Supporting Information

Design, Synthesis, and Biological Evaluation of 1-Methyl-1,4-dihydroindeno[1,2-*c*]pyrazole Analogues as Potential Anticancer Agents Targeting Tubulin Colchicine Binding Site

Yan-Na Liu,[†,§] Jing-Jing Wang,[‡,§] Ya-Ting Ji,[†] Guo-Dong Zhao,[†] Long-Qian Tang,[†] Cheng-Mei Zhang,[†] Xiu-Li Guo,[‡] and Zhao-Peng Liu*[†]*

[†]Institute of Medicinal Chemistry, Key Laboratory of Chemical Biology (Ministry of Education), School of Pharmaceutical Sciences, Shandong University, Jinan 250012, P. R. China

[‡]Department of Pharmacology, School of Pharmaceutical Sciences, Shandong University, Jinan 250012, P. R. China

Table of contents

1. Tubulin polymerization inhibitory assay

CA-4 (**3**), ABT-751 (**5**), **6b** and **6k** were evaluated by in vitro tubulin polymerization assay. CA-4, ABT-751 and **6b** produced a concentration-dependent inhibition of tubulin polymerization (Figure 1S), with calculated IC_{50} values of 2.48 μM, 2.80 μM and 22.0 μM, respectively (Table 1S). However, **6k** had poor solubility and precipitated in the test solution at the concentration > 30 nM. **6k** exhibited no effect on tubulin polymerization.

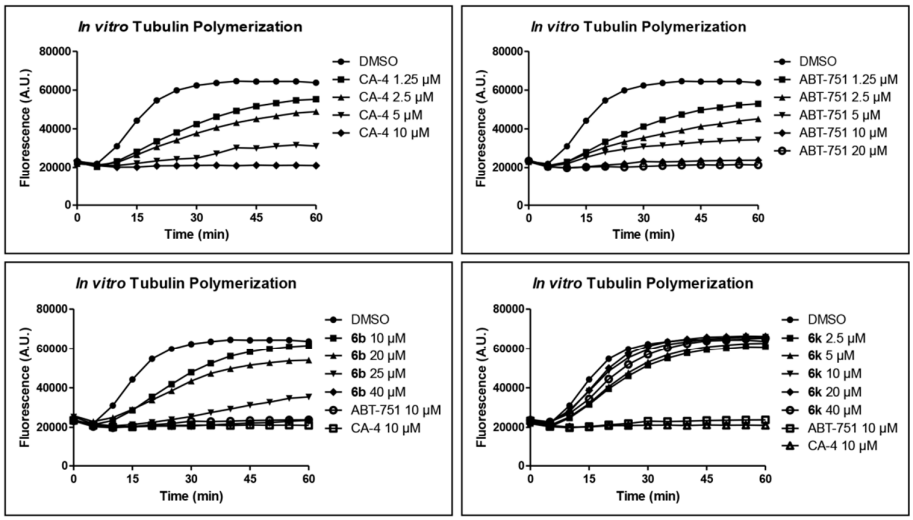


Figure 1S. Effect of CA-4, ABT-751, **6b** and **6k** on tubulin polymerization in vitro.

Table 1S. Inhibition of Tubulin Polymerization

Compound	IC_{50} (μM)
3	2.48
5	2.80
6b	22.0
6k	> 40

2. Molecular Modeling Studies

Molecular docking study was carried out to elucidate the binding features of the 1-methyl-1,4-dihydroindeno[1,2-*c*]pyrazole derivatives with tubulin. Since the $\alpha\beta$–tubulin heterodimers complexed with different ligands in certain flexibility at the colchicine site, we performed the docking studies of **6a** with five representative crystal structures in which the ligands interacted with tubulin in the typical zones 1 and 2 (PDB code: 1SA0, 1SA1, 3HKE), zones 2 and 3 (PDB code: 3HKD), and all the three zones (PDB code: 3HKC). The ligands of these PDBs were shown in Figure 2S.

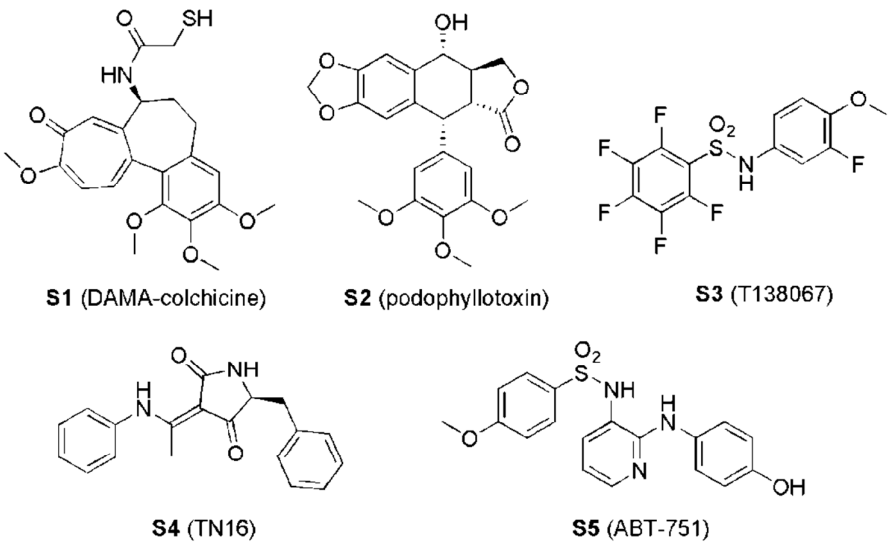


Figure 2S. The ligands for the five representative crystal structures.

SURFLEX-DOCK module of Sybyl–X 1.3 (Tripos, St. Louis, USA) was used to automatically dock all the selected compounds into the binding pocket of tubulin. Surflex is a fully automatic flexible molecular docking algorithm that combines Hammerhead's empirical scoring function with a molecular similarity method to generate putative poses of ligand fragments. The higher scores indicate greater binding affinity and similarity.

Docking process was guided by the protomol generation, preparation of the 3D-structure of the ligands, and docking of the ligands into the colchicine binding pockets.

Protomol generation: The crystal structures of tubulin in complex with different ligands (Figure 2S) were taken from RCSB Protein Data Bank and prepared by Sybyl–X 1.3. After extracting the ligand, hydrogen atoms were added to the crystal. Charges were added to biopolymer by AMBER7 FF99 force field and ligand by Gasteiger-Huckel force field. The extracted ligand was used to generate the protomol with threshold of 0.99 and bloat of 0.

The preparation of the 3D-structure of **6a**: **6a** was constructed by the Sketch module of Sybyl–X 1.3, and the energy minimization was performed using the Tripos force field. Gasteiger-Huckel charge was added to the structure with a NB cutoff distance of 8 Å and a dielectric constant of 1.00.

Docking **6a** into the colchicine binding site: We docked **6a** into the binding pocket of tubulin based on the protomol generated with the angstroms to expand search grid of 6 Å, maximum conformations per fragment of 20 and maximum number of rotatable bonds per molecular of 100. The ligand for each crystal was set to be the reference molecular. Other docking parameters implied in the program were kept at default. The optimal binding mode of **6a** was selected based on the hydrophobic interactions, hydrogen bindings and Surflex scores.

Results and discussions. The docking scores of **6a** with a set of PDBs were listed in Table 2S.

Table 2S. The docking scores of **6a** into a set of PDBs.

	1SA0	1SA1	3HKE	3HKD	3HKC
Total Score	7.8053	5.6405	7.9944	5.4882	9.0857
Crash	–2.0256	–5.8613	–3.5502	–6.7581	–3.8690
Polar	0.0079	0.7848	2.1590	1.0496	1.7930
D_Score	–154.4804	–175.5134	–158.3708	–204.5099	–184.8146
PMF_Score	–39.1896	14.5673	–8.1149	–31.6813	–21.4794
G_Score	–313.8852	–333.9516	–303.6874	–343.5847	–334.8066
ChemScore	–29.8089	–31.8296	–33.7505	–38.8382	–37.6249

The surflex-dock function includes the following terms: hydrophobic, polar, repulsive, entropic, solvation and crash. Surflex-dock scores are expressed as $-\log_{10}(K_d)$. The total score includes the crash score. Crash: the degree of inappropriate penetration by the ligand into the protein and of interpenetration (self-clash) between ligand atoms; Polar: contribution of the polar interactions to the total score; it is a sum over all pairs of complementary polar atoms of a function capturing the effects of hydrogen bonds and salt bridges. This function includes a directionality term that favors hydrogen bonding geometries observed in crystal structures and a term that accounts for favorable interaction between formally charged atoms (if present); D_Score: using only the charge and van der Waals interactions between the protein and the ligand; PMF_Score: Helmholtz free energies of interactions for protein-ligand atom pairs (Potential of Mean Force, PMF); G_Score: using the hydrogen bonding, complex (ligand-protein), and internal (ligand-ligand) energies; ChemScore: hydrogen bonding, metal-ligand interaction, lipophilic contact, and rotational entropy.

1SA0 is complexed with DAMA-colchicine (**S1**) with a resolution of 3.58 Å. 1SA0 is frequently used for docking colchicine site agents. In addition to being accommodated at the zones 1 and 2 that are similar to colchicine, the *m*-ethoxyaniline group in **6a** protrudes into zone 3 and forms hydrogen bonding (2.02 Å) with the key residue Tyr*β*202 (Figure 3S). The Surflex docking score is 7.81.

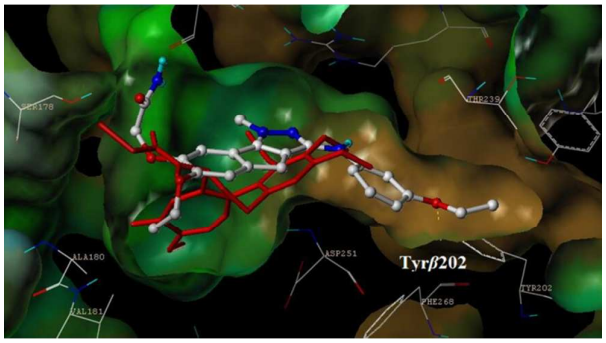


Figure 3S. DAMA-colchicine (red) and binding mode of **6a** (grey).

1SA1 is complexed with podophyllotoxin (**S2**) with a resolution of 4.20 Å. The binding mode of **6a** is quite different with **S2**. The *m*-ethoxyaniline portion of **6a** is accommodated at zone 2. The acetamide substituent at the 7-position of **6a** reaches to α-tubulin and forms hydrogen bonding (2.40 Å) with Asnα101. However, **6a** did not interact with tubulin in zone 1 (Figure 4S). The Surflex docking score is 5.64.

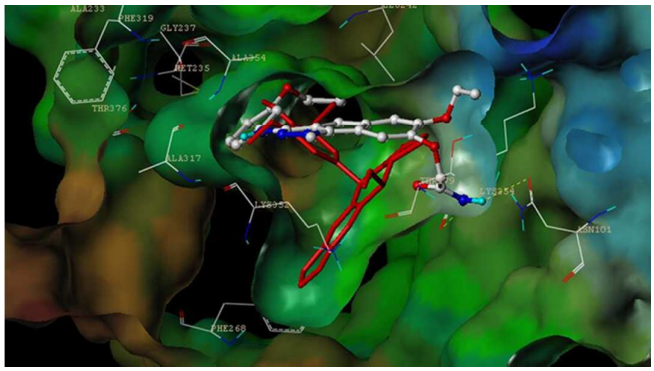


Figure 4S. Podophyllotoxin (red) and binding mode of **6a** (grey).

3HKE is complexed with T138067 (**S3**) with a resolution of 3.60 Å. The binding mode of **6a** is quite different with **S3**. The *m*-ethoxyaniline portion of **6a** protrudes into zone 3 and the ethoxy oxygen forms hydrogen bonding (2.03 Å) with Tyrβ202. The 1-methyl-1,4-dihydroindeno[1,2-*c*]pyrazole portion was accommodated at the zone 2 pocket. The acetamide substituent at the 7-position of **6a** reaches to α-tubulin and forms hydrogen bonding with both Serα178 (2.08 Å) and Thrα179 (1.75 Å). However, **6a** did not interact with tubulin in zone 1 (Figure 5S). The Surflex docking score is 7.99.

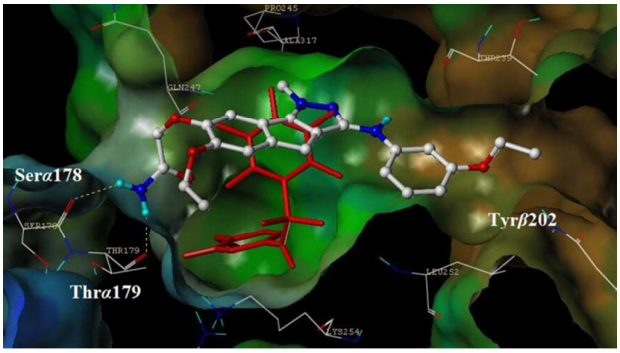


Figure 5S. T138067 (red) and binding mode of **6a** (grey).

3HKD is complexed with TN16 (**S4**) with a resolution of 3.70 Å. As shown in Figure 6S, the *m*-ethoxyaniline portion of **6a** overlaps with the benzyl substituent of **S4** in zone 3, the aniline nitrogen forms hydrogen bonding (2.28 Å) with Tyrβ202. The 1-methyl-1,4-dihydroindeno[1,2-*c*]pyrazole portion was accommodated at the zone 2 pocket. The ethoxy group at the 6-position of **6a** interacts partially with zone 1. The acetamide substituent at the 7-position forms hydrogen bonding (1.97 Å) with Thrα179 (Figure 6S). The Surflex docking score is 5.49.

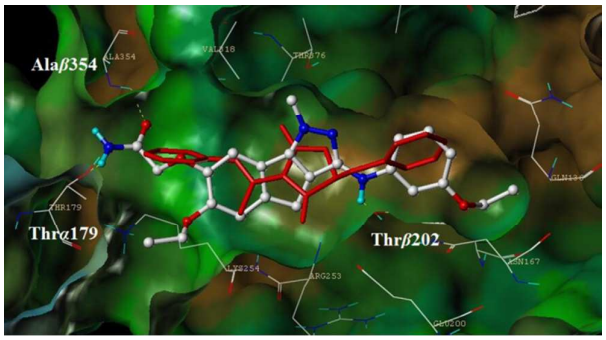


Figure 6S. TN16 (red) and binding mode of **6a** (grey).

