Synthesis and structure-activity relationship study of aldose reductase inhibiting marine alkaloid lukianol A and its derivatives

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Lukianol A (**1a**) and its six derivatives **1b–1g**, in which each hydroxyl groups of **1a** was individually modified, were synthesized via the common intermediate **7a**, which was obtained by condensation of the styryl carbazate **10** with *p*-hydroxyphenylpyruvic acid and subsequent [3,3]-sigmatropic rearrangement. The synthesized lukianol derivatives were evaluated for their ability to inhibit human aldose reductase. 4′-*O*-methyl (**1b**) and 4′-dehydroxy (**1g**) derivatives showed the same level of inhibitory activity as **1a** (IC_{50} 2.2 μM), indicating that the 4′-OH is irrelevant for the activity. In contrast, methylation of the hydroxyl group at the 4‴-position (**1d**) resulted in loss of activity at a concentration of 10 μM and masking the hydroxyl group at the 4″-position (**1e**) caused 9-fold decrease in activity compared with that of **1b**, suggesting that the 4″-OH is an essential group, and the 4‴-OH is required for higher activity.

Keywords: marine alkaloid, lukianol, aldose reductase inhibitor, total synthesis, SAR

The polyol pathway consists of two enzymes, aldose reductase (ALR2, EC 1.1.1.21) and sorbitol dehydrogenase (SDH). The first enzyme ALR2 catalyzes the NADPH-dependent reduction of glucose to sorbitol, and the second enzyme SDH converts sorbitol to fructose using NAD^+ as a co-factor. Under normoglycemic conditions, a small portion of glucose enters the polyol pathway because of the low substrate affinity of ALR2 for glucose. Instead, the majority of cellular glucose is converted to glucose 6-phosphate by hexokinase and metabolized through the glycolytic pathway. Under hyperglycemic conditions, especially in cells where glucose uptake is independent of insulin, such as nerves, lens, retina, and kidney, elevated intracellular glucose levels activate ALR2, resulting in the excess production of sorbitol. Since sorbitol has low membrane permeability, it accumulates in tissues, leading to diabetic complications such as neuropathy, nephropathy, and retinopathy (Yabe-Nishimura 1998; Niimi et al 2021). Therefore, many structurally diverse ALR2 inhibitors have been developed as potential drugs to prevent and treat diabetic complication (Kerru et al 2018; Kousaxidis et al 2020; Thakur et al 2021).

Lukianol A (**1a**) and B (**2**) are unique marine 3,4-diaryl pyrrole alkaloids isolated from a tunicate collected in the lagoon of a palmyra atoll by Scheuer and coworkers in 1992 (Yoshida et al 1992). Lukianol A was reported to have moderate cytotoxicity against KB cells (MIC 1 μg/mL), whereas lukianol B was virtually inactive (MIC 100 μg/mL). In 2006, Fruente and coworkers found that lukianol B displayed high ALR2 inhibitory activity, with an IC_{50} value of 0.6 μM, which was six-fold higher than that of the known ALR2 inhibitor sorbinil (Manzanaro et al 2006). Thus, lukianols could be considered promising lead compounds for the development of new types of ALR2 inhibitors; however, to the best of our knowledge, no structure-activity relationship study of lukianols has been reported.

Lukianols have a unique structure and interesting biological activities; therefore, these marine alkaloids have attracted considerable interest from synthetic chemists (Fürstner et al 1995; Banwell et al 1997; Boger et al 1999; Liu et al 2000; Kim et al 2001; Hinze et al 2007; Lu and Arndtsen 2009; Takamura et al 2013; Satyanarayama et al 2020; Morikawa et al 2020). Most syntheses utilize 3,4-diarylpyrrole-2-carboxylate (**3**) as the key intermediate, since it was used in the first total synthesis of lukianol A and lamellarin O by Fürstner's group. Accordingly, several convenient methods for preparing the Fürstner intermediate have been developed (Gupton et al 1999; Bullington et al 2002; Mathew and Asokan 2005). Recently, Zhou and Ma described a novel synthesis of 3,4,5-trisubstituted pyrrole-2-carboxylic acid **6** via the coupling reaction of *N*-protected *N*-alkenylhydrazine **4** with α-keto acid **5** and subsequent [3,3]-sigmatropic rearrangement

(Scheme 1) (Zhou et al 2014). Here, we describe a convergent synthesis of lukianol A utilizing this method for the preparation of MOM-protected 3,4-diarylpyrrole-2-carboxylate (**7a**) as an alternative to the Fürstner intermediate. Moreover, to clarify the effect of the individual hydroxyl groups of **1a** on the activity, six derivatives of **1a** in which each hydroxyl group was individually modified were synthesized and evaluated for their ability to inhibit ALR2.

Results and Discussion

Synthesis

The *N*-styrenyl hydrazine **10** required for pyrrole synthesis was synthesized by a copper-catalyzed coupling reaction (Zhou et al 2014) between *t*-butylcarbazate and vinyl bromide **9**, which was prepared by the bromodecarboxylation of *O*-benzyl coumaric acid (**8**, Ghafary et al 2018) using Oxone® and sodium bromide (You et al 2001). Acetic acid-mediated condensation of **10** with 4-hydroxyphenylpyruvic acid (Billek 1963) following the protocol of Zhou and Ma (Zhou et al 2014) gave 3,4-diarylpyrrole-2-carboxylic acid **11** in 89% yield. After the hydroxyl and carboxylic groups were protected with methoxymethyl groups, pyrrole **7a** was alkylated with 4′-methoxyphenacyl bromide. When 1.5 equivalent of cesium carbonate was used as the base in the reaction, alkylated product **12a** was obtained in 95% yield, whereas the use of an increased amount of the base (2.0 equivalent) under elevated reaction temperatures directly afforded the cyclized product **13** in 57% yield. This concomitant transesterification proceeded only for the methoxymethyl ester: use of methyl ester **7b** gave non-cyclized product **12b** as the sole product (data not shown). Alternatively, enol-lactone **13** was obtained in 51% yield by a cesium carbonate-catalyzed transesterification reaction of **12a**. The complete deprotection of the hydroxyl protecting groups of **13** using boron tribromide afforded lukianol A (**1a**) in 98% yield, whereas partial deprotection under a milder condition using boron trifluoride etherate and dimethyl sulfide (Fuji et al 1980) gave 4′-*O*-methyl lukianol A (**1b**) in 85% yield. The methylation of **1b** under standard conditions afforded tri-*O*-methyl lukianol A (**1c**).

4′,4‴-Bis-*O*-methyl lukianol A (**1d**) was synthesized via i) demethoxymethylation, ii) methylation, and iii) debenzylation of **13** (Scheme 3). The deprotection/methylation sequence in the reverse order gave 4′,4″-bis-*O*-methyl lukianol A (**1e**). 4″,4‴-Bis-*O*-methyl lukianol A (**1f**) and 4′-dehydroxy lukianol A (**1g**) were synthesized by the same strategy using 4′-mesyloxyphenacyl bromide and phenacyl bromide, respectively, instead of 4′-methoxyphenacyl bromide (Scheme 4).

Biological activity

The lukianol A derivatives **1a–1g** were evaluated in vitro for their ability to inhibit recombinant human aldose reductase (h-ALR2). The enzyme activity was measured spectrophotometrically by determining the decrease in NADPH concentration at 340 nm. Epalrestat was used as a positive control. The inhibitory activity at 10 μM and IC_{50} values of the compounds tested are shown in Table 1. Both the 4′-*O*-methyl (**1b**) and 4′-dehydroxy (**1g**) derivatives of **1a** maintained their inhibitory activity at the same level as **1a**, indicating that the 4′-OH is irrelevant for the activity. In contrast, compounds **1c**, **1d**, and **1f**, in which the hydroxyl groups at the 4‴-position were blocked as methyl ether, were almost inactive at a concentration of 10 μM, indicating that the 4‴-OH is necessary for the activity. Masking the hydroxyl group at the 4″-position of **1b** as **1e** caused 9-fold decrease in activity, suggesting that, although not as significant as the 4‴-OH, the 4″-OH is also required for higher activity. Many phenolic compounds of diverse structure having ALR2 inhibitory activity have been isolated from natural source (de la Fuente et al 2003), most of which have two hydroxyphenyl moieties at both sides of the molecules. Among them, lukianols have close structural similarity to the pyrrolo[2,3-*c*]carbazole-type alkaloids, **22a**, **22b**, and **23a** (Fig. 2), isolated from the dark green sponge *Dictyodendrilla* sp (Sato et al 1993). These alkaloids strongly inhibited bovine lens ALR2 with IC_{50} values of 49 (**22a**), 125 (**22b**), and 112 (**23a**) nM, respectively. Their chemically desulfated compounds **22c** retained the same level of the inhibitory activity (IC_{50} 120 nM), whereas the acetylated derivatives **22d** was substantially inactive (IC_{50} >10 μM), indicating the significance of the phenolic hydroxyl group(s) on the activity. Interestingly, sulfate group at 7-position of **23a** (IC_{50} 112 nM), potentiated the activity (**23b**: IC_{50} 567 nM). Thus, increase of the inhibitory activity of lukianols may be expected by introducing a sulfate group at the hydroxyl group at 4”- or 4‴-position.

Conclusion

In summary, we accomplished the total synthesis of lukianol A in six steps in 33% overall yield using a pyrrole formation reaction by condensation of the styryl carbazate **10** and *p*-hydroxypyruvic acid and subsequent [3,3]-sigmatropic rearrangement as the key reaction. Less cytotoxic lukianol B (**1b**) could also be synthesized via regioselective iodination of **16** using *N*-iodosaccharin (Dolenc 2000; Takamura et al 2013). In a SAR study using seven lukianol A analogs, it was shown that the hydroxyl groups at 4″- and 4‴-positions play an important role in the ALR2 inhibitory activity, whereas that at 4′-position is not involved in the activity.

Experimental

General procedures

NMR spectra were recorded on a Varian System 500PS SN spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C), a JEOL JNM-ECZ400R spectrometer (400 MHz for 1H and 101 MHz for ^{13}C) or a Varian Gemini 300 spectrometer (300 MHz for ^{1}H and 75 MHz for ^{13}C) in $CDCl_3$ unless otherwise noted. Chemical shifts for ^{1}H NMR were expressed in parts per million (ppm) relative to the following internal standards: $CDCl_3$ (tetramethylsilane, δ 0.00 ppm), acetone-d_6 (acetone, δ 2.04 ppm), and DMSO-d_6 (DMSO, δ 2.50 ppm). Chemical shifts for ^{13}C NMR are expressed in ppm relative to the following internal standards: $CDCl_3$ ($CDCl_3$, δ 77.00 ppm), acetone-d_6 (acetone-d_6, δ 29.92 ppm), and DMSO-d_6 (DMSO-d_6, δ 39.50 ppm). High-resolution mass spectra were recorded on a JEOL JMS-T100TD instrument (direct analysis in real-time (DART) or electron spray ionization (ESI) mode) or a JMS-700N instrument (FAB mode). IR spectra were recorded on a Thermo Nicolet Nexus 670 FT-IR spectrometer. Dry THF, DMF, and CH_2Cl_2 were purchased from commercial sources (Kanto Chemical or Wako Pure Chemical Industries).

1-Benzyloxy-4-[(1*E*)-3-bromoprop-1-en-1-yl]benzene (9)

To a stirred solution of 4-benzyloxycinnamic acid (4.23 g, 16.6 mmol) in CH_3CN (170 mL), were added a solution of NaBr (1.80 g, 17.5 mmol), Na_2CO_3 (1.76 g, 16.6 mmol) in water (110 mL). After 10 min, an aqueous (110 mL) solution of oxone® (10.23 g, 16.64 mmol) was added dropwise over 10 min. After 10 min, the reaction was quenched by 10% $Na_2S_2O_3$ solution and the CH_3CN was evaporated. The mixture was extracted twice with CH_2Cl_2 (100 mL × 2), dried (Na_2SO_4), and concentrated. The crude product was chromatographed on silica gel eluted with hexane-EtOAc (9:1) to give **9** (3.70 g, 12.8 mmol, 77%) as white crystals, mp 91-92 °C. ^{1}H NMR (300 MHz) δ 5.07 (2H, s), 5.37 (1H, d, J = 7.3 Hz), 5.98 (1H, d, J = 7.3 Hz), 6.97 (2H, d, J = 8.4 Hz), 7.37-7.48 (7H, m). ^{13}C NMR (75 MHz) δ 70.0, 104.1, 115.1, 127.4, 127.4, 128.0, 128.6, 129.0, 136.5, 136.7, 158.8. IR (KBr) 521, 533, 696, 735, 784, 834, 935, 956, 1026, 1036, 1192, 1265, 1285, 1514, 1609 cm^{-1}. Anal. Calcd for $C_{15}H_{13}BrO$: C, 62.30; H, 4.53. Found: C, 62.22; H, 4.57.

1-(*tert*-Butoxycarbonyl)-1-[(*E*)-2-(4-benzyloxyphenyl)ethenyl]hydrazine (10)

A mixture of **9** (1.46 g, 5.06 mmol), *tert*-butyl carbazate (0.82 g, 6.2 mmol), CuI (0.19 g, 1.0 mmol), and K_2CO_3 (spray dried, 0.98 g, 7.1 mmol) in dry DMSO (15 mL) was stirred

at 80 °C under Ar for 18 h. The mixture was cooled to room temperature, poured into water, and extracted 3 times with EtOAc. The combined organic layers were filtered through a pad of Celite, washed with brine, dried (Na_2SO_4), and concentrated. The crude product was purified by silica gel column chromatography eluted with hexane-EtOAc (5:1 to 2:1) to give **10** (1.64 g, 4.82 mmol, 95%) as pale yellow crystals, mp 116-117 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.48 (9H, s), 4.78 (2H, s), 5.07 (2H, s), 6.22 (1H, d, J = 14.0 Hz), 6.93 (2H, d, J = 8.5 Hz), 7.23 (2H, d, J = 8.5 Hz), 7.28-7.48 (6H, m). ^{13}C NMR (75 MHz) δ 28.3, 70.0, 82.3, 109.0, 115.1, 125.9, 126.4, 127.4, 127.9, 128.5, 130.1, 137.1, 153.3, 157.2. IR (KBr) 697, 750, 839, 941, 999, 1013, 1157, 1233, 1250, 1370, 1510, 1662, 1696 cm^{-1}. HRESIMS *m/z* calcd for $C_{20}H_{24}N_2NaO_3$ $(M+Na)^+$ 363.1685, found 363.1666. Anal. Calcd for $C_{20}H_{24}N_2O_3$: C, 70.57; H, 7.11; N, 8.23. Found: C, 70.54; H, 6.98; N, 8.10.

4-(4-Benzyloxyphenyl)-3-(4-hydroxyphenyl)-1*H*-pyrrole-2-carboxylic acid (11)

A solution of **10** (1.50 g, 4.41 mmol), 4-hydroxyphenylpyruvic acid (Billek 1963) (0.79 g, 4.4 mmol), AcOH (1.26 mL) in EtOH (35 mL) was heated at reflux for 4 h. The mixture was concentrated and the solid was recrystallized from 50% aqueous EtOH (40 mL) to give **11** (0.97 g, 2.5 mmol, 57%) as white crystals, mp 212-214 °C. The mother liquid was concentrated and chromatographed on silica gel eluted with hexane-EtOAc (2:3 to 0:1) to give another crop of **11** (0.55 g, 1.4 mmol, 32%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.01 (2H, s), 6.66 (2H, d, J = 8.8 Hz), 6.81 (2H, d, J = 8.8 Hz), 6.95 (2H, d, J = 8.8 Hz), 6.97 (2H, d, J = 8.8 Hz), 7.09 (1H, d, J = 2.7 Hz), 7.28-7.42 (5H, m), 9.27 (1H, br), 11.73 (1H, d, J = 2.7 Hz), 12.01 (1H, br). ^{13}C NMR (101 MHz, DMSO-d_6) δ 69.6, 114.89, 114.91, 120.2, 121.0, 125.1, 126.1, 128.2, 128.3, 128.5, 128.9 (2C), 129.3, 132.2, 137.7, 156.4, 156.9, 162.6. IR (KBr) 742, 839, 1181, 1249, 1271, 1356, 1485, 1512, 1670, 3275 (br) cm^{-1}. HRESIMS *m/z* calcd for $C_{24}H_{19}NNaO_4$ $(M+Na)^+$ 408.1212, found 408.1236.

Methoxymethyl 4-(4-benzyloxyphenyl)-3-(4-(methoxymethoxy)phenyl)-1*H*-pyrrole-2-carboxylate (7a)

To a cooled (0 °C) and stirred suspension of *t*-BuOK (0.92 g, 8.2 mmol) in dry THF (20 mL), was added dropwise a solution of **11** (1.54 g, 4.00 mmol) in THF (20 mL) under Ar atmosphere. After 15 min, chloromethyl methyl ether (0.63 mL, 8.4 mmol) dissolved in THF (10 mL) was dropwise added. The cooling bath was removed and the whole was stirred for 1.5 h before being quenched with 2% NH_4OH solution. The mixture was

extracted twice with EtOAc, washed with brine, dried (Na_2SO_4), and concentrated. The residual solid was recrystallized from ether to give a first crop of **7a** (0.53 g, 1.1 mmol, 28%) as white crystals, mp 111-112 °C. The mother liquid was concentrated and chromatographed on silica gel eluted with hexane-EtOAc (3:2) to give a second crop of **7a** (1.01 g, 2.14 mmol, 54%). ^{1}H NMR (300 MHz) δ 3.27 (3H, s), 3.48 (3H, s), 4.99 (2H, s), 5.17 (2H, s), 5.28 (2H, s), 6.81 (2H, d, J = 8.8 Hz), 6.97 (2H, d, J = 8.8 Hz), 7.03 (2H, d, J = 8.8 Hz), 7.04 (1H, d, J = 2.9 Hz), 7.21 (2H, d, J = 8.8 Hz), 7.29-7.44 (5H, m), 9.46 (1H, br s). ^{13}C NMR (75 MHz) δ 55.9, 57.4, 70.0, 90.1, 94.5, 114.5, 115.3 115.6, 119.2, 120.7, 126.5, 127.1, 127.4, 127.8, 128.5, 129.3, 129.5, 131.8, 137.0, 156.2, 157.3, 160.5. IR (KBr) 736, 838, 907, 982, 1016, 1024, 1083, 1125, 1146, 1237, 1257, 1377, 1422, 1539, 1688 cm^{-1}. HRESIMS *m/z* calcd for $C_{28}H_{27}NNaO_6$ $(M+Na)^+$ 496.1736, found 496.1698.

Methoxymethyl 4-(4-benzyloxyphenyl)-3-(4-(methoxymethoxy)phenyl)-1-[2-(4-methoxyphenyl)-2-oxoethyl]pyrrole-2-carboxylate (12a)

A mixture of **7a** (278 mg, 0.587 mmol), 4′-methoxyphenacyl bromide (336 mg, 1.47 mmol), and Cs_2CO_3 (287 mg, 0.881 mmol) in dry THF (10 mL) was heated under reflux for 7 hr. The mixture was then poured into 10% NH_4Cl solution, extracted twice with EtOAc, dried over Na_2SO_4, and concentrated. The crude product was purified by medium pressure liquid chromatography on silica gel using toluene-EtOAc (19:1) as eluant to give **12a** (345 mg, 0.544 mmol, 95%) as a viscous oil. ^{1}H NMR (400 MHz) δ 2.99 (3H, s), 3.47 (3H, s), 3.88 (3H, s), 4.98 (2H, s), 5.03 (2H, s), 5.17 (2H, s), 5.75 (2H, s), 6.79 (2H, d, J = 8.9 Hz), 6.94-7.03 (7H, m), 7.21 (2H, d, J = 8.7 Hz), 7.28-7.42 (5H, m), 8.01 (2H, d, J = 8.9 Hz). ^{13}C NMR (101 MHz) δ 55.48, 55.53, 55.9, 57.1, 69.9, 89.8, 94.5, 114.1, 114.4, 115.3, 119.5, 124.8, 127.1, 127.5, 127.7, 127.8, 127.9, 128.5, 129.3, 129.5, 130.3, 131.5, 131.9, 137.0, 156.0, 157.1, 161.0, 164.0, 191.7. IR (KBr) 834, 993, 1051, 1078, 1171, 1237, 1601, 1693, 2926 cm^{-1}. HRESIMS *m/z* calcd for $C_{37}H_{35}NNaO_8$ $(M+Na)^+$ 644.2260, found 644.2234.

7-(4-Benzyloxyphenyl)-3-(4-methoxyphenyl)-8-[4-(methoxymethoxy)phenyl]-1*H*-pyrrolo[2,1-*c*][1,4]oxazin-1-one (13)

Method A

A mixture of **12a** (102 mg, 0.164 mmol), Cs_2CO_3 (60 mg, 0.19 mmol), and MS4A (1 g, beads, ca. 2 mm, Nakarai tesque) in dry THF (25 mL) was heated at reflux for 100 min.

More of Cs_2CO_3 (54 mg, 0.16 mmol), and MS4A (0.8 g) were added and the heating was continued for additional 80 min. The mixture was then diluted with EtOAc and filtered. The filtrate was washed with 10% NH_4Cl solution, and the aqueous layer was extracted twice with EtOAc. After drying (Na_2SO_4) and removing the solvent, the crude product was chromatographed on silica gel eluted with toluene-EtOAc (9:1) to give **13** (46 mg, 0.083 mmol, 51%) as pale yellow crystals, mp 178-181 °C.

Method B

A mixture of **7a** (104 mg, 0.220 mmol), Cs_2CO_3 (145 mg, 0.444 mmol), 4′-methoxyphenacyl bromide (126 mg, 0.550 mmol) in dry DME (2 mL) was heated at 90 °C (bath temp.) in a screw-sealed tube under Ar atmosphere for 6 h. The mixture was then diluted with EtOAc (20 mL), filtered through a short column of silica gel, and concentrated. The crude product was purified by silica gel column chromatography eluted with toluene-EtOAc (9:1) to give **13** (70 mg, 0.13 mmol, 57%) as pale yellow crystals. ^{1}H NMR (400 MHz) δ 3.50 (3H, s), 3.84 (3H, s), 5.03 (2H, s), 5.19 (2H, s), 6.87 (2H, d, J = 8.8 Hz), 6.94 (2H, d, J = 8.8 Hz), 6.99 (2H, d, J = 9.0 Hz), 7.08 (2H, d, J = 8.8 Hz), 7.18 (1H, s), 7.28 (2H, d, J = 8.8 Hz), 7.30 (1H, s), 7.38-7.41 (5H, m), 7.64 (2H, d, J = 9.0 Hz). ^{13}C NMR (101 MHz) δ 55.4, 56.1, 70.0, 94.5, 102.7, 112.9, 114.2, 114.8, 115.5, 119.0, 123.0, 125.8, 126.0, 127.5, 128.0, 128.1, 128.6, 129.5, 129.8, 132.0, 136.9, 142.0, 154.3, 156.7, 157.8, 160.5 (one quaternary carbon signal is overlapping). IR (KBr) 799, 836, 1005, 1039, 1177, 1248, 1429, 1536, 1608, 1720 cm^{-1}. HRESIMS *m/z* calcd for $C_{35}H_{29}NNaO_6$ $(M+Na)^+$ 582.1893, found 582.1910.

3,7,8-Tris(4-hydroxyphenyl)-1*H*-pyrrolo[2,1-*c*][1,4]oxazin-1-one (lukianol A, 1a)

To a solution of **13** (50.5 mg, 0.0902 mmol) in dry CH_2Cl_2 (5.5 mL) was added 1M hexane solution of BBr_3 (0.63 mL, 0.63 mmol) at −78 °C under Ar atmosphere. The mixture was stirred at −78 °C (1 h), 0 °C (3 h), and room temperature (30 min), before being quenched with saturated aqueous $NaHCO_3$ (4 mL). The mixture was vigorously stirred for 45 min and extracted twice with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated. The crude product was chromatographed on silica gel eluted with hexane-EtOAc (2:3~0:1) to give **1a** (36.2 mg, 0.0880 mmol, 98%) as pale yellow crystals. Recrystallization from EtOH-ether gave light gray crystals, mp 211-214 °C. ^{1}H NMR (400 MHz, acetone-d_6) δ 6.73 (2H, d, J = 8.6 Hz), 6.78 (2H, d, J = 8.6 Hz), 6.94 (2H, d, J = 8.8 Hz), 7.03 (2H, d, J = 8.6 Hz), 7.16 (2H, d, J

= 8.6 Hz), 7.52 (1H, s), 7.63 (2H, d, J = 8.8 Hz), 7.90 (1H, s), 8.35 (1H, s), 8.37 (1H, s), 8.77 (1H, s). ^{13}C NMR (101 MHz, acetone-d_6) δ103.7, 113.4, 115.3, 116.0, 116.6, 120.5, 123.2, 125.0, 125.8, 126.5, 128.8, 130.2, 130.6, 132.9, 142.5, 154.4, 157.2, 157.5, 159.3. IR (KBr) 791, 836, 1041, 1173, 1204, 1240, 1420, 1506, 1518, 1610, 1696, 3330 cm^{-1}. HRESIMS *m/z* calcd for $C_{25}H_{17}NNaO_5$ $(M+Na)^+$ 434.1004, found 434.0998.

