Supporting Information

Design, synthesis, and antiviral activities of Myricetin derivatives containing pyridazinone

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1 Crystal characterization data

Table 1 Crystal data of **A14** (CCDC: 2247966)

Compound	**A14**
Sum Formula	$C_{34}H_{35}N_2O_{10}$
Formula Weight	631.64
Temperature [K]	273.15
Space group	$P2_1/c$
Crystal system	monoclinic
a [Å]	13.780(2)
b [Å]	13.421(2)
c [Å]	17.928(3)
α [°]	90
β [°]	112.60
γ [°]	90
V[Å^3]	3061.0(8)
Color/shape	Colorless/rodlike
Radiation [Å]	MoKα (λ = 1.54178)
Z	4
Density (calculated) [mg/m^3]	1.371
Absorption coefficient [mm^{-1}]	0.845
F(000)	1332.0
Theta Min-Max [°]	6.948 to 117.572
h,k,l	$-15 \le h \le 15$, $-10 \le k \le 14$, $-18 \le l \le 17$
Measured reflections	15612
Independent reflections	4182 [R_{int} = 0.5297, R_{sigma} = 0.3533]
Data/Restraints/ParametePsa	4182/1/420
Goodness-of-fit on F^2	1.191
Final R indices [I >2sigma(I)]	R_1 = 0.1421, wR_2 = 0.3449
R indices (all data)	R_1 = 0.2199, wR_2 = 0.4020

2 biological activity testing

The bioactivity of the target compounds against tobacco mosaic virus was tested using the half-leaf blotch assay as follows.

2.1 Extraction of Tobacco mosaic virus (TMV)

Three weeks after the virus was infiltrated in common cigarette, the leaves were taken and cut into pieces to remove the meridians, and placed in a mortar and pestle with liquid nitrogen to be finely ground and weighed; 0.2 mol/L phosphate buffer (pH 7.2, containing 1 % mercaptoethanol) was added according to the ratio of mass to volume of 1:1.5/1:2, stirred in an ice bath for 10 min, and then filtered through a double layer of gauze, and the volume of filtrate was retained and measured (primary extract); 8 % of *n*-butanol by volume was added into the primary extract, stirred in an ice bath for 20 min, and then centrifuged for 20 min (4 ℃, 8000 rpm), and the supernatant was collected and measured. *n*-butanol by volume was added to the primary extract, stirred for 20 min in an ice bath and then centrifuged for 20 min (4 ℃, 8000 rpm), the supernatant was collected and its volume was measured; NaCl and polyethylene glycol were added to the primary extract at 4 % of the volume of supernatant, stirred for 1 h in an ice bath and then centrifuged for 20 min (4 ℃, 8000 rpm), the precipitate was collected; homogenization was performed by adding 0.01 mol/L phosphate buffer, and then centrifuged for 20 min (4 ℃, 8000 rpm), the precipitates were collected. The supernatant was collected by centrifugation for 20 min (4 ℃, 8000 rpm). The purified virus was obtained by combining the supernatant three times.

2.2 *In viv*o anti-TMV activity testing of target compounds

2.2.1 Solution Configuration of Compounds to be Tested

Accurately weigh 2 mg of the target compound, add 30 μL DMSO to dissolve and then add 4 mL of 1 % Tween water (Tween-80) to formulate a 500 μg/mL solution.

2.2.2 Curative activity studies of target compounds against tobacco mosaic virus

Select the same leaves of heartleaf tobacco, remove the top and keep 3-5 healthy leaves, sprinkle a small amount of uniform adamantine on each leaf, inoculate all the leaves with virus by using a row pen dipped in 500 times diluted TMV solution, wait for the virus to infest for 30 min to rinse off the adamantine on the surface of the leaves, naturally dry the water, dip a brush into the solution and evenly apply it on the right side of the leaf, and the left leaf was coated with DMSO as a blank control. The treated tobacco plants were placed in the artificial intelligence climate

greenhouse for 2-3 d. After the spots appeared on the leaves, the number of left and right sides were counted and the inhibition rate was calculated, and the experiment was repeated three times.

2.2.2.1 Inhibitory rate calculation formula

$$I = (L-R)/L \times 100\%$$

I: the inhibition rate;

L: the number of black spots on the left half of the leaf;

R: the number of black spots on the right half of the leaf.

2.2.3 Studies on the protective activity of target compounds against tobacco mosaic virus

The heart leaves of the same leaves were selected, and 3-5 healthy leaves were retained after topping. The right leaves were evenly smeared with a brush with a concentration of 500 μg/mL, and the left side was smeared with DMSO as a blank control. After 24 h, the left and right sides of the leaves were evenly spread with emery, and the left and right sides of the leaves were inoculated with 500 times diluted virus with a pencil. After waiting for 30 min, the emery was washed away and placed in the artificial intelligence climate greenhouse for 2-3 d. After the spots on the leaves, the number of left and right sides was counted, the inhibition rate was calculated (the calculation formula was the same as above), and the parallel test was carried out three times.

2.2.4 Studies on the passivating activity of target compounds against tobacco mosaic virus

After removing the top, 3-5 healthy leaves were retained, and a small amount of emery was evenly sprinkled. 1 mL of TMV solution diluted 250 times was added to 1 mL of 500 μg/mL solution to passivate 0.5 h. The right side of the leaf was inoculated with a row of pens, and the TMV solution diluted 500 times was inoculated on the left side of the leaf. After waiting for 30 min, the emery was washed away and placed in the artificial intelligence climate greenhouse for 2-3 d. After the spots on the leaves were counted, the number of left and right sides was counted, and the inhibition rate was calculated (the calculation formula was the same as above), and the parallel test was carried out three times.

2.2.5 Determination of EC_{50} values of the target compounds against tobacco mosaic virus

The compounds were successively prepared into solutions of 500, 250, 125, 62.5 and 31.25 μg/mL. According to the treatment and protection methods, the drugs were administered and the virus was inoculated. The number of spots was counted and the inhibition rate was calculated. The corresponding EC_{50} value was calculated, and the parallel test was performed three times.

3 Microscale thermophoresis (MST) experiment

3.1 Preparation of target compounds

Weigh m (mg) = M (relative molecular mass) × 4 ÷ 1000 in a 200 μL PE tube, dissolve it with 100 μL DMSO, take 10 μL of the drug solution in a 200 μL PE tube and add 190 μL of SEC buffer solution, and this tube of the drug solution is called the mother liquor. The mother liquor was sequentially prepared into 16 specimens with concentration gradients of 10 μL each.

3.2 MST Test

Add 10 μL of TMV CP-labeled protein to each of the solutions 1-16, mix well, and there should be no air bubbles in the PE tube; inhale the specimen into a capillary tube and operate on a Monolith NT.115 instrument to determine the K_d values of the compounds and TMV CP and record them.

4 Molecular docking experiments

Molecular docking performs virtual screening of drugs by predicting affinity and binding patterns of drugs and proteins. Molecular docking was performed using LibDock in Discovery Studio (2019) and visualization analysis was done in this software. TMV-CP (PDB code: **1EI7**) was obtained from the Protein Data Bank (PDB https://www.rcsb.org). All parameters were defaulted during the docking process.

5 Determination of chlorophyll content

Taking **A26** as an example, the changes of chlorophyll content in tobacco leaves after treatment were determined. The CK group, TMV group, drug group and drug + TMV group were set up, and three parallel experiments were set up in each group. The operation steps are as follows :

5.1 Tobacco handling and sampling

The tobacco leaves with the same growth cycle were selected, and 5-6 leaves with the same shape and size were retained after topping. The liquid with a concentration of 500 μg/mL was dipped in a brush and evenly applied to the tobacco leaves of the corresponding groups. The CK group was smeared with DMSO solution for the same operation. After 24 hours, the virus was inoculated. The leaf samples were collected at 1, 3, 5 and 7 d after inoculation, and the group markers were made.

5.2 Determination of chlorophyll content

The collected samples were removed from the meridians to retain the leaves, and 20 mg of each tobacco leaf was accurately weighed and ground into a mortar, and 2 mL of mixed solution (85 % ethanol : 85 % acetone = 1:1) was added. After rapid grinding, it was transferred to a 2 mL

centrifuge tube and incubated in the dark for 1 h. After centrifugation for 5 min (4 ℃, 4000 rpm), the extract was used as a blank control, and the absorbance OD645 and OD663 at 645 and 663 nm were measured respectively.

5.3 Result calculation

5.3.1The experimental results are calculated according to the following formula :

$$Ca\ (mg/L) = 9.784\ OD663 - 0.990\ OD645$$

$$Cb\ (mg/L) = 21.426\ OD645 - 4.650\ OD663$$

$$Ct\ (mg/L) = Ca + Cb = 5.134\ OD663 + 20.436\ OD645$$

Chlorophyll content (mg/g) = concentration (mg/L) * total extract (mL) / leaf weight (g)

6 Determination of malondialdehyde content

The treatment of pre-tobacco leaves was the same as that of chlorophyll, and the collected samples were removed from the meridian, put into a mortar and pestle, and fully ground by adding liquid nitrogen. After grinding, the samples were put into 2 mL PE tubes and labeled, and then stored in a refrigerator at -80 ℃. Later, the determination was carried out according to the instructions that came with the malondialdehyde assay kit.

7 ^{1}H NMR, ^{13}C NMR, ^{19}F NMR and HRMS spectrum of the title compounds

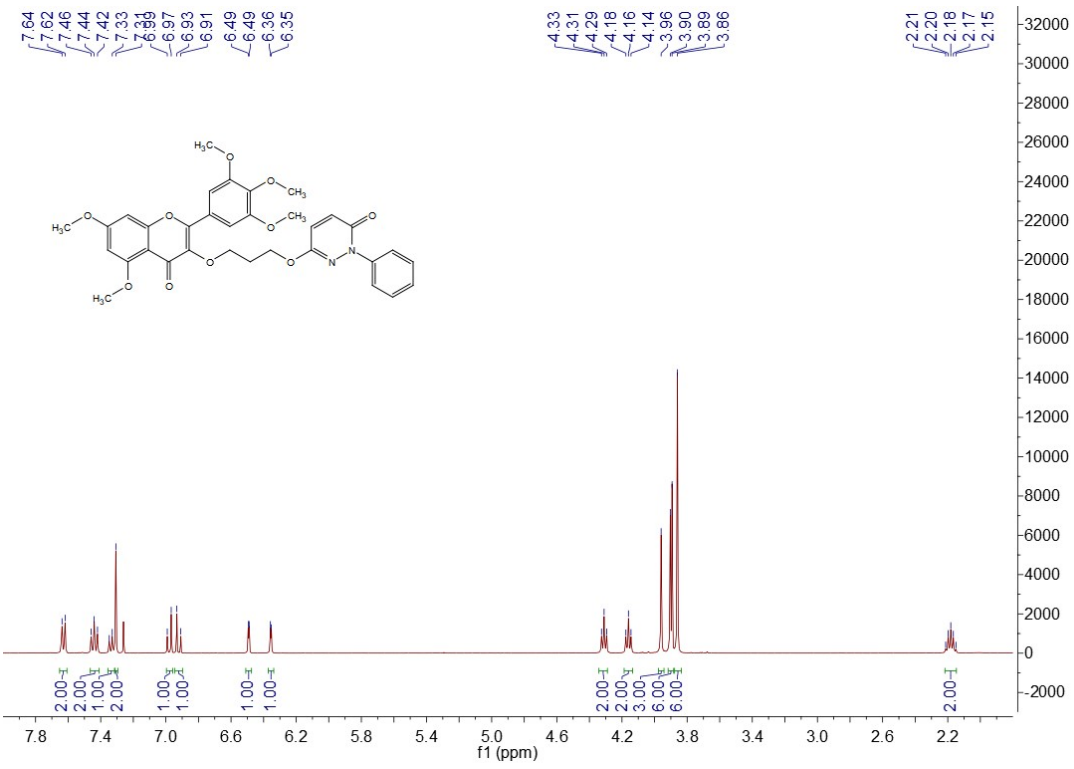
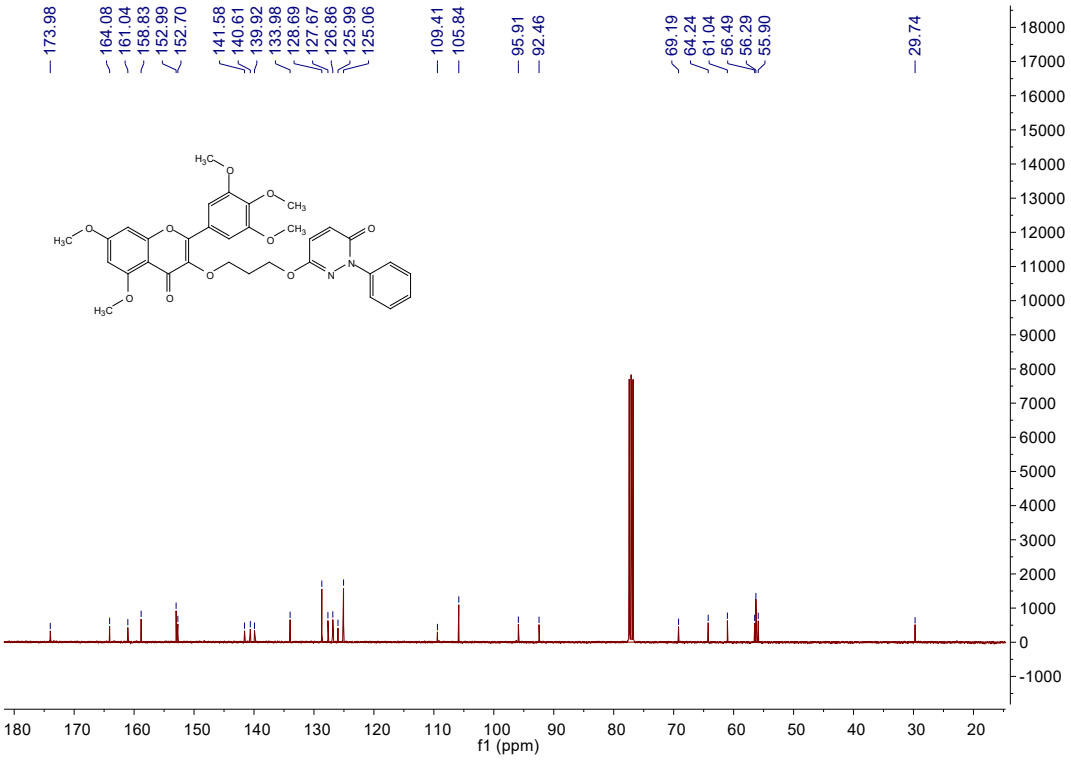
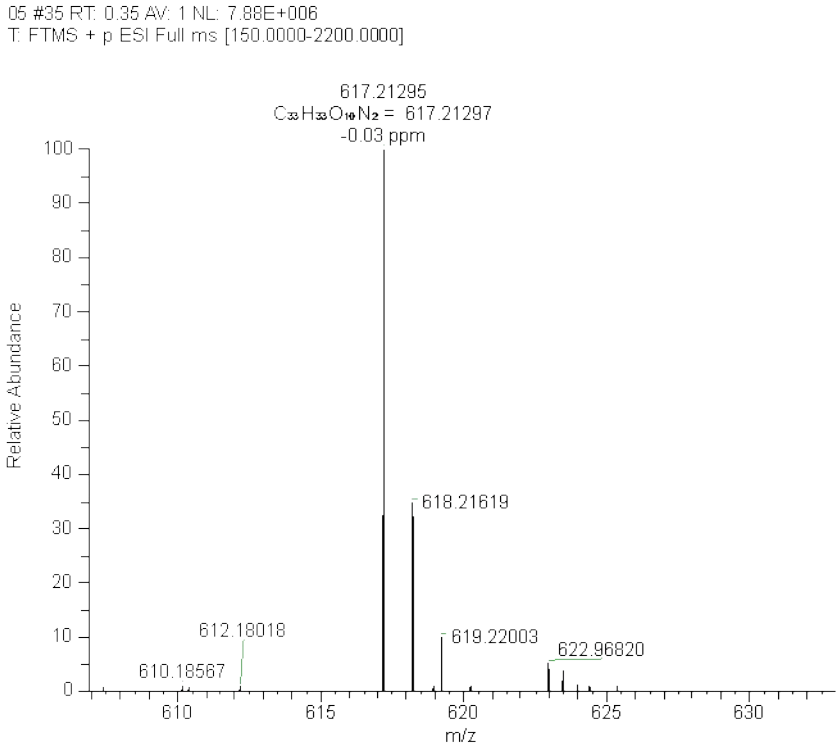


Fig. 1 ^{1}H NMR spectrum of **A1**



124

125 **Fig. 2** ^{13}C NMR spectrum of **A1**



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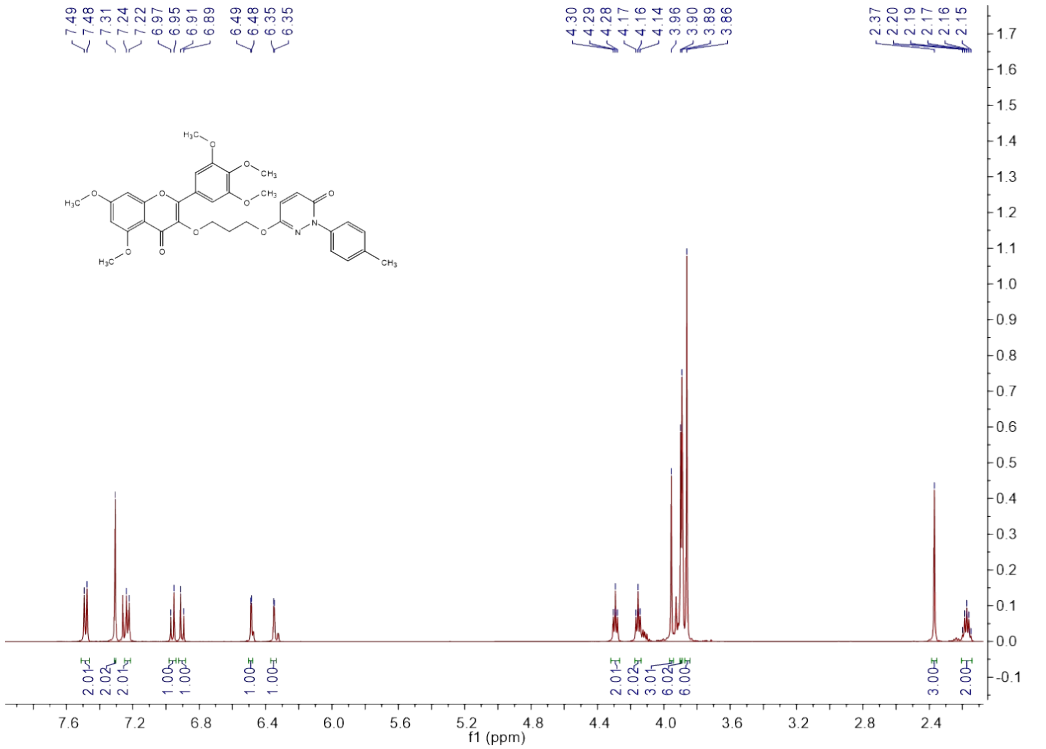
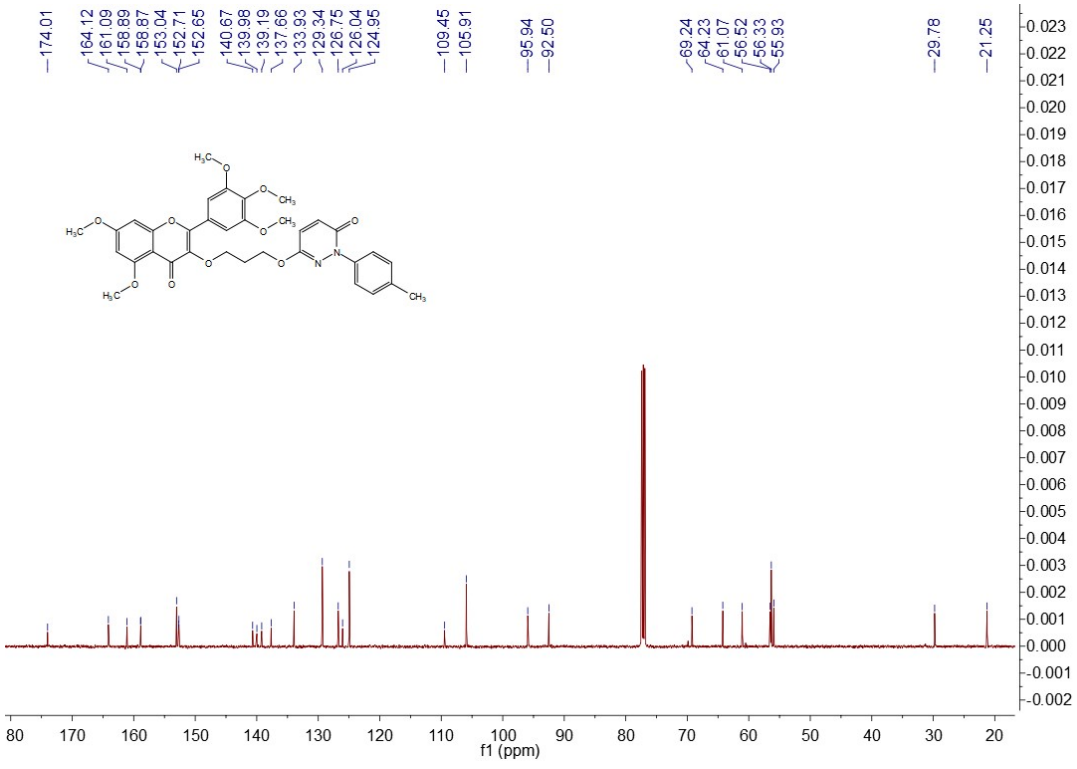
127 **Fig. 3** HRMS spectrum of **A1**



128

129 **Fig. 4** ^{1}H NMR spectrum of **A2**



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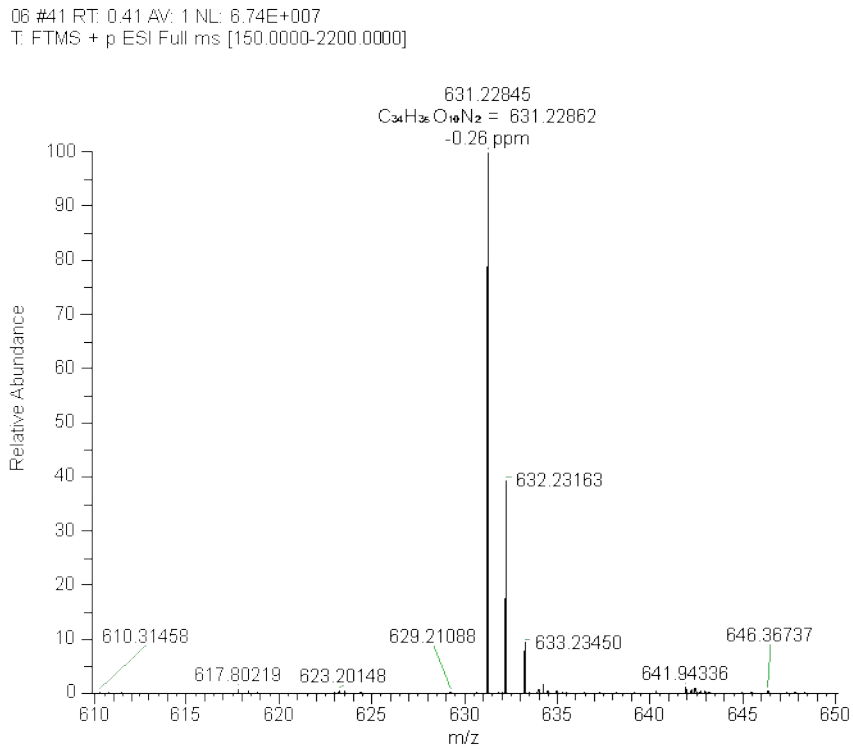
131 **Fig. 5** ^{13}C NMR spectrum of **A2**



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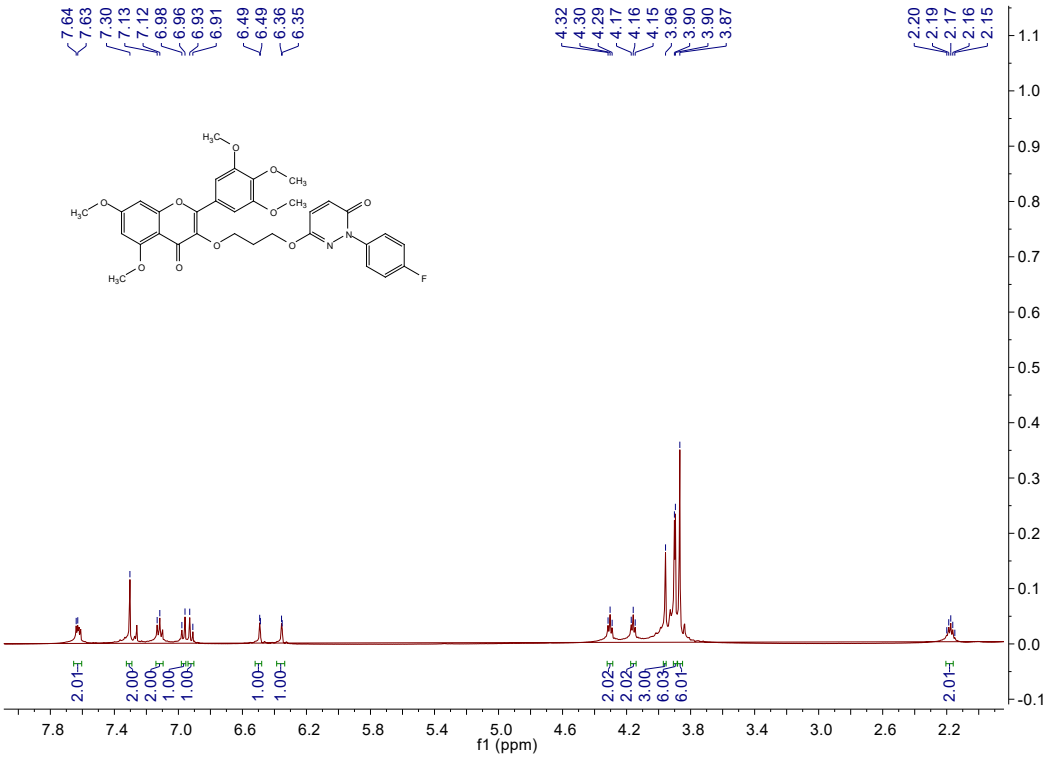
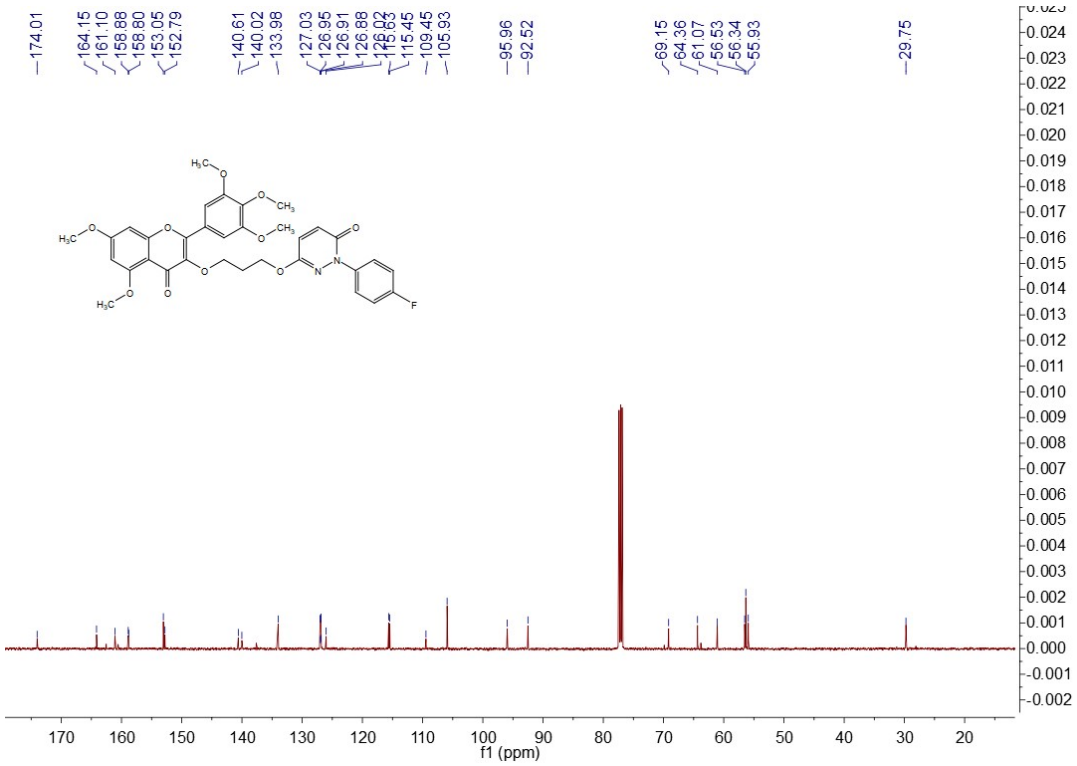
133 **Fig. 6** HRMS spectrum of **A2**