3HKC is complexed with ABT-751 (**S5**) with a resolution of 3.80 Å. As shown in Figure 7S, the binding mode of **6a** similar to that **S5**. The *m*-ethoxyaniline group of **6a** superimposed with the phenol substituent of **S5**, protruded into zone 3 more deeply, and formed the same hydrogen bonding (2.65 Å) with Tyr*β*202. Like the *o*-aminopyridine core in **S5**, the 1-methyl-1,4-dihydroindeno[1,2-*c*]pyrazole portion was accommodated at the zone 2 pocket. In addition, the amino group in the *m*-ethoxyaniline moiety formed hydrogen bonding (2.02 Å) with Val*β*238 in zone 2. Though the ethoxy substituent at the 6-position in **S5** occupied only partial hydrophobic cavity in zone 1, the acetamide substituent at the 7-position interacted additionally with tubulin in an open cavity up to the *α*-tubulin subunit in the interfacial region, and its amide nitrogen formed a critical hydrogen bonding (2.13 Å) with Ser*α*178 at the interfacial surface. The Surflex docking score is 9.09.

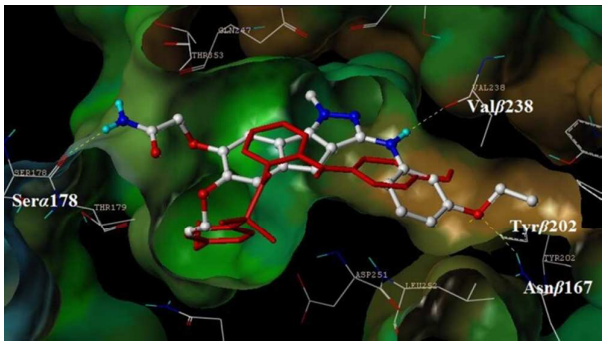


Figure 7S. ABT-751 (red) and binding mode of **6a** (grey).

In summary, with the full consideration of the hydrophobic interactions with the three zones in the tubulin binding pocket, the hydrogen bindings, and the Surflex docking scores, we concluded that **6a** could bind to tubulin in the similar way as that of ABT-751.

The medium potent compound **6b** showed similar binding modes as that of **6a**. In comparison with **6a**, the amino group in the *m*-ethoxyaniline moiety of **6b** did not have hydrogen bonding with Val*β*238 in zone 2 (Figure 8S).

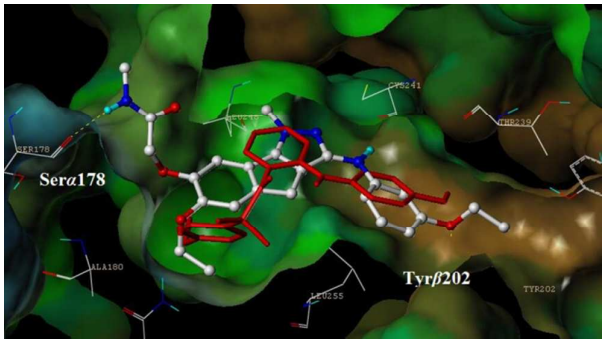


Figure 8S. ABT-751 (red) and binding mode of **6b** (grey).

In comparison with **6a**, the amide nitrogen of **6k** at the 7-position could not form hydrogen bonding with Serα178. Moreover, the cyclopropylmethyl portion protruded out of the interfacial surface (Figure 9S).

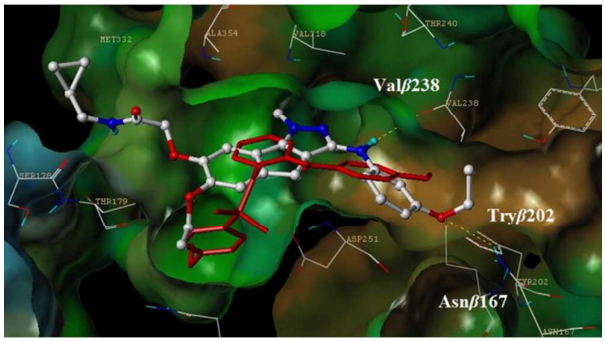
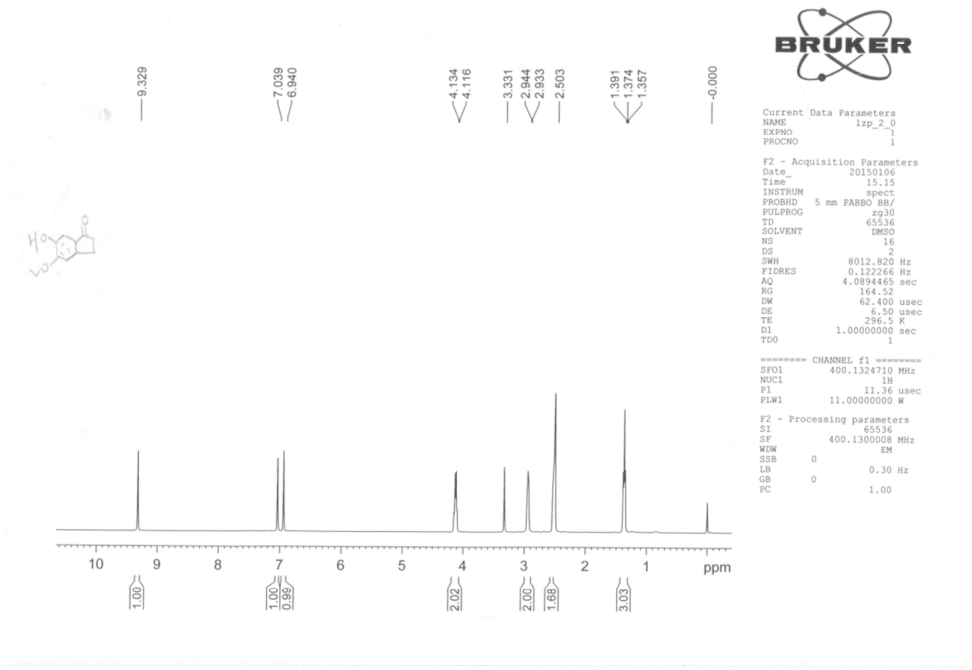


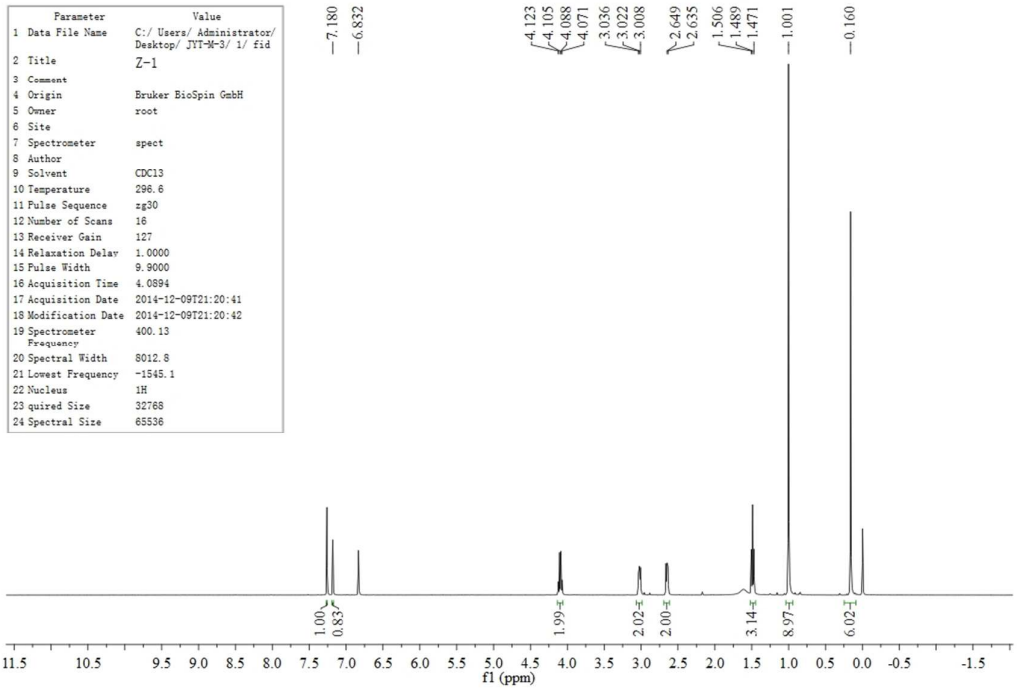
Figure 9S. ABT-751 (red) and binding mode of **6k** (grey).

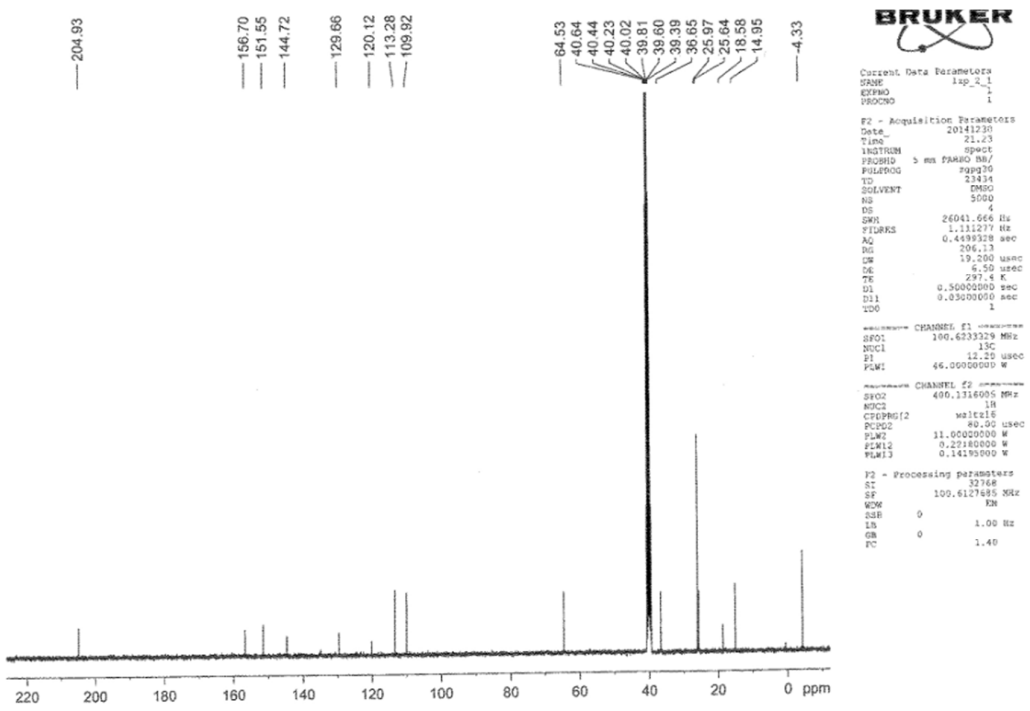
3. ^{1}H and ^{13}C NMR spectra for all the new compounds

5-Ethoxy-6-hydroxyl-1-indanone (8).

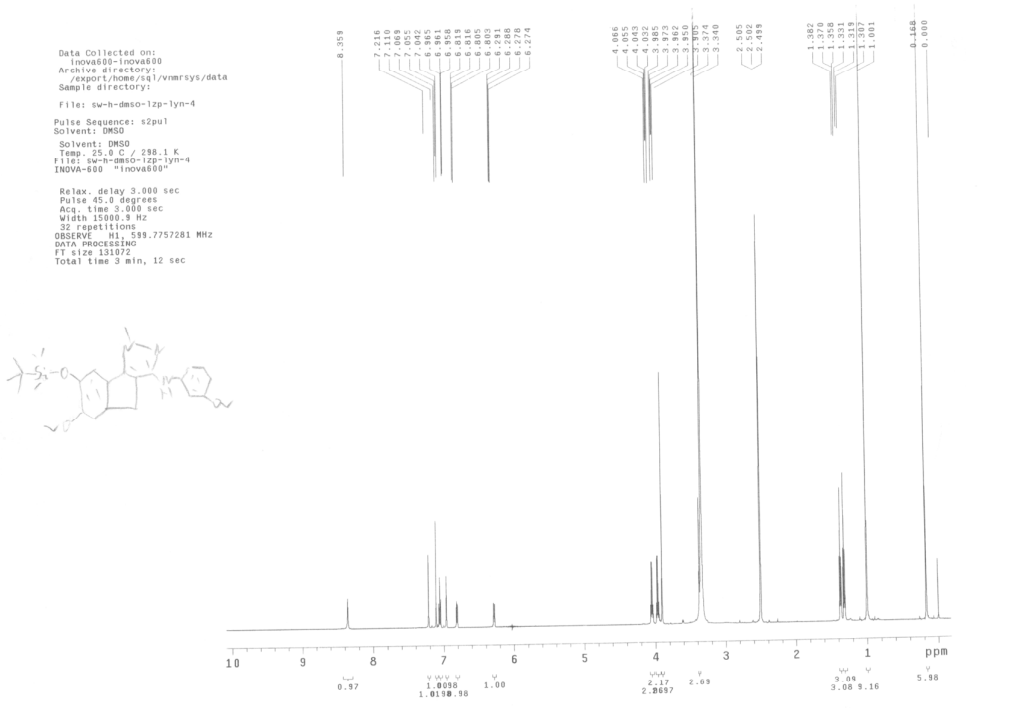


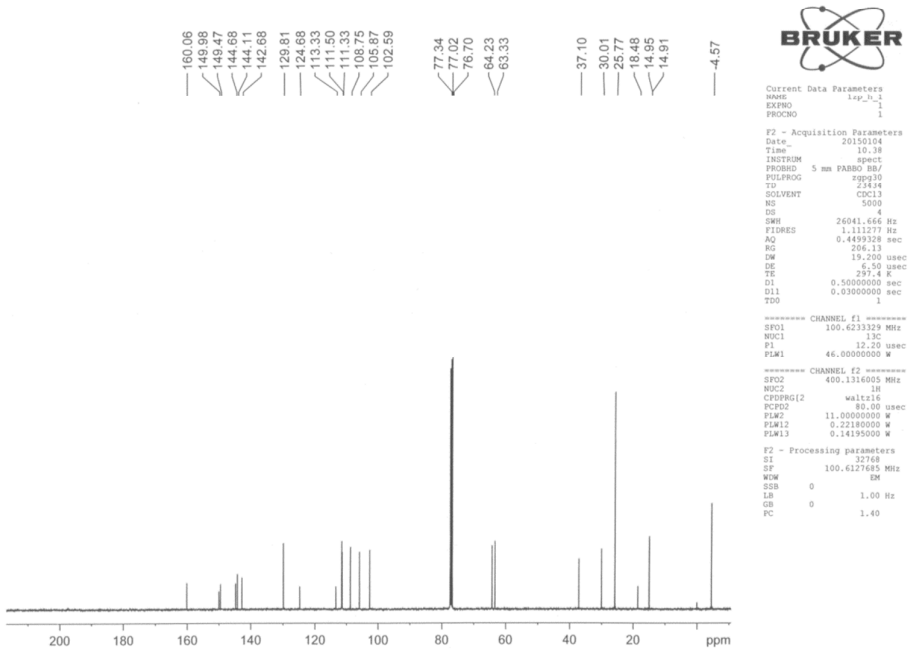
5-Ethoxy-6-*tert*-butyldimethylsiloxy-1-indanone (9).



