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Graphical abstract

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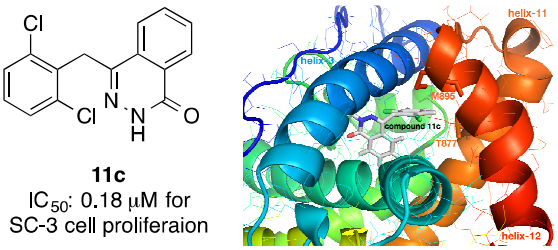

Design and synthesis of 4-benzyl-1-(2*H*)-phthalazinone derivatives as novel androgen receptor antagonists

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Abstract

The androgen receptor (AR) plays important roles in multiple physiological functions, including differentiation, growth, and maintenance of male reproductive organs, and also has effects on hair and skin. In this paper, we report the synthesis of nonsteroidal AR antagonists having a 4-benzyl-1-(2*H*)-phthalazinone skeleton. Among the synthesized compounds, **11c** with two *ortho*-substituents on the phenyl group potently inhibited SC-3 cell proliferation (IC_{50}: 0.18 μM) and showed high wt AR-binding affinity (IC_{50}: 10.9 μM), comparable to that of hydroxyflutamide (**3**). Compound **11c** also inhibited proliferation of LNCaP cells containing T877A-mutated AR. Docking study of **11c** with the AR ligand-binding domain indicated that the benzyl group is important for the antagonism. These phthalazinone derivatives may be useful for investigating potential clinical applications of AR antagonists.

Keywords: Androgen receptor, Antagonist, Phthalazinone, Prostate cancer

1. Introduction

Androgen receptor (AR) is a ligand-inducible transcriptional factor belonging to the nuclear receptor superfamily, and the endogenous ligands (androgens) are testosterone and its active metabolite, dihydrotestosterone (DHT).[1,2] AR modulates multiple physiological phenomena, including differentiation, growth, and maintenance of male reproductive organs, and also has effects on hair and skin (androgenic effects).[3,4] Since androgen plays an important role in progression of prostate cancer,[5] various AR antagonists have been investigated for treatment of prostate cancer, and some of them have been brought into clinical use, including bicalutamide (**1**, Figure 1)[6] and flutamide (**2**).[7] Although hormone therapy using AR antagonist or combined androgen blockade therapy is effective for most prostate cancer patients, its efficacy decreases after a few years of treatment, with the development of so-called castration-resistant prostate cancer (CRPC).[8,9] A major cause of CRPC is mutations of AR,[10-12] such as T877A, which is the most common mutation of AR in CRPC. Hydroxyflutamide (**3**), an activated metabolite of flutamide (**2**), acts as agonists toward T877A AR, and exacerbates the cancer.[3]

Figure 1

AR antagonists can be classified into two types, that is, steroidal and nonsteroidal compounds. The nonsteroidal AR antagonists in clinical use have the common pharmacophore of an anilide structure with nitro, cyano, or related functional groups on the phenyl ring, as in bicalutamide (**1**) and flutamide (**2**). Most of the nonsteroidal AR antagonists so far known are anilide derivatives developed by using the structures of bicalutamide (**1**) and flutamide (**2**) as lead compounds, including second-generation AR antagonists such as MDV3100 (**4**, Figure 1), which are effective against CRPC.[4] In the structure of MDV3100, the bulky aryl ring on the heterocyclic linker is important for the antagonism. Although development of the same pharmacophore as in bicalutamide (**1**) and flutamide (**2**) is thus an effective strategy to develop AR antagonists with high potency, development of antagonists bearing other pharmacophore(s) (i.e. non anilide-type molecules) is also a promising approach to overcome CRPC, and some AR antagonists bearing a unique skeleton or pharmacophore have been reported.[15,16] These compounds have a basic amino or phenolic hydroxyl group on the aromatic ring as the necessary polar group, instead of the cyano- or nitrophenyl group of the anilide-type antagonists.

For example, we have reported two AR antagonists without the anilide-type pharmacophore, i.e., (*Z*)-4-(4-diethylaminophenylmethylene)-3-phenyl-5(4*H*)-isoxazolone (**5**, Figure 2)[17] and 4-(*N*-benzyl-*N*-nitrophenyl)aminopyrrole-2-carboxamide (**6**).[18] These compounds showed high binding affinity for wild-type (wt) AR, and were effective in LNCaP cells bearing T877A-mutated AR. Compounds **5** and **6** contain a substituted phenyl group, a

heterocyclic ring (isoxazolone for **5** and pyrrole for **6**), and a linking atom (methyne for **5** and nitrogen for **6**). Structure-activity relationship studies showed that the polar functional group on the aromatic ring is important for binding to AR, and the bulky substituent on isoxazolone for **5** or on the linking nitrogen atom for **6** is important for the antagonistic activity, serving to disturb formation of the active conformation of holo-AR, especially the proper positioning and orientation of helix-12, the key α-helix motif for ligand-dependent receptor activation. Based on these considerations, we introduced a phthalazinone as the heterocyclic part instead of phenylisoxazolone for **5** and pyrrolecarbonylpyrrolidine for **6**. Phthalizinone is one of the useful heterocyclic building blocks for bioactive molecules,[19] and we assumed that the fused ring structure could improve the activity. Thus, we designed 4-benzyl-1-(2*H*)-phthalazinone as a candidate scaffold for AR antagonists. Here, we report the synthesis and biological activity of 4-benzyl-1-(2*H*)-phthalazinone and its derivatives (general formula shown in Figure 2) as candidate new-generation AR antagonists with a novel pharmacophore.

Figure 2

2. Chemistry

4-Benzyl-1-(2*H*)-phthalazinone (**7**) was synthesized according to the literature[19b] (Scheme 1). Nucleophilic substitution reaction of benzaldehyde and phthalide using sodium methoxide as a base in ethyl propionate and methanol gave diketone **12**. Treatment of **12** with hydrazine monohydrate afforded compound **7**. Then, derivatives **8** and **9** bearing a nitrogen-containing functional group on the phenyl group, corresponding to the diethylamino group of **5** and nitro group of **6**, were synthesized. Nitration of **7** with potassium nitrate in trifluoroacetic acid afforded *para*- (43%) and *ortho*-nitrated (26%) compounds (Scheme 1). Each nitro compound was hydrogenated to afford the amino derivative, which was further converted to *N*-acyl, *N*-mesyl, and *N*-alkylated derivatives (Scheme 2).

Scheme 1

Scheme 2

Various 4-benzyl-1-(2*H*)-phthalazinone derivatives with *ortho*-substituents were similarly synthesized from *ortho*-substituted benzaldehyde and phthalide in two steps (Scheme 3). In the case of compounds bearing two *ortho*-substituents, the reaction of intermediates **14** with hydrazine afforded the phthalazines in rather low yield. 4-Benzyl-1-(2*H*)-phthalazinone derivative **10f** bearing an *ortho*-carboxyl group was synthesized from homophthalic acid.[20,21] Reaction with phthalic anhydride using sodium acetate as a base at 200°C afforded deoxybenzoin derivative **15** (27%), which was reacted

with hydrazine monohydrate to give **10f** (Scheme 3).

Scheme 3

3. Results and Discussion

3.1. Biological Activity

AR agonistic and antagonistic activities of the synthesized compounds were first evaluated in terms of growth-inhibitory activity toward SC-3 cells bearing wt AR.[22] These cells show androgen-dependent cell proliferation. These phthalazinone derivatives alone did not affect the proliferation of SC-3, which means they did not act as AR agonists. The antagonistic activity of the test compounds was examined in terms of their effect on 1 nM DHT-dependent proliferation of SC-3 cells (Figure S2 in Supplementary data); the IC_{50} values are shown in Table 1. Parent compound **7** without any substituent on the phenyl ring showed only weak inhibitory activity (IC_{50}: 5.37 μM) on SC-3 cell proliferation. Introduction of a nitrogen-containing functional group at the *para* position (compounds **8**) caused loss of the activity, while compounds **9** bearing *ortho*-substituents showed inhibitory activity. Compounds **9a** with an *o*-nitro group and **9b** with an *o*-amino group showed IC_{50} values of 0.53 μM and 0.22 μM, respectively, and their activity is comparable to that of hydroxyflutamide (**3**, IC_{50} value: 0.18 μM). Modification of the amino group of **9b**, such as acylation, or mesylation, decreased the activity. Interestingly, *N*-mono- or *N*,*N*-dialkylation of aniline moiety of **9b** also decreased the activity. Among compounds **10** with various functional groups at the *ortho* position, **10d** bearing a chlorine atom showed potent inhibitory activity (IC_{50} value: 1.26 μM). The requirement of the substituent in the electronic and steric properties for high activity is unknown, introduction of the the proper substituent at *ortho* position is effective. Next, we examined the activity of compounds **11** with two *ortho*-substituents. Compound **11a** with *o*,*o*-dimethyl groups showed moderate activity (IC_{50} value: 1.38 μM), stronger than that of compound **10a** with an *o*-monomethyl group (IC_{50} value: 9.54 μM). Similar increases in activity was observed among *o*,*o*-dihalogenated compounds, and *o*,*o*-dichloro derivative **11c** (IC_{50} value: 0.18 μM) showed most potent activity in this series, being as active as hydroxyflutamide (**3**). Then, we examined whether the effect of **11c** on SC-3 proliferation depended on the concentration of DHT. Since 10 nM of DHT afforded maximum response to SC-3 proliferations, we examined the conditions with 0.3 – 10 nM of DHT. In this concentration range, the effect of **11c**, as well as hydroxyflutamide (**3**), showed the inhibitory activity toward SC-3 cells depending on the concentration of DHT (Figure S3 in Supplementary data).

Table 1

In order to clarify whether the inhibitory activity of phthalazinone derivatives toward SC-3 cell proliferation is AR-dependent, selected compounds were subjected to competitive AR binding assay (Table 2). All the phthalazinone derivatives examined showed AR binding affinity, and the binding potency correlated well with the activity in SC-3 assay. Thus, compound **11c** showed most potent binding affinity to AR, comparable to that of hydroxyflutamide (**3**). Further, phthalazinone derivatives did not inhibit the proliferation of androgen-independent prostate cancer cell line PC-3 (Figure S1 in Supplementary data). These results indicated that the inhibition of SC-3 cell proliferation by phthalazinone derivatives would result from their AR agonistic activity.

Table 2

We then investigated the antagonistic activity of selected compounds toward human prostate cancer cells LNCaP, bearing T877A-mutated AR (Table 3).[23,24] All phthalazinone derivatives examined inhibited DHT-stimulated proliferation of LNCaP cells. Among them, compound **11c** showed a lower IC_{50} value than bicalutamide (**1**). These compounds also inhibited PSA production in LNCaP cells, like bicalutamide (**1**) (Figure 3). Thus, our novel phthalazinone derivatives acted as AR antagonists not only toward wt AR, but also toward T877A-mutated AR.

Table 3

Figure 3

3.2. Conformational analysis

The structure-activity relationships of phthalazinone derivatives (Table 1) indicate that the substituent effect of the phenyl group of this series is different from that of compounds **5** and **6**, as well as anilide-type AR antagonists. In the case of phthalazinone derivatives, introduction of polar substituents at the *para* position (diethylamino group for **5** and nitro group for **6**) or electron-withdrawing groups (trifluoromethyl and cyano groups for bicalutamide, and trifluoromethyl and nitro groups for flutamide) was not effective, and introduction of *ortho*-substituents increased the AR antagonistic activity. Since there is no remarkable electronic effect of an *ortho*-substituent, it seemed likely that a change of the molecular shape caused by the *ortho*-substituents would be significant for the antagonistic activity. Thus, in order to clarify the mechanism through which phthalazinone derivatives show AR antagonistic activity, we examined the binding of phthalazinone derivatives to the AR ligand-binding domain (LBD). First, we examined the crystal structures of phthalazinone derivatives **10d** and **11c** (Figure 4), focusing on the dihedral angles between phthalazinone

and phenyl groups (Table 4). In particular, introduction of chlorine at both *ortho* positions increased the torsion angle (90.64°) around the phenyl-CH_2 bond, Thus, a molecular shape with large dihedral and torsion angles due to the steric effect of the *ortho*-substituent on the 4-benzyl-1-(2*H*)-phthalazinone skeleton appears to be important for the AR binding affinity.

Figure 4

Table 4

3.3. Docking simulation

Next, we considered possible docking structures between compound **11c** and the hAR LBD. To dock the compound to hAR LBD, we employed a newly developed in-house procedure rather than using conventional methods. Our method fully utilizes the large amount of protein-ligand interaction data available in PDB[25]. Briefly, we first gathered all the known AR LBD-ligand (mainly a steroid molecule) complex structures, computed the distributions of all atom types of ligands in the AR LBD, and placed all the atoms of compound **11c** to match the empirically obtained atom distributions. By this method, we can indirectly incorporate an entropic term in the docking procedure through the observed distribution of the poses of the ligands against the protein. The detail of the method to analyze the distribution of the atoms was given in ref 26. During this procedure, we allowed the conformation of the compound to vary by rotating the torsion angles. A docking structure that satisfies the empirically well-observed locations of nitrogen, oxygen and chloride atoms was then generated on the ligand-activated hAR LBD (PDB ID: 1xow[27]), energy minimization was carried out using GROMACS,[28] and serious steric clash was released. After the energy minimization, a slight relocation and deformation of helix-12 was observed (Figure 5). One of the possible factors of the relocation seemed repulsive interactions between a chloride atom of compound **11c** and the sulfur atom of M895 on helix-12. Since the relative location of helix-12 is known to be a key factor for enhancing/suppressing the receptor activity,[29] the interactions found in the docking study are suggestive in relation to the antagonistic mechanism of compound **11c**. The interaction between the benzylic moiety and helix-12 and that between one of the chlorine atoms on the compound and the sulfur atom of methionine on helix-12 seem to be critical factors for the antagonist activity. The docking study also demonstrated that the location of compound **11c** is distant (> 6Å) from the resistant mutation site T877A, and this may be the reason why the effect of compound **11c** on AR is rather independent of this mutation. Thus, the bulky aryl ring on the heterocyclic ring of compound

11c appears to be important for the AR antagonism, as may also be the case for MDV3100, an effective antagonist for CRPC.

Figure 5

4. Conclusion

4-Benzyl-1-(2*H*)-phthalazinone derivatives were designed and synthesized as novel AR antagonist candidates with a different pharmacophore from that of conventional AR antagonists. 4-Benzyl-1-(2*H*)-phthalazinone derivatives that were *ortho*-substituted on the phenyl ring showed AR antagonistic activity. Since an *ortho*-substituent has no marked electronic effect, distortion of the overall molecular shape by the substituent may be important for the activity. Among the synthesized compounds, compound **11c** potently inhibited SC-3 cell proliferation and showed high binding affinity to wt AR; its potency is similar to that of hydroxyflutamide (**3**). Further, compound **11c** acted as an AR antagonist toward LNCaP cells bearing T877A-mutated AR. Docking study indicated that the benzyl group of compound **11c** would disturb the conformation of helix-3 of the hAR LBD, which in turn might result in an inactive conformation of helix-12, thereby inactivating the whole receptor. Our phthalazinone derivatives discussed here have a unique pharmacophore that may be available for development of clinically useful AR antagonists, including drugs to treat CRPC.

5. Experimental

5.1 Chemistry

All reagents were purchased from Sigma-Aldrich Chemical Co., Tokyo Kasei Kogyo Co. Wako Pure Chemical Industries, and Kanto Kagaku Co., Inc. Silica gel for column chromatography was purchased from Kanto Kagaku Co., Inc. ^{1}H and ^{13}C NMR spectra were recorded on JEOL ECS 400 spectrometer or Bruker 600 spectrometer. Mass spectral data was obtained on a Bruker Daltonics microTOF-2focus or the MStation JMS-700 in the positive ion detection modes. Melting points were determined on a RFS-30 melting point apparatus (Round Science). Elemental analysis was performed on MT-6 elemental analyzer (Yanagimoto). Infrared spectra were obtained on FT/IR-6100 spectrometer (JASCO).

Preparation of compounds

1,3-Dioxo-2-phenylindane (**12**): A solution of sodium methoxide in methanol (28%, 2.196 g, 11.4 mmol) was added to a suspension of benzaldehyde (307 mg, 2.89 mmol) and phthalide (387 mg, 2.89 mmol) in ethyl propionate (1.2 mL) at 30°C, and the mixture was heated under reflux for 1 h. The reaction mixture was diluted with methanol and, was further heated for 1 h. After concentration *in vacuo*, water was added to the residue, and was washed with diethyl

ether. After acidified with acetic acid, the suspension was stirred for 15 min. The product was collected by filtration, washed with water, and dried *in vacuo* to afford **12** (331 mg, 52%) as colorless powder. ^{1}H NMR (400 M Hz, $CDCl_3$) δ 8.08 (dd, *J* = 2.9 Hz, 2 H), 7.91 (dd, *J* = 2.9 Hz, 2 H), 7.35-7.26 (m, 3 H), 7.19 (dd, *J* = 8.8, 2.0 Hz, 2 H), 4.27 (s, 1 H).

4-Benzyl-1-(2*H*)-phthalazinone (**7**): **12** (51 mg, 0.227 mmol) was suspended in hydrazine hydrate (300 mg, 6.00 mmol) and was heated at 100°C for 6 h. The reaction mixture was cooled to 20°C. The product was collected by filtration, washed with water, and dried *in vacuo* to afford **7** (30 mg, 56%) as colorless powder. mp 204-207°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.13 (br, 1 H), 8.45 (dt, *J* = 9.3, 2.0 Hz, 1 H), 7.77-7.70 (m, 3 H), 7.33-7.21 (m, 5 H), 4.30 (s, 2 H); ^{13}CNMR (150 MHz, $CDCl_3$) δ 160.8, 146.6, 137.7, 133.6, 131.5, 130.0, 128.9, 128.6, 128.5, 127.2, 127.0, 125.6, 39.0; HRMS calcd for $C_{15}H_{13}N_2O$ $(M+H)^+$ 237.1022, found 237.1017; Anal. calcd for $C_{15}H_{12}N_2O$: C, 76.25; H, 5.12; N, 11.86. found: C, 76.16; H, 5.35; N, 11.91.

4-(4-Nitorophenyl)methyl-1-(2*H*)-phthalazinone (**8a**) and 4-(2-Nitorophenyl)methyl-1-(2*H*)-phthalazinone (**9a**): Potassium nitrate (37 mg, 0.363 mmol) was added to a suspension of **7** (91 mg, 0.383 mmol) in trifluoroacetic acid (4 mL) at 0°C. and the mixture was stirred at 0°C for 20 h. The reaction mixture was basified with 2 M sodium hydroxide, and was extract with ethyl acetate. The organic layer was dried over $MgSO_4$, and evaporated. The residue was purified by column chromatography (silica gel, ethyl acetate / *n*-hexane = 1/1 then 2/1) to afford **8a** (43%) and **9a** (26%). **8a**: pale pink powder; mp 213.3-215.6°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.77 (br, 1 H), 8.48-8.46 (m, 1 H), 8.18 (d, *J* = 8.2 Hz, 2 H), 7.79-7.77 (m, 2 H), 7.68-7.66 (m, 1 H), 7.45 (d, *J* = 8.2 Hz, 2 H), 4.39 (s, 2 H); ^{13}CNMR (150 MHz, $CDCl_3$) δ 160.6, 147.1, 145.2, 145.1, 133.9, 131.9, 129.7, 129.6, 128.5, 127.5, 124.9, 124.1, 38.6; HRMS calcd for $C_{15}H_{12}N_3O_3$ $(M+H)^+$ 282.0873, found 282.0878; Anal. calcd for $C_{15}H_{11}N_3O_3$: C, 64.05; H, 3.94; N, 14.94. found: C, 63.86; H, 4.26; N, 14.68. **9a**: pale pink powder; mp 235.2-237.1°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.61 (br, 1 H), 8.47 (d, *J* = 7.8 Hz, 1 H), 8.10 (dd, *J* = 7.8, 1.4 Hz, 1 H), 7.88-7.79 (m 3 H), 7.58 (dt, *J* = 7.3, 1.4 Hz, 1 H), 7.48 (dt, *J* = 7.8, 1.4 Hz, 1 H), 7.33 (dd, *J* = 7.8, 1.4 Hz, 1 H), 4.68 (s, 2 H); ^{13}CNMR (150 MHz, $CDCl_3$) δ 160.2, 149.4, 144.8, 134.0, 133.5, 132.6, 132.3, 131.9, 129.8, 128.4, 128.3, 127.4, 125.3, 124.6, 35.9; HRMS calcd for $C_{15}H_{12}N_3O_3$ $(M+H)^+$ 282.0873, found 282.0878; Anal. calcd for $C_{15}H_{11}N_3O_3$: C, 64.05; H, 3.94; N, 14.94. found: C, 64.00; H, 3.94; N, 14.94.

4-(4-Aminophenyl)methyl-1-(2*H*)-phthalazinone (**8b**): A solution of **8a** (51 mg, 0.182 mmol)

in methanol (15 mL) was hydrogenated with 10% Pd/C (9.8 mg) under hydrogen atmosphere for 1 h at room temperature. After filtration over celite, and the filtrate were evaporated. The residue was purified by column chromatography (silica gel, ethyl acetate / *n*-hexane = 4/1, 5/1, then ethyl acetate only) to afford **8b** (86%). green powder; mp 231.3-232.0°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.52 (br, 1 H), 8.22 (dd, *J* = 7.8, 1.4 Hz, 1 H), 7.90 (d, *J* = 7.8 Hz, 1 H), 7.84 (dt, *J* = 7.3, 1.4 Hz, 1 H), 7.79 (dt, *J* = 7.8, 1.4 Hz, 1 H), 6.94 (d, *J* = 8.2 Hz, 2 H), 6.46 (d, *J* = 8.7 Hz, 2 H), 4.90 (s, 2 H), 4.08 (s, 2 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 159.9, 147.6, 146.3, 133.7, 131.7, 129.7, 129.3, 128.4, 126.4, 126.3, 125.4, 114.5, 37.5; HRMS calcd for $C_{15}H_{14}N_3O$ $(M+H)^+$ 252.1131, found 252.1132; Anal. calcd for $C_{15}H_{13}N_3O$: C, 71.70; H, 5.21; N, 16.72. found: C, 71.64; H, 5.36; N, 16.95.

4-(2-Aminophenyl)methyl-1-(2*H*)-phthalazinone (**9b**) was prepared by the same procedure for **8b**. Yield: 86%; green powder; mp 208.9-210.5°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.72 (br, 1 H), 8.44 (dd, *J* = 7.8, 1.4 Hz, 1 H), 7.99 (d, *J* = 7.8 Hz, 1 H), 7.82 (dt, *J* = 7.3, 1.4 Hz, 1 H), 7.76 (dt, *J* = 7.8, 1.4 Hz, 1 H), 7.16 (d, *J* = 7.8 Hz, 1 H), 7.08 (d, *J* = 7.8, 1.4 Hz, 1 H), 6.73 (dt, *J* = 7.3, 1.4 Hz, 1 H), 6.70 (d, *J* = 7.8 Hz, 1 H), 4.18 (s, 2 H), 4.12 (br, 2 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.8, 146.0, 133.9, 131.8, 130.6, 129.9, 128.4, 127.3, 125.4, 121.4, 118.6, 116.6, 35.6; HRMS calcd for $C_{15}H_{14}N_3O$ $(M+H)^+$ 252.1131, found 252.1128; Anal. calcd for $C_{15}H_{13}N_3O$: C, 71.70; H, 5.21; N, 16.72. found: C, 71.59; H, 5.31; N, 16.54.

4-(4-Acetaminophenyl)methyl-1-(2*H*)-phthalazinone (**8c**): Acetyl chloride (40 μL, 0.570 mmol) was added to a solution of **8b** (48 mg, 0.189 mmol) and triethylamine (40 mg, 0.390 mmol) in dichloromethane (15 mL) at 0°C, and the mixture was stirred at room temperature for 1 h. 2 M Hydrochrolic acid was added to the mixture, extracted with ethyl acetate. The organic layer was dried over $MgSO_4$, and evaporated. The residue was purified by column chromatography (silica gel, ethyl acetate / *n*-hexane = 2/1) to afford **8c** (41.7 mg, 75%) as colorless powder. mp 240.3-242.8°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.57 (br, 1 H), 9.86 (s, 1 H), 8.24 (d, *J* = 7.8 Hz, 1 H), 7.90 (d, *J* = 7.8 Hz, 1 H), 7.86 (dt, *J* = 7.3, 1.4 Hz, 1 H), 7.80 (dt, *J* = 7.8, 1.4 Hz, 1 H), 7.46 (d, *J* = 8.8 Hz, 2 H), 7.21 (d, *J* = 8.8 Hz, 2 H), 4.23 (s, 2 H), 1.99 (s,3 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 168.6, 159.9, 145.8, 138.2, 133.9, 133.1, 131.9, 129.6, 129.2, 128.4, 126.5, 126.2, 119.7, 37.6, 24.4; HRMS calcd for $C_{17}H_{16}N_3O_2$ $(M+H)^+$ 294.1237, found 294.1246.

4-(2-Acetaminophenyl)methyl-1-(2*H*)-phthalazinone (**9c**) was prepared by the same procedure for **8c**. Yield 75%; colorless powder; mp 265.1-265.9°C; ^{1}H NMR (400 MHz,

$CDCl_3$) δ 9.86 (br, 1 H), 8.51 (br, 1 H), 8.46 (d, *J* = 7.8 Hz, 1 H), 8.04 (d, *J* = 8.2 Hz, 1 H), 7.94 (d, *J* = 8.2 Hz, 1 H), 7.88 (dt, *J* = 7.3, 1.4 Hz, 1 H), 7.81 (dt, *J* = 7.8, 1.4 Hz, 1 H), 7.35 (d, *J* = 7.3 Hz, 1 H), 7.11 (t, *J* = 7.8 Hz, 1 H), 4.27 (s, 2 H), 2.24 (s, 3 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.1, 160.6, 146.9, 146.8, 137.3, 134.1, 132.3, 130.4, 129.6, 129.2, 128.4, 127.5, 125.4, 125.1, 124.2, 35.5, 24.8; HRMS calcd for $C_{17}H_{16}N_3O_2$ $(M+H)^+$ 294.1237, found 294.1237.

4-(4-Trifluoroacetaminophenyl)methyl-1-(2*H*)-phthalazinone (**8d**) was prepared by the similar procedure for **8c** using trifluoroacetyl chloride: Yield 59%; colorless powder; mp 269.5-270.4°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.58 (br, 1 H), 11.19 (br, 1 H), 8.25 (d, *J* = 7.8 Hz, 1 H), 7.92 (d, *J* = 7.3 Hz, 1 H), 7.86 (dt, *J* = 7.3, 1.5 Hz, 1 H), 7.81 (dt, *J* = 6.8, 0.98 Hz, 1 H), 7.56 (d, *J* = 8.3 Hz, 2 H), 7.33 (d, *J* = 8.8 Hz, 2 H), 4.29 (s, 2 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 159.5, 154.5, 145.2, 135.7, 134.7, 133.5, 131.6, 129.2, 128.0, 126.1, 125.7, 121.4, 116.8, 114.9, 37.2; HRMS calcd for $C_{17}H_{13}F_3N_3O_2$ $(M+H)^+$ 348.0954, found 348.0946; Anal. calcd for $C_{17}H_{12}F_3N_3O_2$: C, 58.79; H, 3.58; N, 12.10. found: C, 58.76; H, 3.61; N, 12.10.

