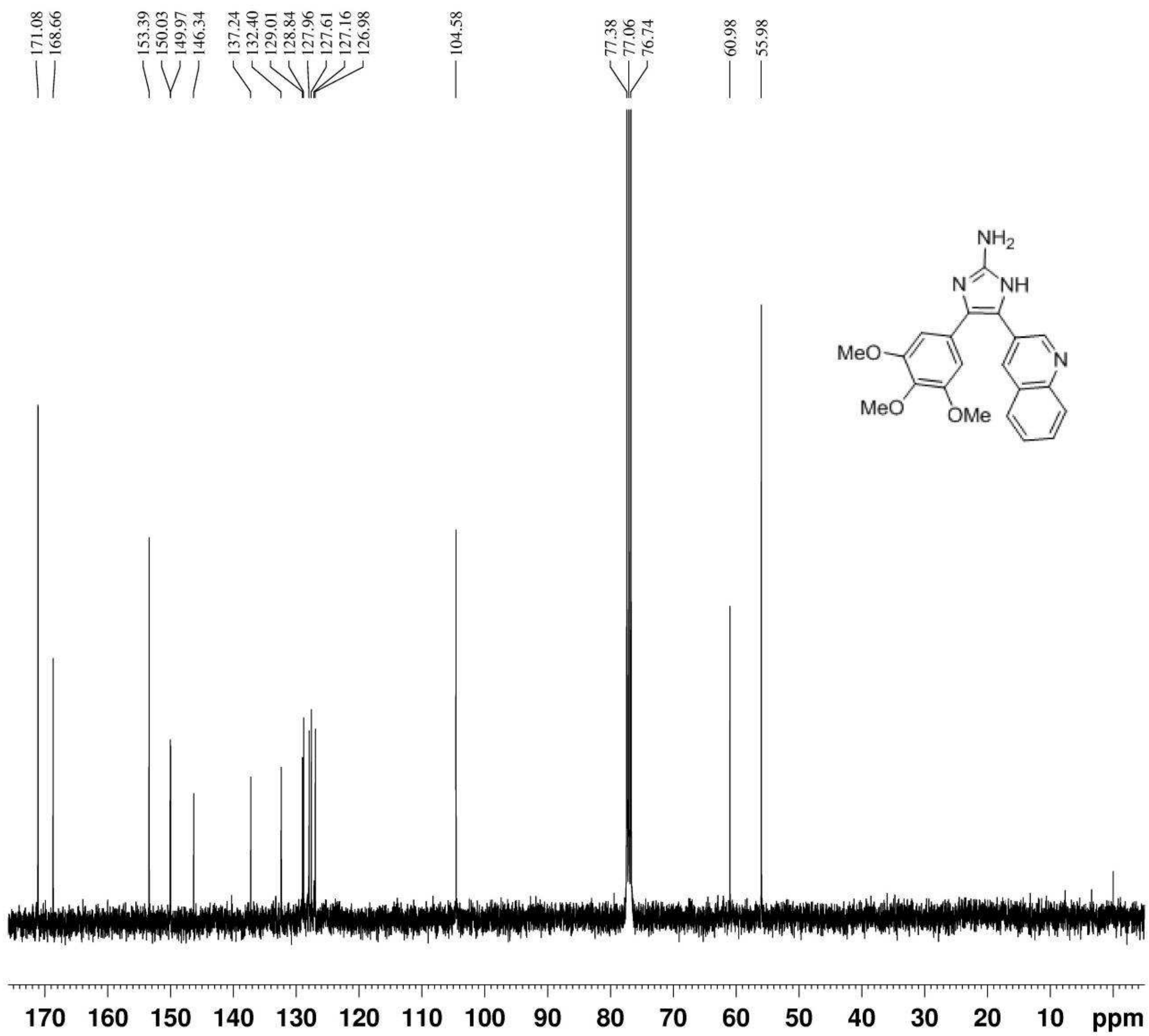


153.29
149.65
147.93
147.25
137.17
134.68
129.25
127.70
127.33
123.22
115.85
104.49
77.36
77.25
77.04
76.73
60.95
55.90
-0.00
NH2
N
NH
N
MeO
MeO
OMe
150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