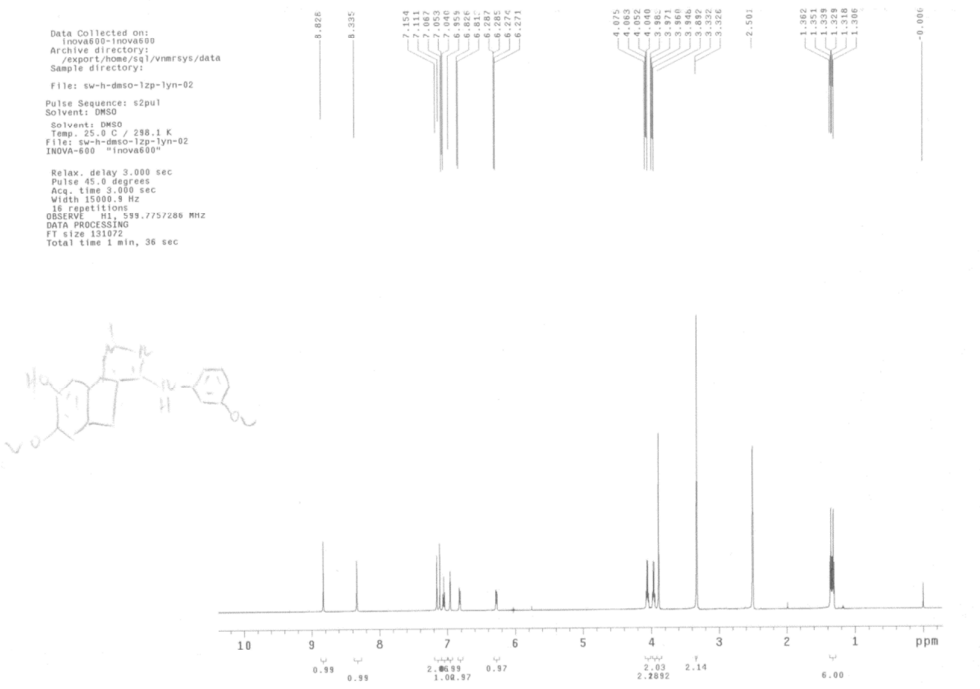


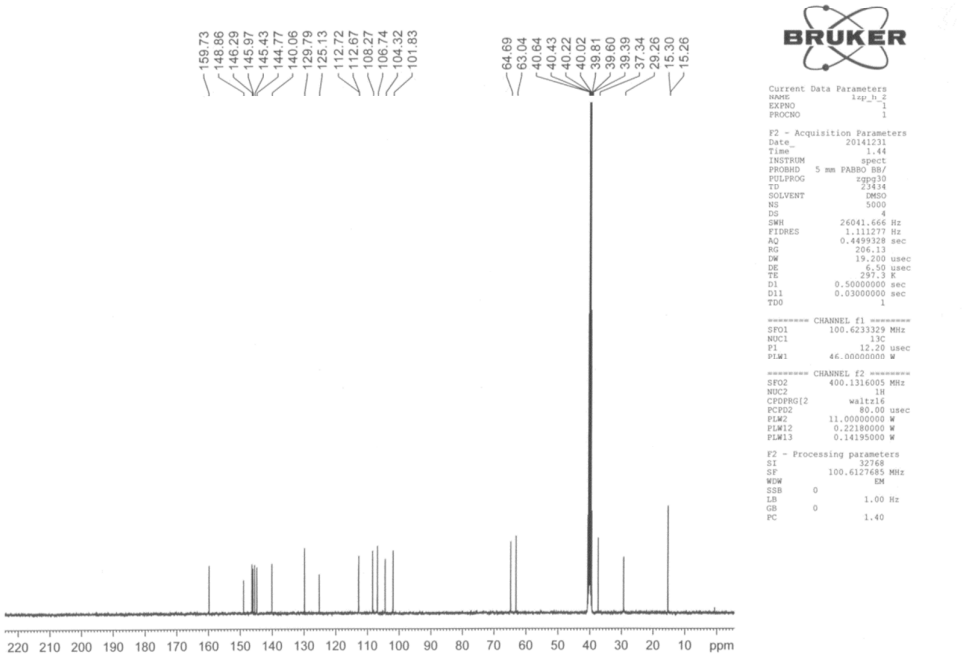
7-(*tert*-Butyldimethylsilyloxy)-6-ethoxy-N-(3-ethoxyphenyl)-1-methyl-1,4-dihydroindeno[1,2-*c*]pyrazol-3-amine (10).



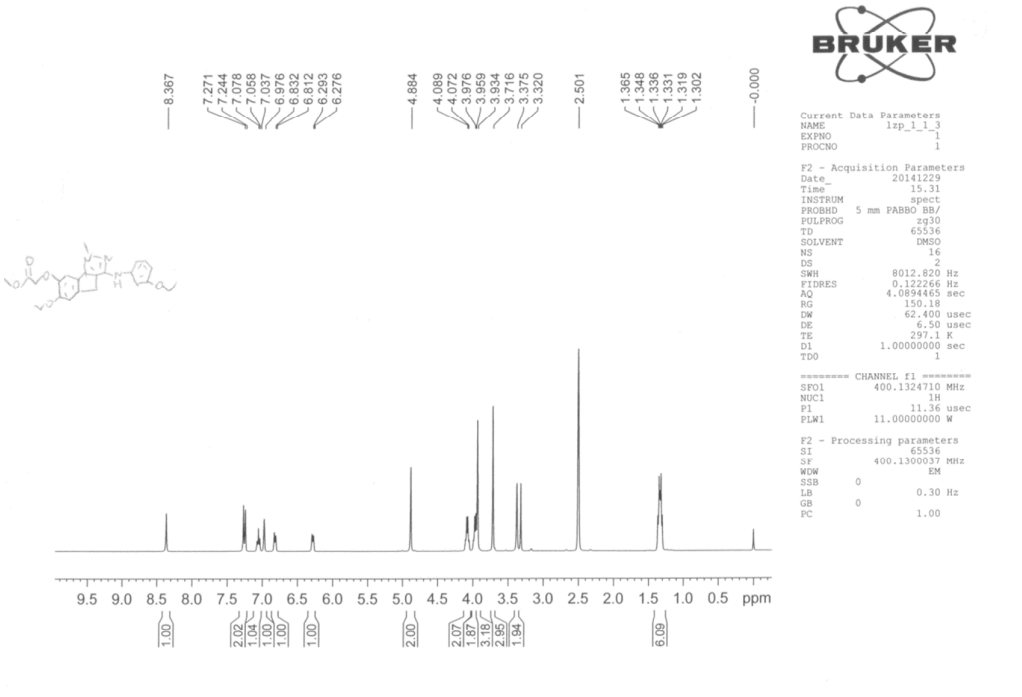


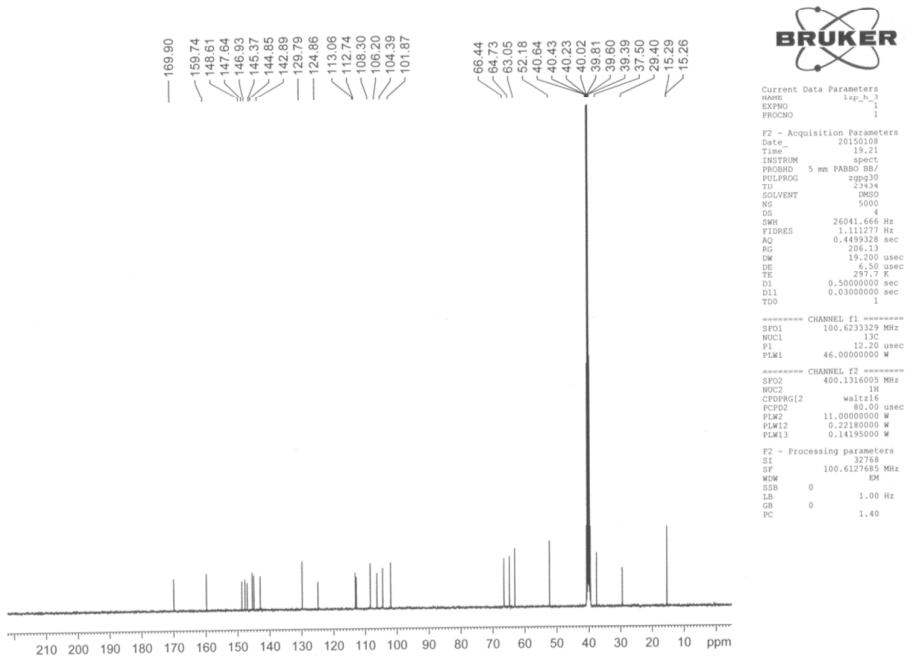
6-Ethoxy-3-(3-ethoxyphenylamino)-1-methyl-1,4-dihydroindeno[1,2-*c*]pyrazol-7-ol (11).



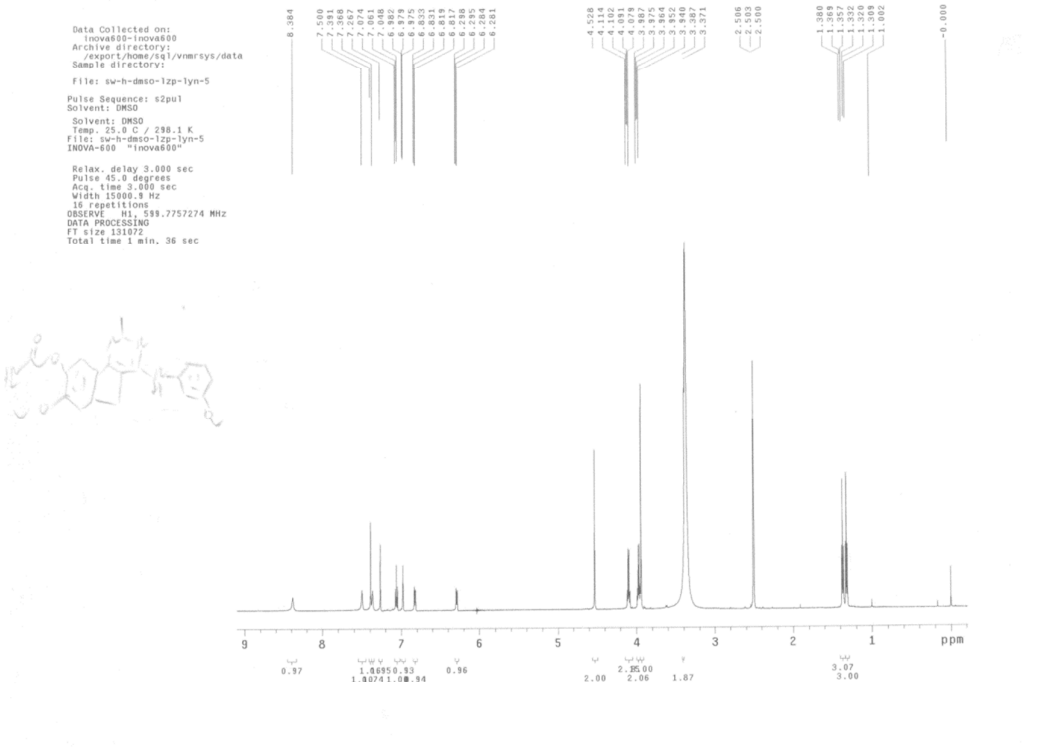


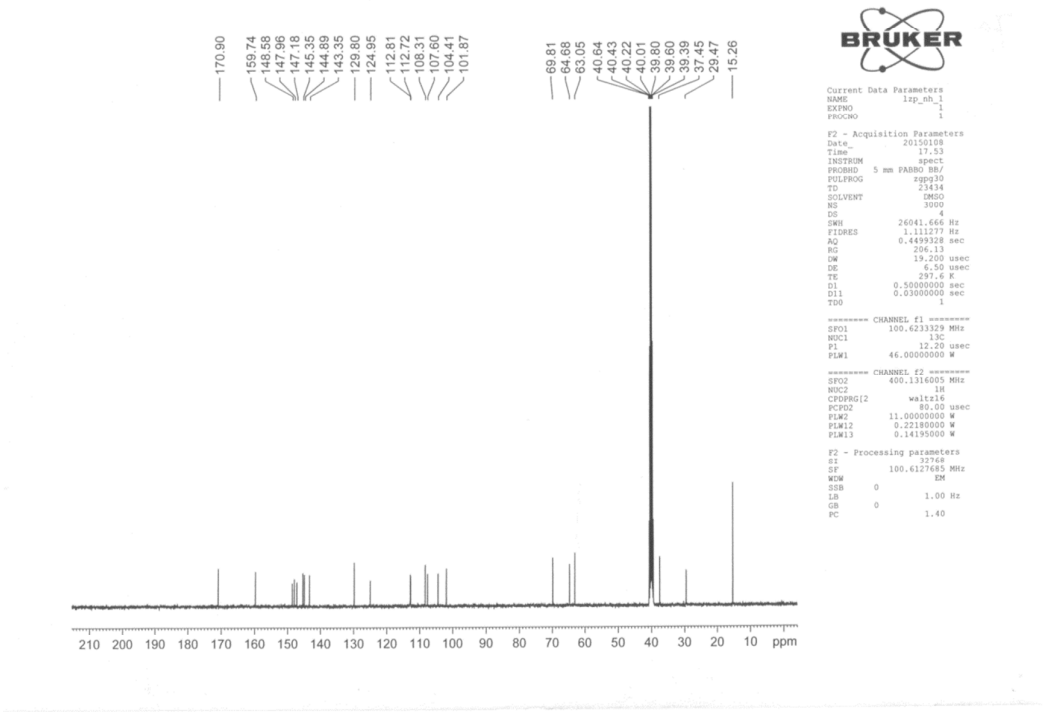
Methyl 2-(6-ethoxy-3-(3-ethoxyphenylamino)-1-methyl-1,4-dihydroindeno[1,2-*c*]pyrazol-7-yloxy)acetate (12).