7,8-Bis(4-hydroxyphenyl)-3-(4-methoxyphenyl)-1*H*-pyrrolo[2,1-*c*][1,4]oxazin-1-one (1b)

To a solution of **13** (39.6 mg, 0.0659 mmol) and Me_2S (0.24 mL, 3.2 mmol) in dry CH_2Cl_2 (1 mL), was added $BF_3{\cdot}OEt_2$ (0.176 mL, 1.35 mmol) at room temperature. After the mixture had been stirred for 5 h, the reaction was quenched with water and the whole was extracted twice with a mixture of EtOAc and THF (1:1). The organic layers were combined, washed with 5% $NaHCO_3$, dried over Na_2SO_4, and concentrated. The residue was triturated with EtOAc to give **1b** (23.7 mg, 0.0558 mmol, 85%) as pale yellow crystals, mp >300 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.81 (3H, s), 6.66 (2H, d, J = 8.6 Hz), 6.70 (2H, d, J = 8.6 Hz), 6.95 (2H, d, J = 8.6 Hz), 7.05 (2H, d, J = 8.6 Hz), 7.06 (2H, d, J = 8.8 Hz), 7.59 (1H, s), 7.66 (2H, d, J = 8.8 Hz), 8.15 (1H, s), 9.43 (1H, s, OH), 9.48 (1H, s, OH). ^{13}C NMR (101 MHz, DMSO-d_6) δ 55.4, 103.7, 112.0, 114.5, 114.6, 115.3, 119.6, 122.9, 123.1, 123.9, 125.4, 127.5, 128.5, 129.5, 131.8, 140.4, 153.6, 156.4, 156.6, 159.9. IR (KBr) 763, 837, 1024, 1041, 1184, 1120. 1231, 1255, 1413, 1430, 1516, 1611, 1691, 3240, 3448 cm^{-1}. HRESIMS *m/z* calcd for $C_{26}H_{19}NNaO_5$ $(M+Na)^+$ 448.1161, found 448.1151.

3,7,8-Tris(4-methoxyphenyl)-1*H*-pyrrolo[2,1-*c*][1,4]oxazin-1-one (1c)

A mixture of **1b** (5.3 mg, 0.12 mmol) and powdered K_2CO_3 (55.0 mg, 0.399 mmol) in dry DMF (1 mL) was stirred for 10 min. MeI (25 μL, 0.40 mmol) was then added and the whole was stirred at room temperature for 16 h. The mixture was diluted with EtOAc (5 mL), filtered, and the filtrate was concentrated. The crude product was purified by a silica gel chromatography eluted with hexane-EtOAc (1:1) to give **1c** (5.5 mg, 0.012 mmol, 100%) as yellow crystals, mp 181-183 °C. ^{1}H NMR (400 MHz) δ 3.80 (3H, s), 3.84 (3H, s), 3.86 (3H, s), 6.82 (2H, d, J = 8.9 Hz), 6.88 (2H, d, J = 8.9 Hz), 6.97 (2H, d, J = 8.9 Hz), 7.10 (2H, d, J = 8.9 Hz), 7.22 (1H, s), 7.30 (2H, d, J = 8.9 Hz), 7.34 (1H, s), 7.67 (2H, d, J = 8.9 Hz). ^{13}C NMR (101 MHz) δ 55.1, 55.2, 55.4, 102.7, 113.0, 113.3, 113.9, 114.3, 118.9, 123.0, 123.1, 124.7, 125.9, 128.2, 129.7, 129.8, 132.0, 142.0, 154.3, 158.6,

158.9, 160.5. IR (KBr) 795, 836, 1034, 1179, 1251, 1430, 1506, 1516, 1609, 1733, 2922 cm^{-1}. HRESIMS *m/z* calcd for $C_{28}H_{23}NNaO_5$ $(M+Na)^+$ 476.1474, found 476.1511.

7-(4-Benzyloxyphenyl)-3,8-bis(4-methoxyphenyl)-1*H*-pyrrolo[2,1-*c*][1,4]oxazin-1-one (14)

A solution of **13** (39.3 mg, 0.0703 mmol) in EtOH (20 mL) containing one drop of conc. HCl was heated at reflux for 45 min. The mixture was then cooled to room temperature, neutralized with one drop of Et_3N, and concentrated. The crude product was purified by silica gel column chromatography eluted with hexane-EtOAc (1:1) to give **14** (34.2 mg, 0.0663 mmol, 94%) as pale yellow crystals, mp 247-249 °C. ^1H NMR (500 MHz, DMSO-d_6-acetone-d_6) δ 3.81 (3H, s), 5.06 (2H, s), 6.72 (2H, d, J = 8.8 Hz), 6.93 (2H, d, J = 8.8 Hz), 7.04-7.11 (6H, m), 7.31-7.35 (1H, m), 7.37-7.41 (2H, m), 7.42-7.46 (2H, m), 7.65 (1H, s), 7.68 (2H, d, J = 8.9 Hz), 8.17 (1H, s), 9.49 (1H, s). ^{13}C NMR (125 MHz, DMSO-d_6-acetone-d_6) δ 55.3, 69.2, 103.7, 112.1, 114.5, 114.69, 114.71, 120.2, 122.9, 123.0, 125.4, 125.8, 127.0, 127.8, 127.9, 128.4, 129.0, 129.4, 131.7, 137.0, 140.5, 153.5, 156.7, 157.3, 160.0. IR (KBr) 785, 831, 1046, 1181, 1251, 1417, 1430, 1506, 1518, 1615, 1700 cm^{-1}. HRESIMS *m/z* calcd for $C_{33}H_{25}NNaO_5$ $(M+Na)^+$ 538.1630, found 538.1649.

7-(4-Hydroxyphenyl)-3,8-bis(4-methoxyphenyl)-1*H*-pyrrolo[2,1-*c*][1,4]oxazin-1-one (1d)

A mixture of **14** (48.6 mg, 0.0942 mmol), K_2CO_3 (130.0 mg, 0.942 mmol), and MeI (0.050 mL, 0.80 mmol) in dry acetone (5 mL) was heated at 50 °C. The progress of the reaction was monitored by TLC and some additional 0.050 mL portions of MeI were added until the TLC indicated complete consumption of the starting material. The mixture was then cooled to room temperature, diluted with EtOAc (10 mL), filtered through a pad of Celite, and concentrated to give crude **15** (66.1 mg).

The crude product was dissolved in CH_2Cl_2 (2 mL) and Me_2S (0.350 mL, 4.73 mmol) and $BF_3{\cdot}OEt_2$ (0.232 mL, 1.88 mmol) were added. After 4 h at room temperature, the reaction was quenched by addition of 5% $NaHCO_3$ solution and the volatiles were evaporated by a current of N_2. The mixture was then extracted twice with EtOAc, washed with brine, dried (Na_2SO_4), and concentrated. The crude product was purified by silica gel chromatography eluted with hexane-EtOAc (2:1~1:1) to first afford **15** (8.1 mg, 0.015 mmol, 16%), followed by **1d** (14.7 mg, 0.0334 mmol, 36%).

15: pale yellow crystals, mp 208-210 °C. ^1H NMR (400 MHz) δ 3.83 (3H, s),

3.85 (3H, s), 5.03 (2H, s), 6.87 (4H, d, J = 8.8 Hz), 6.95 (2H, d, J = 9.0 Hz), 7.08 (2H, d, J = 8.8 Hz), 7.20 (1H, s), 7.28 (2H, d, J = 8.8 Hz), 7.32 (1H, s), 7.32-7.44 (5H, m), 7.65 (2H, d, J = 8.8 Hz). ^{13}C NMR (101 MHz) δ 55.1, 55.4, 70.0, 102.7, 112.9, 113.3, 114.3, 114.8, 118.9, 123.1, 124.3, 124.6, 125.8, 126.1, 127.5, 128.0, 128.1, 128.6, 129.8, 132.0, 136.9, 140.9, 154.3, 157.8, 158.9, 160.5. IR (KBr) 787, 839, 1032, 1176, 1249, 1431, 1506, 1517, 1609, 1728 cm^{-1}. HRESIMS *m/z* calcd for $C_{34}H_{27}NNaO_5$ $(M+Na)^+$ 552.1787, found 552.1820.

1d: pale yellow crystals, mp >300 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.77 (3H, s), 3.80 (3H, s), 6.65 (2H, d, J = 8.7 Hz), 6.88 (2H, d, J = 8.8 Hz), 6.94 (2H, d, J = 8.7 Hz), 7.05 (2H, d, J = 8.9 Hz), 7.17 (2H, d, J = 8.8 Hz), 7.60 (1H, s), 7.66 (2H, d, J = 8.9 Hz), 8.16 (1H, s), 9.43 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ55.0, 55.3, 103.7, 112.1, 113.1, 114.5, 115.3, 120.1, 122.9, 123.8, 124.8, 125.4, 127.6, 128.4, 129.5, 131.8, 140.5, 153.5, 156.4, 158.4, 160.0. IR (KBr) 797, 825, 1029, 1178, 1257, 1428, 1508, 1516, 1611, 1698, 3441 (br) cm^{-1}. HRESIMS *m/z* calcd for $C_{27}H_{21}NNaO_5$ $(M+Na)^+$ 462.1317, found 462.1325.

7-(4-Hydroxyphenyl)-3-(4-methoxyphenyl)-8-[4-(methoxymethoxy)phenyl]-1*H*-pyrrolo[2,1-*c*][1,4]oxazin-1-one (16)

Compound **13** (151 mg, 0.270 mmol) was hydrogenated over 10% $Pd(OH)_2$/C (27 mg) in THF (10 mL) at 13 °C under H_2 balloon atmosphere for 25 min. The mixture was then filtered, concentrated, and chromatographed on silica gel eluted with CH_2Cl_2-acetone (19:1) to afford **16** (110 mg, 0.235 mmol, 87%) as pale yellow crystals, mp 234-235 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.42 (3H, s), 3.81 (3H, s), 5.19 (2H, s), 6.65 (2H, d, J = 8.7 Hz), 6.94 (4H, d, J = 8.7 Hz), 7.03 (2H, d, J = 8.8 Hz), 7.18 (2H, d, J = 8.7 Hz), 7.57 (1H, s), 7.66 (2H, d, J = 8.8 Hz), 8.08 (1H, s), 9.26 (1H, s). ^{13}C NMR (101 MHz, $CDCl_3$-DMSO-d_6) δ 53.3, 53.8, 91.9, 101.7, 110.2, 112.4, 113.3, 113.4, 118.2, 120.9, 121.8, 123.5, 124.1, 125.8, 126.3, 127.6, 129.8, 138.6, 151.6, 154.1, 154.5, 158.0. IR (KBr) 844, 987, 1155, 1175, 1201, 1255, 1413, 1431, 1505, 1517, 1610, 1700, 1711, 3344 (br) cm^{-1}. HRESIMS *m/z* calcd for $C_{28}H_{23}NNaO_6$ $(M+Na)^+$ 492.1432, found 492.1384.

8-(4-Hydroxyphenyl)-3,7-bis(4-methoxyphenyl)-1*H*-pyrrolo[2,1-*c*][1,4]oxazin-1-one (1e)

Title compound was synthesized by subsequent methylation and demethoxymethylation

of **16** in 81% yield as described in the synthesis of **15** and **14**. **1e**: pale yellow crystals, mp 289-290 °C. ^{1}H NMR (300 MHz, acetone-d_6) δ 3.76 (3H, s), 3.85 (3H, s), 6.78 (2H, d, J = 8.7 Hz), 6.82 (2H, d, J = 8.7 Hz), 7.03 (2H, d, J = 9.0 Hz),7.12 (2H, d, J = 8.7 Hz), 7.15 (2H, d, J = 8.7 Hz), 7.56 (1H, s), 7.72 (2H, d, J = 9.0 Hz), 7.97 (1H, s), 7.98 (1H, s). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 55.0, 55.3, 103.7, 112.1, 113.9, 114.5, 114.7, 120.2, 122.9, 123.0, 125.4, 125.6, 127.0, 129.0, 129.4, 131.7, 140.5, 153.5, 156.7, 158.1, 160.0. IR (KBr) 794, 838, 1033, 1047, 1178, 1249, 1431, 1519, 1613. 1701, 2963, 3271 (br) cm^{-1}. HRESIMS *m/z* calcd for $C_{27}H_{21}NNaO_5$ $(M+Na)^+$ 462.1317, found 462.1327.

7-(4-Benzyloxyphenyl)-3-(4-methanefulfonyloxyphenyl)-8-[4-(methoxymethoxy)phenyl]-1*H*-pyrrolo[2,1-*c*][1,4]oxazin-1-one (18)

The title compound was synthesized in 39% yield by the same manner as Method B in the synthesis of **13** using DMF instead of DME. **18**: pale yellow crystals, mp 208-209 °C. ^{1}H NMR (300 MHz) δ 3.17 (3H, s), 3.50 (3H, s), 5.03 (2H, s), 5.19 (2H, s), 6.88 (2H, d, J = 8.8 Hz), 6.99 (2H, d, J = 8.8 Hz), 7.08 (2H, d, J = 8.8 Hz), 7.22 (1H, s), 7.27 (2H, d, J = 8.8 Hz), 7.33-7.43 (8H, m), 7.75 (2H, d, J = 8.8 Hz). ^{13}C NMR (101 MHz) δ 37.6, 56.1, 70.0, 94.4, 104.6, 112.9, 114.8, 115.5, 119.4, 122.5, 125.5, 125.7, 125.9, 127.5, 128.0, 128.6, 128.7, 129.8, 130.2, 132.0, 136.8, 140.4, 149.4, 153.8, 156.8, 157.9 (one quaternary carbon signal is overlapping). IR (KBr) 845, 887, 1003, 1048, 1158, 1177, 1199, 1234, 1342, 1429, 1507, 1741 cm^{-1}. HRESIMS *m/z* calcd for $C_{35}H_{29}NNaO_8S$ $(M+Na)^+$ 646.1512, found 646.1545.

7,8-Bis(4-hydroxyphenyl)-3-(4-methanefulfonyloxyphenyl)-1*H*-pyrrolo[2,1-*c*][1,4]oxazin-1-one (20)

The title compound was synthesized from **18** in 52% yield in the same manner as described for the synthesis of **1b**. **20**: pale yellow crystals, mp 164-165 °C. ^{1}H NMR (400 MHz, acetone-d_6) δ 3.32 (3H, s), 6.73 (2H, d, J = 8.6 Hz), 6.79 (2H, d, J = 8.6 Hz), 7.03 (2H, d, J = 8.6 Hz), 7.17 (2H, d, J = 8.6 Hz), 7.46 (2H, d, J = 8.9 Hz), 7.56 (1H, s), 7.89 (2H, d, J = 8.9 Hz), 8.15 (1H, s), 8.34 (1H, s), 8.37 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 37.5, 105.8, 112.1, 114.6, 115.3, 120.3, 122.9, 123.7, 125.5, 127.8, 129.4, 129.5, 129.7, 131.7, 139.1, 149.2, 153.2, 156.4, 156.7 (one quaternary carbon signal is overlapping). IR (KBr) 869, 1051, 1151, 1176, 1219, 1358, 1427, 1508, 1541, 1509, 1541,1653, 1729, 3406 (br) cm^{-1}. HRESIMS *m/z* calcd for $C_{26}H_{19}NNaO_7S$ $(M+Na)^+$

512.0780, found 512.0805.

3-(4-Methanefulfonyloxyphenyl)-7,8-bis(4-methoxyphenyl)-1*H*-pyrrolo[2,1-*c*][1,4]oxazin-1-one (21)

The title compound was synthesized from **20** in 83% yield in the same manner as described for the synthesis of **15**. **20**: pale yellow crystals, mp 207-208 °C. ^{1}H NMR (300 MHz) δ 3.18 (3H, d, J = 0.5 Hz), 3.79 (3H, s), 3.82 (3H, s), 6.80 (2H, d, J = 8.5 Hz), 6.87 (2H, d, J = 8.5 Hz), 7.08 (2H, d, J = 8.5 Hz), 7.22 (1H, s), 7.28 (2H, d, J = 8.5 Hz), 7.35 (2H, d, J = 8.8 Hz), 7.44 (1H, s), 7.75 (2H, d, J = 8.8 Hz). ^{13}C NMR (101 MHz) δ37.6, 55.1, 55.2, 104.6, 112.9, 113.4, 113.9, 119.3, 122.6, 124.3, 125.5, 125.9, 128.7, 129.8, 129.9, 130.4, 132.0, 140.5, 149.4, 153.8, 158.7, 159.0. IR (KBr) 845, 867, 1034, 1158, 1181, 1251, 1366, 1435, 1509, 1738 cm^{-1}. HRESIMS *m/z* calcd for $C_{28}H_{23}NNaO_7S$ $(M+Na)^+$ 540.1093, found 540.1054.

3-(4-Hydroxyphenyl)-7,8-bis(4-methoxyphenyl)-1*H*-pyrrolo[2,1-*c*][1,4]oxazin-1-one (1f)

To a solution of **21** (25.0 mg, 0.0483 mmol) in dry THF (1.5 mL), was added a 1M THF solution of TBAF (0.193 mL, 0.193 mmol) at room temperature under Ar atmosphere (Fox 2002). After 4.5 h, additional portion (0.193 mL) portion of TBAF (0.193 mmol) was added and the mixture was stirred for another 2 h. The reaction was then quenched by 10% NH_4Cl solution, extracted 3 times with EtOAc, dried over Na_2SO_4, and concentrated. The crude product was purified by silica gel column chromatography eluted with CH_2Cl_2-acetone (19:1) to give **1f** (13.5 mg, 0.0307 mmol, 64%) as pale yellow crystals, mp 215-216 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.71 (3H, s), 3.76 (3H, s), 6.83 (2H, d, J = 8.8 Hz), 6.87 (2H, d, J = 8.8 Hz), 6.88 (2H, d, J = 8.8 Hz), 7.06 (2H, d, J = 8.8 Hz), 7.17 (2H, d, J = 8.8 Hz), 7.55 (2H, d, J = 8.8 Hz), 7.64 (1H, s), 8.08 (1H, s), 9.90 (1H, br). ^{13}C NMR (101 MHz, DMSO-d_6) δ 55.0, 55.1, 103.1, 112.2, 113.2, 114.0, 115.8, 120.2, 121.2, 124.8, 125.5, 125.6, 127.0, 128.3, 129.5, 131.8, 141.0, 153.6, 158.2, 158.5 (2C). IR (KBr) 835, 1038, 1176, 1247, 1423, 1505, 1519, 1541, 1611, 1717, 2925, 3321 (br) cm^{-1}. HRESIMS *m/z* calcd for $C_{27}H_{21}NNaO_5$ $(M+Na)^+$ 462.1317, found 462.1320.

7-(4-Benzyloxyphenyl)-8-[4-(methoxymethoxy)phenyl]-3-phenyl-1*H*-pyrrolo[2,1-*c*][1,4]oxazin-1-one (19)

The title compound was synthesized by the same procedure as Method B described in the synthesis of **13** using phenacyl bromide instead of 4'-methoxyphenacyl bromide in 65% yield. **19**: white crystals, mp 163-164 °C. ^{1}H NMR (500 MHz) δ 3.52 (3H, s), 5.05 (2H, s), 5.21 (2H, s), 6.89 (2H, d, J = 8.8 Hz), 7.01 (2H, d, J = 8.8 Hz), 7.10 (2H, d, J = 8.8 Hz), 7.22 (1H, s), 7.30 (2H, d, J = 8.8 Hz), 7.38-7.45 (9H, m), 7.73 (2H, dd, J = 7.1, 1.4 Hz). ^{13}C NMR (125 MHz) δ 56.1, 69.9, 94.4, 103.9, 113.0, 114.7, 115.5, 119.1, 124.2, 125.6, 125.9, 127.5, 127.9, 128.3, 128.5, 128.8, 129.2, 129.8, 130.5, 130.5, 132.0, 136.8, 141.8, 154.1, 156.7, 157.8. IR (KBr) 758, 844, 997, 1033, 1078, 1153, 1175, 1198, 1235, 1426, 1450, 1503. 1536, 1610, 1717 cm^{-1}. HRESIMS *m/z* calcd for $C_{34}H_{27}NNaO_5$ $(M+Na)^+$ 552.1787, found 552.1775.

7,8-Bis(4-hydroxyphenyl)-3-phenyl-1*H*-pyrrolo[2,1-*c*][1,4]oxazin-1-one (1g)

The title compound was synthesized from **19** by the same procedure used for the synthesis of **1b** in 73% yield. **1g**: pale yellow crystals, mp >300 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.66 (2H, d, J = 8.7 Hz), 6.70 (2H, d, J = 8.7 Hz), 6.95 (2H, d, J = 8.7 Hz), 7.06 (2H, d, J = 8.7 Hz), 7.41-7.43 (1H, m), 7.47-7.51 (2H, m), 7.62 (1H, s), 7.72-7.74 (2H, m), 8.29 (1H, s), 9.44 (1H, s), 9.48 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 105.2, 112.1, 114.6, 115.3, 120.3, 123.0, 123.7, 123.8, 127.7, 129.0, 129.2, 129.5, 130.6, 131.8, 140.1, 153.4, 156.4, 156.7 (one quaternary carbon signal is overlapping). IR (KBr) 760, 829, 845, 1059, 1172, 1199, 1235, 1256, 1274, 1371, 1420, 1507, 1552, 1613, 1697, 3196, 3403 (br) cm^{-1}. HRESIMS *m/z* calcd for $C_{25}H_{17}NNaO_4$ $(M+Na)^+$ 418.1055, found 418.1069.

In vitro Aldose Reductase Inhibition Assay

The ALR2 activity assay was performed in a 96-well plate following standard protocols (Nishimura et al 1991; Mylari et al 2003; Saito et al 2009). In brief, a reaction mixture containing 25 μL of sample solution in MeOH containing DMSO (<10% v/v), 25 μL of 1.25 mM β-NADPH (Oriental Yeast, Osaka, Japan), 20 μL of 50 mg/mL human recombinant aldose reductase (ATGen, Seongnam, South Korea) in 155 μL of 100 mM pottasium phosphate buffer (pH 6.2) was preincubated at 37 °C for 5 min. The reaction was initiated by adding 25 μL of 20 mM DL-glyceraldehyde (Wako Pure Chemical, Osaka, Japan). The rate of decrease in optical density at 340 nm after 30 min at 37 °C was recorded using a BioTek Cytation 3 microplate reader (BioTek Instruments Inc., USA). To correct for non-enzymatic oxidation of NADPH, a reference blank assay was

performed using the buffer solution instead of the sample and enzyme solutions. The inhibitory activity of the test compound was calculated using following equation:

Inhibitory activity (%) = $((\Delta OD_{contol} - \Delta OD_{sample})/ (\Delta OD_{control} - \Delta OD_{blank})) \times 100$

The experiment was performed at least in triplicates. The IC_{50} values of the test compounds were calculated using log linear regression analysis of the log dose-inhibition curves.

Acknowledgements

We thank Mr. Kotaro Shutoh for his support in the synthesis of lukianol derivatives. We would like to thank Editage (www.editage.com) for English language editing. This work was the result of using research equipment shared in MEXT Project for promoting public utilization of advanced research infrastructure（Program for supporting introduction of the new sharing system）Grant Number JPMXS0422500320.

Supplementary material

Supplementary material is available at Bioscience, Biotechnology, and Biochemistry online.

Data availability

The data underlying this article are available in the article and in its online supplementary material.

Author Contribution

F.I. designed this study; T.F. contributed to the discussion; F.I., T.K., and M.S. performed the synthesis; F.I. and S.Z. performed the bioassay; M.U. performed the data analysis; F.I. wrote the manuscript with assistance from T.F. and M.U.

Funding

This work was supported in part by a Grant-in-Aid for Scientific Research from Nagasaki University, Japan.

Disclosure Statement

The authors declare there are no conflicts of interest.

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552 **References**

553 Banwell MG, Flynn BL, Hamel E et al. Convergent synthesis of the pyrrolic marine
554 natural products lamellarin-O, lamellarin-Q, lukianol-A and some more highly
555 oxygenated congeners. Chem Commun 1997: 207−208.

556 Billek G. p-Hydroxyphenylpyruvic acid. Org Synth 1963;43:49-53.

557 Boger DL, Boyce CW, Labroli MA et al. Total syntheses of ningalin A, lamellarin O,
558 lukianol A, and permethyl storniamide A utilizing heterocyclic azadiene Diels -
559 Alder reactions. J Am Chem Soc 1999;121:54−62.

560 Bullington JL, Wolff RR, Jackson PF. Regioselective preparation of 2-substituted 3,4-
561 diaryl pyrroles: A concise total synthesis of ningalin B. J Org Chem
562 2002;67:9439−9442.