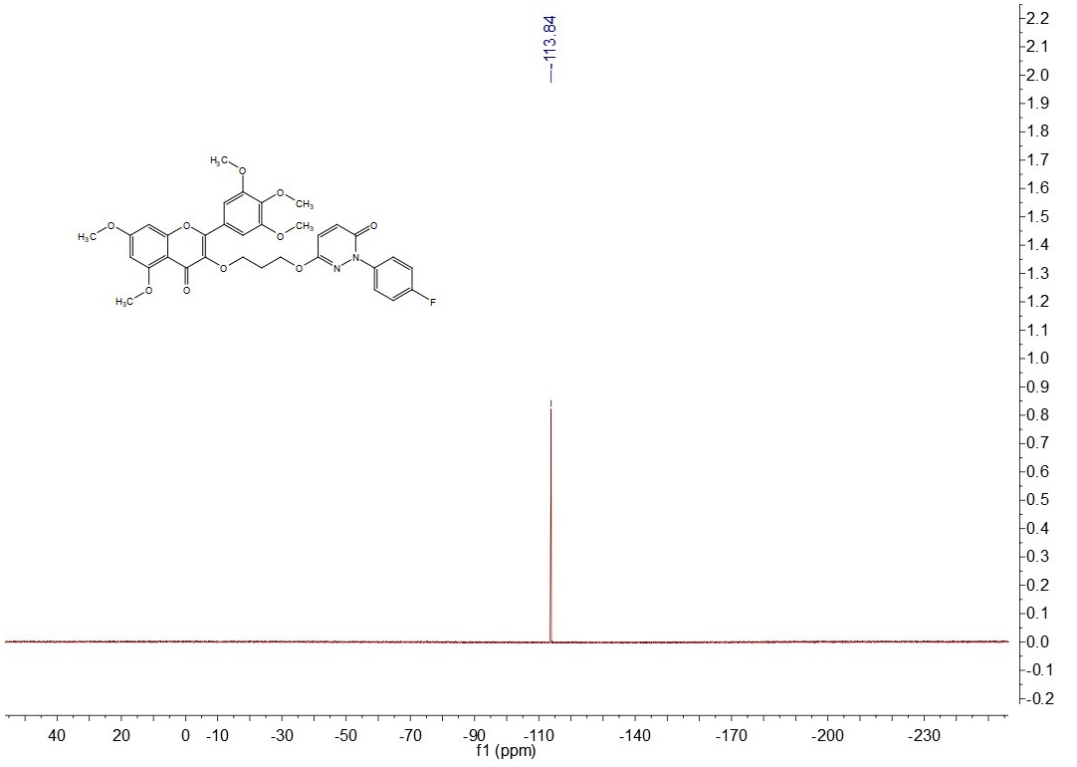
134

135 **Fig. 7** ^{1}H NMR spectrum of **A3**



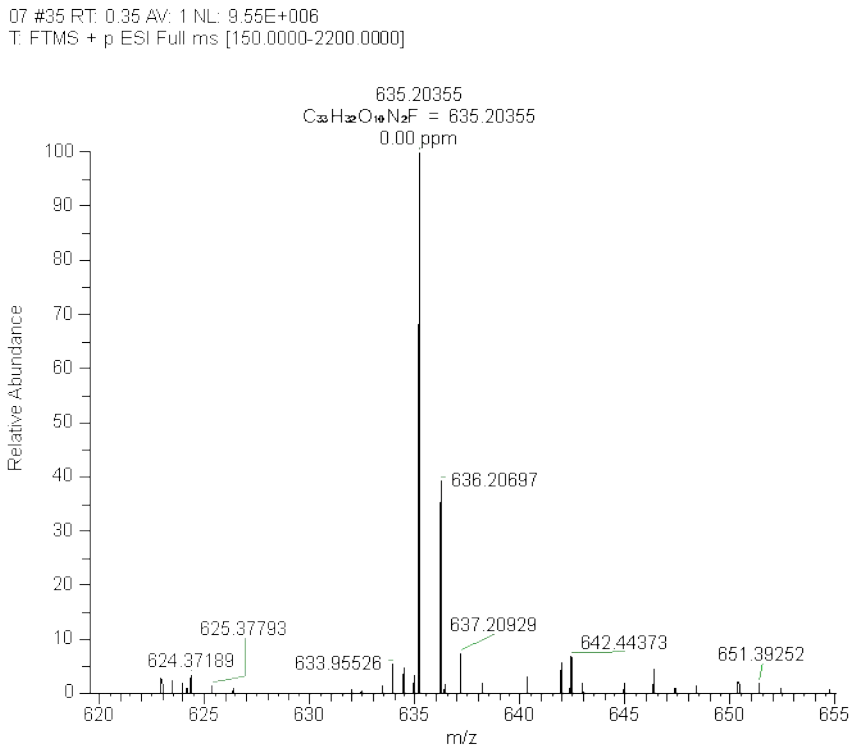
136

137 **Fig. 8** ^{13}C NMR spectrum of **A3**



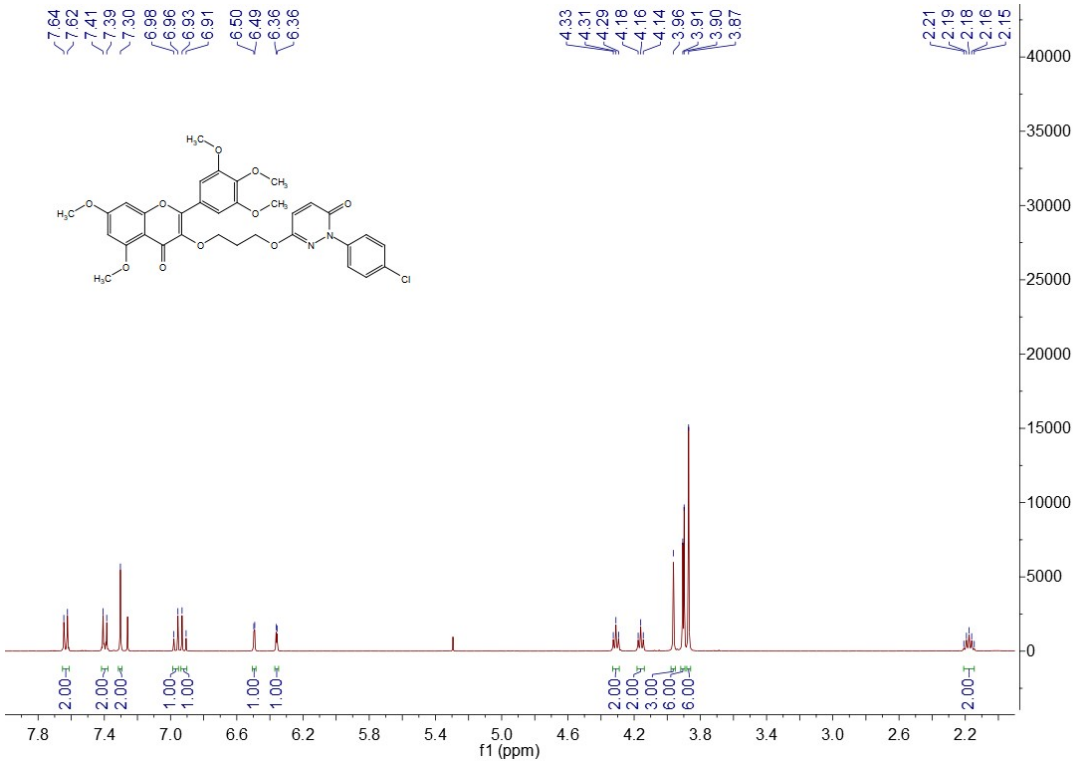
138

139 **Fig. 9** ^{19}F NMR spectrum of **A3**



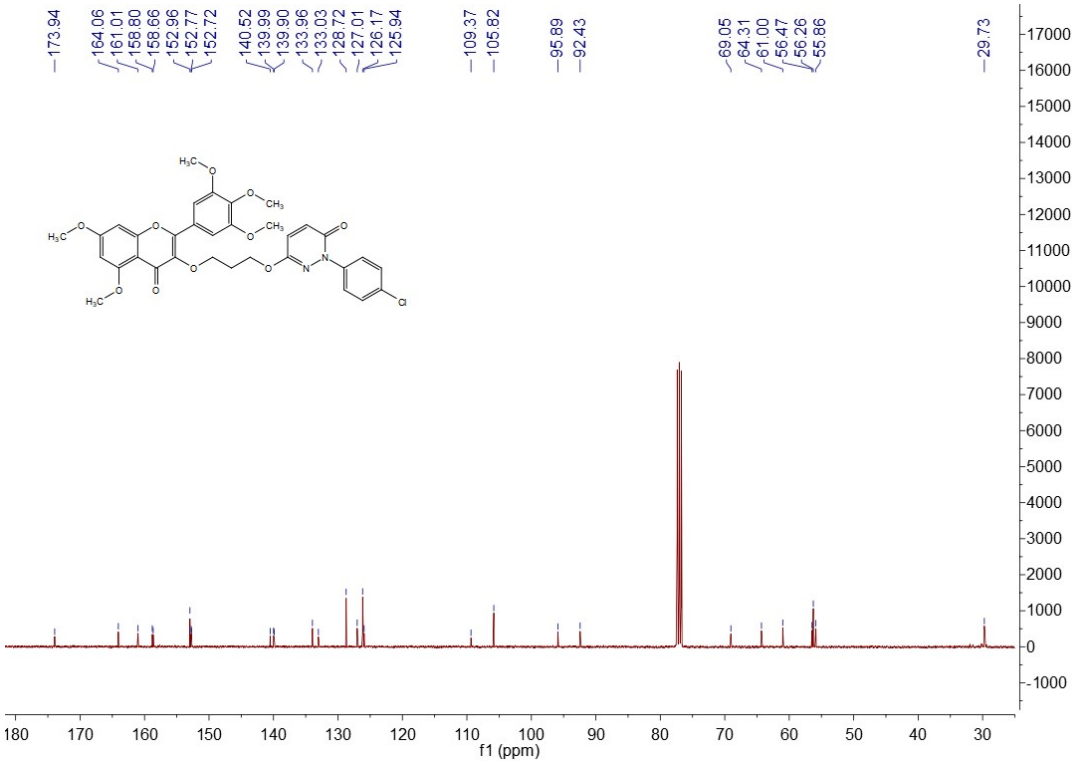
140

141 **Fig. 10** HRMS spectrum of **A3**



142

143 **Fig. 11** ^{1}H NMR spectrum of **A4**



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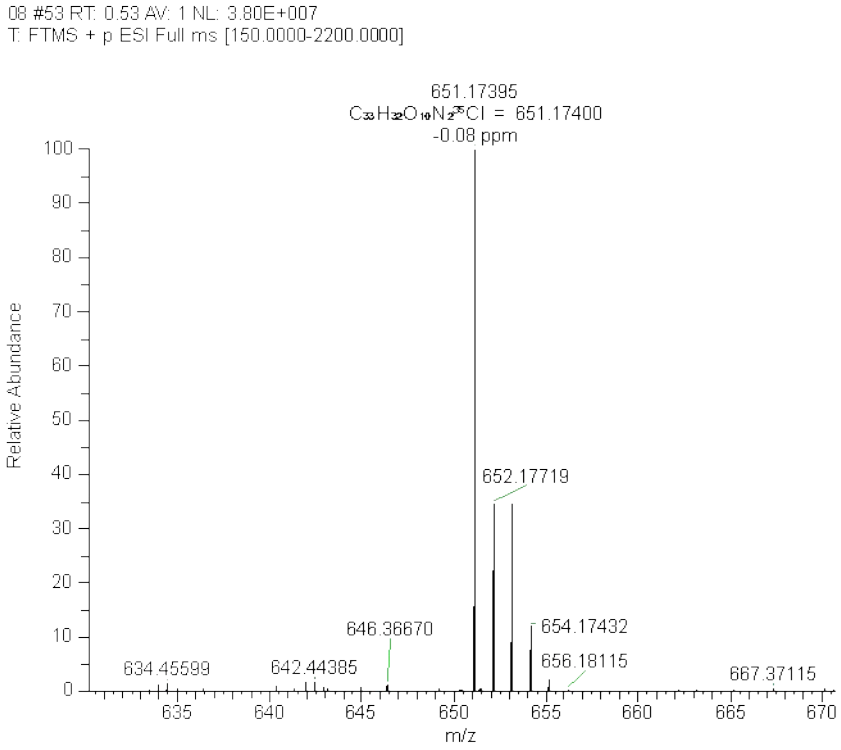
145 **Fig. 12** ^{13}C NMR spectrum of **A4**



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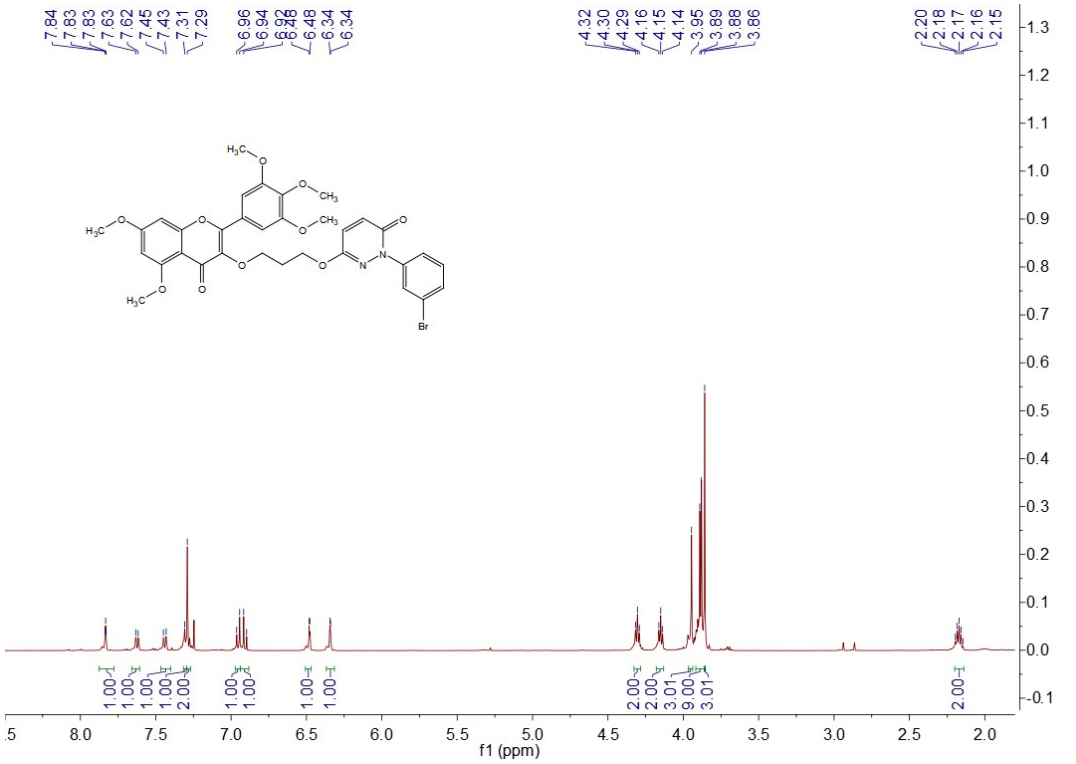
147 **Fig. 13** HRMS spectrum of **A4**



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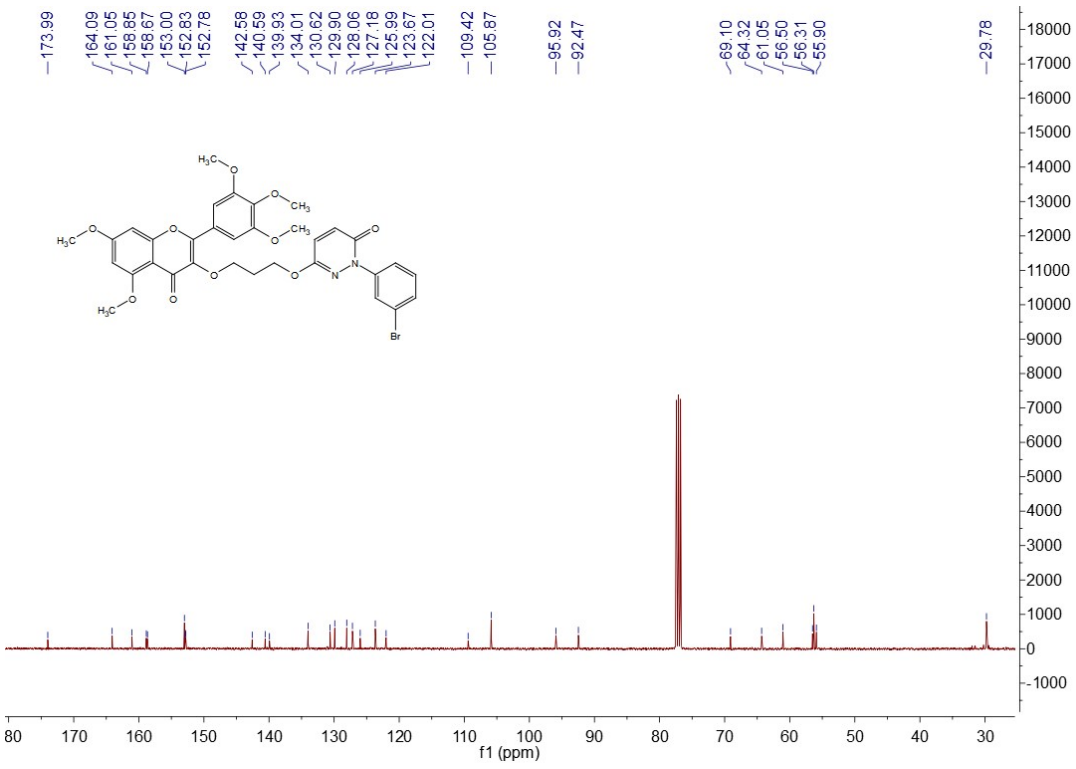
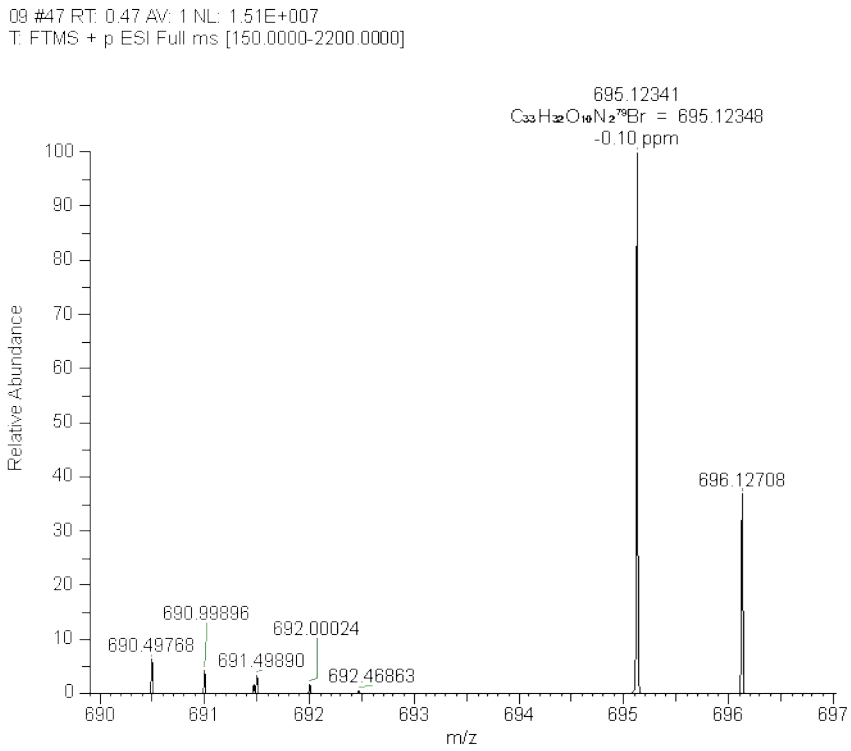
149 **Fig. 14** ^{1}H NMR spectrum of **A5**



150

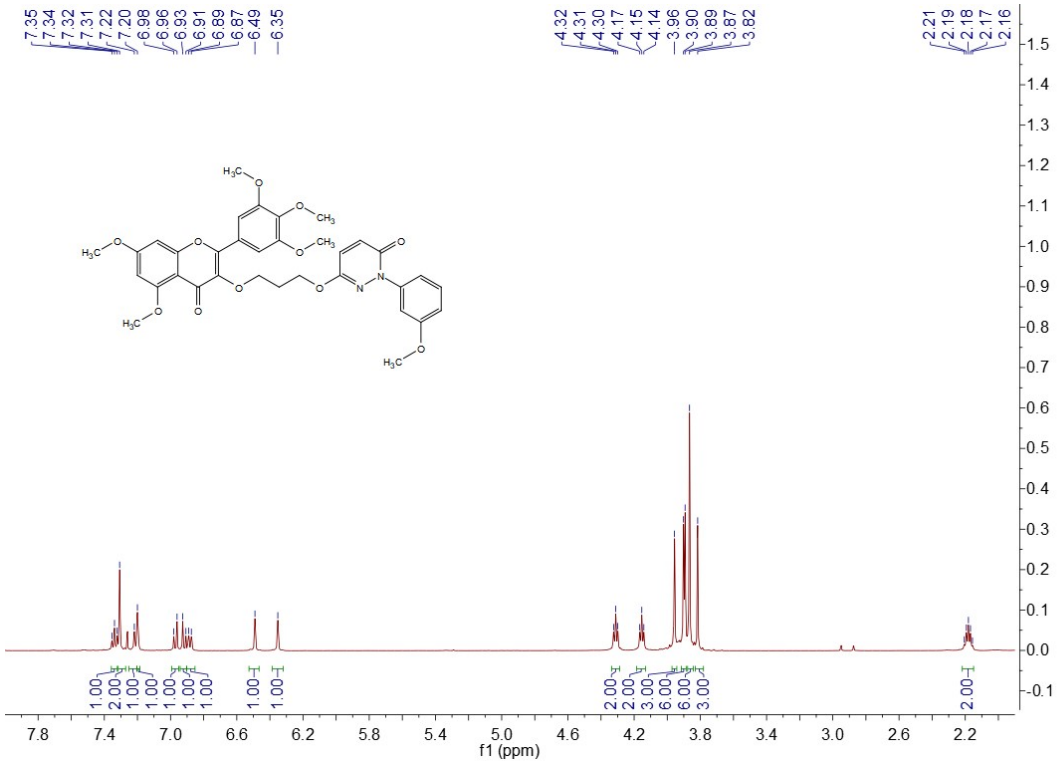
151 **Fig. 15** ^{13}C NMR spectrum of **A5**

152



153

154 **Fig. 16** HRMS spectrum of **A5**



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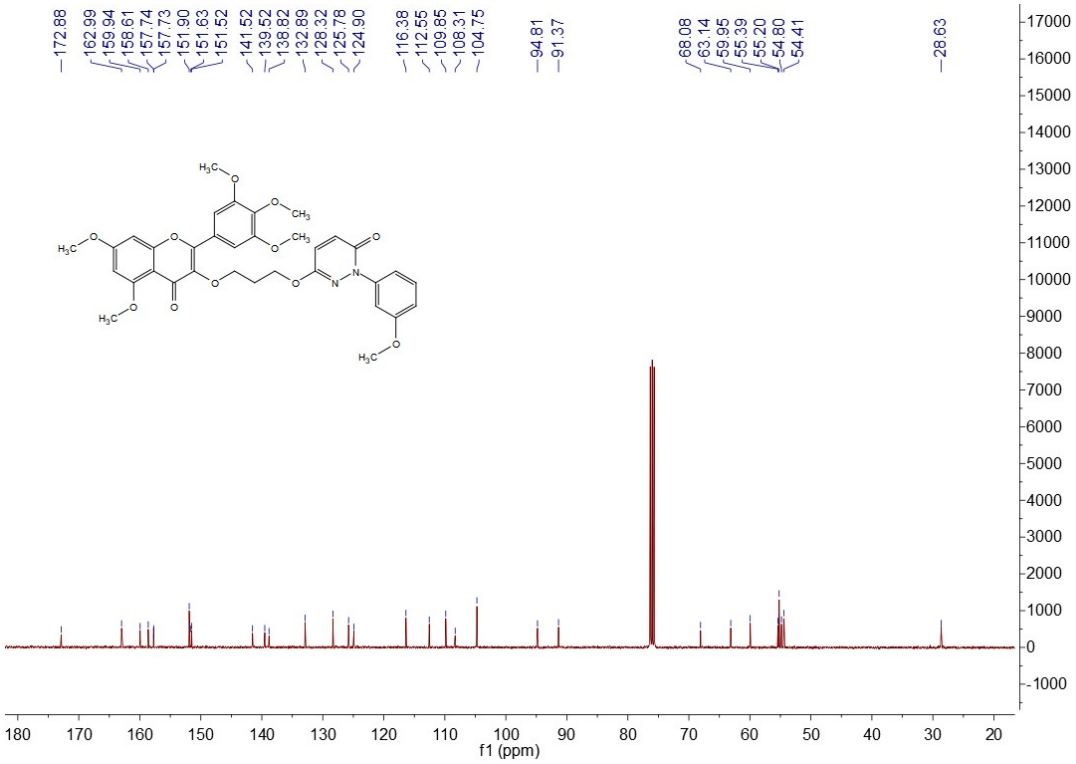
156 **Fig. 17** ^{1}H NMR spectrum of **A6**