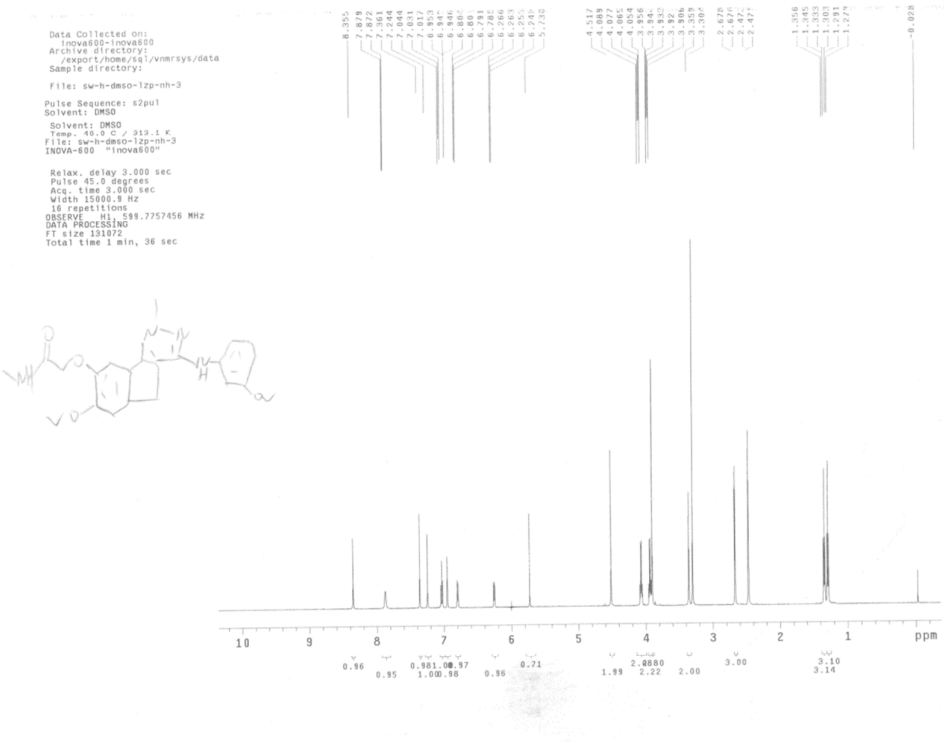


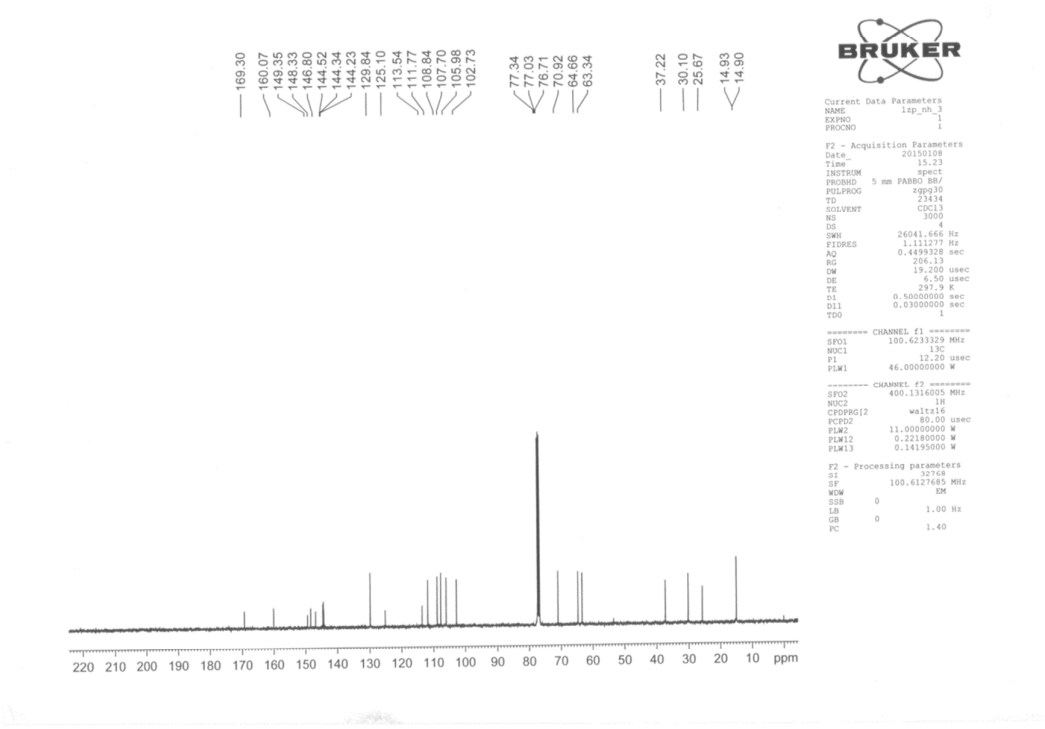
2-(6-Ethoxy-3-(3-ethoxyphenylamino)-1-methyl-1,4-dihydroindeno[1,2-*c*]pyrazol-7-yloxy)acetamide (6a).



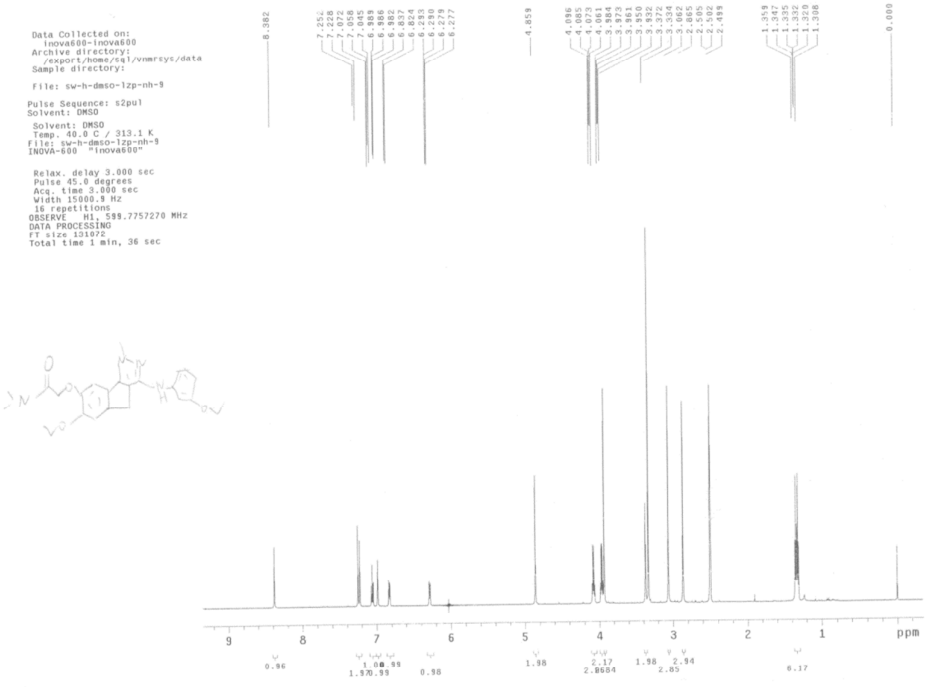


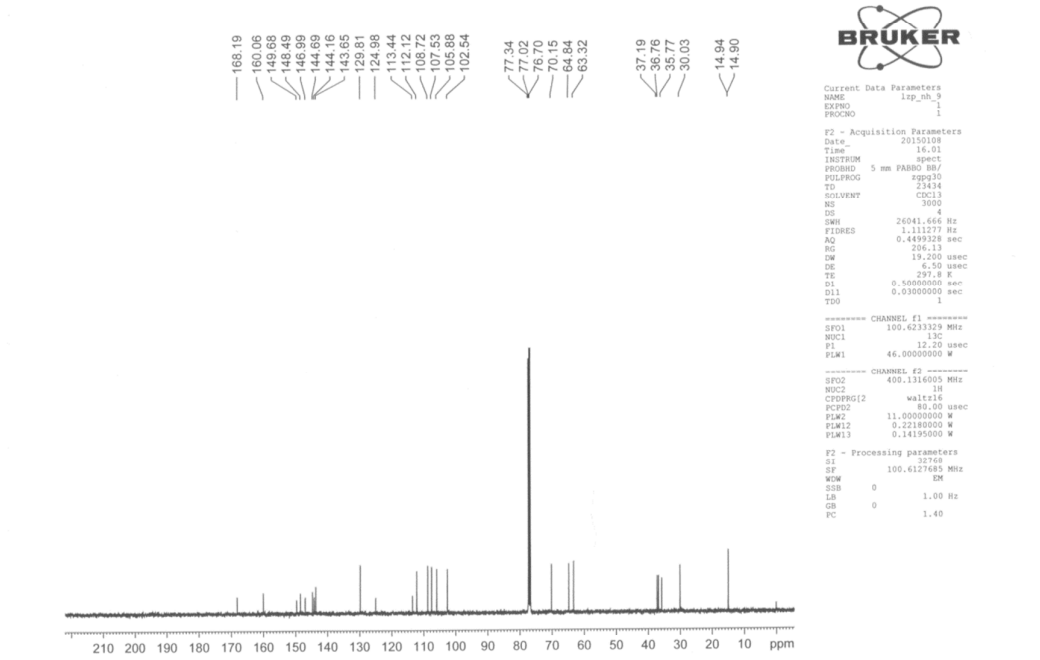
2-(6-Ethoxy-3-(3-ethoxyphenylamino)-1-methyl-1,4-dihydroindeno[1,2-*c*]pyrazol-7-yloxy)-*N*-methylacetamide (6b).



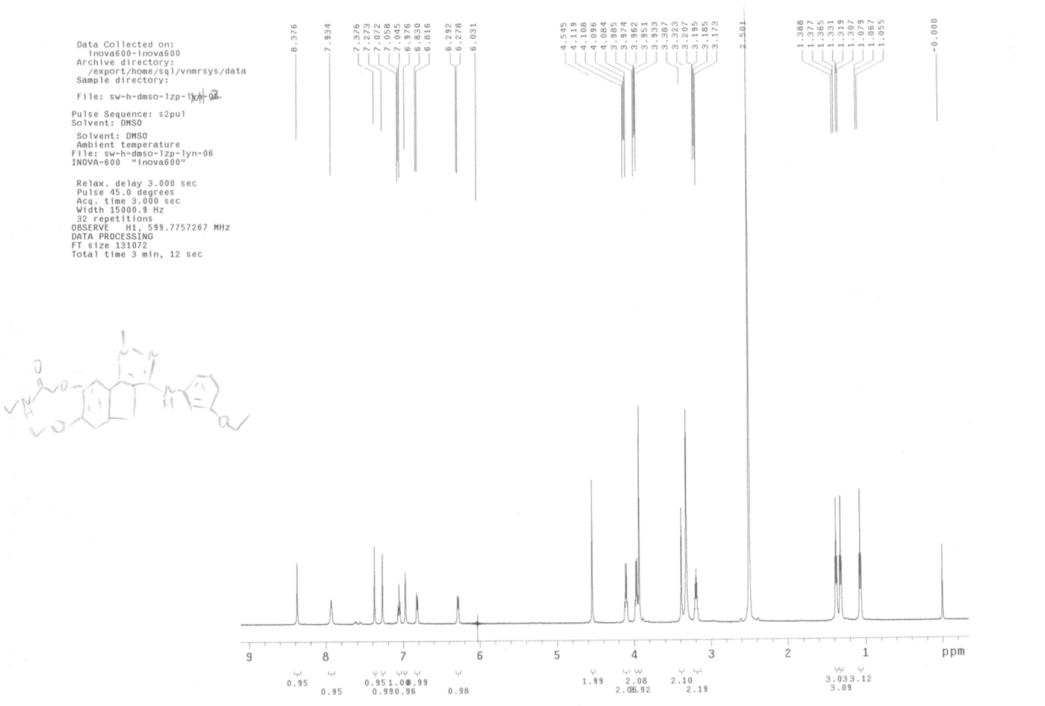


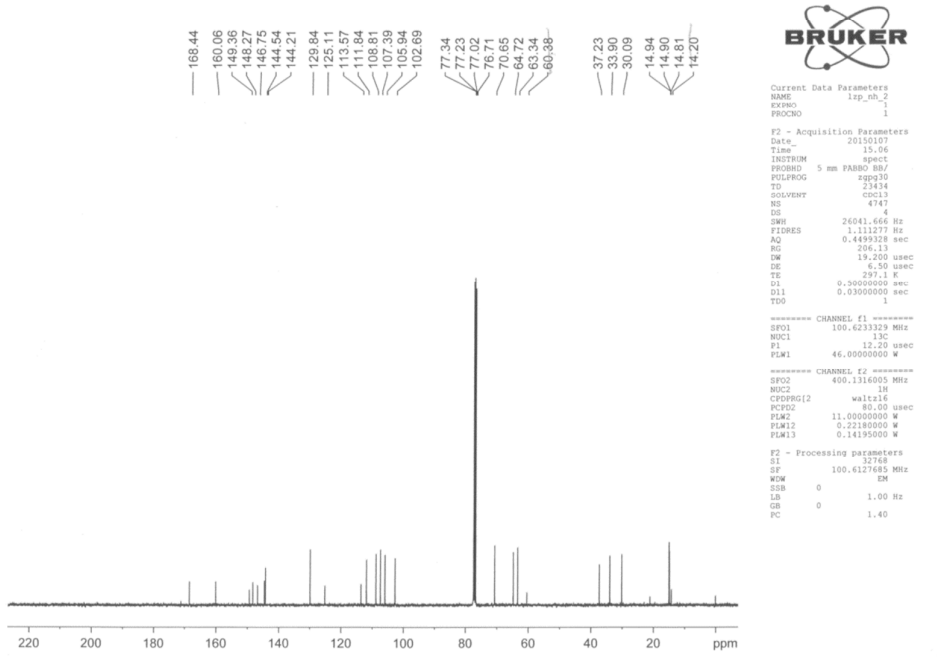
2-(6-Ethoxy-3-(3-ethoxyphenylamino)-1-methyl-1,4-dihydroindeno[1,2-*c*]pyrazol-7-yloxy)-*N*,*N*-dimethylacetamide (6c).