563 de la Fuente JÁ, Manzanaro S. Aldose reductase inhibitors from natural sources. Nat Prod
564 Rep 2003;20:243-251.

565 Dolenc D. N-Iodosaccharin – a new reagent for iodination of alkenes and activated
566 aromatics. Synlett 2000: 544-546.

567 Fox E, Lennon IC, Meek G. A novel synthesis of 5-hydroxy-2,2-dimethyl-10-propyl-2*H*-
568 pyrano[2,3-*f*]chromen-8-one. Tetrahedron Lett 2002;43;2899−2902.

569 Fuji K, Kawabata T, Fujita E. Hard acid and soft nucleophile system. IV. Removal of
570 benzyl protecting group with boron trifluoride etherate and dimethyl sulfide. Chem
571 Pharm Bull 1980;28:3662−3664.

572 Fürstner A, Weintritt H, Hupperts A. A new, titanium-mediated approach to pyrroles:
573 First synthesis of lukianol A and lamellarin O dimethyl ether. J Org Chem 1995;60:
574 6637−6641.

575 Ghafary S, Najafi Z, Mohammadi-Khanaposhtani, M et al. Novel cinnamic acid–
576 tryptamine hybrids as potent butyrylcholinesterase inhibitors: Synthesis,
577 biologicalevaluation, and docking study. Arch Pharm Chem Life Sci
578 2018;351:e1800115.

579 Gupton JT, Sikorski JA. The application of vinylogous iminium salt derivatives to a
580 regiocontrolled and efficient relay synthesis of lukianol A and related marine

natural products. Tetrahedron 1999;55:14515−14522.

Hinze C, Kreipi A, Terpin A et al. Synthesis of simple 3,4-diarylpyrrole-2,5-dicarboxylic acid and lukianol A by oxidative condensation of 3-arylpyruvic acid with ammonia. Synthesis 2007: 608−612.

Kerru N, Singh-Pillay A, Awolade P et al. Current anti-diabetic agents and their molecular targets: A review. Eur J Med Chem 2018;152:436−488.

Kim S, Son S, Kang H. Efficient syntheses of 2-carbomethoxy-3,4-disubstituted pyrroles by the condensation of vinylogous amides with aminomalonate. Bull Korean Chem Soc 2001;22:1403−1406

Kousaxidis A, Petrou A, Lavrentaki V et al. Aldose reductase and protein tyrosine phosphatase 1B inhibitors as a promising therapeutic approach for diabetes mellitus. Eur J Med Chem 2020;207:112742.

Liu JH, Yang QC, Mak TCW et al. Highly regioselective synthesis of 2,3,4-trisubstituted 1*H*-pyrroles: A formal total synthesis of lukianol A. J Org Chem 2000;65:3587−3595.

Lu Y, Arndtsen BA. A direct phosphine-mediated synthesis of pyrroles from accid chlorides and α,β-unsaturated imines. Org Lett 2009;11:1369-1372.

Manzanaro S, Salvá, de la Fuente JA. Phenolic marine natural products as aldose reductase inhibitors. J Nat Prod 2006;69:1485−1487.

Mathew P, Asokan CV. Cyclization of functionalized ketene-N,S-acetals to substituted pyrroles: application in the synthesis of marine pyrrole alkaloids. Tetrahedron Lett 2005;46:475-478.

Morikawa D, Morii K, Yasuda Y et al. Convergent total synthesis of lamellarins and their congeners. J Org Chem 2020;85:8603-8617.

Mylari BL, Armento SJ, Beebe DA et al. A highly selective, non-hydantoin, non-carboxylic acid inhibitor of aldose reductase with potent oral activity in diabetic rat models: 6-(5-Chloro-3-methylbenzofuran- 2-sulfonyl)-2-H-pyridazin-3-one. J Med Chem 2003;46:2283−2286

609 Niimi N, Yako H, Takaku S et al. Aldose reductase and the polyol pathway in Schwann
610 cells: Old and new problems. Int J Mol Sci 2021;22:1031−1045.
611 Nishimura C, Yamaoka T, Mizutani M et al. Purification and characterization of the
612 recombinant human aldose reductase expressed in baculovirus system. Biochim
613 Biophys Acta 1991;1078:171−178.
614 Saito R, Tokita M, Uda K et al. Synthesis and in vitro evaluation of botryllazine B
615 analogues as a new class of inhibitor against human aldose reductase. Tetrahedron
616 2009;65:3019−3026.
617 Sato A, Morishita T, Shiraki T et al. Aldose reductase inhibitors from a marine sponge,
618 *Dictyodendrilla* sp. J Org Chem 1993;58:7632-7634.
619 Satyanarayana I, Yang DY, Liou TJ. Synthesis of lamellarin R, lukianol A, lamellarin O
620 and their analogues. RSC Adv 2020;10:43168−43174.
621 Takamura K, Matsuo H, Tanaka A et al. Total synthesis of the marine natural products
622 lukianols A and B. Tetrahedron 2013;69:2782−2788.
623 Thakur S, Gupta SK, Ali V et al. Aldose Reductase: a cause and a potential target for the
624 treatment of diabetic complications. Arch Pharm Res 2021;44:655−667
625 Yabe-Nishimura Aldose reductase in glucose toxicity: A potential target for the
626 prevention of diabetic complications. Pharmacol Rev 1998;50:21−34.
627 Yoshida WY, Lee KK, Carroll AR et al. A complex pyrrolo-oxazinone and its iodo
628 derivative isolated from a tunicate. Helv Chim Acta 1992;75:1721−1725.
629 You HW, Lee KL. Halodecarboxylation of α,β-unsaturated carboxylic acids bearing aryl
630 and styrenyl group at β-carbon with oxone® and sodium halide. Synlett 2001:105−
631 107.
632 Zhou C, Ma D. A copper-catalyzed coupling reaction of vinyl halides and carbazates:
633 application in the assembly of polysubstituted pyrroles. Chem Commun
634 2014;50:3085−3088.

635

Figure caption/legend

Fig. 1. Structure of lukianol A (**1**), B (**2**), and Fürstner interemediate (**3**).

Scheme 1. Zhou and Ma's polysubstituted pyrrole synthesis

Scheme 2. Synthesis of lukianol A (**1a**), 4′-*O*-methyl lukianol A (**1b**), and tri-*O*-methyl lukianol A (**1c**). (a) NaBr, Na_2CO_3, Oxone®, aq. CH_3CN (77%); (b) $BocNHNH_2$, CuI, K_2CO_3, DMSO, 80 °C (95%); (c) AcOH, EtOH, reflux (89%); (d) MOMCl, *t*-BuOK, THF (82%); (e) Cs_2CO_3, THF, reflux (95%); (f) Cs_2CO_3, THF, reflux (51%); (g) BBr_3, CH_2Cl_2, -78 °C (1 h), 0 °C (3 h), and rt (30 min), (**1a**: 98%); (h) $BF_3{\cdot}OEt_2$, Me_2S, CH_2Cl_2 (**1b**: 85%); (i) MeI, K_2CO_3, DMF (100%).

Scheme 3. Synthesis of 4″-*O*-methyl lukianol A (**1d**) and 4‴-*O*-methyl lukianol A (**1e**). (a) HCl, EtOH, reflux, (**14**: 94%, **1e**: 81% (2 steps)); (b) MeI, K_2CO_3, acetone, reflux; (c) $BF_3{\cdot}OEt_2$, Me_2S, CH_2Cl_2, 36% (**1c**, 2 steps); (d) H_2, $Pd(OH)_2$-C, THF, 87%.

Scheme 4. Synthesis of 4′,4‴-bis-*O*-methyl lukianol A (**1f**) and 4′-dehydroxy lukianol A (**1g**). (a) Cs_2CO_3 (**18**: in DMF, 39%, **19**: in DME, 69%); (b) $BF_3{\cdot}OEt_2$, Me_2S, CH_2Cl_2, (**20**: 52%, **1g**: 73%); (c) MeI, K_2CO_3, acetone, reflux (83%); (d) Bu_4NF, THF (64%) (Fox 2002).

Fig. 2. Aldose reductase inhibitors isolated from a marine sponge, *Dictyodendrilla* sp.

672

673 **Graphical abstract caption**

674

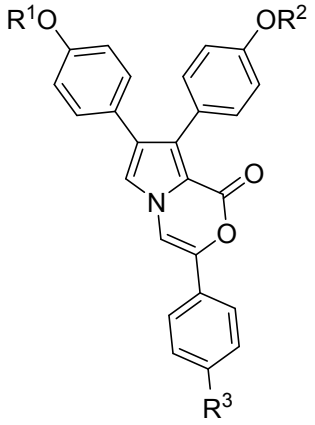
675 Lukianol A (**1a**) and its derivatives **1b**–**1g** were synthesized and evaluated for their ability
676 to inhibit human aldose reductase (Table 1).

677

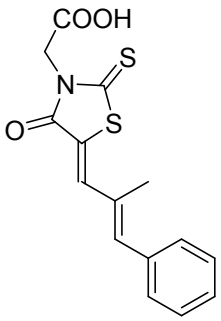
678

679 Table 1. In vitro human ALR2 inhibitory activity of lukianol A derivatives **1a**-**1g** and

680 epalrestat.



1a~1g



epalrestat

681

Compound	R^1	R^2	R^3	Concentration (μM)	Inhibition (%)[a]	IC_{50} (μM)[b]	R^2
1a	H	H	OH	50	79.4±8.1	2.2	0.981
				10	62.3±7.4		
				2	47.0±7.9		
				0.83	43.9±6.2		
1b	H	H	OMe	50	79.8±10.7	5.3	0.989
				10	46.1±8.7		
				2	36.8±8.7		
				0.83	25.6±6.7		
1c	Me	Me	OMe	10	6.1±4.9	nd[c]	
1d	H	Me	OMe	10	1.7±0.6	nd[c]	
1e	Me	H	OMe	50	55.5±3.4	47.8	0.937
				10	22.4±4.1		
				2	10.7±0.5		
				0.83	3.0±2.1		
1f	Me	Me	OH	10	1.3±1.0	nd[c]	

1g	H	H	H	50	85.9±5.6	3.2	0.999
				10	64.3±13.7		
				2	44.1±9.9		
				0.83	32.0±6.6		
epalrestat				10	67.2±6.0	0.8	0.981

[a]Each value represents the mean±SEM (n=3).

[b]IC_{50} values were calculated from the least-square regression line of the logarithmic concentration plotted against inhibitory activity.

[c]not determined

687

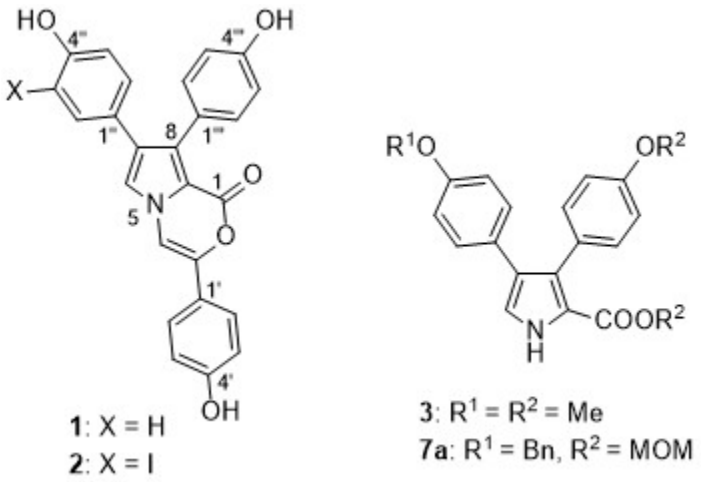
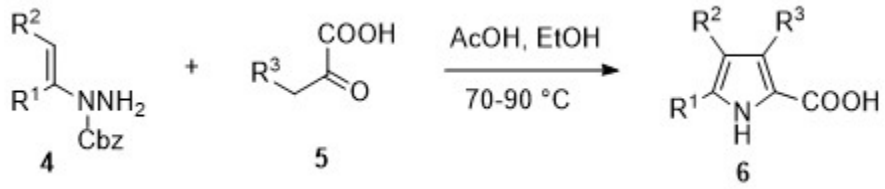


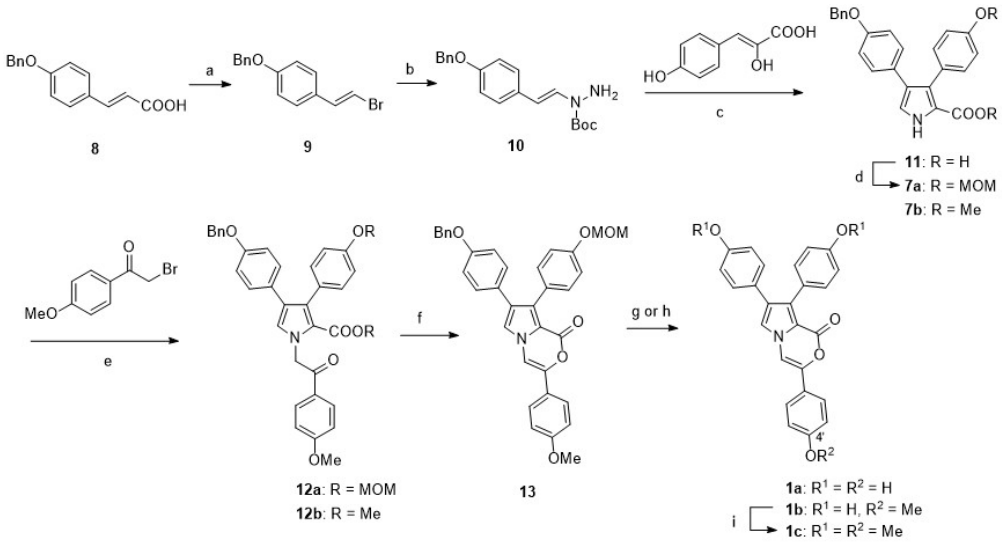
Fig. 1. Structure of lukianol A (**1**), B (**2**), and Fürstner interemediate (**3**).

92x64mm (96 x 96 DPI)



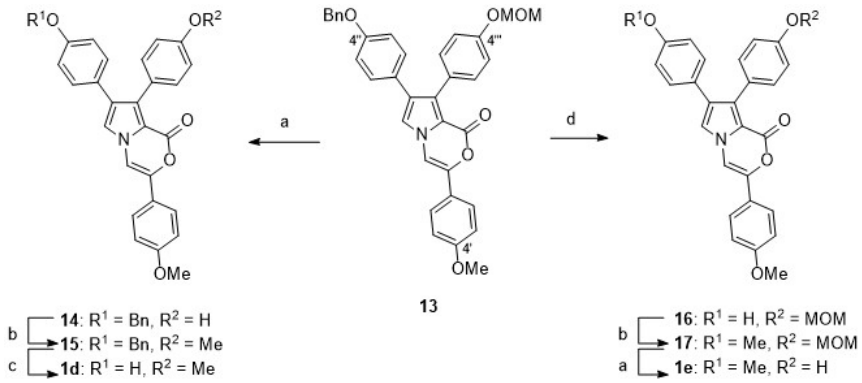
Scheme 1. Zhou and Ma's polysubstituted pyrrole synthesis.

116x26mm (96 x 96 DPI)



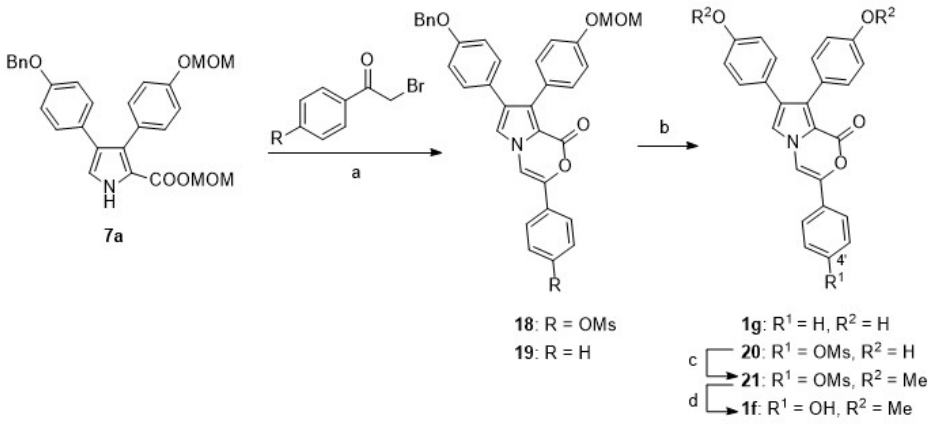
Scheme 2. Synthesis of lukianol A (**1a**), 4′-*O*-methyl lukianol A (**1b**), and tri-*O*-methyl lukianol A (**1c**). (a) NaBr, Na_2CO_3, Oxone®, aq. CH_3CN (77%); (b) $BocNHNH_2$, CuI, K_2CO_3, DMSO, 80 °C (95%); (c) AcOH, EtOH, reflux (89%); (d) MOMCl, *t*-BuOK, THF (82%); (e) Cs_2CO_3, THF, reflux (95%); (f) Cs_2CO_3, THF, reflux (51%); (g) BBr_3, CH_2Cl_2, -78 °C (1 h), 0 °C (3 h), and rt (30 min), (**1a**: 98%); (h) $BF_3{\cdot}OEt_2$, Me_2S, CH_2Cl_2 (**1b**: 85%); (i) MeI, K_2CO_3, DMF (100%).

228x124mm (96 x 96 DPI)



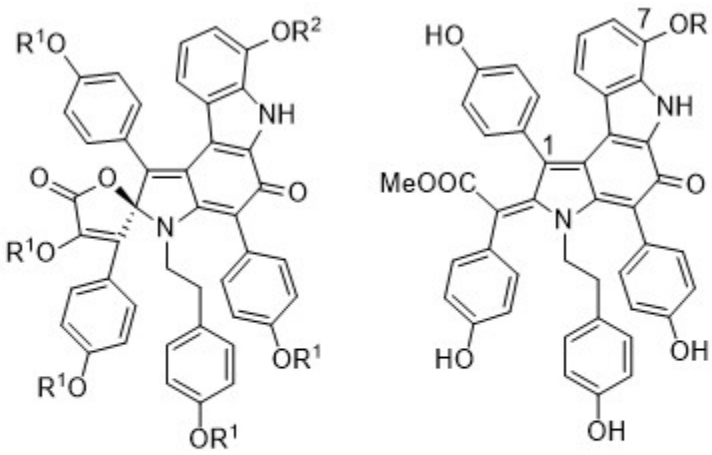
Scheme 3. Synthesis of 4''-*O*-methyl lukianol A (**1d**) and 4'''-*O*-methyl lukianol A (**1e**). (a) HCl, EtOH, reflux, (**14**: 94%, **1e**: 81% (2 steps)); (b) MeI, K_2CO_3, acetone, reflux; (c) $BF_3{\cdot}OEt_2$, Me_2S, CH_2Cl_2, 36% (1c, 2 steps); (d) H_2, $Pd(OH)_2$-C, THF, 87%.

202x78mm (96 x 96 DPI)



Scheme 4. Synthesis of 4′,4′′′-bis-*O*-methyl lukianol A (**1f**) and 4′-dehydroxy lukianol A (**1g**). (a) Cs_2CO_3 (**18**: in DMF, 39%, **19**: in DME, 69%); (b) $BF_3{\cdot}OEt_2$, Me_2S, CH_2Cl_2, (**20**: 52%, **1g**: 73%); (c) MeI, K_2CO_3, acetone, reflux (83%); (d) Bu_4NF, THF (64%) (Fox 2002).

194x83mm (96 x 96 DPI)



22a: R^1 = H, R^2 = SO_3Na
22b: R^1 = H, R^2 = SO_3H
22c: R^1 = R^2 = H
22d: R^1 = R^2 = Ac

23a: R = SO_3H
23b: R = H

Fig. 2. Aldose reductase inhibitors isolated from a marine sponge, Dictyodendrilla sp.

94x83mm (96 x 96 DPI)

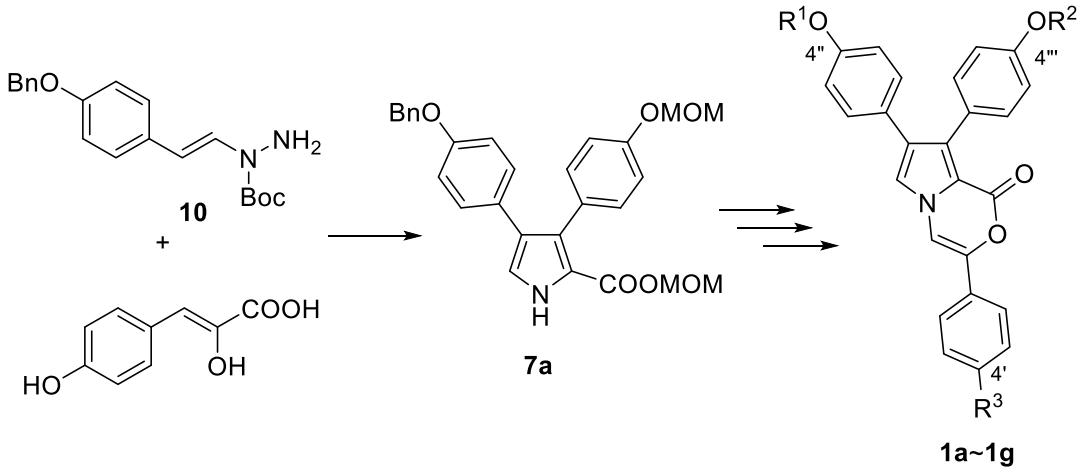


Table1. h-ALR2 inhibitory activity of **1a-1g**

comp.	R^1	R^2	R^3	IC_{50} (μM)
1a	H	H	OH	2.2
1b	H	H	OMe	5.3
1c	Me	Me	OMe	na*
1d	H	Me	OMe	na*
1e	Me	H	OMe	48
1f	Me	Me	OH	na*
1g	H	H	H	3.2

*no activity at 10 μM.