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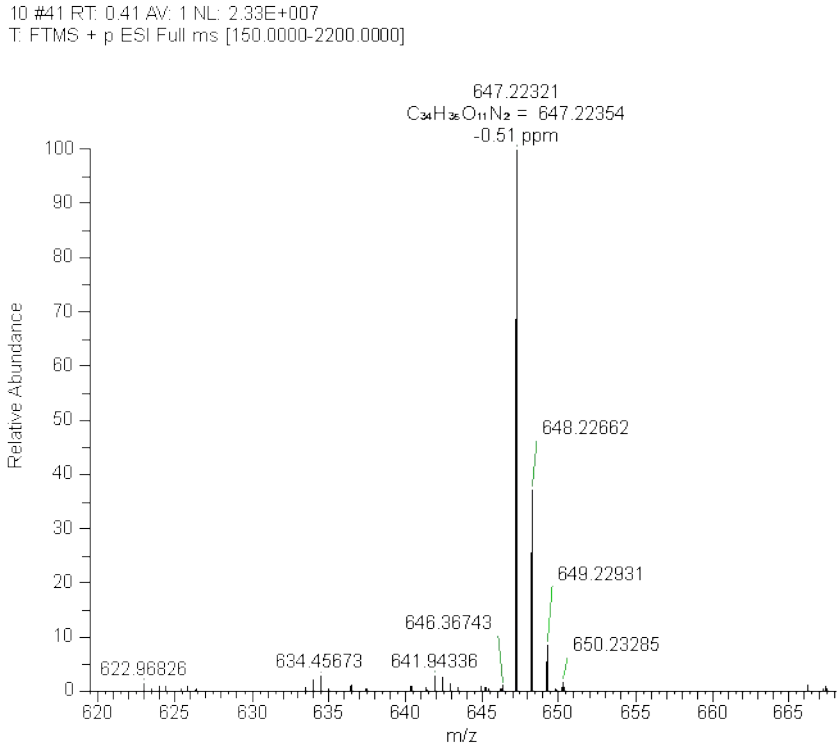
158 **Fig. 18** ^{13}C NMR spectrum of **A6**



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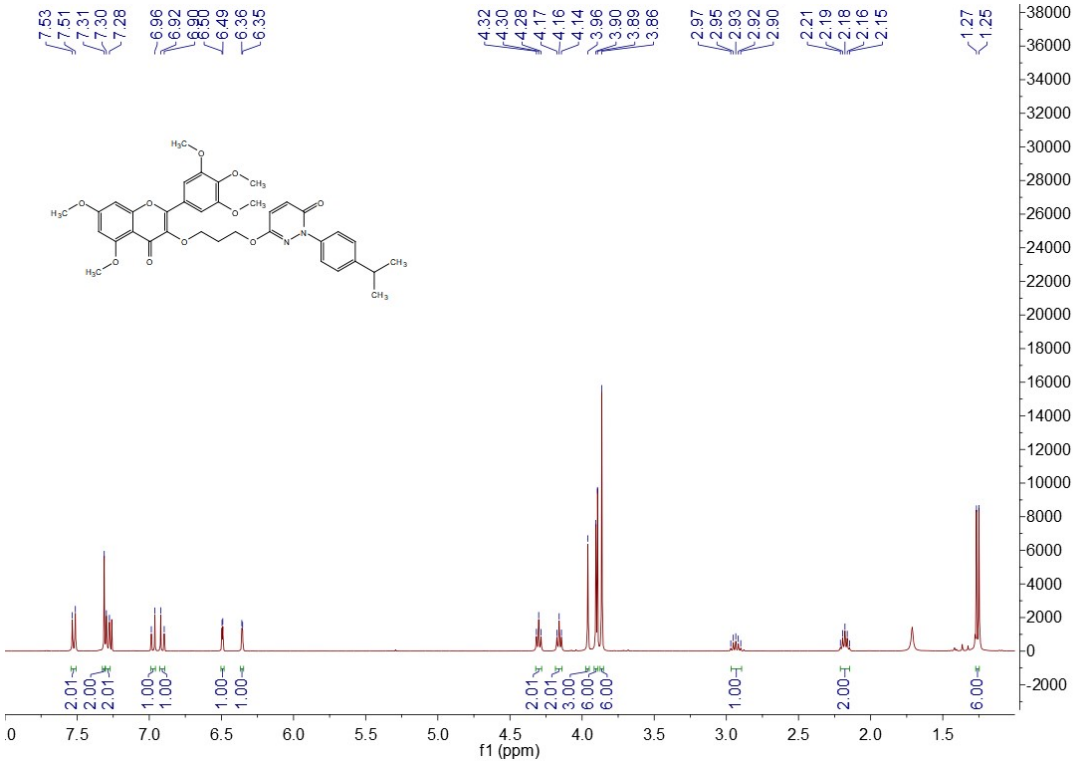
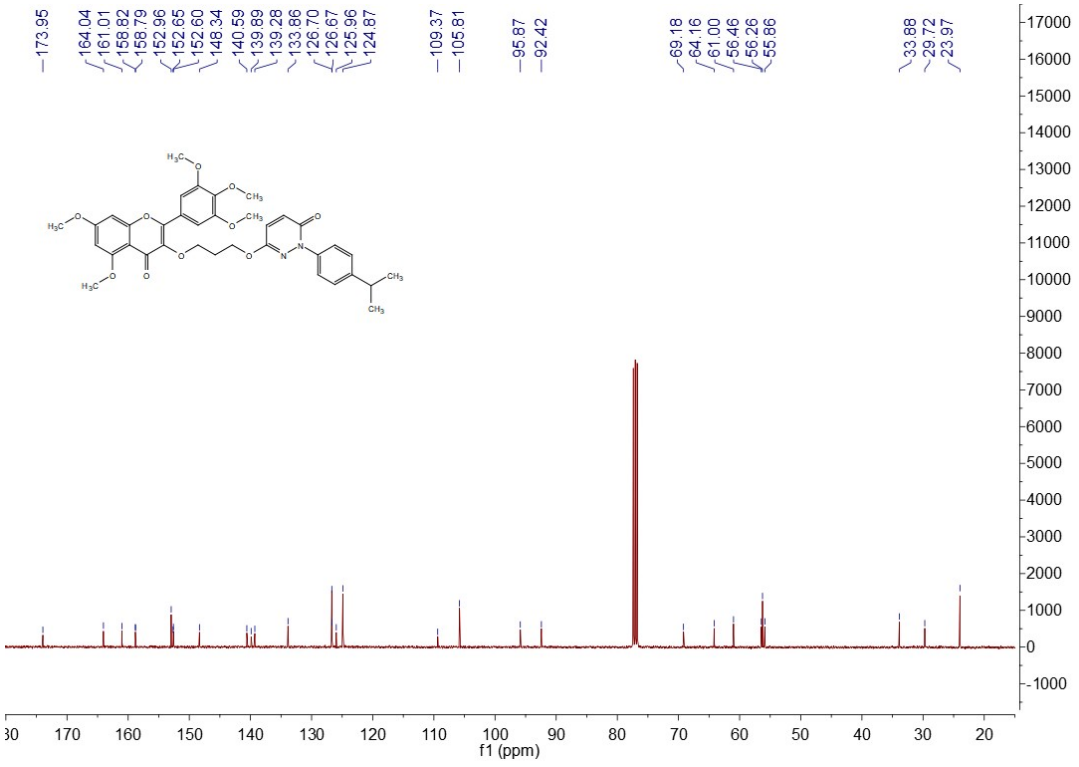
160 **Fig. 19** HRMS spectrum of **A6**



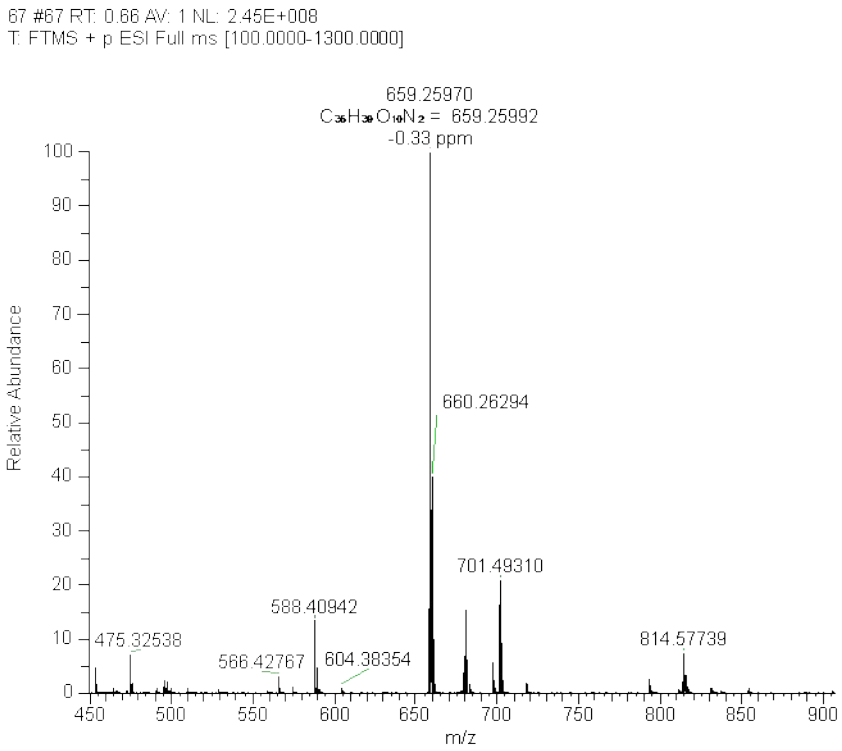
161

162 **Fig. 20** ^{1}H NMR spectrum of **A7**



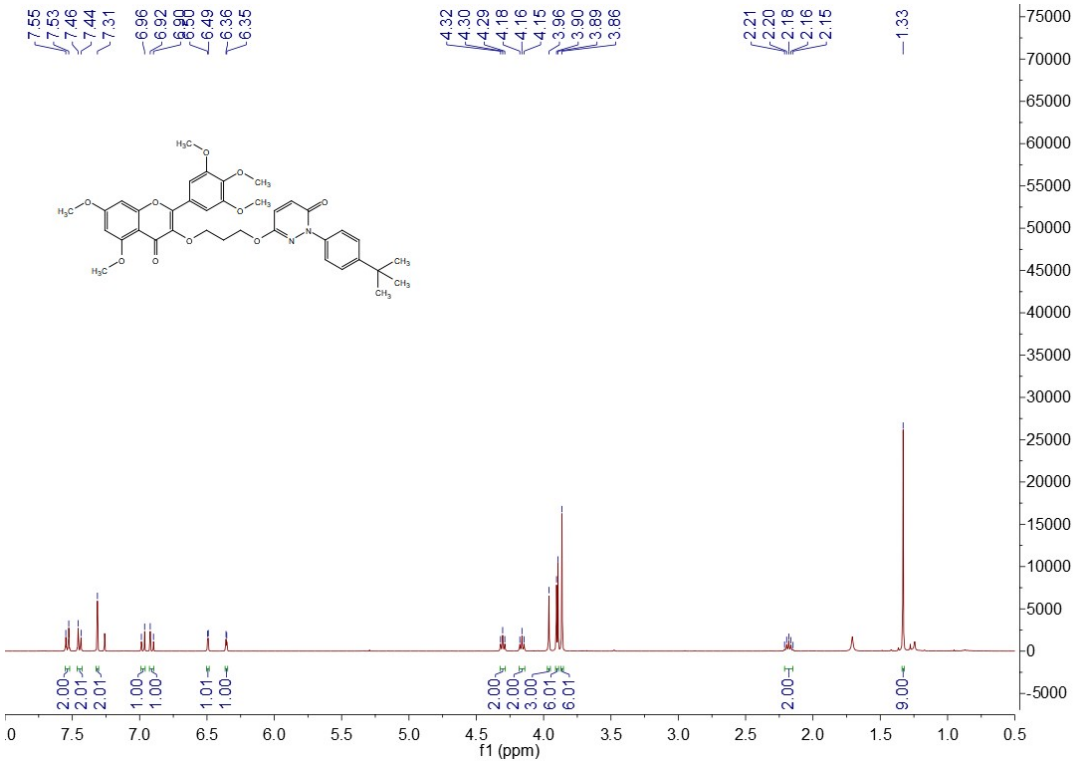
163

164 **Fig. 21** ^{13}C NMR spectrum of **A7**



165

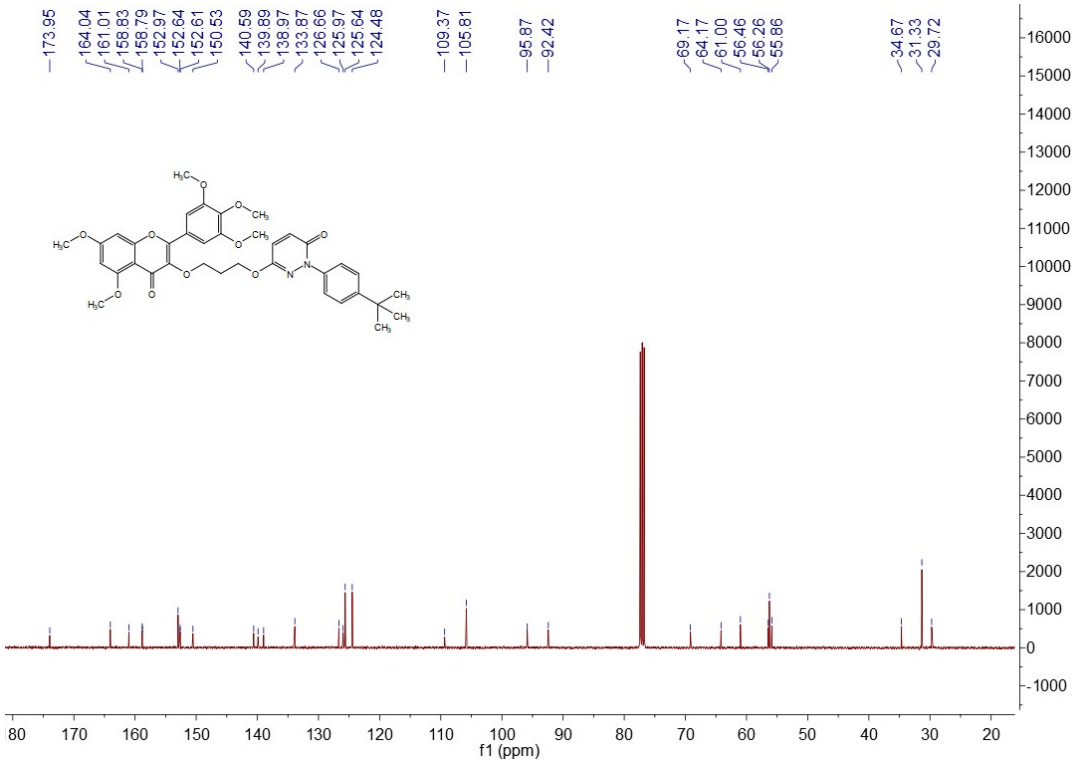
166 **Fig. 22** HRMS spectrum of **A7**



167

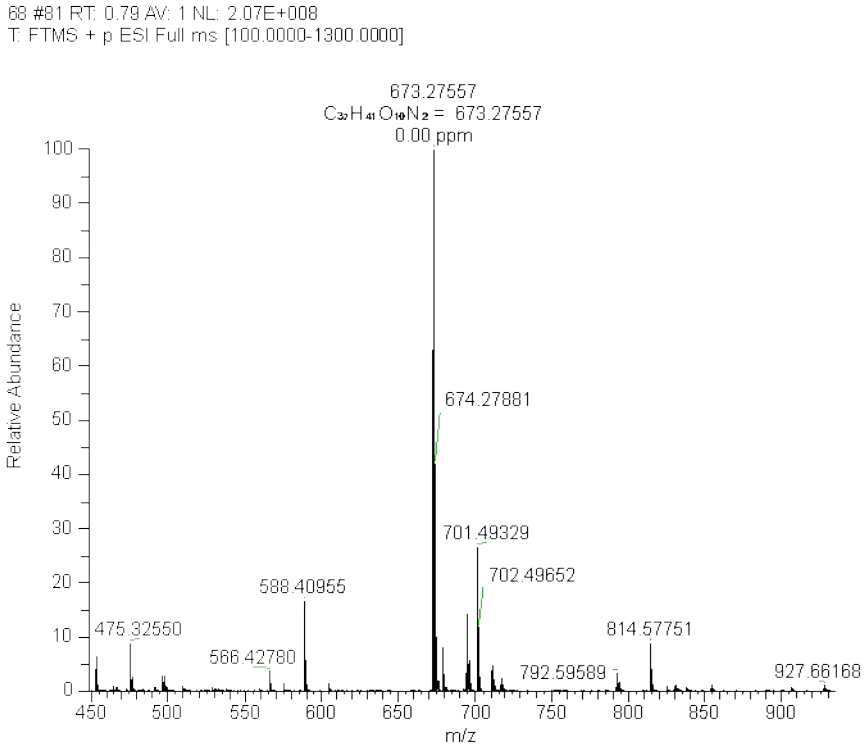
168 **Fig. 23** ^{1}H NMR spectrum of **A8**

169



170

171 **Fig. 24** ^{13}C NMR spectrum of **A8**



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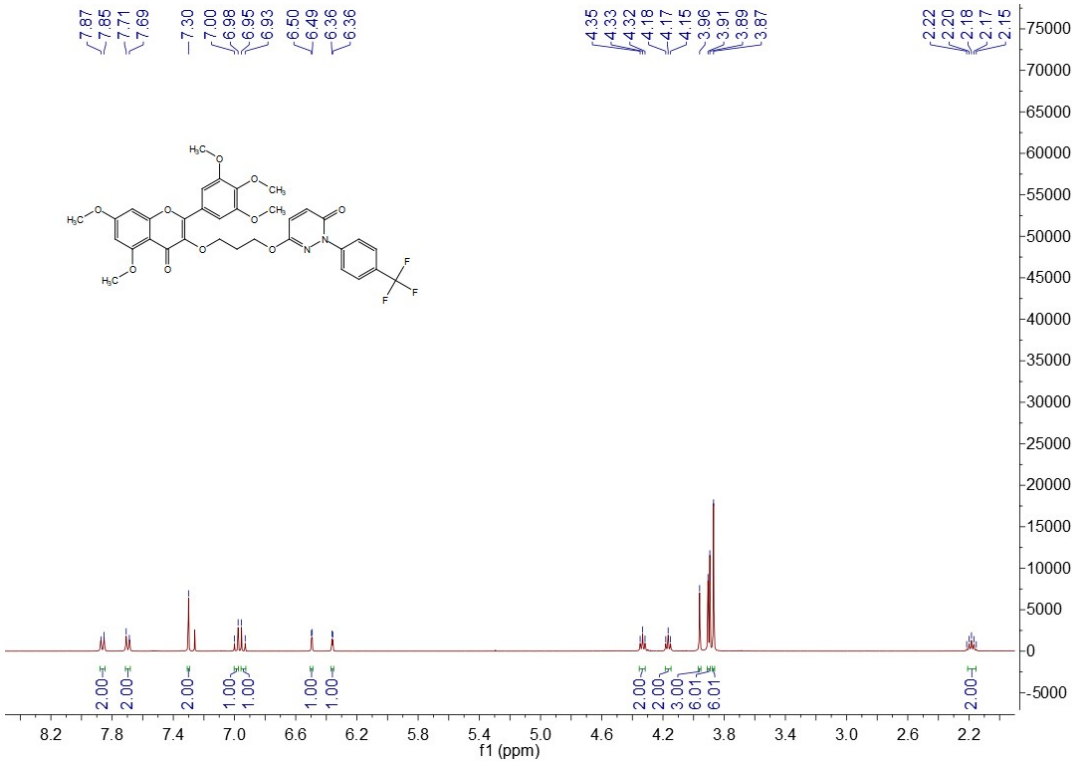
173 **Fig. 25** HRMS spectrum of **A8**



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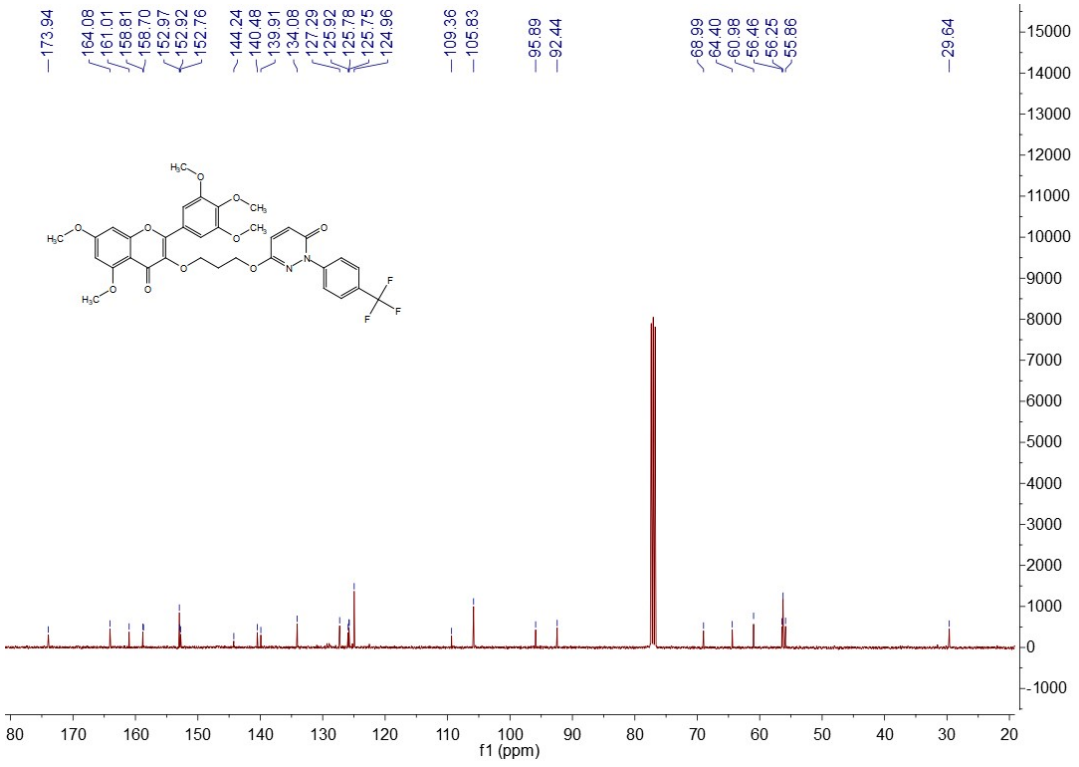
175 **Fig. 26** ^{1}H NMR spectrum of **A9**



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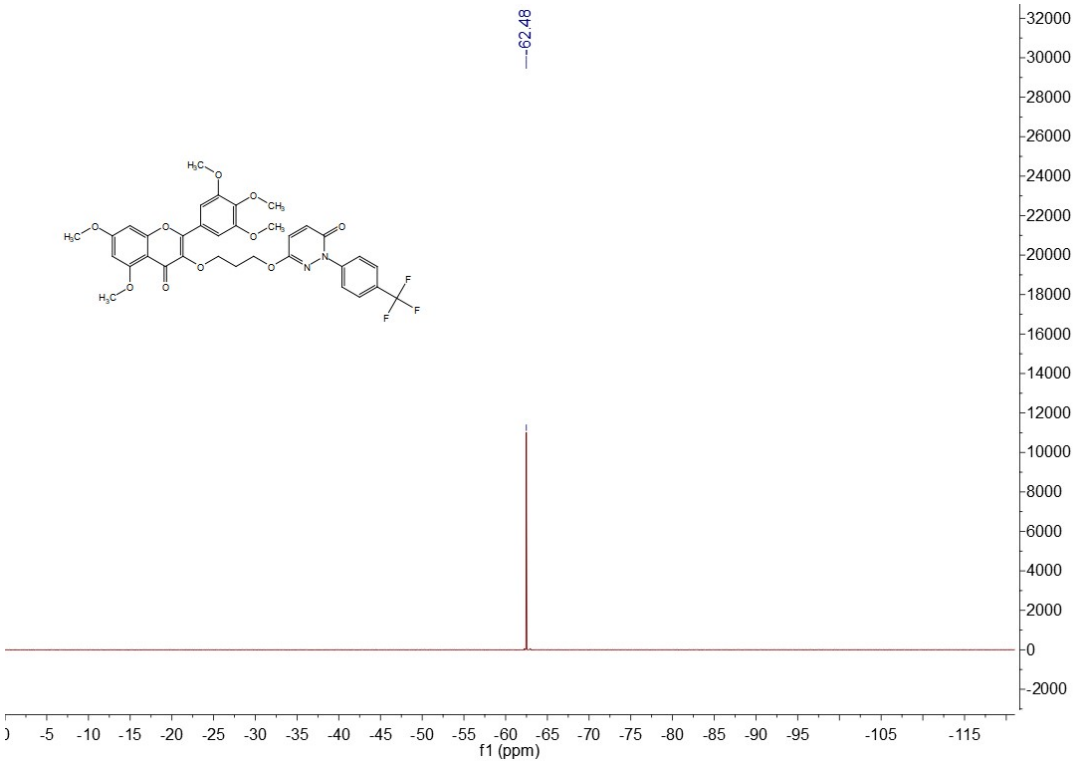
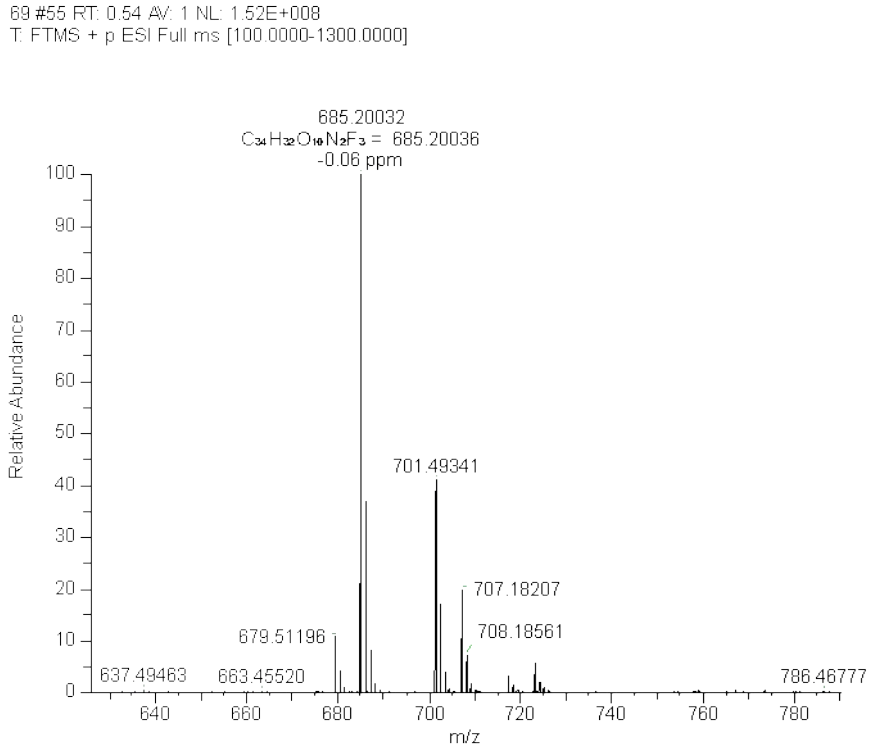
177 **Fig. 27** ^{13}C NMR spectrum of **A9**