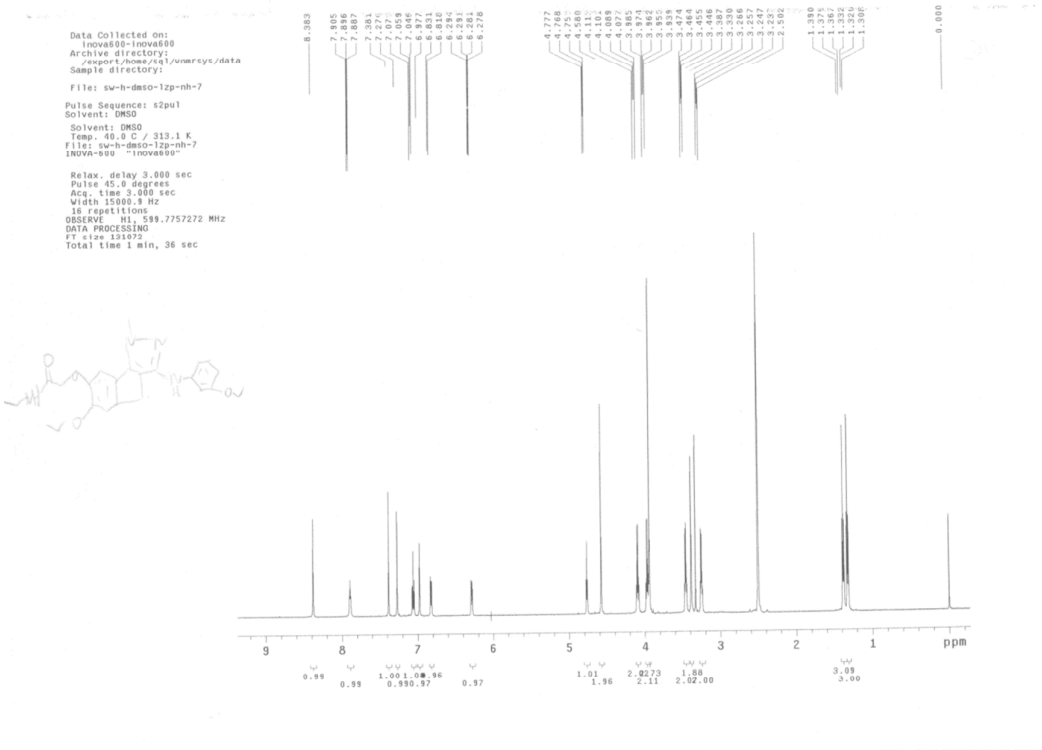


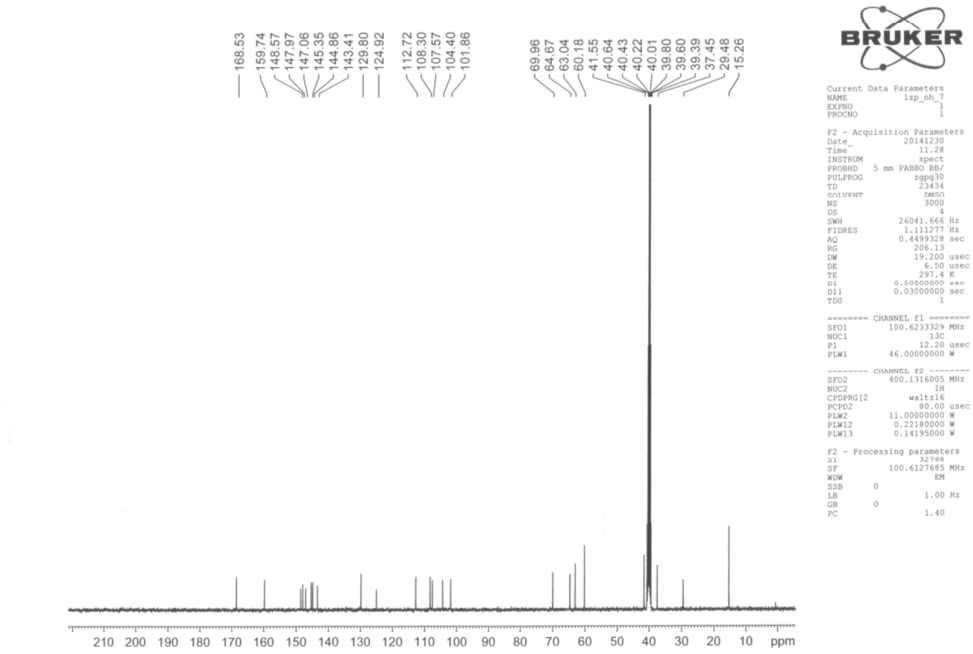
2-(6-Ethoxy-3-(3-ethoxyphenylamino)-1-methyl-1,4-dihydroindeno[1,2-*c*]pyrazol-7-yloxy)-*N*-ethylacetamide (6d).



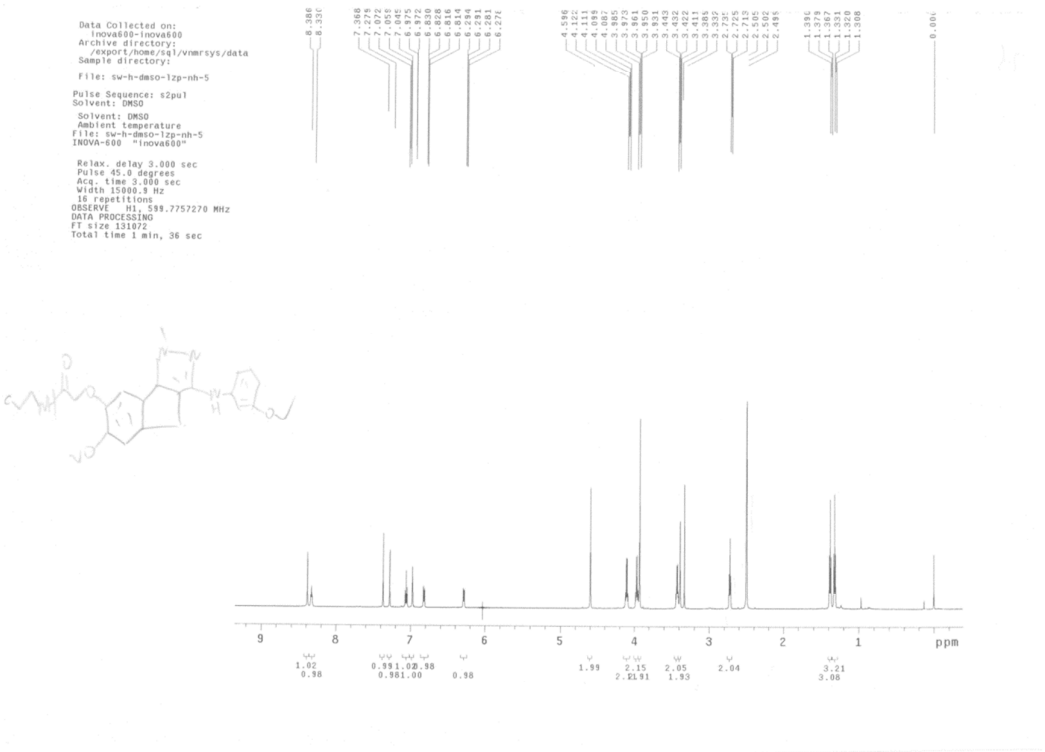


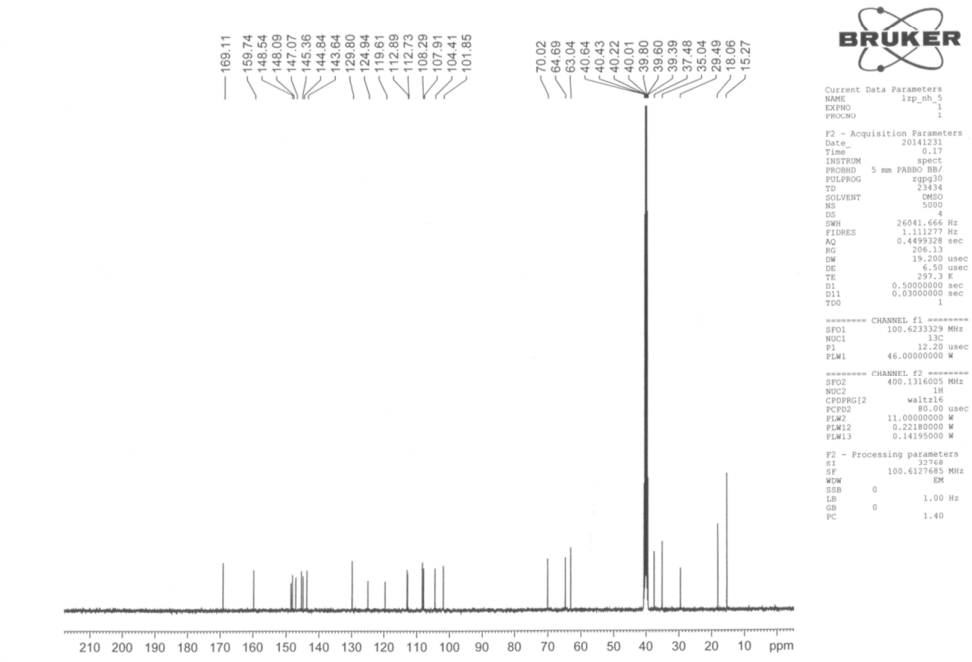
2-(6-Ethoxy-3-(3-ethoxyphenylamino)-1-methyl-1,4-dihydroindeno[1,2-*c*]pyrazol-7-yloxy)-*N*-(2-hydroxyethyl)acetamide (6e).



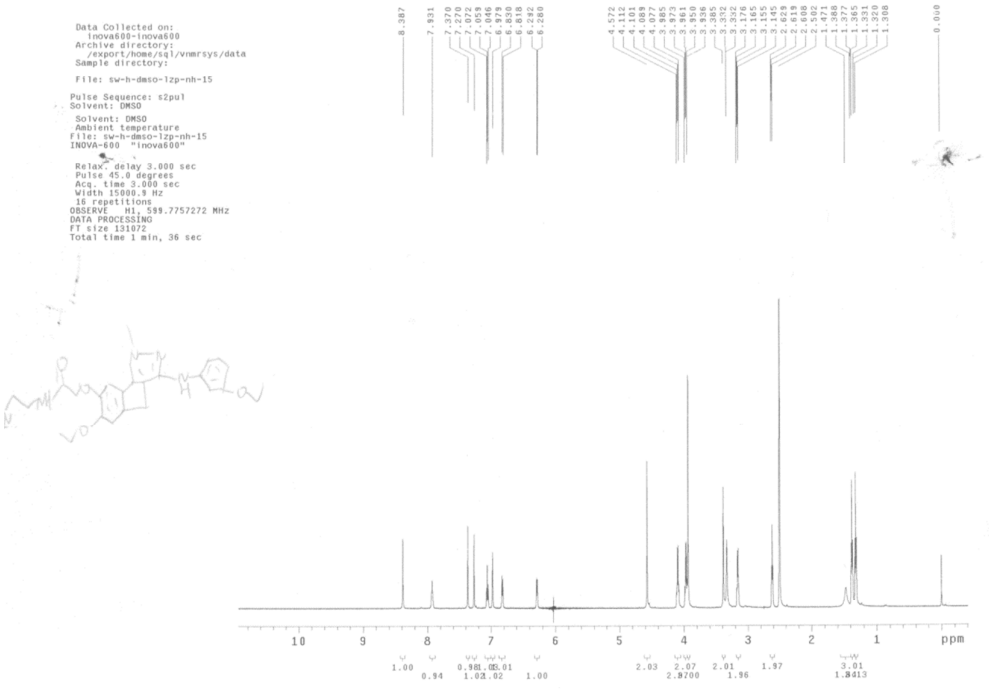


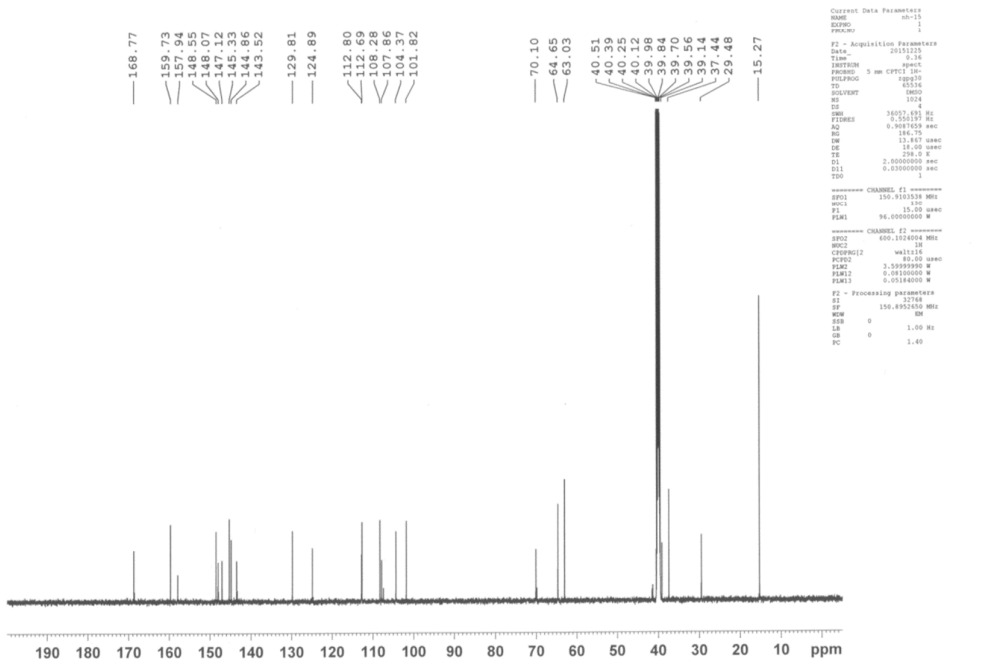
***N*-(2-Cyanoethyl)-2-(6-ethoxy-3-(3-ethoxyphenylamino)-1-methyl-1,4-dihydroindeno[1,2-*c*]pyrazol-7-yloxy)acetamide (6f).**