Supplemental materials

Synthesis and structure-activity relationship study of the aldose reductase inhibiting marine alkaloid lukianol A and its derivatives

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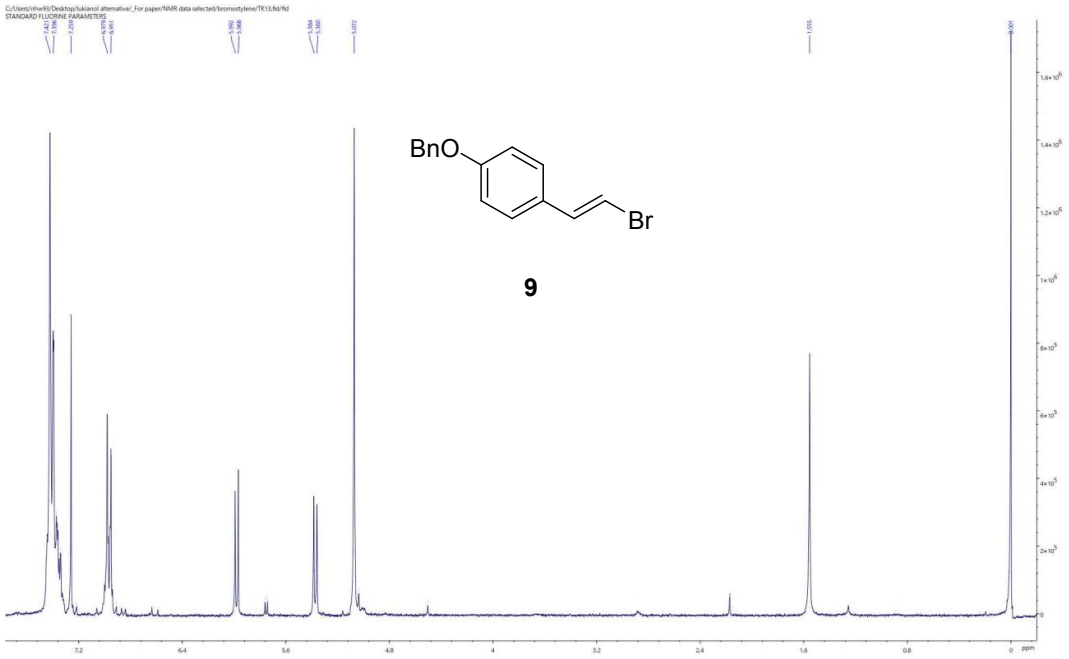
[1]Graduate School of Fisheries and Environmental Sciences, and [2]Environmental Protection Center, Nagasaki University, 1-14 Bunkyo-Machi, Nagasaki, Japan

[3]School of Earth and Environment, Anhui University of Science and Technology, Huainan 232001, China

Contents

Fig. 1. NMR spectra of compound **9**

^{1}H NMR spectrum of compound **9** (300 MHz, $CDCl_3$)



^{13}C NMR spectrum of compound **9** (75 MHz, $CDCl_3$)

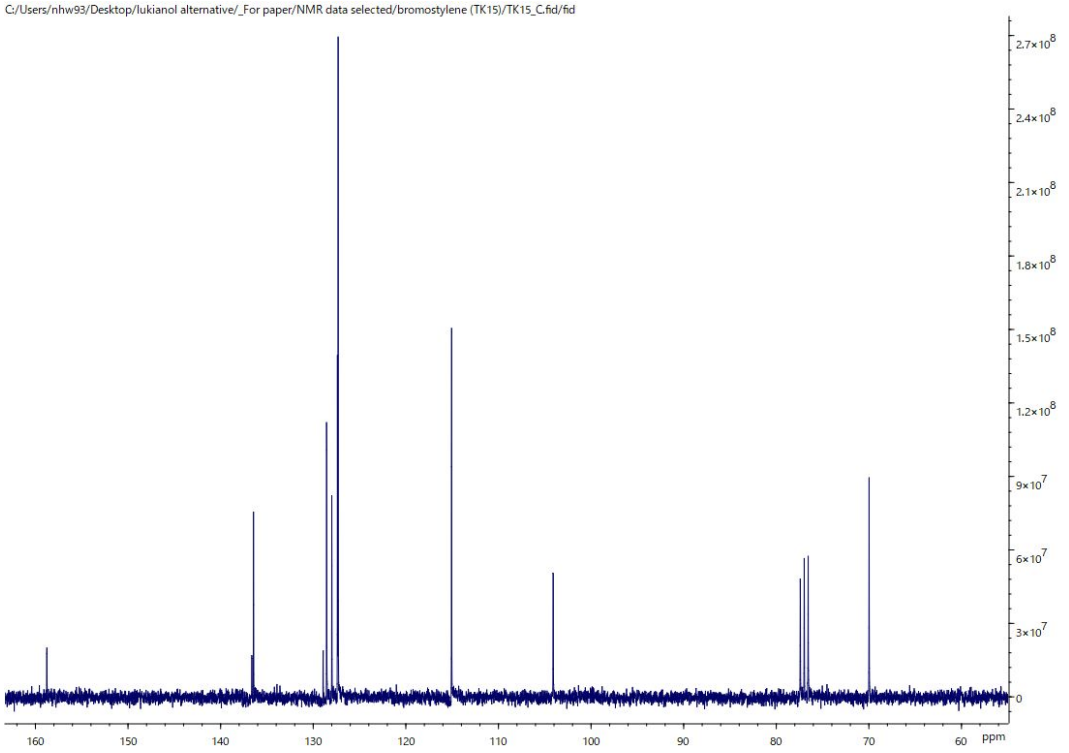
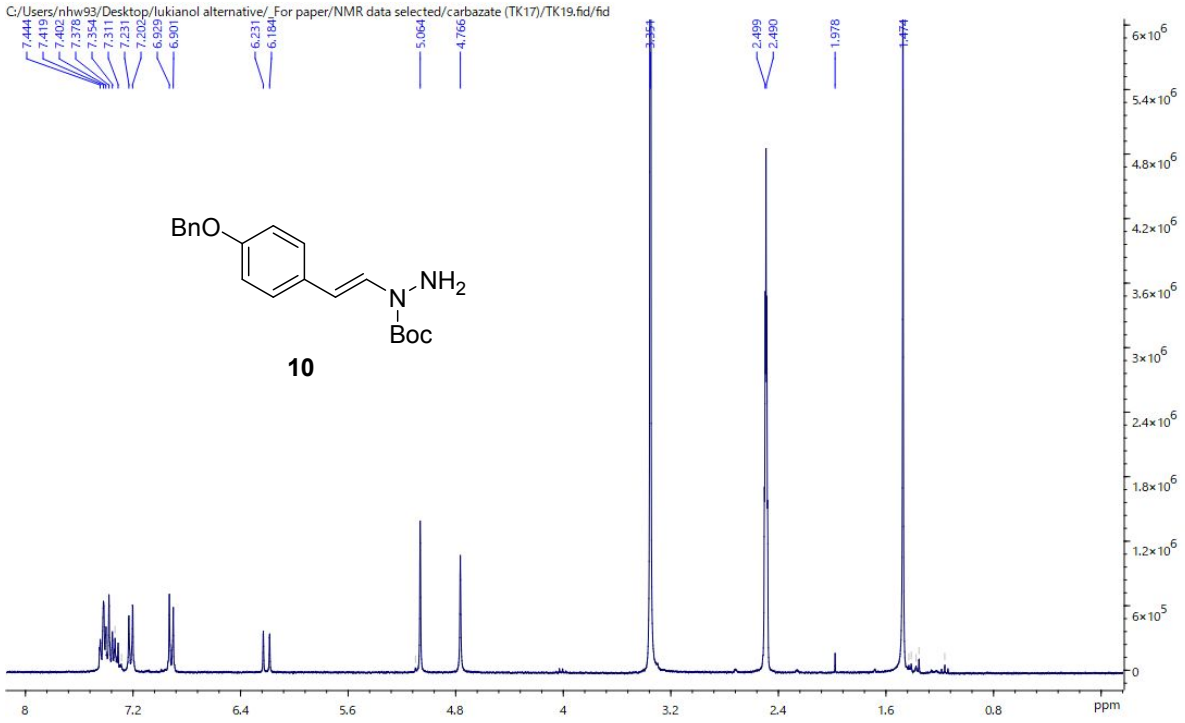


Fig. 2. NMR spectra of compound **10**

^{1}H NMR spectrum of compound **10** (300 MHz, DMSO-d_6)



^{13}C NMR spectrum of compound **10** (75 MHz, $CDCl_3$)

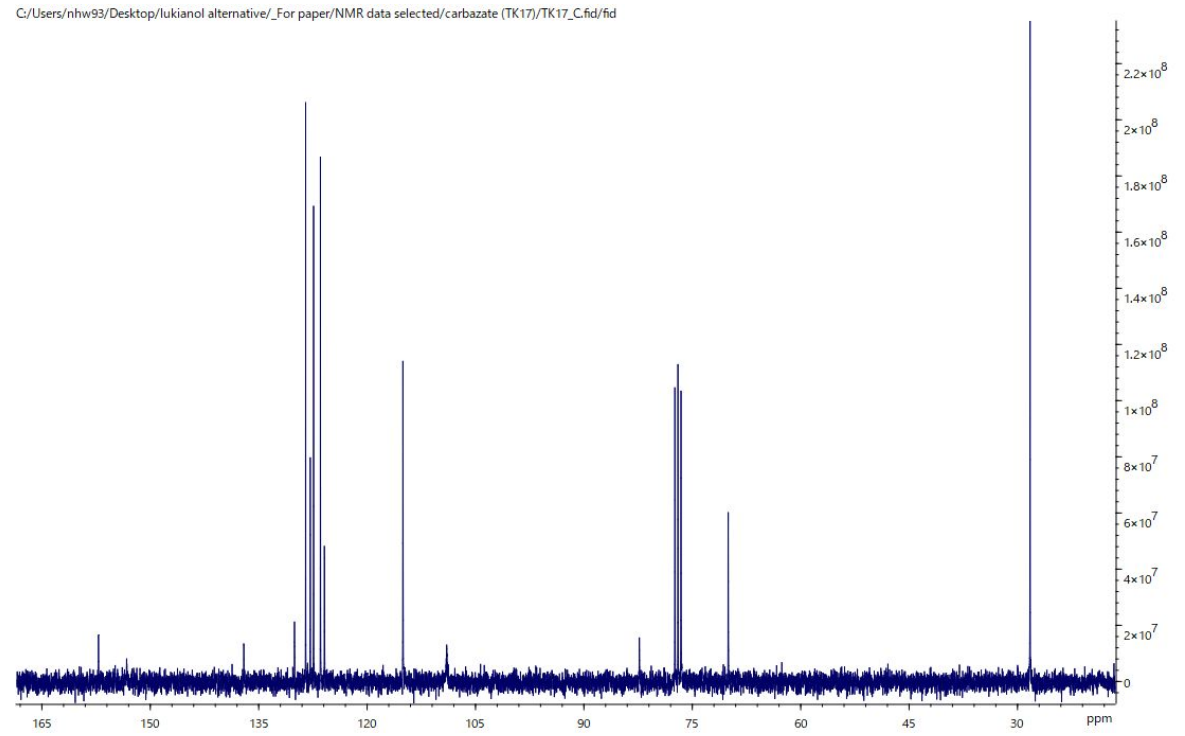
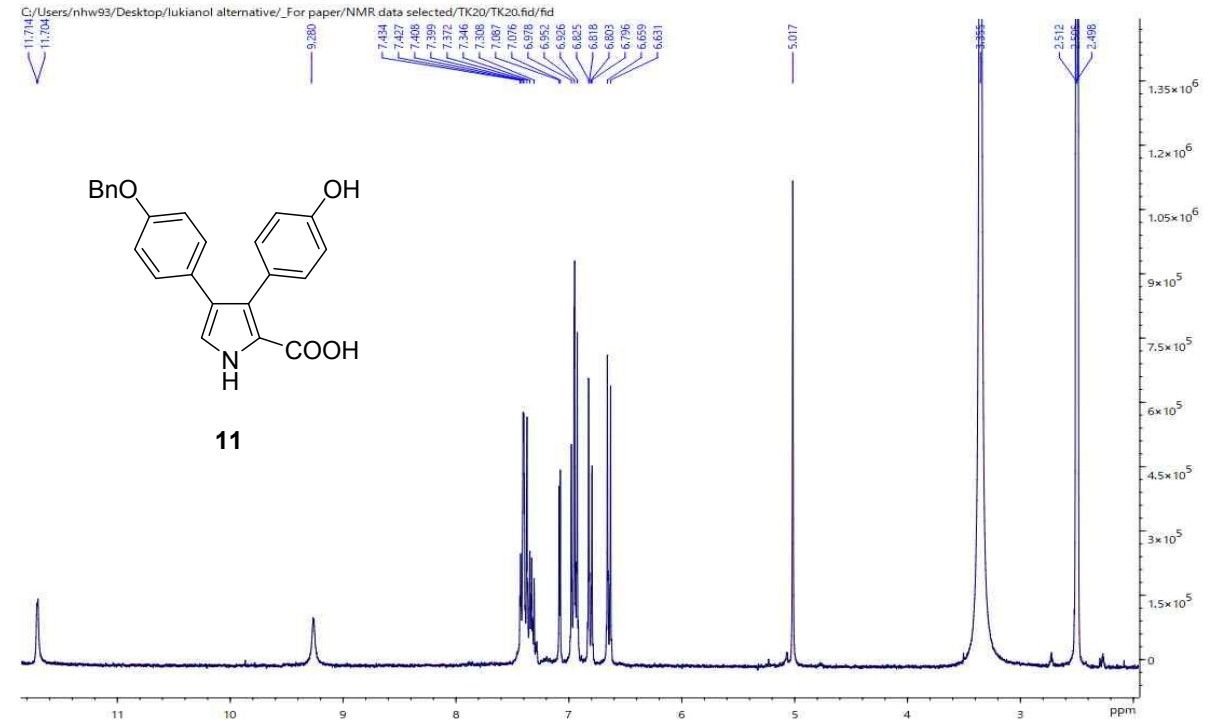


Fig. 3. NMR spectra of compound **11**

^{1}H NMR spectrum of compound **11** (300 MHz, DMSO-d_6)



^{13}C NMR spectrum of compound **11** (101 MHz, DMSO-d_6)

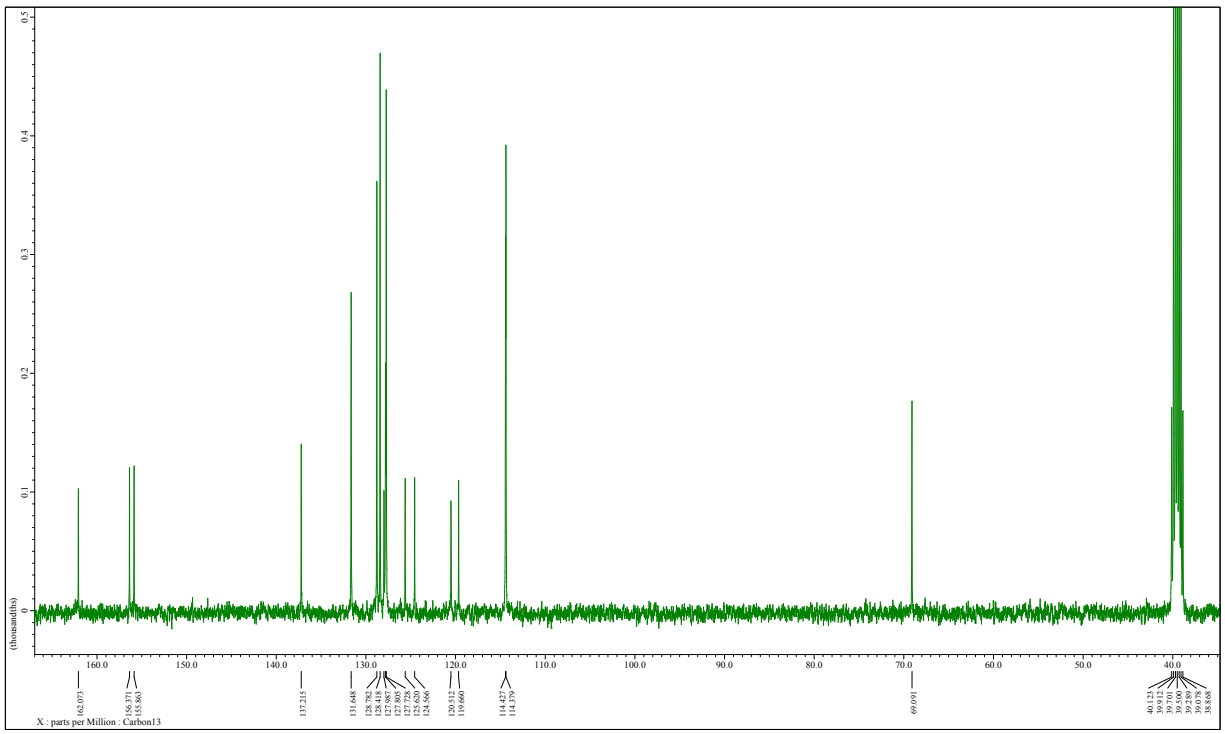
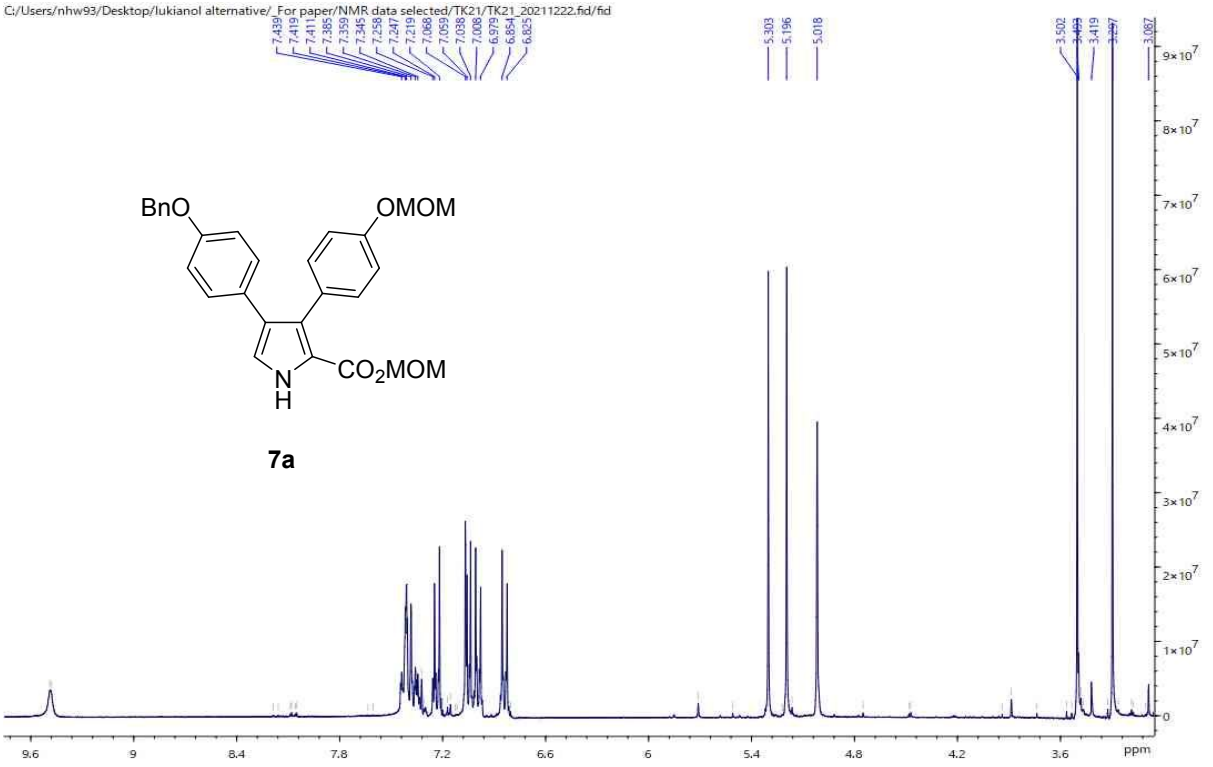


Fig. 4. NMR spectra of compound **7a**

^{1}H NMR spectrum of compound **7a** (300 MHz, $CDCl_3$)



^{13}C NMR spectrum of compound **7a** (75 MHz, $CDCl_3$)

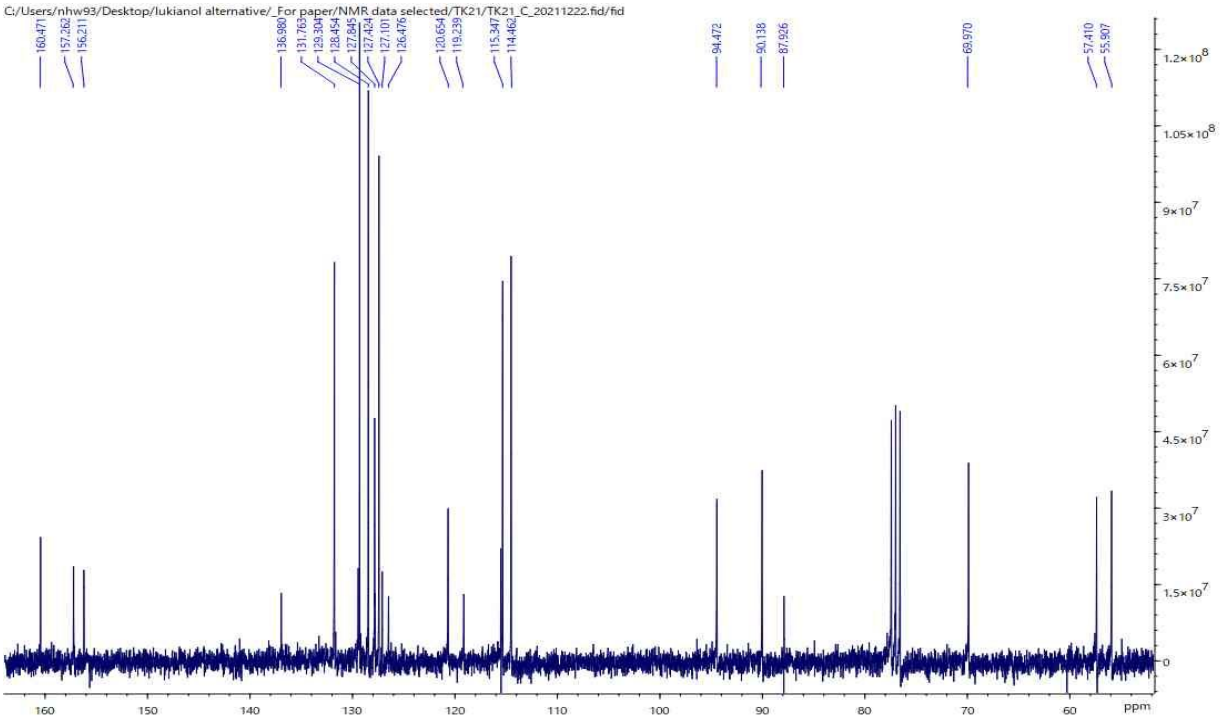
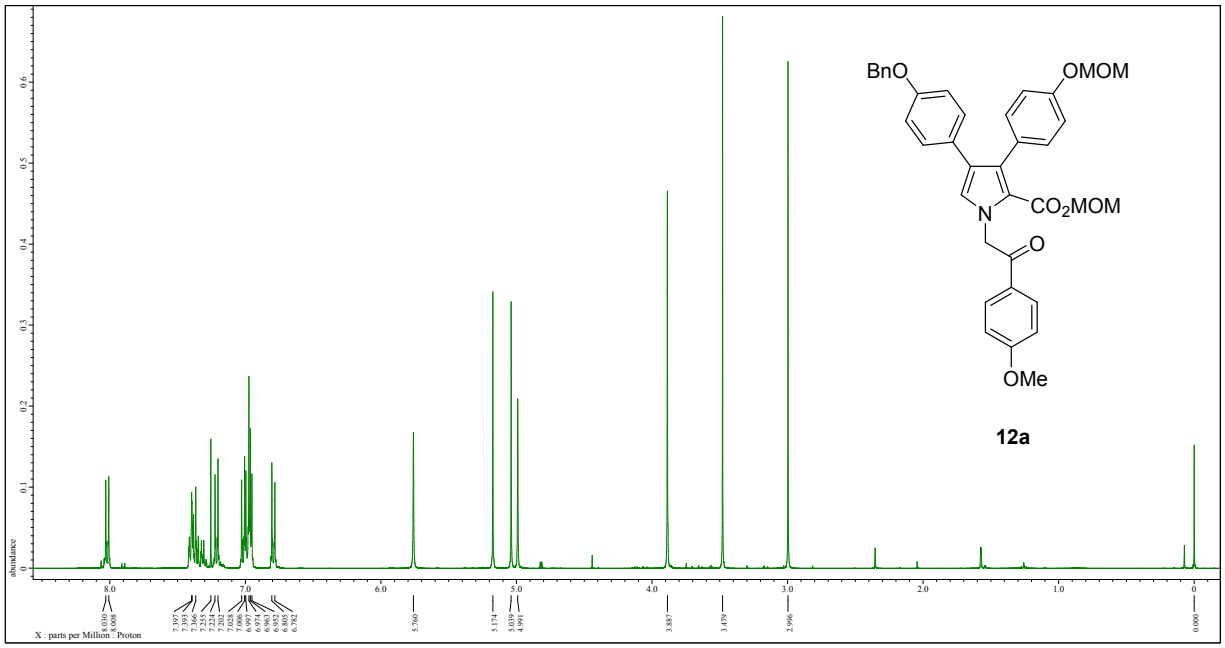


Fig. 5. NMR spectra of compound **12a**

^{1}H NMR spectrum of compound **12a** (400 MHz, $CDCl_3$)



^{13}C NMR spectrum of compound **12a** (101 MHz, $CDCl_3$)