4-(2-Trifluoroacetaminophenyl)methyl-1-(2*H*)-phthalazinone (**9d**) was prepared by the similar procedure for **8c** using trifluoroacetyl chloride. Yield 45%; colorless powder; mp 206.5-207.0°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.03 (br, 1 H), 10.07 (br, 1 H), 8.50 (d, *J* = 7.8 Hz, 1 H), 8.13 (d, *J* = 8.2 Hz, 1 H), 8.00 (t, *J* = 8.7 Hz, 1 H), 7.97 (dd, *J* = 7.8, 0.9 Hz, 1 H), 7.89 (dt, *J* = 7.3, 0.9 Hz, 1 H), 7.45 (d, *J* = 7.3 Hz, 1 H), 7.35 (t, *J* = 7.3 Hz, 1 H), 7.21 (dt, *J* = 7.3, 1.4 Hz, 1 H), 4.33 (s, 2 H), 2.24 (s, 3 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.1, 146.9, 135.2, 134.2, 132.6, 130.8, 129.2, 128.8, 128.7, 128.3, 127.8, 126.7, 125.0, 124.1, 34.8; HRMS calcd for $C_{17}H_{13}F_3N_3O_2$ $(M+H)^+$ 348.0954, found 348.0954.

4-(4-Trifluoroacetaminophenyl)methyl-1-(2*H*)-phthalazinone (**8e**) was prepared by the similar procedure for **8c** using methanesulfonyl chloride: Yield 22%; colorless powder; mp 258.5-260.7°C; ^{1}HNMR (400MHz, DMSO-d_6) δ 12.57 (br, 1 H), 9.63 (br, 1 H), 8.25 (d, *J* = 7.8 Hz, 1 H), 7.93 (d, *J* = 7.8 Hz, 1 H), 7.87 (dt, *J* = 7.8, 1.5 Hz, 1 H), 7.81 (dt, *J* = 7.8, 0.98 Hz, 1 H), 7.27 (d, *J* = 8.3 Hz, 2 H), 7.33 (d, *J* = 8.3 Hz, 2 H), 4.25 (s, 2 H), 2.93 (s, 3 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 159.4, 145.2, 136.7, 133.7, 133.4, 131.4, 129.4, 129.1, 127.9, 126.0, 125.6, 120.2, 40.0, 36.9; HRMS calcd for $C_{16}H_{16}N_3O_3S$ $(M+H)^+$ 330.0907, found 330.0905; Anal. calcd for $C_{16}H_{15}N_3O_3S$: C, 58.34; H, 4.59; N, 12.76. found: C, 58.36; H, 4.73; N, 12.46.

4-(2-Trifluoroacetaminophenyl)methyl-1-(2*H*)-phthalazinone (**9e**) was prepared by the similar procedure for **8c** using methanesulfonyl chloride: Yield 36%; colorless powder; mp 228.9-230.1°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.96 (br, 1 H), 8.46 (d, *J* = 7.8 Hz, 1 H), 8.16 (br, 1 H), 8.02 (d, *J* = 8.2 Hz, 1 H), 7.89 (dt, *J* = 8.2, 1.4 Hz, 1 H), 7.81 (dt, *J* = 7.3, 0.9 Hz, 1 H), 7.54 (dt, *J* = 8.2, 0.9 Hz, 1 H), 7.40 (dd, *J* = 7.8, 0.9 Hz, 1 H), 7.29 (dt, *J* = 7.8, 0.9 Hz, 1 H), 7.17 (dt, *J* = 7.3, 1.4 Hz, 1 H), 4.32 (s, 2 H), 3.04 (s, 3 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 159.4, 144.9, 135.5, 134.3, 133.4, 131.5, 130.1, 129.4, 127.8, 127.4, 126.4, 126.2, 126.0, 125.4, 40.1, 33.6; HRMS calcd for $C_{16}H_{16}N_3O_3S$ $(M+H)^+$ 330.0907, found 330.0907; Anal. calcd for $C_{16}H_{15}N_3O_3S$: C, 58.34; H, 4.59; N, 12.76. found: C, 58.31; H, 4.52; N, 12.67.

4-(4-Diethylaminophenyl)methyl-1-(2*H*)-phthalazinone (**8f**) and 4-(4-ethylaminophenyl)methyl-1-(2*H*)- phthalazinone (**8g**): Iodoethane (80 μL, 0.96 mmol) was added to a solution of **8b** (59 mg, 0.233 mmol) in DMF (2.0 mL), and the mixture was stirred for 1 d at 40°C. After the solvent was removed *in vacuo*, the residue was purified by column chromatography (silica gel, ethyl acetate / *n*-hexane = 2/1) to afford **8f** (13 mg, 18%) and **8g** (12 mg, 19%). **8f**: colorless powder; mp 194.0-195.8°C;^{1}H NMR (400 MHz, DMSO-d_6) δ 12.53 (br, 1 H), 8.23 (d, *J* = 7.3 Hz, 1 H), 7.95 (d, *J* = 7.3 Hz, 1 H), 7.85 (dt, *J* = 7.3, 1.4 Hz, 1 H), 7.79 (dt, *J* = 7.3, 0.9 Hz, 1 H), 7.07 (d, *J* = 8.7 Hz, 2 H), 6.55 (d, *J* = 8.7 Hz, 2 H), 4.12 (s, 2H), 3.25 (q, *J* = 7.3 Hz, 4 H), 1.02 (t, *J* = 6.9 Hz, 6 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.5, 147.4, 146.8, 133.6, 131.4, 130.1, 129.5, 128.5, 127.0, 125.9, 123.9, 112.1, 44.4, 38.0, 12.7; HRMS calcd for $C_{19}H_{22}N_3O$ $(M+H)^+$ 308.1757, found 308.1751. **8g**: colorless powder; mp 203.8-205.8°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.52 (br, 1 H), 8.23 (dd, *J* = 7.3, 0.9 Hz, 1 H), 7.92 (d, *J* = 7.8 Hz, 1 H), 7.84 (dt, *J* = 6.9, 1.4 Hz, 1 H), 7.79 (dt, *J* = 7.8, 1.4 Hz, 1 H), 7.00 (d, *J* = 8.2 Hz, 2 H), 6.45 (d, *J* = 8.7 Hz, 2 H), 5.37 (t, *J* = 5.5 Hz, 1 H), 4.10 (s, 2 H), 2.94 (q, *J* = 5.5 Hz, 2 H), 1.10 (t, *J* = 6.9 Hz, 3 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ□160.7, 147.4, 147.2, 133.5, 131.3, 130.1, 129.5, 128.5, 127.0, 126.0, 125.8, 113.1, 38.6, 38.2, 15.0; HRMS calcd for $C_{17}H_{18}N_3O$ $(M+H)^+$ 280.1444, found 280.1438.

4-(4-Dimethylaminophenyl)methyl-1-(2*H*)-phthalazinone (**8h**) and 4-(4-methylaminophenyl)methyl-1-(2*H*)-phthalazinone (**8i**) were prepared by the similar procedure for **8f** and **8g**. **8h**: Yield 9%; colorless powder; mp 249.3-250.3°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.54 (br, 1 H), 8.23 (d, *J* = 8.2 Hz, 1 H), 7.93 (d, *J* = 8.2 Hz, 1 H), 7.85 (t, *J* = 8.2 Hz, 1 H), 7.79 (t, *J* = 7.8 Hz Hz, 1 H), 7.11 (d, *J* = 8.7 Hz, 2 H), 6,63 (d, *J* = 8.7 Hz, 2

H), 4.15 (s, 2 H), 2.81 (s, 6 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.5, 149.6, 147.3, 133.6, 131.4, 130.1, 129.3, 128.5, 127.1, 125.8, 125.2, 113.0, 38.1, 30.8; HRMS calcd for $C_{17}H_{18}N_3O$ $(M+H)^+$ 280.1444, found 280.1436. **8i**: Yield 12%; colorless powder; mp 196.4-198.2°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.53 (br, 1 H), 8.23 (d, *J* = 6.4 Hz, 1 H), 7.91 (d, *J* = 7.8 Hz, 1 H), 7.84 (dt, *J* = 7.3, 1.4 Hz, 1 H), 7.78 (dt, *J* = 8.2, 0.9 Hz, 1 H), 7.01 (d, *J* = 8.2 Hz, 2 H), 6.43 (d, *J* = 8.2 Hz, 2 H), 5.48 (q, *J* = 5.0 Hz, 1 H), 4.11 (s, 2 H), 2.59 (d, *J* = 5.0 Hz, 3 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.4, 147.2, 133.6, 131.4, 130.1, 129.7, 129.5, 128.5, 127.1, 126.1, 125.8, 112.9, 38.2, 31.0; HRMS calcd for $C_{16}H_{16}N_3O$ $(M+H)^+$ 266.1288, found 266.1284.

4-(2-Diethylaminophenyl)methyl-1-(2*H*)-phthalazinone (**9f**) and 4-(2-ethylaminophenyl)methyl-1-(2*H*)-phthalazinone (**9g**) were prepared from **9b** by the similar procedure for **8f** and **8g**. **9f**: Yield 3%; colorless powder; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.97 (br, 1 H), 8.40 (dd, *J* = 8.2, 1.4 Hz, 1 H), 7.74 (dd, *J* = 7.3, 1.4 Hz, 1 H), 7.68 (dt, *J* = 7.3, 1.4 Hz, 1 H), 7.63 (dt, *J* = 7.8, 1.4 Hz, 1 H), 7.20-7.11 (m, 3 H), 6.93 (dt, *J* = 7.8, 1.8 Hz, 1 H), 4.35 (s, 2 H), 3.08 (q, *J* = 6.9 Hz, 4 H), 1.09 (t, *J* = 6.9 Hz, 6 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ□160.4, 149.1, 148.1, 134.5, 133.4, 131.4, 130.2, 129.8, 128.3, 127.2, 126.8, 126.2, 123.8, 122.6, 47.7, 33.5, 12.5; HRMS calcd for $C_{19}H_{22}N_3O$ $(M+H)^+$ 308.1757, found 308.1763. **9g**: Yield 23%; colorless powder; mp 184.9-185.7°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.08 (br, 1 H), 8.43 (dd, *J* = 7.3, 0.92 Hz, 1 H), 8.00 (d, *J* = 7.8 Hz, 1 H), 7.80 (dt, *J* = 7.3, 1.8 Hz, 1 H), 7.75 (dt, *J* = 7.3, 1.4 Hz, 1 H), 7.18 (dd, *J* = 7.3, 1.4 Hz, 1 H), 7.16 (dt, *J* = 7.8, 1.4 Hz, 1 H), 6.67 (dt, *J* = 7.3, 0.9 Hz, 1 H), 6.65 (dd, *J* = 8.2 Hz, 1 H), 4.16 (s, 2 H), 3.15 (q, *J* = 7.3 Hz, 2 H), 1.29 (t, *J* = 7.3 Hz, 3 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.4, 147.5, 146.0, 137.7, 133.7, 131.7, 130.5, 130.0, 128.9, 128.7, 128.6, 127.2, 125.7, 120.7, 116.8, 38.5, 36.1, 14.9; HRMS calcd for $C_{17}H_{18}N_3O$ $(M+H)^+$ 280.1444, found 280.1443.

4-(2-Dimethylaminophenyl)methyl-1-(2*H*)-phthalazinone (**9h**) and 4-(2-methylaminophenyl)methyl-1-(2*H*)-phthalazinone (**9i**) were prepared from **9b** by the similar procedure for **8f** and **8g**. **9h**: Yield 16%; white powder; mp 234.5-234.9°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.78 (br, 1 H), 8.39 (dd, *J* = 1.8 Hz, 1 H), 7.78 (dd, *J* = 2.7 Hz, 1 H), 7.69-7.66 (m, 2 H), 7.20-7.17 (m, 2 H), 7.12 (d, *J* = 6.9 Hz, 1 H), 6.93 (dt, *J* = 6.4, 2.3 Hz, 1 H), 4.36 (s, 2 H), 2.79 (s, 6 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ□160.7, 152.1, 148.0, 133.6, 132.6, 131.4, 130.2, 129.9, 128.9, 127.8, 126.8, 126.0, 123.7, 119.7, 45.2, 33.4; Anal. calcd for $C_{17}H_{17}N_3O$: C, 73.10; H, 6.13; N, 15.04. found: C, 73.22; H, 6.09; N, 15.21. **9i**: Yield 9%; white powder; mp 194.8-195.7°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.78 (br, 1 H), 8.43 (dd, *J* =

7.8, 1.4 Hz, 1 H), 8.99 (d, *J* = 7.3 Hz, 1 H), 7.81 (dt, *J* = 7.3, 1.4 Hz, 1 H), 7.75 (dt, *J* = 7.3, 0.9 Hz, 1 H), 7.21-7.16 (m, 2 H), 6.69 (d, *J* = 7.3 Hz, 1 H), 6.65 (d, *J* = 8.2 Hz, 1 H), 4.60 (br, 1 H), 4.15 (s, 2 H), 2.87 (s, 3 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.1, 148.3, 146.0, 133.8, 131.8, 130.4, 130.0, 128.6, 128.6, 127.3, 125.6, 121.0, 117.0, 110.6, 35.9, 30.8; HRMS calcd for $C_{16}H_{16}N_3O$ $(M+H)^+$ 266.1288, found 266.1285.

Compounds **13a – e** and **14a – d** were prepared by the same procedure for **12**.
2-(2-Methylphenyl)-1,3-dioxoindane (**13a**): Yield 86%; yellow powder; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (dd, *J* = 3.2 Hz, 2 H), 7.91 (dd, *J* = 2.7 Hz, 2 H), 7.24 (d, *J* = 7.3 Hz, 1 H), 7.21 (dt, *J* = 7.3, 1.4 Hz, 1 H), 7.13 (dt, *J* = 7.3, 1.8 Hz, 1 H), 6.88 (d, *J* = 7.3 Hz, 1 H), 4.52 (s, 1 H), 2.33 (s, 3 H).

2-(2-Methoxyphenyl)-1,3-dioxoindane (**13b**): Yield 85%; yellow powder; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (dd, *J* = 2.7 Hz, 2 H), 7.87 (dd, *J* = 2.7 Hz, 2 H), 7.31 (dt, *J* = 8.2, 2.7 Hz, 1 H), 7.28 (dd, *J* = 8.2, 2.7 Hz, 1 H), 7.00 (dt, *J* = 8.2, 2.7 Hz, 1 H), 6.79 (d, *J* = 8.2 Hz, 1 H), 4.17 (s, 1 H), 3.44 (s, 3 H).

2-(2-Fluorophenyl)-1,3-dioxoindane (**13c**): Yield 71%; yellow powder; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (dd, *J* = 3.2 Hz, 2 H), 7.91 (dd, *J* = 3.2 Hz, 2 H), 7.36-7.30 (m, 1 H), 7.21 (dt, *J* = 7.8, 2.3 Hz, 1 H), 7.16 (dt, *J* = 7.3, 0.9 Hz, 1 H), 7.05 (dt, *J* = 8.7, 0.9 Hz, 1 H), 4.39 (s, 1 H).

2-(2-Chlorophenyl)-1,3-dioxoindane (**13d**): Yield 36%; yellow powder; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (dd, *J* = 3.2 Hz, 2 H), 7.90 (dd, *J* = 3.2 Hz, 2 H), 7.40-7.38 (m, 1 H), 7.31-7.29 (m, 2 H), 7.22-7.20 (m, 1 H), 4.56 (s, 1 H).

1,3-Dioxo-2-(2-triflooromethylphenyl)indane (**13e**): Yield 72%; yellow powder; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (dd, *J* = 3.2 Hz, 2 H), 7.92 (dd, *J* = 3.2 Hz, 2 H), 7.77 (dd, *J* = 7.3 Hz, 1.8 Hz, 1 H), 7.50-7.42 (m, 2 H), 6.94 (d, *J* = 6.9 Hz, 1 H), 4.76 (s, 1 H).

2-(2,6-Dimethylphenyl)-1,3-dioxoindane (**14a**): Yield 70%; yellow powder; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.58 (br, 1 H), 8.49 (dd, *J* = 7.3 Hz, 1 H), 7.99 (dd, *J* = 7.9 Hz, 1 H), 7.93 (dt, *J* = 6.9, 1.4 Hz, 1 H), 7.83 (dt, *J* = 8.2 Hz, 1 H), 7.38 (d, *J* = 8.2 Hz, 2 H), 7.22 (t, *J* = 8.2 Hz, 1 H), 4.60 (s, 2 H).

2-(2,6-Difluorophenyl)-1,3-dioxoindane (**14b**): Yield quant; yellow powder; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (dd, *J* = 3.2 Hz, 2 H), 7.92 (dd, *J* = 3.2 Hz, 2 H), 7.35-7.23 (m, 1 H), 6.93 (t, *J* = 8.7 Hz, 1 H), 4.70 (s, 1 H).

2-(2,6-Dichlorophenyl)-1,3-dioxoindane (**14c**): Yield 53%; yellow powder; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (dd, *J* = 3.2 Hz, 2 H), 7.89 (dd, *J* = 3.2 Hz, 2 H), 7.45 (dt, *J* = 4.1, 1.3 Hz, 1 H), 7.25 (dd, *J* = 5.9, 1.3 Hz, 2 H), 5.28 (s, 1 H).

1,3-Dioxo-2-(2,3,6-trichlorophenyl)indane (**14d**): Yield 34%; yellow powder; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (q, *J* = 3.2 Hz, 2 H), 7.92 (q, *J* = 3.2 Hz, 2 H), 7.42 (t, *J* = 2.3 Hz, 1 H), 7.20 (d, *J* = 8.7 Hz, 1 H), 5.33 (d, *J* = 29 Hz, 1 H).

Compounds **10a – e** and **11a – d** were prepared by the same procedure for **7**.
4-(2-Toluyl)methyl-1-(2*H*)-phthalazinone (**10a**): Yield 27%; colorless powder; mp 209.9-210.8°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.91 (br, 1 H), 8.47 (dd, *J* = 3.2, 1.4Hz, 1 H), 7.79-7.76 (m, 2 H), 7.72 (dd, *J* = 3.2, 1.3 Hz, 1 H), 7.23 (d, *J* = 6.9 Hz, 1 H), 7.18 (t, *J* = 7.3 Hz, 1 H), 7.11 (t, *J* = 7.3 Hz, 1 H), 6.99 (d, *J* = 7.8 Hz, 1 H), 4.27 (s, 1 H), 2.38 (s, 3 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.7, 146.2, 136.4, 135.9, 133.7, 131.5, 130.5, 130.2, 129.0, 128.3, 127.2, 127.0, 126.3, 125.2, 36.3, 20.0; HRMS calcd for $C_{16}H_{15}N_2O$ $(M+H)^+$ 251.1179, found 266.1173.

4-(2-Methoxyphenyl)methyl-1-(2*H*)-phthalazinone (**10b**): Yield 63%; colorless powder; mp 196.3-197.6°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.80 (br, 1 H), 8.45-8.43 (m, 1 H), 7.85-7.83 (m, 1 H), 7.77-7.92 (m, 2 H), 7.23 (t, *J* = 6.9 Hz, 1 H), 7.08 (d, *J* = 7.3 Hz, 1 H), 6.93 (d, *J* = 8.2 Hz, 1 H), 6.86 (t, *J* = 7.3 Hz, 1 H), 4.29 (s, 2 H), 3.90 (s, 3 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.5, 156.9, 147.0, 133.6, 131.4, 130.5, 130.0, 128.3, 128.2, 127.0, 126.0, 125.6, 120.8, 110.7, 55.7, 32.1; HRMS calcd for $C_{16}H_{14}N_2NaO_2$ $(M+Na)^+$ 289.0947, found 289.0946; Anal. calcd for $C_{16}H_{14}N_2O_2$; C, 72,16; H, 5.30; N, 10.52. found: C, 72.12; H, 5.24; N, 10.75.

4-(2-Fluorophenyl)methyl-1-(2*H*)-phthalazinone (**10c**): Yield 62%; colorless powder; mp 234.7-235.4°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.77 (br, 1 H), 8.45 (dd, *J* = 6.9, 1.4Hz, 1 H), 7.82-7.74 (m, 3 H), 7.19 (dt, *J* = 7.3, 1.4 Hz, 1 H), 7.11 (d, *J* = 8.7 Hz, 1 H), 7.06 (dt, *J* = 7.3, 1 H), 4.31 (s, 2 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.7, 145.7, 133.8, 131.7, 130.8, 130.7, 129.8, 128.9, 128.8, 128.3, 127.2, 125.1, 124.5, 115.6, 31.2; HRMS calcd for $C_{15}H_{11}FN_2NaO$

$(M+Na)^+$ 277.0748, found 277.0743; Anal. calcd for $C_{15}H_{11}FN_2O$: C, 70.86; H, 4.36; N, 11.02. found: C, 70.97; H, 4.48; N, 11.10.

4-(2-Chlorophenyl)methyl-1-(2*H*)-phthalazinone (**10d**): Yield 76%; colorless powder; mp 222.5-223.4°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.03 (br, 1 H), 8.47 (dd, *J* = 6.4, 2.3 Hz, 1 H), 7.82-7.73 (m, 3 H), 7.44 (dd, *J* = 7.7, 1.4 Hz, 1 H), 7.21 (dt, *J* = 7.2, 1.8 Hz, 1 H), 7.16 (dt, *J* = 7.7, 1.8 Hz, 1 H), 7.12 (dd, *J* = 7.2, 1.8 Hz, 1 H), 4.41 (s, 2 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.6, 145.7, 135.4, 134.0, 133.8, 131.7, 130.5, 129.9, 129.8, 128.4, 128.4, 127.2, 127.2, 125.2, 35.8; HRMS calcd for $C_{15}H_{12}ClN_2O$ $(M+H)^+$ 271.0633, found 271.0630; Anal. calcd for $C_{15}H_{11}ClN_2O$: C, 66.55; H, 4.10; N, 10.35. found: C, 66.66; H, 4.03; N, 10.30.

4-(2-Trifluoromethylphenyl)methyl-1-(2*H*)-phthalazinone (**10e**): Yield 65%; colorless powder; mp 196.9-197.5°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.83 (br, 1 H), 8.47 (dd, *J* = 3.6 Hz, 1 H), 7.78-7.73 (m, 3 H), 7.64 (dd, *J* = 3.6 Hz, 1 H), 7.42 (dt, *J* = 7.3 Hz, 1 H), 7.36 (t, *J* = 7.3 Hz, 1 H), 7.13 (d, *J* = 7.3 Hz, 1 H), 4.49 (s, 2 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.9, 145.5, 136.1, 133.9, 132.2, 131.7, 130.6, 129.9, 128.6, 128.4, 127.2, 127.1, 126.4, 126.4, 125.1, 35.1; HRMS calcd for $C_{16}H_{12}F_3N_2O$ $(M+H)^+$ 305.0896, found 305.0899; Anal. calcd for $C_{16}H_{11}F_3N_2O$: C, 63.16; H, 3.64; N, 9.21. found: C, 62.99; H, 3.67; N, 9.30.

4-(2,6-Dimethylphenyl)methyl-1-(2*H*)-phthalazinone (**11a**): Yield 9%; colorless powder; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.60 (br, 1 H), 8.49 (dd, *J* = 8.2, 0.9 Hz, 1 H), 8.02 (d, *J* = 7.8 Hz, 1 H), 7.92 (dt, *J* = 7.3, 1.4 Hz, 1 H), 7.83 (dt, *J* = 7.3, 0.9 Hz, 1 H), 7.16 (dd, *J* = 8.6, 2.7 Hz, 1 H), 7.10 (d, *J* = 7.8 Hz, 2 H), 4.30 (s, 2 H), 2.25 (s, 6 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.0, 144.7, 137.4, 133.9, 133.6, 131.7, 130.3, 128.2, 128.1, 127.3, 127.3, 124.3, 32.0, 20.4; HRMS calcd for $C_{17}H_{17}N_2O$ $(M+H)^+$ 265.1335, found 265.1334.

4-(2,6-Difluorophenyl)methyl-1-(2*H*)-phthalazinone (**11b**): Yield 20%; colorless powder; mp 270.7-271.0°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.73 (br, 1 H), 8.47 (dd, *J* = 7.8, 0.92 Hz, 1 H), 7.95 (d, *J* = 7.8 Hz, 1 H), 7.88 (dt, *J* = 7.3, 1.4 Hz, 1 H), 7.80 (dt, *J* = 8.2, 1.4 Hz, 1 H), 7.31-7.24 (m, 1 H), 6.94 (t, *J* = 7.7 Hz, 1 H), 4.34 (s, 2 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 162.6, 161.0, 160.1, 143.9, 133.8, 131.7, 129.9, 129.0, 128.2, 127.3, 124.4, 111.5, 25.6; HRMS calcd for $C_{15}H_{11}F_2N_2O$ $(M+H)^+$ 273.0834, found 273.0835; Anal. calcd for $C_{15}H_{10}F_2N_2O$: C, 66.17; H, 3.70; N, 10.27. found: C, 66.12; H, 3.78; N, 10.31.

4-(2,6-Dichlorophenyl)methyl-1-(2*H*)-phthalazinone (**11c**): Yield 20%; colorless powder; mp

276.6-278.4°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.91 (br, 1 H), 8.47 (dd, *J* = 3.2, 1.4Hz, 1 H), 7.79-7.76 (m, 2 H), 7.72 (dd, *J* = 3.2, 1.3 Hz, 1 H), 7.23 (d, *J* = 6.9 Hz, 1 H), 7.18 (t, *J* = 7.3 Hz, 1 H), 7.11 (t, *J* = 7.3 Hz, 1 H), 6.99 (d, *J* = 7.8 Hz, 1 H), 4.27 (s, 1 H), 2.38 (s, 3 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.3, 160.1, 143.4, 136.5, 133.8, 133.4, 131.7, 130.0, 128.9, 128.3, 128.2, 127.4, 124.2, 33.7; HRMS calcd for $C_{15}H_{11}Cl_2N_2O$ $(M+H)^+$ 305.0243, found 305.0241; Anal. calcd for $C_{15}H_{10}Cl_2N_2O$; C, 59.04; H, 3.30; N, 9.18. found: C, 58.94; H, 3.60; N, 9.05.

4-(2,3,6-Trichlorophenyl)methyl-1-(2*H*)-phthalazinone (**11d**): Yield 14%; colorless powder; mp 274.8-275.7°C: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.56 (br, 1 H), 8.49 (d, *J* = 7.8 Hz, 1 H), 7.97 (t, *J* = 8.2 Hz, 1 H), 7.92 (d, *J* = 7.8 Hz, 1 H), 7.85 (t, *J* = 7.8 Hz, 1 H), 7.40 (d, *J* = 8.7 Hz, 1 H), 7.33 (d, *J* = 8.7 Hz, 1 H), 4.64 (s, 2 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 159.2, 141.9, 136.1, 134.1, 134.0, 133.8, 132.1, 130.9, 129.8, 129.2, 129.0, 127.5, 126.2, 124.9, 34.6; HRMS calcd for $C_{15}H_{10}Cl_3N_2O$ $(M+H)^+$ 338.9853, found 338.9857; Anal. calcd for $C_{15}H_9Cl_3N_2O$: C, 53.05; H, 2.67; N, 8.25. found: C, 53.29; H, 2.90; N, 8.25.

2-[2-(2-Carboxyphenyl)acetyl]benzoic acid (**15**): A mixture of phthalic anhydride (604 mg, 4.08 mmol), homophthalic acid (605 mg, 3.36 mmol), and anhydrous sodium acetate (56 mg, 0.686 mmol) was heated at 200-210°C for 5 h. After cooling, orange mass was warmed with 10% aqueous KOH solution (10 mL) until most of them dissolved, and the resulting deep red solution was filtered. The filtrate was acidified with 2 M hydrochloric acid, and the precipitates were collected, which was recrystallized from ethyl acetate to afford **15** (311 mg, 27%) as yellow crystal. ^{1}H NMR (400 MHz, DMSO-d_6, 105°C) δ 7.85 (d, *J* = 7.6 Hz, 1 H), 7.78 (d, *J* = 7.4 Hz, 1 H), 7.63 (dt, *J* = 7.4, 0.72 Hz, 1 H), 7.54 (dt, *J* = 7.6, 0.90 Hz, 1 H), 7.46 (t, *J* = 6.2 Hz, 2 H), 7.34 (t, *J* = 7.7, 1 H), 7.31 (d, *J* = 7.6 Hz, 1 H), 4.43 (br, 2 H); Anal. calcd for $C_{16}H_{12}O_5$: C, 67.60; H, 4.25. found: C, 67.52; H, 4.53.