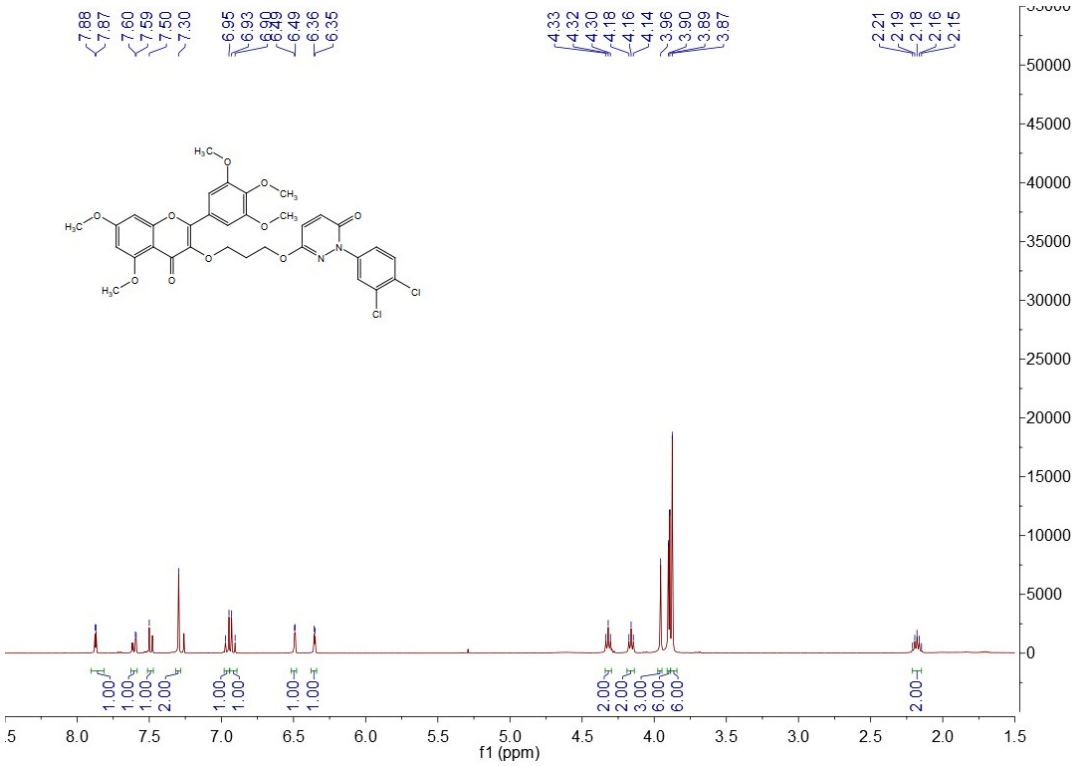
178

179 **Fig. 28** ^{19}F NMR spectrum of **A9**



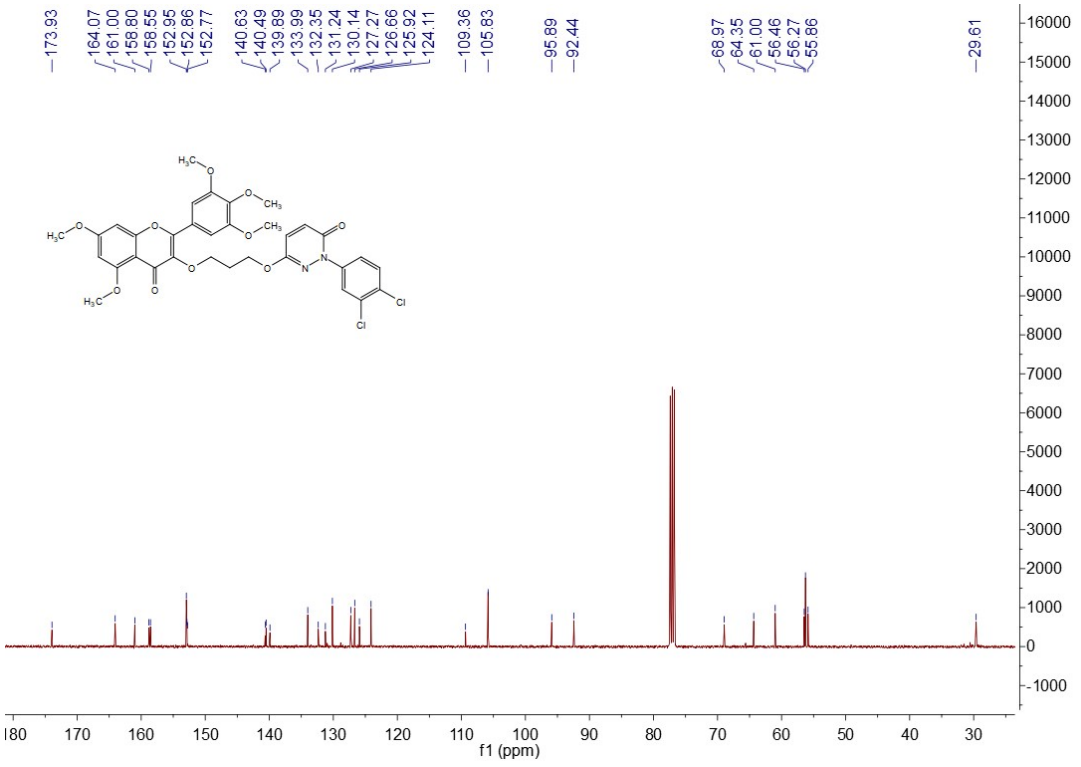
180

181 **Fig. 29** HRMS spectrum of **A9**



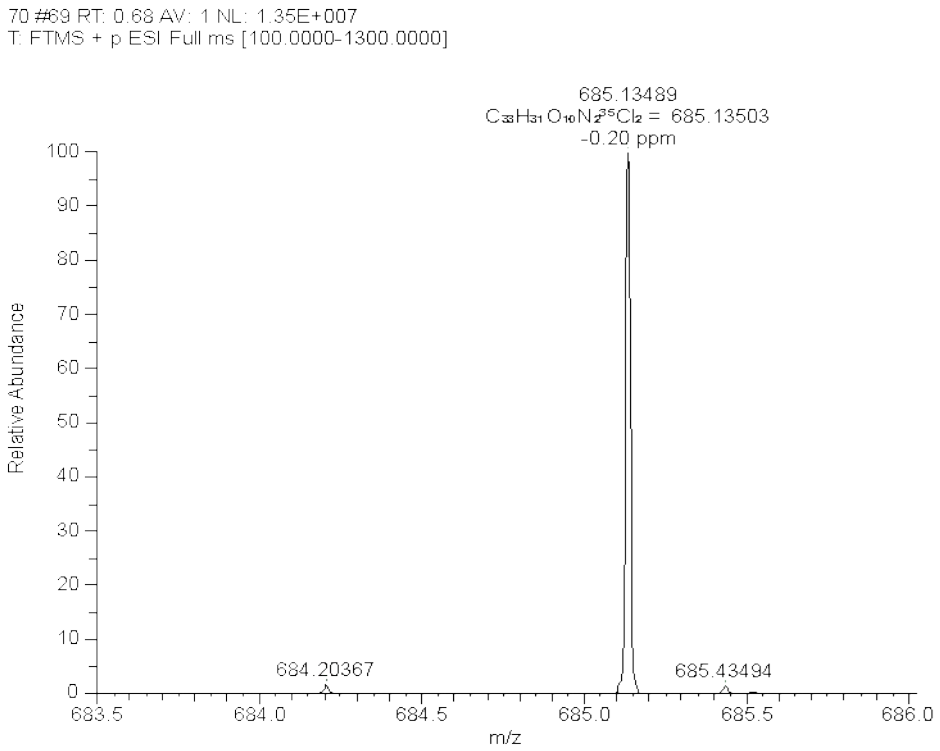
182

183 **Fig. 30** ^{1}H NMR spectrum of **A10**



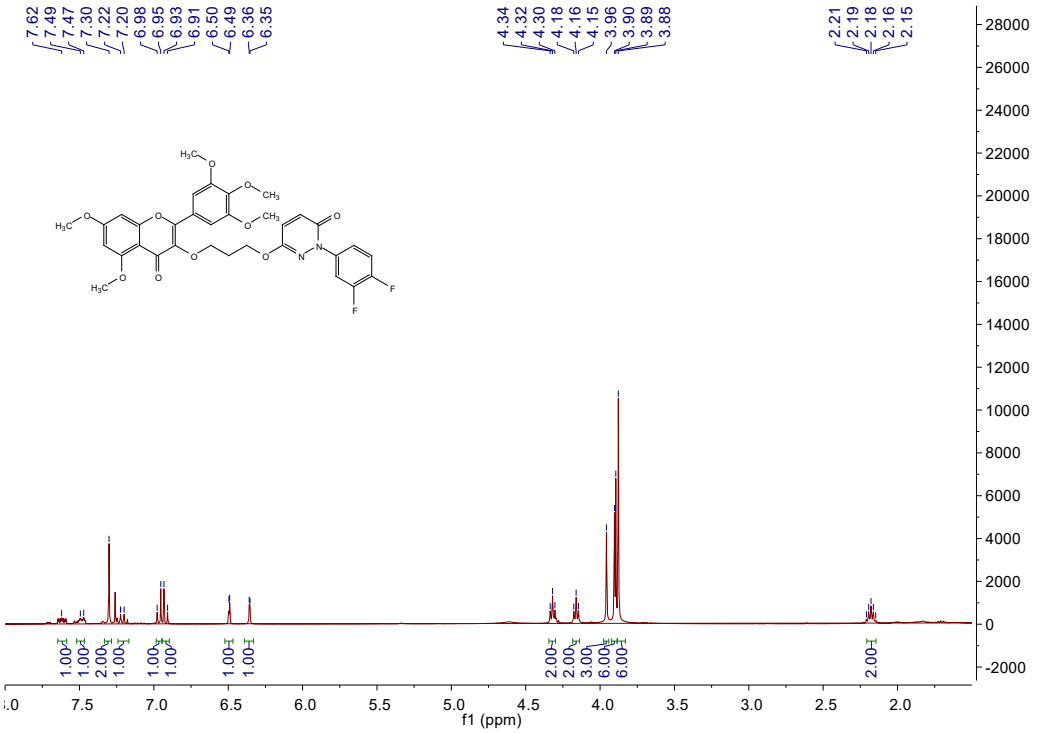
184

185 **Fig. 31** ^{13}C NMR spectrum of **A10**



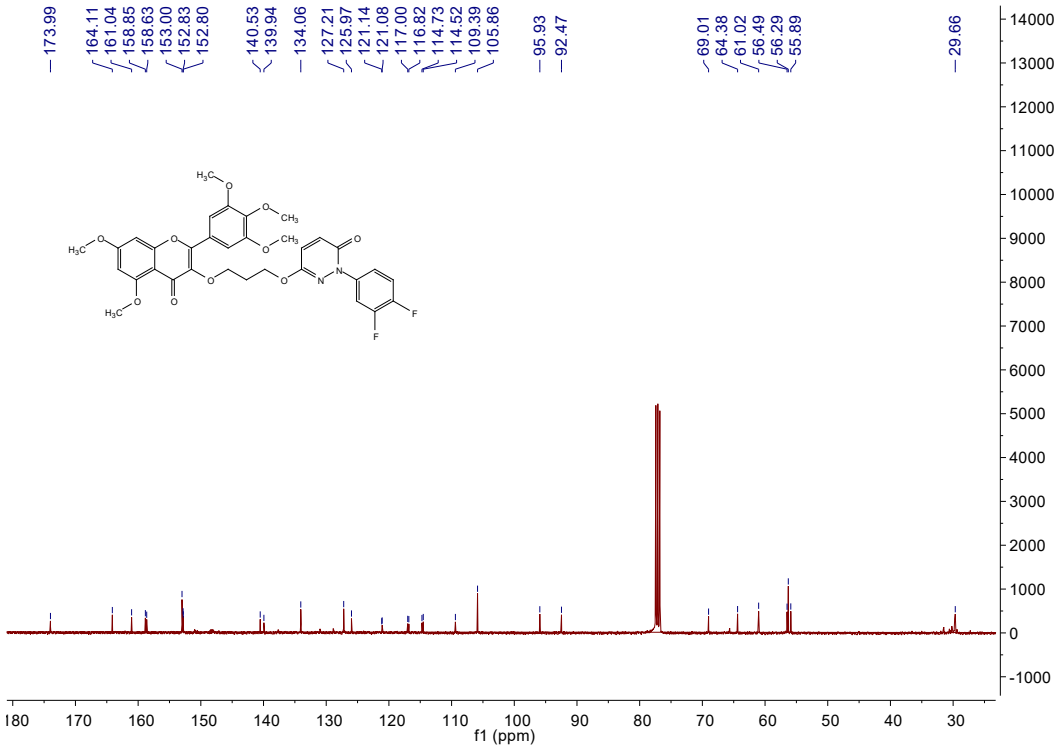
186

187 **Fig. 32** HRMS spectrum of **A10**



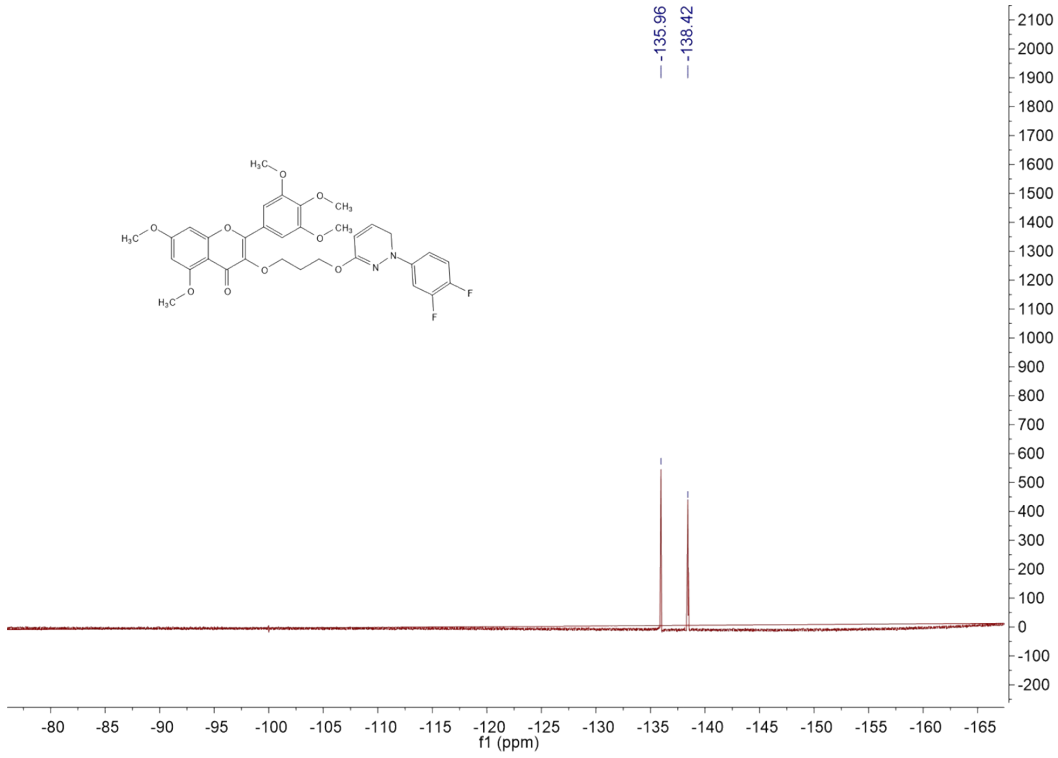
188

189 **Fig. 33** ^{1}H NMR spectrum of **A11**



190

191 **Fig. 34** ^{13}C NMR spectrum of **A11**



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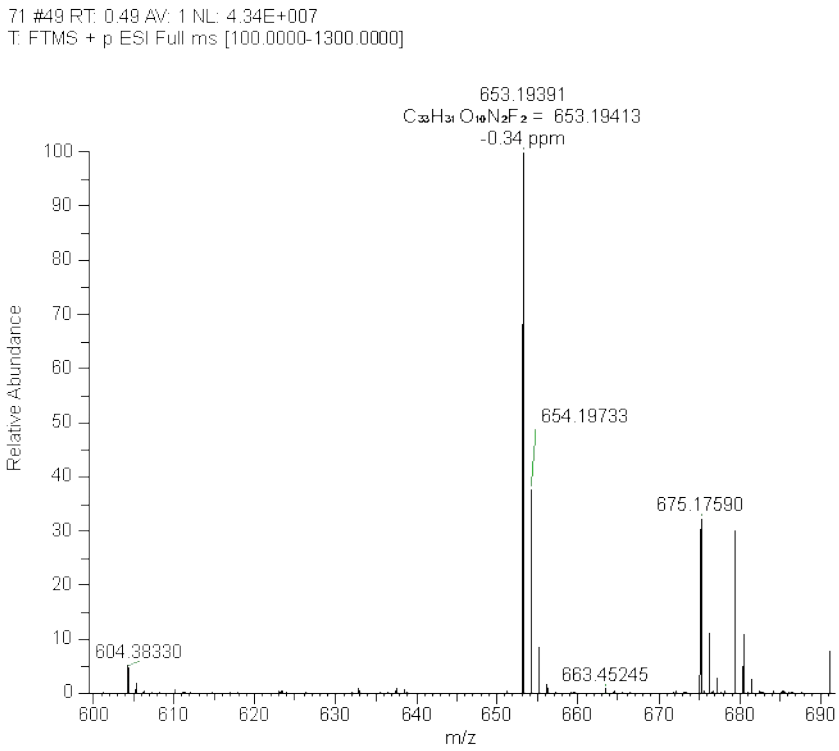
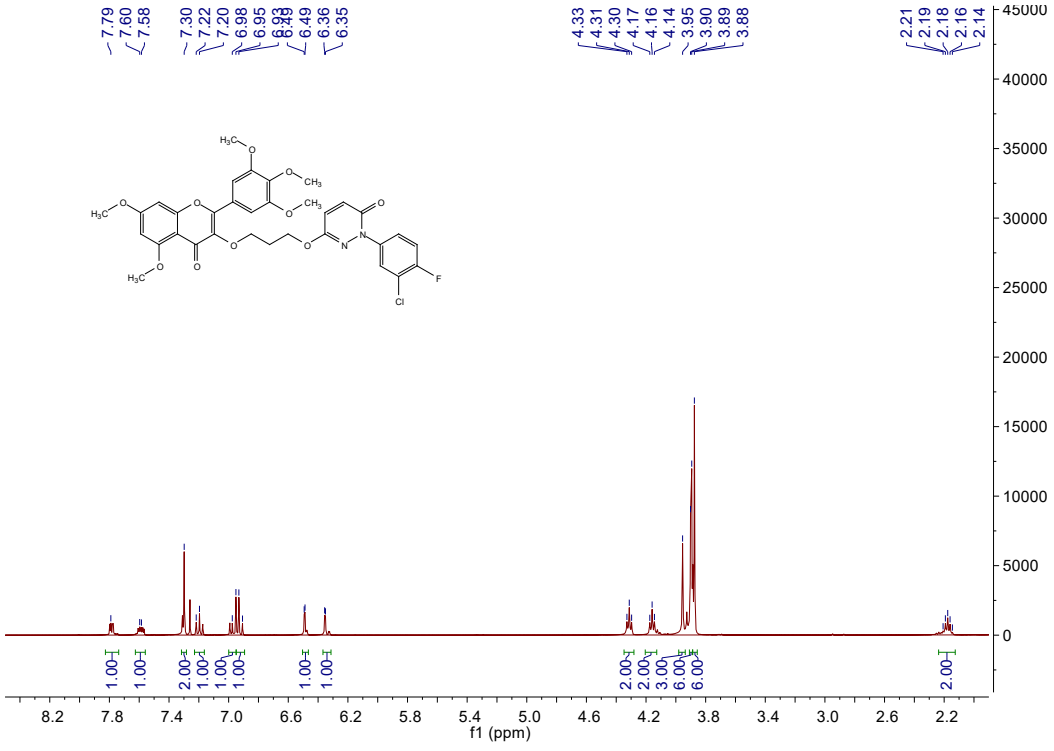
193 **Fig. 35** ^{19}F NMR spectrum of **A11**



