Accepted Manuscript

Synthesis and Molecular Docking Studies of Potent #-Glucosidase Inhibitors Based on Biscoumarin Skeleton

Khalid Mohammed Khan, Fazal Rahim, Abdul Wadood, Naveen Kosar, Muhammad Taha, Salima Lalani, Aisha Khan, Muhammad Imran Fakhri, Muhammad Junaid, Wajid Rehman, Momin Khan, Shahnaz Perveen, Muhammad Sajid, M. Iqbal Choudhary

PII: S0223-5234(14)00424-3

DOI: 10.1016/j.ejmech.2014.05.010

Reference: EJMECH 6966

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 6 January 2014

Revised Date: 4 March 2014

Accepted Date: 2 May 2014

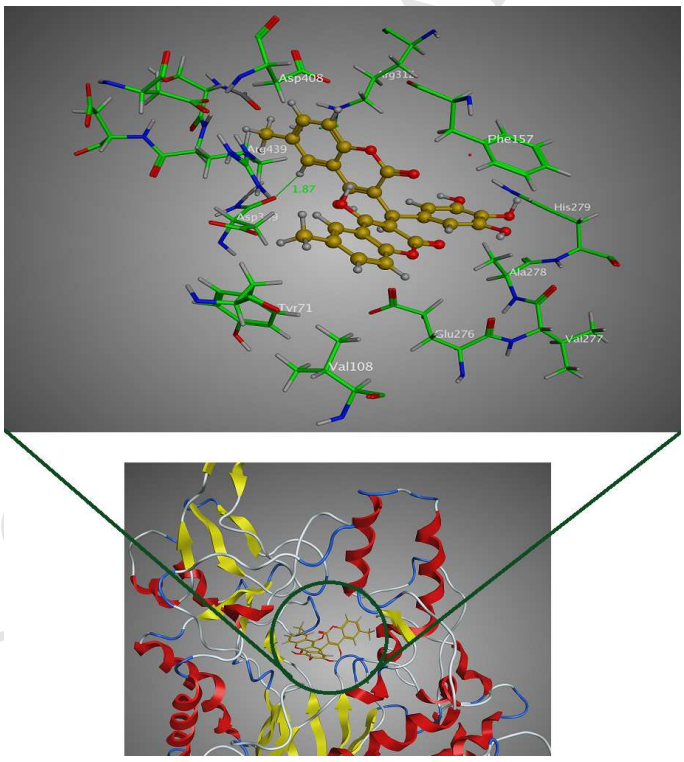
Please cite this article as: K.M. Khan, F. Rahim, A. Wadood, N. Kosar, M. Taha, S. Lalani, A. Khan, M.I. Fakhri, M. Junaid, W. Rehman, M. Khan, S. Perveen, M. Sajid, M.I. Choudhary, Synthesis and Molecular Docking Studies of Potent #-Glucosidase Inhibitors Based on Biscoumarin Skeleton, *European Journal of Medicinal Chemistry* (2014), doi: 10.1016/j.ejmech.2014.05.010.

Synthesis and Molecular Docking studies of Potent *α*-Glucosidase Inhibitors Based on Biscoumarin Skeleton

Khalid Mohammed Khan,[a*] Fazal Rahim,[b] Abdul Wadood,[c] Naveen Kosar,[b] Muhammad Taha, [d,h] Salima Lalani,[a] Aisha Khan,[b] Muhammad Imran Fakhri,[a] Muhammad Junaid,[c] Wajid Rehman,[c] Momin Khan,[e] Shahnaz Perveen,[f] Muhmmad Sajid,[g] and M. Iqbal Choudhary[a]

[a]H. E. J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan
[b]Department of Chemistry, Hazara University, Mansehra, Pakistan
[c]Computational Medicinal Chemistry Laboratory, Department of Biochemistry, Abdul Wali Khan University, Mardan, Mardan-23200, Pakistan
[d]Atta-ur-Rahman Institute for Natural Product Discovery, Universiti Teknologi MARA (UiTM), Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor, Malaysia
[e]Department of Chemistry, Abdul Wali Khan University, Mardan, Mardan-23200, Pakistan
[f]PCSIR Laboratories Complex, Karachi, Shahrah-e-Dr. Salimuzzaman Siddiqui, Karachi 75280, Pakistan
[g]Depatment of Biochemistry, Hazara University, Mansehra, Pakistan
[h]Faculty of Applied Science UiTM, 40450 Shah Alam, Selangor, Malaysia

*Corresponding author : khalid.khan@iccs.edu



Synthesis and Molecular Docking Studies of Potent α-Glucosidase Inhibitors Based on Biscoumarin Skeleton

Khalid Mohammed Khan,[a*] Fazal Rahim,[b] Abdul Wadood,[c] Naveen Kosar,[b] Muhammad Taha,[d,h] Salima Lalani,[a] Aisha Khan,[b] Muhammad Imran Fakhri,[a] Muhammad Junaid ,[c] Wajid Rehman,[b] Momin Khan,[e] Shahnaz Perveen,[f] Muhammad Sajid[g] and M. Iqbal Choudhary[a]

[a]H. E. J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan
[b]Depatment of Chemistry, Hazara University, Mansehra, Pakistan
[c]Computational Medicinal Chemistry Laboratory, Department of Biochemistry, Abdul Wali Khan University, Mardan, Mardan-23200, Pakistan
[d]Atta-ur-Rahman Institute for Natural Product Discovery, Universiti Teknologi MARA (UiTM), Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor, Malaysia
[e]Department of Chemistry, Abdul Wali Khan University, Mardan, Mardan-23200, Pakistan
[f]PCSIR Laboratories Complex, Karachi, Shahrah-e-Dr. Salimuzzaman Siddiqui, Karachi 75280, Pakistan
[g]Depatment of Biochemistry, Hazara University, Mansehra, Pakistan
[h]Faculty of Applied Science Universiti Tecknologi MARA, 40450 Shah Alam,Selangor D. E. Malaysia
*Corresponding author: khalid.khan@iccs.edu

Abstract:

In our effort directed toward the discovery of new anti-diabetic agent for the treatment of diabetes, a library of biscoumarin derivative **1-18** was synthesized and evaluated for α-glucosidase inhibitory potential. All eighteen (**18**) compounds displayed assorted α-glucosidase activity with IC_{50} values 16.5-385.9 μM, if compared with the standard acarbose (IC_{50} = 906 ± 6.387 μM). In addition, molecular docking studies were carried out to explore the binding interactions of biscoumarin derivatives with the enzyme. This study has identified a new class of potent α-glucosidase inhibitors.