2-[[1-(2*H*)-Phthalazinon-4-yl]methyl]benzoic acid (**10f**): A suspension of **15** (244 mg, 0.858 mmol) in hydrazine hydrate (1846 mg, 36.9 mmol) was heated at 100°C for 6 h. After cooling, the solution was diluted with water, washed with ethyl acetate, and acidified with 2 M hydrochloric acid. The resulting pale yellow suspension was stirred for 10 min, and the precipitates were collected, which was washed with water to afford **10f** (197 mg, 68%) as yellow powder. mp 247.8°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.39 (s, 1 H), 8.26 (d, *J* = 7.8 Hz, 1 H), 8.02 (d, *J* = 7.9 Hz, 1 H), 7.92 (t, *J* = 7.8 Hz, 2 H), 7.85 (t, *J* = 7.8 Hz, 1 H), 7.48 (t, *J* = 7.3 Hz, 1 H), 7.35 (t, *J* = 7.3, 1 H), 7.28 (d, *J* = 7.8 Hz, 1 H), 4.69 (s, 2 H); ^{13}C NMR (150

MHz, DMSO) δ 169.5, 160.4, 147.1, 139.0, 134.5, 132.4, 132.1, 131.8, 131.1, 130.2, 127.8, 127.4, 126.6, 125.8, 36.5; HRMS calcd for $C_{16}H_{12}FN_2NaO_3$ $(M+Na)^+$ 303.0740, found 303.0740.

5.2 Biology

SC-3 Growth Inhibition Assay: The assay was examined by the same procedure described in our previous report.[16] SC-3 cells were cultured in MEMα (Wako Co.) supplemented with 2% FBS and 1 nM DHT at 37°C under 5% CO_2. All experiments were performed in triplicate or more. Cells were trypsinized and diluted to 20,000 cells/mL with MEMα supplemented with 2% charcoal-stripped FBS. This cell suspension was seeded in 96-well plates at a volume of 100 μL and incubated at 24 h. After removal of 10μL of medium from each well, 10μL of the drug solution, which was supplemented with serial dilutions of the test compounds or DMSO as a dilution control in the presence of 1 nM DHT, was added. Then the plates were incubated at 37°C under 5% CO_2 for 3 d, and the cell number was determined using a Cell Counting Kit-8 (DOJINDO). A 10μL aliquot of WST-8 was added to each well of microcultures, and the cells were incubated for 2 h. The absorbance at 450 nm was measured with a microplate reader. This parameter is related to the number of living cells in the culture. IC_{50} values were determined as the concentration that the compound reduces half amount of DHT-induced cell growth, calculated by linear approximation of two points adjacent to IC_{50} values.

Competitive Binding Assay using hAR-LBD: The assay was examined by the same procedure described in our previous report.[16] A hAR-LBD expression plasmid vector which encodes GST-hARLBD (627-919 aa, EF domain) fusion protein under the lac promoter was transfected into *Escherichia coli* strain HB-101. An overnight culture (10 mL) of the bacteria was added to 1 L of LB medium and incubated at 27°C until its optical density reached 0.6-0.7 at 600 nm. Following the addition of IPTG to a concentration of 1 mM, incubation was continued for an additional 4.5 h. Cells were harvested by centrifugation at 4000g at 4°C for 15 min and stored at -80°C until use. All subsequent operations were performed at 4°C. The bacterial pellet obtained from 40 mL of culture was resuspended in 1 mL of ice-cold TEGDM buffer (10 mM Tris-HCl pH 7.4, 1 mM EDTA, 10% glycerol, 10 mM DTT, 10 mM sodium molybdate). This suspension was subjected to sonication using 10 × 10s bursts on ice, and crude GST-hARLBD fraction was prepared by centrifugation of the suspension at 12,000g for 30 min at 4°C. This crude receptor fraction was diluted with buffer (20 mM Tris-HCl pH 8.0, 0.3 M KCl, 1 mM EDTA) to a protein concentration of 0.3-0.5 mg/mL and used in binding assays as hAR-LBD fraction. Aliquots of the hARLBD fraction were

incubated in the dark at 4 °C with [3H]-DHT (PerkinElmer, 4 nM final concentration), triamcinolone acetonide (1 μM final concentration), and reference or test compounds (dissolved in DMSO). Nonspecific binding was assessed by addition of a 200-fold excess of nonradioactive DHT. Thus, binding of 4 nM of [3H]-DHT to hAR LBD without non-labeled DHT reached radioactivity count of 28100 cpm. When the excess amount of non-labeled DHT was added, the cpm counts decreased in 2800. The subtract count (25300) was considered as specific binding of labeled DHT to the DHT binding site. After 15 h, a Dextran T-70/γ-globulin-coated-charcoal suspension was added to the ligand/protein mixture (1% Norit A, 0.05%γ-globulin, 0.05% Dextran T-70 final concentration each) and the whole was incubated at 4 °C for 10 min. The charcoal was removed by centrifugation for 10 min at 1,300g, and the radioactivity of the supernatant was measured in scintillation cocktail (Ultima Gold®, PerkinElmer) by using a liquid scintillation counter. All experiments were performed in duplicate or more.

LNCaP cell proliferation and PSA assay: The cell proliferation assay was examined by the same procedure described in our previous report.[16] The human prostate adenocarcinoma cell line, LNCaP was routinely cultivated in RPMI-1640 supplemented with 10% FBS at 37 °C in a 5% CO_2 humidified incubator. All experiments were performed in triplicate or more. Cells were trypsinized and diluted to 20,000 cells/mL with RPMI-1640 supplemented with 10% charcoal-stripped FBS. This cell suspension was seeded in 96-well plates at a volume of 100 μL and incubated at 24 h. 10 μL of the medium was removed and 10 μL of the drug solution, supplemented with serial dilutions of the test compounds or DMSO as dilute control in the presence or absence of 10 nM DHT, was added to each well. Cells were incubated for 6 d, and half of the media was removed and medium with the test compounds or DMSO as dilute control in the presence or absence of 10 nM DHT was replaced once after 3 d. At the end of the incubation time, proliferation was evaluated by using the WST-8. 10 μM of WST-8 was added to wells, and cells were incubated for 2 h. The absorbance at 450 nm was measured. This parameter relates to the number of living cells in the culture. The PSA assay was examined according to procedure described in our previous report.[18] After the incubation, the supernatant was collected and the amount of PSA contained in the supernatant was quantified by the use of f-PSA Enzyme immunoassay test kit (IMMUNOSPEC Corp.) according to the manufacturer's instructions.

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Appendix A. Supplementary data

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List of Schemes and Figures

Figure 1

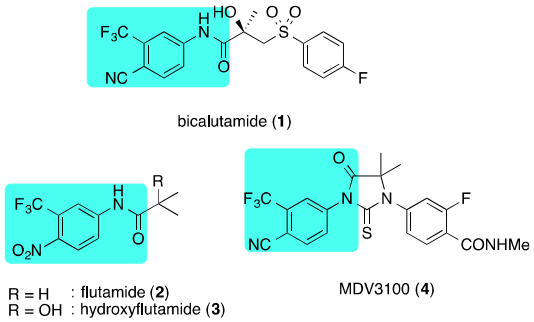


Figure 2

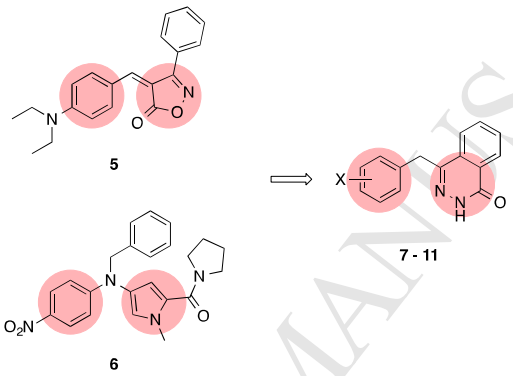
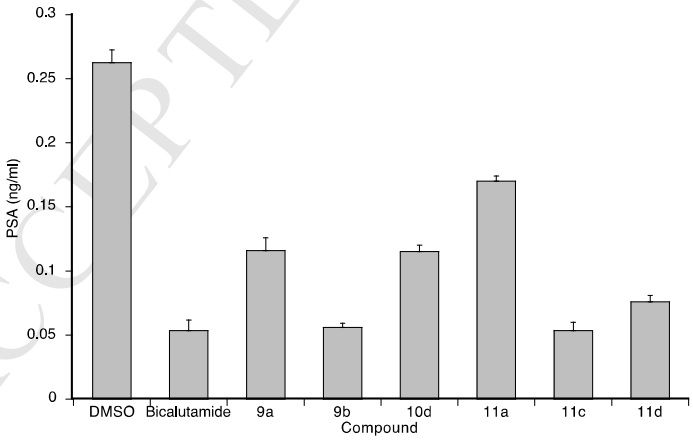


Figure 3



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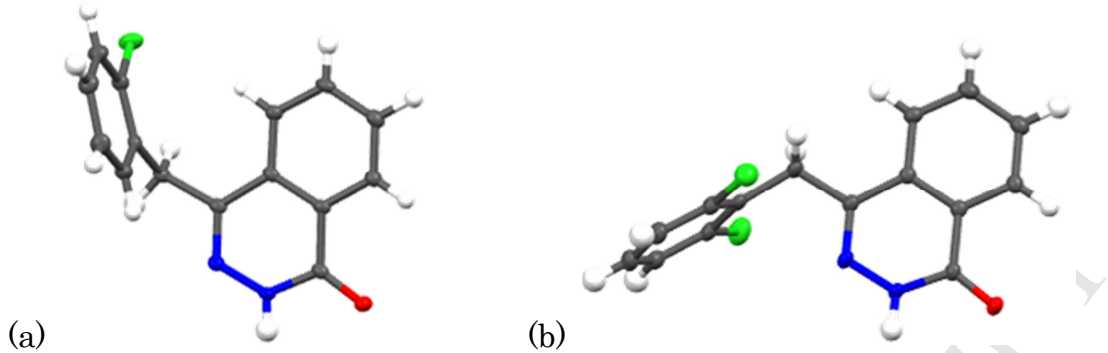
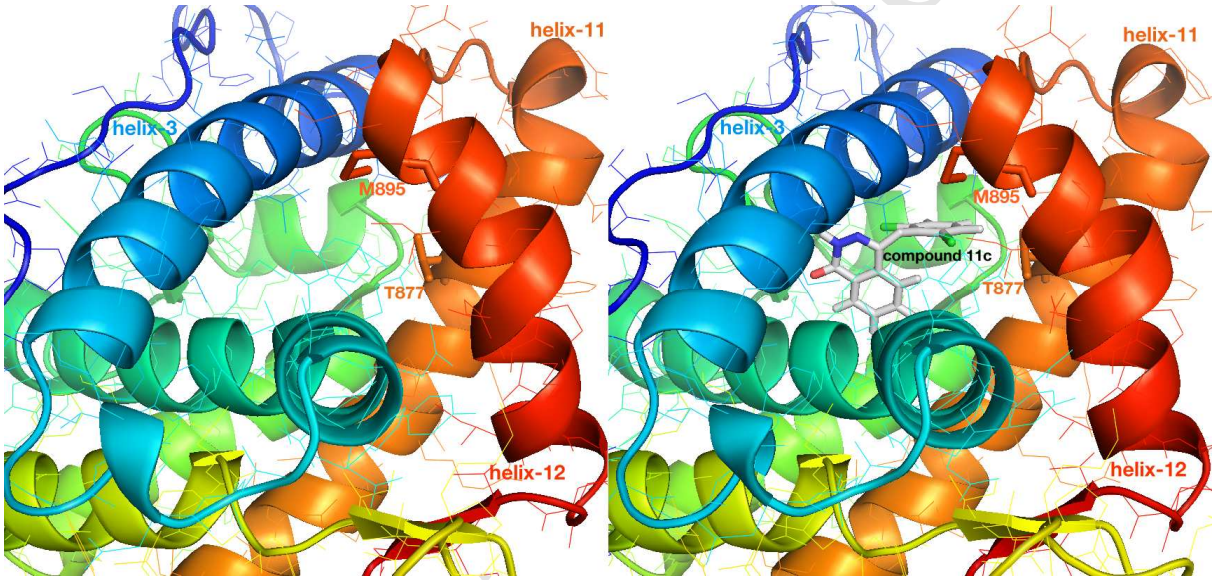
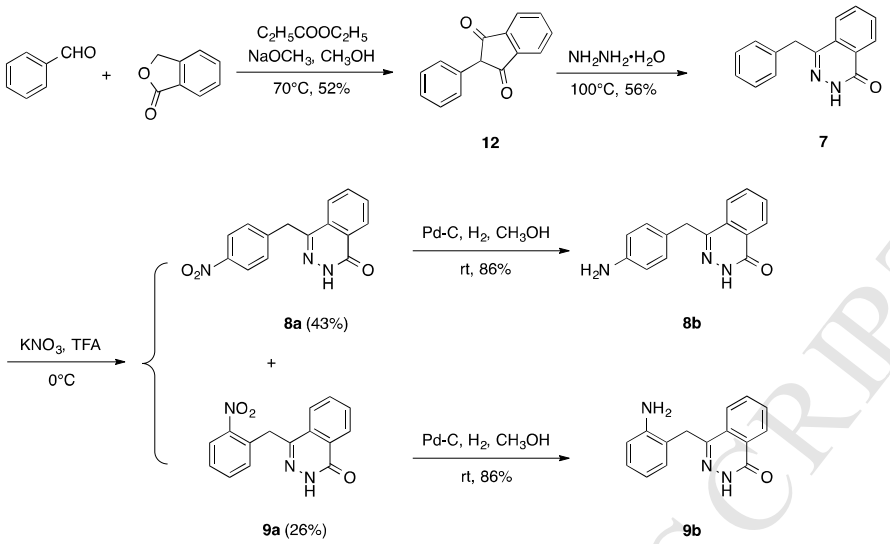


(a) (b)

Figure 5



Scheme 1



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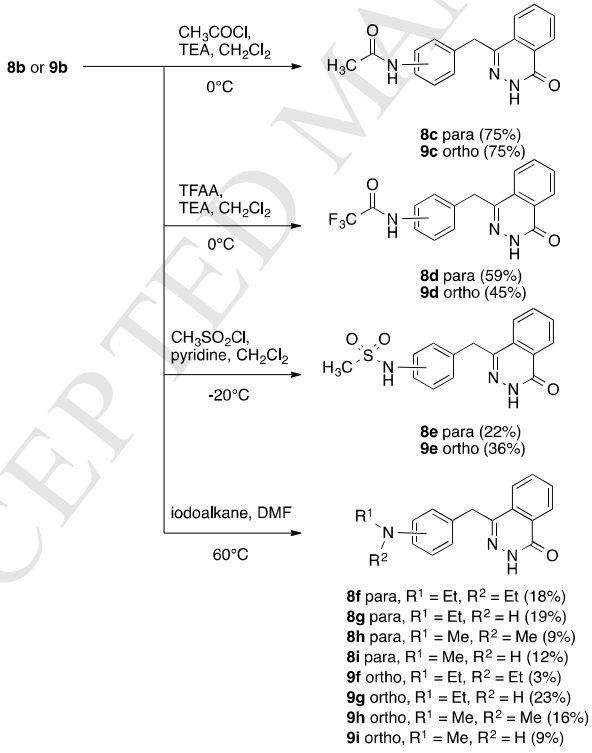

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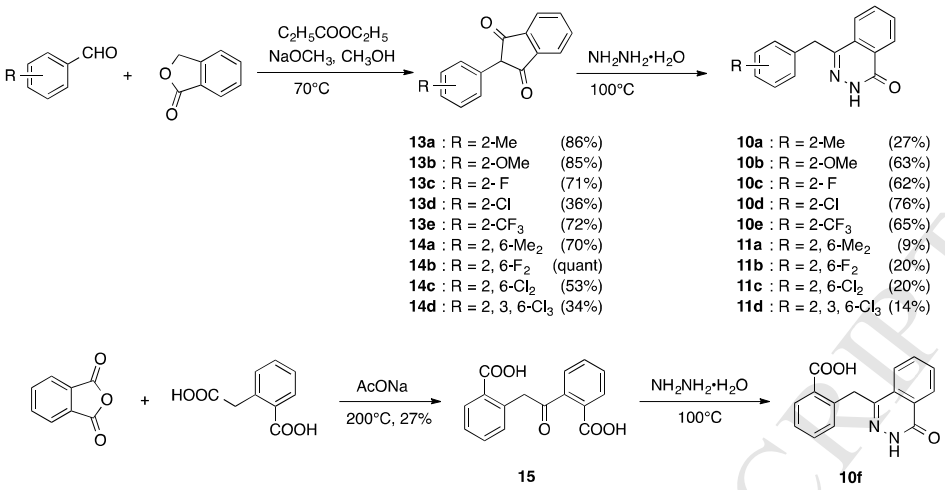


13a : R = 2-Me (86%)
13b : R = 2-OMe (85%)
13c : R = 2- F (71%)
13d : R = 2-Cl (36%)
13e : R = 2-CF_3 (72%)
14a : R = 2, 6-Me_2 (70%)
14b : R = 2, 6-F_2 (quant)
14c : R = 2, 6-Cl_2 (53%)
14d : R = 2, 3, 6-Cl_3 (34%)

10a : R = 2-Me (27%)
10b : R = 2-OMe (63%)
10c : R = 2- F (62%)
10d : R = 2-Cl (76%)
10e : R = 2-CF_3 (65%)
11a : R = 2, 6-Me_2 (9%)
11b : R = 2, 6-F_2 (20%)
11c : R = 2, 6-Cl_2 (20%)
11d : R = 2, 3, 6-Cl_3 (14%)

Table. 1. Inhibition of SC-3 cell proliferation by phthalazinone derivatives

Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
Hydroxyflutamide (**3**)	0.18 ± 0.03		
7	5.37 ± 2.06		
8a	inactive	**9a**	0.53 ± 0.18
8b	inactive	**9b**	0.22 ± 0.03
8c	inactive	**9c**	inactive
8d	inactive	**9d**	1.01 ± 0.16
8e	inactive	**9e**	inactive
8f	inactive	**9f**	1.66 ± 0.82
8g	inactive	**9g**	3.71 ± 2.01
8h	inactive	**9h**	1.95 ± 0.27
8i	inactive	**9i**	9.02 ± 2.96
10a	9.54 ± 3.24	**11a**	1.38 ± 0.25
10b	9.59 ± 1.94	**11b**	3.76 ± 0.71
10c	10.5 ± 1.0	**11c**	0.18 ± 0.03
10d	1.26 ± 0.65	**11d**	0.92 ± 0.31
10e	3.67 ± 0.77		
10f	inactive		

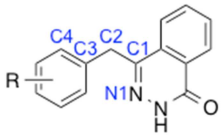
Table 2. Binding affinity of phthalazinone derivatives to wt AR

Compound	IC_{50} (μM)
Hydroxyflutamide (**3**)	7.69
9a	> 100
9b	45.2
10d	85.4
11a	84.3
11c	10.9
11d	56.7

Table 3. Inhibitory activities of phthalazinone derivatives on proliferation of LNCaP cells

Compound	IC_{50} (μM)
Bicalutamide (**1**)	0.85 ± 0.24
9a	1.46 ± 2.83
9b	0.99 ± 0.36
10d	0.86 ± 0.15
11a	3.42 ± 5.12
11c	0.55 ± 0.14
11d	0.92 ± 0.62

Table 4. Crystal data for **10d** and **11c**



	10d (R = 2-Cl)	**11c** (R = 2,6-Cl_2)
Empirical formula	$C_{15}H_{11}ClN_2O$	$C_{15}H_{10}Cl_2N_2O$
Crystal system	Triclinic	Triclinic
Space group	$P\ \bar{1}$	$P\ \bar{1}$
Unit cell dimensions	a = 7.300(4) Å	a = 7.9804(5) Å
	b = 8.038(4) Å	b = 9.0057(6) Å
	c = 11.462(6) Å	c = 11.2287(7) Å
	α = 82.165(7)°	α = 91.1510(10)°
	β = 84.373(6)°	β = 93.6230(10)°
	γ = 66.395(6)°	γ = 100.1800(10)°
Final R indices [I>2σ(I)]	R_1 = 0.0465, wR_2 = 0.1144	R_1 = 0.0345, wR_2 = 0.1042
Dihedral angle		
Ph-phthalazinone	88.19°	87.93°
Tosion angle		
N1-C1-C2-C3	114.12	3.02
C1-C2-C3-C4	-53.40	90.64

Highlights

Ms Ref. No. EJMECH-D-14-02570
Title: Design and synthesis of 4-benzyl-1-(2*H*)-phthalazinone derivatives as novel androgen receptor antagonists
Corresponding author: Aya Tanatani

- A series of 4-benzyl-1-(2*H*)-phthalazinone derivatives were synthesized.
- 4-Benzyl-1-(2*H*)-phthalazinone with *ortho*-substituent was a novel scaffold of AR antagonist.
- Compound **11c** exhibited potent AR antagonistic activity towards both wt and T877A-mutated ARs.
- Docking simulation suggested the AR binding feature and mechanism of AR antagonism of **11c**.

Supplementary data

Design and synthesis of 4-benzyl-1-(2*H*)-phthalazinone derivatives as novel androgen receptor antagonists

Kazumi Inoue, Ko Urushibara, Misae Kanai, Kei Yura, Shinya Fujii, Yuichi Hashimoto, Mari Ishigami-Yuasa, Shuichi Mori, Emiko Kawachi, Mio Matsumura, Tomoya Hirano, Hiroyuki Kagechika, Aya Tanatani*

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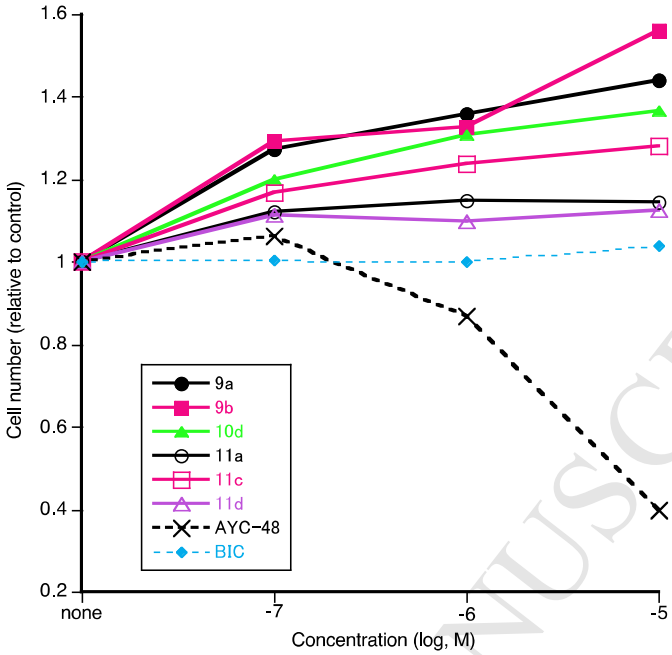
Figure S1. Effect of phthalazinone derivatives on AR-independent PC cell growth



The assay was examined by the same procedure described in our previous report (reference 16). The human prostate cancer cell line PC-3 was routinely cultivated in DMEM medium supplemented with 10% FBS at 37°C in a 5% CO_2 humidified incubator. Cells were trypsinized and diluted to 20,000 cells/mL with DMEM supplemented with 10% charcoal-stripped FBS. This cell suspension was seeded in 96-well plates at a volume of 100 μL and incubated at 24 h. 10 μL of the medium was removed and 10 μL of the drug solution, supplemented with serial dilutions of the test compounds or DMSO as dilute control was added to each well. Cells were incubated for 3 days, and at the end of the incubation time, proliferation was evaluated by using the WST-8. 10 μM of WST-8 was added to triplicate wells, and cells were incubated for 2 h. The absorbance at 450 nm was measured. This parameter relates to the number of living cells in the culture. BIC is bicartamide, and AYC-48 is positive control with the inhibitory activity of PC-3 cell proliferation (reference 16).