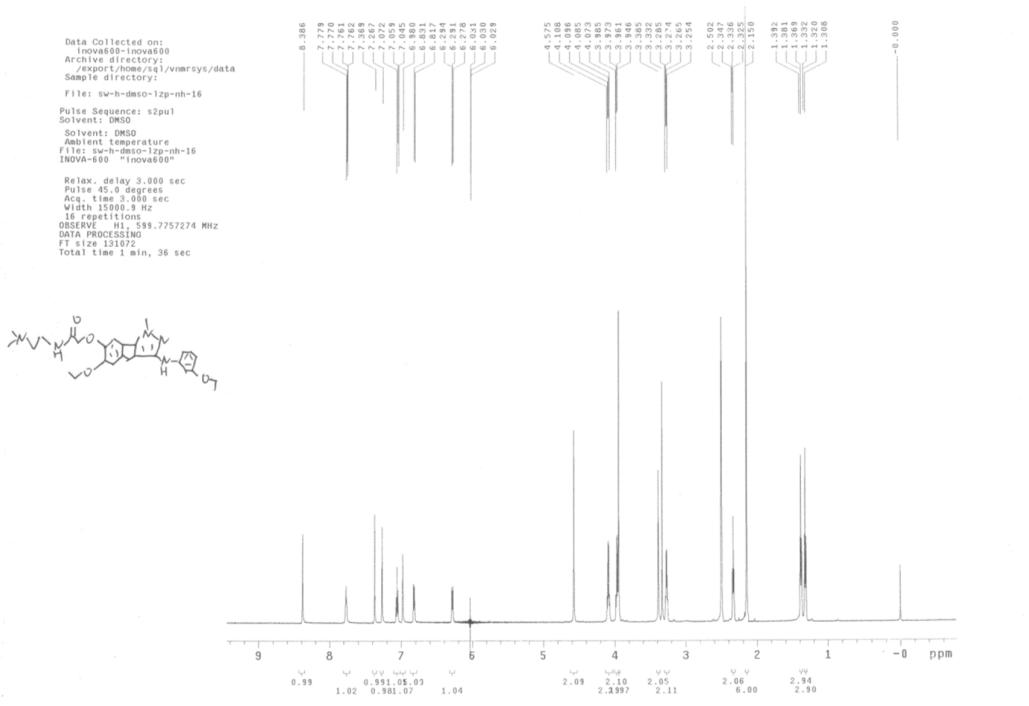


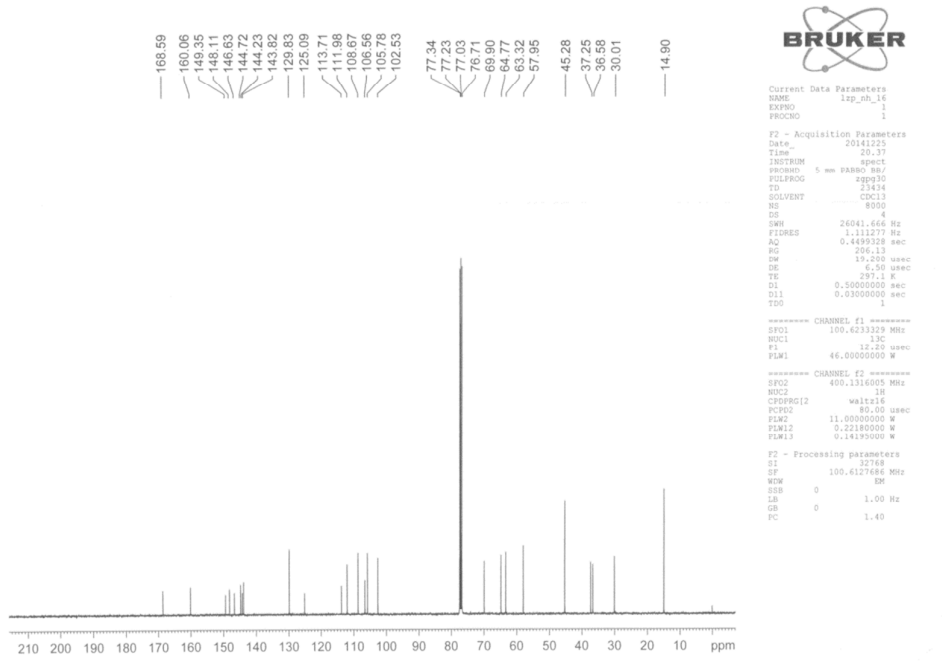
***N*-(2-Aminoethyl)-2-(6-ethoxy-3-(3-ethoxyphenylamino)-1-methyl-1,4-dihydroindeno[1,2-c]pyrazol-7-yloxy)acetamide (6g).**



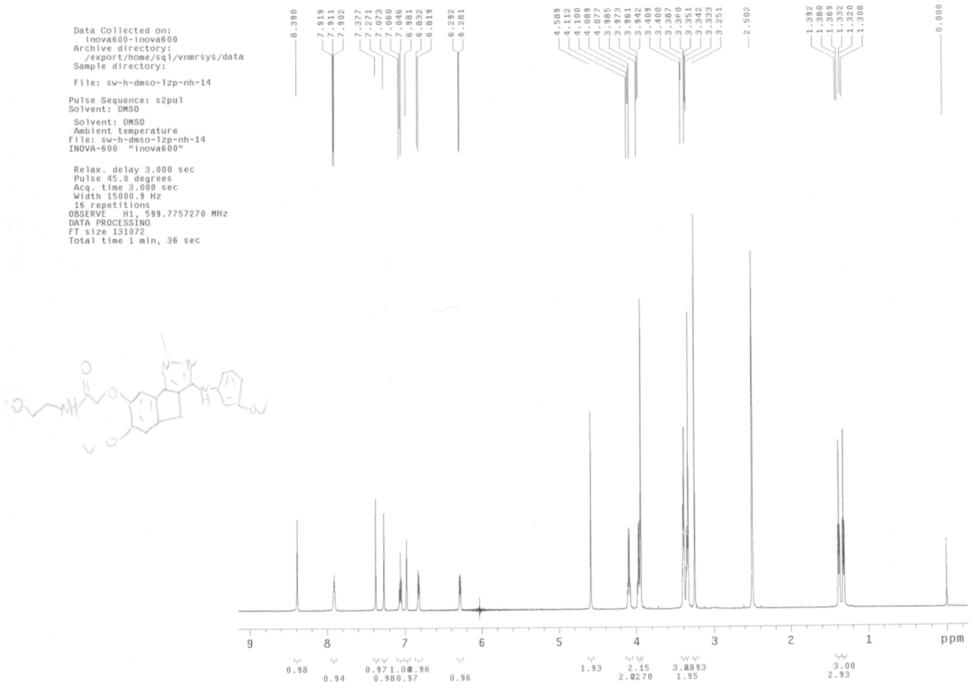


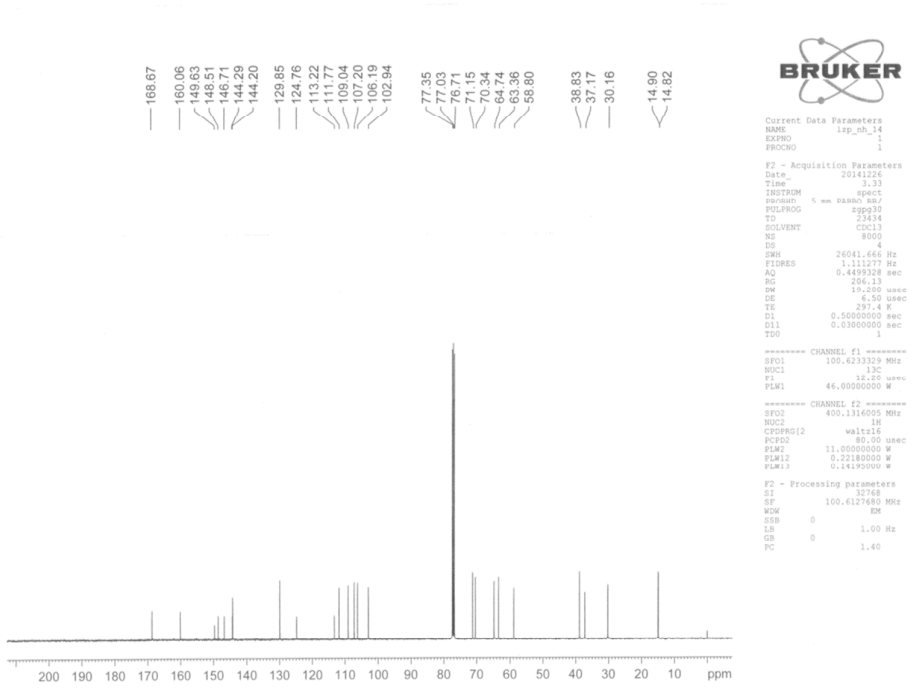
***N*-(2-(Dimethylamino)ethyl)-2-(6-ethoxy-3-(3-ethoxyphenylamino)-1-methyl-1,4-dihydroindeno[1,2-*c*]pyrazol-7-yloxy)acetamide (6h).**



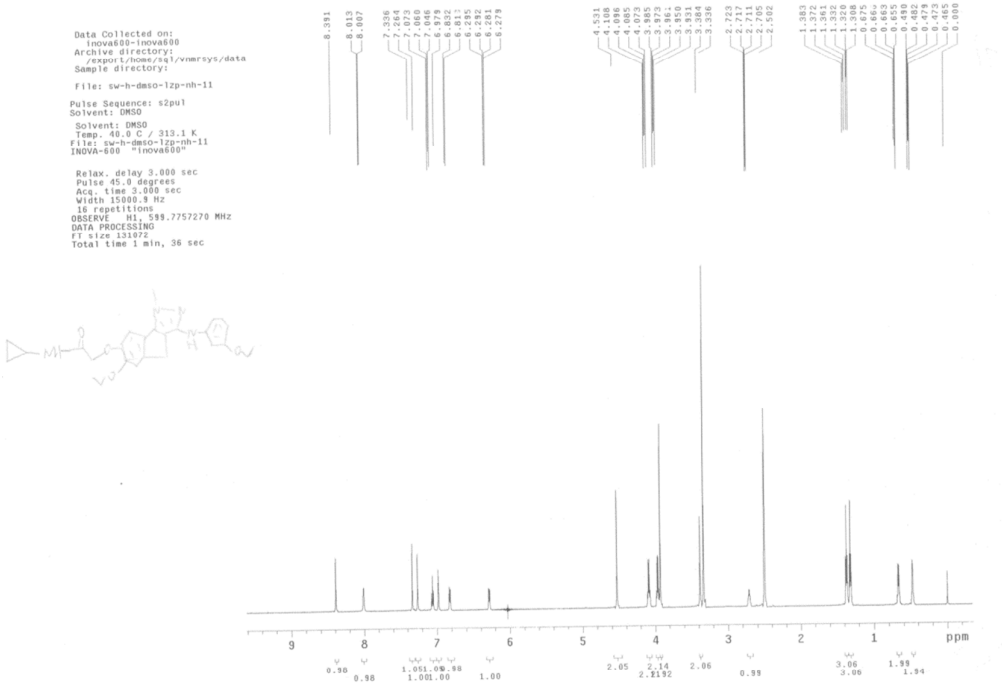


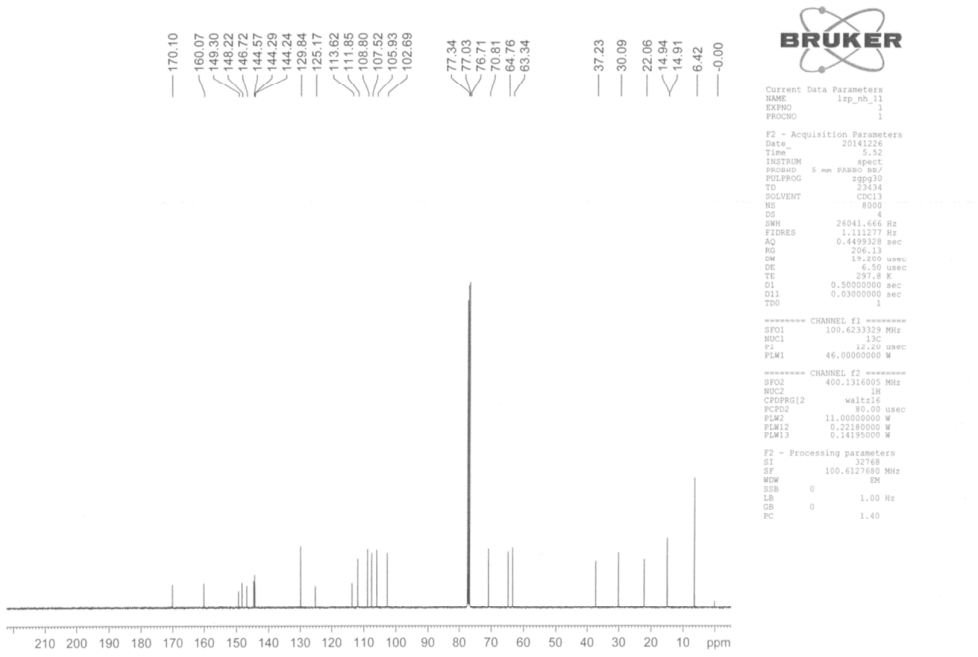
2-(6-Ethoxy-3-(3-ethoxyphenylamino)-1-methyl-1,4-dihydroindeno[1,2-*c*]pyrazol-7-yloxy)-*N*-(2-methoxyethyl)acetamide (6i).