Keywords: Biscoumarin, α-glucosidase inhibition, molecular docking

*Coressponding authors: Tel.: (+92-21) 34824910, E-mail: khalid.khan@iccs.edu and hasssaan2@super.net.pk (Khalid Mohammed Khan)

1. Introduction

Mammalian *α*-glucosidases inhibitors, which impede with enzymatic action in small intestine, might slow the release of D-glucose from oligosaccharides and disaccharides, causing in delaying glucose absorption and decreasing postprandial blood glucose levels [1]. The acarbose [2] and voglibose [3] from microorganisms, and nojirimycin [4] and 1-deoxynojirimycin [5] from plants have been reported as potent α-glucosidase inhibitors. The effects of all these compounds on blood glucose levels after food uptake have been reported [6-9].

Glucosidases are liable for the catalytic cleavage of α-glycosidic bond with specificity depending on the position of cleavage site, the number of monosaccharides, and the configuration of the hydroxyl groups on the substrate [10]. The pharmaceutical research community has a special interest in *α*-glucosidases (EC 3.2.1.20) because the inhibition of its catalytic activity caused in the impedance of glucose absorption and decreases the postprandial blood glucose level. Thus, effective α-glucosidases inhibitors may help as chemotherapeutic agents for clinical use in the treatment of obesity and diabetes [11-13]. The *α*-glucosidase inhibitors has also been well appreciated as a therapeutic target for the other carbohydrate mediated diseases including viral infections [14,15], cancer [16] and hepatitis [17].

In the course of our efforts in the development of biologically important synthetic compounds, we observed *α*-glucosidase inhibitory potential in substituted new biscoumarin derivatives. It is obvious that polyphenols well intermingle with proteins and lead to inhibit enzyme activities [18]. Natural as well as synthetic biscoumarin have become an important class of oxygenated heterocycle mainly due to their wide variety of biological activities such as urease inhibition, anti-inflammatory, antioxidant, CYP3A and antifungal activities [19-21].

There is few reported synthetic and natural coumarin derivatives showed potent *α*-glucosidase inhibition [22-25]. Surprisingly no biscoumarin was tested for *α*-glucosidase inhibition. Therefore, we designed our project to synthesize biscoumarin and test them for *α*-glucosidase inhibitory properties.

In the continuation of our work, on biological potent small molecules [26] and their evaluation for enzyme inhibition [27], we herein report synthesis of substituted biscoumarin analogs, their *α*-glucosidase inhibitory potentials. The molecular docking was also performed in order to study their binding affinity.

2. Results and Discussion

2.1 Chemistry

In synthesis of biscoumarin analogs **1-18** (Scheme-1), to a stirred mixture of coumarin derivatives (4.0 mmol) and substituted aromatic aldehydes (2.0 mmol) in water and a catalytic amount of tetraethylammonium bromide (TEAB) was added. The reaction mixture was stirred at 60 °C for 1-2 h. Completion of the reaction was monitored by periodic TLC. After completion of reaction, it was filtered and washes with distilled water affording a pure product in high yields. In some cases the compounds purified through column chromatography using 3:7 acetone and *n*-hexane as eluent afforded pure products in good yield. The structures of compounds **1-18** were deduced by using different spectroscopic techniques such as ^{1}H NMR and EI mass spectroscopy. All compounds gave a satisfactory elemental analysis.

Insert Scheme 1 here

Insert table 1 here

2.2 Pharmacology

Glucosidase are responsible for the catalytic cleavage of a glycosidic bond of complex molecules of carbohydrates. Enzyme inhibition is one of the most significant tools in pharmaceutical research as well as in the field of drug discovery. During this study, we have synthesized eighteen (**18**) derivatives and evaluated for α-glucosidase enzyme inhibitory activity. All compounds showed a potent inhibition superior to the standard inhibitor of α-glucosidase.

Compounds **1-18** exhibited a varying degree of α-glucosidase inhibitory activity with IC_{50} values between 16.54 ± 0.36-385.99 ± 0.65 μM when compared with standard acarbose (IC_{50} = 906 ± 6.387 μM). Although the main skeleton for all compounds are same, the slight difference in their inhibitory potential might be due to the different substitution pattern on benzaldehyde.

Compound 3,3′-((2-4-Dichlorophenyl)methylene)*bis*(6-chloro-4-hydroxy-2*H*-chromen-2-one) (**12**) showed an excellent activity (IC_{50} = 16.54 ± 0.36 μM) 54-fold more active than the standard acarbose (IC_{50} = 906 ± 6.387 μM). Similarly, compound 3,3′-((3-nitrophenyl) methylene)*bis*(6-chloro-4-hydroxy-2*H*-chromen-2-one) (**13**) showed an excellent activity with an IC_{50} value of 27.07 ± 0.13 μM, 33-fold better than the standard. The remaining all compounds also exhibited potent inhibitory activities.

To know the mechanism of α-glucosidase inhibition and binding mode of biscoumarin analogs inside the binding pocket of *α*-glucosidases, molecular docking studies were performed.

2.2 Molecular Docking studies

The molecular docking study was carried out to explore the binding mode of biscoumarin derivatives within the binding pocket of *α*-glucosidase and to understand their structure activity relationship using MOE-Dock as docking software (www.chemcomp.com). As a means of testing the adopted protocols, the known inhibitor acarbose (the first α-glucosidase inhibitor approved for type 2 diabetes treatments) was docked into the binding pocket of a developed homology model, the acarbose fit well in the binding pocket and showed interaction to the important active site residues (**Figure-1**).

Although the x-ray crystallographic structures of *α*-glucosidase have been reported from some bacteria, the three-dimensional structural information is still not available for the eukaryotic *α*-glucosidase enzyme from *Sacchromyces species* (the enzyme use in our biological assay). However, only a few homology models have been reported for this enzyme previously [28-30]. The three dimensional coordinates of none of these model are publically available. Therefore, we construct the 3D structure of *α*-glucosidase by homology modeling using the same protocol as described by Burke *et al* [31], based on the crystal structure of *Saccrhromyces cerevisiae* (3AJ7.pdb) [26].