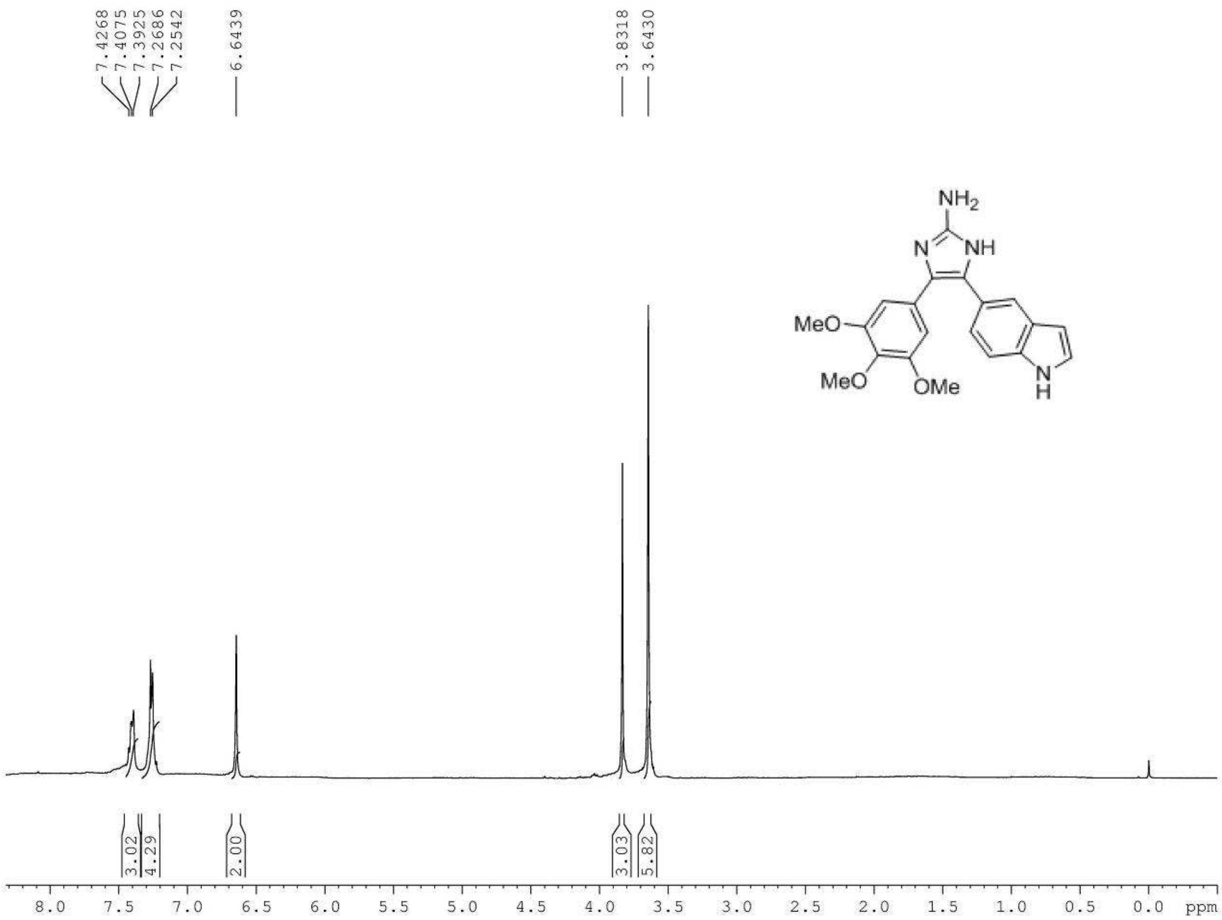
12: ^{1}H NMR


9.0258
9.0205
8.2614
8.2575
8.0034
7.9824
7.6774
7.6572
7.6403
7.6370
7.6230
7.6195
7.6020
7.5987
7.4946
7.4925
7.4748
7.4571
7.4548
7.4162
7.2835
6.6782
3.8420
3.6220
-0.0001
NH2
N
NH
MeO
MeO
OMe
N
1.02
1.00
1.01
1.01
1.10
1.35
3.16
2.00
3.33
6.11
9
8
7
6
5
4
3
2
1
0
ppm