Figure S2. Dose-responsive data of inhibitory activity of phthalazinone derivatives on SC-3 cell proliferation

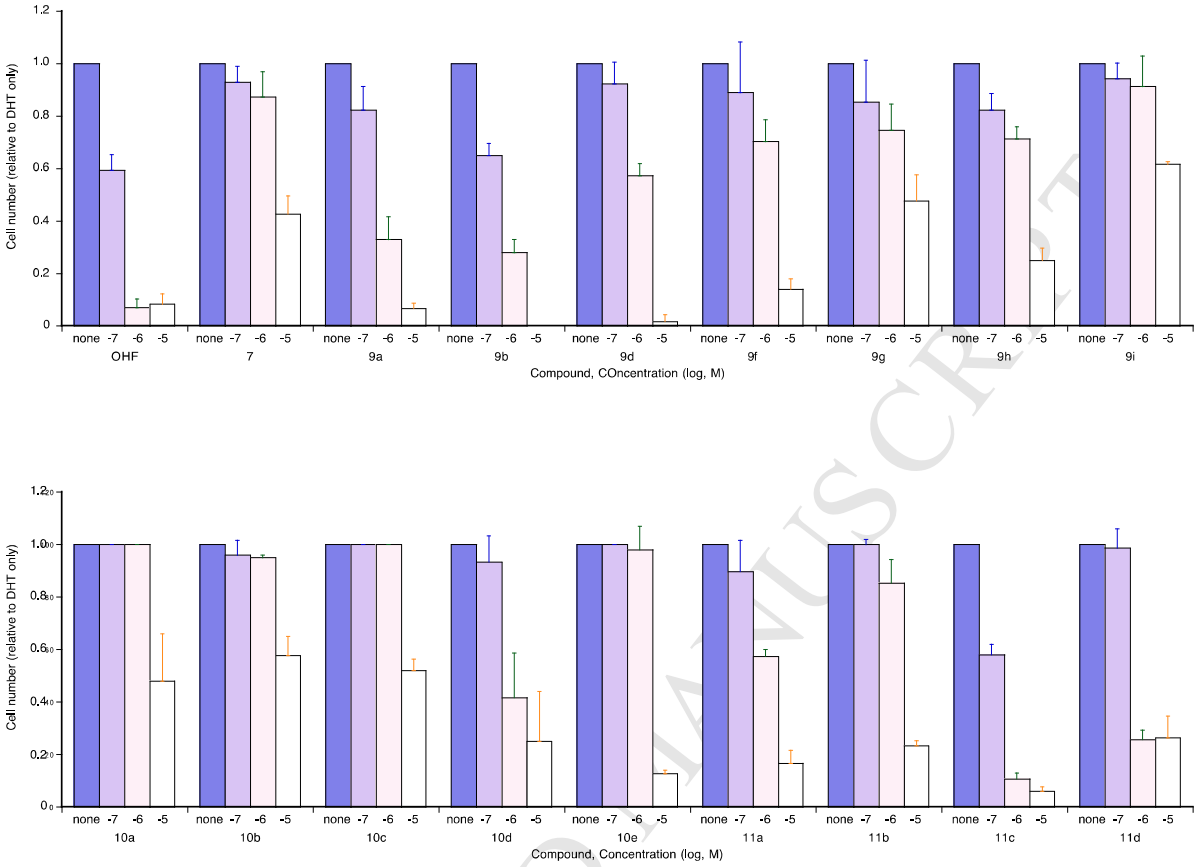
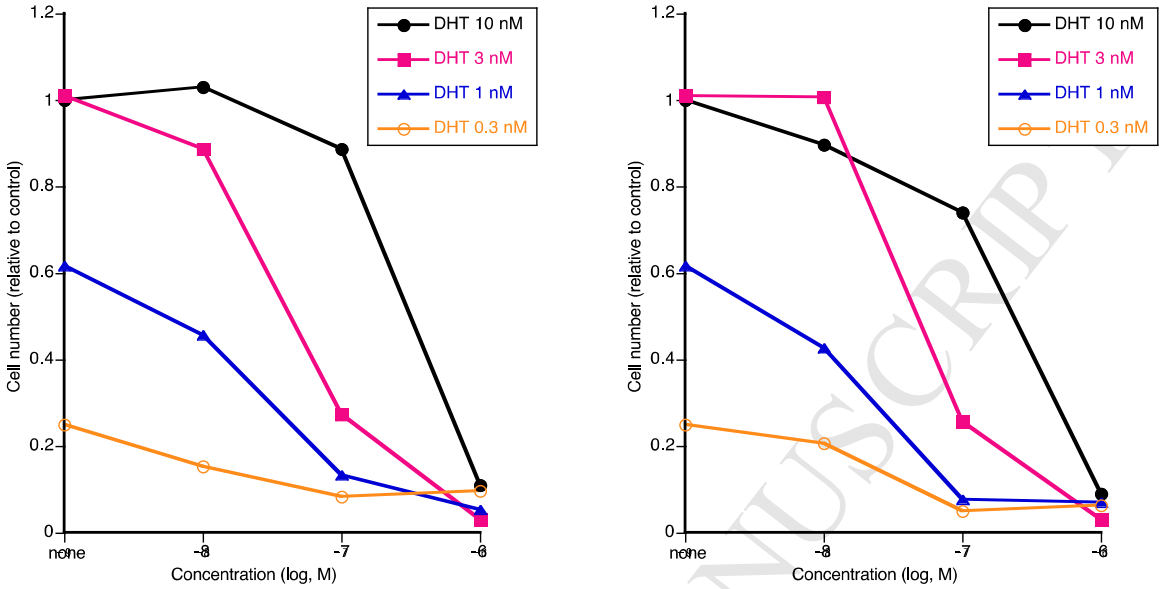


Figure S3. Dose-responsive curves of (a) Hydroxyflutamide (**3**) and (b) **11c** in the presence of various concentrations of DHT in SC-3 Assay

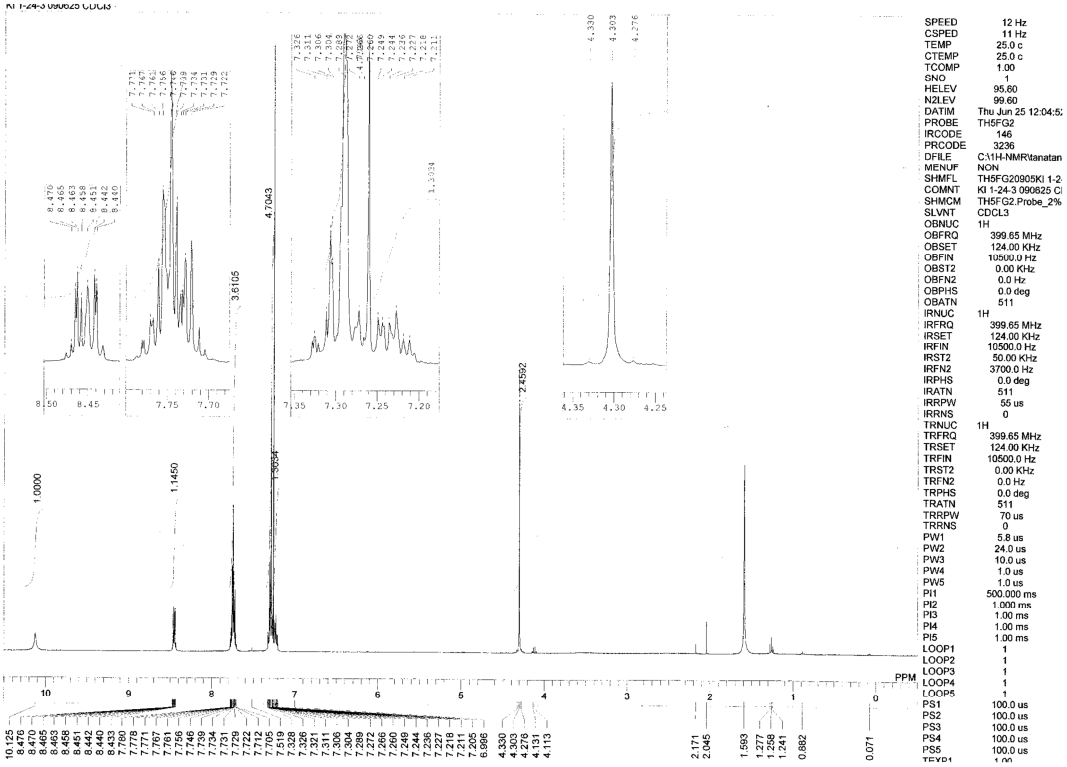
(a) (b)



Vertical scale is cell number relative to control (DHT: 10 nM, test compound: none). Horizontal scale is concentration of (a) hydroxyflutamide (**3**) and (b) **11c**.

Figure S4. ^{1}H & ^{13}C NMR data of **7**

^{1}H NMR



^{13}C NMR

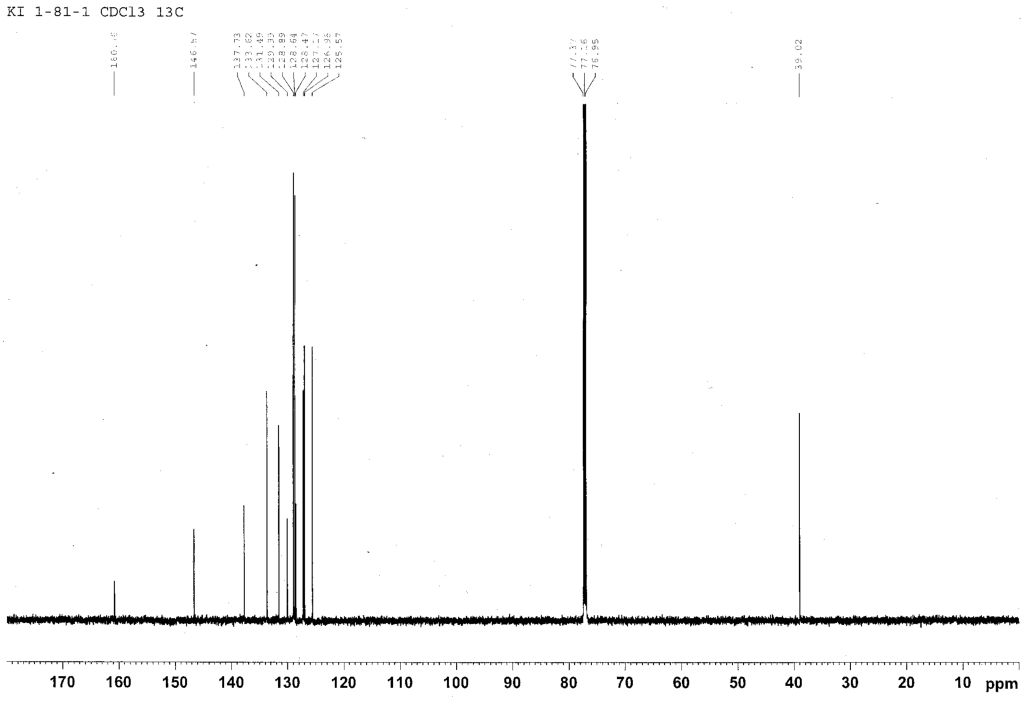
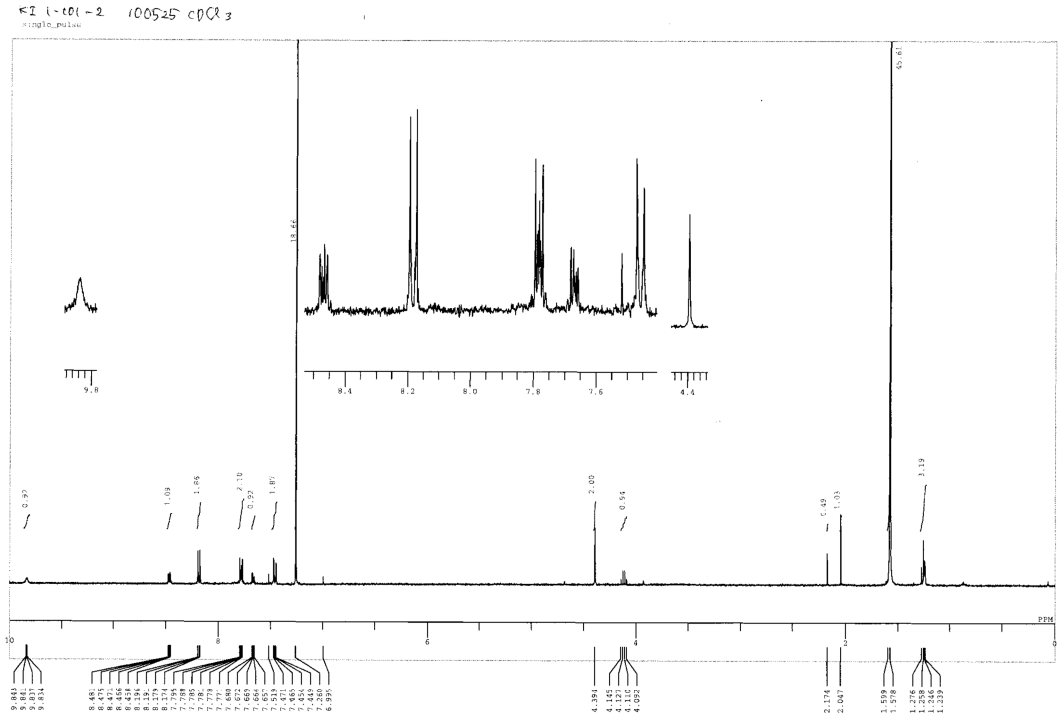


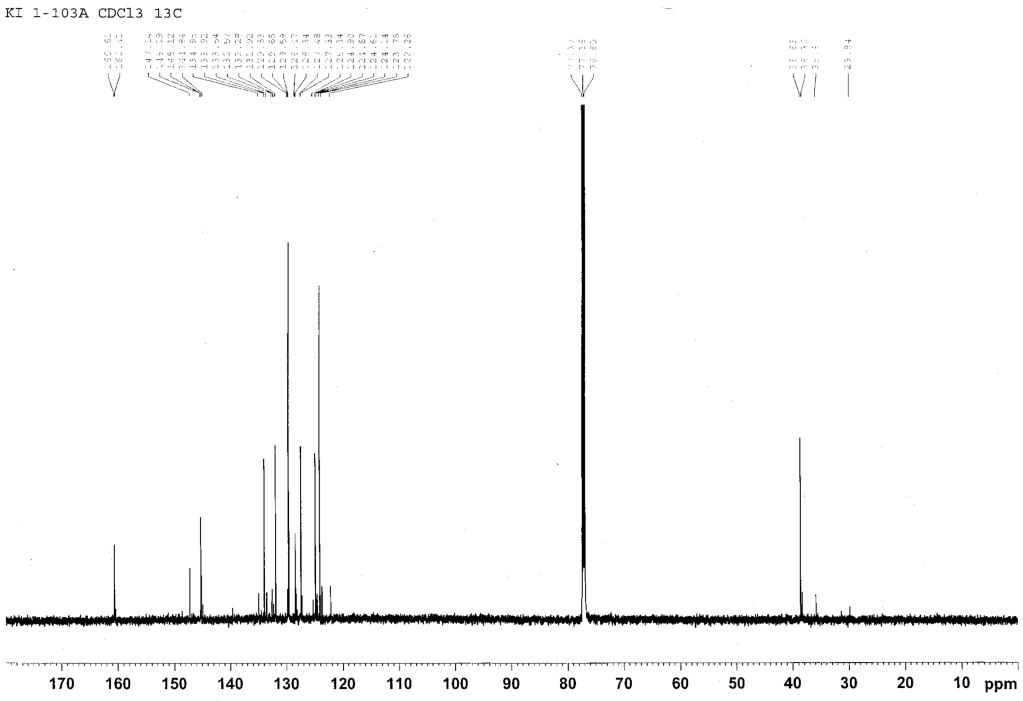
Figure S5. ^{1}H & ^{13}C NMR data of **8a-i**