All the compounds were docked into the binding pocket of a developed homology model of α-glucosidase enzyme. From the molecular docking it was observed that the top ranked conformation of the most active compound **12** (IC_{50} =16.54 ± 0.36 μM) (**Figure-2**) established six hydrogen bonds between the hydroxyl group on coumarin ring of the compound and the

active site residues (Asp 214, Glu 276, Arg 312, Asp 408 and Arg 439). Additionally the aryl group of the compound formed an arene-cation interaction with the Arg 439. Furthermore, several hydrophobic interactions were observed between the compound and the active site residues, *e.g.*, Val 108, Phe 157, Phe 177 and Phe 300 are the other residues that stabilized the binding of the compound **12** in the active site of *α*-glucosidase. The strong hydrogen bonding network observed for compound **12** by the hydroxyl groups attached to the coumarin ring might be due to chloro groups, particularly dichlorobenzene in which the chloride moiety has strong electron withdrawing inductive effect. This effect of chloride moieties increases the ionizing ability of hydroxyl group that might be one of the reasons for its highest activity showed in the series (**Table-1**). These observations can be verified in case of compounds **2** (**Figure-3**), **15** (**Figure-4**) and **17** (**Figure-5**) as they have low biological activities as well as less interaction with active site residue as compared to compound **12**. As these compounds have groups with electron donating inductive effect instead of chloride present as in compound **12**. Similarly compounds **6** to **11** have two side chain chloride groups, but lacking the dichlorobenzene group that makes them slightly less active as compare to compound **12**. From the docking conformation of compounds **13** it was observed that its *m*-nitrobenzene showed almost similar behavior as dichlorobenzene in compound **12** because the nitro group has also electron withdrawing inductive effect.

Insert Figure 1 here

Insert Figure 2 here

Insert Figure 3 here

Insert Figure 4 here

Insert Figure 5 here

2.3 Molecular docking

Homology modeling of α-glucosidase for *Saccharomyces cerevisiae* was performed to predict its three dimensional (3D) structure. The amino acid sequence of α-glucosidase from *Saccharomyces cerevisiae* was retrieved from UniProt protein resource data bank (http://www.uniprot.org/) under the access code P53341. Similarity search was carried out in MOE-2010.11 using default parameter against PDB Databank implemented in MOE2010.11. The crystallographic structure of *Saccharomyces cerevisiae* isomaltase (PDB code 3AJ7, 1.30 Å resolution) with 72.4% of sequence identity with the target was selected as the template. 3D structure was built using homology modeling tool implemented in MOE. The developed structure was subjected to energy minimization up to 0.05 RMS gradients and the minimized structure was then refined by MD simulation up to 500 picoseconds. The final refine structure was then used for the molecular docking purpose.

Prior to docking ligand and protein were prepared using MOE2010.11. All synthetic compounds were modeled using Builder program implemented in MOE, finally a database was created in which all the compound structures were present in 3D format. Subsequently, their energies were minimized up to 0.05 Gradient, using the MMFF94x force field. Energy minimization of compound database was followed by the preparation of protein for docking purposes. Most of macromolecular crystal structures contain little or no hydrogen coordinate data due to limited resolution, thus protonation should be done prior to docking using the Protonate 3D Option. The protonation was followed by energy minimization up to 0.05 gradient, using Amber99 force field. The database was docked into the binding pocket of a protein using the Triangular Matching docking method and 30 conformations for each ligand protein complex were generated with docking score. Each complex was analyzed for interactions and the 3D image was taken.

2.4 α-Glucosidase Inhibitory Assay

Rat intestinal acetone powder in typical saline (100:1; w/v) was sonicated appropriately and the supernatant was used as a source of basic intestinal *α*-glucosidase after centrifugation. In short, 10 mL of test samples of 5 mg/mL in DMSO solution were reconstituted in 100 mL of 100 mM-phosphate buffer at pH 6.8 in 96-well micro-plate and incubated with 50 mL of basic intestinal α-glucosidase for 5 min before 50 mL substrate (5 mM, *p*-nitrophenyl-α-D-glucopyranoside prepared in same buffer) was added. *p*-Nitrophenol released was measured at 405 nm spectrophotometrically (SpectraMax plus384), Molecular Devices Corporation, Sunnyvale, CA, USA) 5 min after incubation with the substrate. Individual blanks for test samples were prepared to accurate background absorbance where the substrate was changed with 50 mL of buffer. Control sample contained 10 mL DMSO beside test samples. Percentage of enzyme inhibition was measured as (1 - B/A) x100 where [A] represents absorbance of control exclusive of test samples, and [B] corresponding to absorbance in presence of test samples [33].

3. Conclusions

Synthesis of biscoumain analogs and their *α*-glucosidase inhibitory potential was evaluated. All these eighteen (18) derivatives showed potent α-glucosidase inhibitory potential. Consequently, *in silico* studies were performed to recognize the binding mode of these compounds. The planned scaffold of *α*-glucosidase inhibitors offers the possibility of expedient additional modifications that could give rise to lead structures with enhanced inhibitory activity and selectivity towards the enzyme.

4. Material and Methods

NMR experiments were performed on an Avance Bruker AM 300 MHz machine. CHN Analyses were carried out a Carlo Erba Strumentazion-Mod-1106, Italy. Electron impact mass spectra (EI MS) were recorded on a Finnigan MAT-311A (Germany) mass spectrometer. Thin layer chromatography (TLC) was carried out on pre-coated silica gel aluminum plates (Kieselgel 60, 254, E. Merck, Germany). Chromatograms were visualized by iodine vapors or UV at 254 and 365 nm.

4.1 General procedure for the synthesis of compounds 1-18

In synthesis of biscoumarin analogs **1-18** (Scheme-1), to a stirred mixture of coumarin derivatives (4.0 mmol) and substituted aromatic aldehyde (2.0 mmol) in water and 1o mol% triethylammonium bromide (TEAB) was added. The reaction mixture was stirred at 60 °C for 1-2 h. Completion of reaction was monitored by periodic TLC. After completion of reaction, it was filtered, and then washed with distilled water affording a pure product in high yields. In some cases pure products were obtained through column chromatography (silica gel) using 3:7 acetone and *n*-hexane as eluent. The structures of compounds **1-18** were deduced by using different spectroscopic techniques, including ^{1}H NMR and EI mass spectroscopy. All synthetic compounds **1-18** gave satisfactory elemental analyses.

4.1.1 3,3'-((4-Nitrophenyl)methylene)*bis*(4-hydroxy-6-methyl-2*H*-chromen-2-one) (1)

Yield: 0.23 g (84%); ^{1}H-NMR: (DMSO-d_6, 300 MHz): δ 8.05 (d, 2H, $J_{3'',2''/6'',5''}$ = 8.7 Hz, H-3''/5''), 7.59 (br s, 2H, H-5/5'), 7.47 (d, 2H, $J_{2'',3''/6'',5''}$ = 8.6 Hz, H-2''/6''), 7.34 ((dd, 1H, $J_{7,8/7',8'}$ = 8.1 , $J_{7,5/7',5'}$ = 2.6 Hz, H-7/7'), 7.17 (d, 1H, $J_{8,7/8',7'}$ = 8.2 Hz, H-8/8'), 6.31 (s, 1H, Ar_3CH), 2.32 (s, 6H, 2 x CH_3); Anal. Calcd for $C_{27}H_{19}NO_8$, C = 66.80, H = 3.95, N = 2.89, Found C = 66.78, H = 3.97, N = 2.91; EI-MS: *m/z* (rel. int. %): 485 (M^+, 42), 308 (15), 292(45), 134 (100), 106 (20).