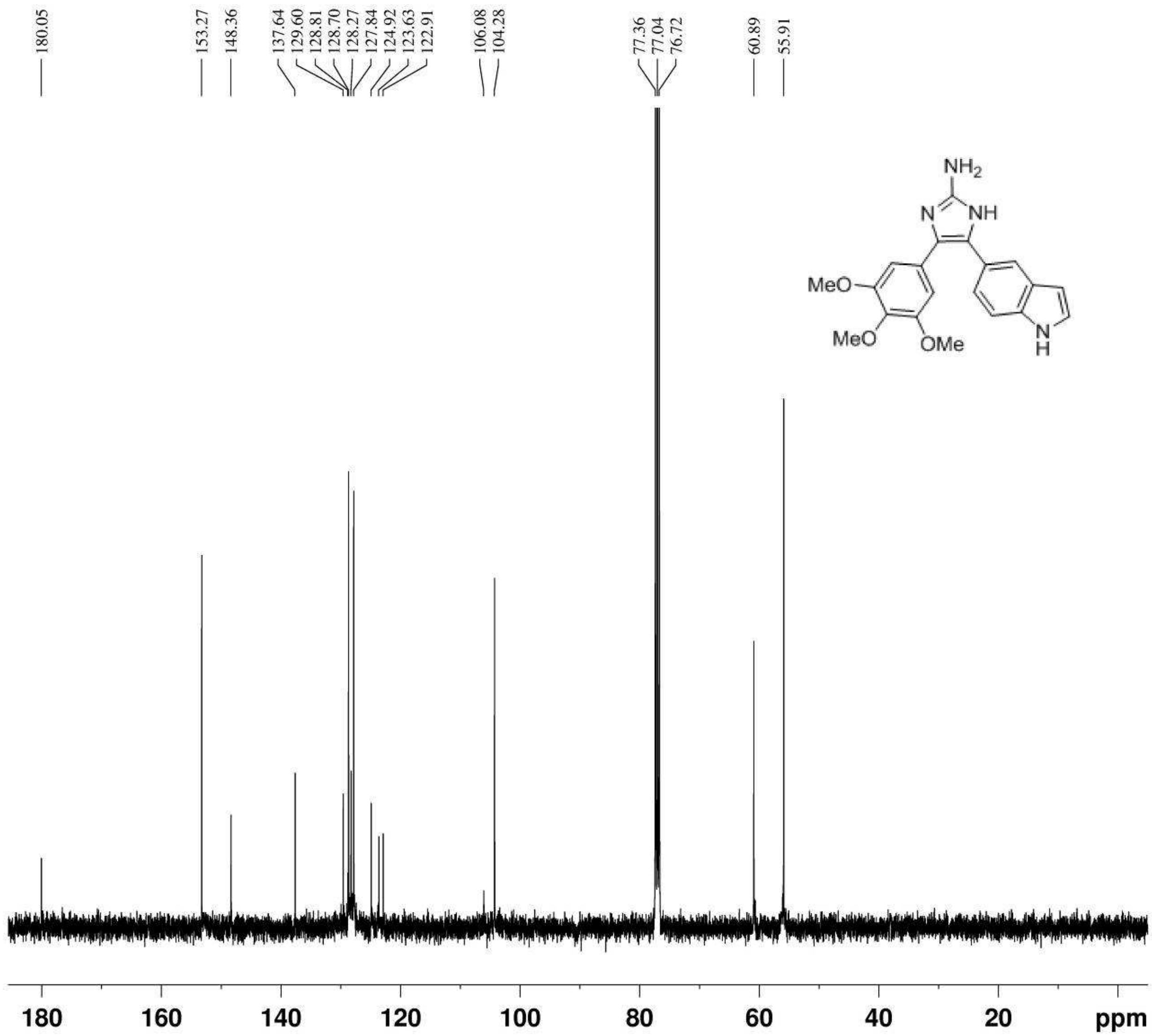
12: ^{13}C NMR


171.08
168.66
153.39
150.03
149.97
146.34
137.24
132.40
129.01
128.84
127.96
127.61
127.16
126.98
104.58
77.38
77.06
76.74
60.98
55.98
NH2
N
NH
MeO
MeO
OMe
N
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