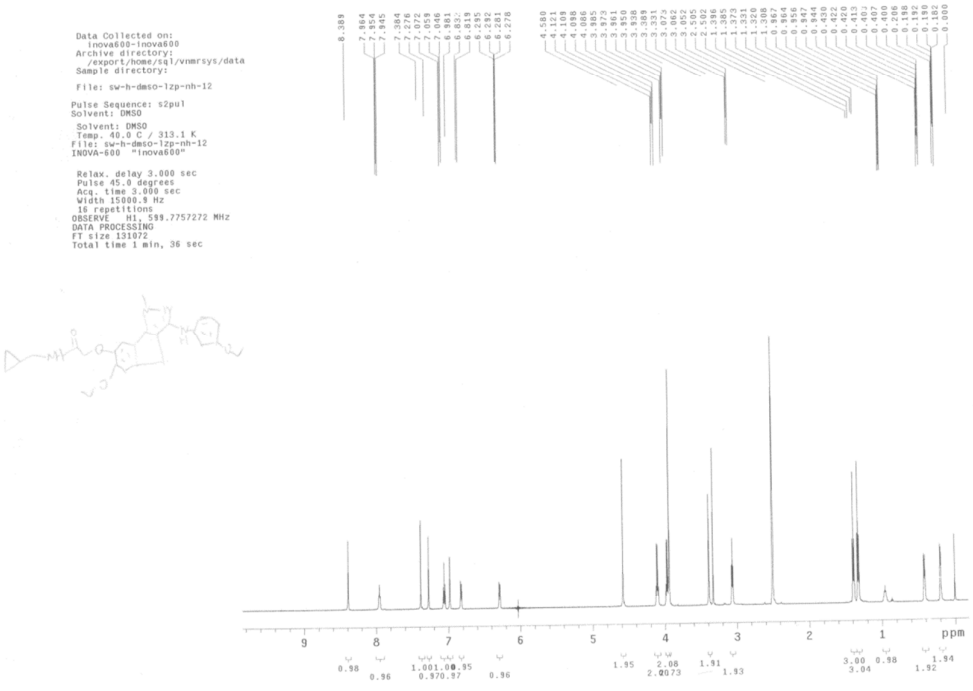


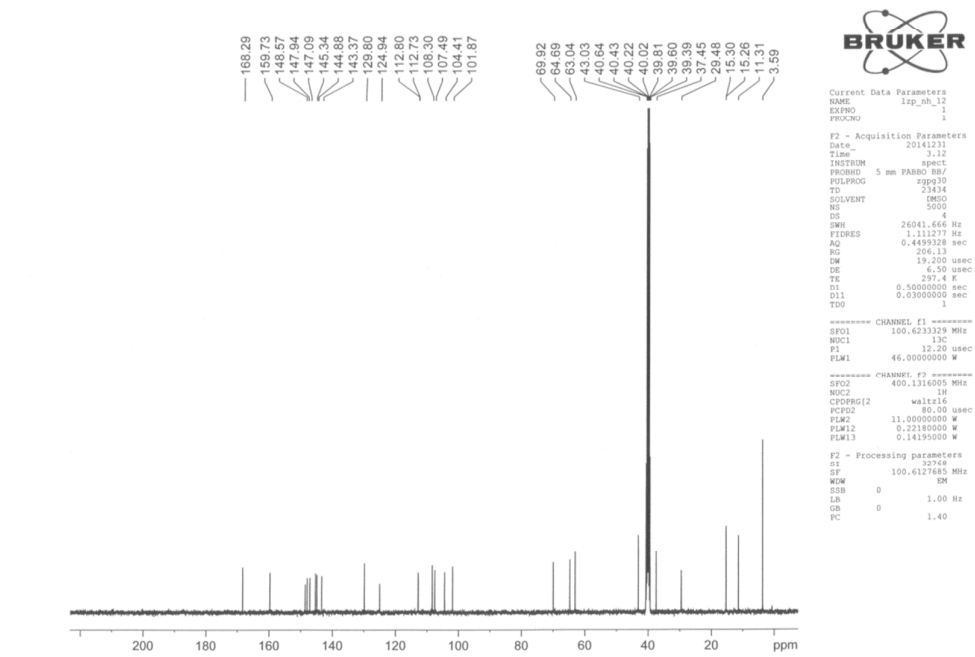
_N_-Cyclopropyl-2-(6-ethoxy-3-(3-ethoxyphenylamino)-1-methyl-1,4-dihydroindeno[1,2-*c*]pyrazol-7-yloxy)acetamide (6j).



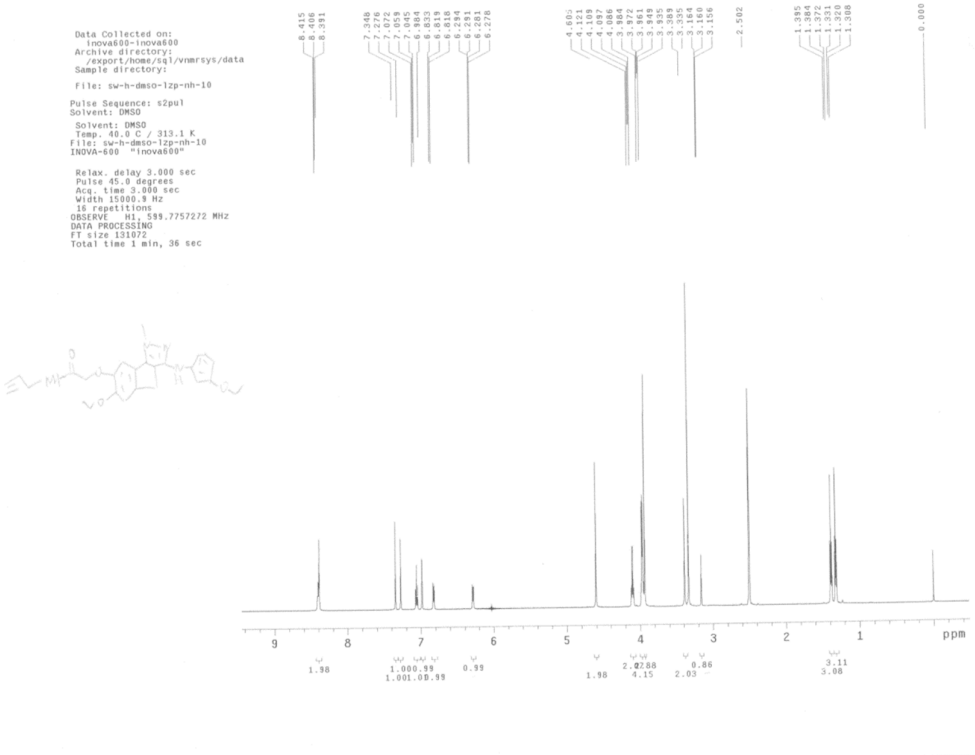


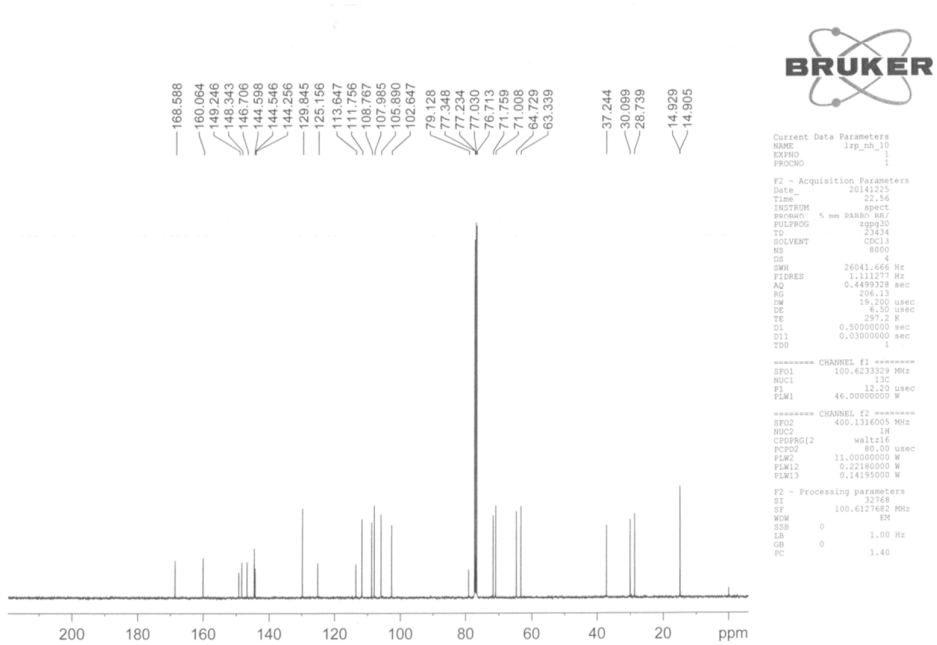
_N_-(Cyclopropylmethyl)-2-(6-ethoxy-3-(3-ethoxyphenylamino)-1-methyl-1,4-dihydroindeno[1,2-*c*] pyrazol-7-yloxy)acetamide (6k).



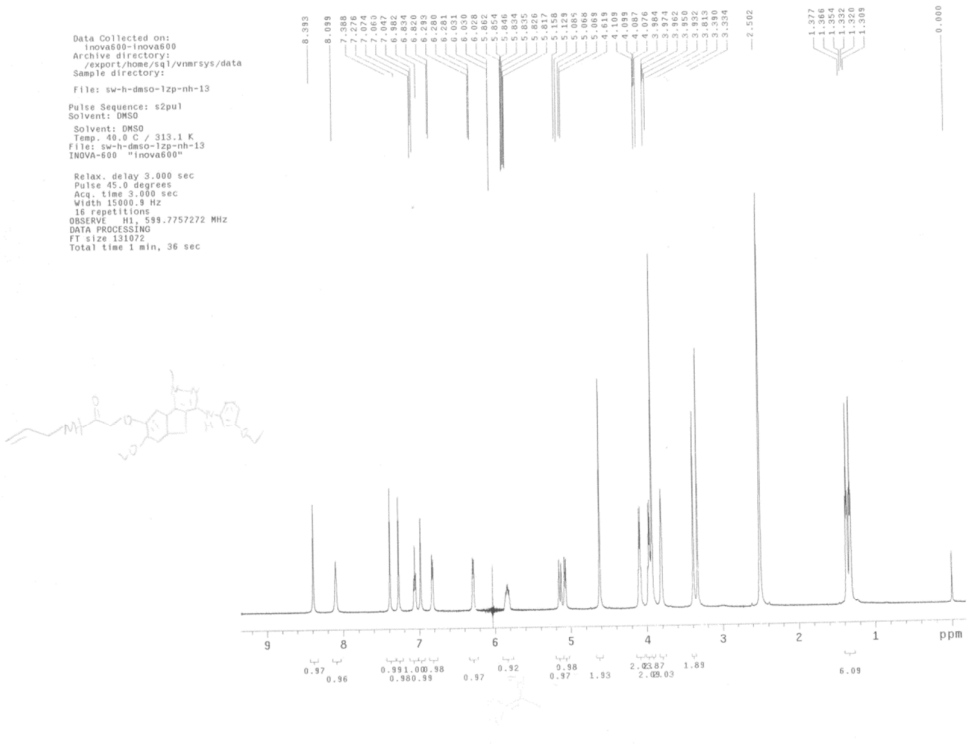


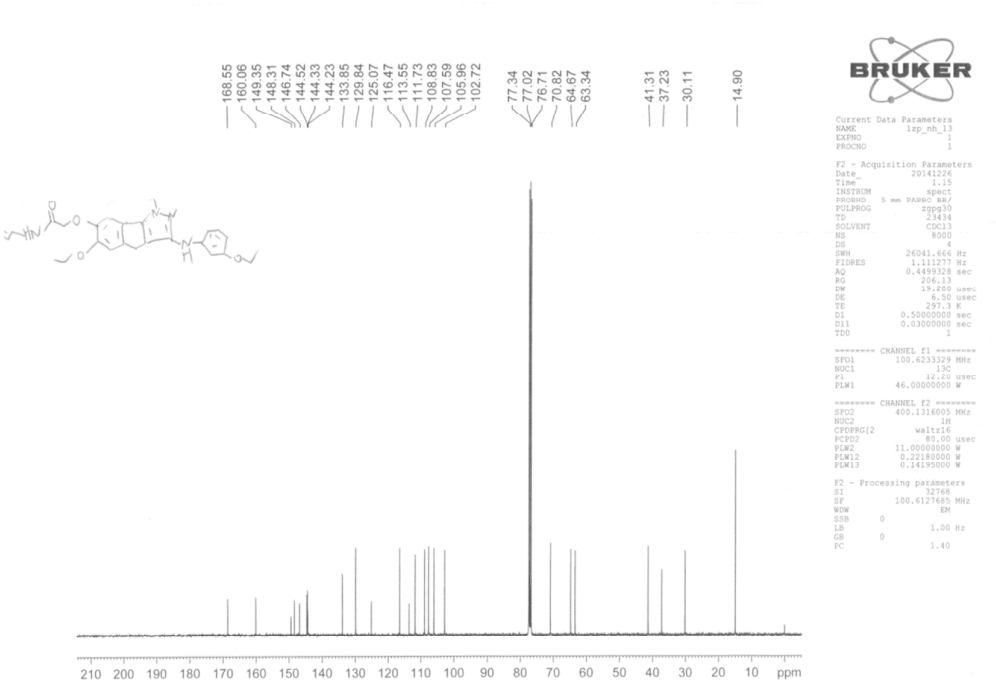
2-(6-Ethoxy-3-(3-ethoxyphenylamino)-1-methyl-1,4-dihydroindeno[1,2-c]pyrazol-7-yloxy)-*N*-(prop-2-ynyl)acetamide (6l).