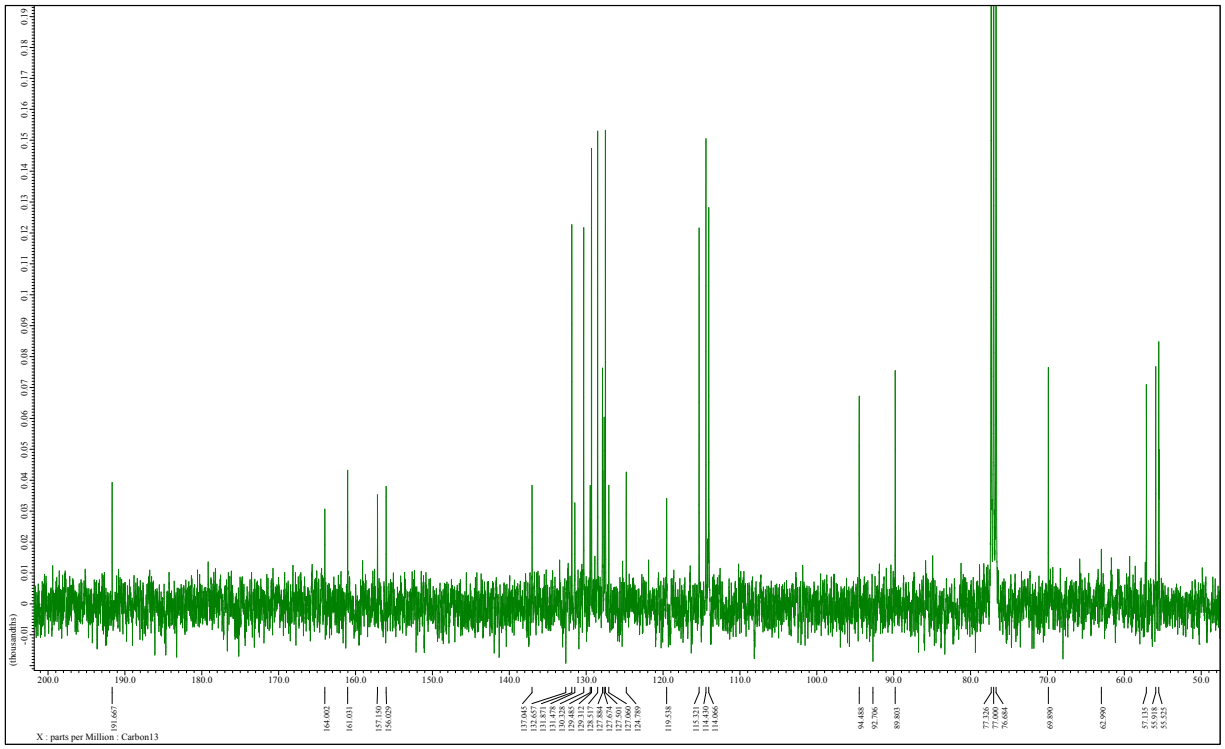
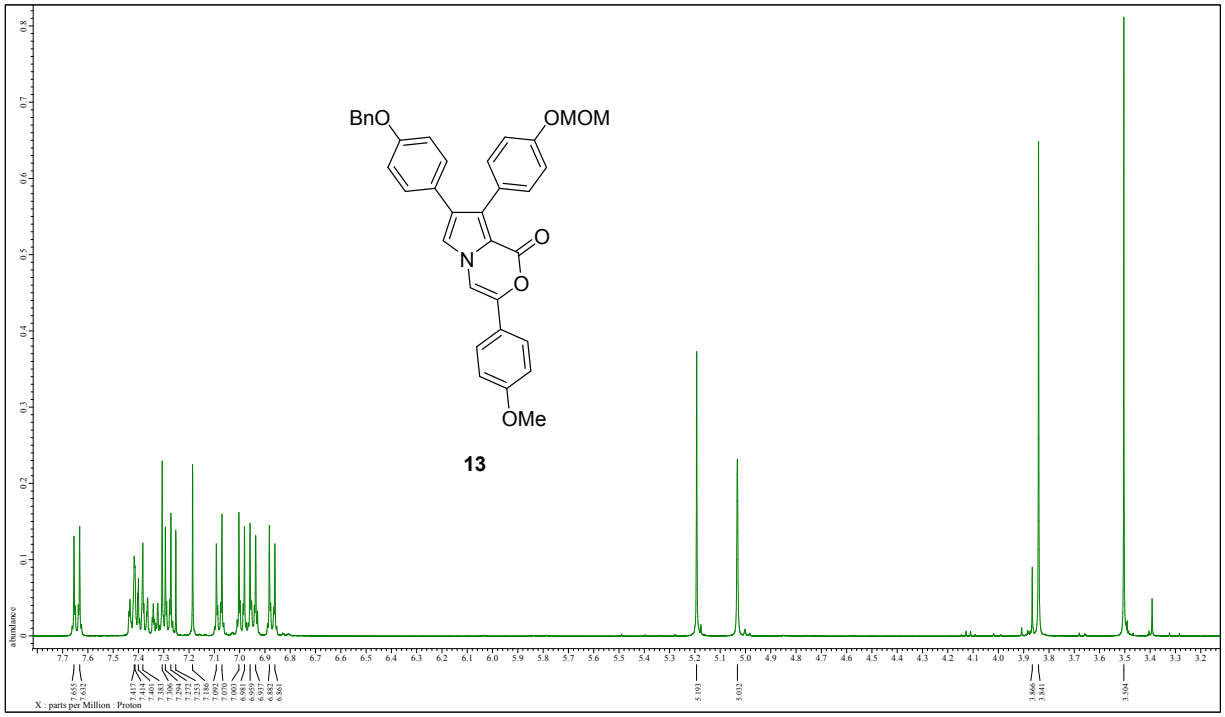


Fig. 6. NMR spectra of compound **13**

^{1}H NMR spectrum of compound **13** (400 MHz, $CDCl_3$)



^{13}C NMR spectrum of compound **13** (101 MHz, $CDCl_3$)

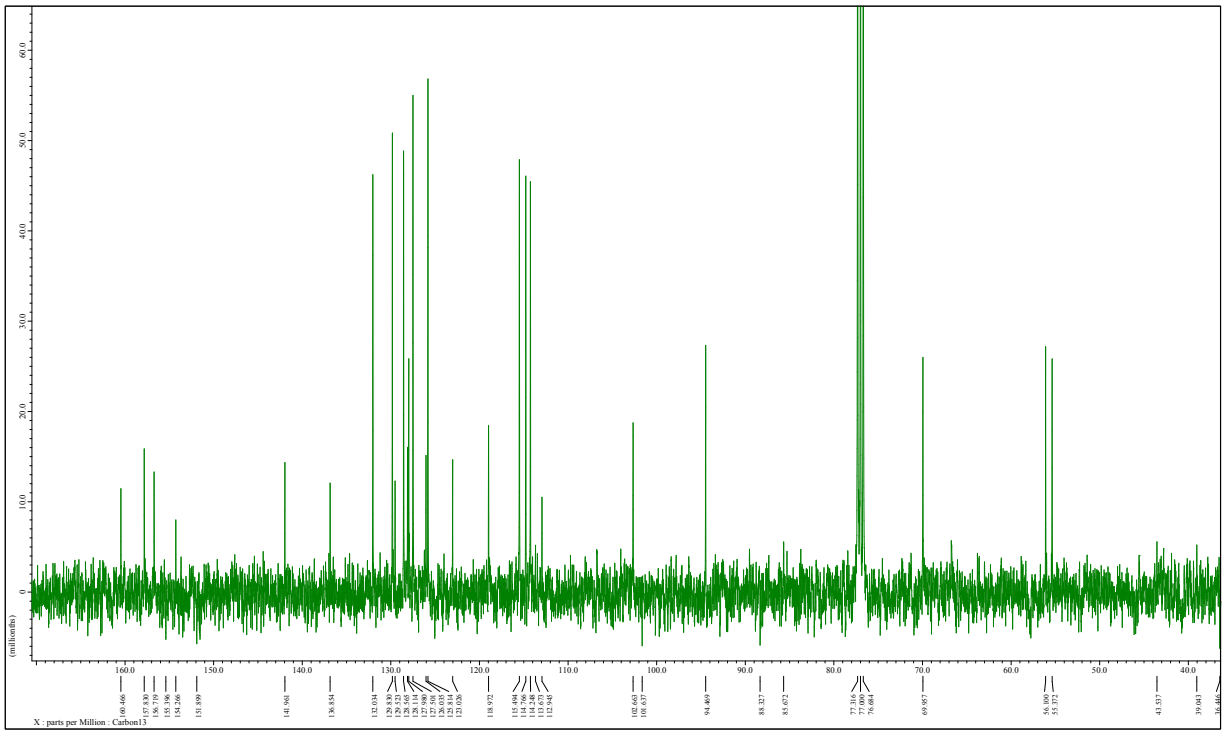
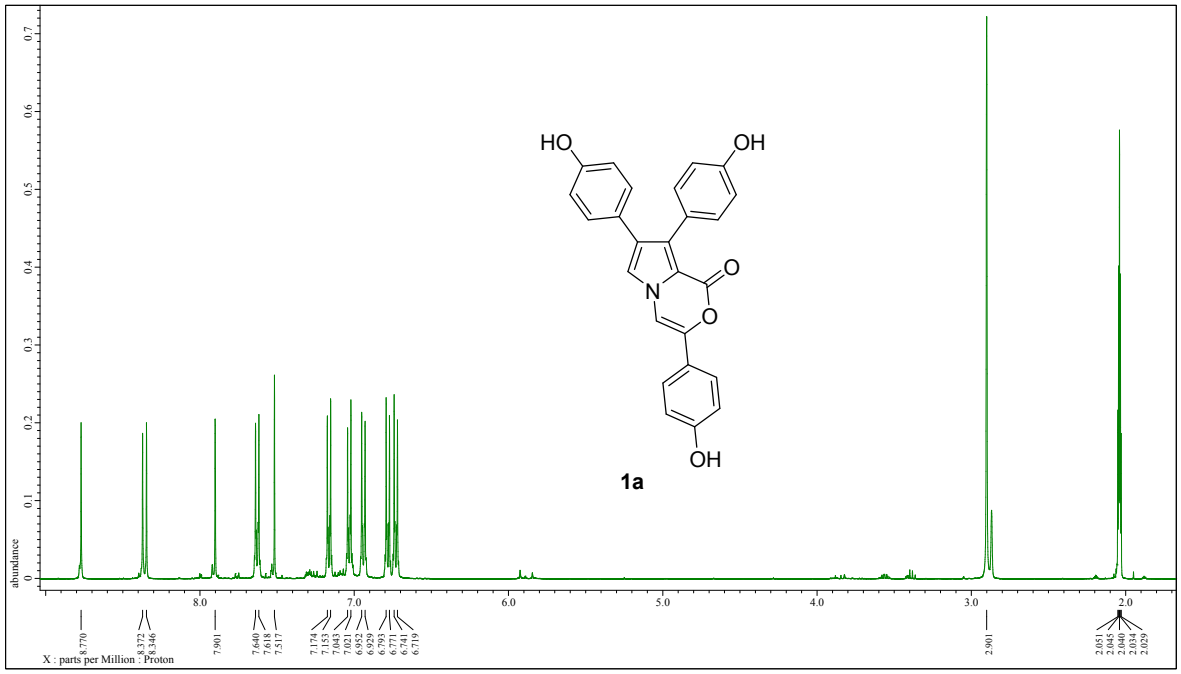


Fig. 7. NMR spectra of compound **1a**

^{1}H NMR spectrum of compound **1a** (400 MHz, acetone-d_6)



^{13}C NMR spectrum of compound **1a** (101 MHz, acetone-d_6)

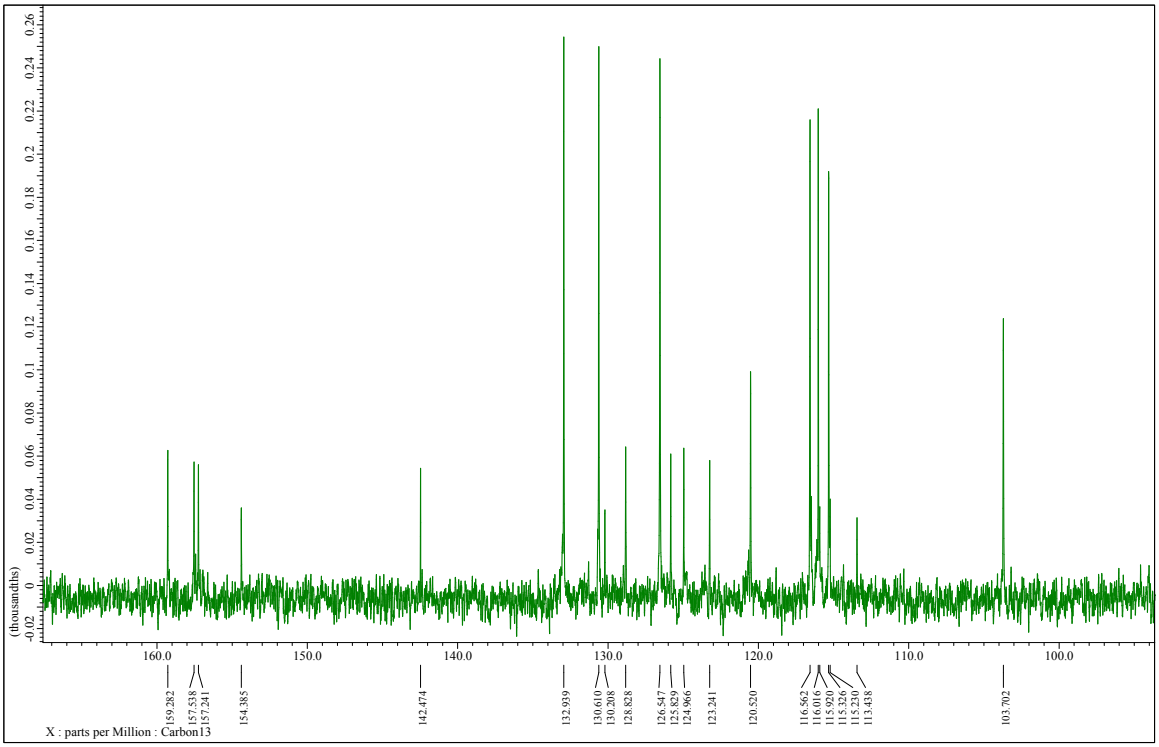
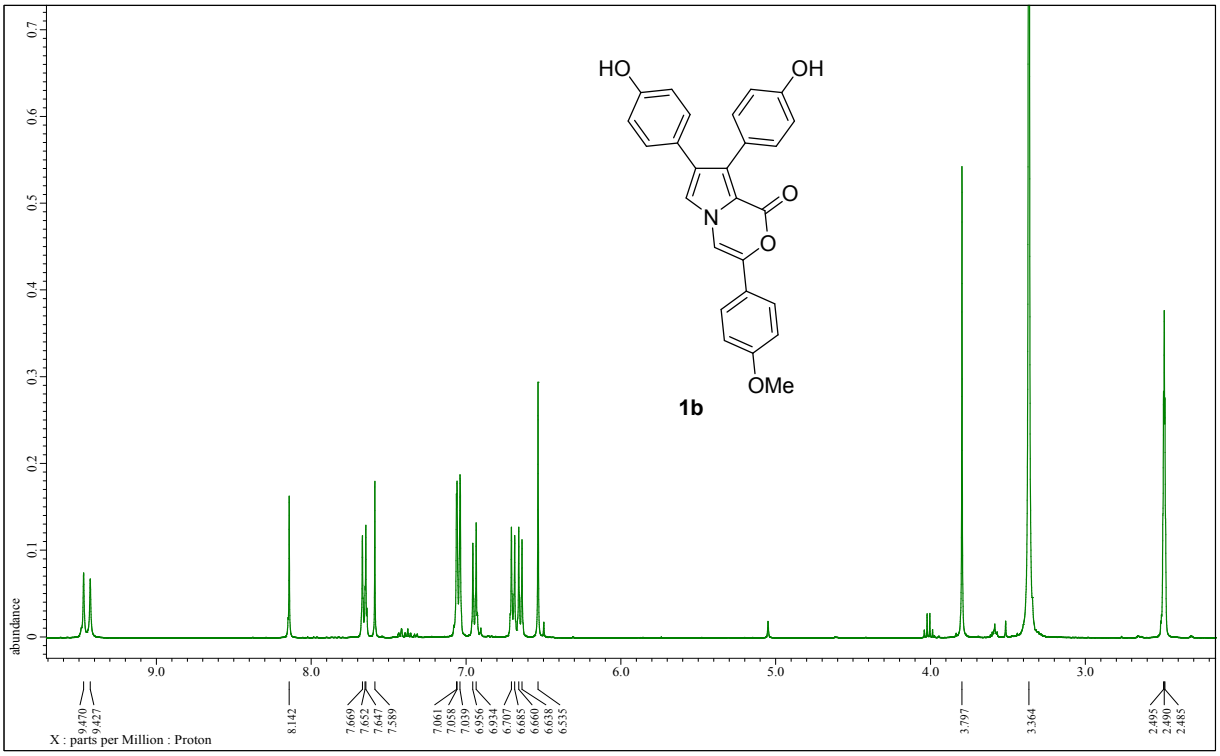
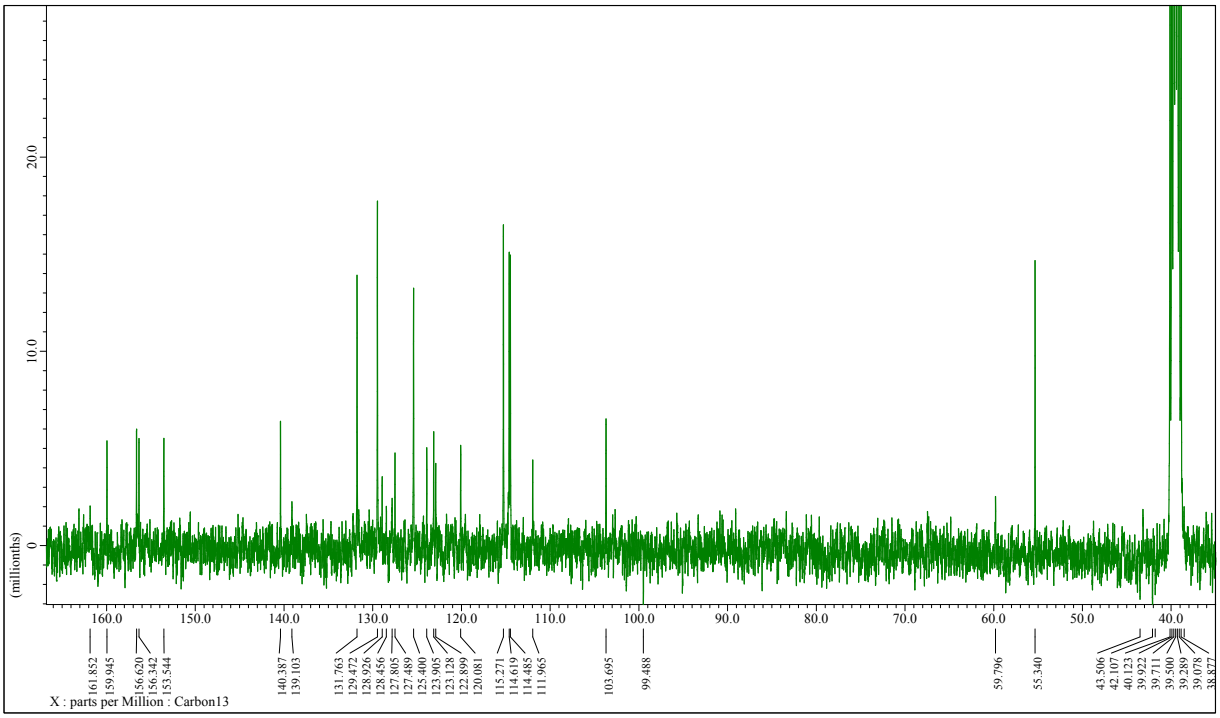


Fig. 8. NMR spectra of compound **1b**

^{1}H NMR spectrum of compound **1b** (400 MHz, DMSO-d_6)



^{13}C NMR spectrum of compound **1b** (101 MHz, DMSO-d_6)



HSQC spectrum of compound **1b**

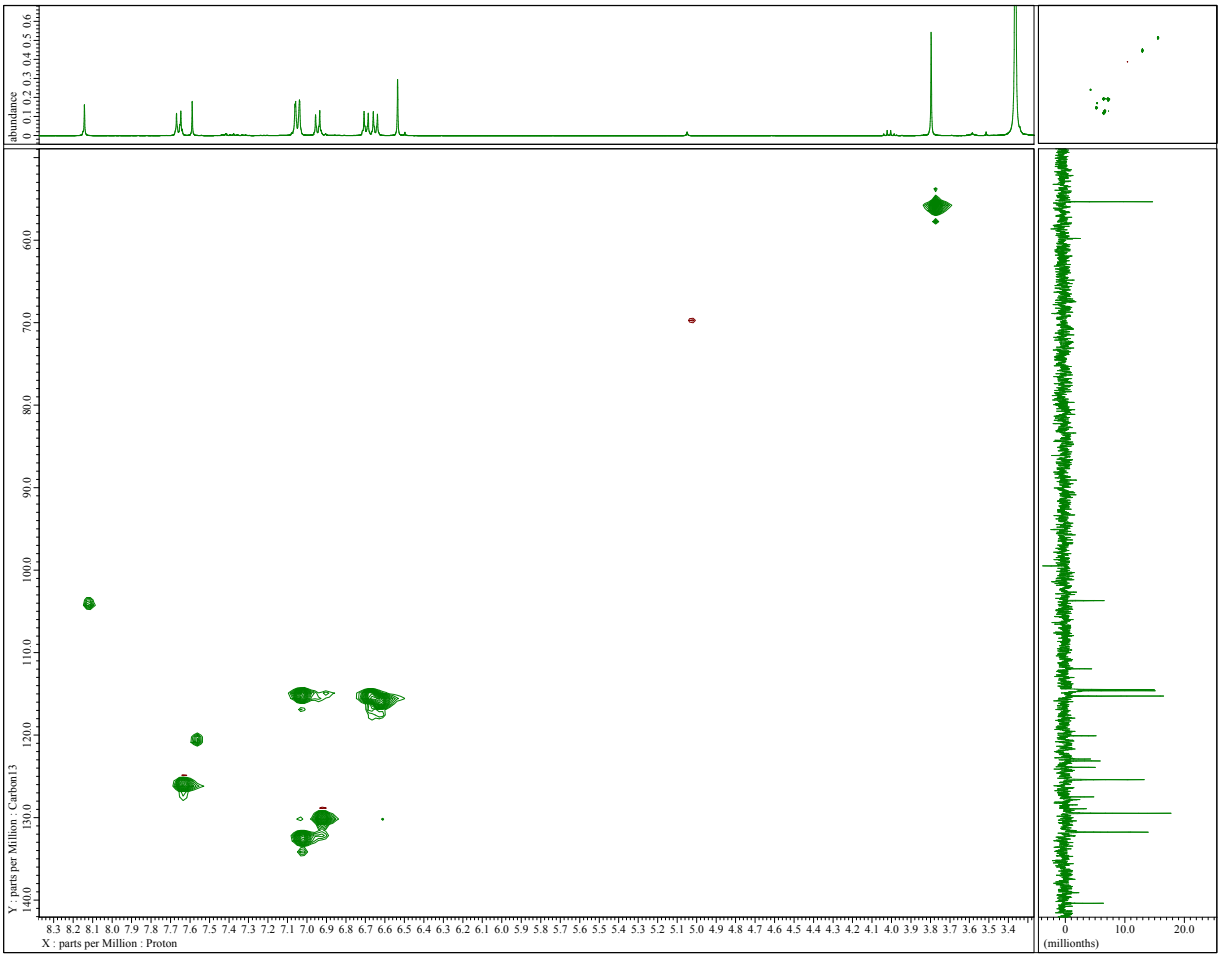
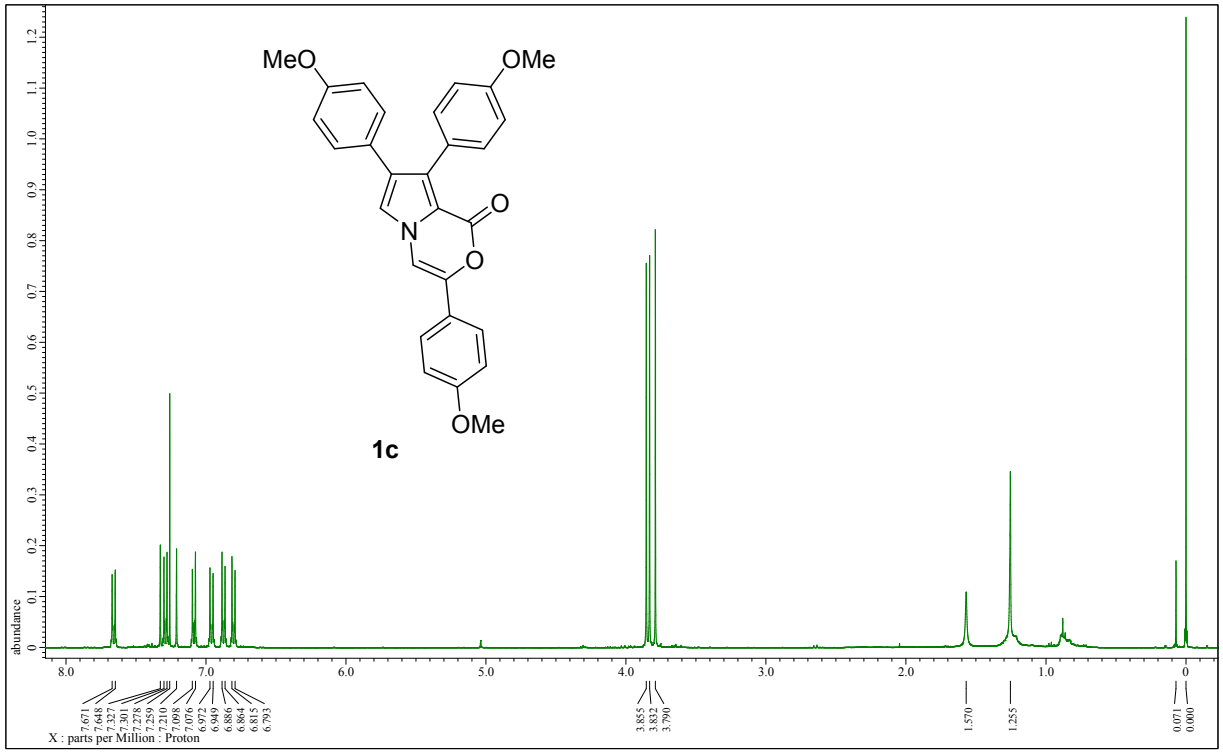

abundance
0 0.1 0.2 0.3 0.4 0.5 0.6
Y : parts per Million : Carbon13
60.0 70.0 80.0 90.0 100.0 110.0 120.0 130.0 140.0
8.3 8.2 8.1 8.0 7.9 7.8 7.7 7.6 7.5 7.4 7.3 7.2 7.1 7.0 6.9 6.8 6.7 6.6 6.5 6.4 6.3 6.2 6.1 6.0 5.9 5.8 5.7 5.6 5.5 5.4 5.3 5.2 5.1 5.0 4.9 4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4
X : parts per Million : Proton
0 10.0 20.0
(millionths)