(a) Compound **8a**

^{1}H NMR

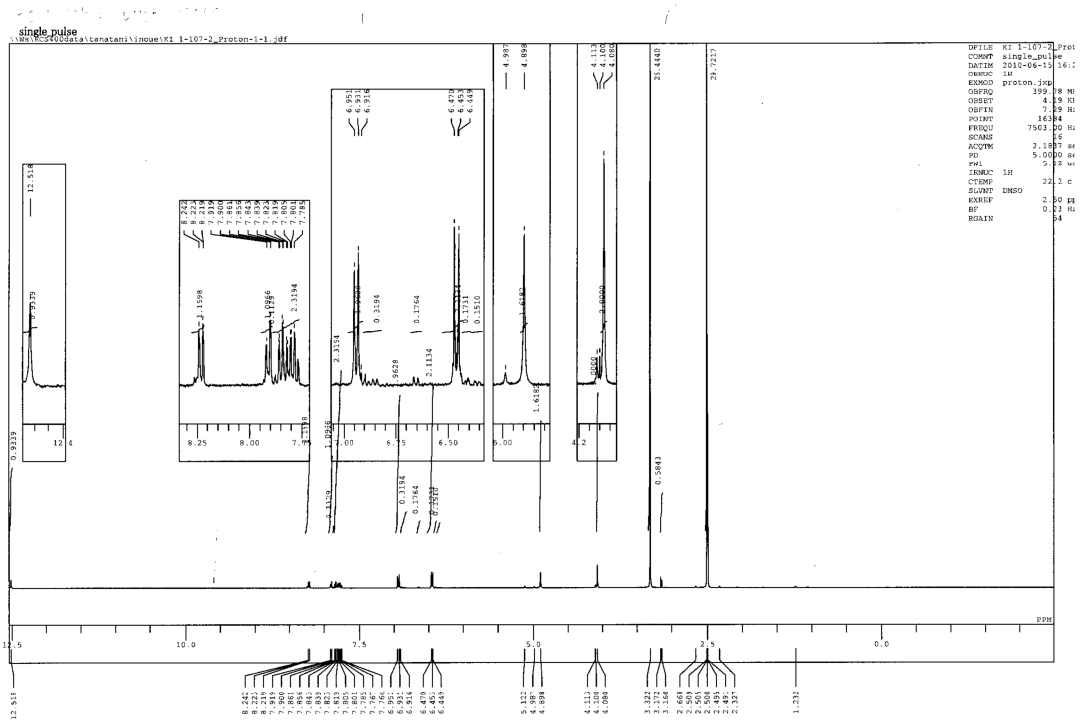


^{13}C NMR

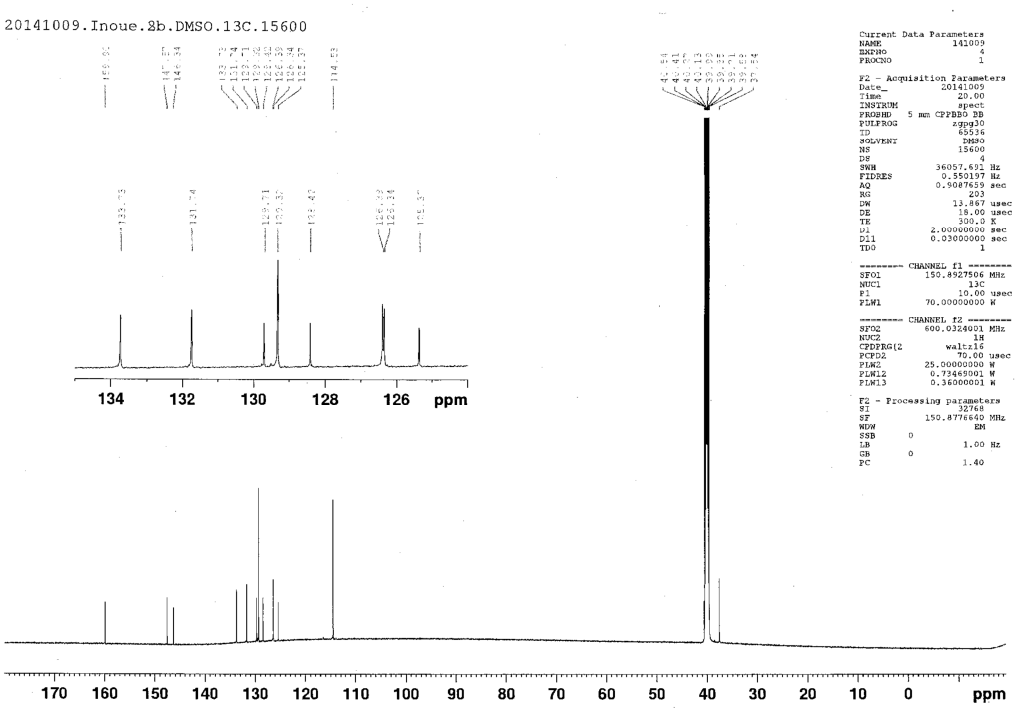


(b) Compound **8b**

^{1}H NMR

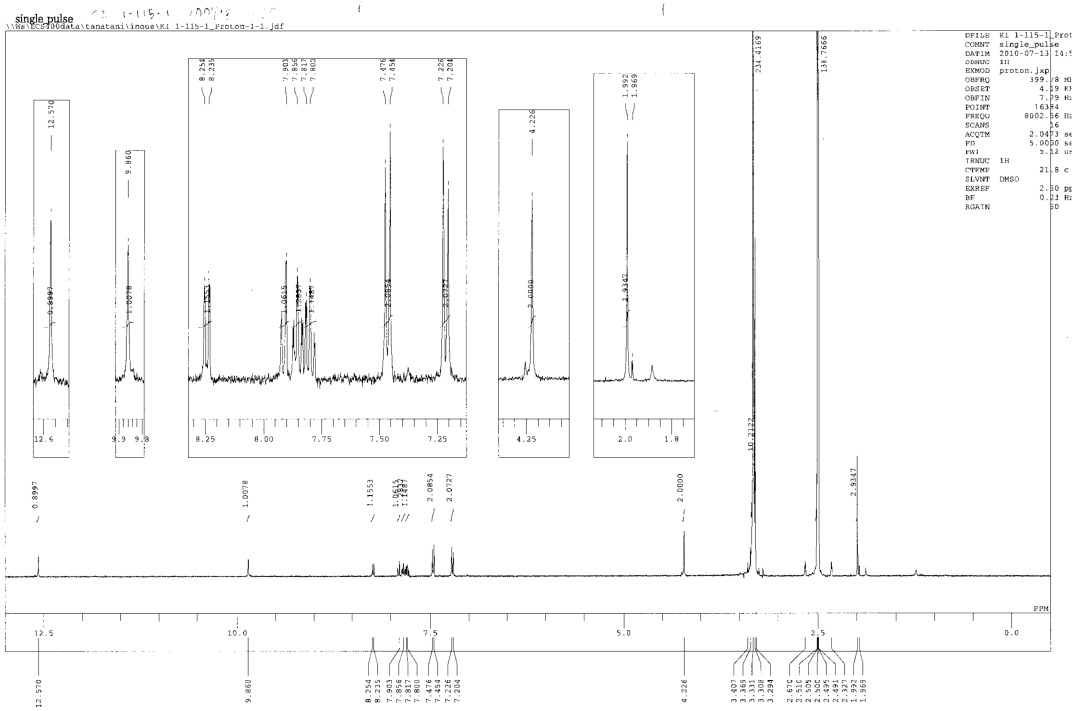


^{13}C NMR

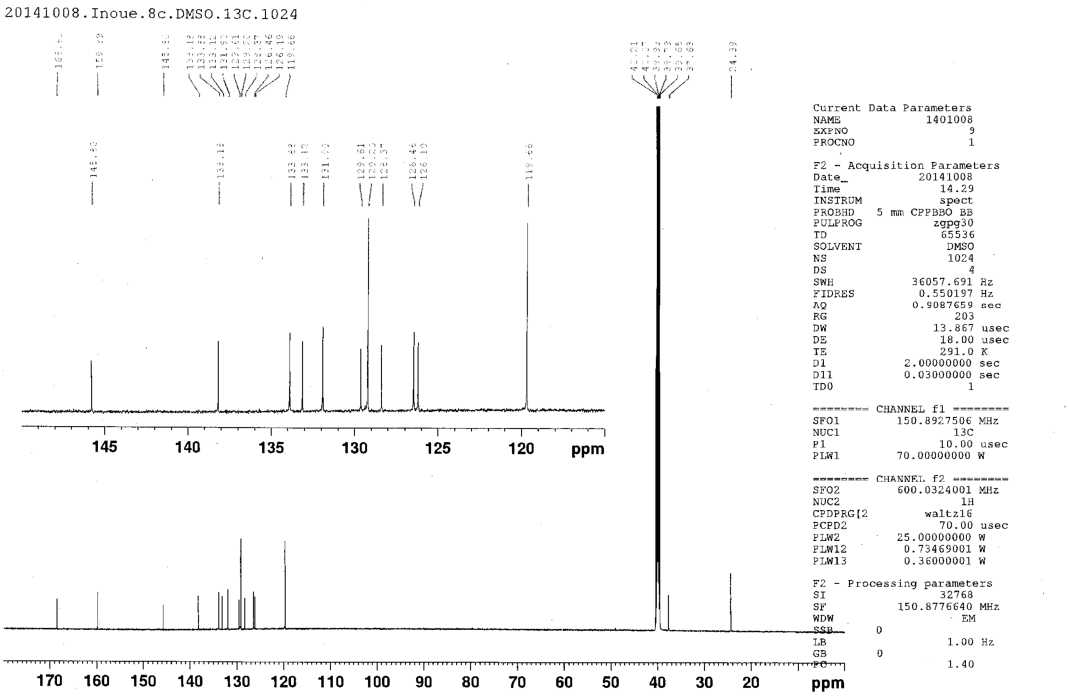


(c) Compound **8c**

^{1}H NMR

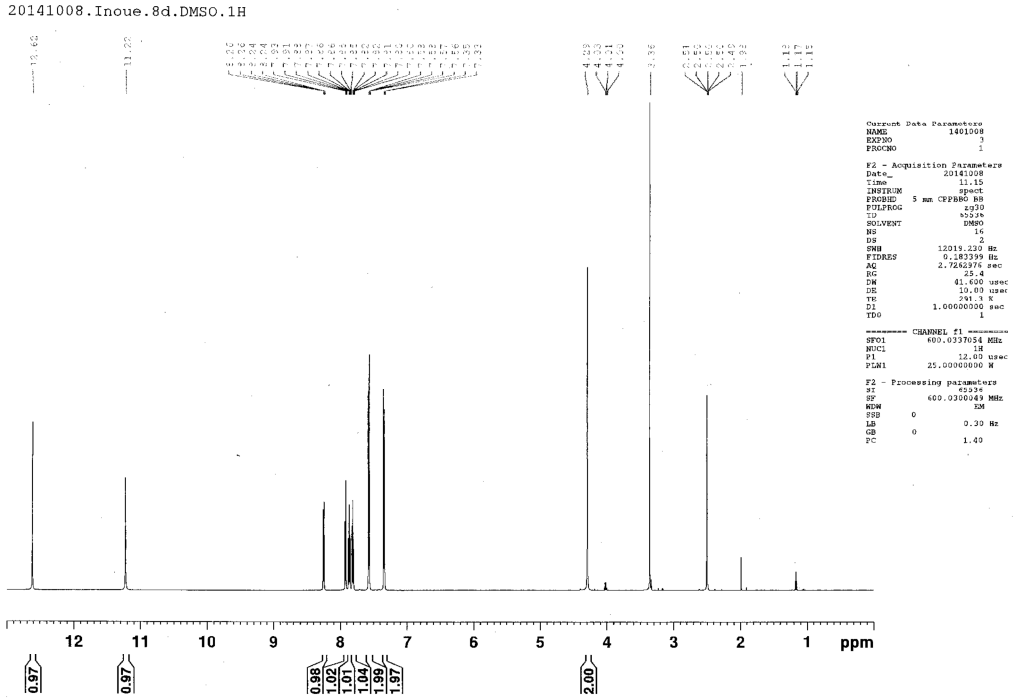


^{13}C NMR

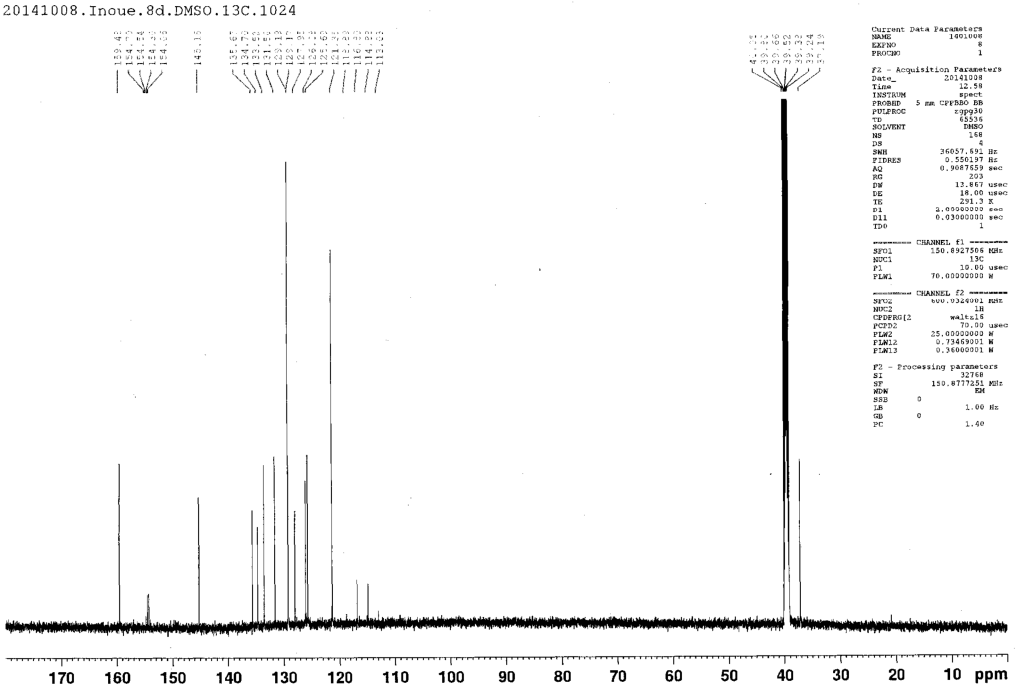


(d) Compound **8d**

^{1}H NMR

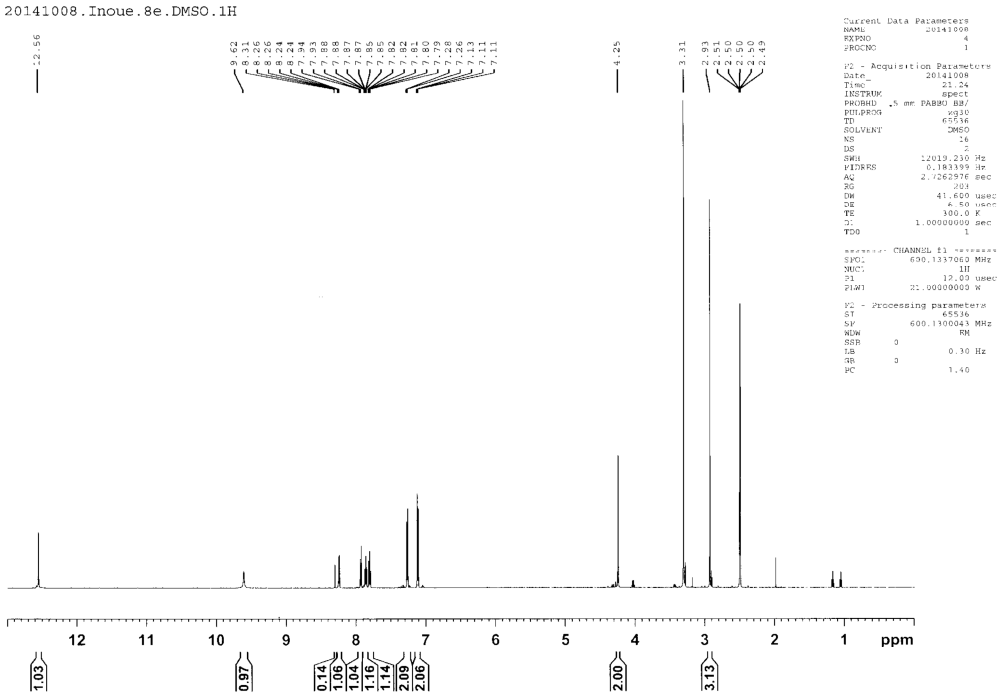


^{13}C NMR

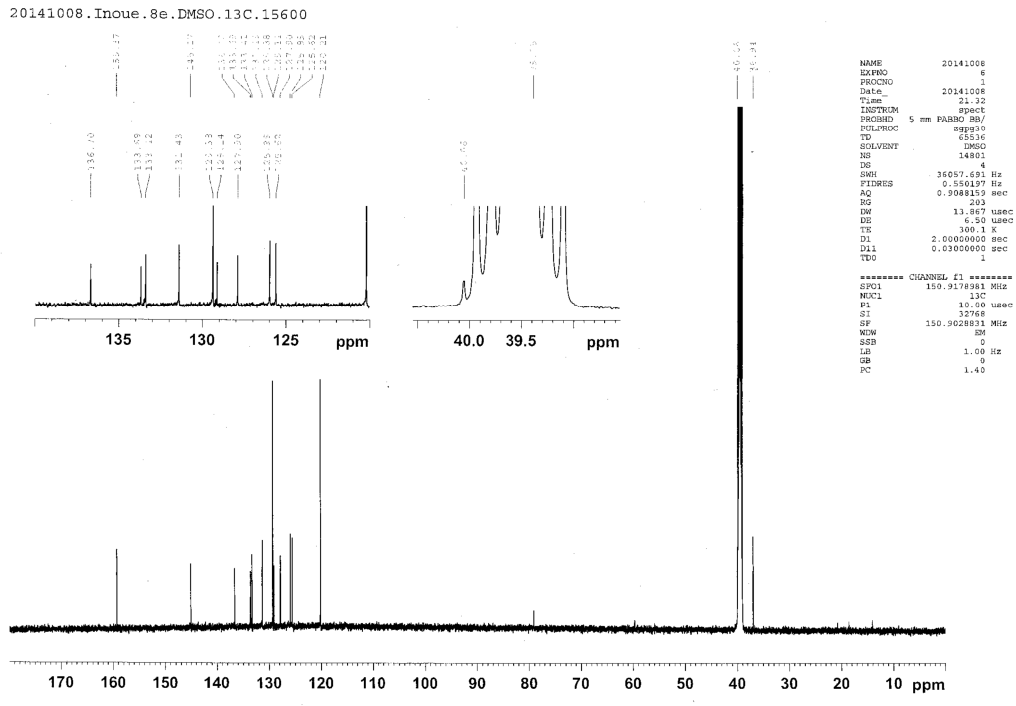


(e) Compound **8e**

^{1}H NMR

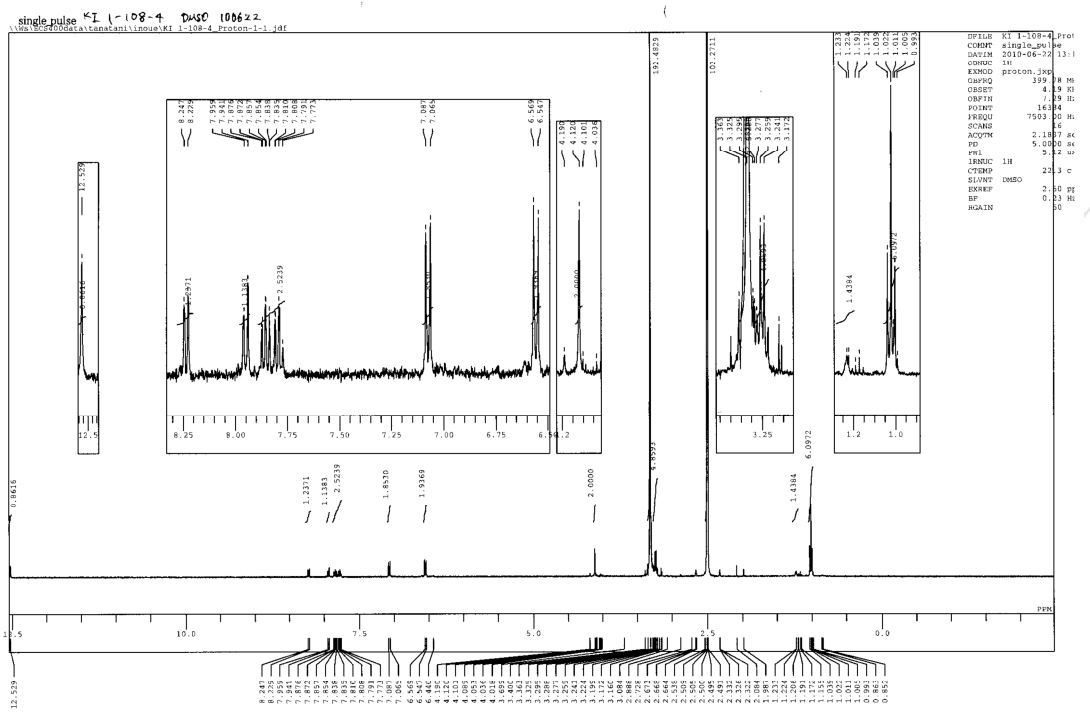


^{13}C NMR

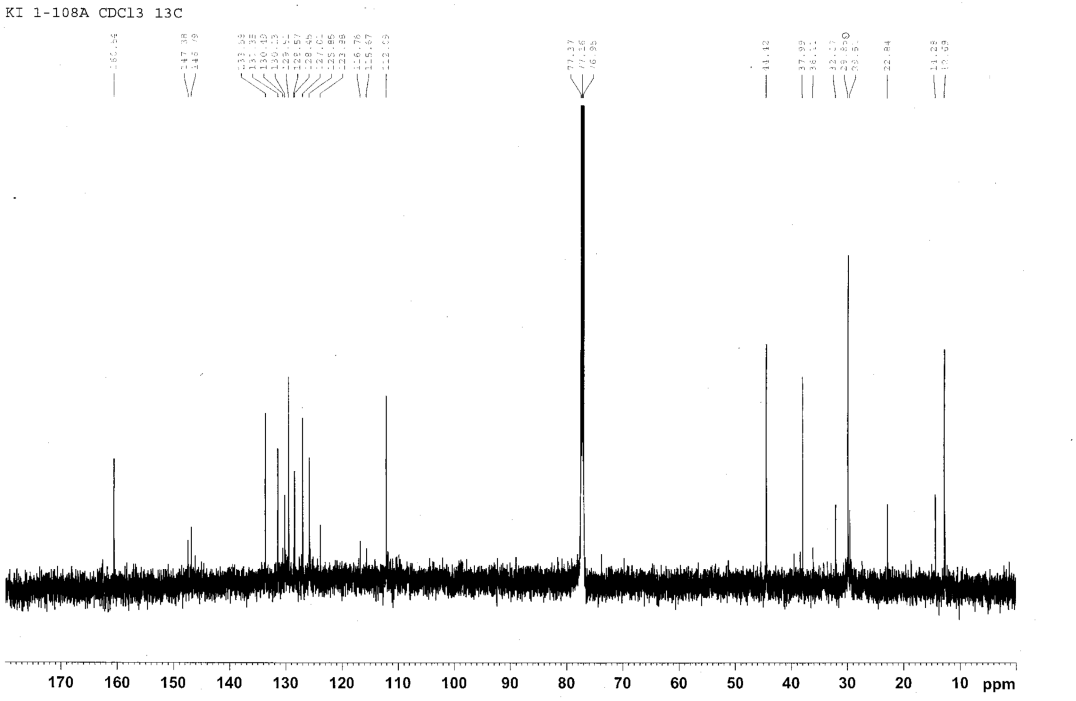


(f) Compound **8f**

^{1}H NMR

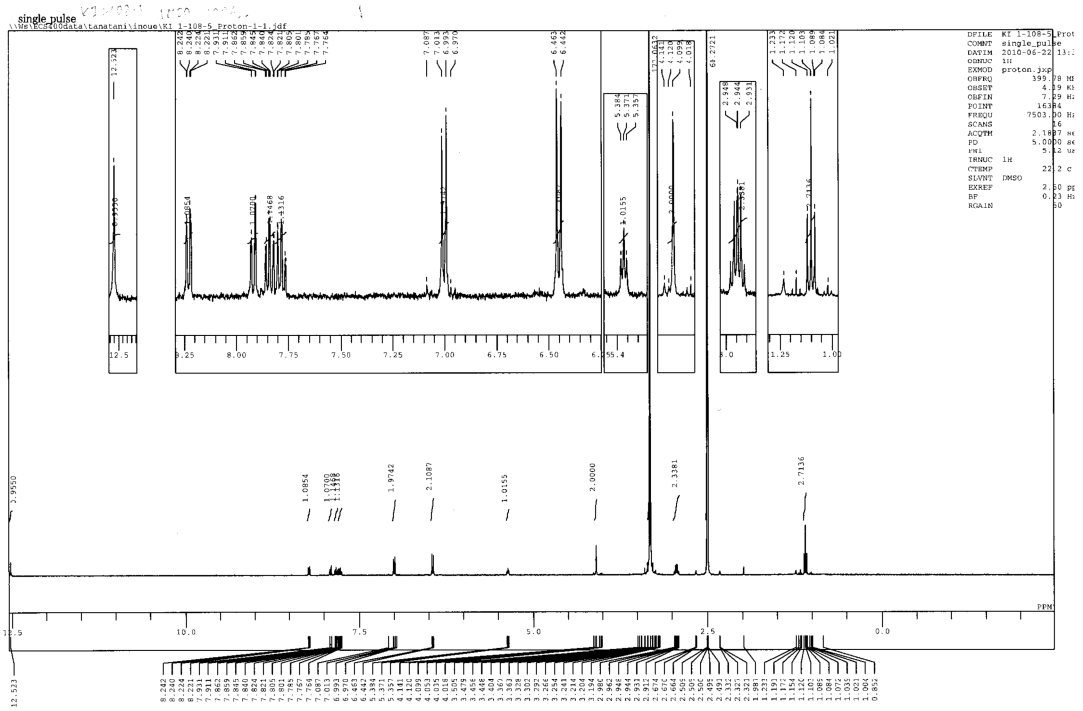


^{13}C NMR

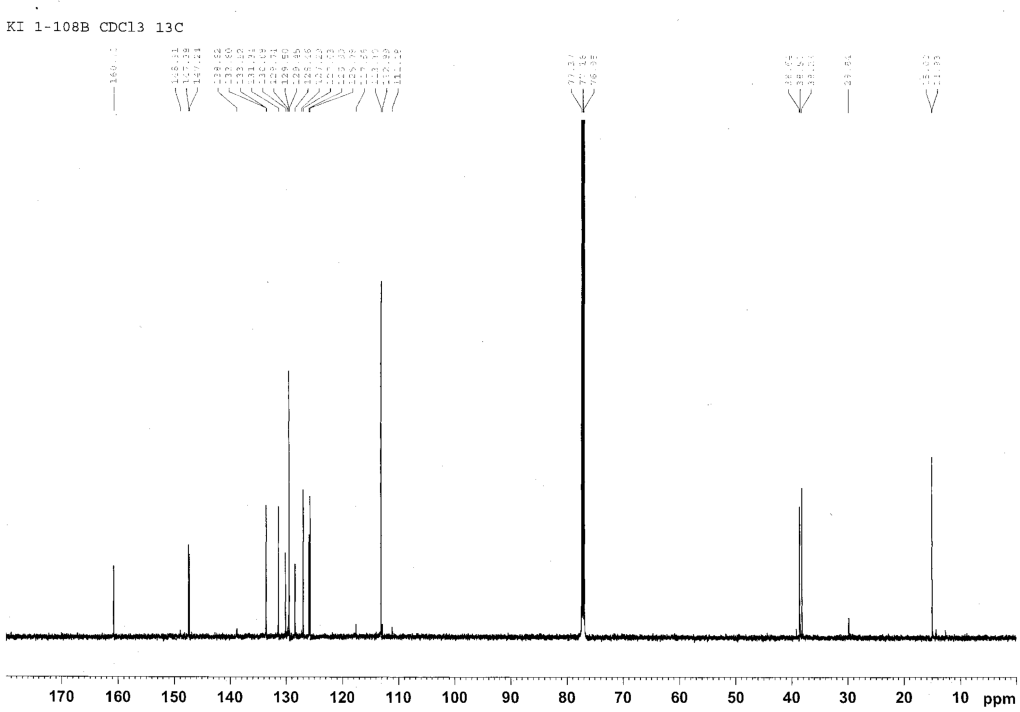


(g) Compound **8g**

^{1}H NMR

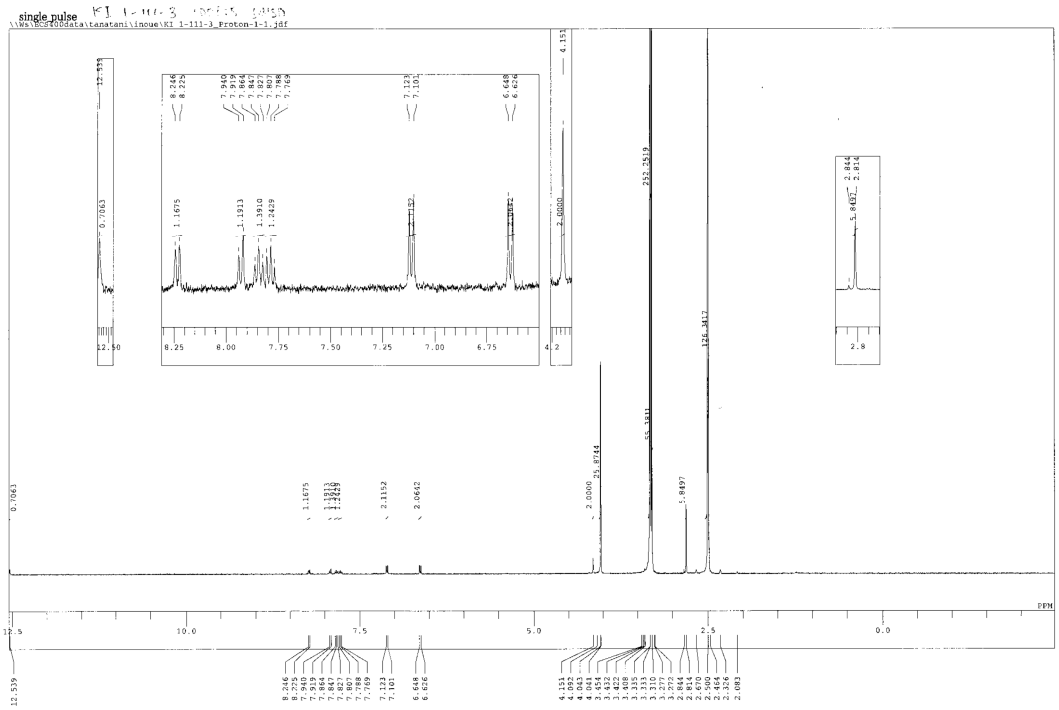


^{13}C NMR

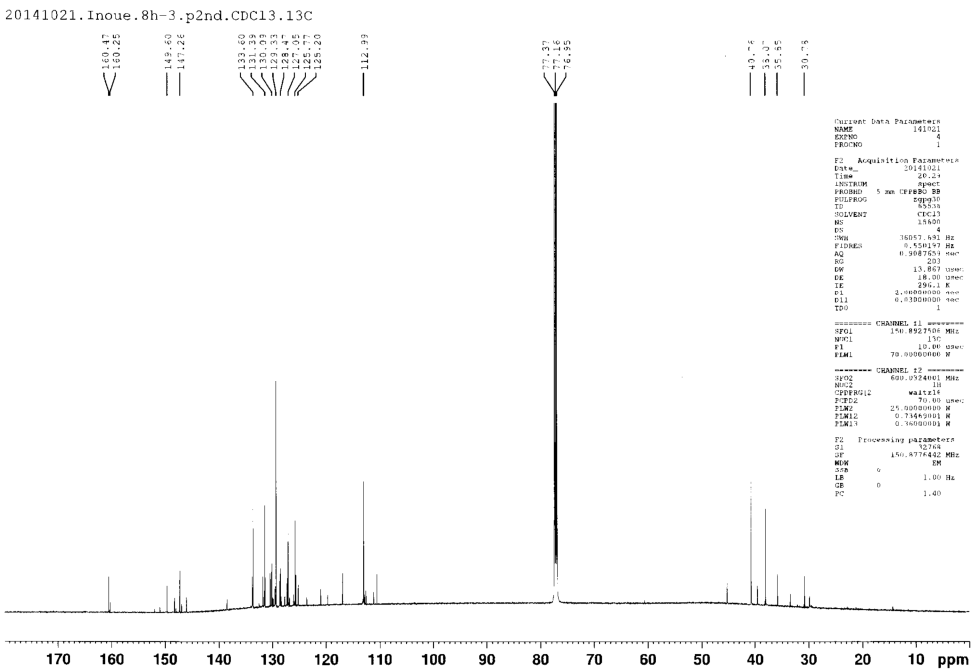


(h) Compound **8h**

^{1}H NMR

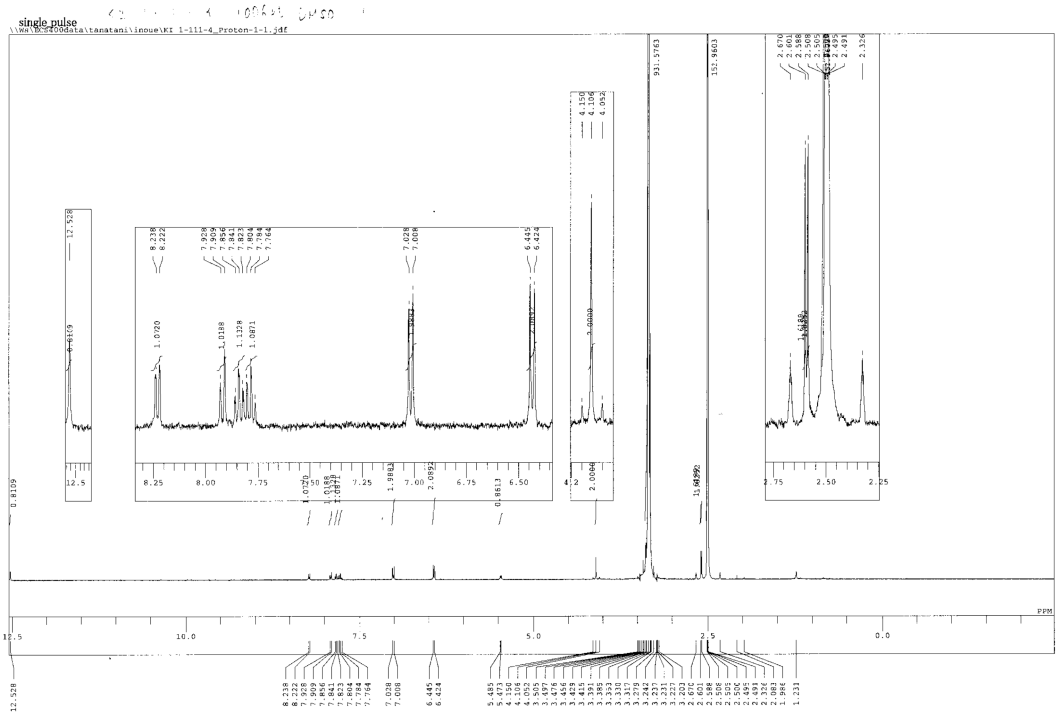


^{13}C NMR



(i) Compound **8i**

^{1}H NMR



^{13}C NMR

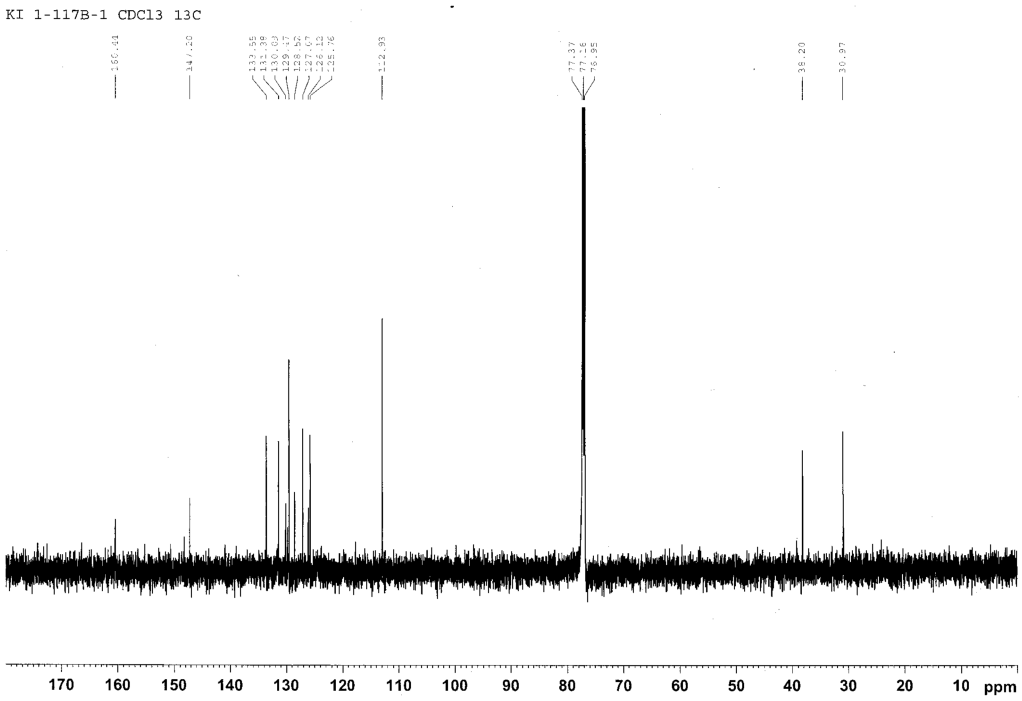
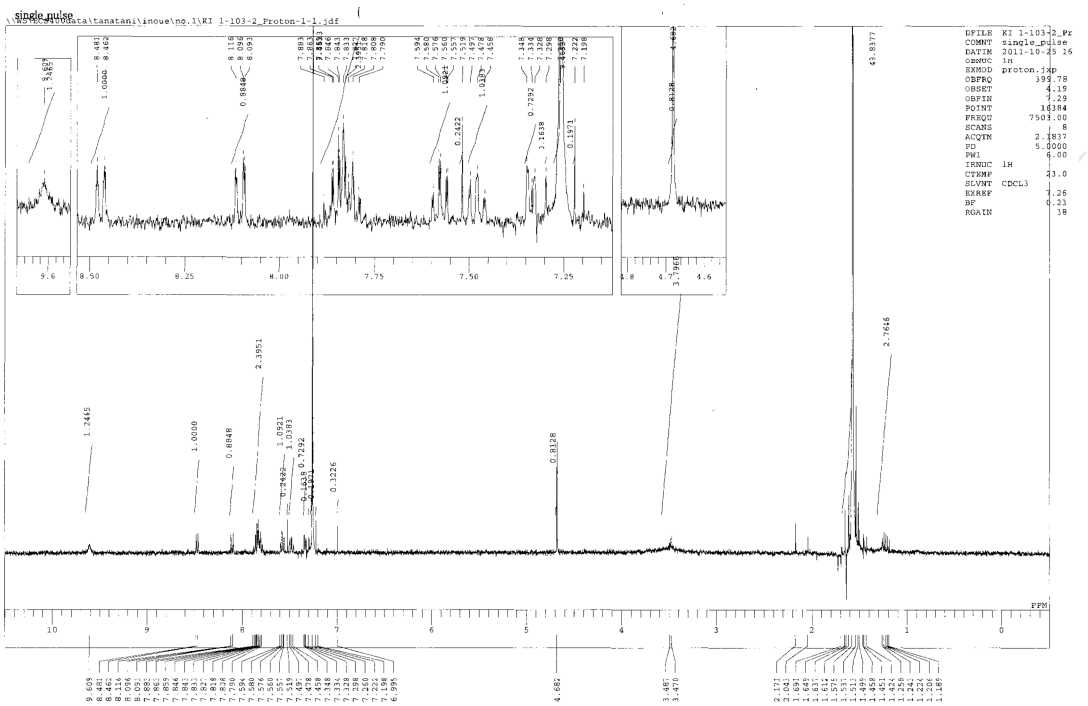


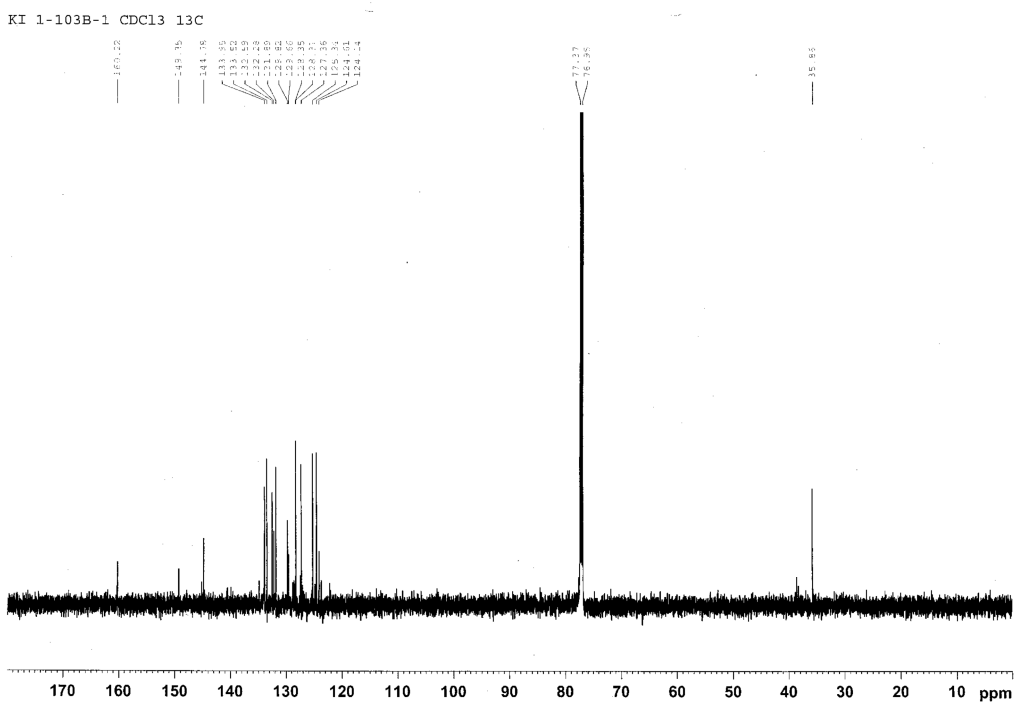
Figure S6. ^{1}H & ^{13}C NMR data of **9a-i**