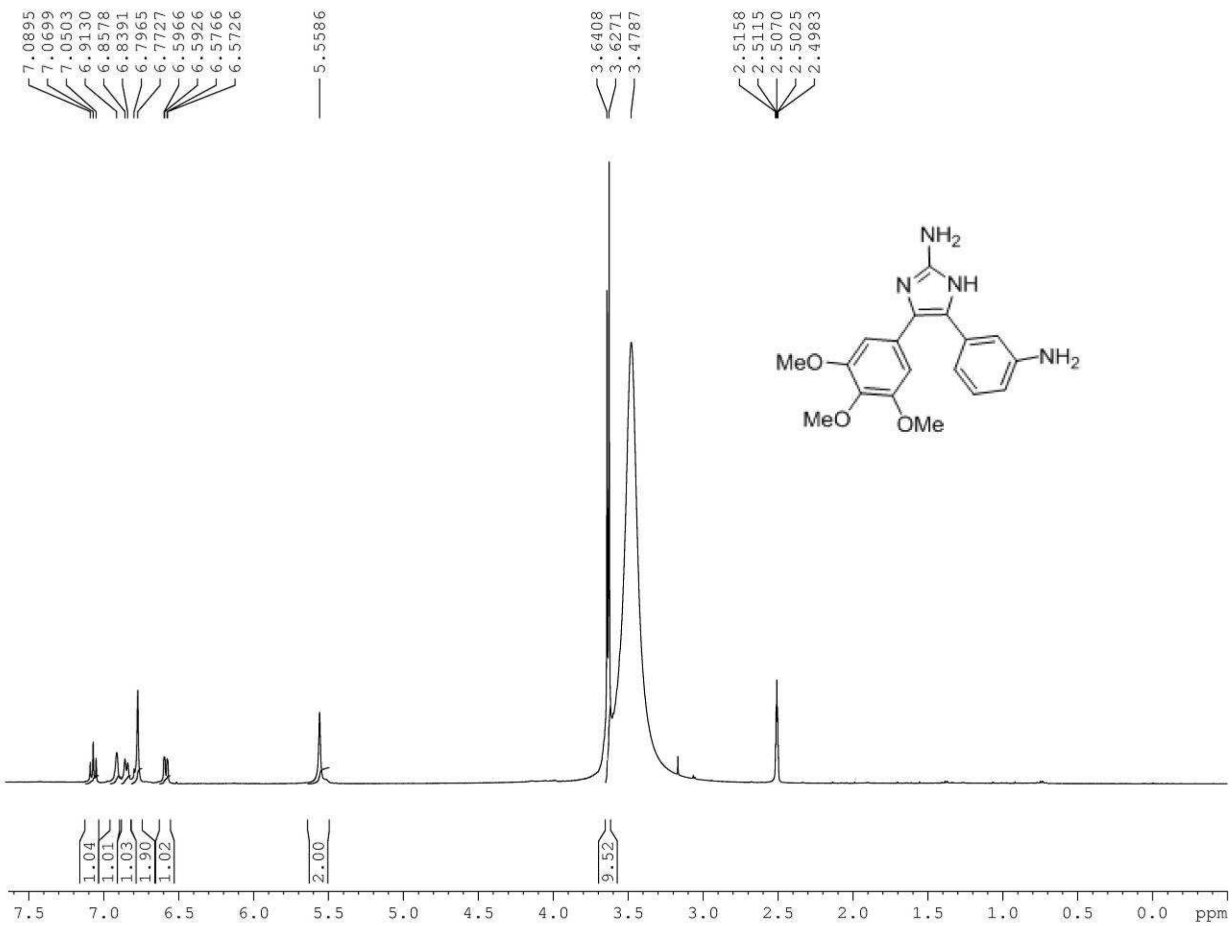
13: ^{1}H NMR


7.4268
7.4075
7.3925
7.2686
7.2542
6.6439
3.8318
3.6430
NH2
N
NH
MeO
MeO
OMe
N
H
3.02
4.29
2.00
3.03
5.82
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm

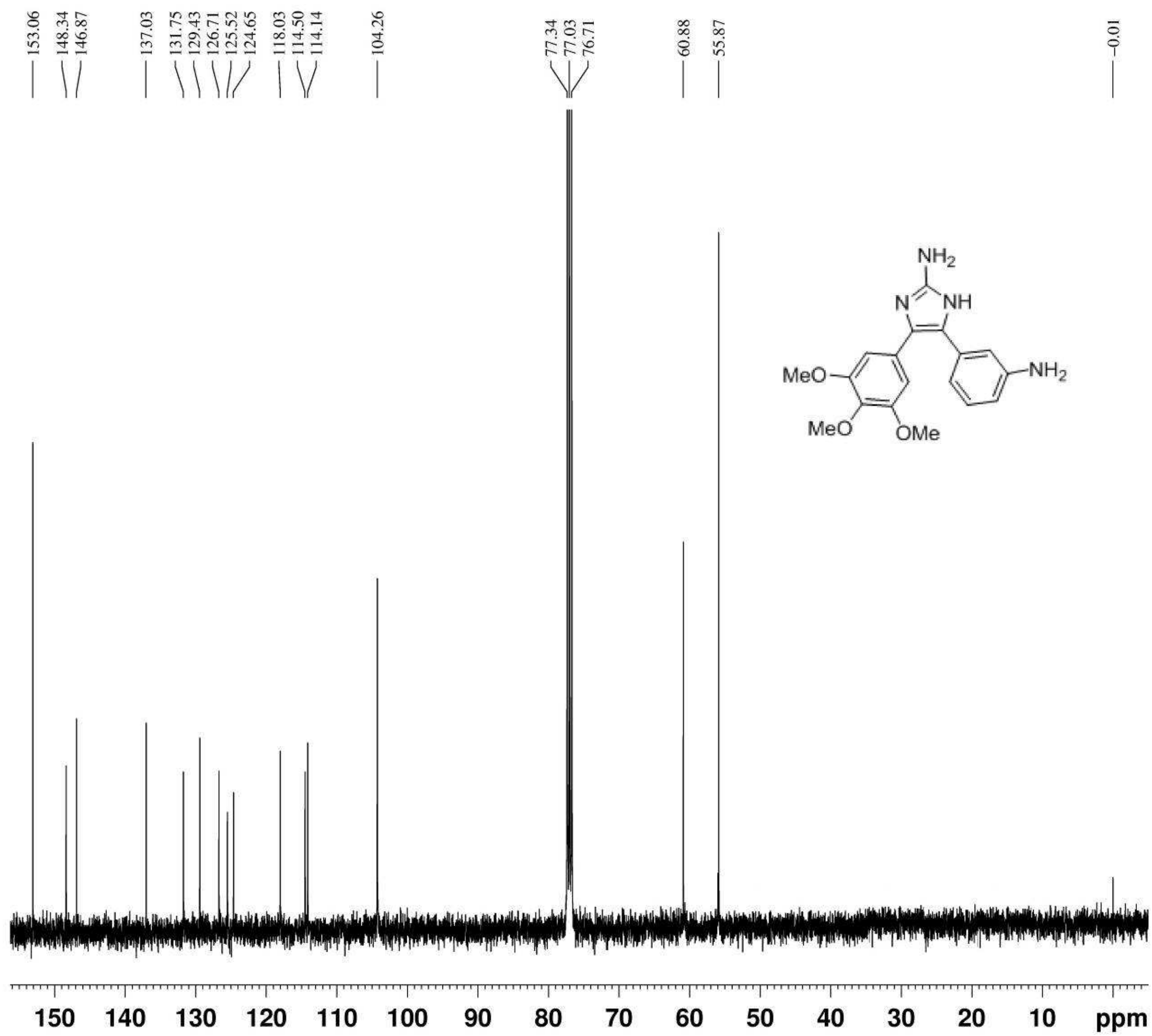
13: ^{13}C NMR


180.05
153.27
148.36
137.64
129.60
128.81
128.70
128.27
127.84
124.92
123.63
122.91
106.08
104.28
77.36
77.04
76.72
60.89
55.91
NH2
N
NH
MeO
MeO
OMe
N
H
180
160
140
120
100
80
60
40
20
ppm