Fig. 9. NMR spectra of compound **1c**

^{1}H NMR spectrum of compound **1b** (400 MHz, $CDCl_3$)



^{13}C NMR spectrum of compound **1c** (101 MHz, $CDCl_3$)

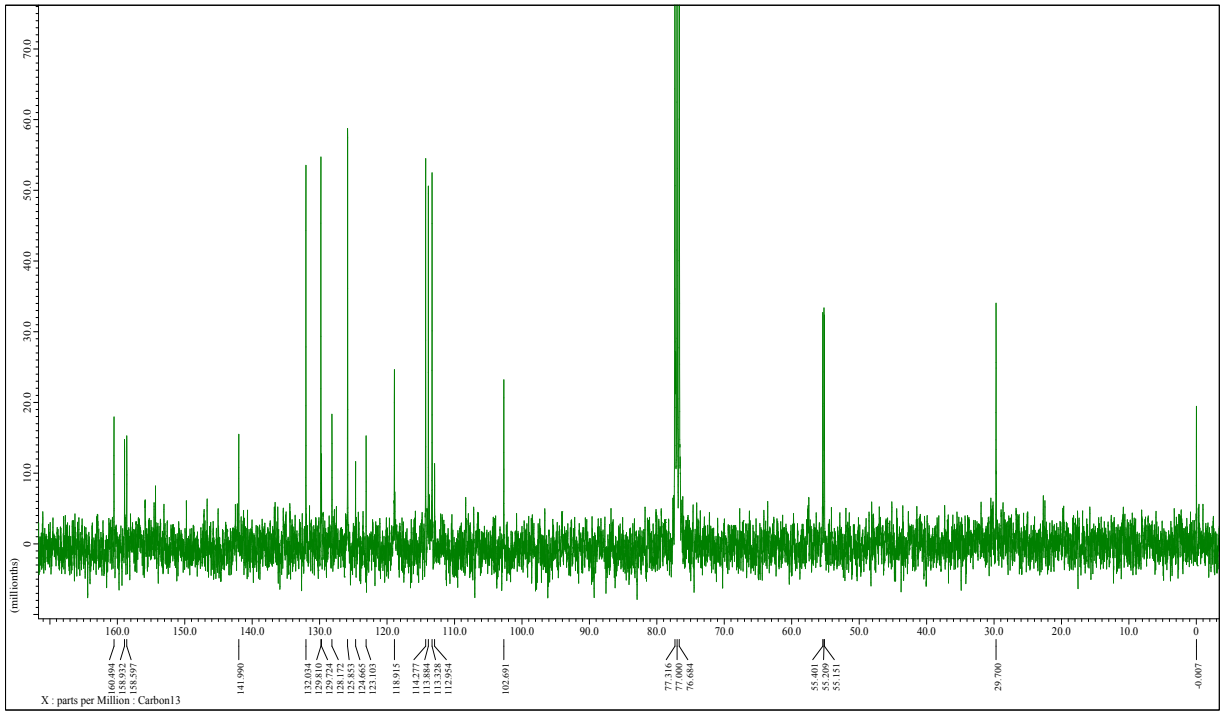
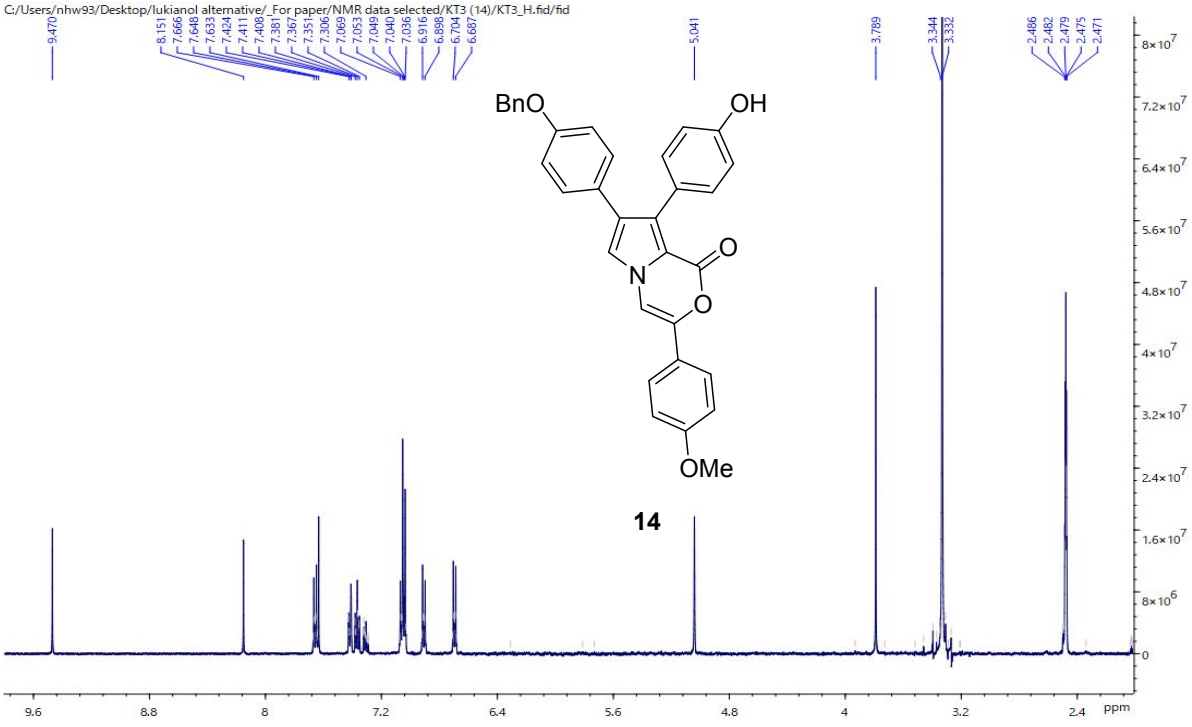


Fig. 10. NMR spectra of compound **14**

^{1}H NMR spectrum of compound **14** (300 MHz, DMSO-d_6)



^{13}C NMR spectrum of compound **14** (75 MHz, DMSO-d_6)

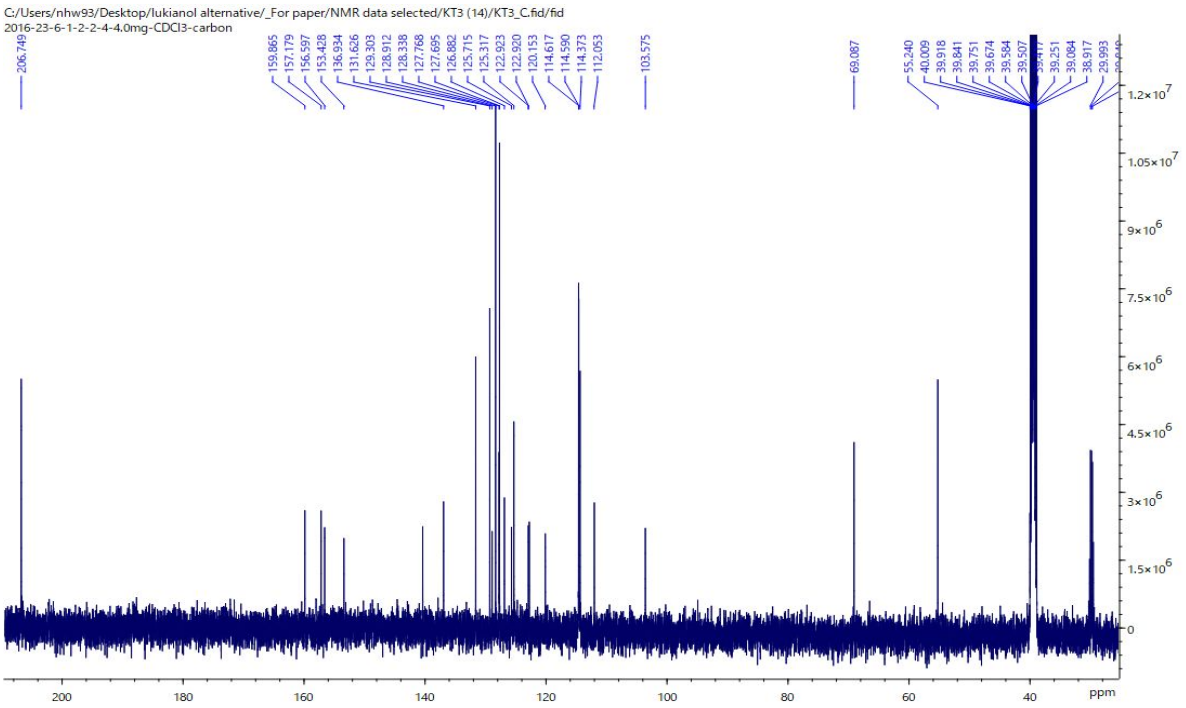
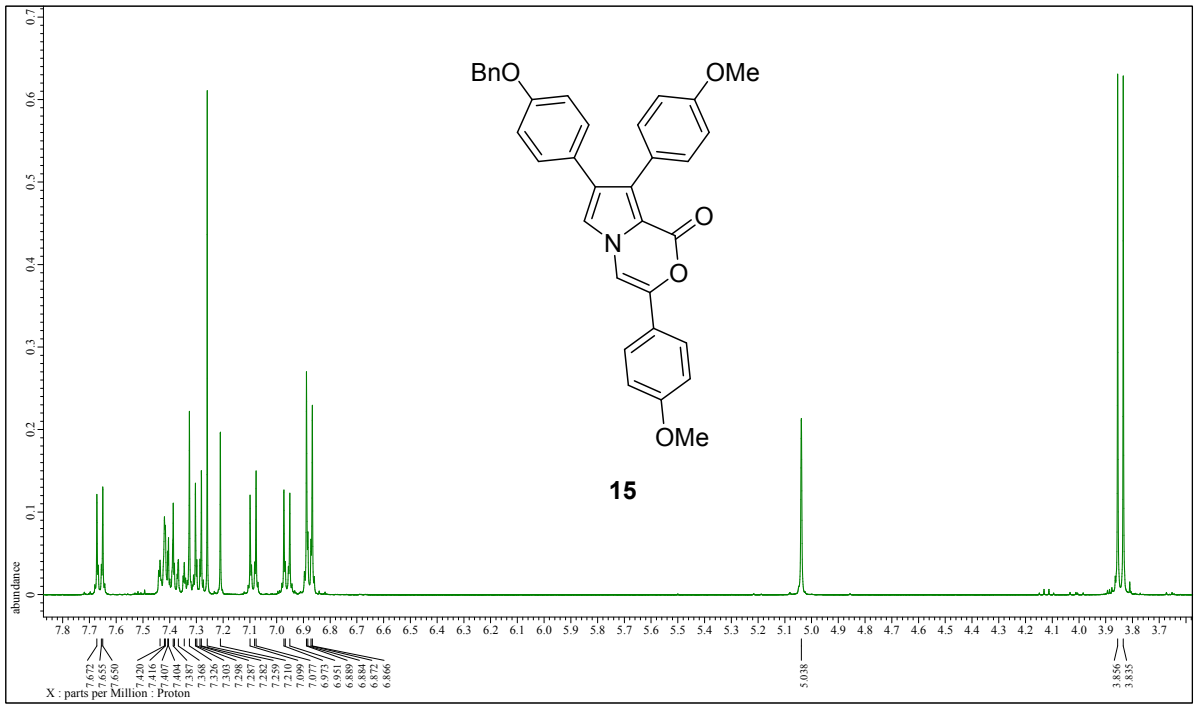


Fig. 11. NMR spectra of compound **15**

^{1}H NMR spectrum of compound **15** (400 MHz, $CDCl_3$)



^{13}C NMR spectrum of compound **15** (101 MHz, $CDCl_3$)

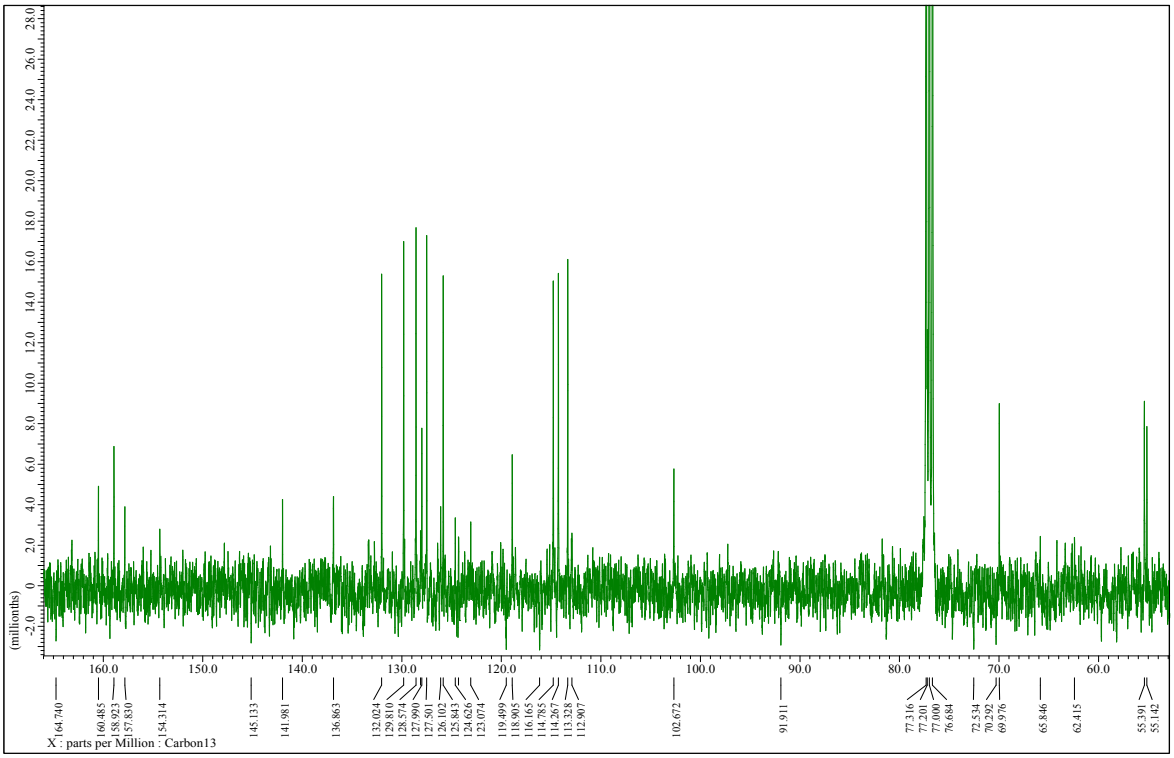
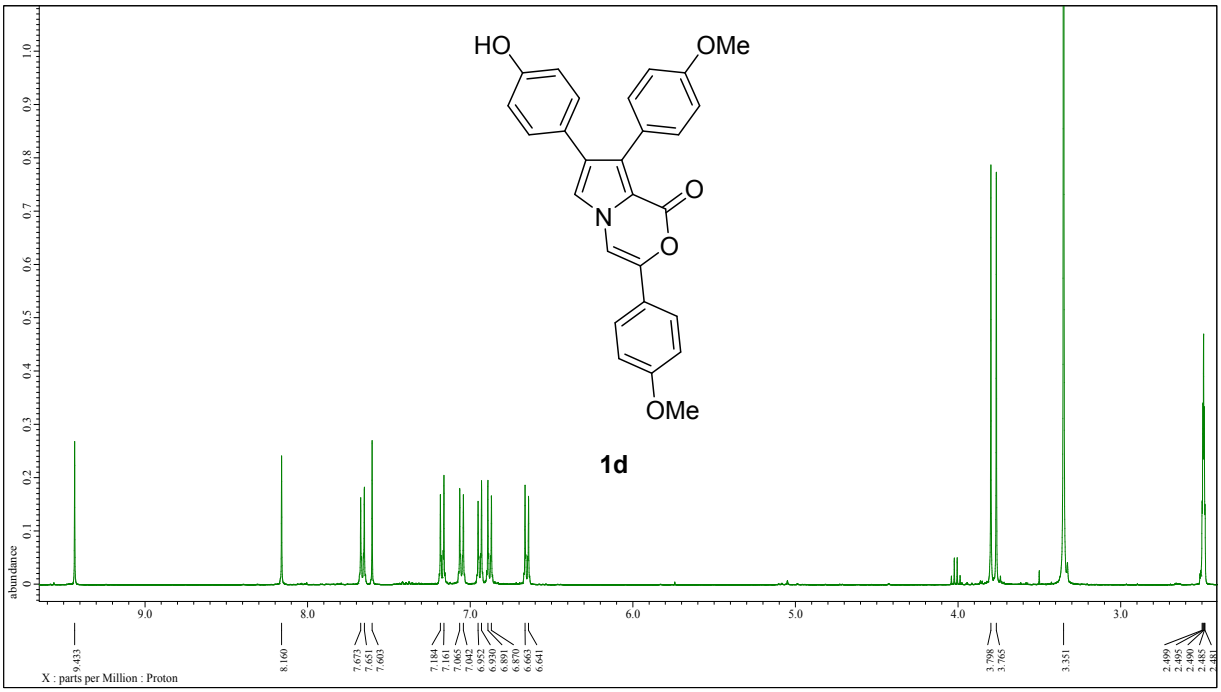


Fig. 12. NMR spectra of compound **1d**

^{1}H NMR spectrum of compound **1d** (400 MHz, DMSO-d_6)



^{13}C NMR spectrum of compound **1d** (101 MHz, DMSO-d_6)

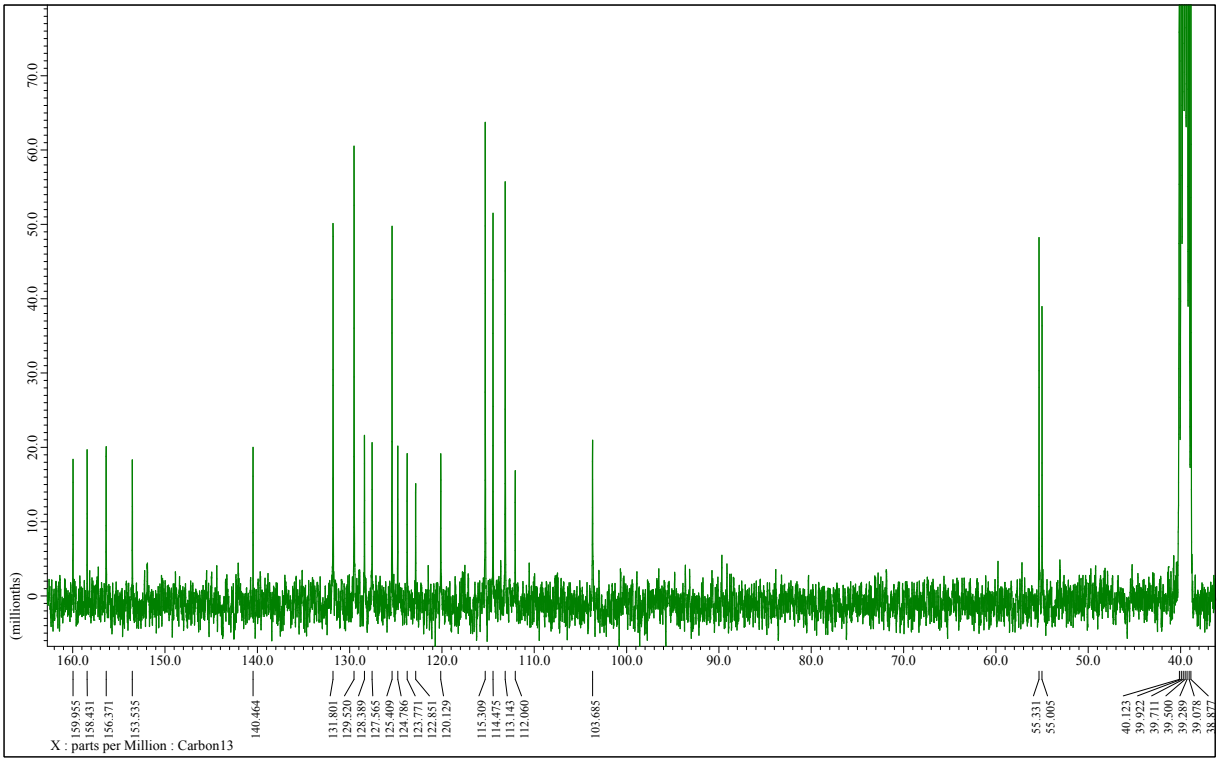
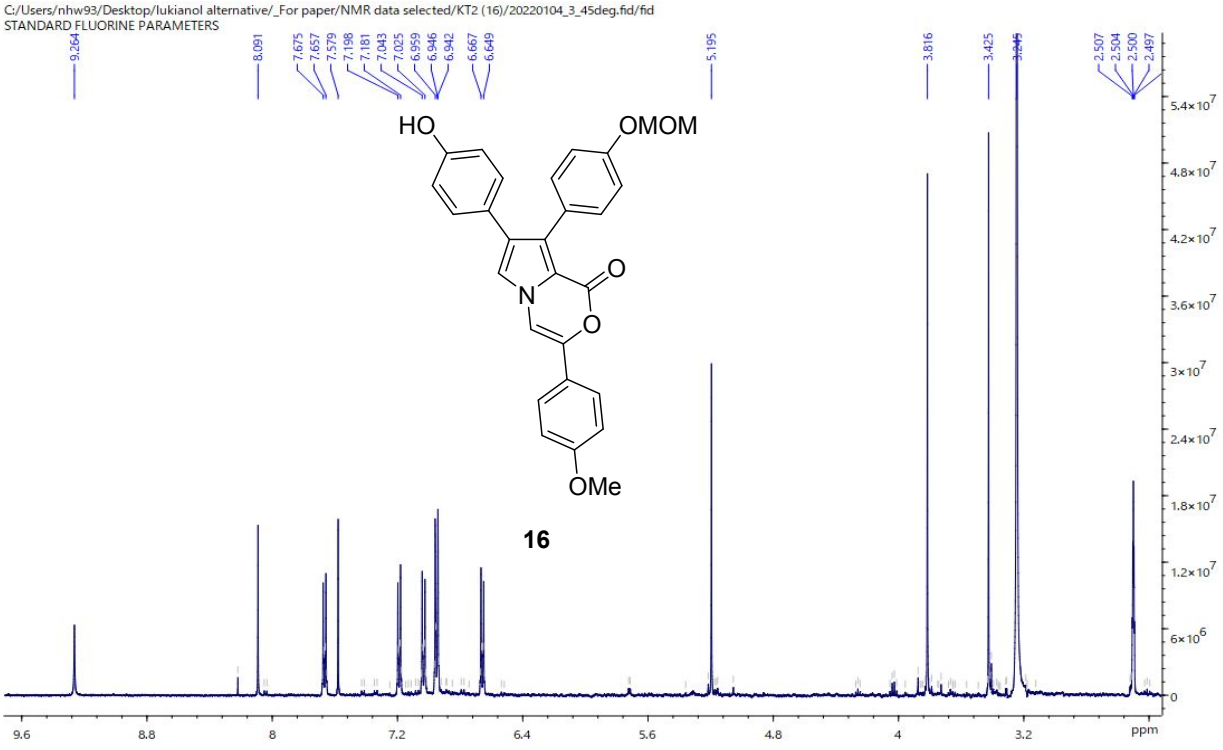


Fig. 13. NMR spectra of compound **16**

^{1}H NMR spectrum of compound **16** (500 MHz, DMSO-d_6)



^{13}C NMR spectrum of compound **16** (101 MHz, DMSO-d_6)