4.1.2 3,3′-((3,4,5-Trimethoxyphenyl)methylene)*bis*(6-methyl-4-hydroxy-2*H*-chromen-2-one (2)

Yield: 0.22 g (82%); ^{1}H-NMR: (DMSO-d_6, 300 MHz,): δ 7.64 (br, s, 2H, H-5/5'), 7.35 (dd, 2H, $J_{7,8/7',8'}$ = 8.4 , $J_{7,5/7',5'}$ = 1.8 Hz, H-7/7'), 7.19 (d, 2H, $J_{8,7/8',7'}$ = 8.1 Hz, H-8/8'), 6.39 (s, 2H, H-2''/6''), 6.21 (s, 1H, Ar_3CH), 3.61 (s, 3H, OCH_3), 3.54 (s, 6H, OCH_3), 2.33 (s, 6H, 2 x CH_3); Anal. Calcd for $C_{30}H_{26}O_9$, C = 67.92, H = 4.94, Found C = 67.94, H = 4.96; EI-MS: *m/z* (rel. int. %): 530 (M^+, 65), 353 (63), 322 (100), 175 (28), 134 (13).

4.1.3 3,3′-((4-(Methylthio)phenyl)methyl)6-methyl-*2H*-chromen-2-one) (3)

Yield: 0.23 g (84%); ^{1}H-NMR: (DMSO-d_6, 300 MHz): δ 7.61 (br. s, 2H, H-5/5'), 7.35 (dd, 2H, $J_{7,8/7',8'}$ = 8.4 Hz, $J_{7,5/7',5'}$ = 1.8 Hz, H-7/7'), 7.19 (d, 2H, $J_{8,7/8',7'}$ = 8.4 Hz, H-8/8'), 7.09 (m, 4H, H-2''/3''/5''/6''), 6.21 (s, 1H, Ar_3CH), 2.39 (s, 3H, -SCH_3), 2.33 (s, 6H, CH_3); Anal. Calcd for

$C_{28}H_{22}O_6S$, C = 69.12, H = 4.56, S = 6.59, Found C = 69.14, H = 4.55, S = 6.57; EI-MS: *m/z* (rel. int. %): 486 (M^+, 42), 308 (59), 263 (100), 176 (42), 134 (61).

4.1.4 3,3′-((3-Hydroxyphenyl)methylene)*bis*(6-methyl-4-hydroxy-2*H*-chromen-2-one) (4)

Yield: 0.24 g (86%); ^{1}H-NMR: (DMSO-d_6 300 MHz): δ 7.58 (br.s, 2H, H-5/5'), 7.30 (dd, 2H, $J_{7,8/7',8'}$ = 8.7, $J_{7,5/7',5'}$ = 2.1 Hz, H-7/7'), 7.13 (d, 2H, $J_{8,7}$ = 8.1 Hz, H-8/8'), 6.92 (t, 1H, $J_{5''(4'',6'')}$ = 7.8 Hz, H-5''), 6.52 (m, 3H, H-2''/4''/6''), 6.14 (s, 1H, Ar_3CH), 2.32 (s, 6H, 2 x CH_3); Anal. Calcd for $C_{27}H_{20}O_7$, C = 71.05, H = 4.42, Found C = 71.06, H = 4.40; EI-MS: *m/z* (rel. int. %): 456 (M^+, 42), 345 (18), 279 (37), 176 (52), 134 (100);

4.1.5 3,3′-((2-Nitrophenyl)methylene)*bis*(6-chloro-4-hydroxy-2*H*-chromen-2-one) (5)

Yield: 0.21 g (80%); ^{1}H-NMR (DMSO-d_6, 300 MHz); δ 7.69 (d, 2H, $J_{5,7/5',7'}$ = 2.7 Hz, H-5/5'), 7.52 (m, 4H, H-7/7'/3''/6''), 7.33 (m, 4H, H-8/8'/4''/5''), 6.42 (s, 1H, Ar_3CH); Anal. Calcd for $C_{25}H_{13}Cl_2NO_8$, C = 57.05, H = 2.49, Found C = 57.07, H = 2.51; EI-MS *m/z* (rel. int. %): 526 (M^+, 42), 298 (67), 283 (58), 196 (27), 154 (100);

4.1.6 3,3′-((3-Methoxy,4-hydroxyphenyl)methylene)*bis*(6-chloro-4-hydroxy-2*H*-chromen-2-one) (6)

Yield: 0.23 g (84%); ^{1}H-NMR (DMSO-d_6, 300 MHz); δ 7.73 (d, 2H, $J_{5,7/5',7'}$ = 2.7Hz, H-5/5'), 7.54 (dd, 2H, $J_{7,8/7',8'}$ = 8.7, $J_{7,5/7',5'}$ = 2.4 Hz, H-7/7'),7.31 (d, 2H, $J_{8,7/8',7'}$ = 8.7 Hz, H-8/8'), 6.59 (d, 1H, $J_{6'',5''}$ = 6.0 Hz, H-6''), 6.54 (s, 1H, H-2''),6.49 (d, 1H, $J_{5'',6''}$ = 8.1Hz, H-5''), 6.12(s, 1H, Ar_3CH), 5.61(s, 1H, OH), 3.53 (s, 3H, OCH_3); Anal. Calcd for $C_{26}H_{16}Cl_2O_8$, C = 59.22, H = 3.06, Cl = 13.45, Found C = 59.23, H = 3.04; EI-MS *m/z* (rel. int. %): 527 (M^+, 42), 329 (100), 313 (56), 299(73), 196 (51).