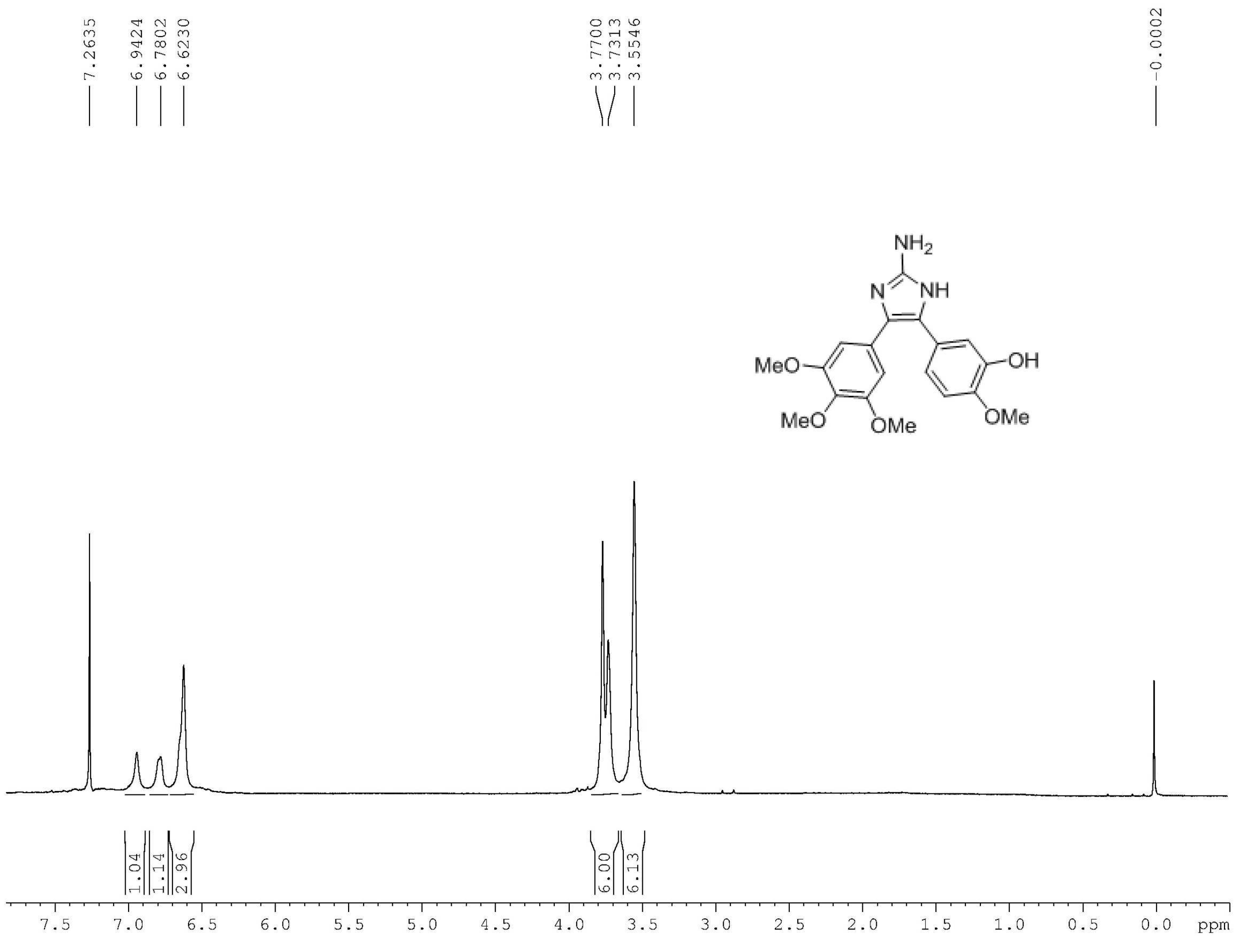
14: ^{1}H NMR


7.0895
7.0699
7.0503
6.9130
6.8578
6.8391
6.7965
6.7727
6.5966
6.5926
6.5766
6.5726
5.5586
3.6408
3.6271
3.4787
2.5158
2.5115
2.5070
2.5025
2.4983
NH2
N
NH
MeO
NH2
MeO
OMe
1.04
1.01
1.03
1.90
1.02
2.00
9.52
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm

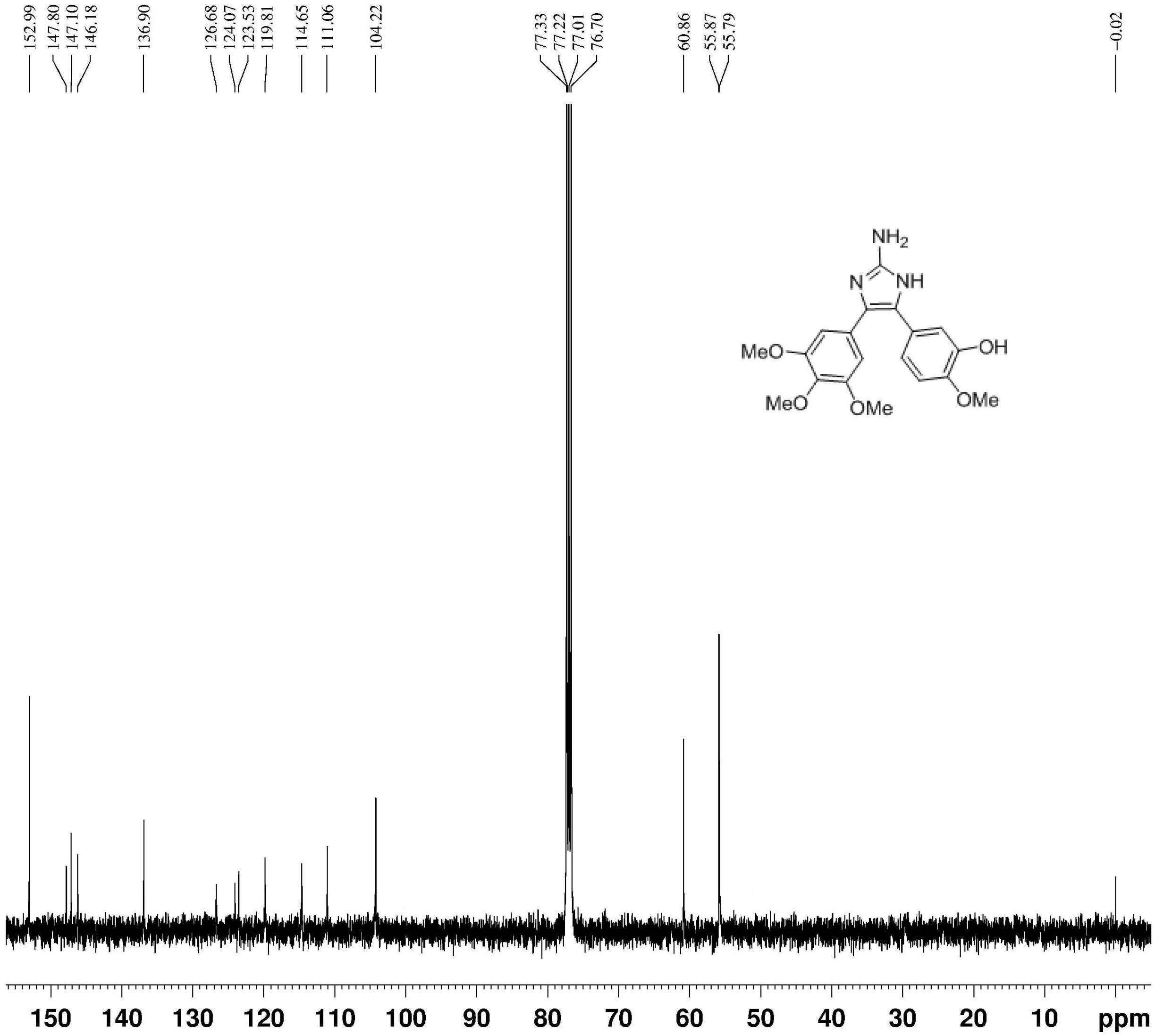
14: ^{13}C NMR

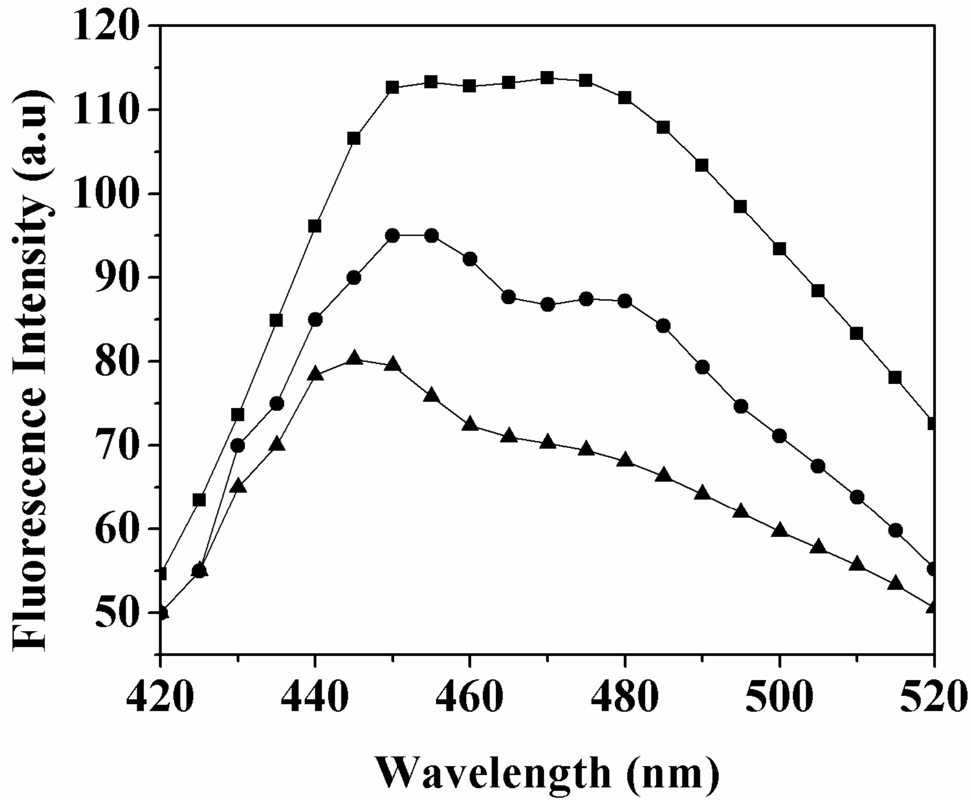

153.06
148.34
146.87
137.03
131.75
129.43
126.71
125.52
124.65
118.03
114.50
114.14
104.26
77.34
77.03
76.71
60.88
55.87
-0.01
NH2
N
NH
MeO
NH2
MeO
OMe
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