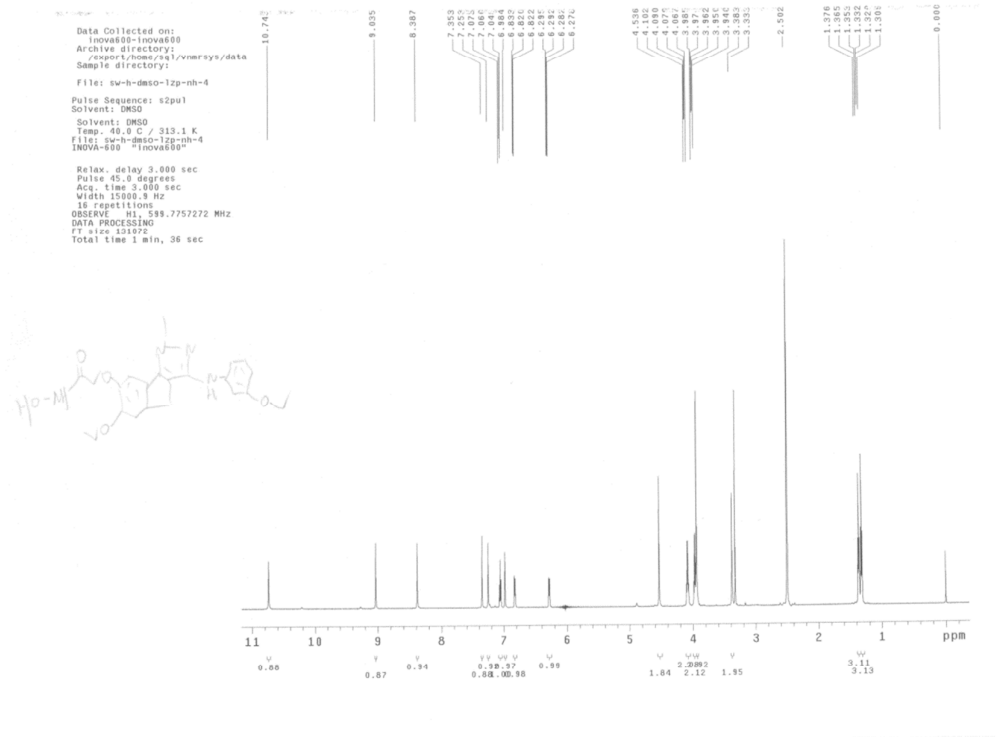


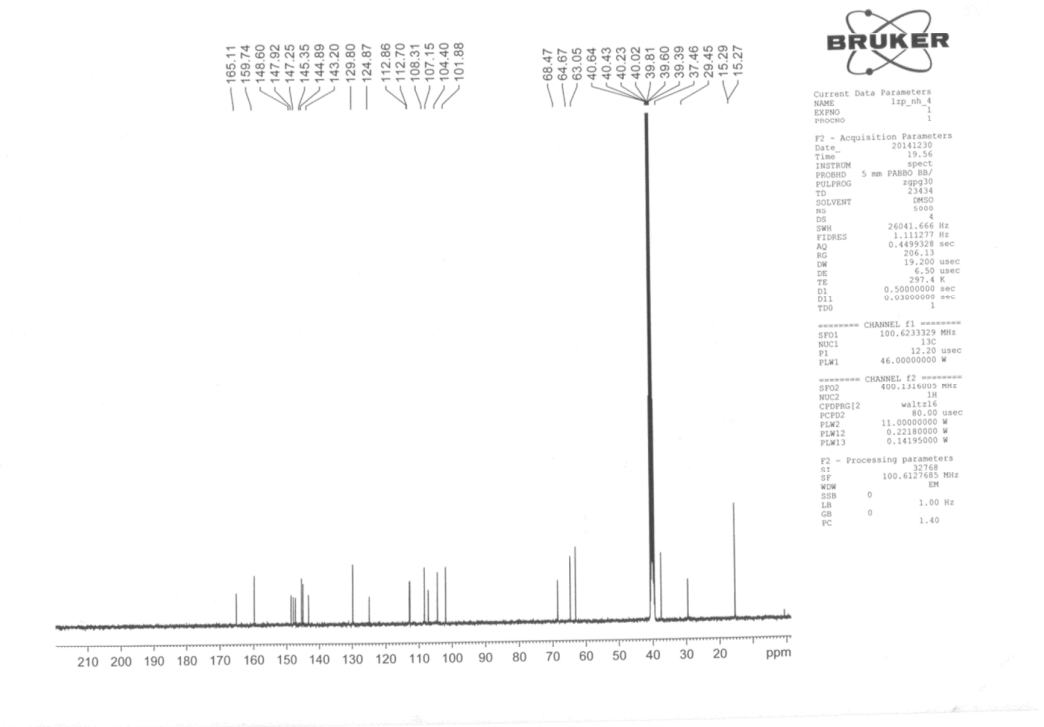
_N_-Allyl-2-(6-ethoxy-3-(3-ethoxyphenylamino)-1-methyl-1,4-dihydroindeno[1,2-*c*]pyrazol-7-yloxy) acetamide (6m).



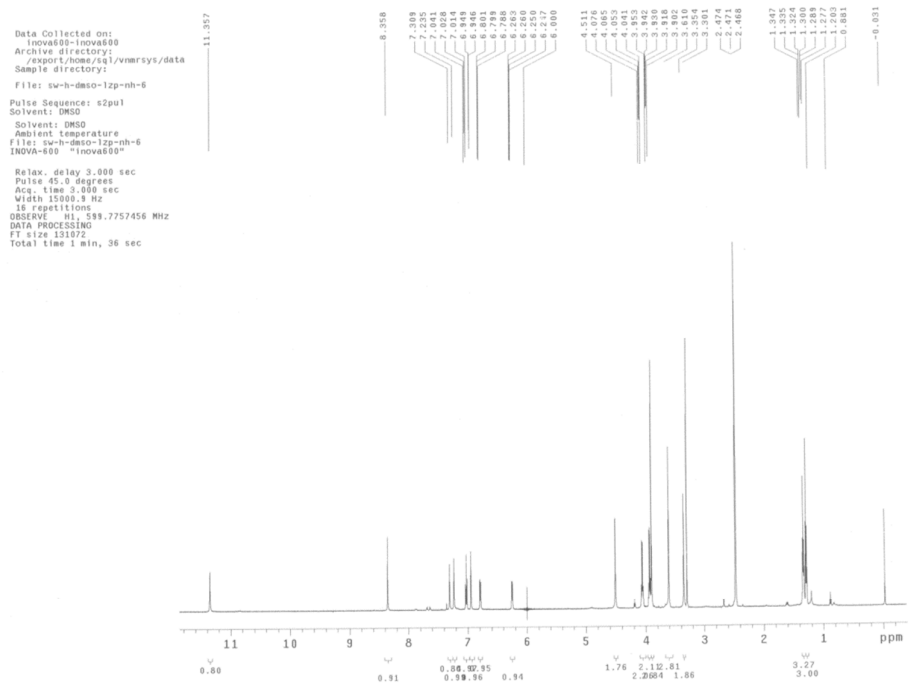


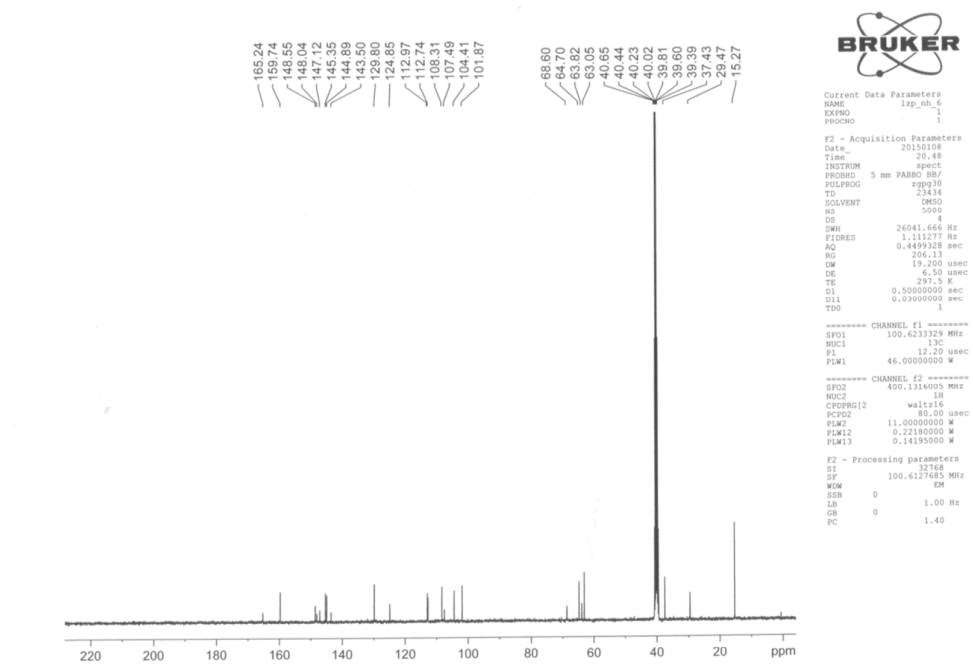
2-(6-Ethoxy-3-(3-ethoxyphenylamino)-1-methyl-1,4-dihydroindeno[1,2-*c*]pyrazol-7-yloxy)-*N*-hydroxyacetamide (6n).



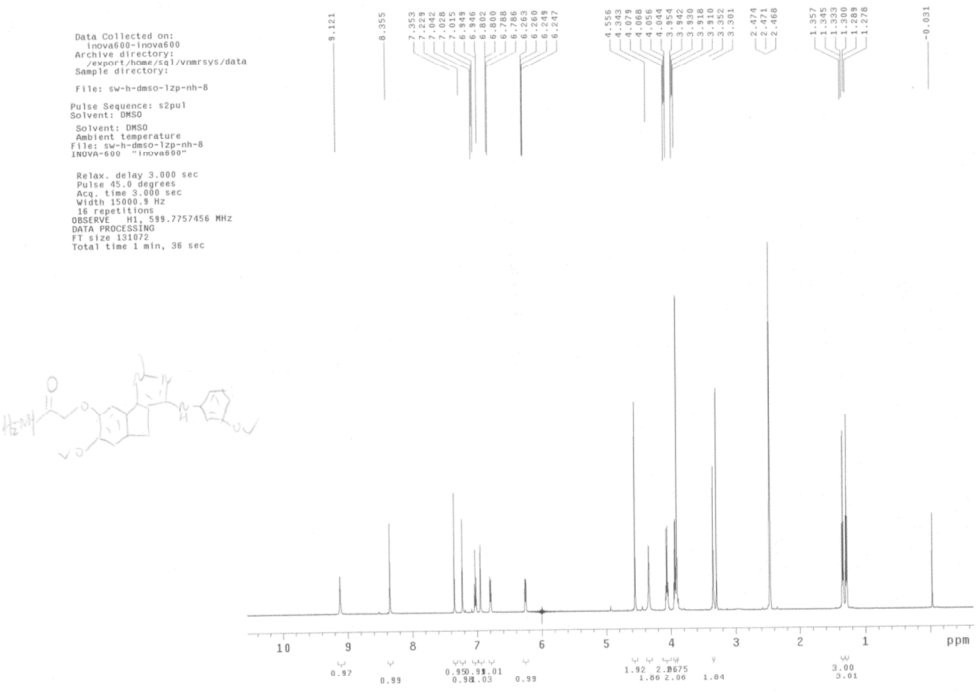


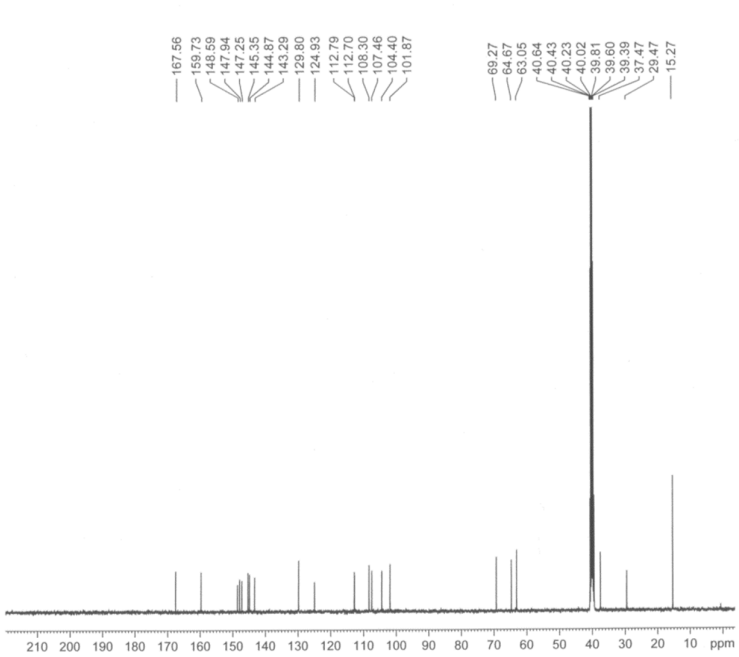
2-(6-Ethoxy-3-(3-ethoxyphenylamino)-1-methyl-1,4-dihydroindeno[1,2-*c*]pyrazol-7-yloxy)-*N*-methoxyacetamide (6o).





2-(6-Ethoxy-3-(3-ethoxyphenylamino)-1-methyl-1,4-dihydroindeno[1,2-*c*]pyrazol-7-yloxy)acetohydrazide (6p).





BRUKER

```
Current Data Parameters
NAME                  lzp_nh_8
EXPNO                        1
PROCNO                       1

F2 - Acquisition Parameters
Date_              20141230
Time                  22.50
INSTRUM                spect
PROBHD  5 mm PABBO BB/
PULPROG               zgpg30
TD                     23434
SOLVENT                DMSO
NS                      5000
DS                         4
SWH               26041.666 Hz
FIDRES             1.111277 Hz
AQ                0.4499328 sec
RG                    206.13
DW                   19.200 usec
DE                    6.50 usec
TE                   297.3 K
D1                0.50000000 sec
D11               0.03000000 sec
TD0                        1

======== CHANNEL f1 ========
SFO1             100.6233329 MHz
NUC1                     13C
P1                     12.20 usec
PLW1             46.00000000 W

======== CHANNEL f2 ========
SFO2             400.1316005 MHz
NUC2                     1H
CPDPRG[2              waltz16
PCPD2                  80.00 usec
PLW2             11.00000000 W
PLW12             0.22180000 W
PLW13             0.14195000 W

F2 - Processing parameters
SI                       32768
SF               100.6127685 MHz
WDW                         EM
SSB          0
LB                      1.00 Hz
GB           0
PC                      1.40
```