(a) Compound **9a**

^{1}H NMR

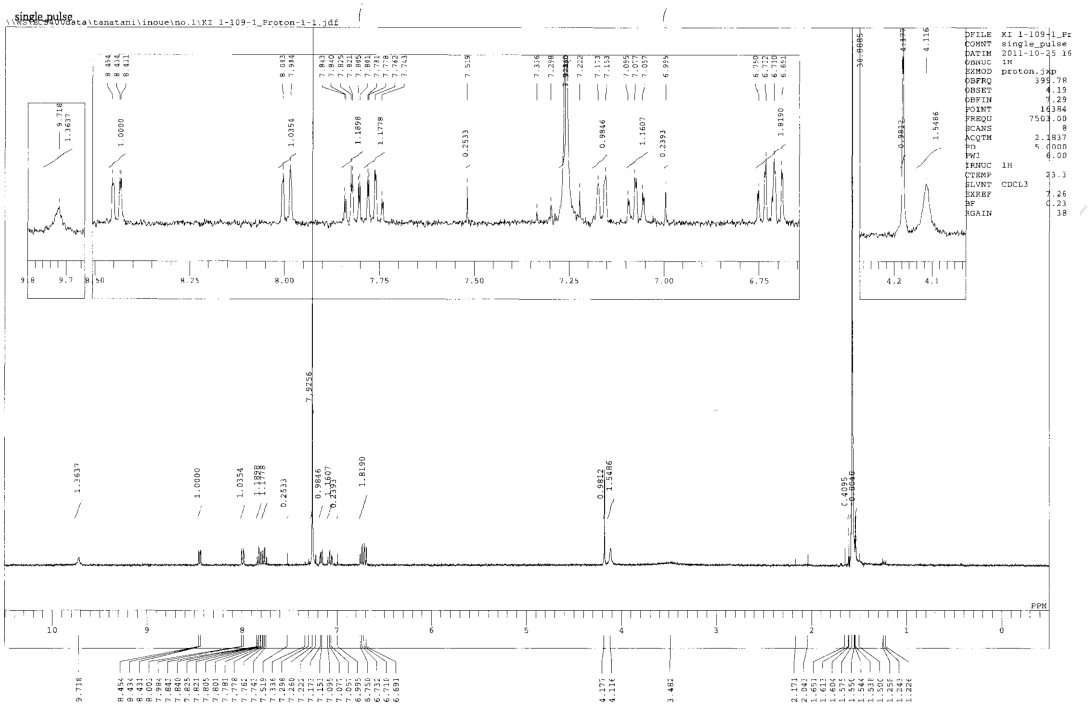


^{13}C NMR

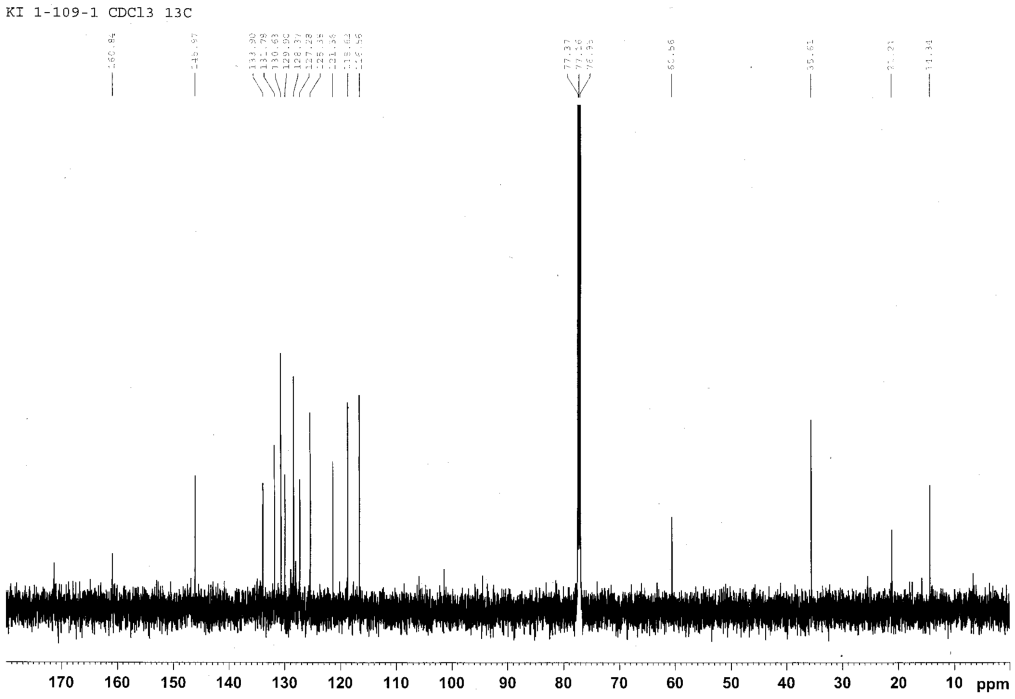


(b) Compound **9b**

^{1}H NMR

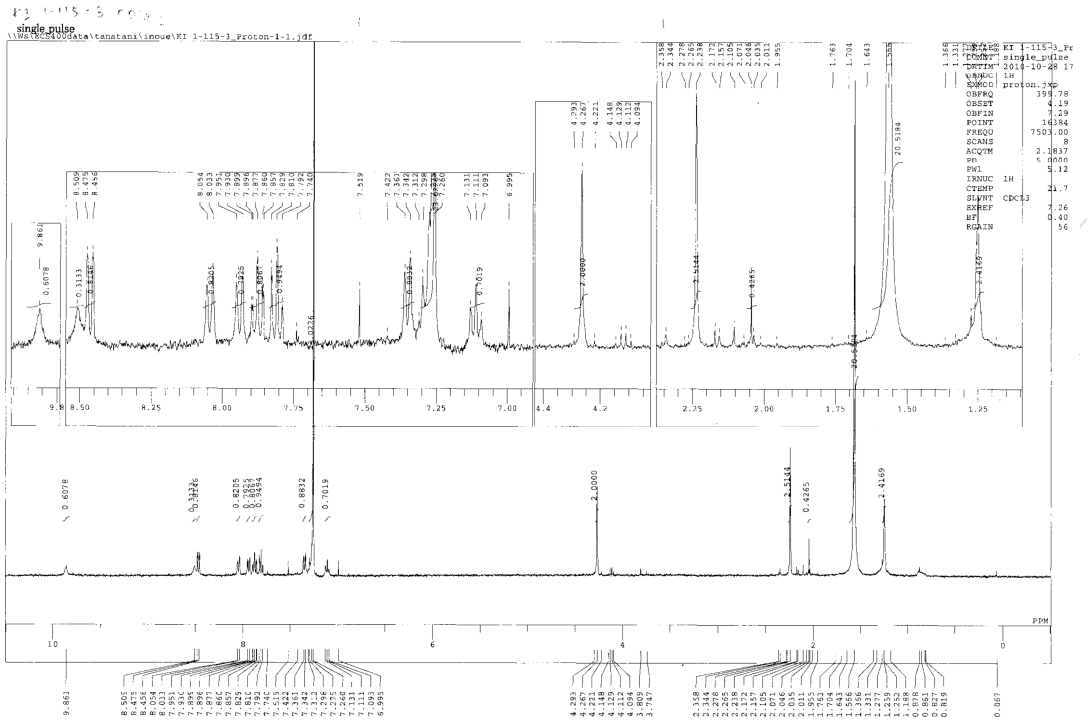


^{13}C NMR

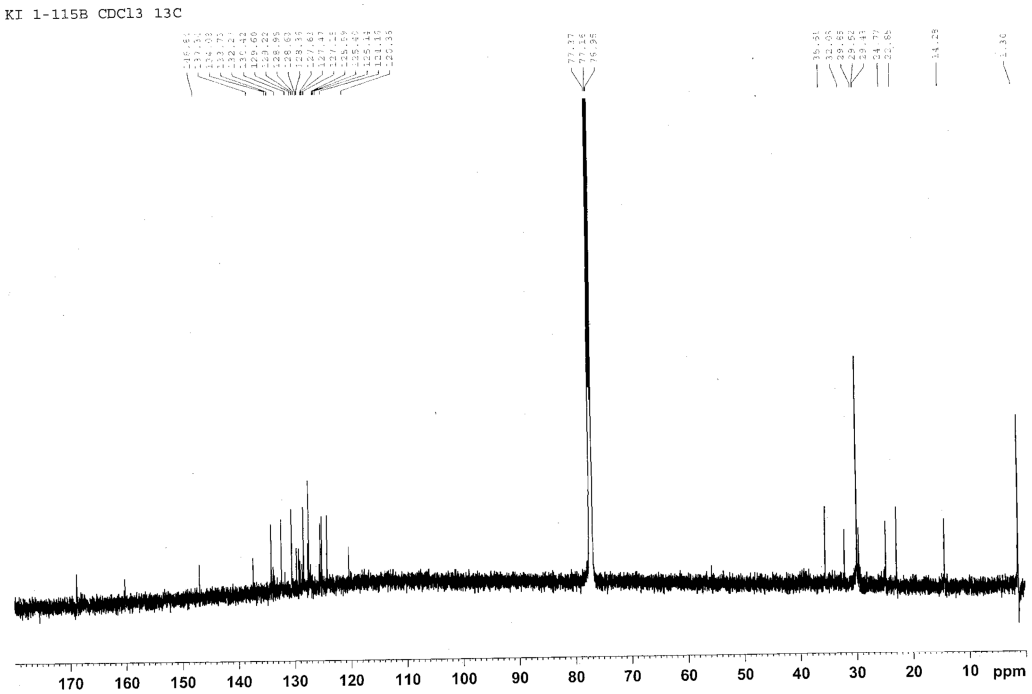


(c) Compound **9c**

^{1}H NMR

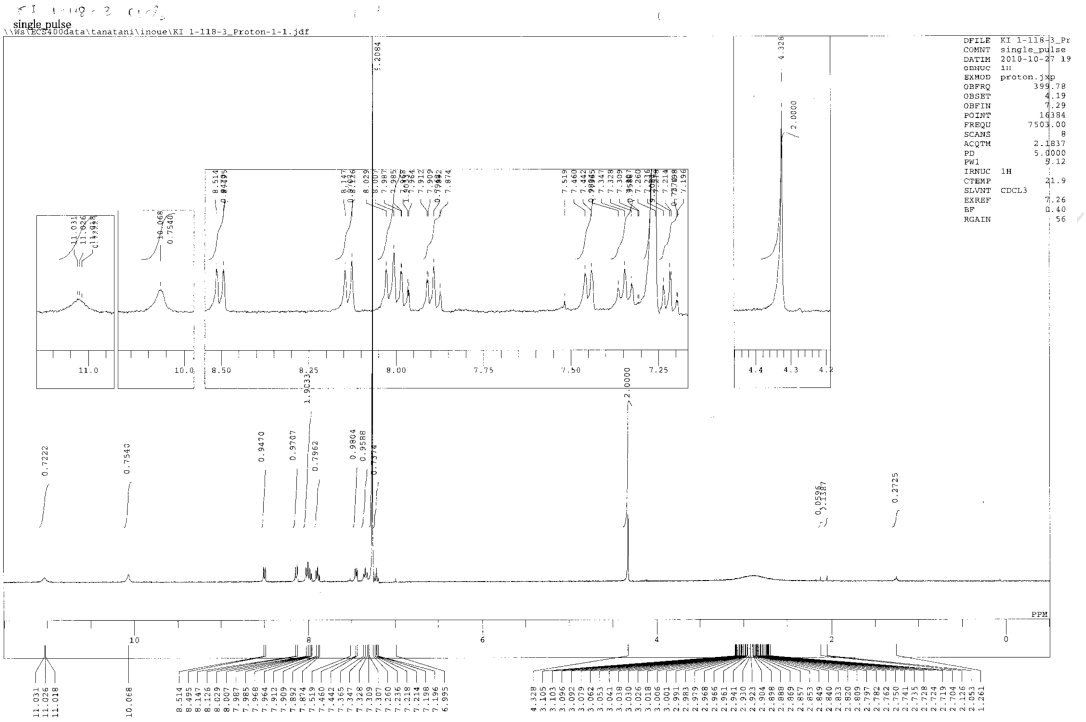


^{13}C NMR

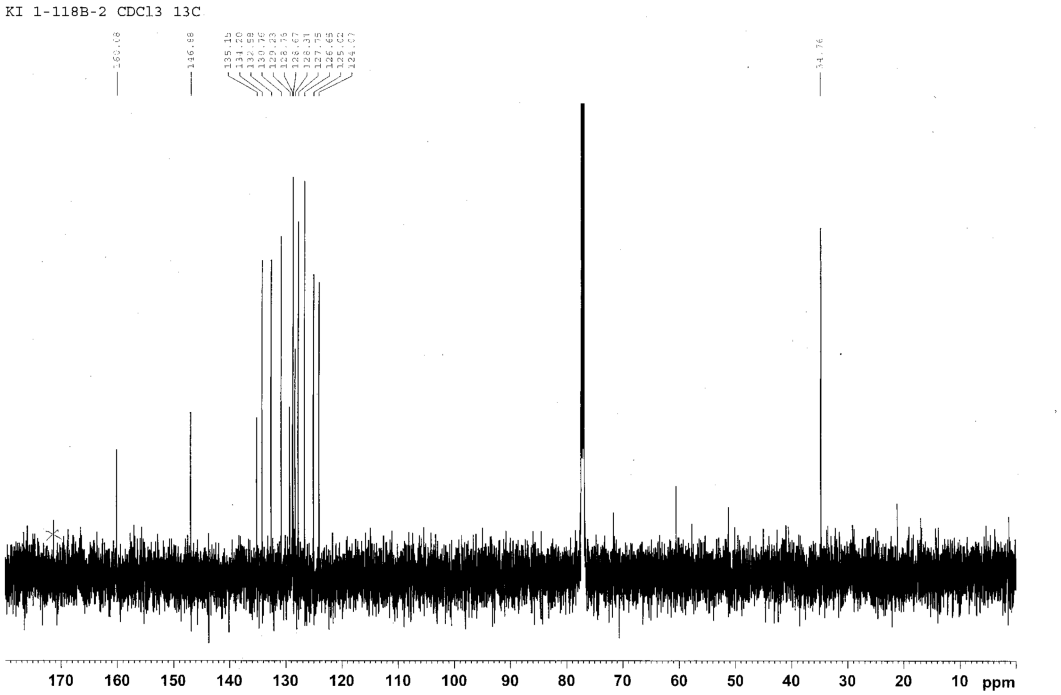


(d) Compound **9d**

^{1}H NMR

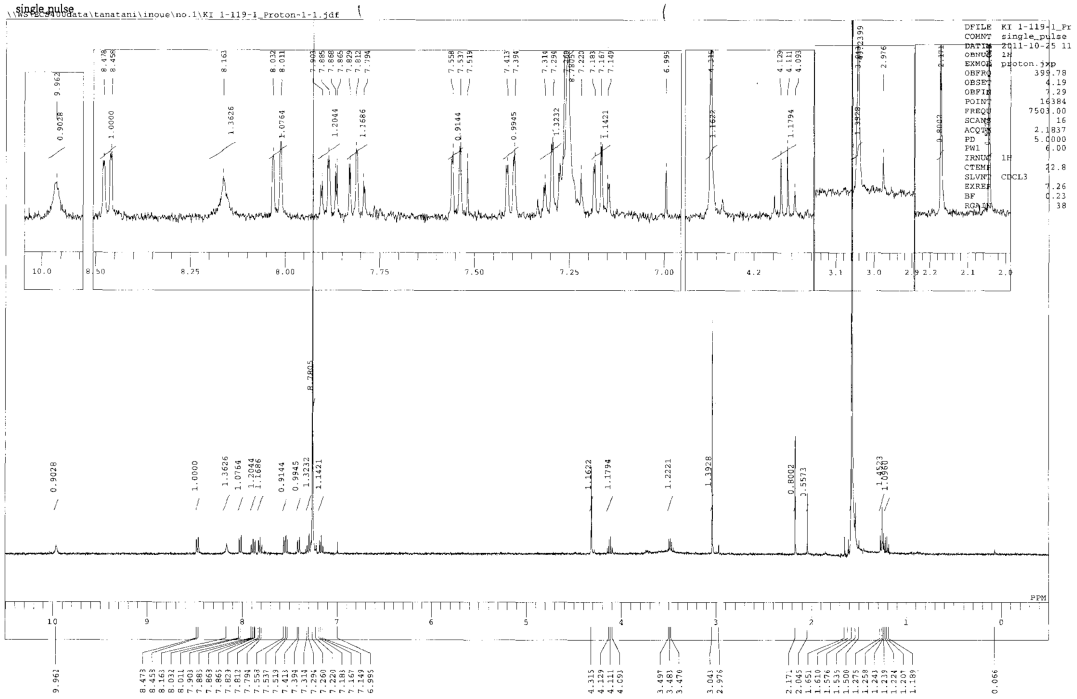


^{13}C NMR

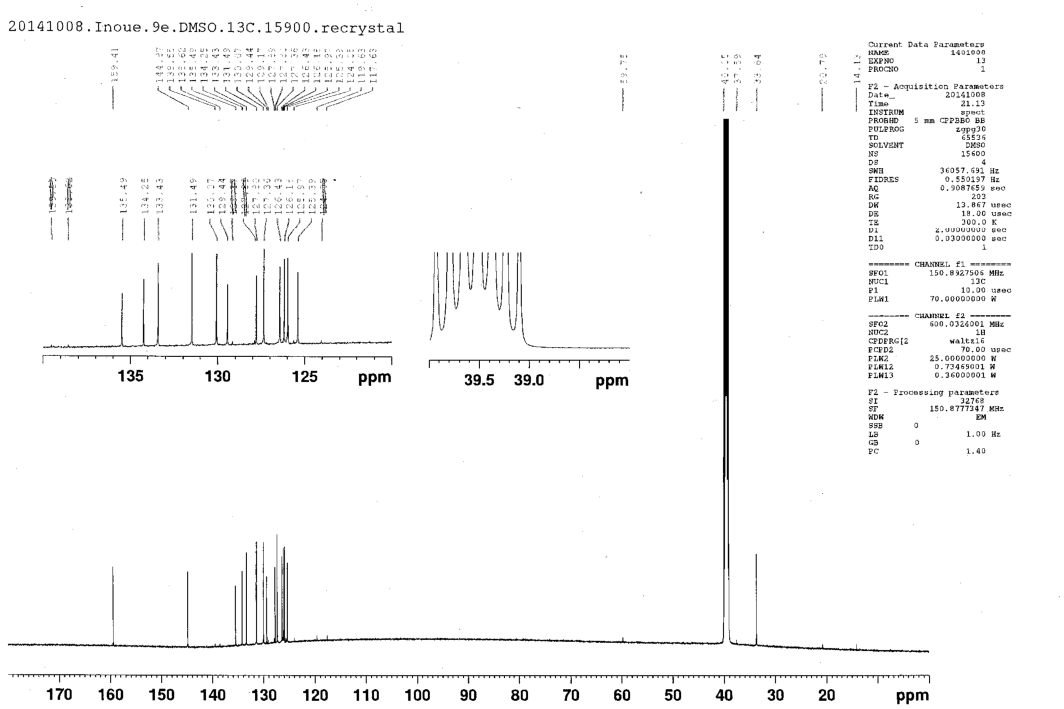


(e) Compound **9e**

^{1}H NMR

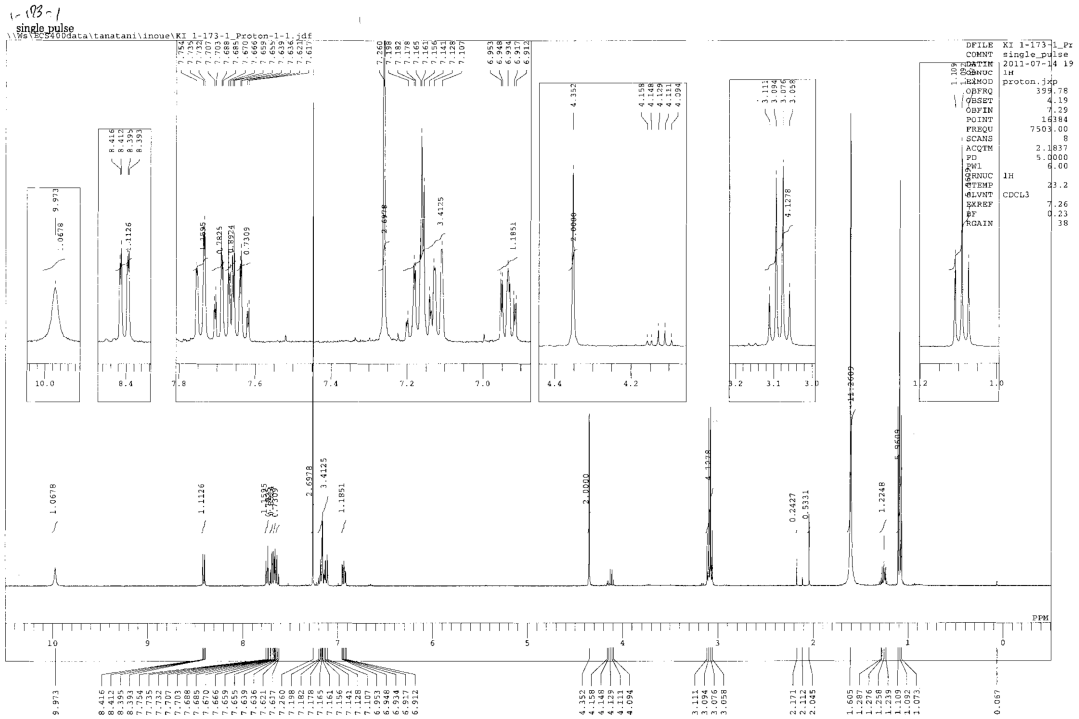


^{13}C NMR