4.1.7 3,3′-((3-Hydroxyphenyl) methylene)*bis*(6-chloro-4-hydroxy-2*H*-chromen-2-one) (7)

Yield: 0.22 g (82%); ^{1}H-NMR (DMSO-d_6, 300 MHz); δ 7.74 (d, 2H, $J_{5,7/5',7'}$ = 2.7 Hz, H-5/5'), 7.55 (dd, 2H, $J_{7,8/7',8'}$ = 8.7, $J_{7,5/7',5'}$ = 2.7 Hz, H-7/7'), 7.32 (d, 2H, $J_{8,7/8',7'}$ = 8.7 Hz, H-8/8'), 6.92 (t, 1H, $J_{5''(4'',6'')}$ = 7.8 Hz, H-5''), 6.51 (m, 3H, H-2''/4''/6''), 6.14 (s, 1H, Ar_3CH); Anal. Calcd for $C_{25}H_{14}Cl_2O_7$, C = 60.38, H = 2.84, Found C = 60.36, H = 2.82; EI-MS *m/z* (rel. int. %): 497 (M^+, 42), 299 (95), 283 (74), 196 (41), 154 (100).

4.1.8 3,3′-((3-Hydroxy-4-methoxyphenyl)methylene)*bis*(6-chloro-4-hydroxy-2*H*-chromen-2-one) (8)

Yield: 0.23 g (84%); ^{1}H-NMR (DMSO-d_6, 300 MHz): δ 7.73 (d, 2H, $J_{5,7/5',7'}$ = 2.7 Hz, H-5/5'), 7.54 (dd, 2H, $J_{7,8/7',8'}$ = 8.7, $J_{7,5/7',5'}$ = 2.7 Hz, H-7/7'),7.32 (d, 2H, $J_{8,7/8',7'}$ = 9.0 Hz, H-8/8'), 6.69 (d, 1H, $J_{5'',6''}$ = 8.4 Hz, H-5''),6.52 (s, 1H, H-2''), 6.43 (d, 1H, $J_{6'',5''}$ = 8.4 Hz H-6''), 6.09 (d, 1H, Ar_3CH); Anal. Calcd for $C_{26}H_{16}Cl_2O_8$, C = 59.22, H = 3.06, Found C = 59.24, H = 3.04; EI-MS *m/z* (rel. int. %): 527 (M^+, 42), 329 (89), 313 (100), 299 (53), 196 (48).

4.1.9 3,3'-((3-Ethoxy-4-hydroxyphenyl)methylene)*bis*(6-chloro-4-hydroxy-2*H*-chromen-2-one) (9)

Yield: 0.22 g (82%); ^{1}H-NMR (DMSO-d_6, 300 MHz): δ 7.73 (d, 2H, $J_{5,7/5',7'}$ = 2.4 Hz, 2 x H-5), 7.54 (dd, 2H, $J_{7,8/7',8'}$ = 8.7, $J_{7,5/7',5'}$ = 2.7 Hz, H-7/7'),7.31 (d, 2H, $J_{8,7/8',7'}$ = 8.7 Hz, H-8/8'), 6.58 (d, 2H, $J_{5'',6''/2'',6''}$ = 8.1 Hz, H-5''/2''),6.48 (d, 1H, $J_{6'',5''}$ = 8.1 Hz, H-6''), 6.10 (s, 1H, Ar_3CH), 3.80 (q, 2H, O**C**$\mathbf{H_2}$$CH_3$), 1.17 (t, 3H, OCH_2**C**$\mathbf{H_3}$); Anal. Calcd for $C_{27}H_{18}Cl_2O_8$, C = 59.91, H = 3.35, Found C = 59.92, H = 3.34; EI-MS *m/z* (rel. int. %):541 (M^+, 42), 299 (100), 287 (25), 154 (74), 126 (38).

4.1.10 3,3'-((3,4,5-Trimethoxyphenyl)methylene)*bis*(6-chloro-4-hydroxy-2*H*-chromen-2-one) (10)

Yield: 0.23 g (84%); ^{1}H-NMR (DMSO-d_6, 300 MHz): δ 7.75 (d, 2H, $J_{5,7/5',7'}$ = 2.7 Hz, 2 x H-5), 7.54 (dd, 2H, $J_{7,8/7',8'}$ = 8.7, $J_{7,5/7',5'}$ = 2.4 Hz, H-7/7'),7.32 (d, 2H, $J_{8,7/8',7'}$ = 8.7 Hz, H-8/8'), 6.37 (s, 2H, H-2''/6''), 6.16 (s, 1H, Ar_3CH), 3.60 (s, 3H, OCH_3), 3.54 (s, 6H, 2 x OCH_3); Anal. Calcd for $C_{28}H_{20}Cl_2O_9$, C = 58.86, H = 3.53; Found C = 58.84, H = 3.54; EI-MS *m/z* (rel. int. %): 571 (M^+, 42), 374 (89), 343 (100), 196 (37), 154 (61).

4.1.11 3,3'-((3,4-Dimethoxyphenyl)methylene)*bis*(6-chloro-4-hydroxy-2*H*-chromen-2-one) (11)

Yield: 0.23 g (84%); ^{1}H-NMR (DMSO-d_6, 300 MHz): δ 7.74 (d, 2H, $J_{5,7/5',7'}$ = 1.5 Hz, H-5/5'), 7.54 (dd, 2H, $J_{7,8/7',8'}$ = 5.1, $J_{7,5/7',5'}$ = 1.5 Hz, H-7/7'), 7.32 (d, 2H, $J_{8,7/8',7'}$ = 5.4 Hz, 2 x H-8), 6.75 (d, 1H, $J_{5'',6''}$ = 5.1Hz, H-5''), 6.63 (s, 1H, H-2''), 6.61 (d, 1H, $J_{6'',5''}$ = 5.1 H-6''), 6.16 (s, 1H, Ar_3CH), 3.67 (s, 3H, 3-OCH_3), 3.52 (s, 3H, 4-OCH_3); Anal. Calcd for $C_{27}H_{18}Cl_2O_8$, C = 59.91, H = 3.35; Found C = 59.93, H = 3.33; EI-MS *m/z* (rel. int. %): 541 (M^+, 42), 343 (34), 313 (100), 196 (36), 154 (65).

4.1.12 3,3'-((2-4-Dichlorophenyl)methylene)*bis*(6-chloro-4-hydroxy-2*H*-chromen-2-one) (12)

Yield: 0.23 g (84%); ^{1}H-NMR (DMSO-d_6, 300 MHz): δ 7.76 (s, 1H, H-3''), 7.73 (d, 2H, $J_{5,7/5',7'}$ = 2.4 Hz, H-5/5'), 7.54 (dd, 2H, $J_{7,8/7',8'}$ = 8.7, $J_{7,5/7',5'}$ = 2.4 Hz, H-7/7'), 7.36 (d, 2H, $J_{8,7/8',7'}$ = 8.7Hz, 2 x H-8/8'), 7.40 (m, 2H, H-5''/6''), 6.07 (s, 1H, Ar_3CH); Anal. Calcd for $C_{25}H_{12}Cl_4O_6$, C = 54.58, H = 2.20, Found C = 54.56, H = 2.19; EI-MS *m/z* (rel. int. %):550 (M^+, 42), 317 (100), 196 (37), 154 (72), 126 (37).