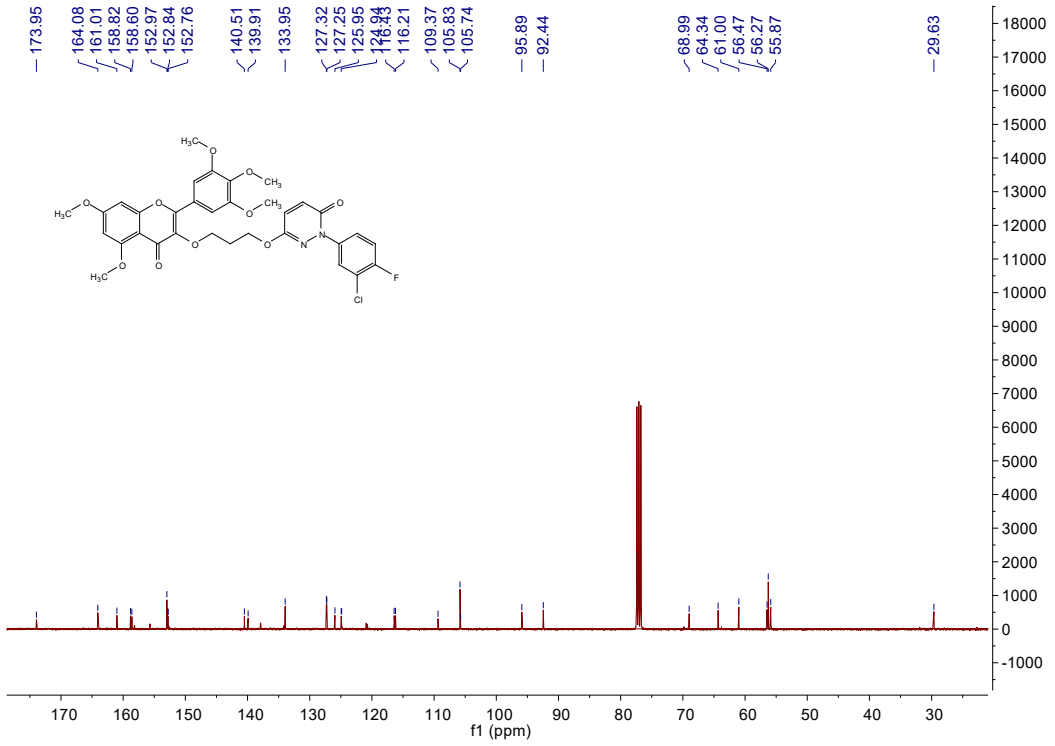
194

195 **Fig. 36** HRMS spectrum of **A11**



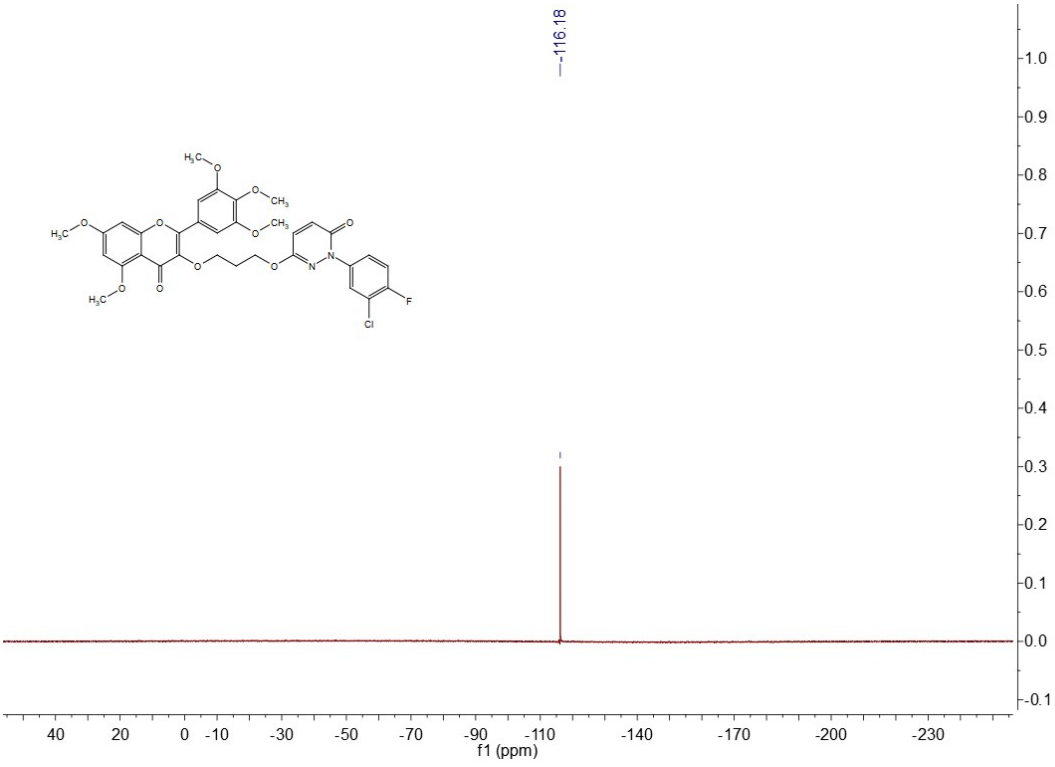
196

197 **Fig. 37** ^{1}H NMR spectrum of **A12**



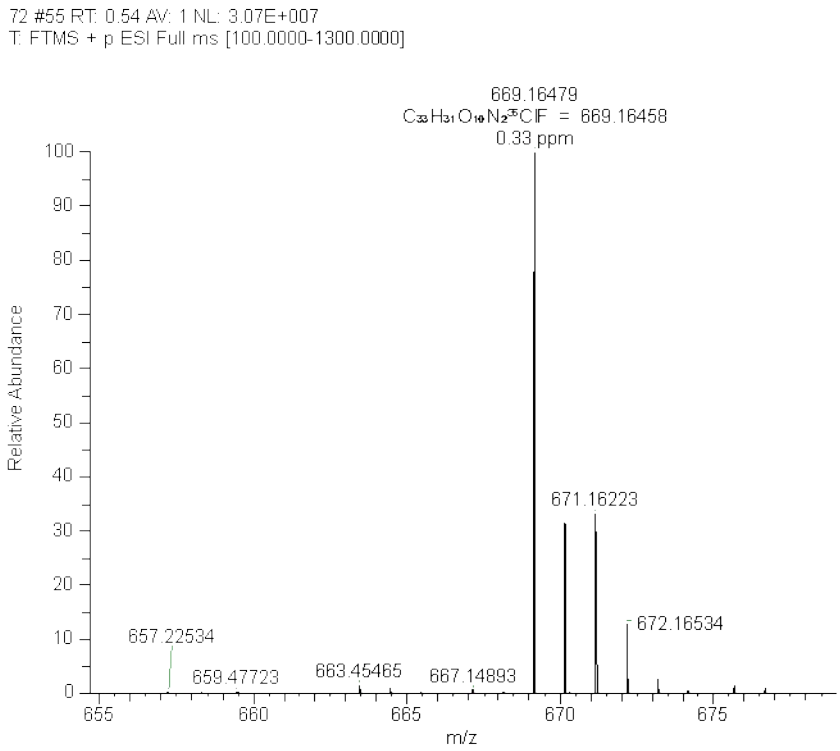
198

199 **Fig. 38** ^{13}C NMR spectrum of **A12**



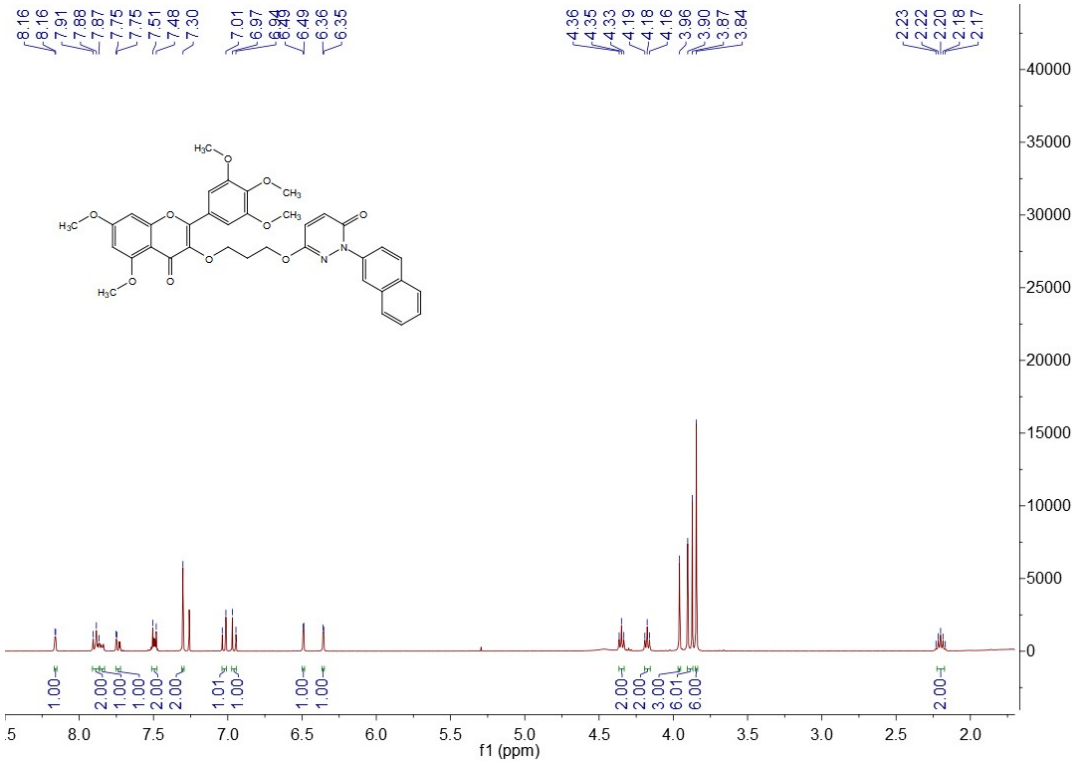
200

201 **Fig. 39** ^{19}F NMR spectrum of **A12**



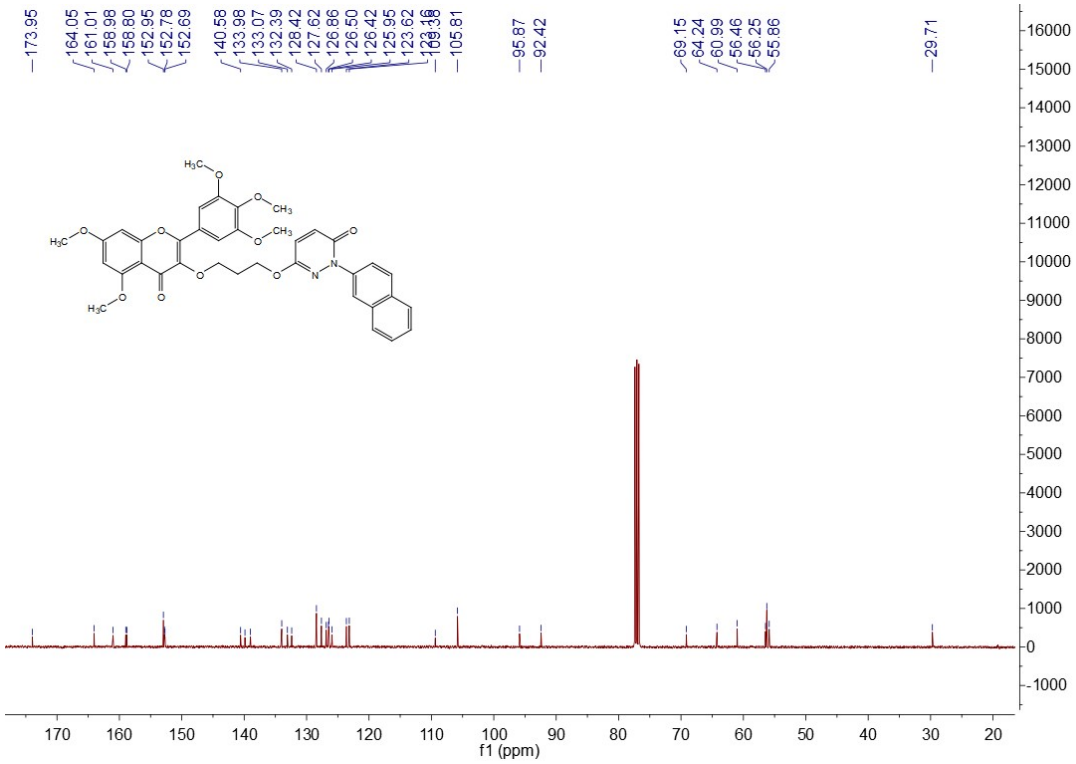
202

203 **Fig. 40** HRMS spectrum of **A12**



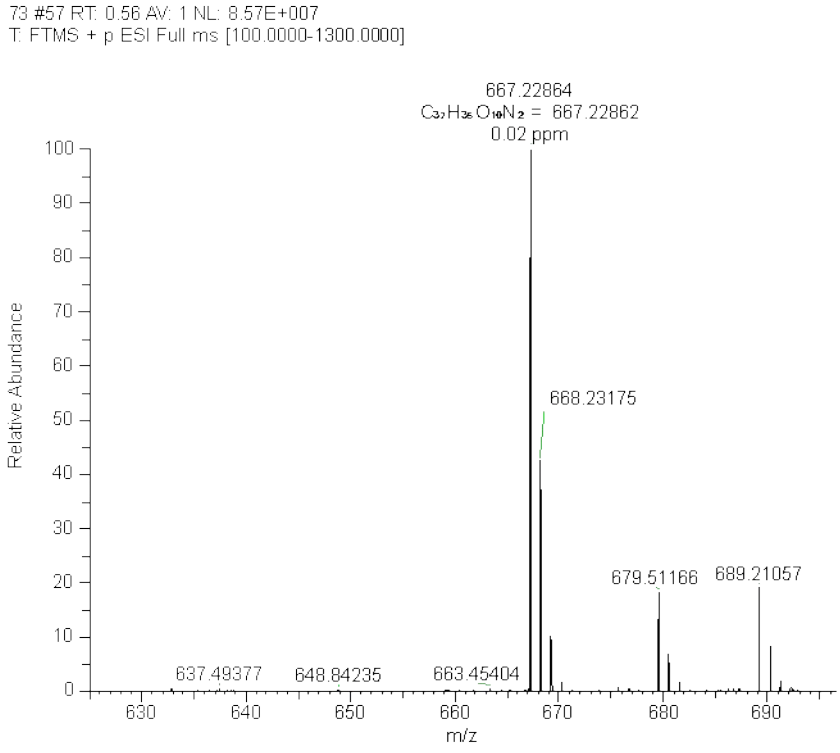
204

205 **Fig. 41** ^{1}H NMR spectrum of **A13**



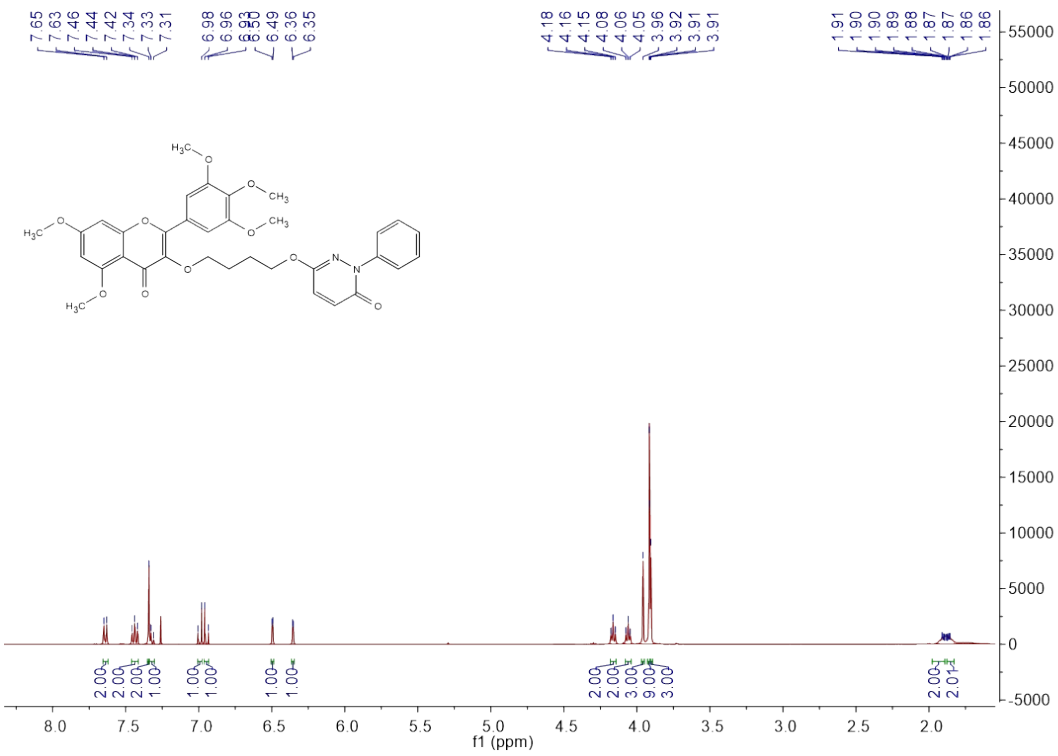
206

207 **Fig. 42** ^{13}C NMR spectrum of **A13**



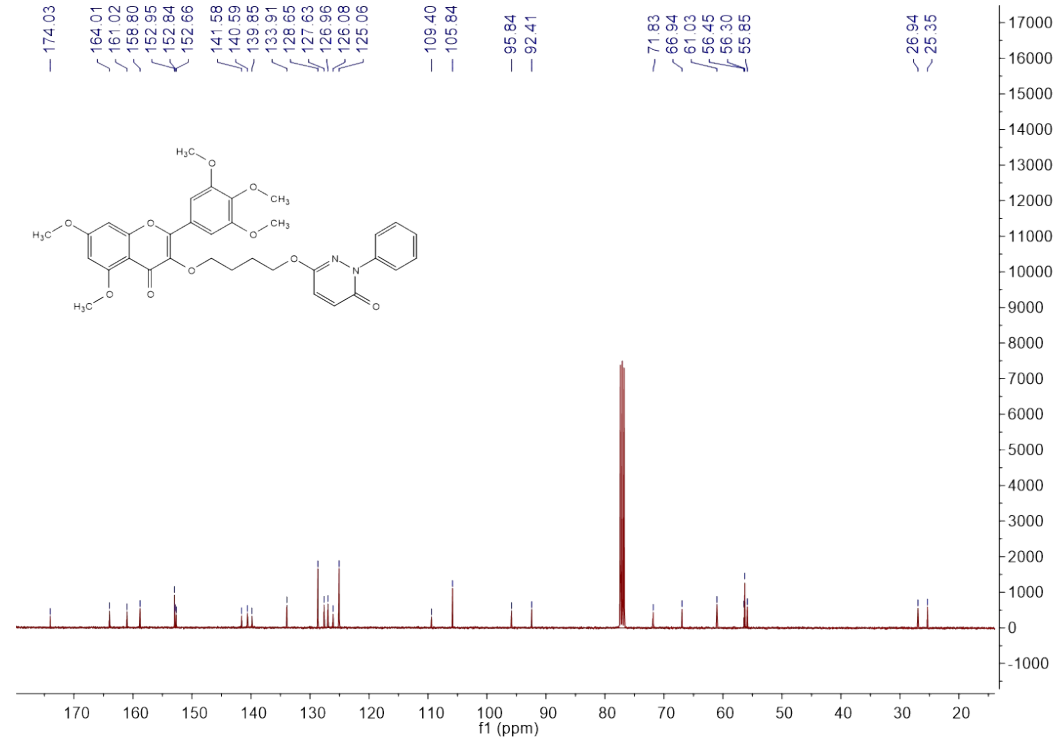
208

209 **Fig. 43** HRMS spectrum of **A13**



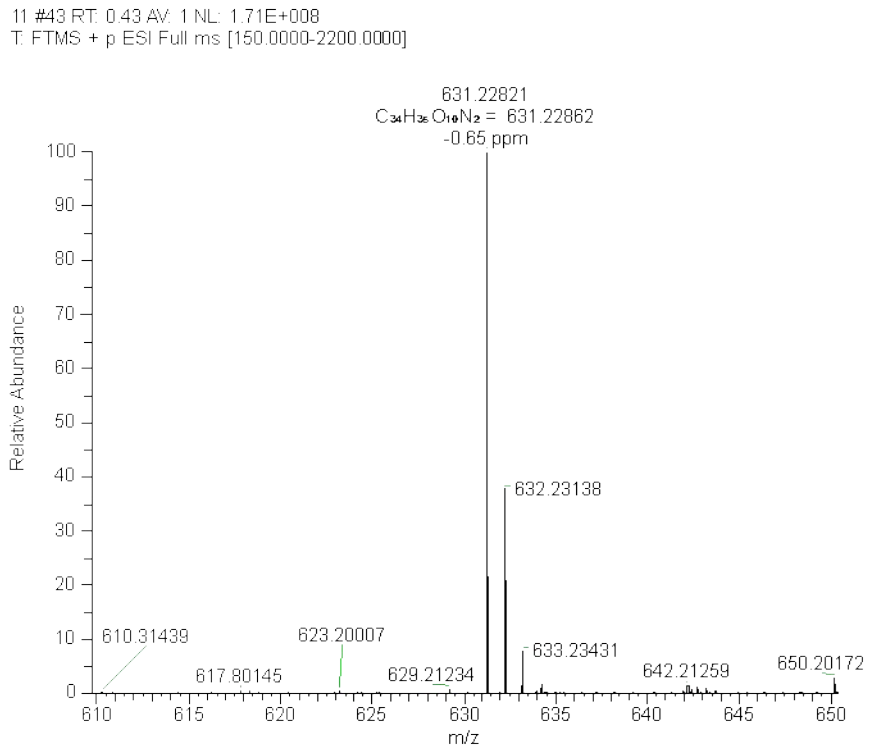
210

211 **Fig. 44** ^{1}H NMR spectrum of **A14**



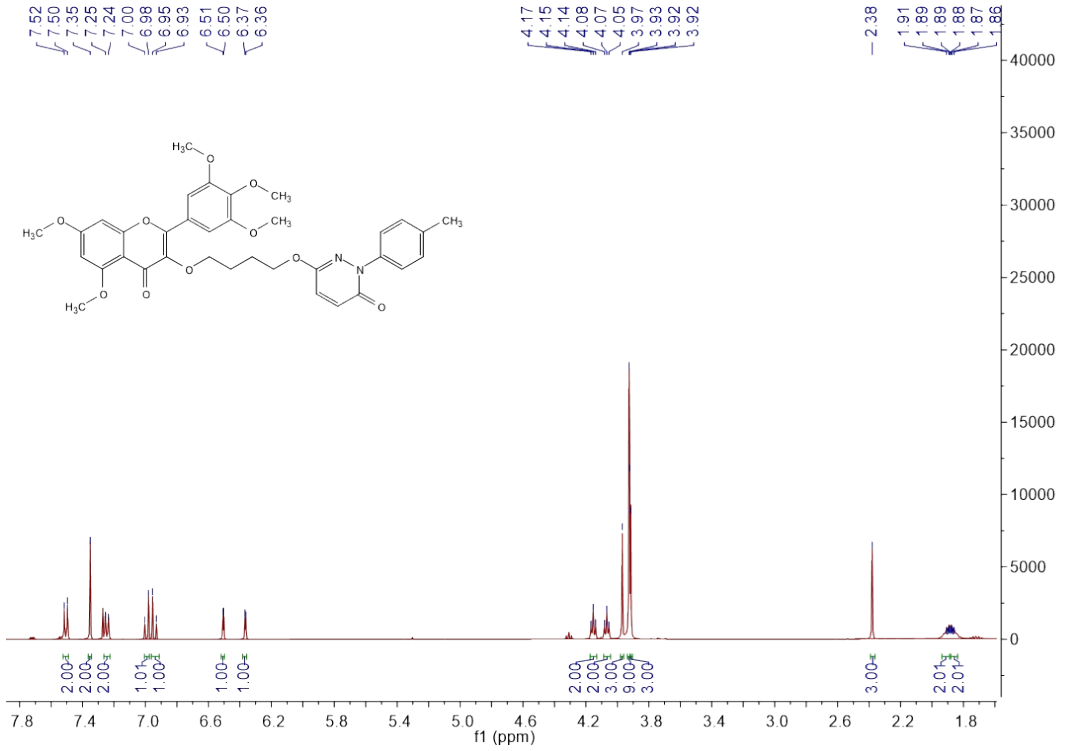
212

213 **Fig. 45** ^{13}C NMR spectrum of **A14**



214

215 **Fig. 46** HRMS spectrum of **A14**



216

217 **Fig. 47** ^{1}H NMR spectrum of **A15**

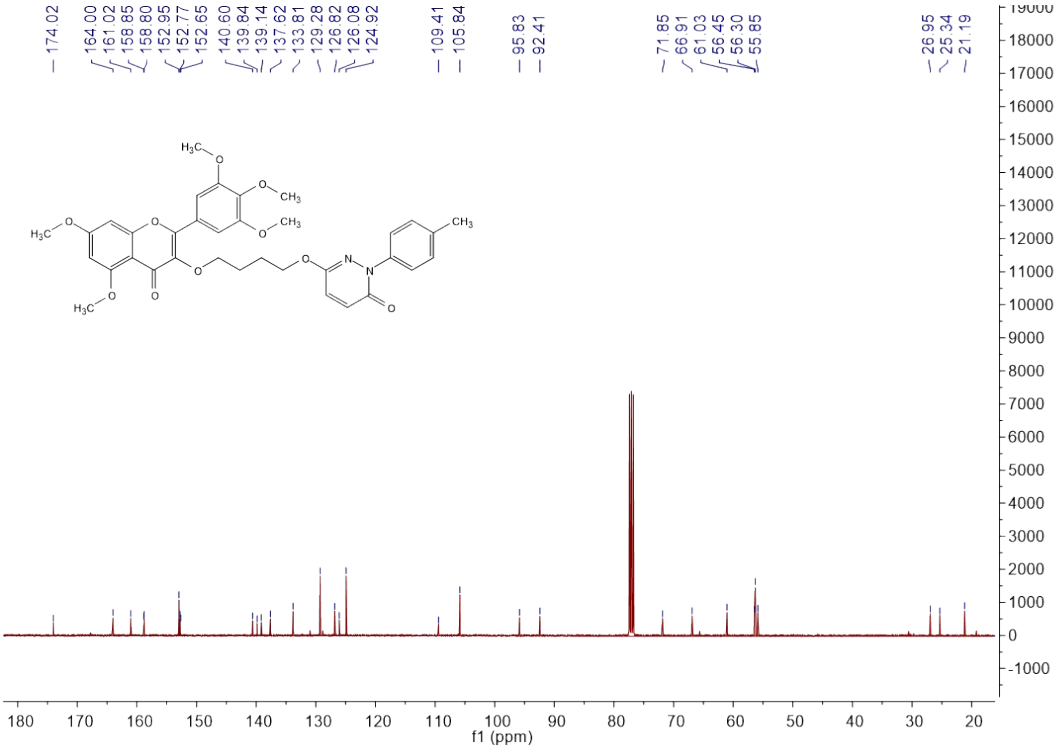


Fig. 48 ^{13}C NMR spectrum of **A15**

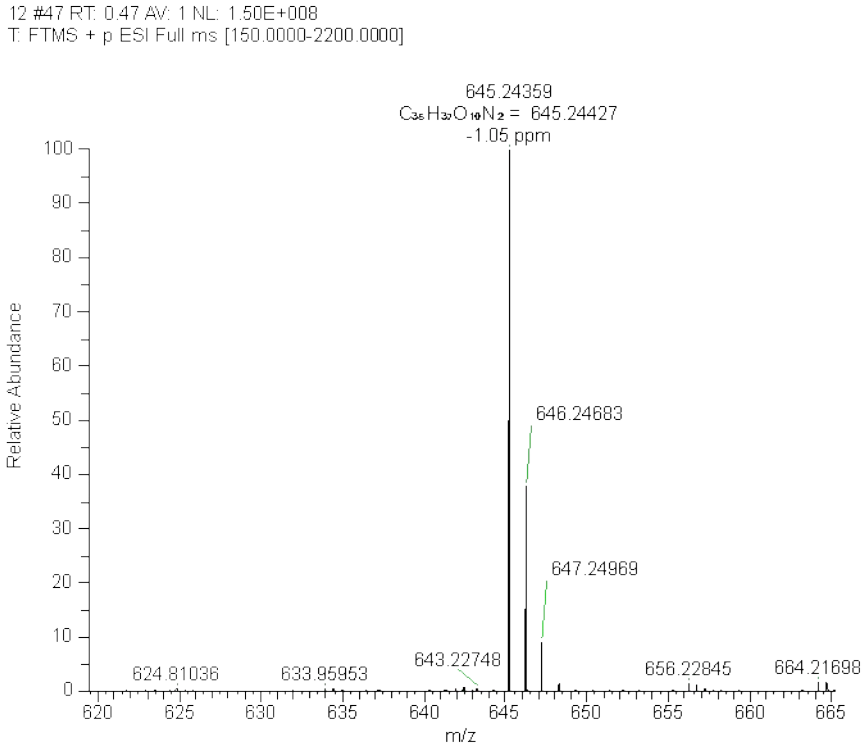
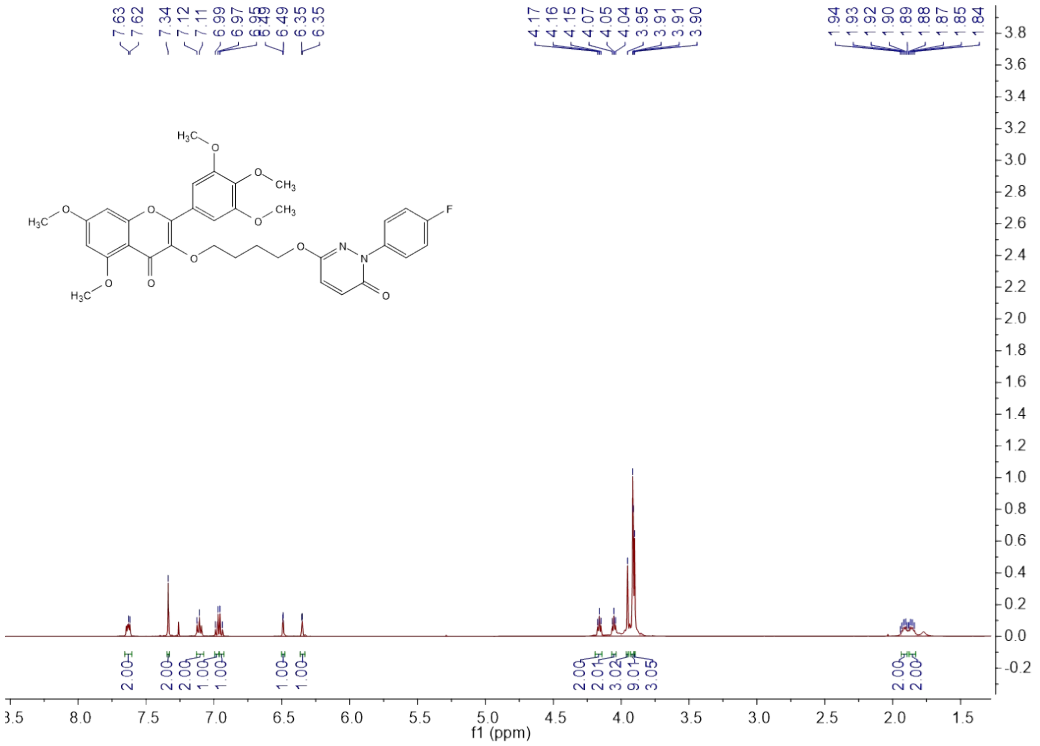
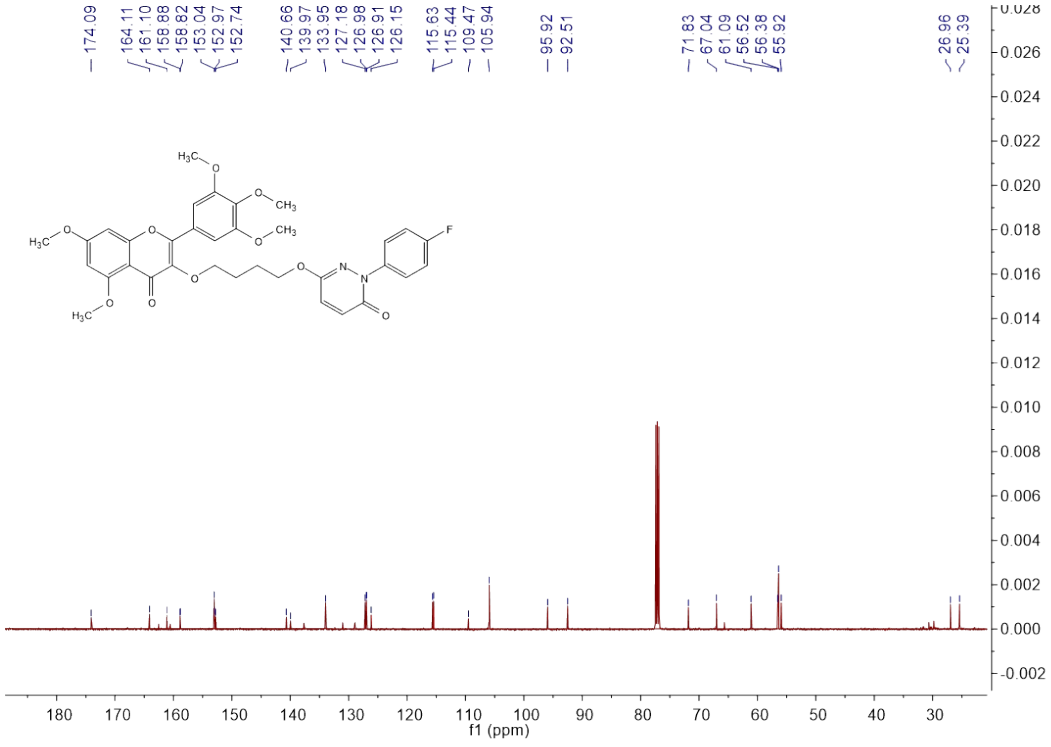