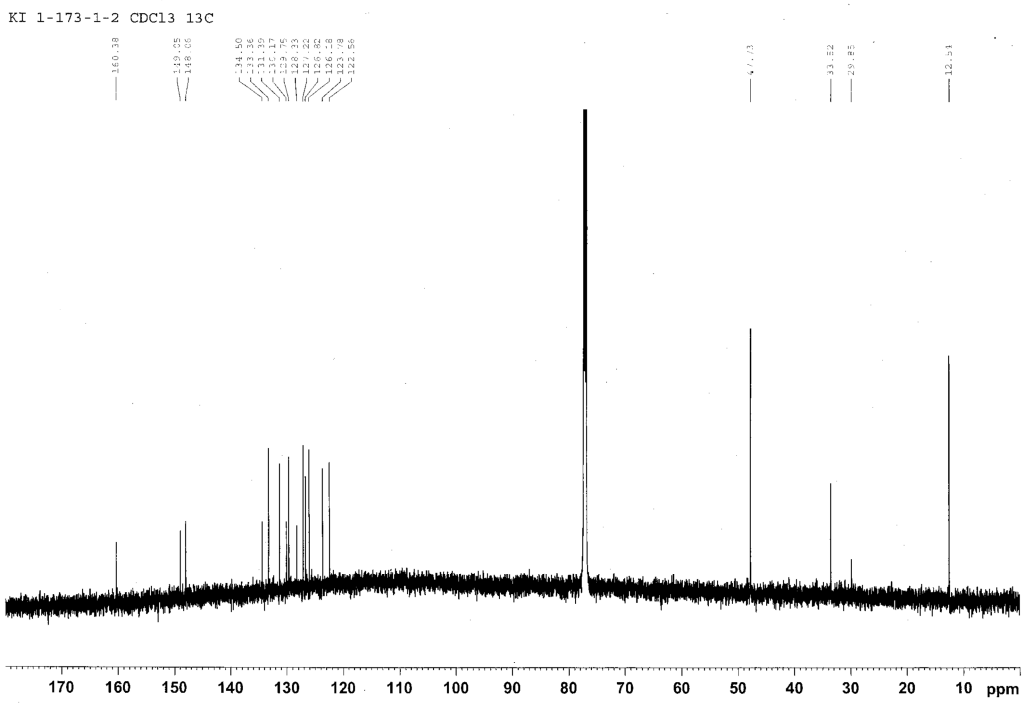


(f) Compound **9f**

^{1}H NMR

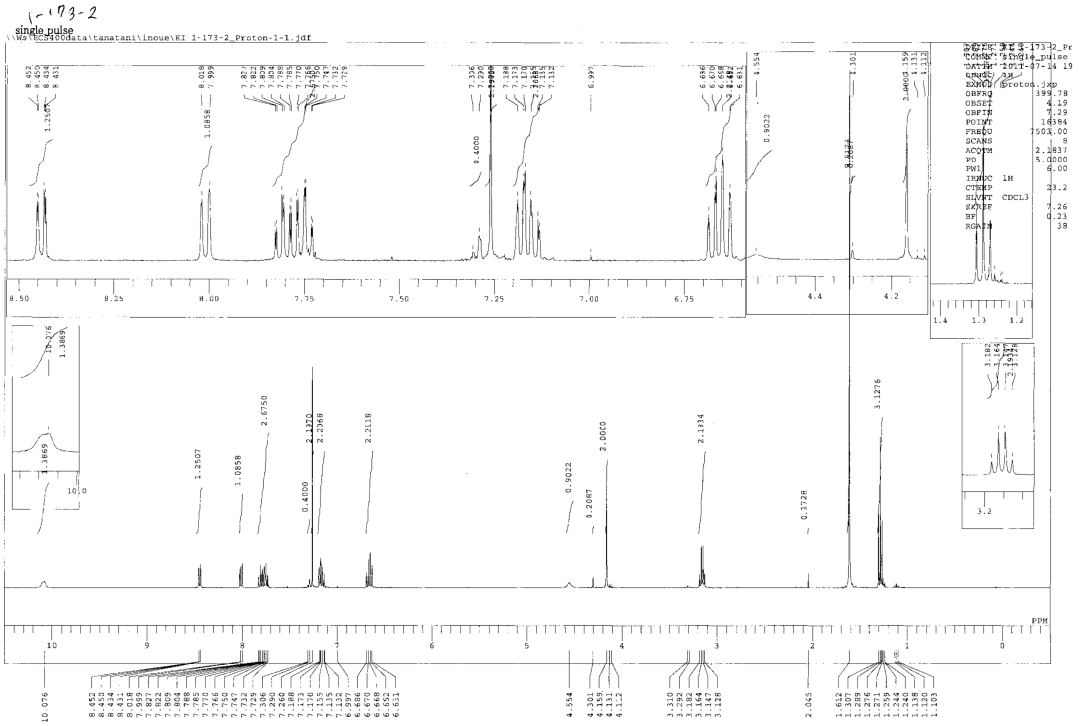


^{13}C NMR

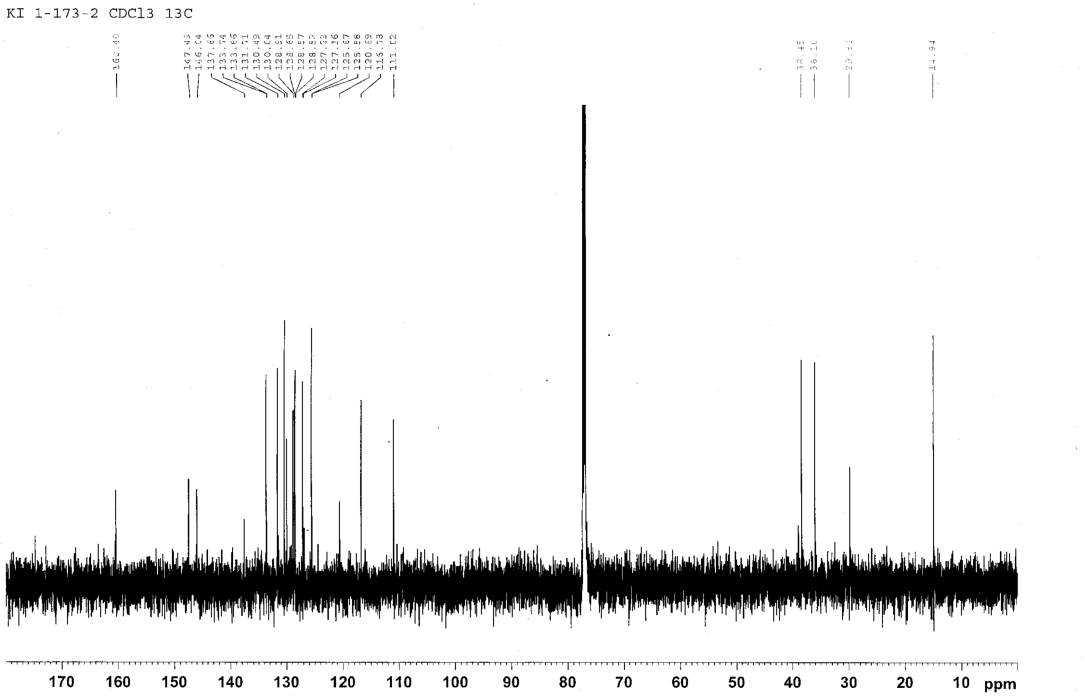


(g) Compound **9g**

^{1}H NMR

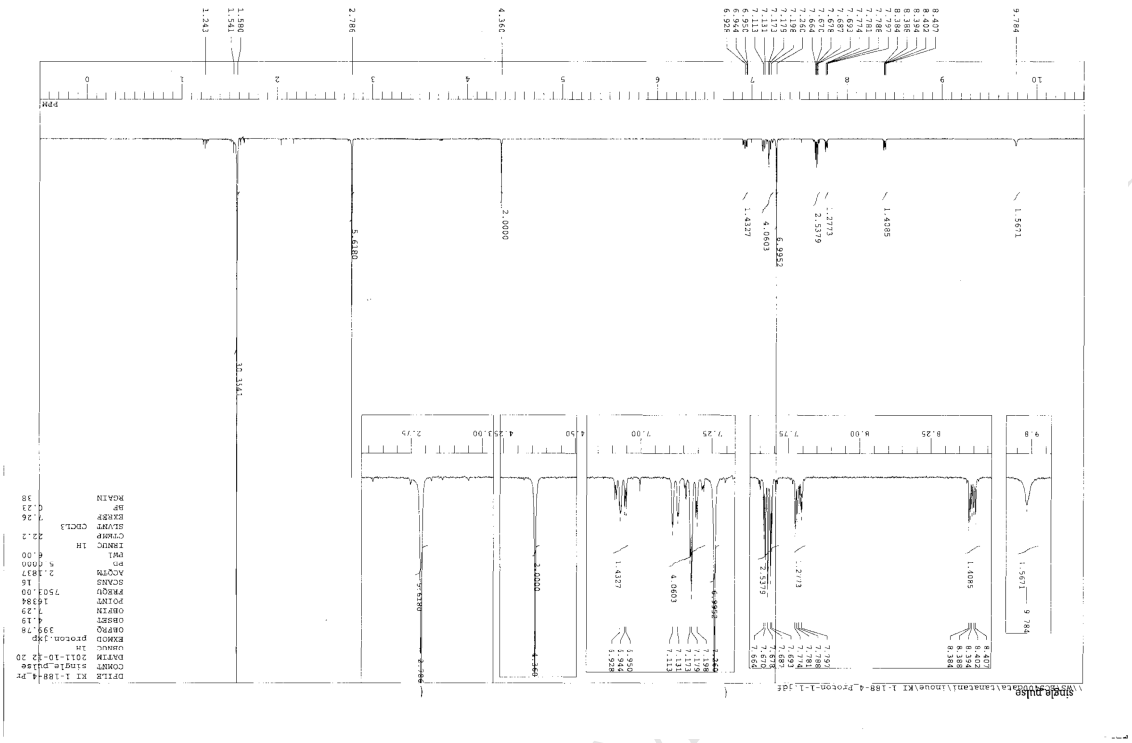


^{13}C NMR

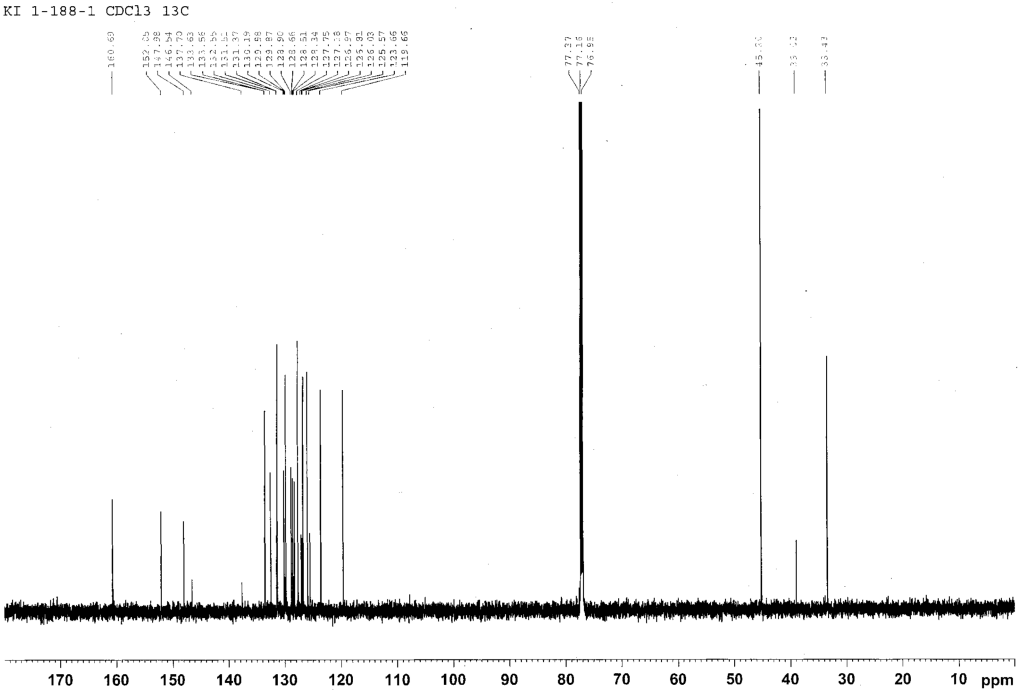


(h) Compound **9h**

^{1}H NMR

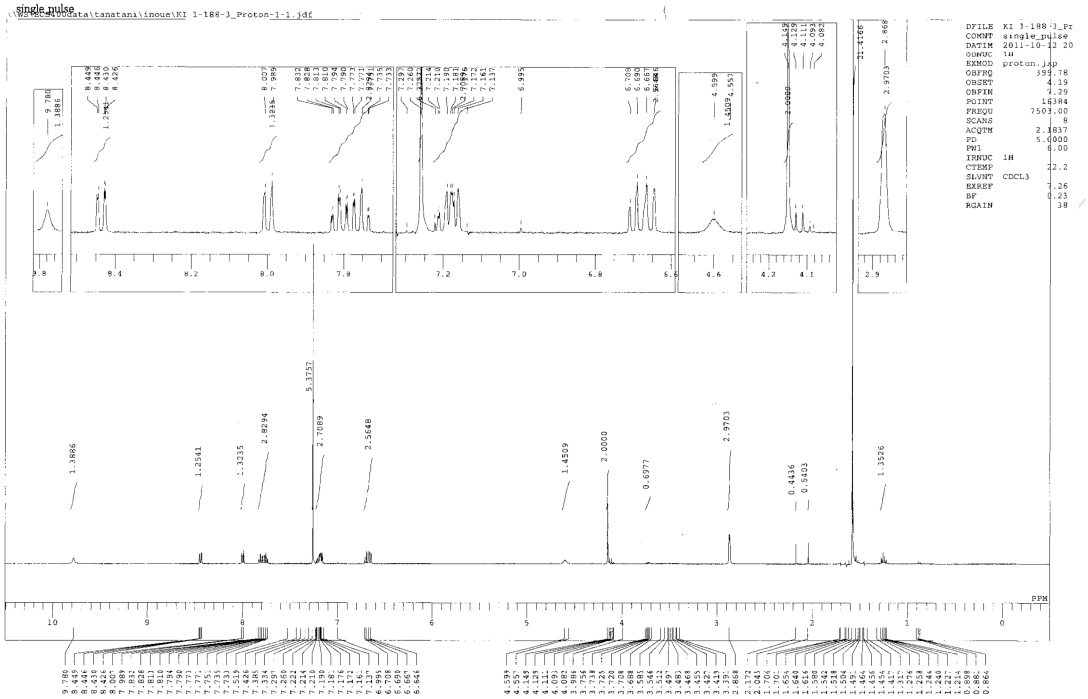


^{13}C NMR



(i) Compound **9i**

^{1}H NMR



^{13}C NMR

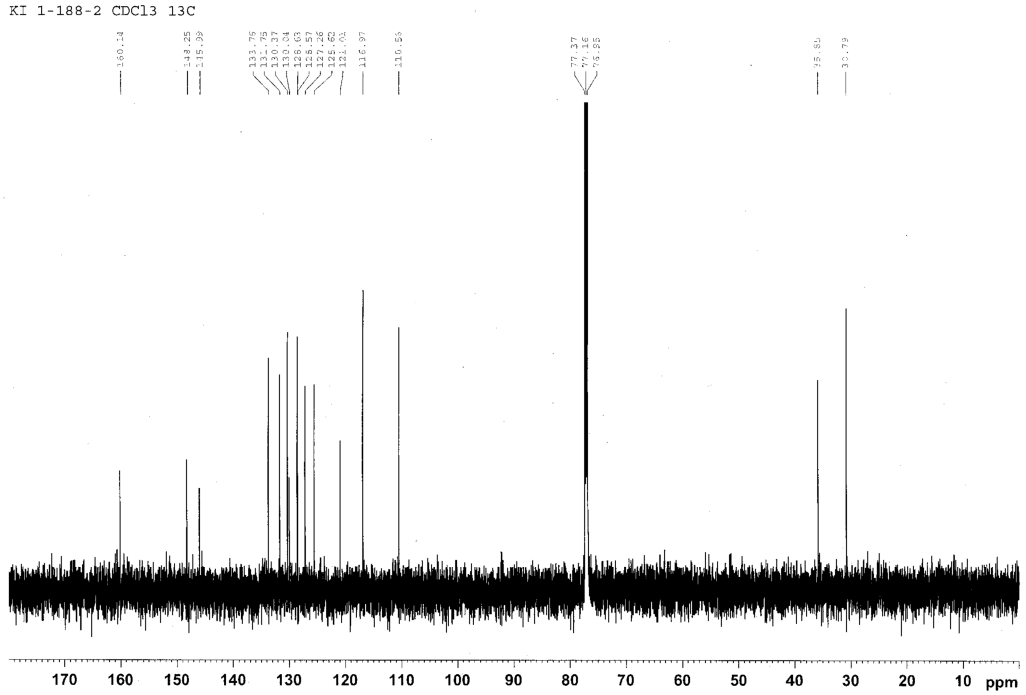
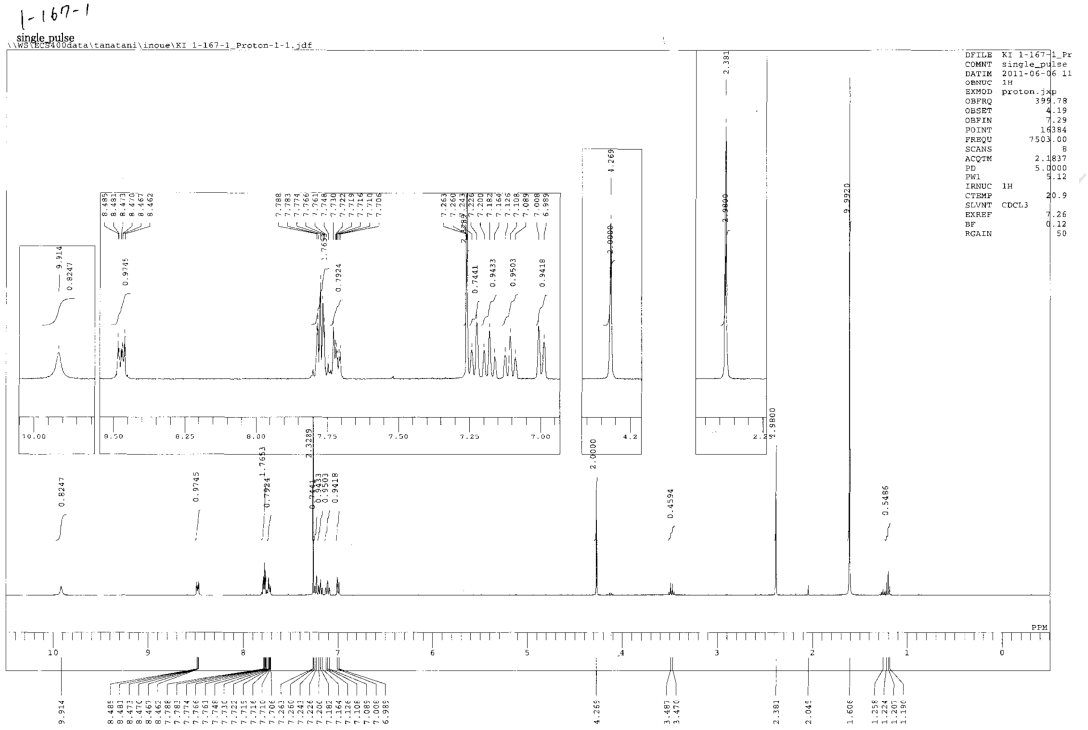


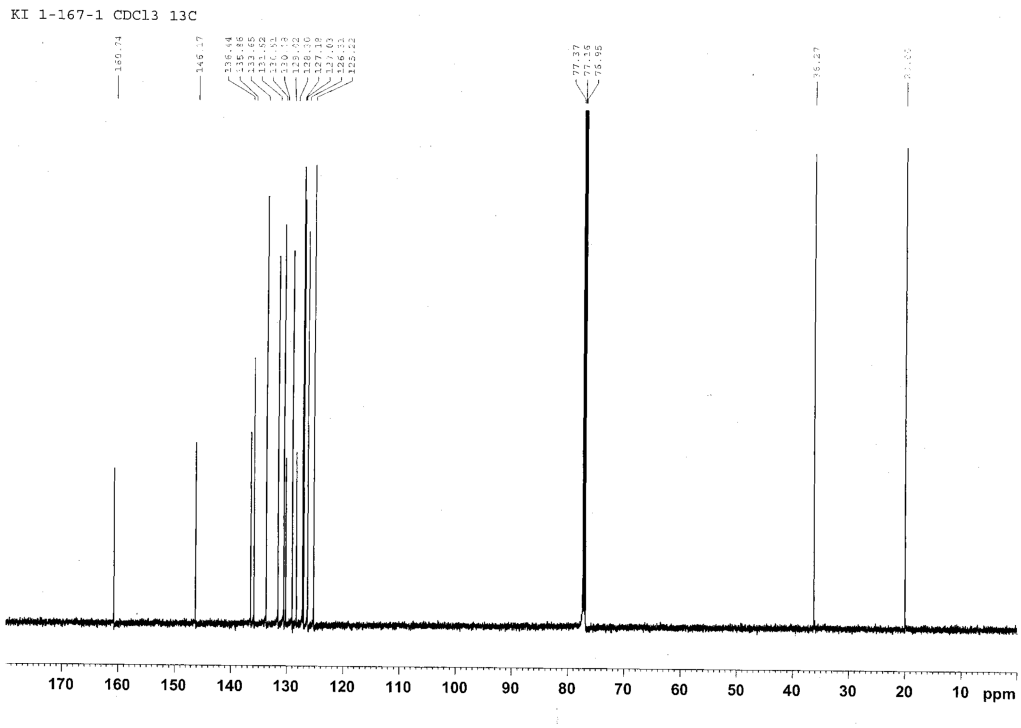
Figure S7. ^{1}H & ^{13}C NMR data of **10a-f**