4.1.13 3,3'-((3-Nitrophenyl) methylene)*bis*(6-chloro-4-hydroxy-2*H*-chromen-2-one) (13)

Yield: 0.22 g (82%); ^{1}H-NMR (DMSO-d_6, 300 MHz): δ 7.73 (d, 2H, $J_{5,7/5',7'}$ = 2.4 Hz, H-5/5'), 7.57 (dd, 2H, $J_{7,8/7',8'}$ = 8.7, $J_{7,5/7',5'}$ = 2.7 Hz, H-7/7'), 7.48 (m, 4H, H-2''/4''/5''/6''), 7.32 (d, 2H, $J_{8,7/8',7'}$ = 8.7Hz, H-8/8'), 6.31 (s, 1H, Ar_3CH); Anal. Calcd for $C_{25}H_{13}Cl_2NO_8$, C = 57.05, H = 2.49, N = 2.66, Found C = 57.07, H = 2.48, N = 2.67; EI-MS *m/z* (rel. int. %): 511 (M^+, 42), 313 (100), 283 (69), 196 (23), 154 (44);

4.1.14 3,3'-((3-Nitrophenyl)methylene)*bis*(4-hydroxy-6-methyl-2*H*-chromen-2-one) (14)

Yield: 0.24 g (86%); ^{1}H-NMR: (DMSO-d_6, 300 MHz): δ 8.00 (d, 1H, $J_{4'',5''}$ = 8.1 Hz, H-4''), 7.86 (br s, 1H, H-2''), 7.61 (s, 2H, H-5/5'), 7.56 (d, 1H, $J_{6'',5''}$ = 7.2 Hz, H-6''), 7.48 (t, 1H, $J_{5''(4'',6'')}$ = 7.2 Hz, H-5''), 7.36 (dd, 2H, $J_{7,8/7',8'}$ = 8.7, $J_{7,5/7',5'}$ = 2.4 Hz, H-7/7'), 7.19 (d, 2H, $J_{8,7/8',7'}$ = 8.4 Hz, 2H-8/8'), 6.33 (s, 1H, Ar_3CH), 2.33 (s, 6H, CH_3); Anal. Calcd for $C_{27}H_{19}NO_8$, C = 66.80, H = 3.95, N = 2.89, Found C = 66.82, H = 3.97, N = 2.87; EI-MS *m/z* (rel. int. %): 485 (M^+, 42), 309 (24), 176 (31), 134 (100), 106 (24);

4.1.15 3,3'-((3-Ethoxy-4-hydroxyphenyl)methylene)*bis*(6-methyl-4-hydroxy-2*H*-chromen-2-one) (15)

Yield: 0.22 g (82%); ^{1}H-NMR: (DMSO-d_6, 300 MHz): δ 7.63 (br s, 2H, H-5/5'), 7.36 (dd, 2H, $J_{7,8/7',8'}$ = 8.4, $J_{7,5/7',5'}$ = 1.5 Hz, H-7/7'), 7.20 (d, 2H, $J_{8,7/8',7'}$ = 8.4 Hz, H-8/8'), 6.61 (d, 2H, $J_{5'',6''/6'',5''}$ = 8.4 Hz, H-5''/6''), 6.50 (d, 1H, $J_{2'',6''}$ = 7.8 Hz, H-3''/5''), 6.16 (s, 1H, Ar_3CH_3), 2.35 (s, 6H, CH_3), 2.31 (q,2H,O**CH_2**CH_3), 1.18 (t,3H,OCH_2**CH_3**); Anal. Calcd for $C_{29}H_{24}O_8$, C = 69.59, H = 4.83, Found C = 69.61, H = 4.84; EI-MS: *m/z* (rel. int. %):500 (M^+, 42), 324 (95), 295 (25), 279(60), 267(100).

4.1.16 3,3′-((2-Nitrophenyl)methylene)*bis*(6-methyl-4-hydroxy-2*H*-chromen-2-one) (16)

Yield: 0.22 g (82%); ^{1}H-NMR: (DMSO-d_6, 300 MHz): δ 7.56 (m, 4H,H-5/5'/3''/6''), 7.37 (m, 4H, H-7/7'/3''/6''), 7.15(d, 2H, $J_{8,7/8',7'}$ = 8.4 Hz, H-8/8'), 6.46 (s, 1H, Ar_3CH), 2.31 (s, 6H, CH_3); Anal. Calcd for $C_{27}H_{19}NO_8$, C = 66.80, H = 3.95, N = 2.89, Found C = 66.82, H = 4.83, N = 2.91; EI-MS: *m/z* (rel. int. %): 485 (M^+, 42), 263 (100), 176 (29), 134 (81), 106 (17).

4.1.17 3,3′-((4-Methoxyphenyl)methylene)*bis*(4-hydroxy-6-methyl-2*H*-chromen-2-one) (17)

Yield: 0.23 g (84%); ^{1}H-NMR: (DMSO-d_6, 300 MHz): 7.65 (br s, 2H, H-5/5'), 7.38 (dd, 2H, $J_{7,8/7',8'}$ = 8.4 , $J_{7,5/7',5'}$ = 2.4 Hz, H-7/7'), 7.22 (d, 2H, $J_{8,7/8',7'}$ = 8.4 Hz, H-8/8'), 7.01 (d, 2H, $J_{2'',3''/6'',5''}$ = 8.4 Hz, H-2/6), 6.75 (d, 2H, $J_{3'',2''/5'',6''}$ = 8.7 Hz, H-3''/5''), 6.22 (s, 1H, Ar_3CH), 3.68 (s, 3H, OCH_3) 2.34 (s, 6H, $2CH_3$); Anal. Calcd for $C_{28}H_{22}O_7$, C = 71.48, H = 4.71, Found C = 71.46, H = 4.73; EI-MS *m/z* (rel. int. %): 470 (M^+, 42), 293 (100), 263 (52), 176(16), 134 (47).