Fig. 49 HRMS spectrum of **A15**



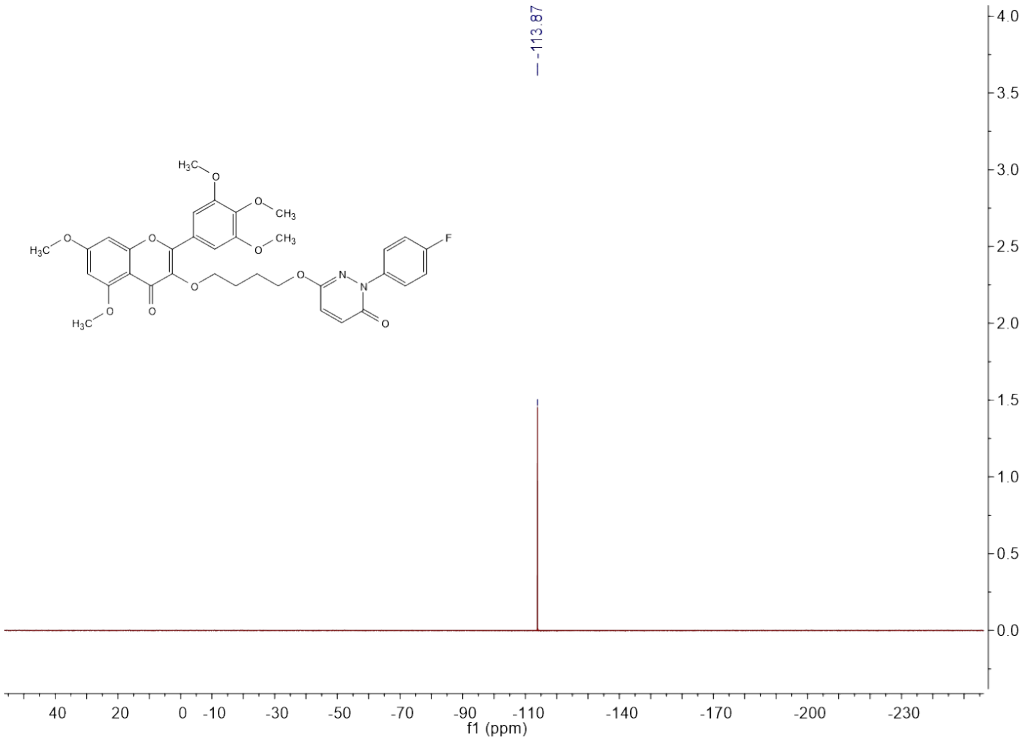
222

223 **Fig. 50** ^{1}H NMR spectrum of **A16**



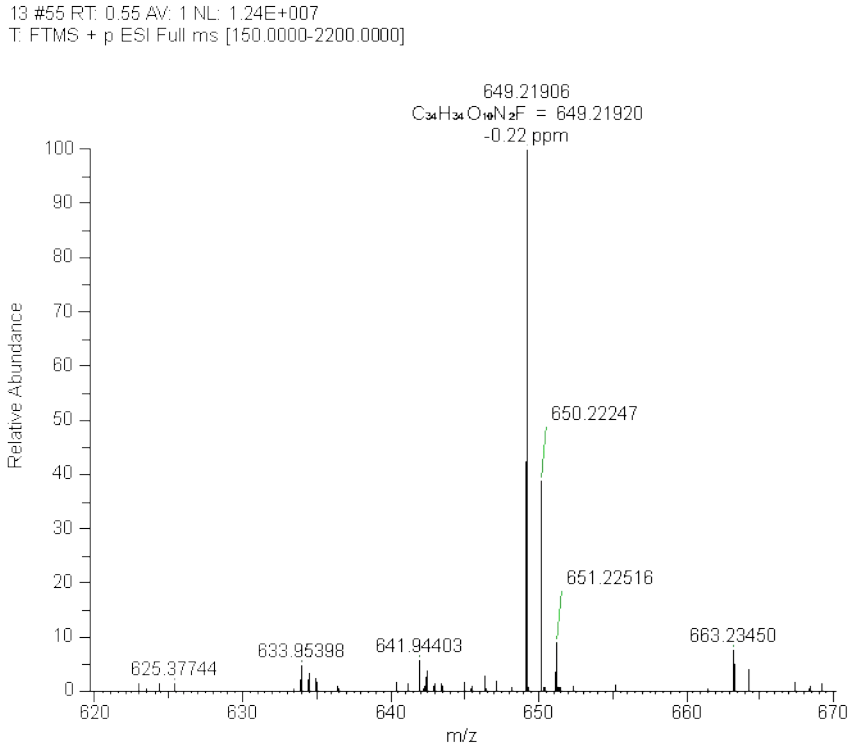
224

225 **Fig. 51** ^{13}C NMR spectrum of **A16**



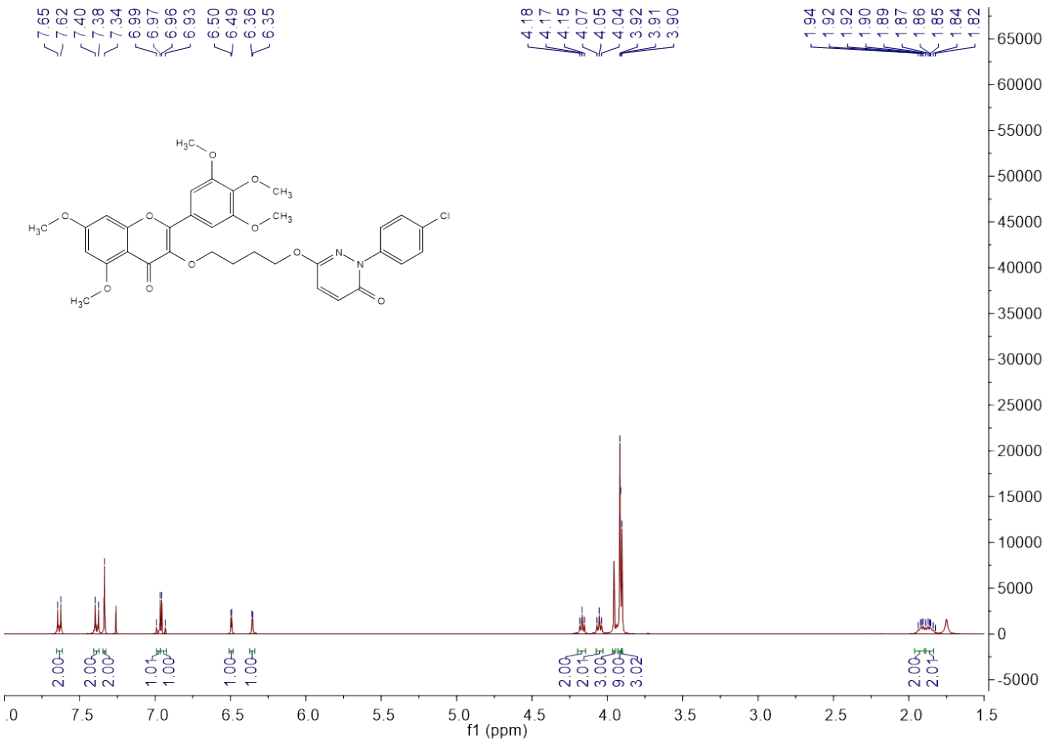
226

227 **Fig. 52** ^{19}F NMR spectrum of **A16**



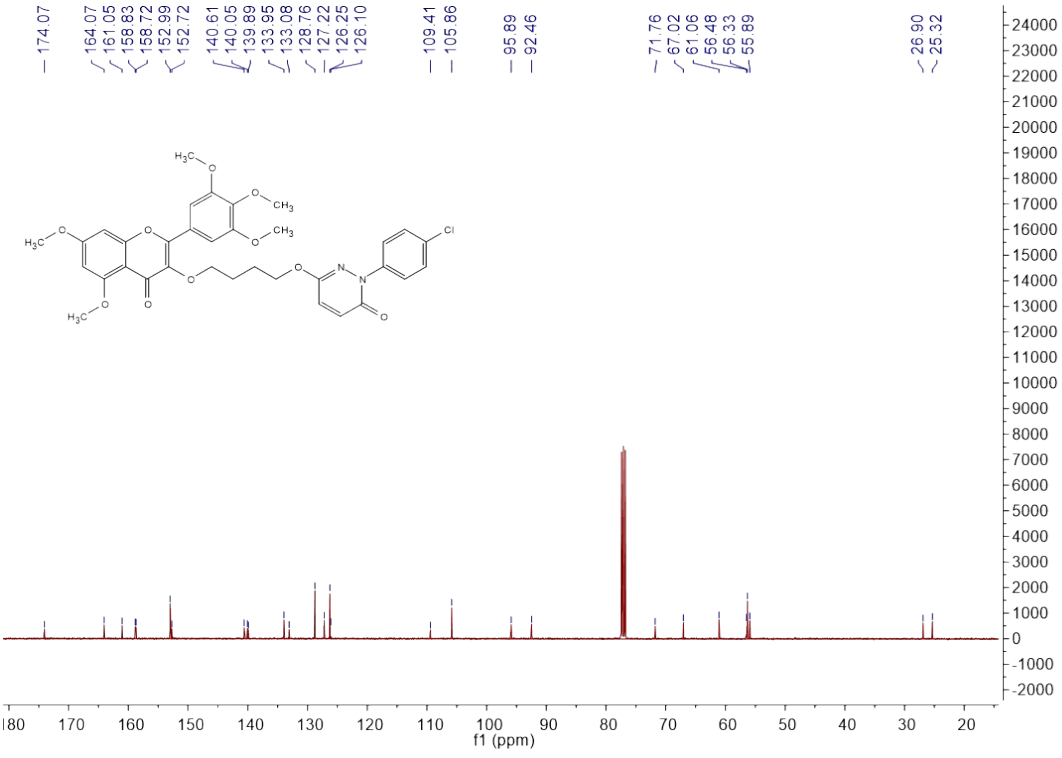
228

229 **Fig. 53** HRMS spectrum of **A16**



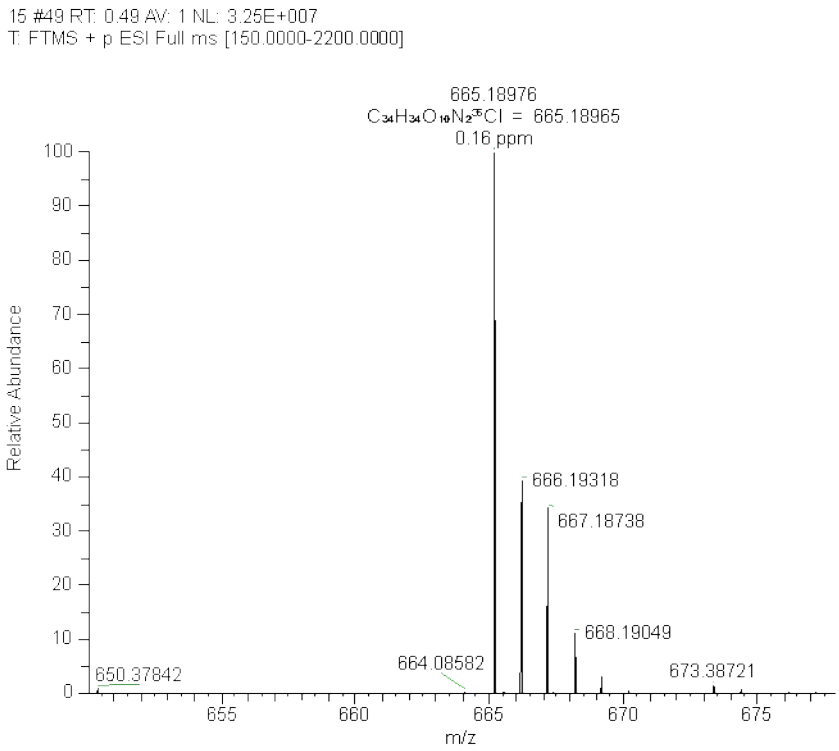
230

231 **Fig. 54** ^{1}H NMR spectrum of **A17**



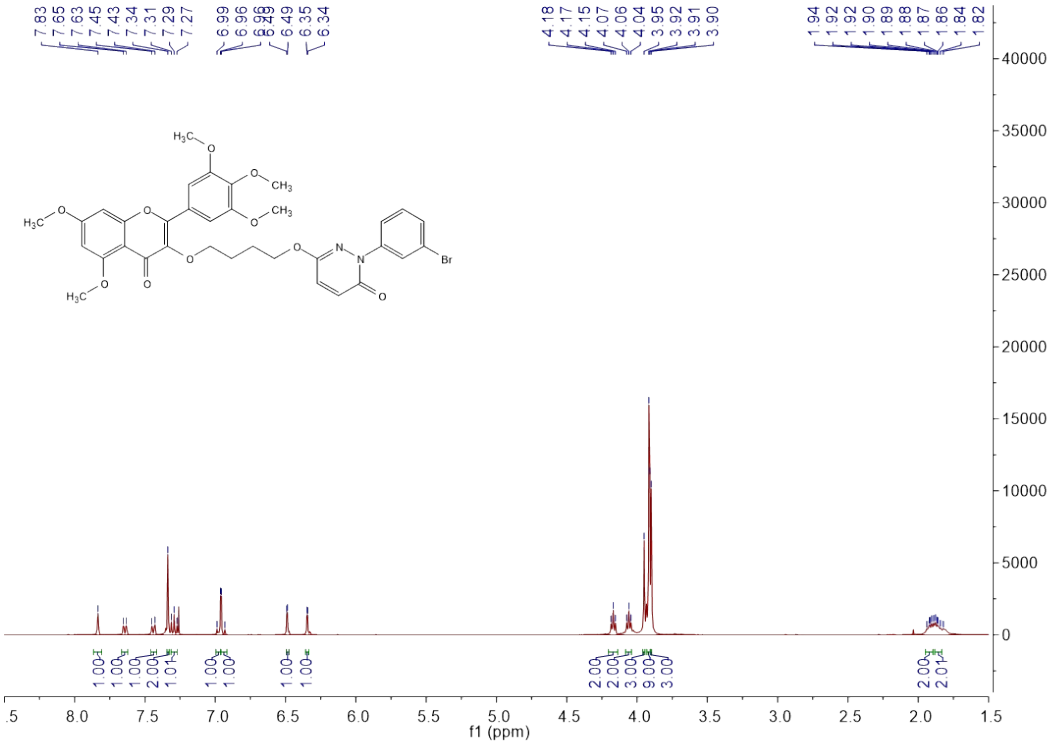
232

233 **Fig. 55** ^{13}C NMR spectrum of **A17**



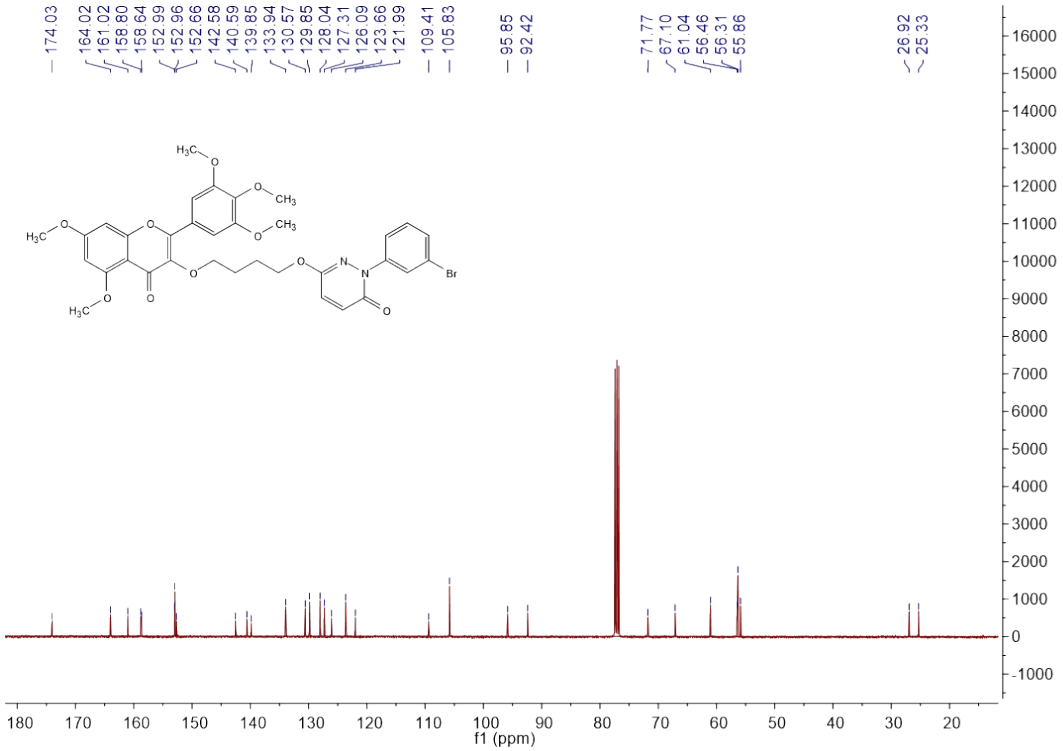
234

235 **Fig. 56** HRMS spectrum of **A17**



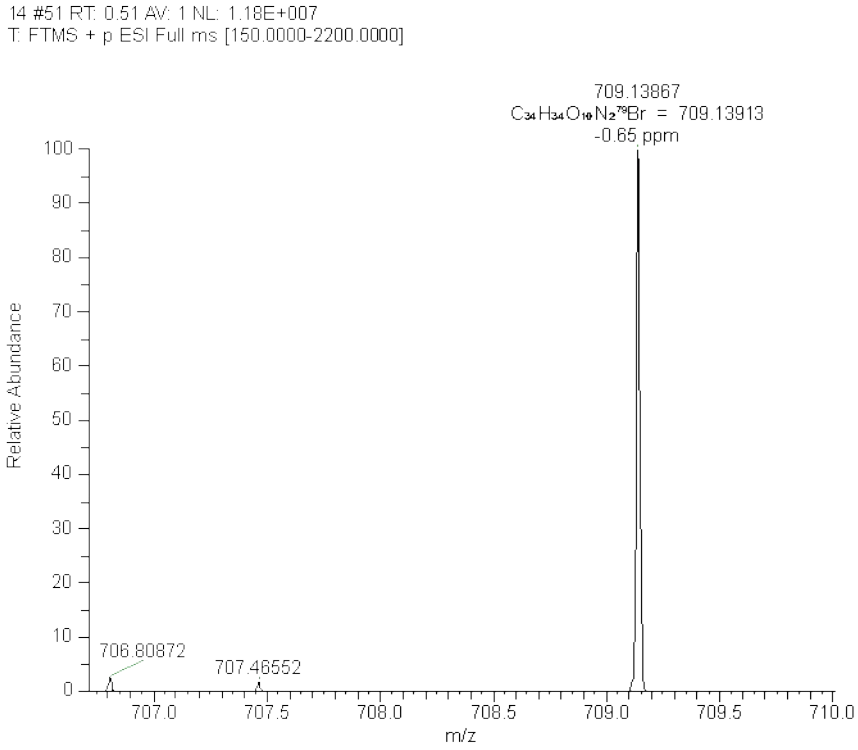
236

237 **Fig. 57** ^{1}H NMR spectrum of **A18**



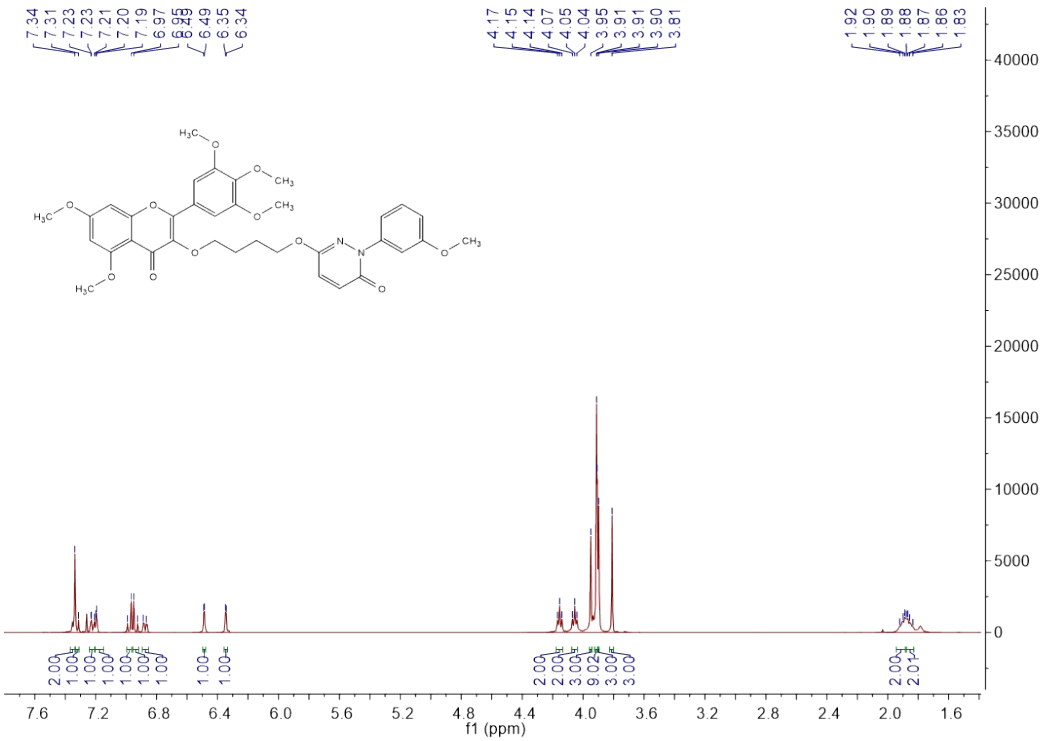
238

239 **Fig. 58** ^{13}C NMR spectrum of **A18**



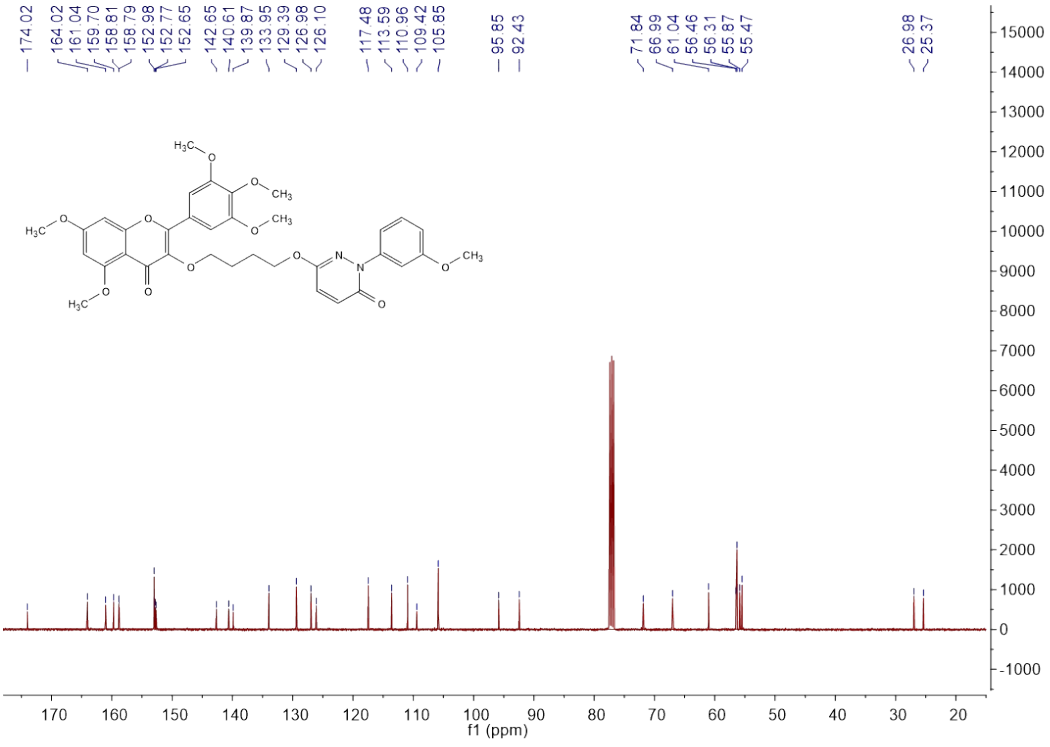
240

241 **Fig. 59** HRMS spectrum of **A18**



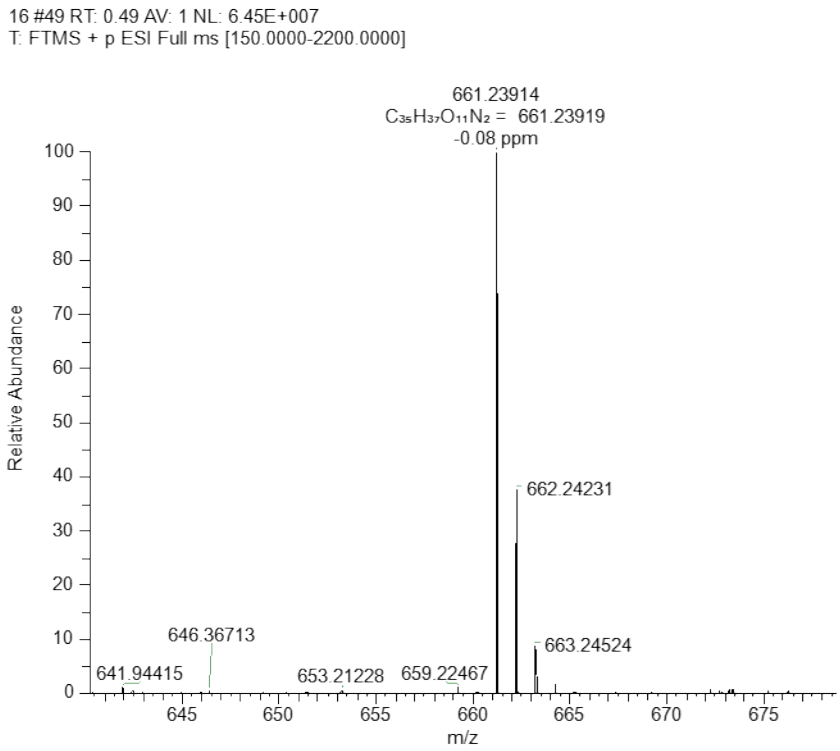