15: ^{1}H NMR

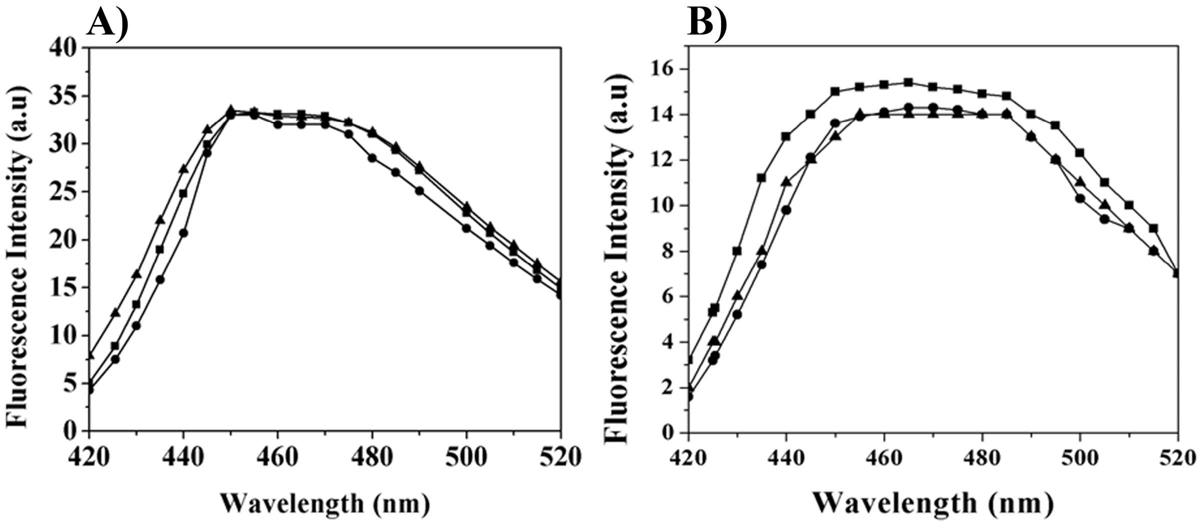

7.2635
6.9424
6.7802
6.6230
3.7700
3.7313
3.5546
-0.0002
NH2
N
NH
MeO
OH
MeO
OMe
OMe
1.04
1.14
2.96
6.00
6.13
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm

15: ^{13}C NMR


152.99
147.80
147.10
146.18
136.90
126.68
124.07
123.53
119.81
114.65
111.06
104.22
77.33
77.22
77.01
76.70
60.86
55.87
55.79
-0.02
NH2
N
NH
MeO
OH
MeO
OMe
OMe
150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm



S1. Compound 12 binds to tubulin in a reversible manner. Tubulin (5 µM) was incubated with 10 µM Compound **12** (■) for 30 minutes. The reaction mixture was then incubated without or with 50 µM of CA-4 (●) and 50 µM of podophyllotoxin (▲) for further 30 minutes in 25 mM PIPES at 25 °C. The spectra of all the reaction mixtures are shown. Three sets of experiments were performed. One of the sets is shown.



S2. Effects of vinblastine and paclitaxel on the binding of compound 12 to tubulin. (A). Tubulin (3 μM) was incubated without (▲) or with different concentrations of 10 μM (●) and 30 μM (■) of vinblastine in 25 mM PIPES for 10 min at 25 °C. Subsequently, the reaction mixtures were incubated with 6 μM compound **12** for another 10 min at 25 °C. An appropriate blank spectrum of 6 μM compound **12** was subtracted from all spectra. Three sets of experiments were performed. One of the sets is shown. (B). Tubulin (3 μM) was incubated without (■) or with different concentrations of 10 μM (●) and 30 μM (▲) of paclitaxel in 25 mM PIPES for 10 min at 25 °C. Subsequently, the reaction mixtures were incubated with 6 μM compound **12** for another 10 min at 25 °C.