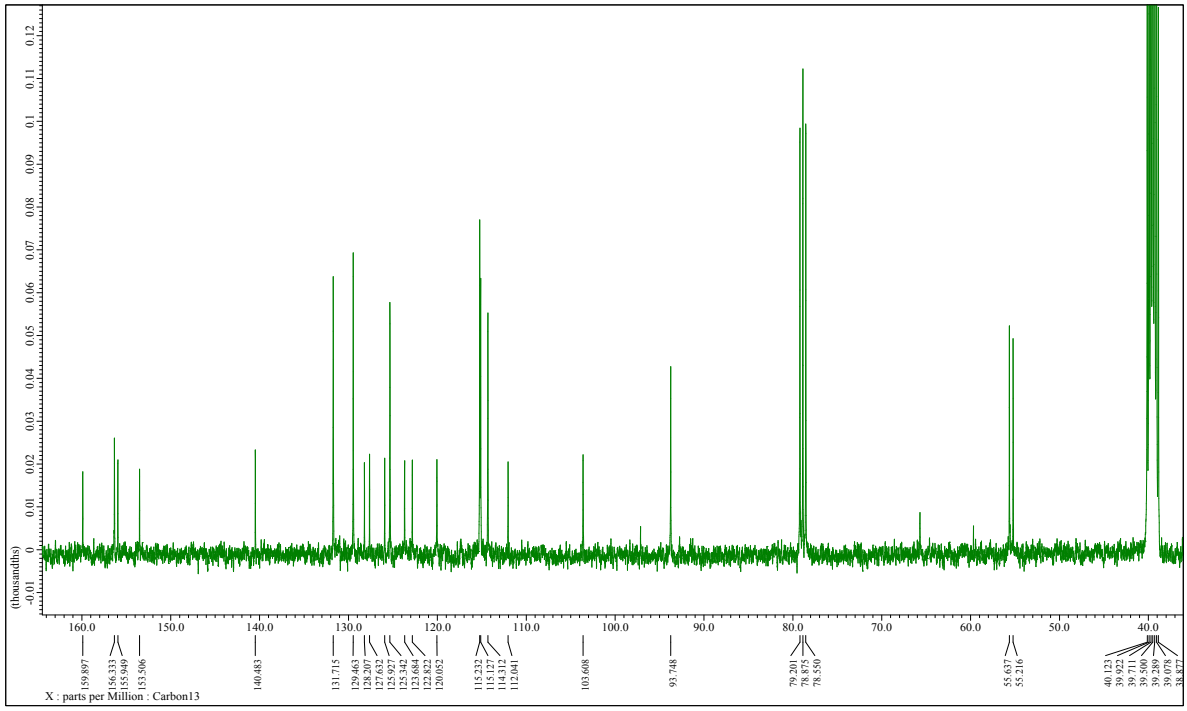
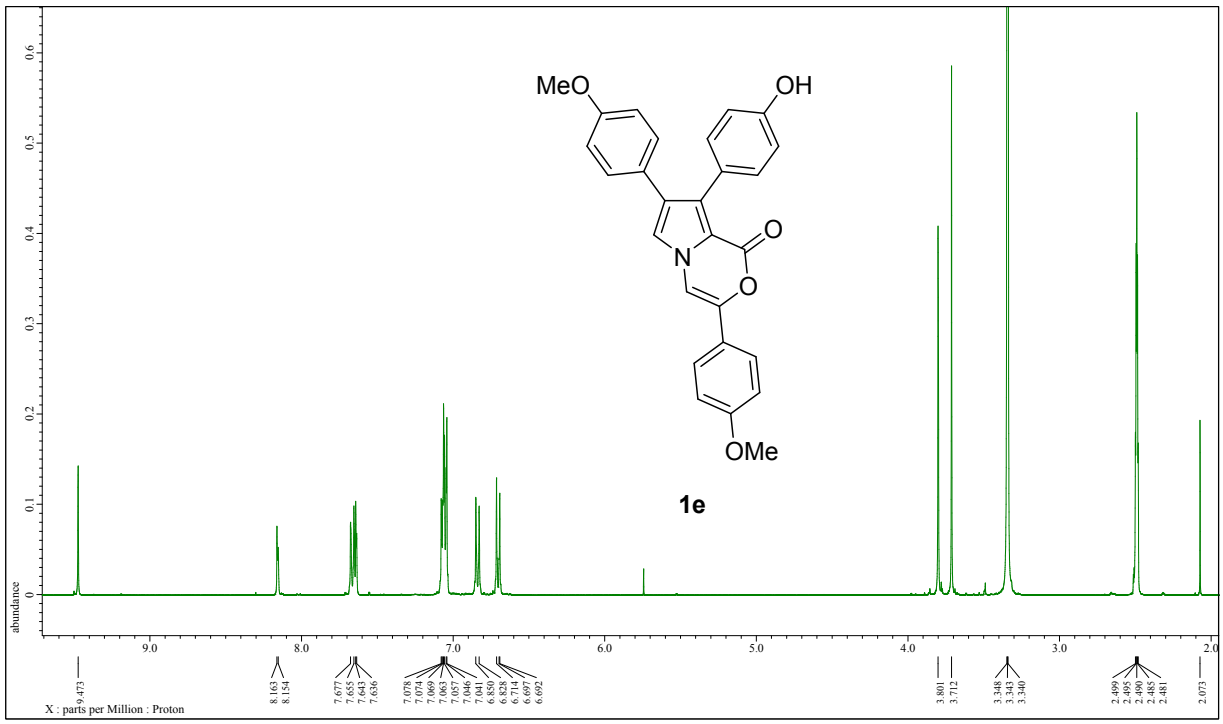


Fig. 14. NMR spectra of compound **1e**

^{1}H NMR spectrum of compound **1e** (400 MHz, DMSO-d_6)



^{13}C NMR spectrum of compound **1e** (101 MHz, DMSO-d_6)

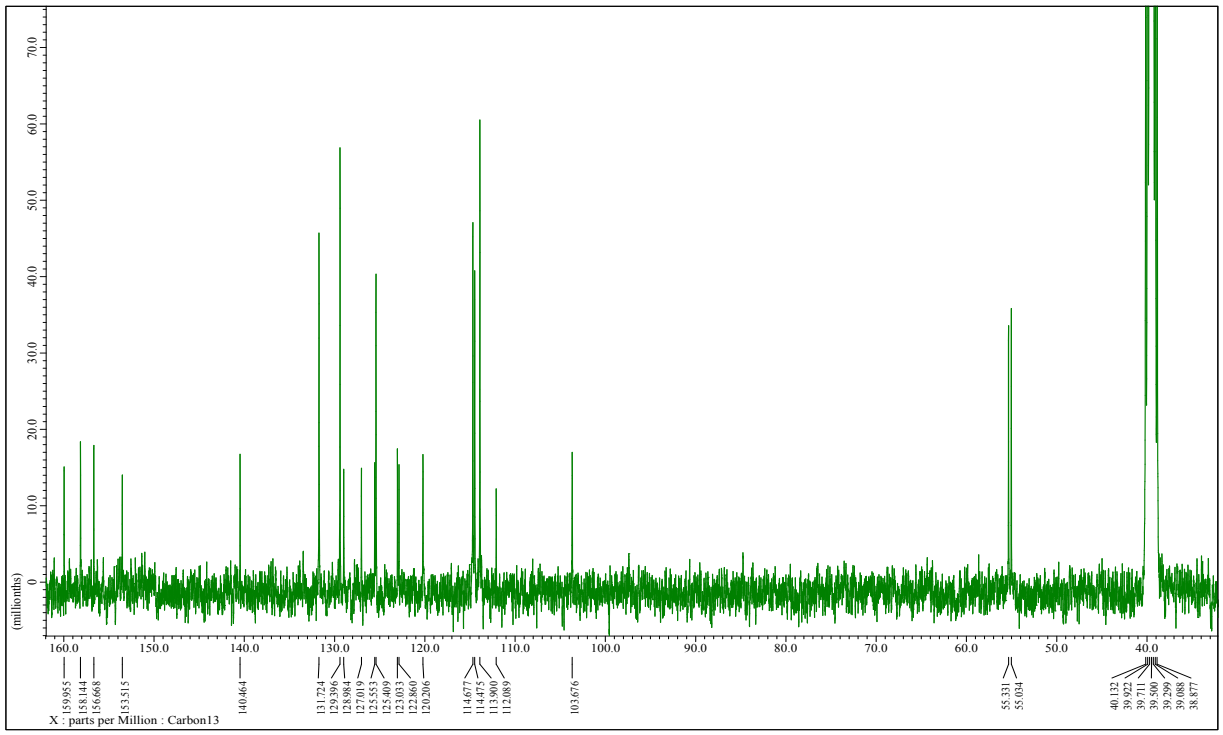
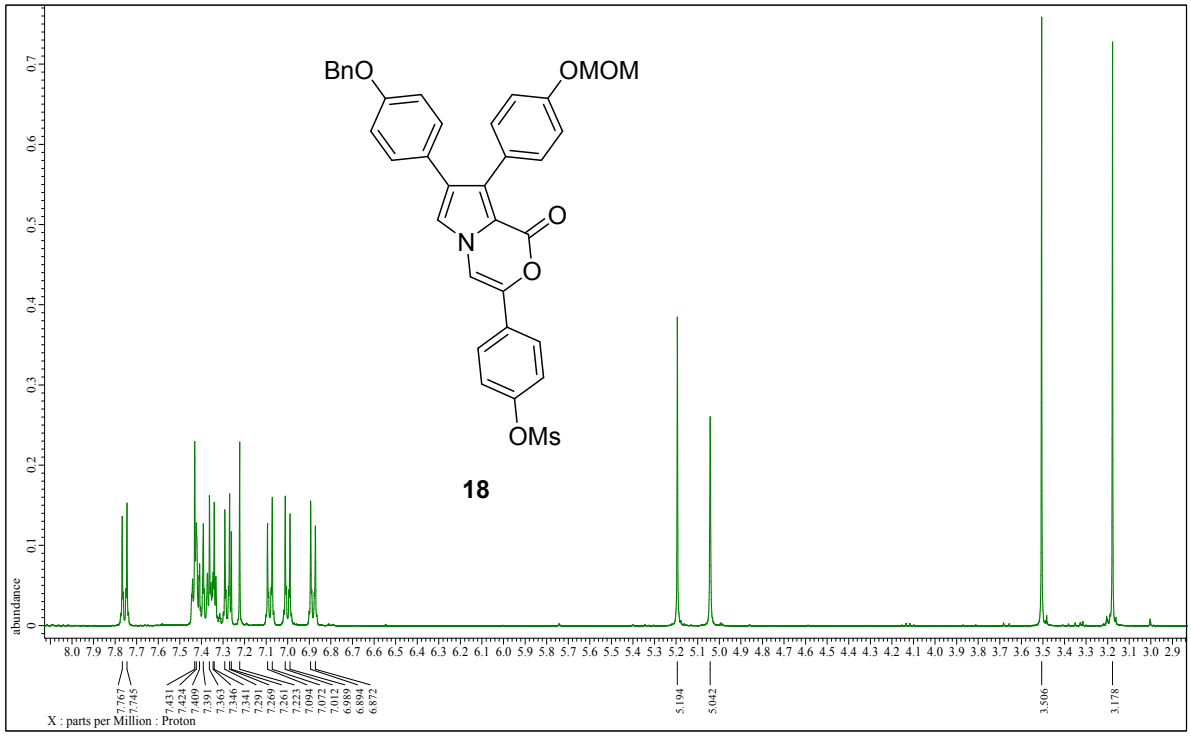


Fig. 15. NMR spectra of compound **18**

^{1}H NMR spectrum of compound **18** (400 MHz, $CDCl_3$)



^{13}C NMR spectrum of compound **18** (101 MHz, $CDCl_3$)

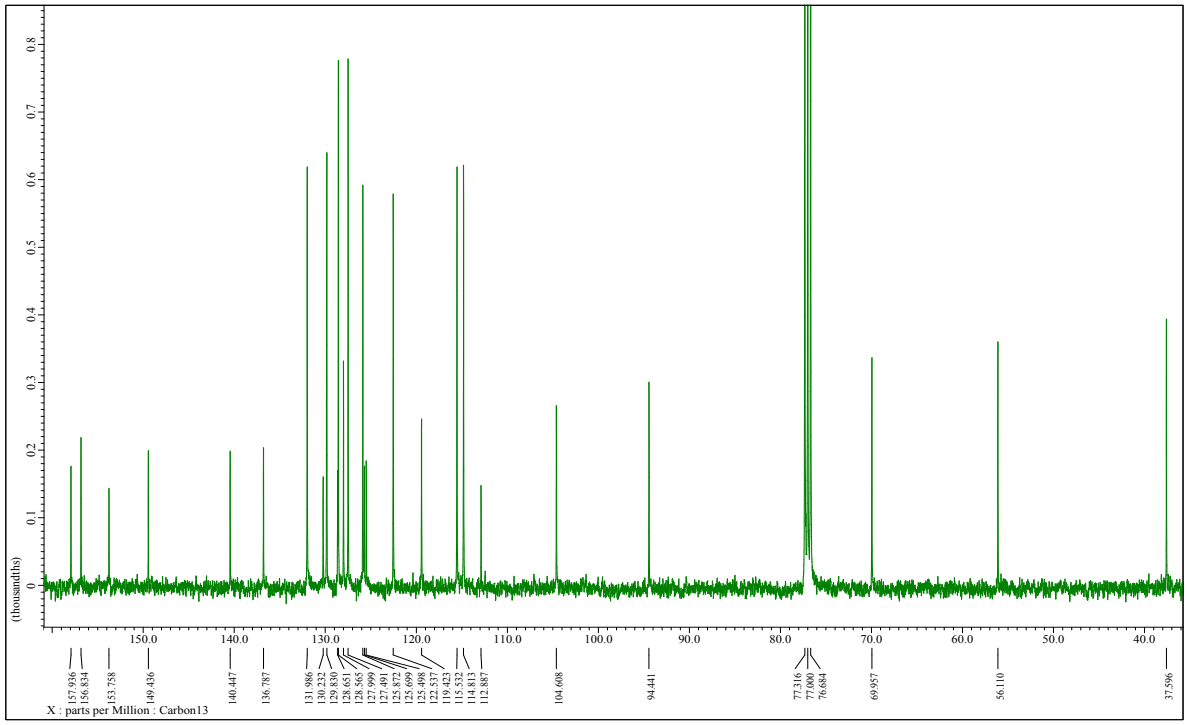
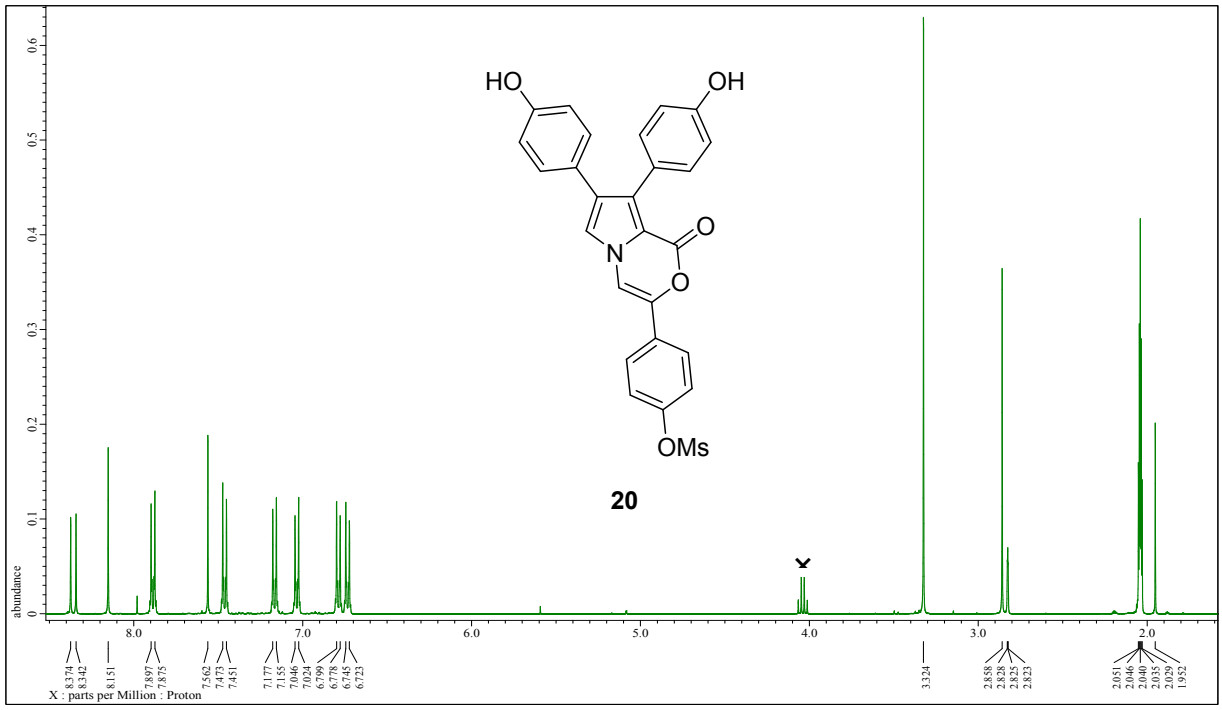


Fig. 16. NMR spectra of compound **20**

^{1}H NMR spectrum of compound **20** (400 MHz, acetone-d_6)



^{13}C NMR spectrum of compound **20** (101 MHz, DMSO-d_6)

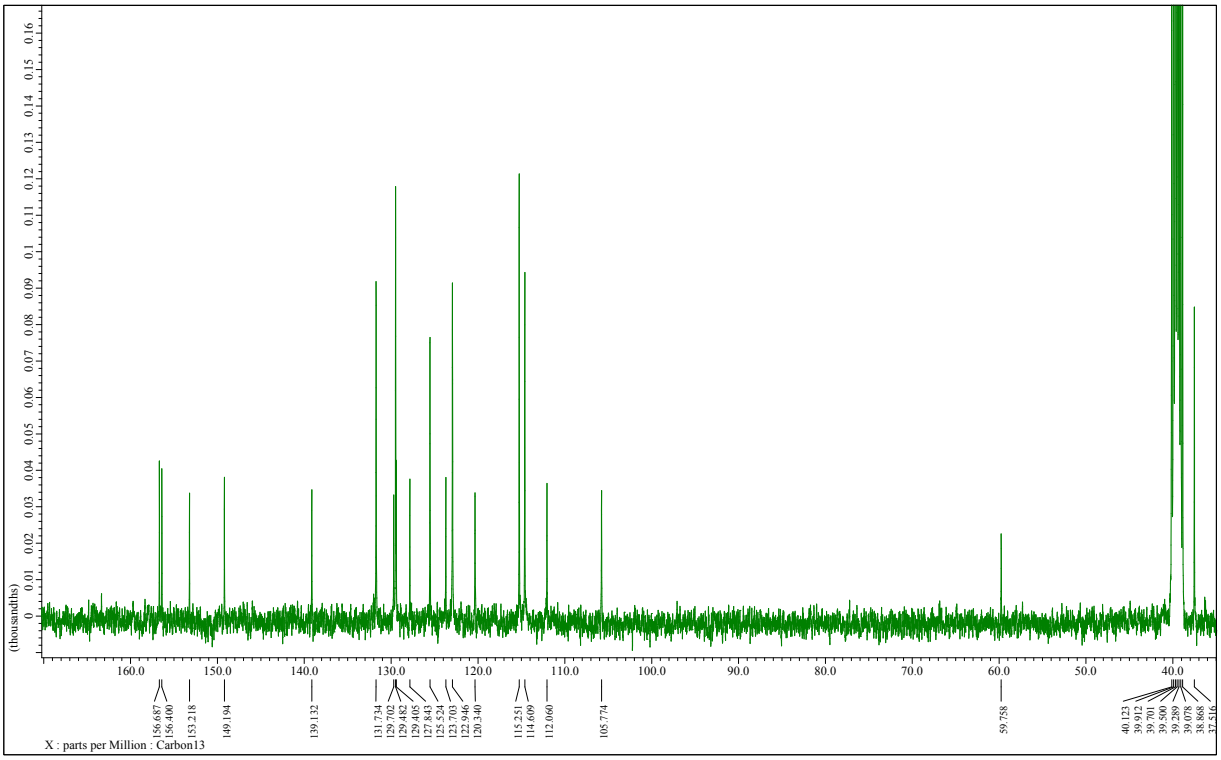
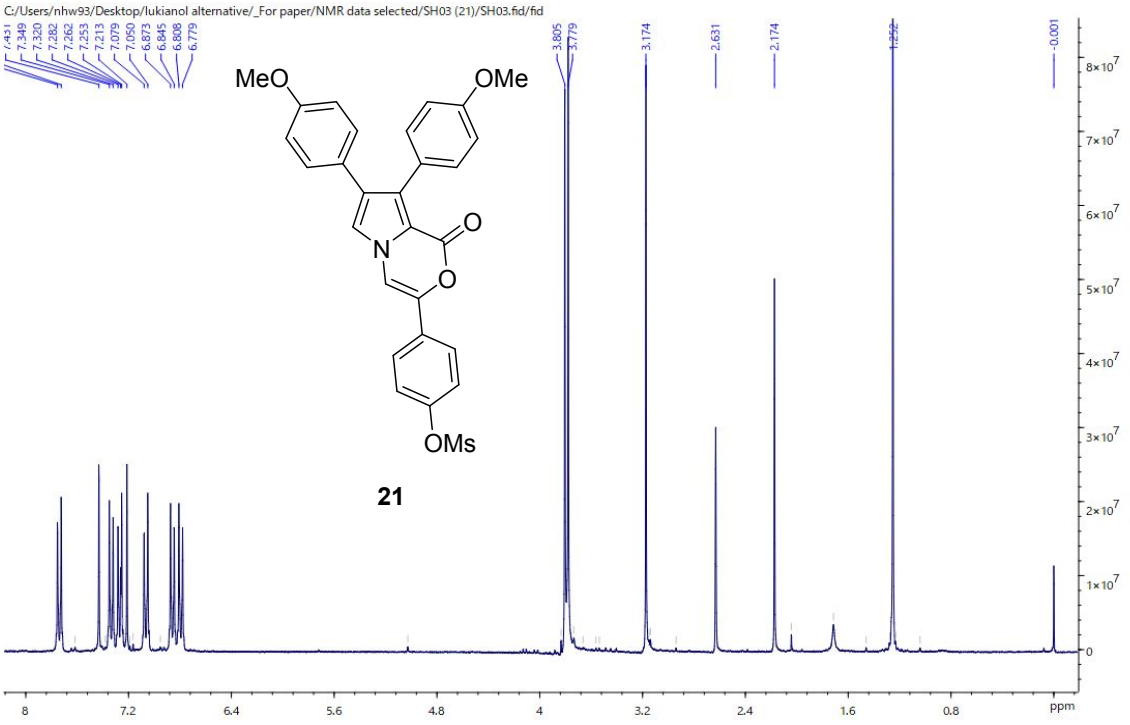


Fig. 17. NMR spectra of compound **21**

^{1}H NMR spectrum of compound **21** (300 MHz, $CDCl_3$)



^{13}C NMR spectrum of compound **21** (101 MHz, $CDCl_3$)

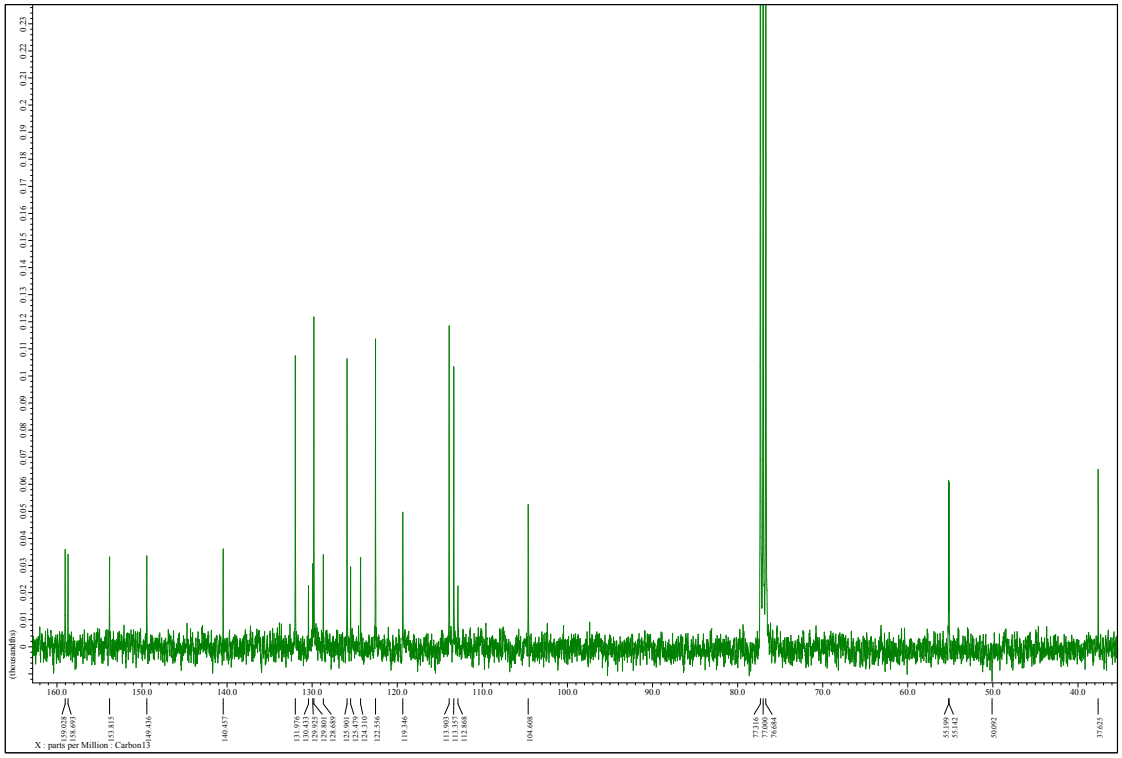
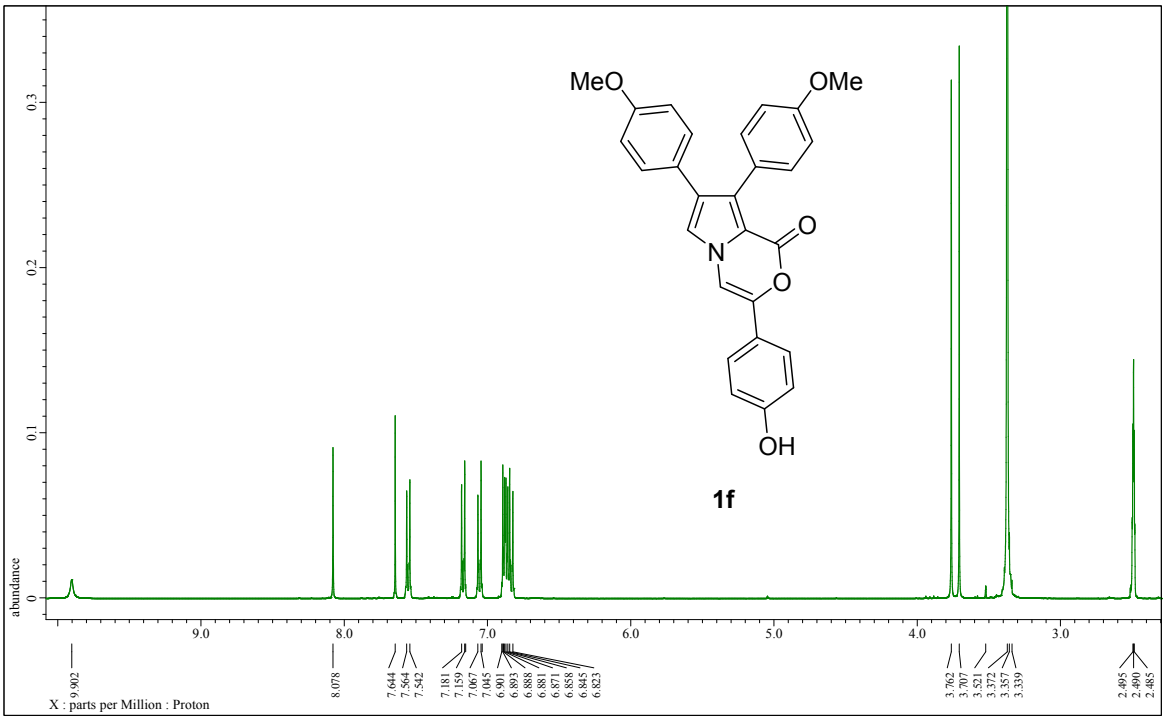