242

243 **Fig. 60** ^{1}H NMR spectrum of **A19**



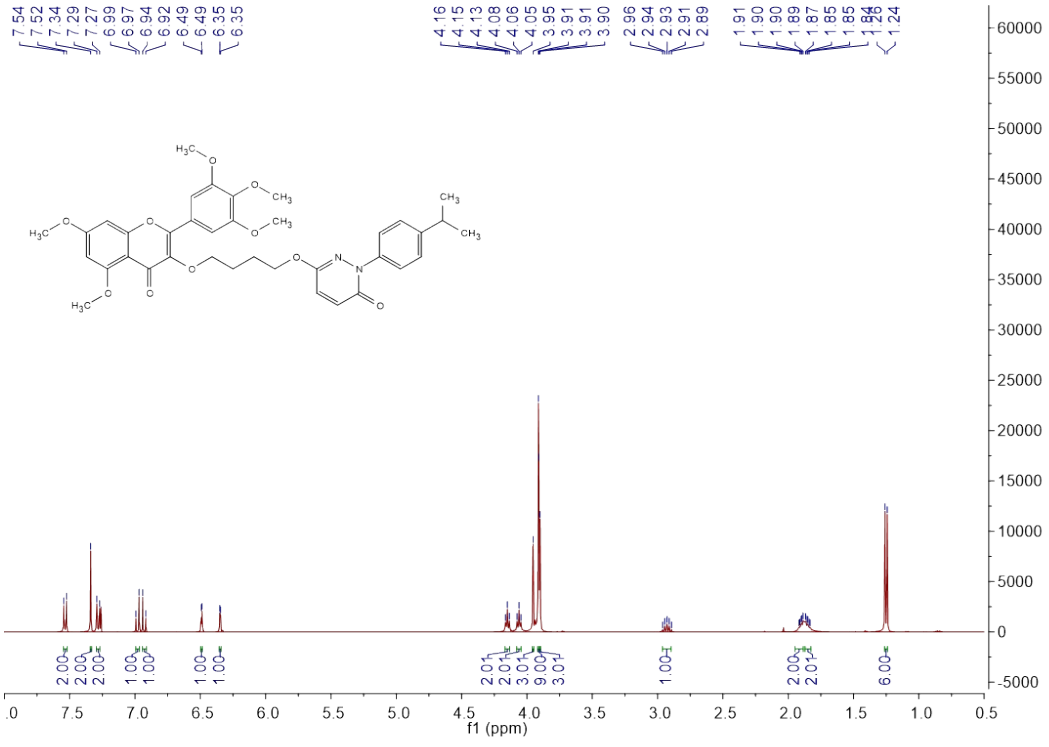
244

245 **Fig. 61** ^{13}C NMR spectrum of **A19**



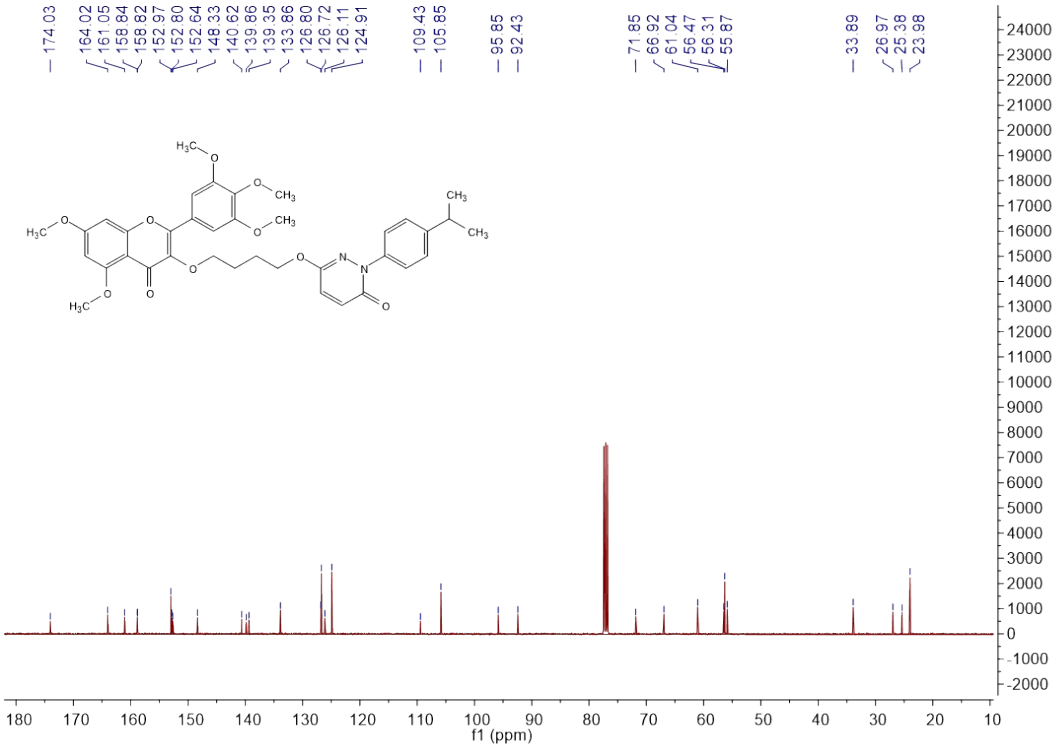
246

247 **Fig. 62** HRMS spectrum of **A19**



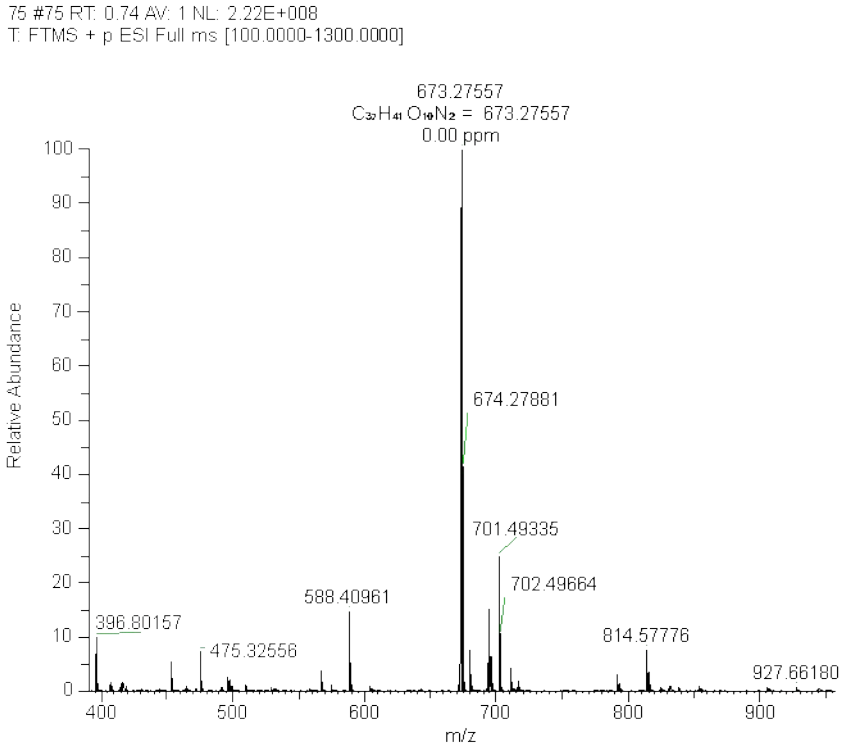
248

249 **Fig. 63** ^{1}H NMR spectrum of **A20**



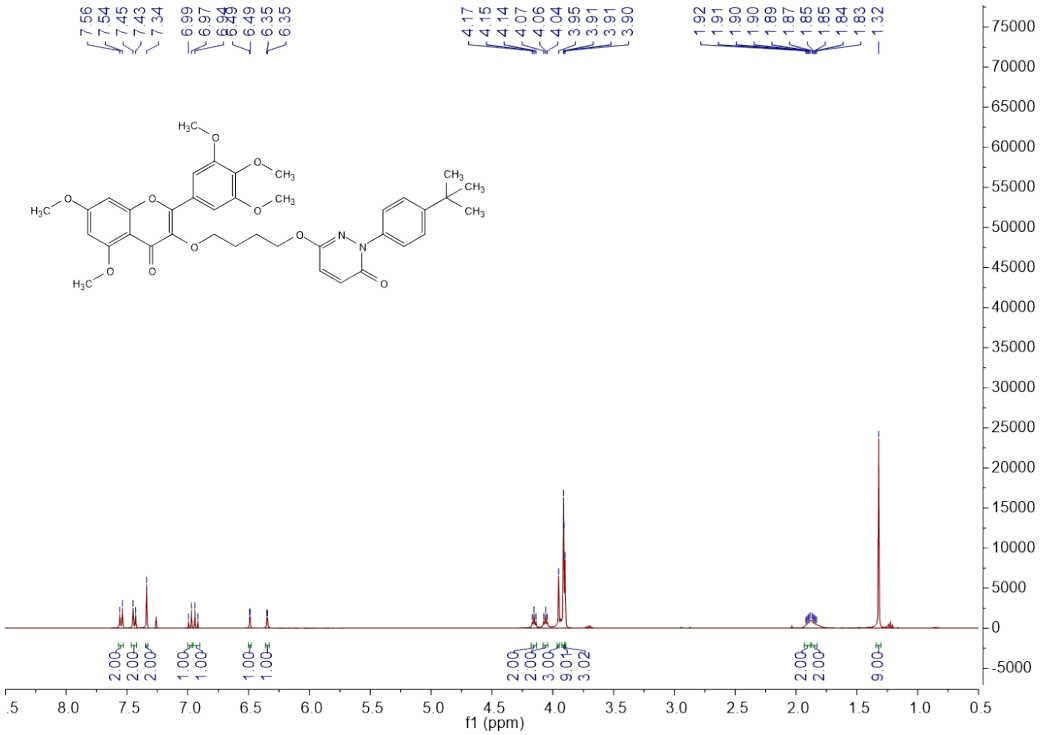
250

251 **Fig. 64** ^{13}C NMR spectrum of **A20**



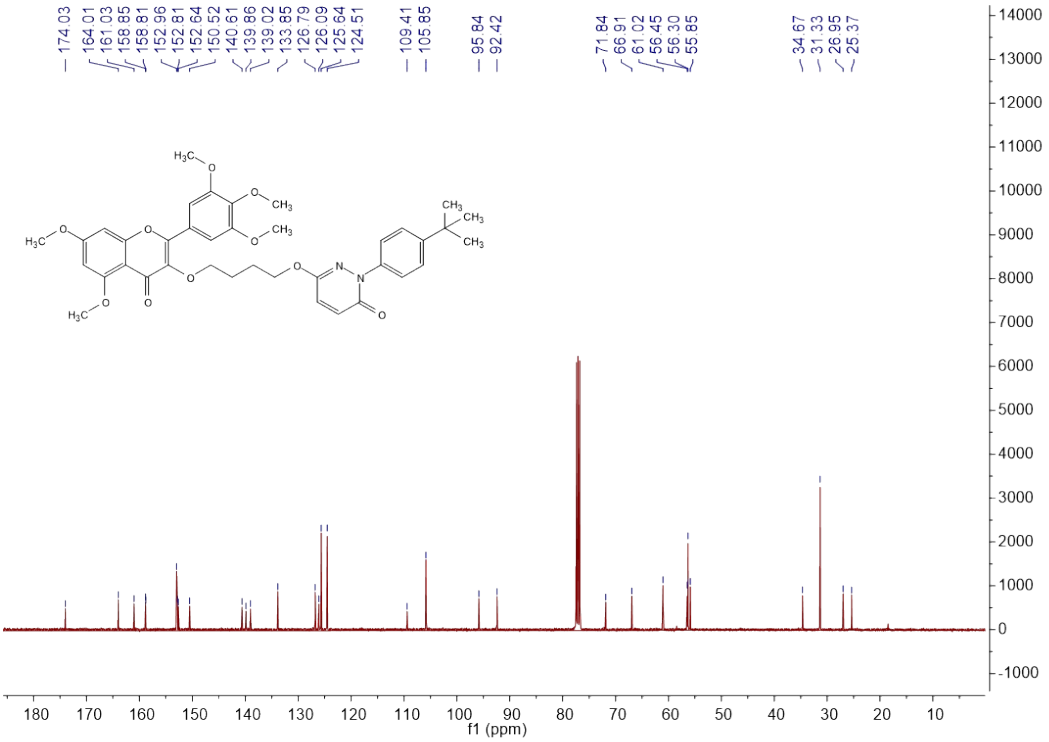
252

253 **Fig. 65** HRMS spectrum of **A20**



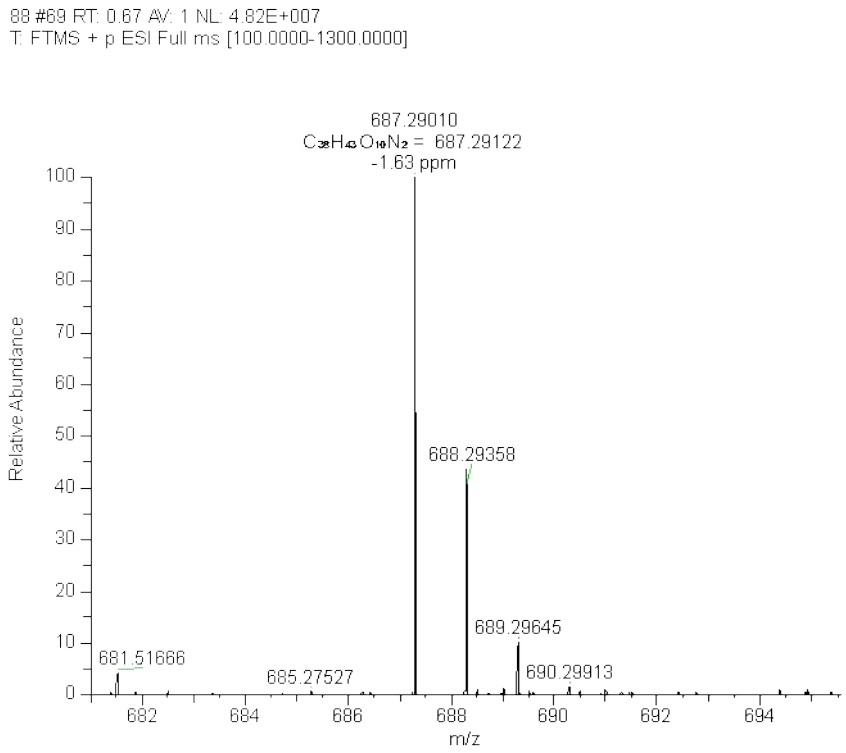
254

255 **Fig. 66** ^{1}H NMR spectrum of **A21**



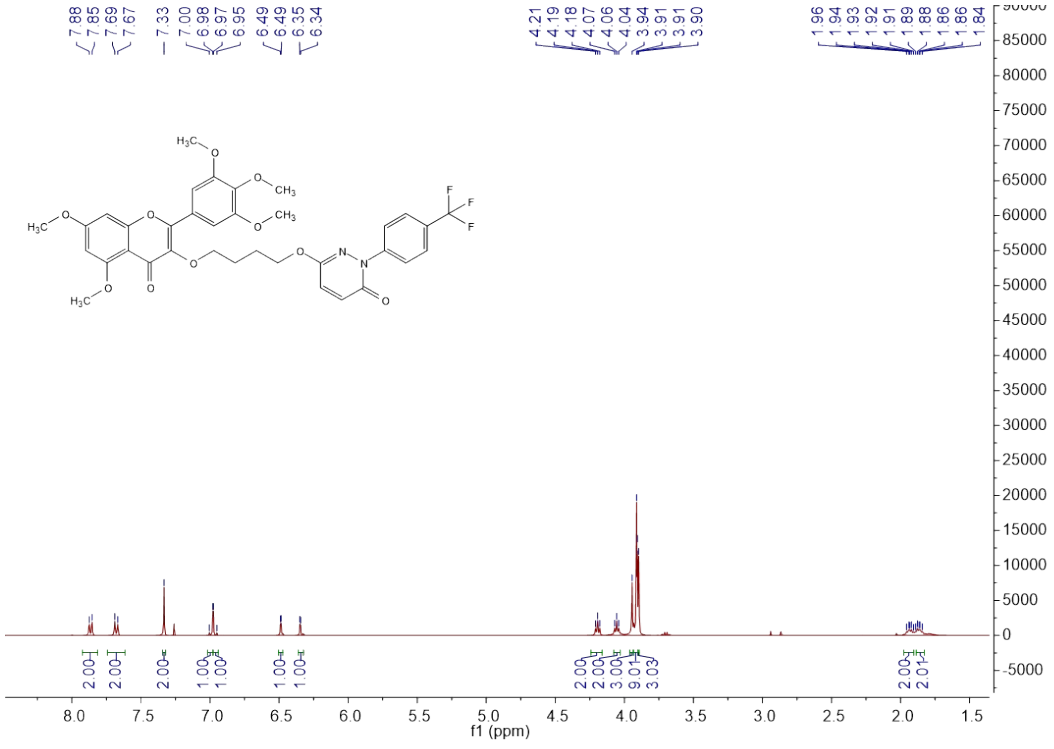
256

257 **Fig. 67** ^{13}C NMR spectrum of **A21**



258

259 **Fig. 68** HRMS spectrum of **A21**



260

261 **Fig. 69** ^{1}H NMR spectrum of **A22**

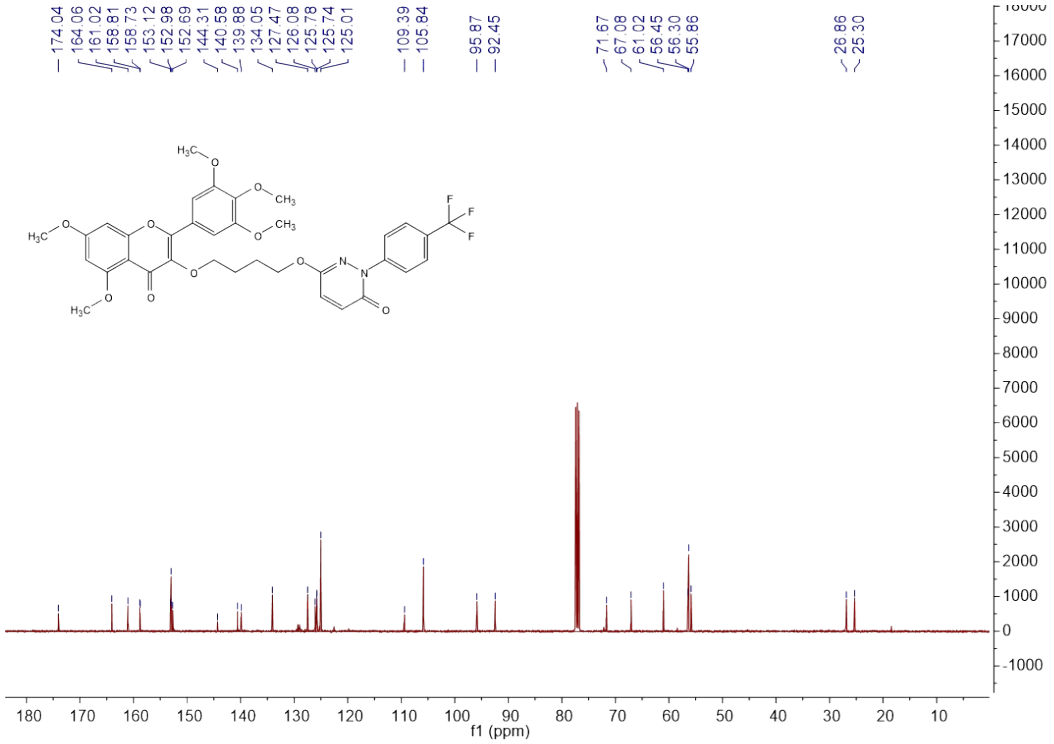


Fig. 70 ^{13}C NMR spectrum of **A22**

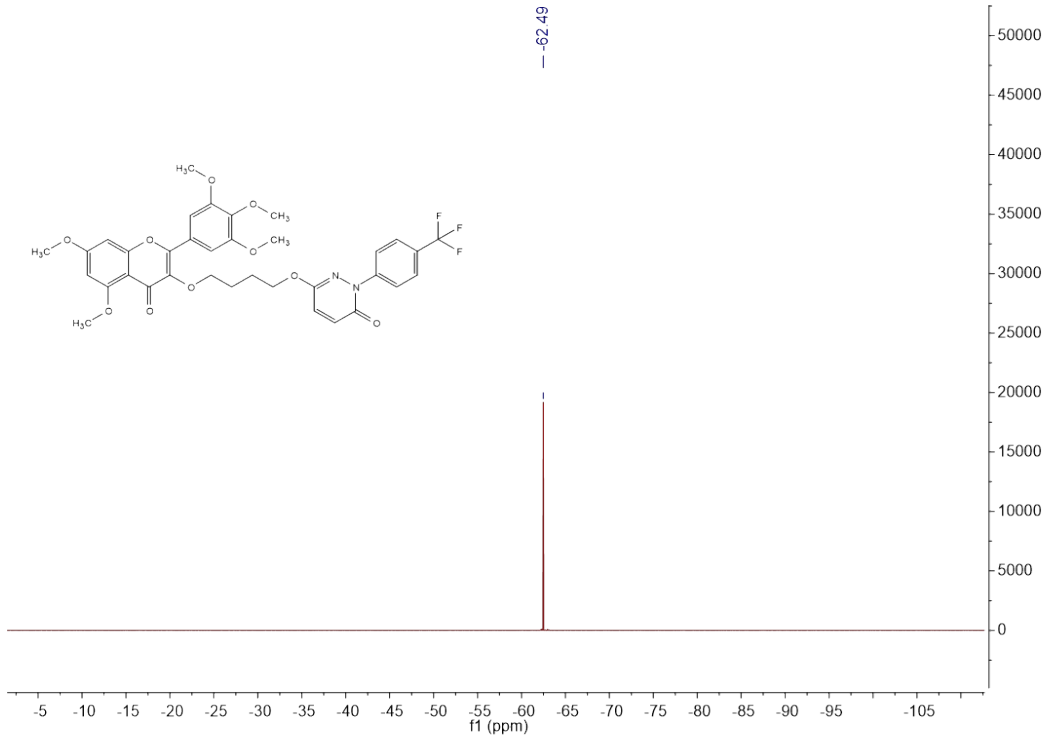
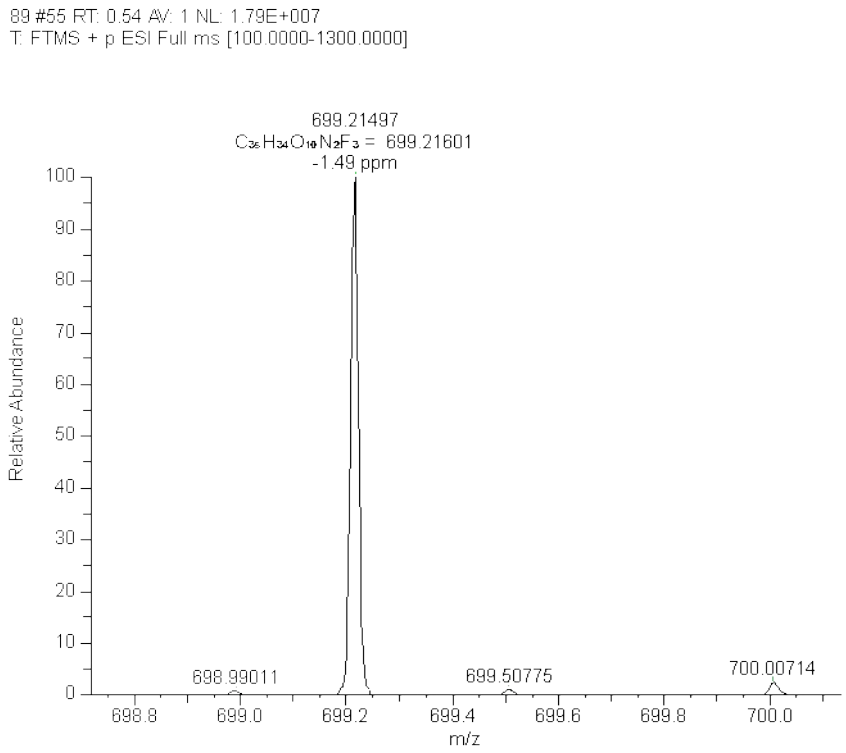
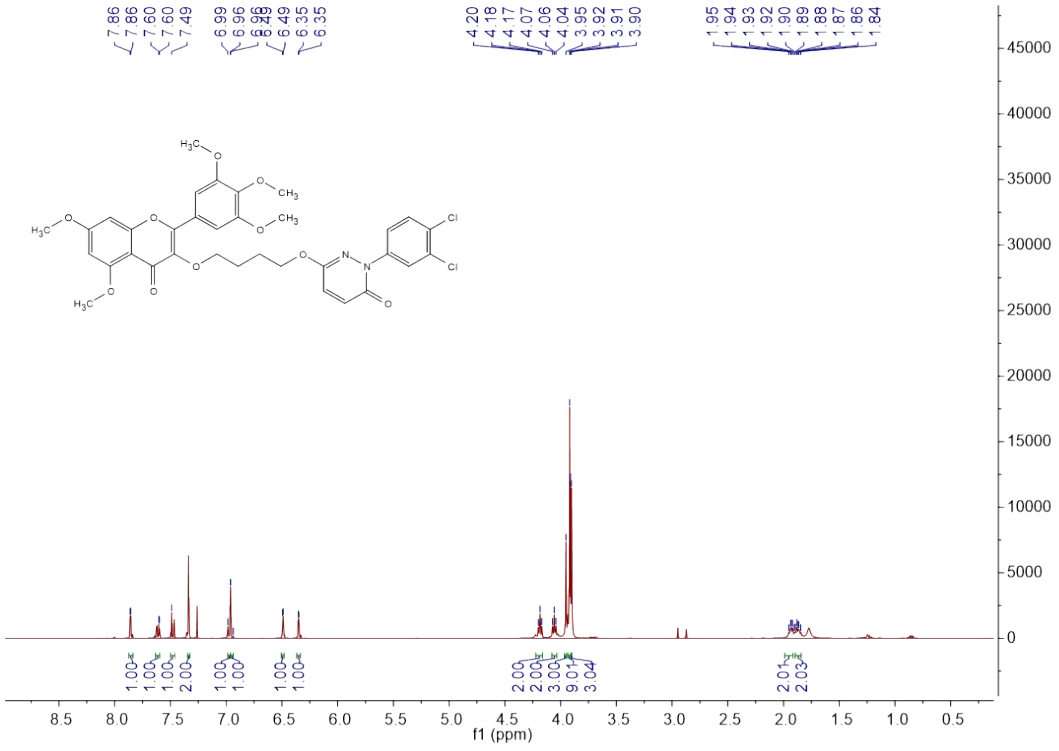