4.1.18 3,3′-((4-Methoxyphenyl)methylene)*bis*(6-chloro-4-hydroxy-2*H*-chromen-2-one) (18)

Yield: 0.23 g (84%); ^{1}H-NMR (DMSO-d_6, 300 MHz): δ 7.73 (d, 2H, $J_{5,7/5',7'}$ = 2.4 Hz, H-5/5'), 7.54 (dd, 2H, $J_{7,8/7',8'}$ = 8.7, $J_{7,5/7',5'}$ = 2.4 Hz, H-7/7'),7.32 (d, 2H, $J_{8,7/8',7'}$ = 8.7Hz, H-8/8'), 6.98 (d, 2H, $J_{2'',3''/6'',5''}$ = 8.1Hz, H-2''/6''),6.73 (d, 2H, $J_{3'',2''/5'',6''}$ = 8.7Hz, H-3''/5''), 6.15 (s, 1H, Ar_3CH), 3.66 (s, 3H,OCH_3); Anal. Calcd for $C_{26}H_{16}Cl_2O_7$, C = 61.07, H = 3.15, Found C = 61.06, H = 3.16; EI-MS *m/z* (rel. int. %): 511 (M^+, 42), 313 (100), 283 (69), 196(23), 154 (44);

Acknowledgement: The authors are thankful to Higher Education Commission (HEC) Pakistan for providing financial support under "National Research Program for Universities" to Project No. 20-1394.

References

[1] H. Lebovitz, Clin. Diabetes 13 (1995) 99-103.

[2] D. Schmidit, W. Frommer, B. Junge, L. Muller, W. Wingender, E. Truscheit, D. Schafer, Naturwissenschaften 64 (1977) 535-536.

[3] T. Matsuo, H. Odaka, H. Ikeda, Am. J. Clin. Nutr. 55 (1992) 314s-17s.

[4] N. Asano, E. Tomioka, H. Kizu, K. Matsui, Carbohydr. Res. 253 (1994) 235-245.

[5] N. Asano, H. Kizu, K. Oseki, E. Tomioka, K. Matsui, J. Med. Chem. 38 (1995) 2349-2356.

[6] J. Hoffmann, M. Spengler, Diabetes Care, 17 (1994) 561-566.

[7] K. Shinozaki, M. Suzuki, M. Ikebuchi, J.; Hirose, Y. Harano, Metabolism 6 (1996) 731-737.

[8] Y. Yoshikuni, Agric. Biol. Chem. 52 (1988) 121-128.

[9] P. H. Joubert, W. J. Bam, N. Manyane, Eur. J. Clin Pharmacol. 30 (1986) 253-2555.

[10] A. Kimura, J.H. Lee, I.S. H.-S. Lee, Lee, K.-H. Park, S. Chiba, D. Kim, Carbohydr. Res. 339 (2004) 1035-1040.

[11] K. M. Robinson, M. E. Begovic, B. L.;Rhinehart, E. W. Heineke, J. B. Ducep, P. R. Kastner, F.N. Marshall, C. Danzin, Diabetes 40 (1991) 825-830.

[12] C. Braun, G. D. Brayer, S. G. J. Withers, Biol. Chem. 270 (1995) 26778-26781.

[13] R.A. Dwek, T.D. Butters, F.M. Platt, N. Nicole Zitzmann, Nat. Rev. Drug Disc. 1 (2002) 65-75.

[14] A. Mehta, N. Zitzmann, P.M. Rudd, T.M. Block, R.A. Dwek, FEBS Lett. 430 (1998) 17-22.

[15] A. Karpas, G.W.J. Fleet, R.A. Dwek, S. Petursson, S.K. Namgoong, N.G. Ramsden, G.S. Jacob, T.W. Rademacher, Proc. Natl. Acad. Sci. U.S.A. 85 (1988) 9229-9233.

[16] M.J. Humphries, K. Matsumoto, S.L. White, K. Olden, Cancer Res. 46 (1986) 5215-5222.

[17] N. Zitzmann, A.S. Mehta, S. Carroue, T.D. Butters, F.M. Platt, J. McCauley, B.S. Blumberg, R.A. Dwek, T.M. Block, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 11878-11882.

[18] Haslam, E. Practical Polyphenols; Cambridge University Press: Cambridge, (1998) pp 168–174.

[19] V.S. Koneni, K. Manoj, K.M. Ram, S. Ravi, B. Gitika, A.K. Khanna, R. Shivika, S. Rakesh, Bioorg. Med. Chem. Lett. 21 (2011) 4480-4484.

[20] M. Taniguchi, Y. Hada, A. Yabu, K. Baba, Y. Q. Xiao, L. Li, L.Q. Guo, Y. Yamazoe, Tennen Yuki Kagobutsu Toronkai Koen Yoshishu 41 (1999) 373-378.

[21] K. Hu, H. Kobayashi, A. Dong, S. Iwasaki, X. Yao, Planta Med. 66 (2000) 564-567.

[22] D. P. K. Q ueiroz, A.G. Ferreira, A. S. Lima, E.S. Lima, M.D. lima, Int. J. Pharm. Pharm. Sci. 5 (2013), 336-339.

[23] M.N. Islam, H.A. Jung, S.H. Sohn, H.M. Kim, J. S. Choi, Arch. Pharm. Res. 36 (2013), (5), 542-552.

[24] H. M. S. Shihabudeen, D.H. Priscilla, K. Thirumurugan Nutrition & Metabolism 8 (2011) 46.

[25] B.S. Jayashree, A. Kumar, A. Pai, Pharmacologyonline 3 (2011) 1061-1076.

[26] K. M. Khan, Z. Shah, V. U. Ahmad, M. Khan, M. Taha, F. Rahim, H. Jahun. S. Perveen, M. I. Choudhary. Med. Chem. 7 (2011**)** 572-580 (b) K. M. Khan, M. Khan, M. Ali, M. Taha, S. Rasheed, S. Perveen, M. I. Choudhary. Bioorg. Med. Chem., 17 (2009) 7795-780, (c) K. M. Khan, M. Taha, F. Rahim, M. I. Fakhri, W. Jamil, M. Khan, S. Rasheed, A. Karim, S. Perveen, M. I. choudhary. J. Pak. Chem Soc. 35 (2013) 929 (d) K. M. Khan F. Rahim, N. Ambreen, M. Taha, M. Khan, H. Jahan, Najeebullah, A. Shaikh, S. Iqbal, S. Perveen, M. I. Choudhary. Med. Chem., 9 (2013) 588. (e) M. Taha, M. S. Baharudin, N. H. Ismail, K. M. Khan, F. M. Jaafar, Samreen, S. Siddiqui, M. I. Choudhary. Bioorg. Med Chem. Lett. 23 (2013) 3463. (f) K. M. Khan, M. Taha, F. Naz, S. Ali, S. Perveen, M. I. Choudhary. *Med. Chem.* 8 (2012) 705-7010.