(a) Compound **10a**

^{1}H NMR

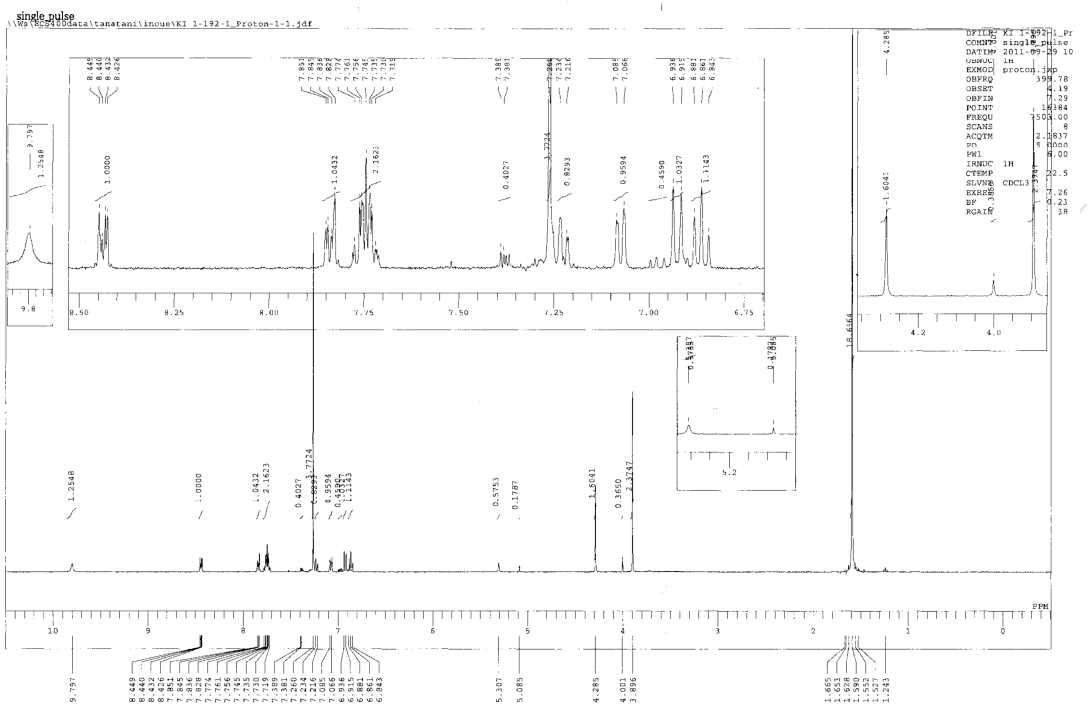


^{13}C NMR

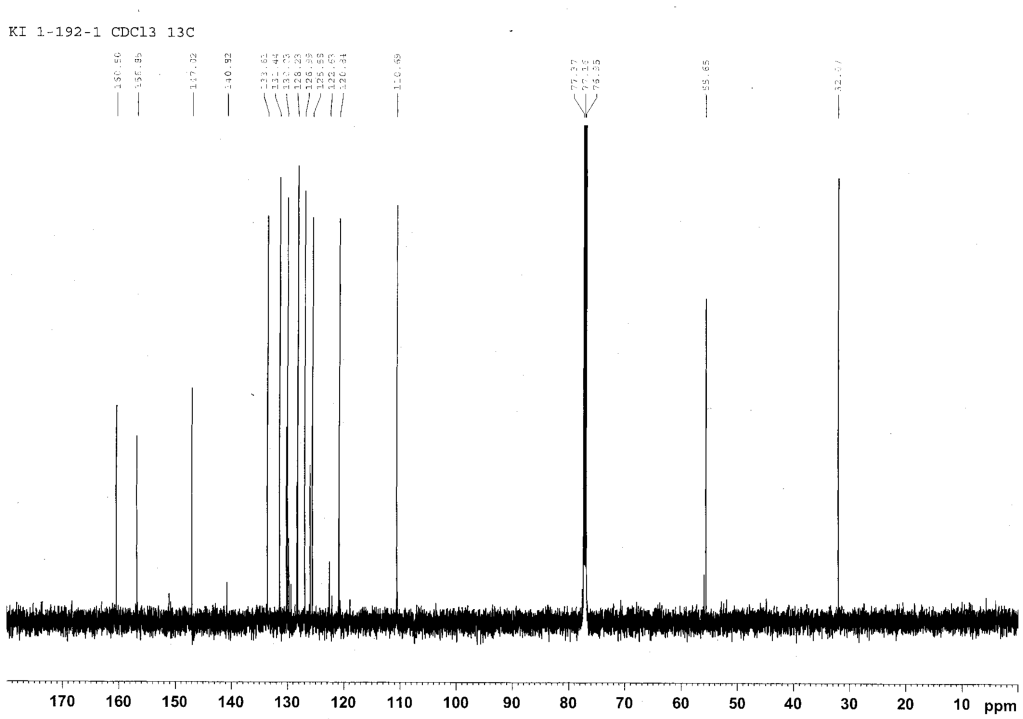


(b) Compound **10b**

^{1}H NMR

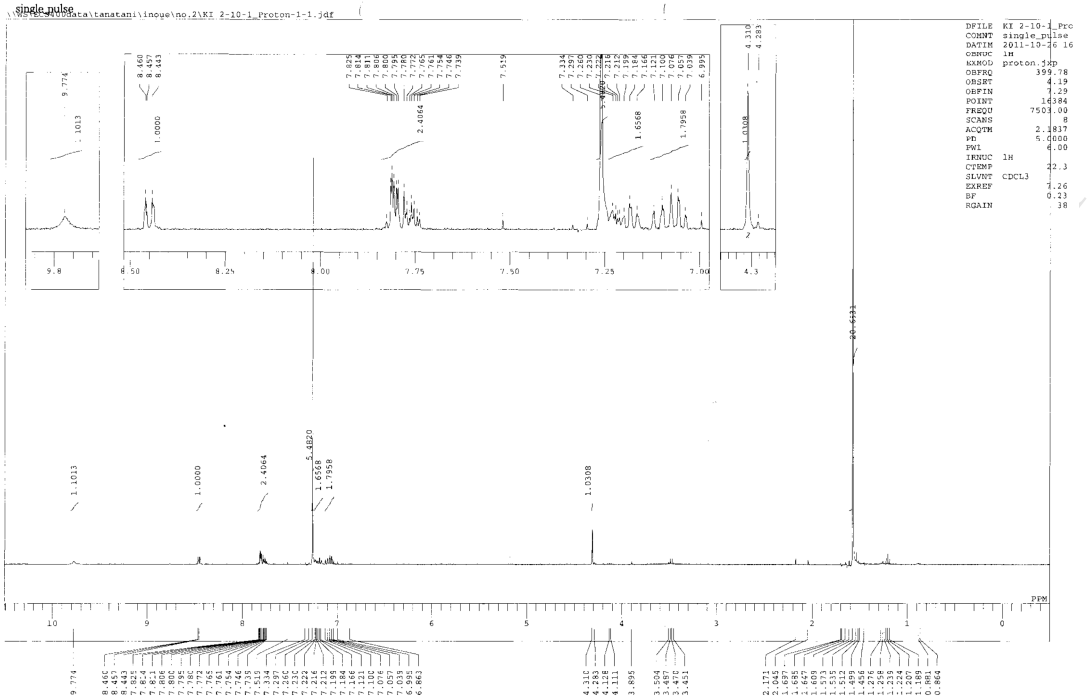


^{13}C NMR

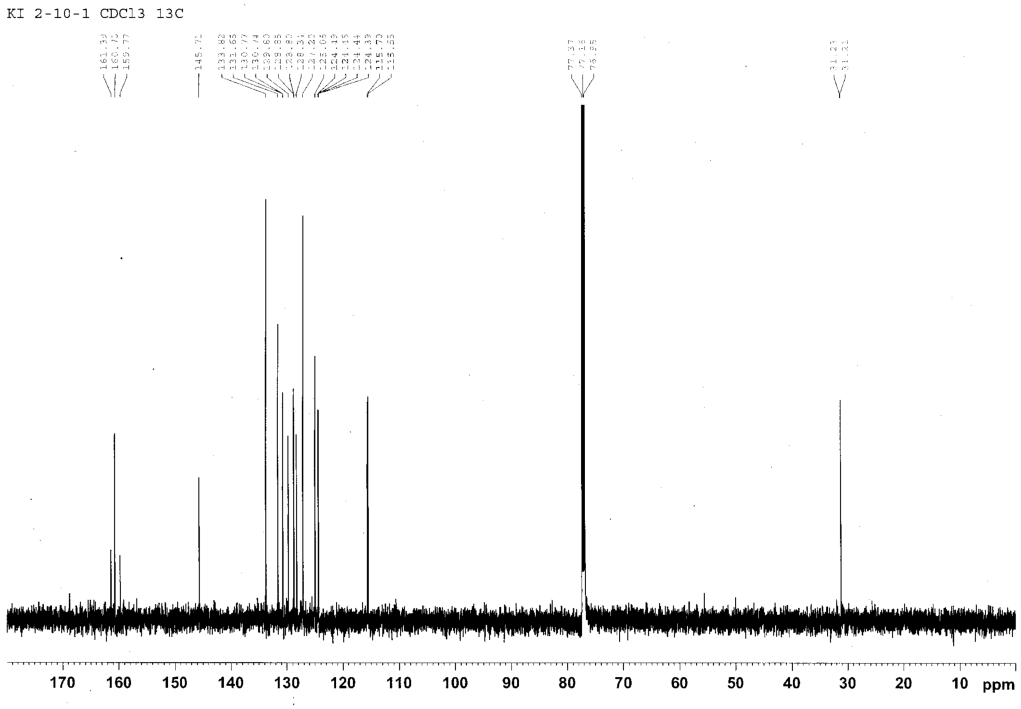


(c) Compound **10c**

^{1}H NMR

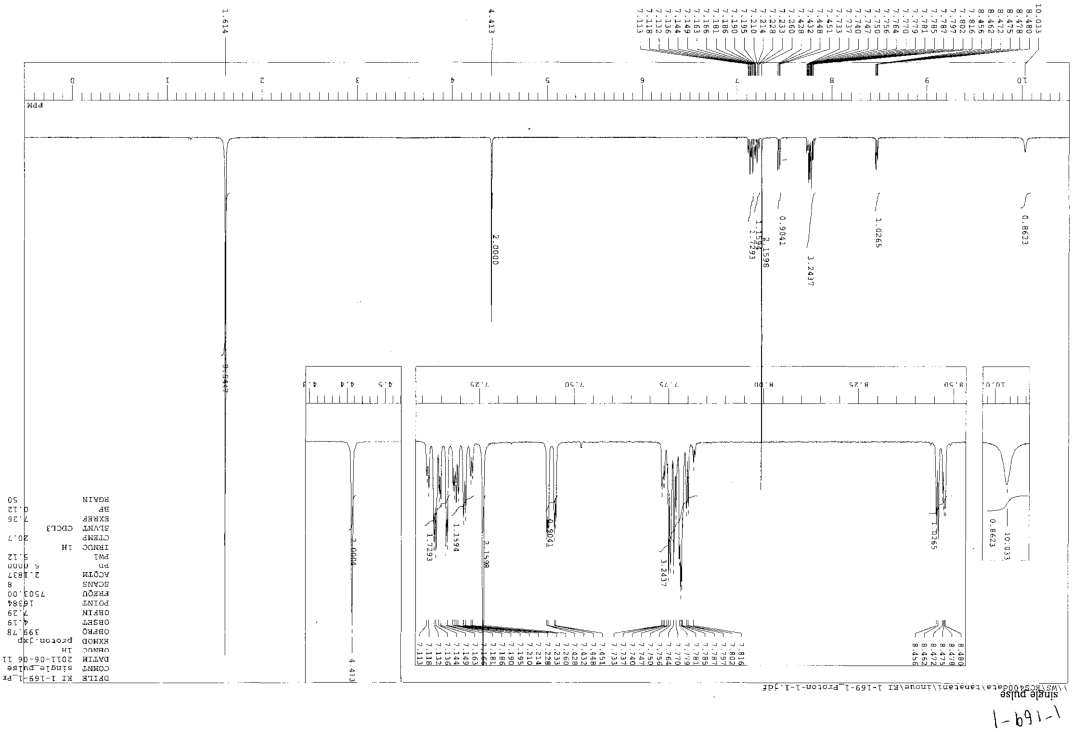


^{13}C NMR

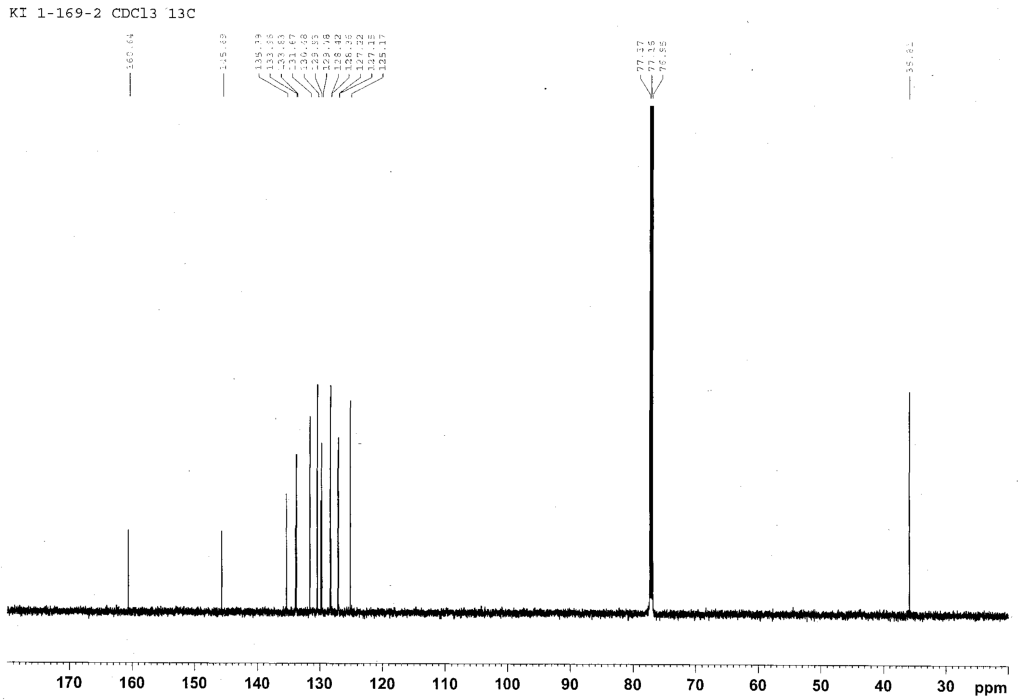


(d) Compound **10d**

^{1}H NMR

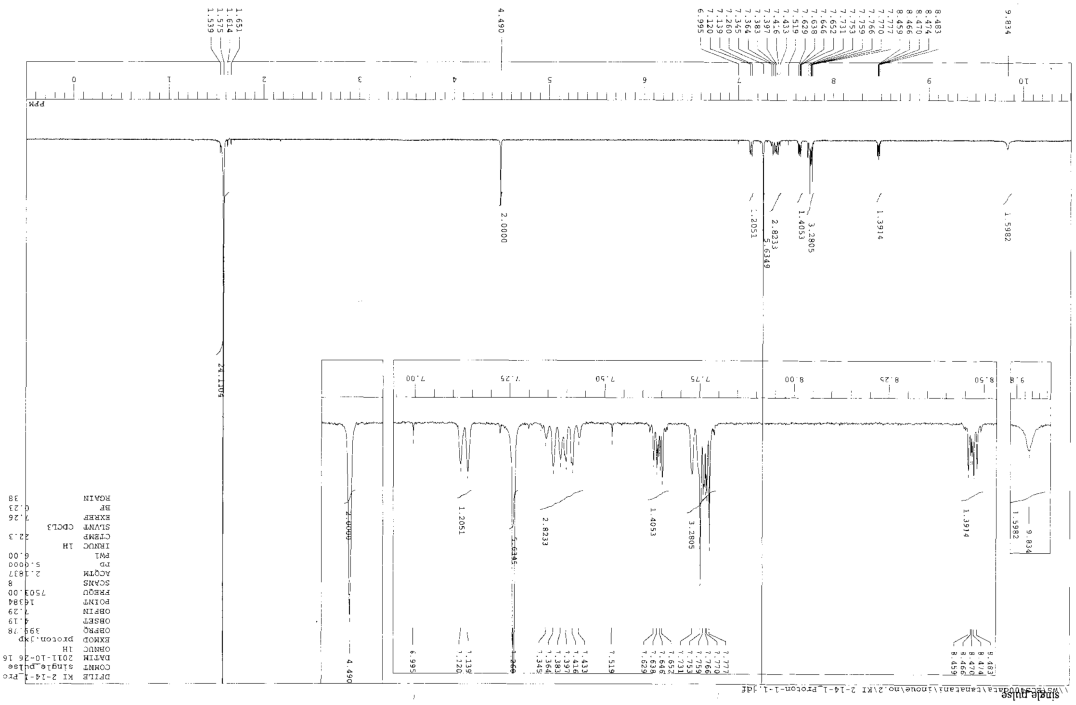


^{13}C NMR

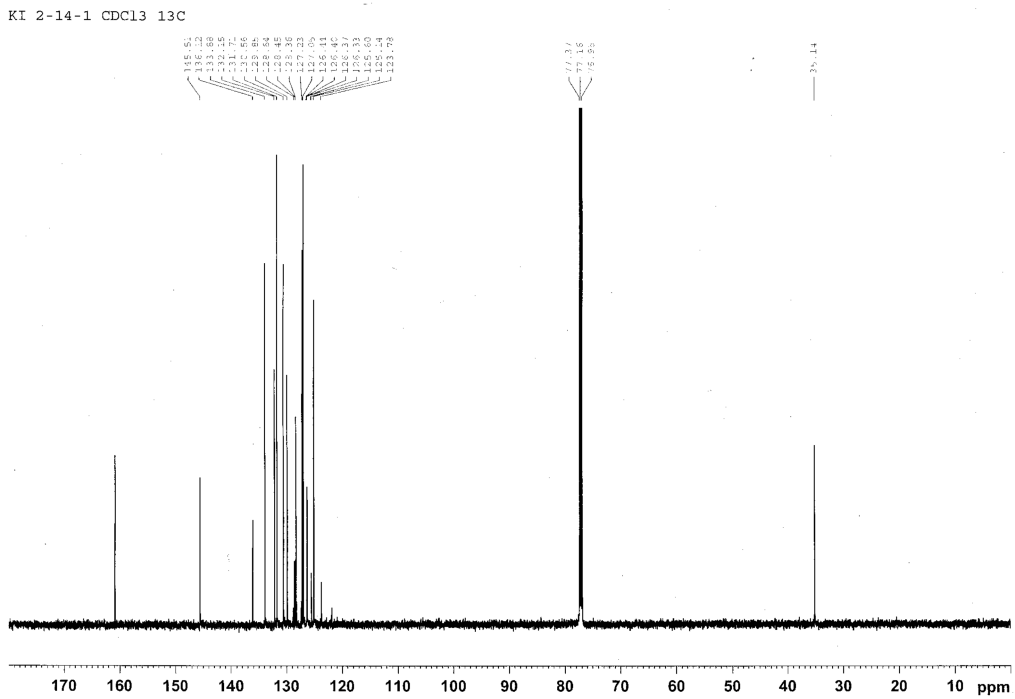


(e) Compound **10e**

^{1}H NMR

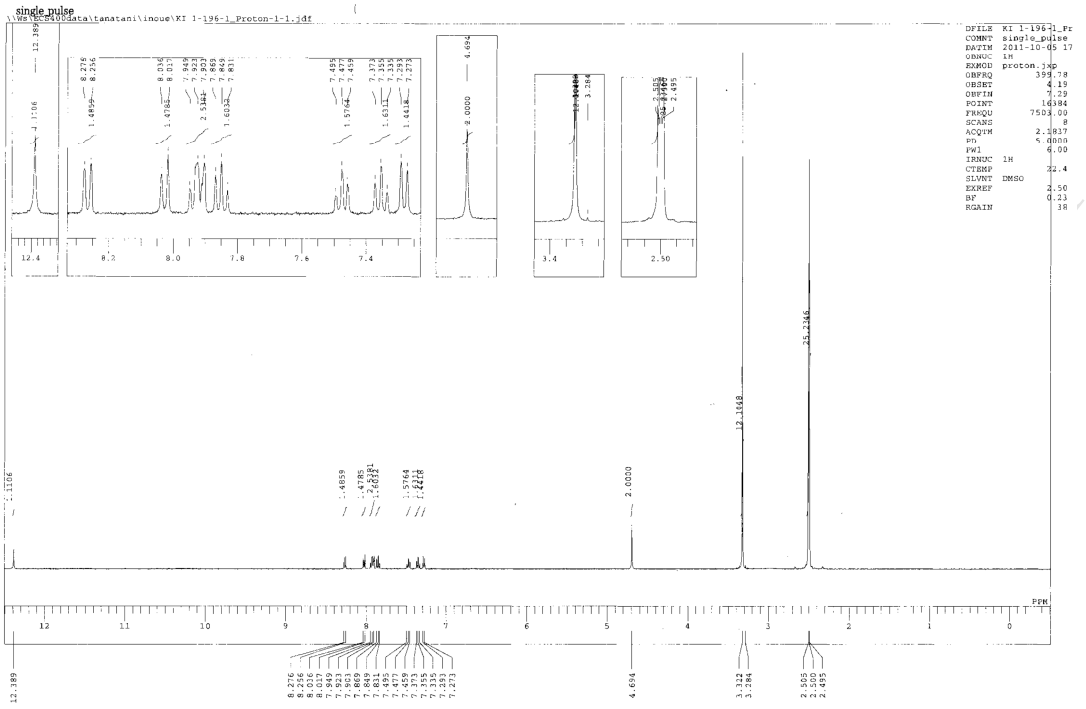


^{13}C NMR



(f) Compound **10f**

^{1}H NMR



^{13}C NMR

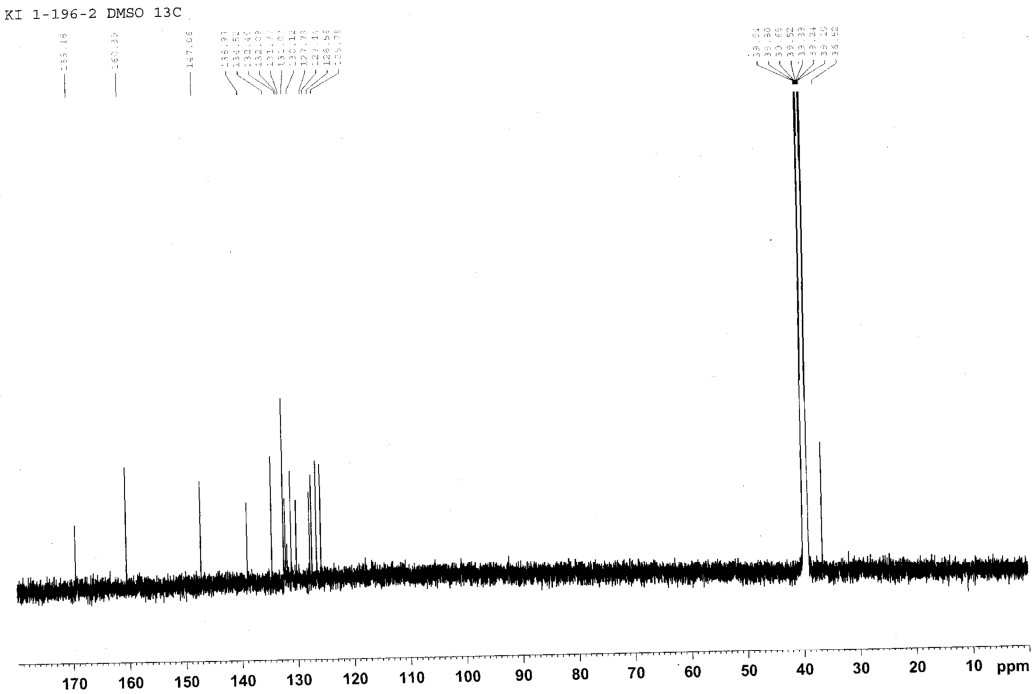
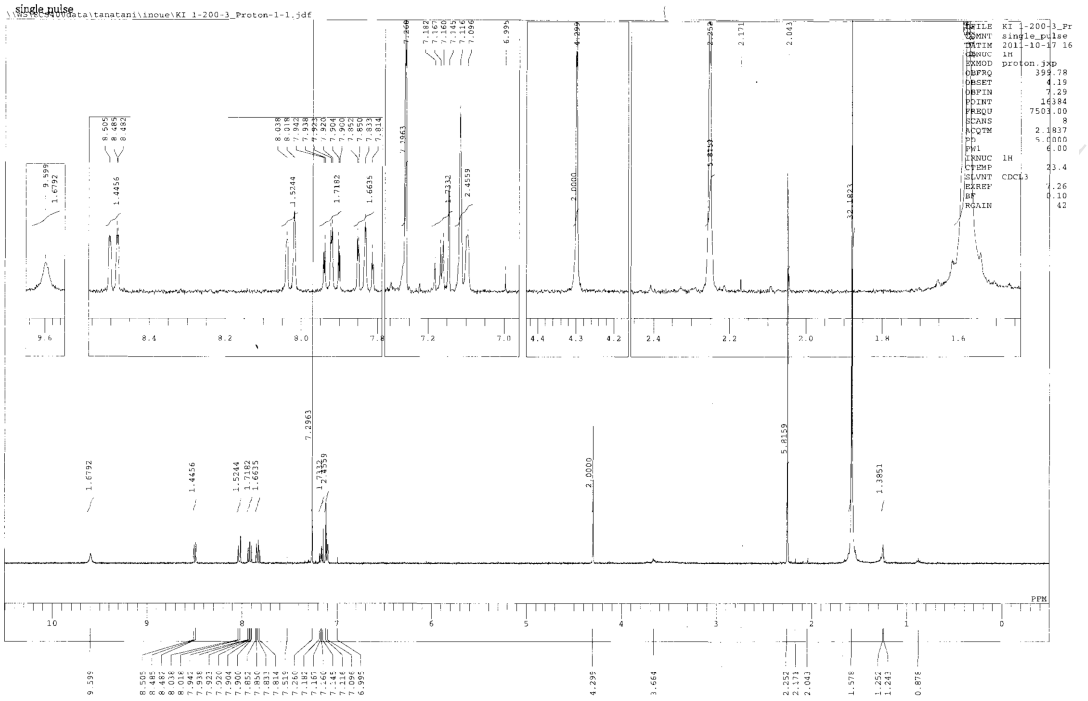


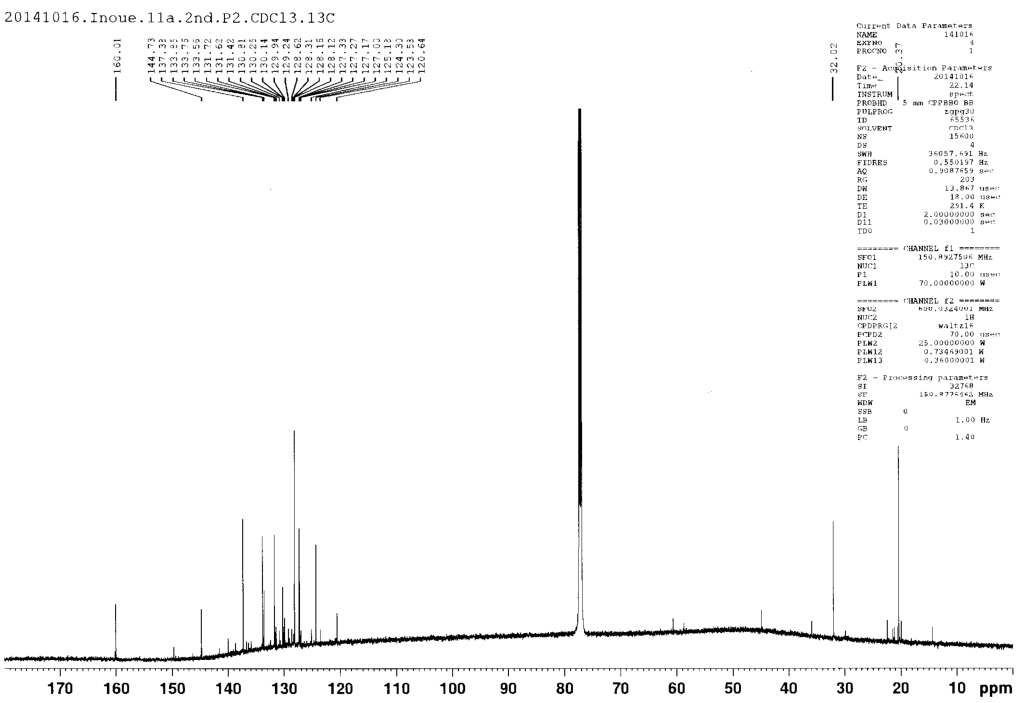
Figure S8. ^{1}H & ^{13}C NMR data of **11a-d**

(a) Compound **11a**

^{1}H NMR

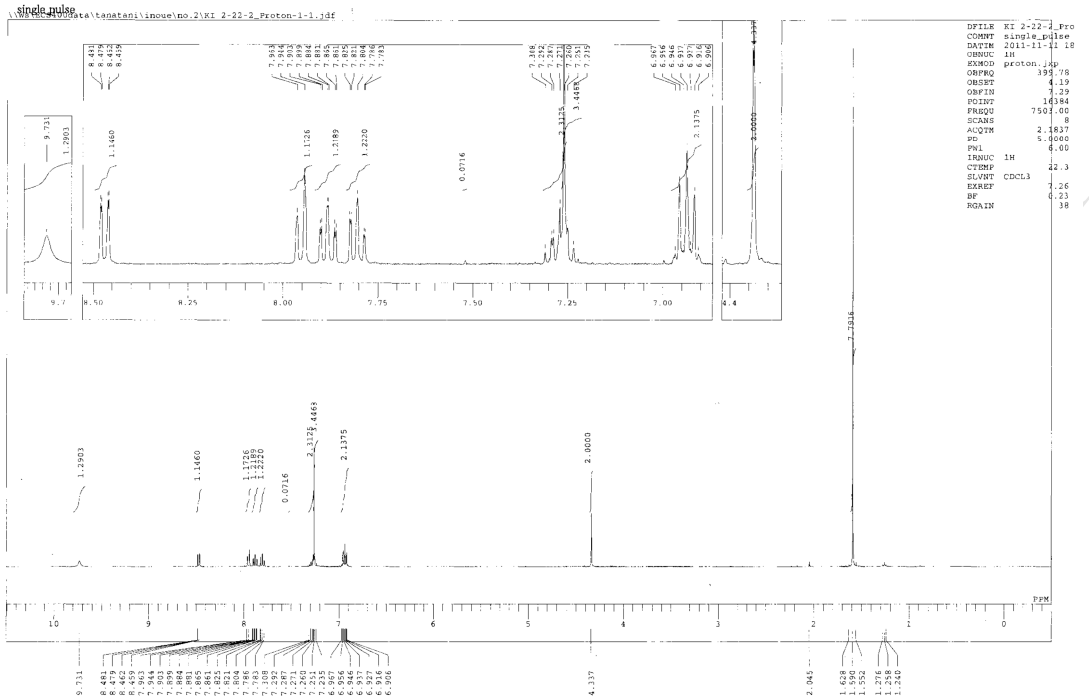


^{13}C NMR

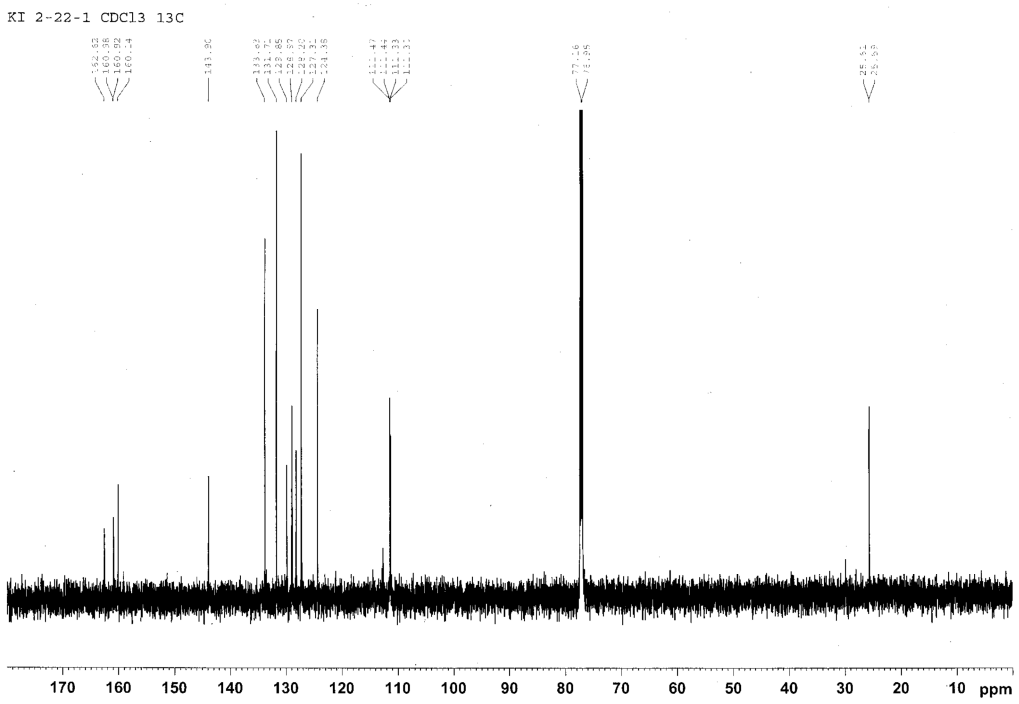


(b) Compound **11b**

^{1}H NMR

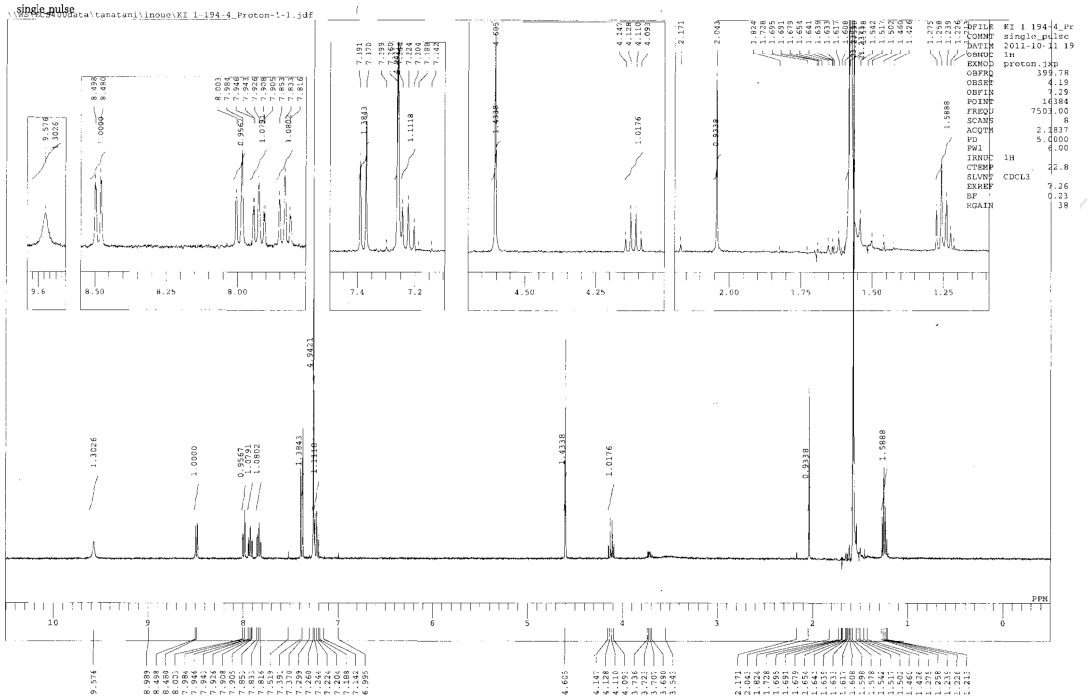


^{13}C NMR

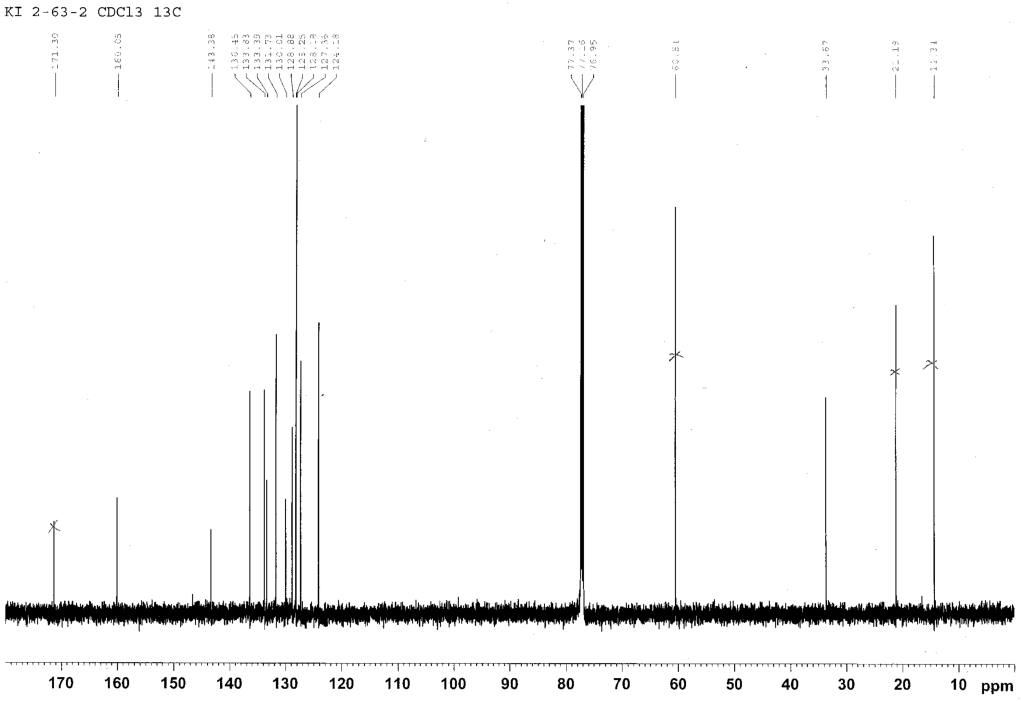


(c) Compound **11c**

^{1}H NMR

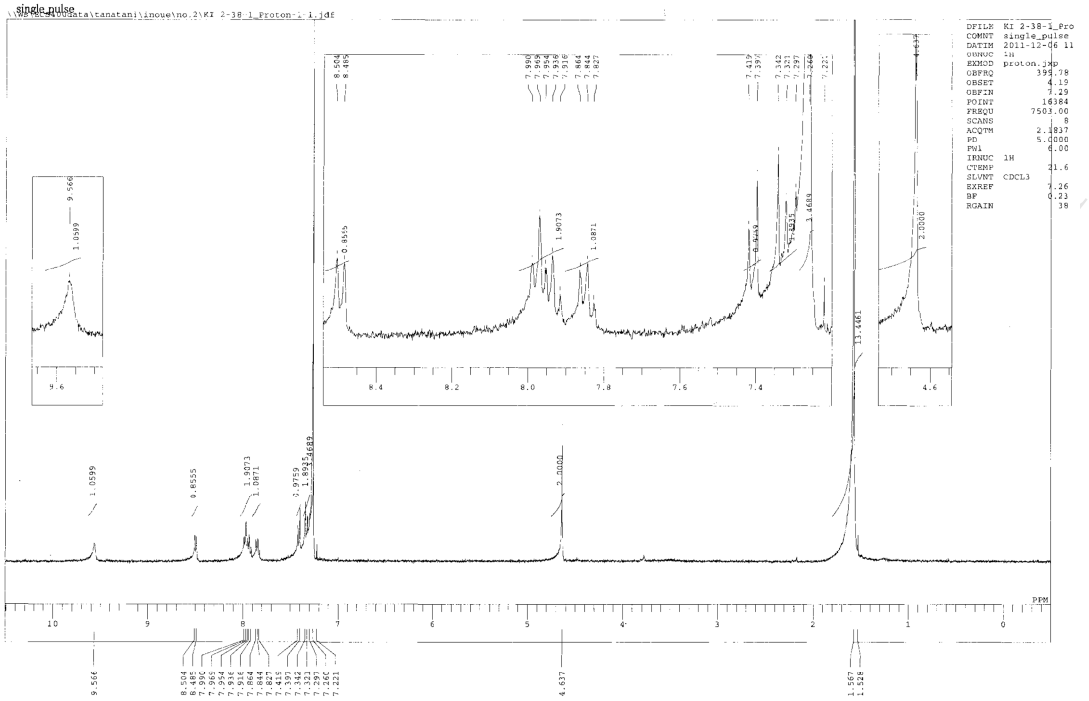


^{13}C NMR



(d) Compound **11d**

^{1}H NMR



^{13}C NMR