Fig. 71 ^{19}F NMR spectrum of **A22**



266

267 **Fig. 72** HRMS spectrum of **A22**



268

269 **Fig. 73** ^{1}H NMR spectrum of **A23**

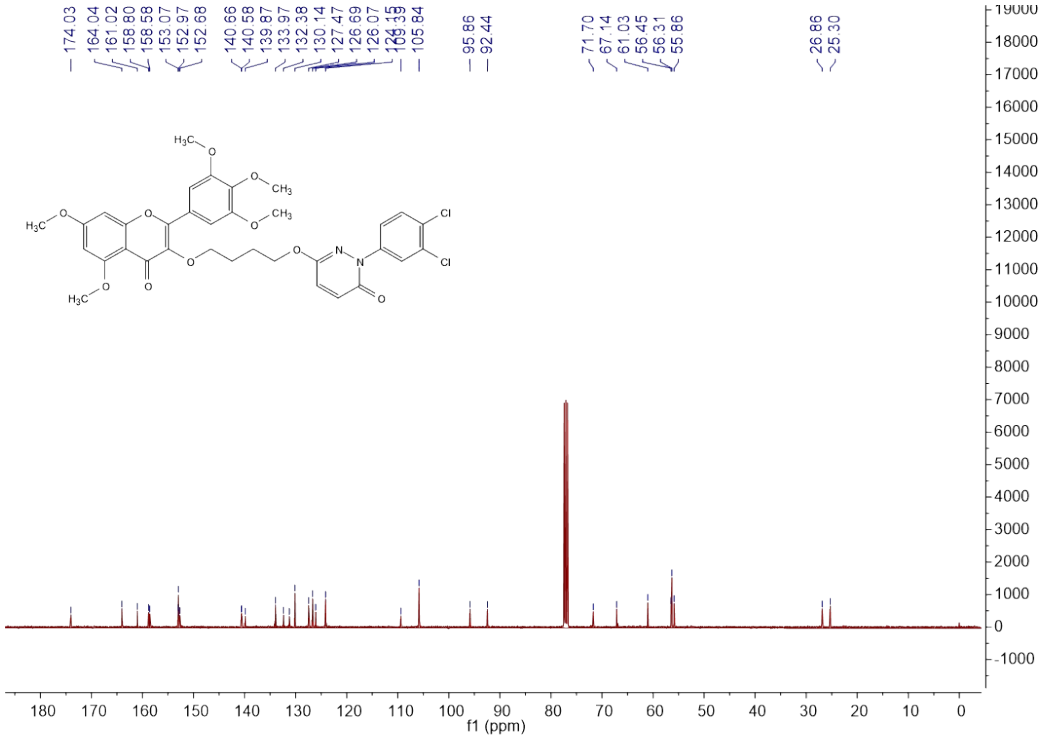


Fig. 74 ^{13}C NMR spectrum of **A23**

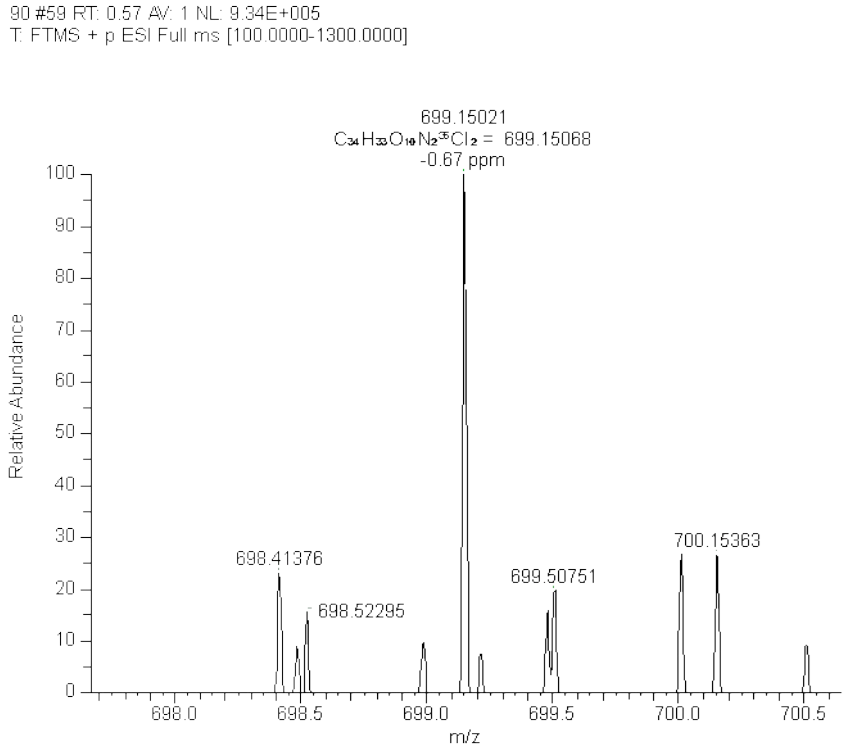
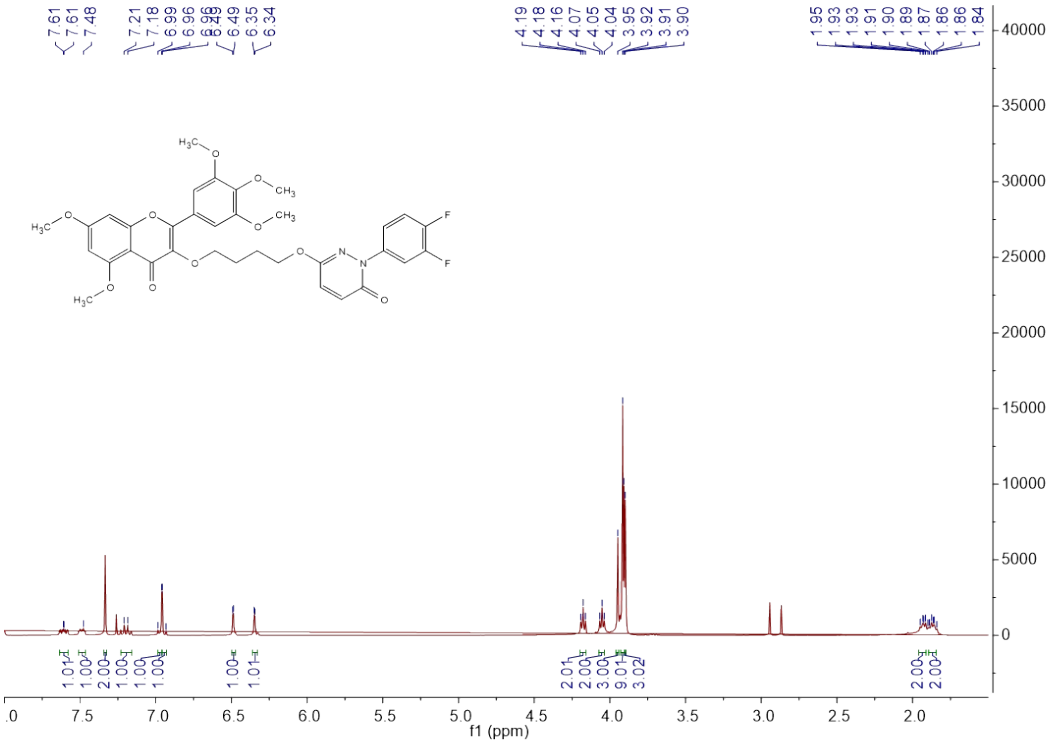
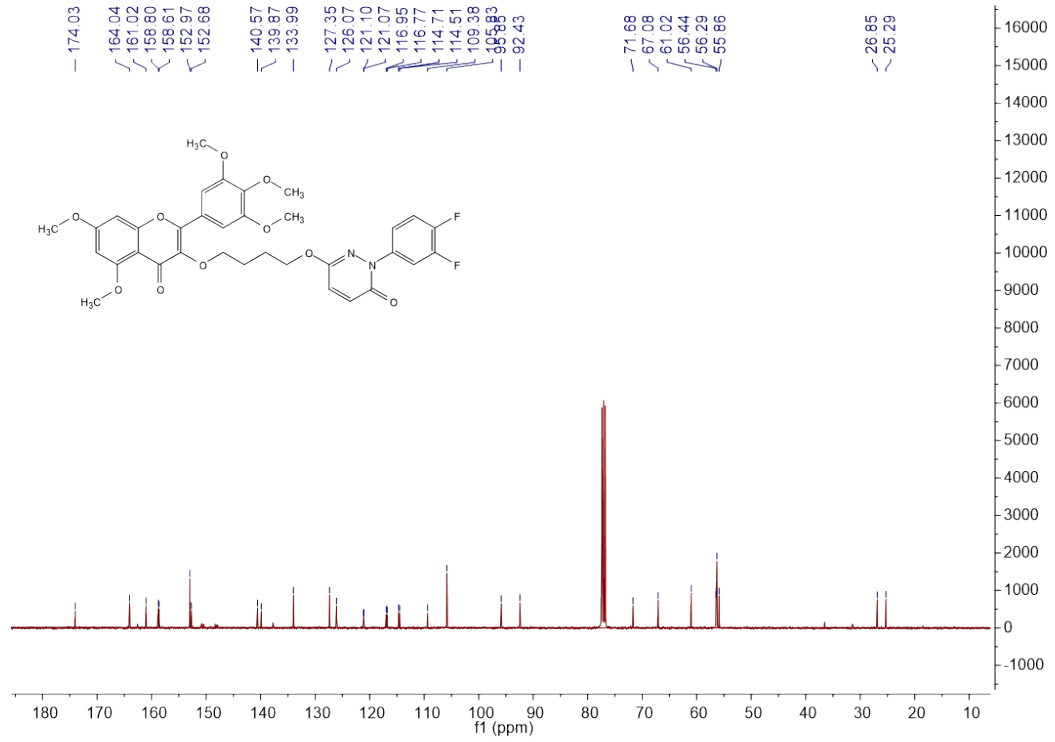


Fig. 75 HRMS spectrum of **A23**



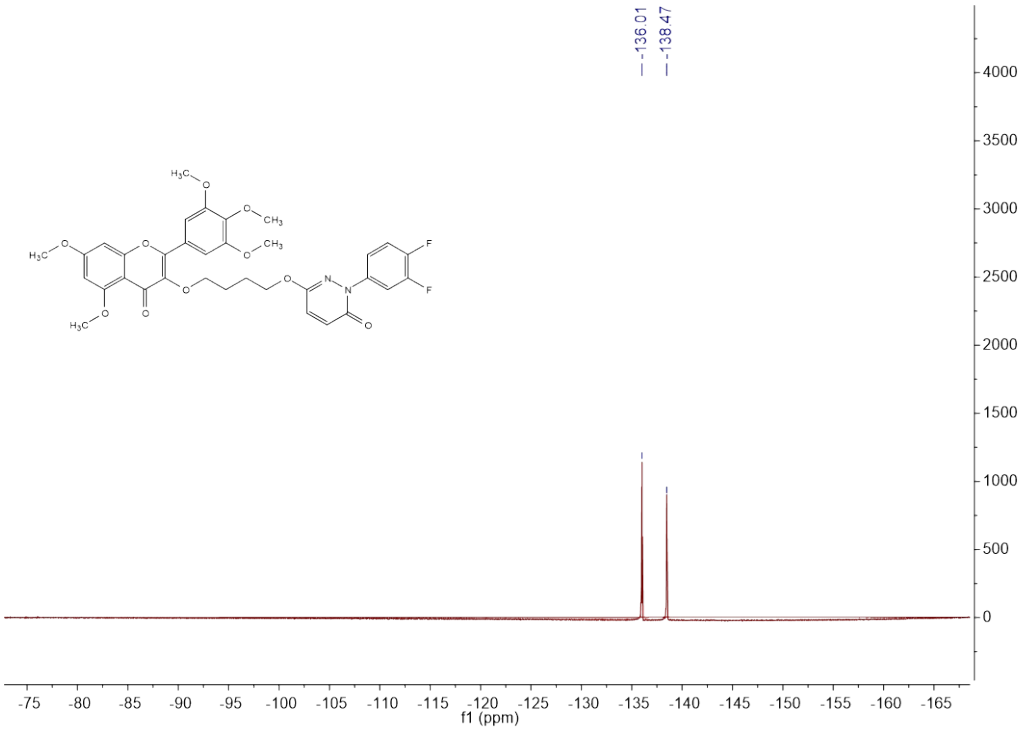
274

275 **Fig. 76** ^{1}H NMR spectrum of **A24**



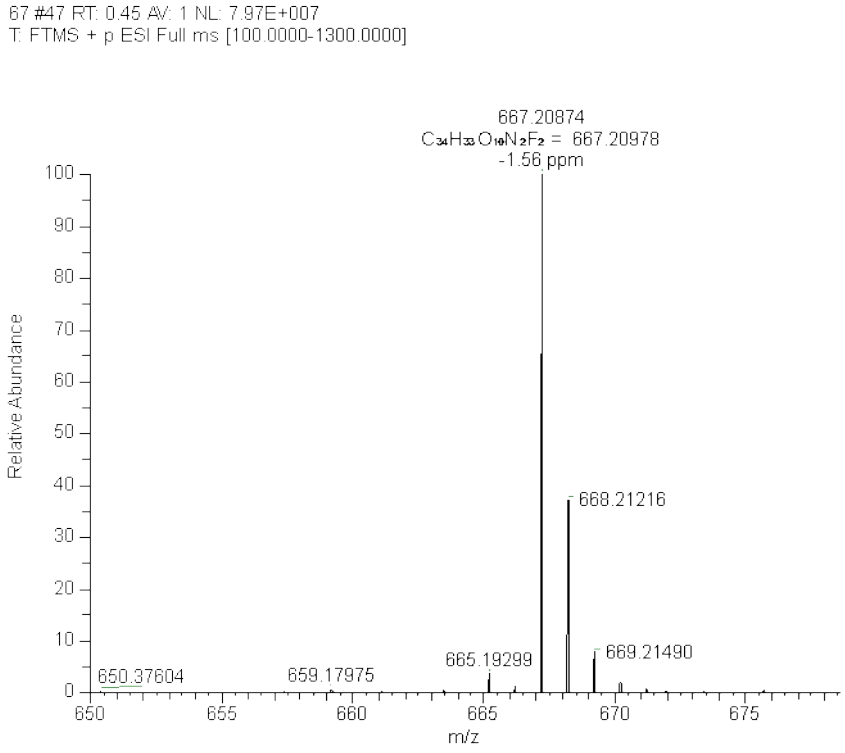
276

277 **Fig. 77** ^{13}C NMR spectrum of **A24**



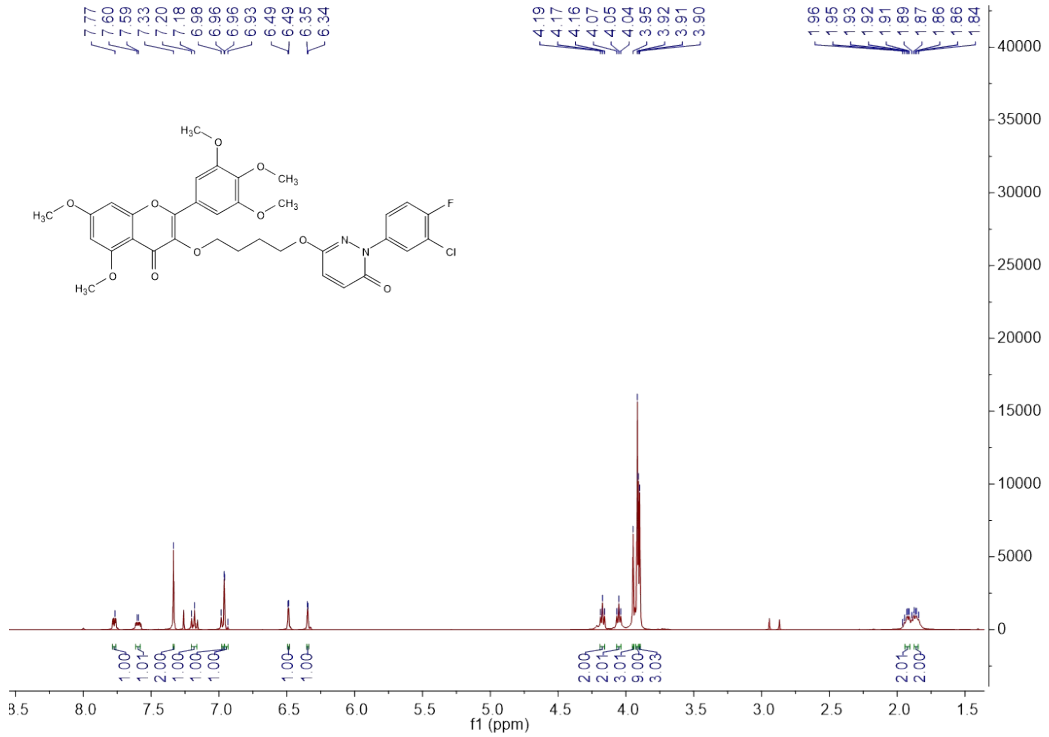
278

279 **Fig. 78** ^{19}F NMR spectrum of **A24**



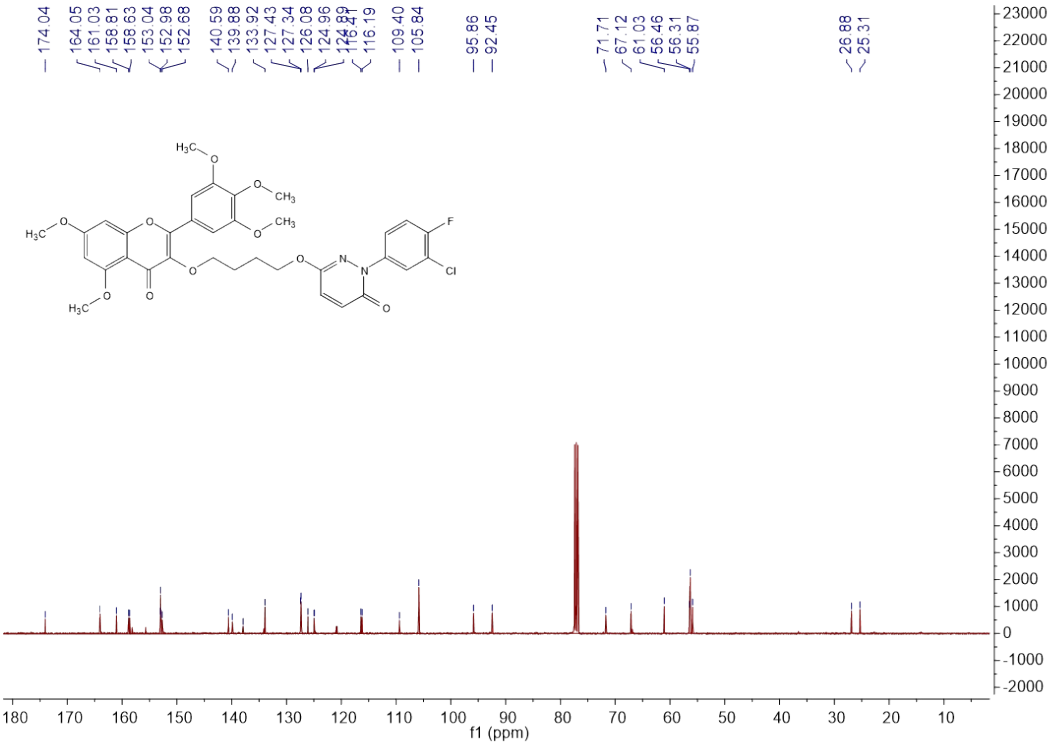
280

281 **Fig. 79** HRMS spectrum of **A24**



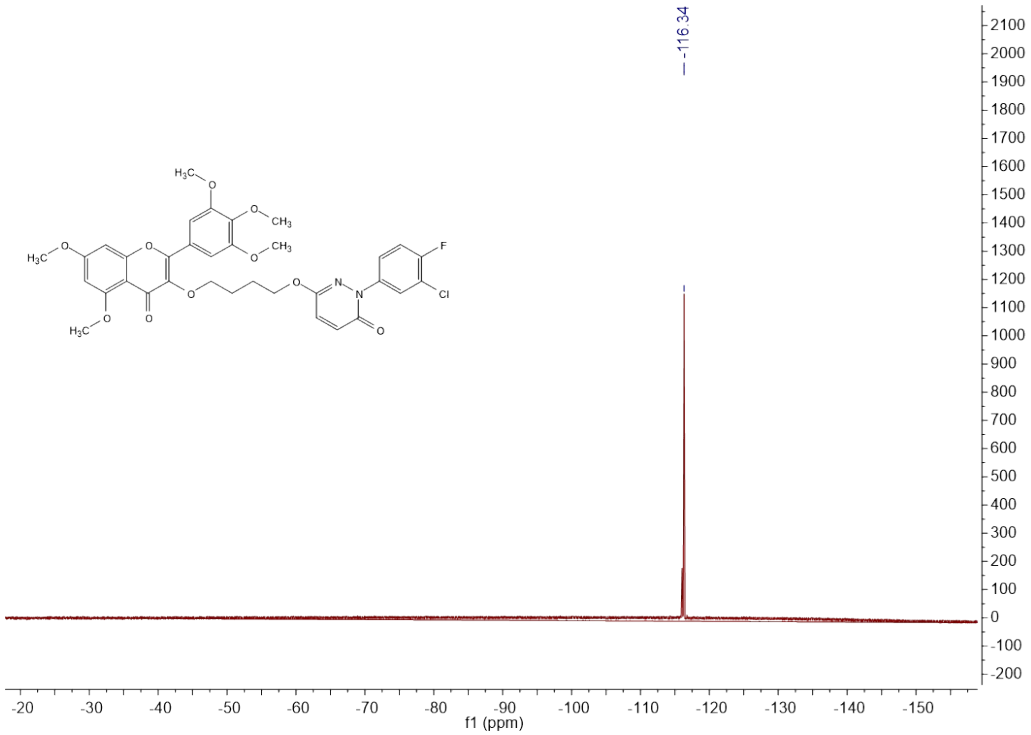
282

283 **Fig. 80** ^{1}H NMR spectrum of **A25**



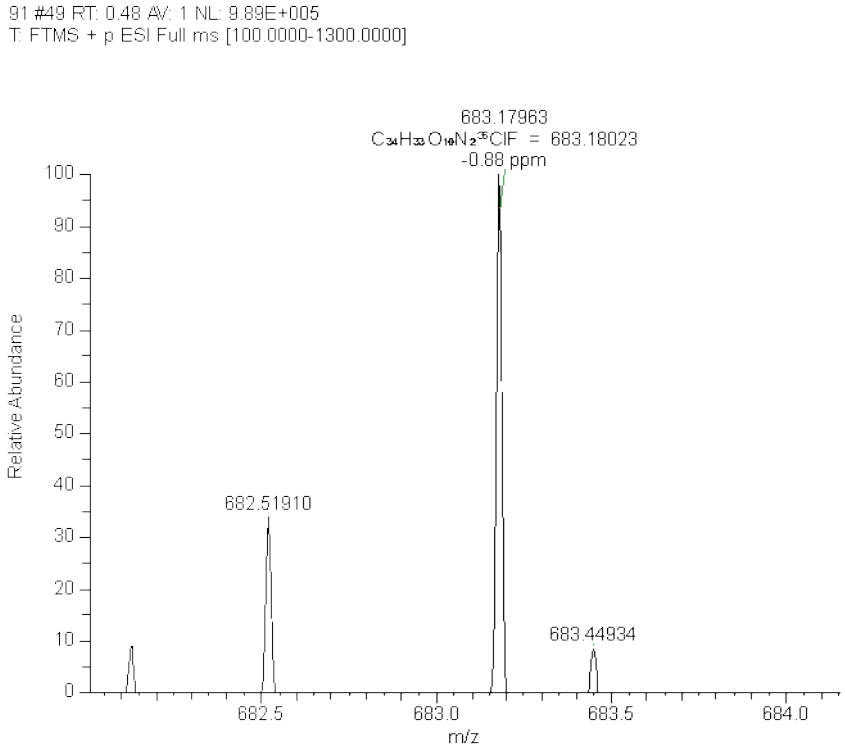
284

285 **Fig. 81** ^{13}C NMR spectrum of **A25**



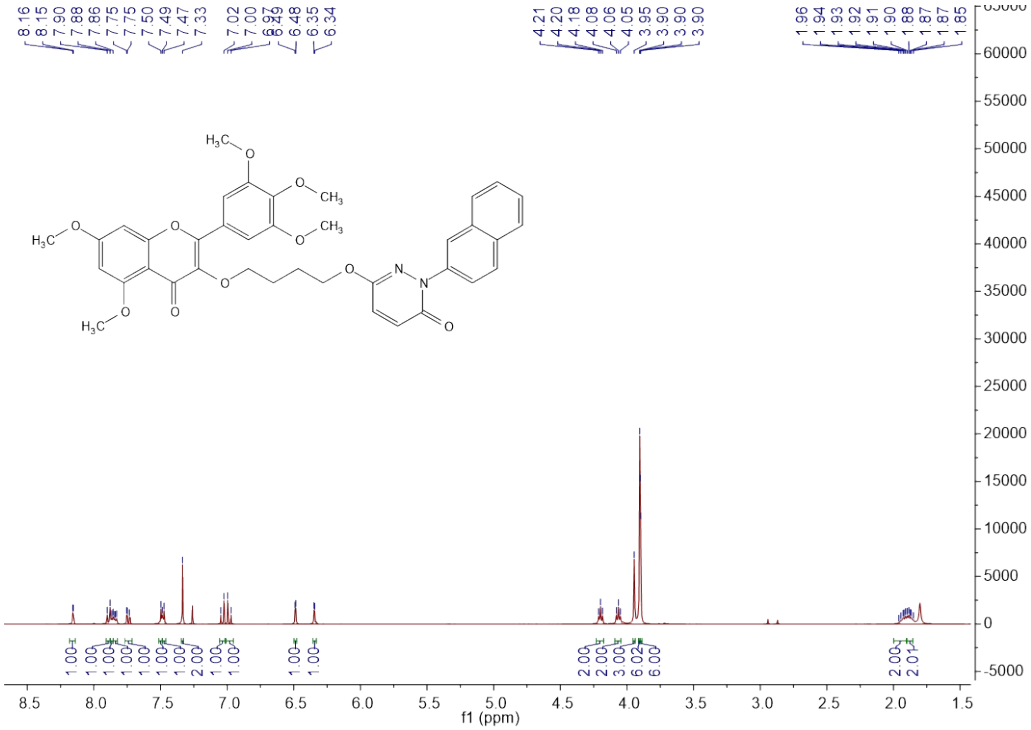
286

287 **Fig. 82** ^{19}F NMR spectrum of **A25**



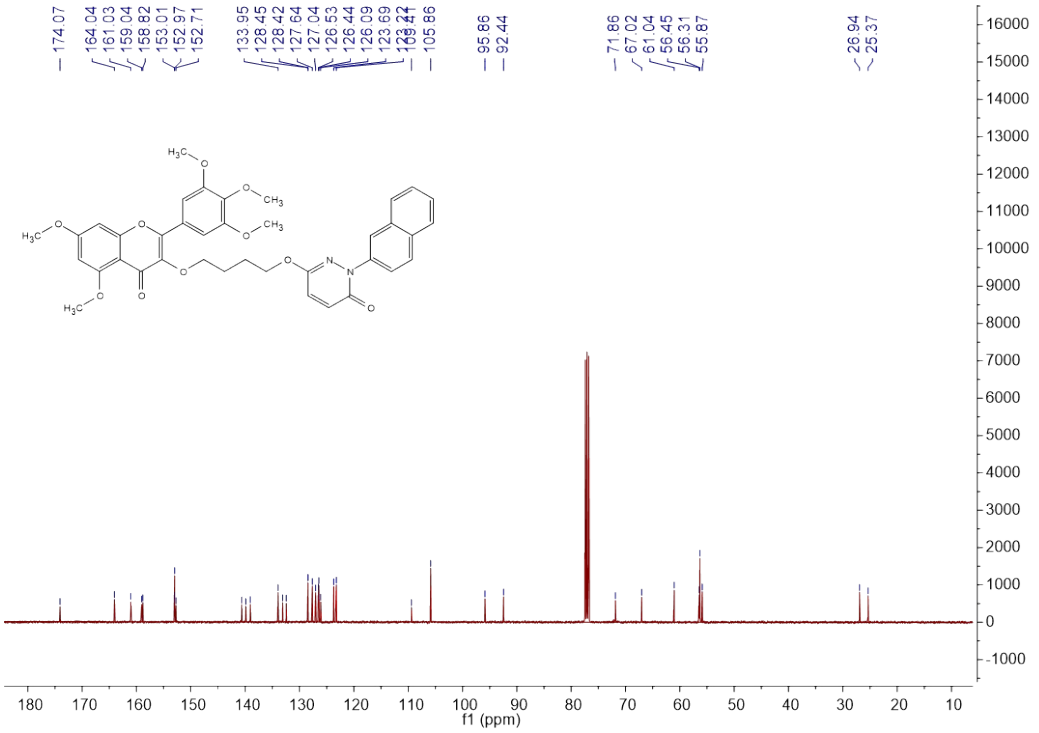
288

289 **Fig. 83** HRMS spectrum of **A25**



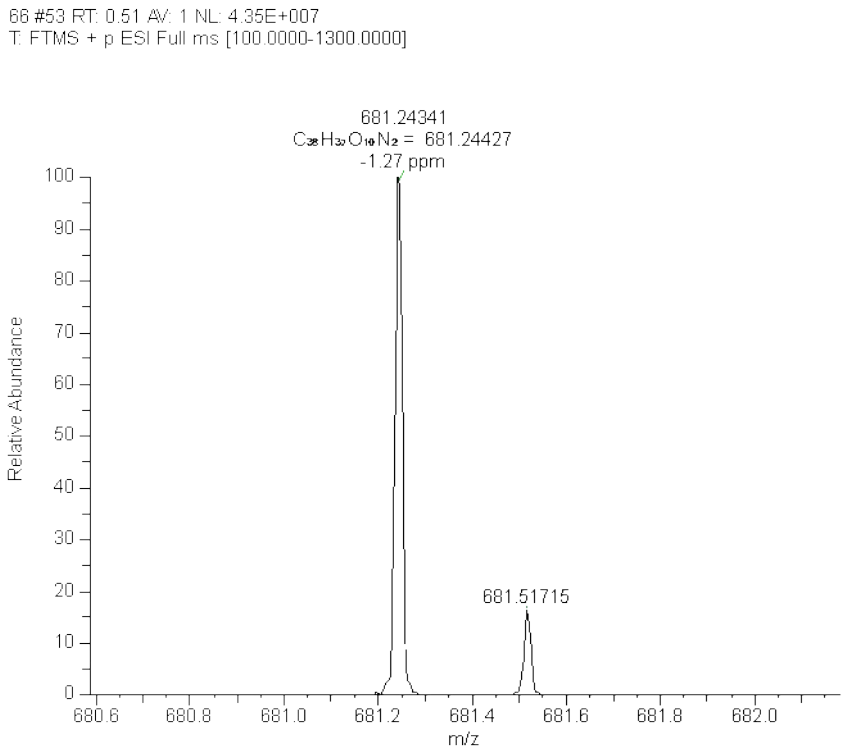
290

291 **Fig. 84** ^{1}H NMR spectrum of **A26**



292

293 **Fig. 85** ^{13}C NMR spectrum of **A26**



294

295 **Fig. 86** HRMS spectrum of **A26**