Fig. 18. NMR spectra of compound **1f**

^{1}H NMR spectrum of compound **1f** (300 MHz, $CDCl_3$)



^{13}C NMR spectrum of compound **1f** (75 MHz, $CDCl_3$)

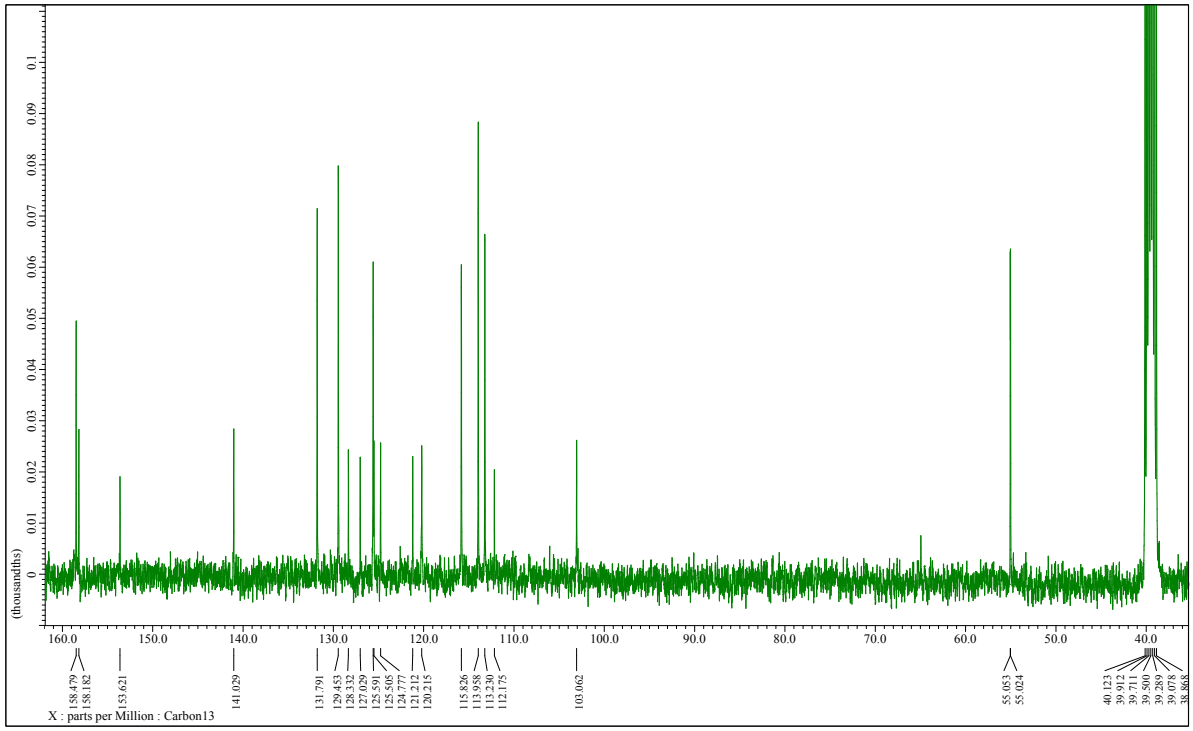
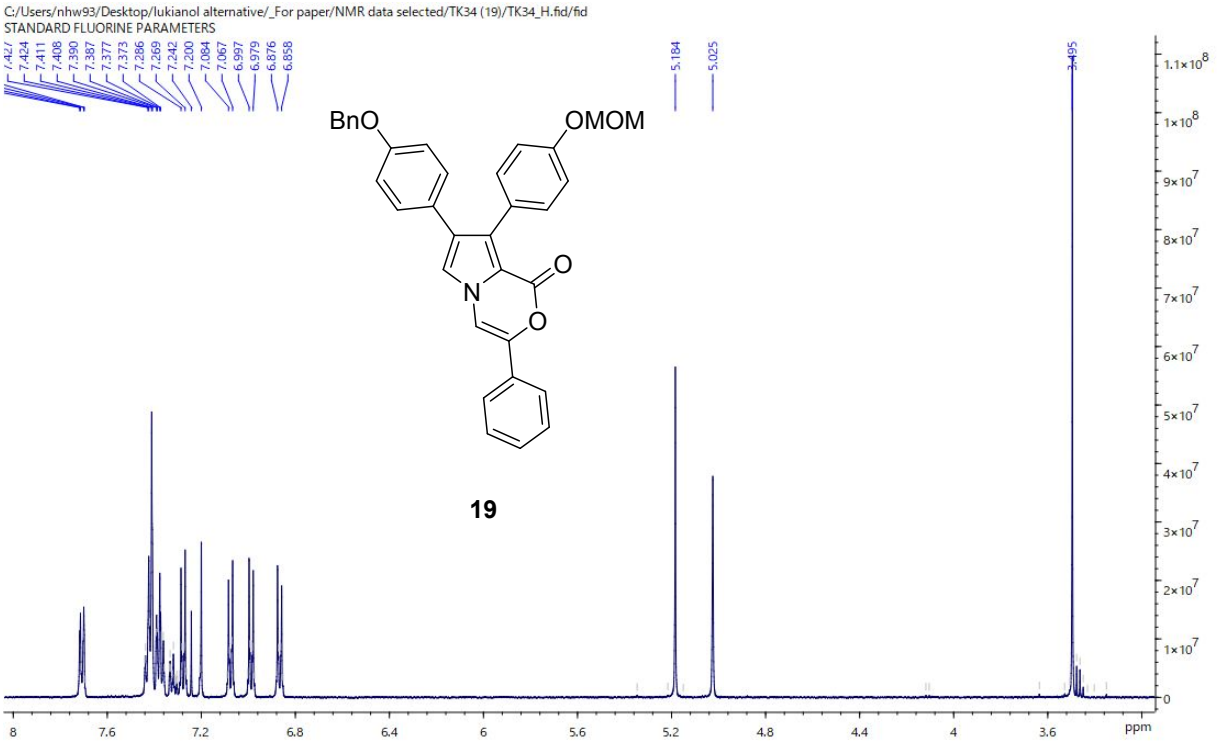


Fig. 19. NMR spectra of compound **19**

^{1}H NMR spectrum of compound **19** (300 MHz, $CDCl_3$)



^{13}C NMR spectrum of compound **19** (75 MHz, $CDCl_3$)

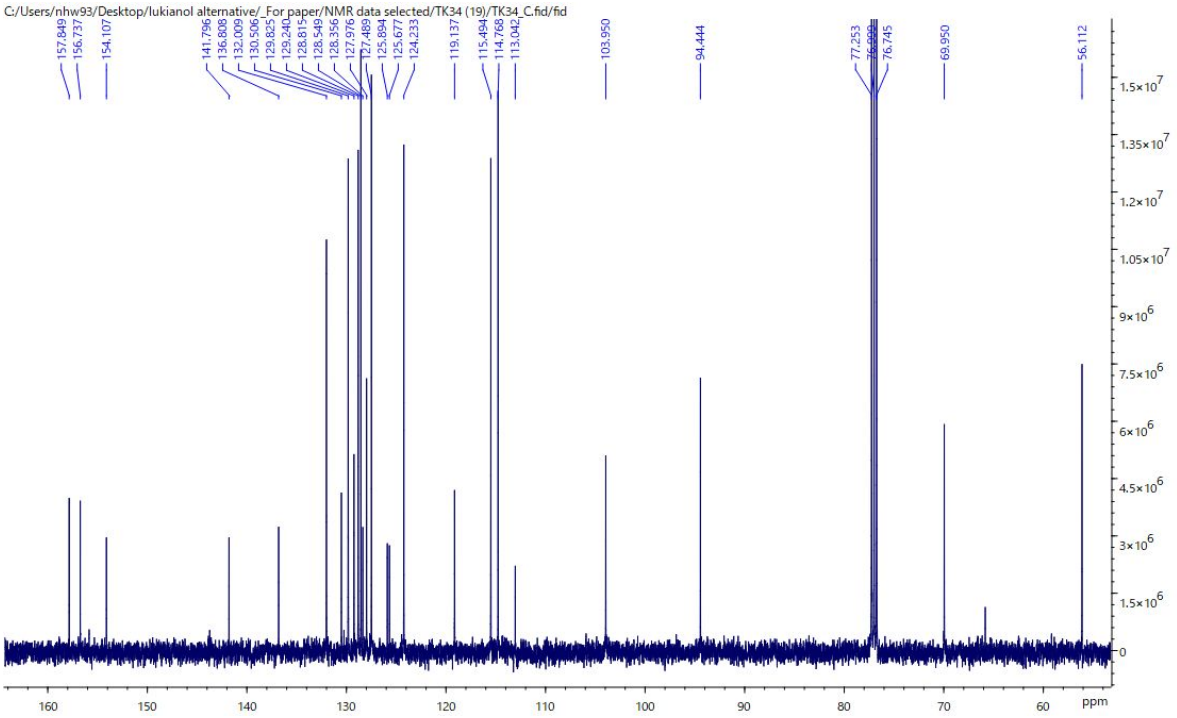
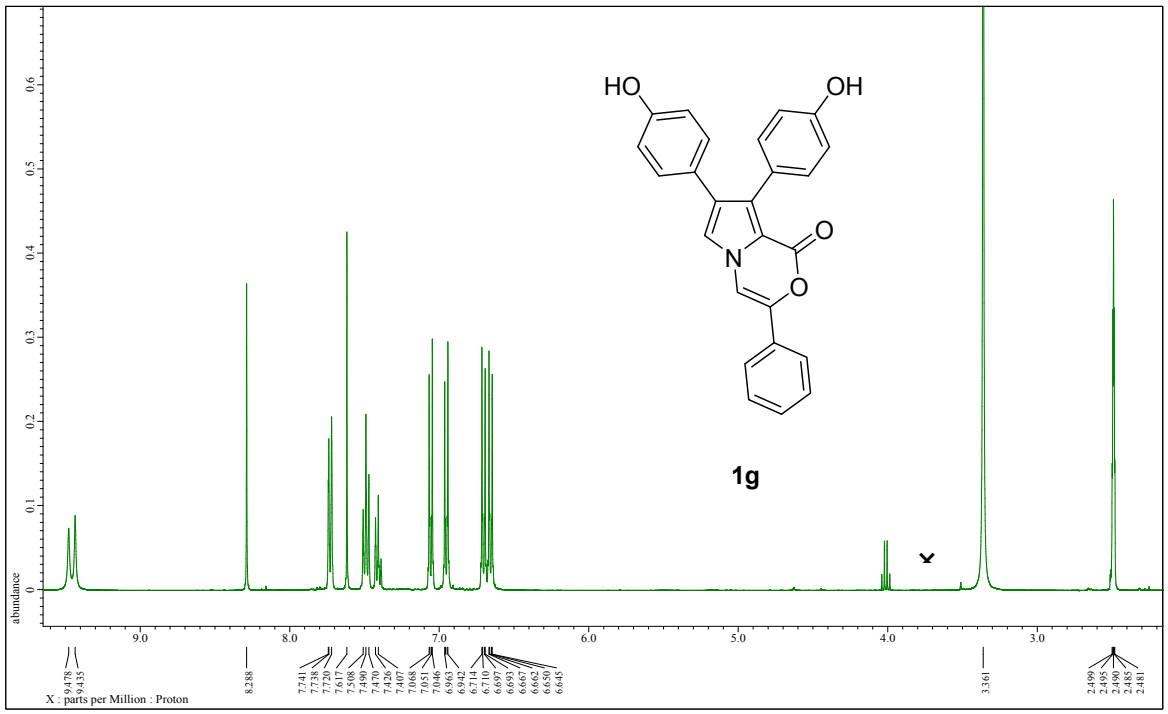


Fig. 20. NMR spectra of compound **1g**

^{1}H NMR spectrum of compound **1g** (400 MHz, DMSO-d_6)



^{13}C NMR spectrum of compound **1g** (101 MHz, DMSO-d_6))

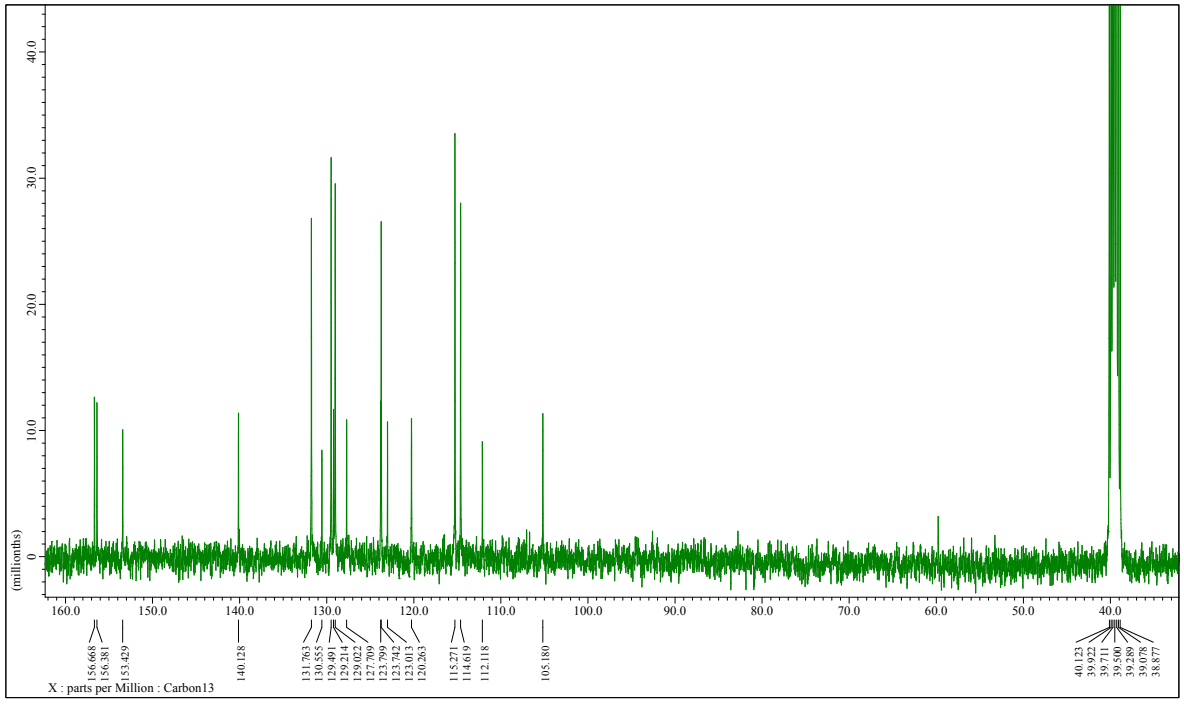
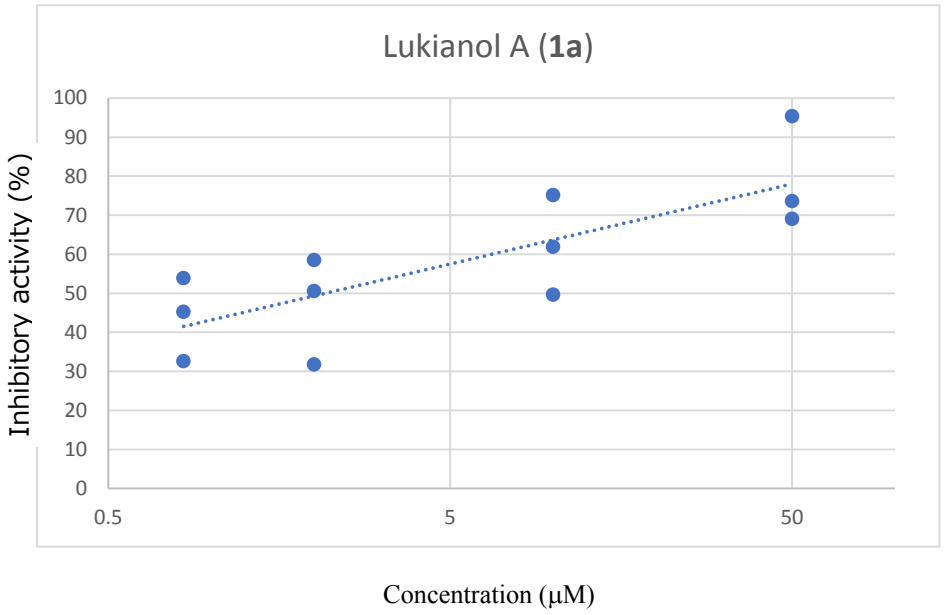


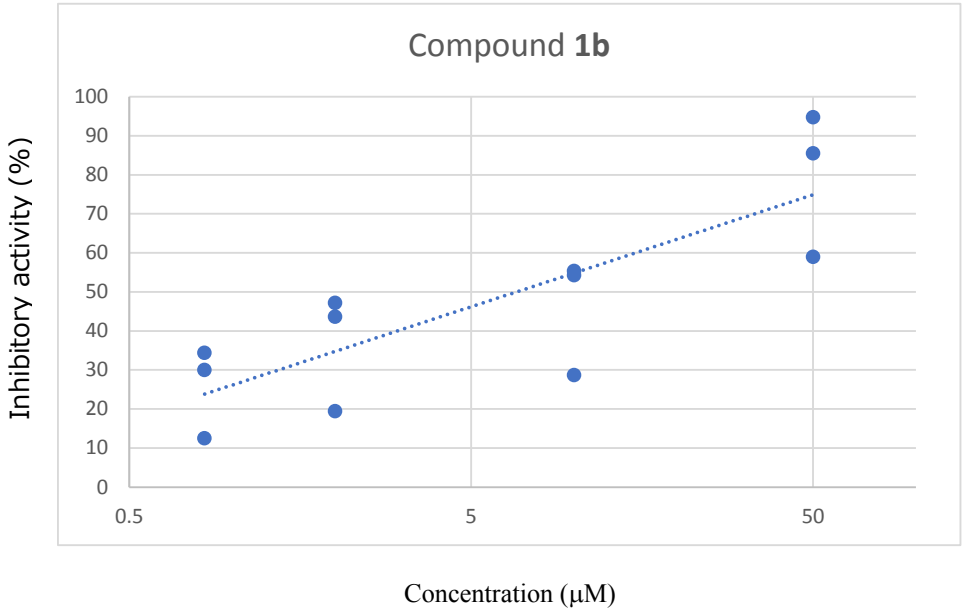
Table 1. Concentration-response curves for inhibitory effect (%) of compounds **1a** (A), **1b** (B), **1e** (C), and **1g** (D)

on h-ALR2.

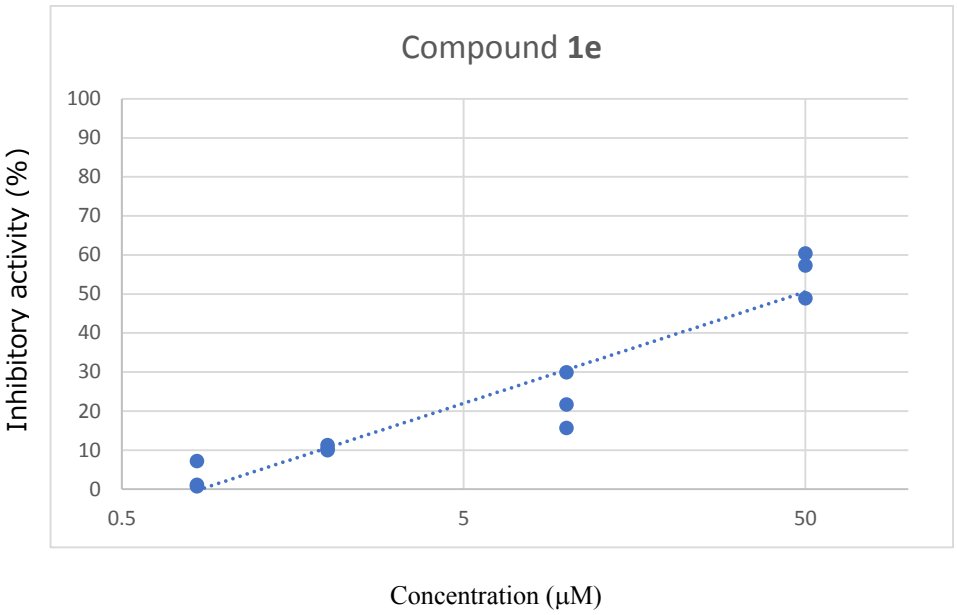
(A)


Lukianol A (1a)
Inhibitory activity (%)
100
90
80
70
60
50
40
30
20
10
0
0.5
5
50
Concentration (μM)

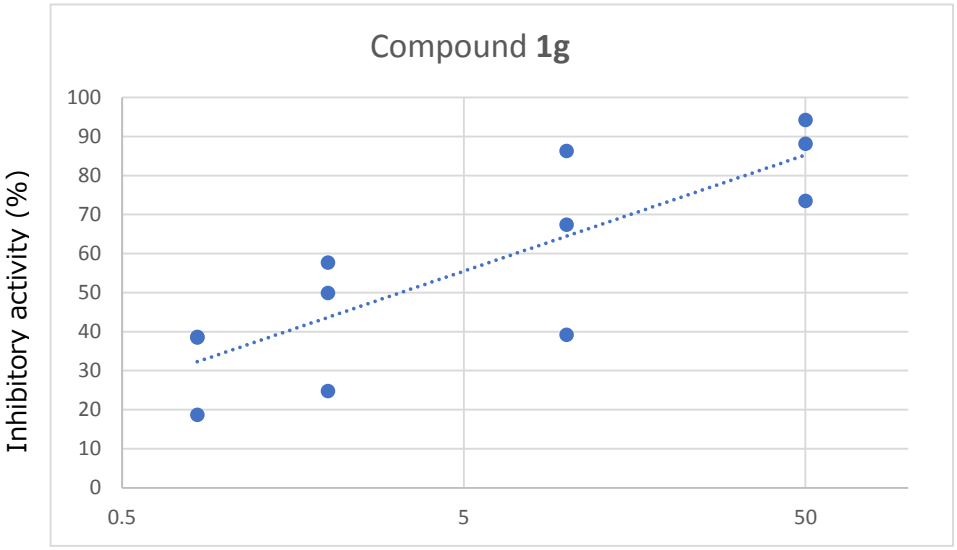
(B)


Compound 1b
Inhibitory activity (%)
100
90
80
70
60
50
40
30
20
10
0
0.5
5
50
Concentration (μM)

(C)


Compound 1e
Inhibitory activity (%)
100
90
80
70
60
50
40
30
20
10
0
0.5
5
50
Concentration (μM)

(D)


Compound 1g
Inhibitory activity (%)
100
90
80
70
60
50
40
30
20
10
0
0.5
5
50
Concentration (μM)