[27] (a) K. M. Khan, F. Naz, M. Taha, A. Khan, S. Perveen, M. I. Choudhary, W. Voelter. Eur J Med Chem. 74 (2014) 314-323; (b) K. M. Khan, M. Khan, A. Karin, M. Taha, N. Ambreen, A. Gojayev, S. Perveen, M.I. Choudhary. J. Pak. Chem Soc. 35 (2013) 495-498; (c) K.M. Khan, M. Khan, M. Saleem, M. Taha, S. Perveen, M.I. Choudhary. Benzimidazoles: J. Pak. Chem Soc. 35 (2013) 901-904; (d) K.M. Khan, Zarbad Shah, V.U. Ahmad, N. Ambreen, M. Khan, M. Taha, F. Rahim, S. Noreen, S. Perveen, M.I. Choudhary, W. Voelter. 6-Nitroben-zimidazole derivatives: Potential phosphodiesterase inhibitors:Synthesis and structure-activity relationship. Bioorg. Med. Chem. 20 (2012) 1521-1526; (e) K.M. Khan, F. Rahim, S. A. Halim, M. Taha, M. Khan, S. Perveen, Zaheer-Ul-Haq, M. A. Mesaik, M.I. Choudhary. Synthesis of novel inhibitors of β-glucuronidase based on benzothiazole skeleton and study of their binding affinity by molecular docking. Bioorg Med Chem. 19 (2011) 4286-4294.

[28] S.B. Ferreira, A.C.R. Sodero, M.F.C. Cardoso, E.S. Lima, C.R. Kaiser, F.P. Silva, V.F. Ferreira, J. Med. Chem. 2010, 53, 2364-2375.

[29] J.-H. Park, S. Ko, H. Park, Bull. Korean Chem. Soc. 29 (2008) 921

[30] Roujeinikova, A.; Raasch, C.; Sedelnikova, S.; Liebl, W.; Rice, W. J. Mol. Biol. 2002, 321(1), 149-162.

[31] L.R. Guerreiro, E.P. Carriero, L. Fernandes, T.A. Cardote, R. Moreira, A.T. Caldeira, R.C. Guedes, A.J. Burke, Bioorg. Med. Chem. 21 (2013) 1911-1917.

[32] K. Yamamoto, H. Miyake, M. Kusunoki, S. Osaki, FEBS J. 277 (2010) 4205-4214

[33] R.R. Ranga, K.T. Ashok, R.P.K. Prabhakar, B.K. Suresh, Z. Amtul, B.K. Ali, J. Madhusudana, R. Madhusudana, Bioorg. Med. Chem. 17 (2009) 5170-5175.

Scheme Caption

Synthesis of biscoumarin derivatives **1-18**

Table Caption

α-Glucosidase inhibitory potential of biscoumarin analogs **1-18**

Figure Captions

(Figure-1) Binding mode of acarbose (known inhibitor) in the binding pocket of a developed homology model of α-glucosidase.

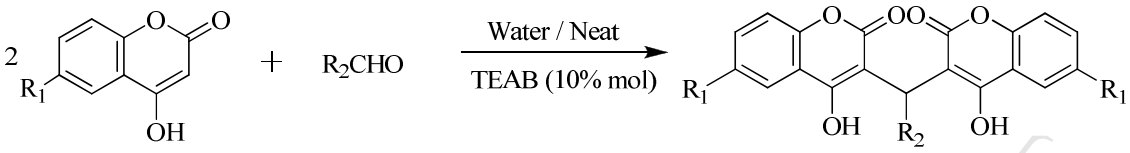
(Figure-2) Binding mode of compound **12** in the binding pocket of developed homology model of α-glucosidase.

(Figure-3) Binding mode of compound **2** in the binding pocket of developed homology model of α-glucosidase.

(Figure-4) Binding mode of compound **15** in the binding pocket of developed homology model of *α*-glucosidase

(Figure-5) Binding mode of compound **17** in the binding pocket of developed homology model of *α*-glucosidase.

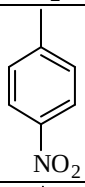
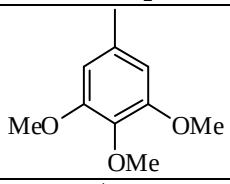
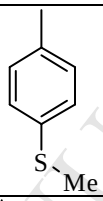
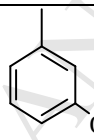
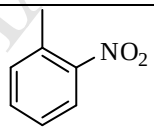
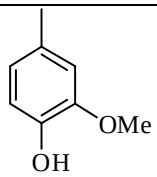
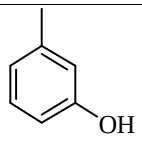
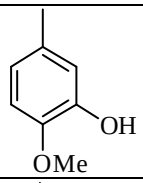
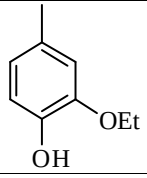
Scheme-1

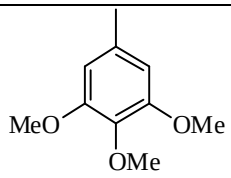
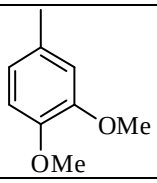
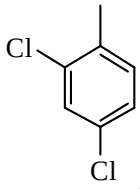
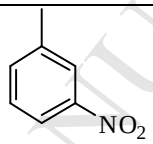
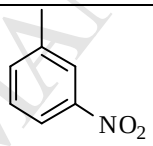
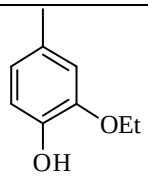
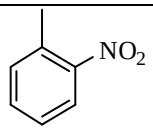
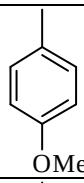
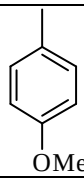


R_1 = Me, Cl

1-18

Table-1:

Compound No.	R_1	R_2	IC_{50} ± SEM[a] (μM)
1	6-Me		79.18 ± 2.7
2	6-Me		385.99 ± 0.65
3	6-Me		37.38 ± 0.69
4	6-Me		52.6 ± 0.21
5	6-Cl		80.94 ± 0.62
6	6-Cl		113.05 ± 3.43
7	6-Cl		84.06 ± 5.7
8	6-Cl		83.64 ± 3.39
9	6-Cl		57.14 ± 0.35

10	6-Cl		128.14 ± 2.04
11	6-Cl		91.36 ± 1.16
12	6-Cl		16.54 ± 0.36
13	6-Cl		27.07 ± 0.13
14	6-Me		67.96 ± 2.44
15	6-Me		221.6 ± 2.47
16	6-Me		128.6 ± 1.16
17	6-Me		106.63 ± 1.61
18	6-Cl		75.74 ± 1.11
Acarbose	-	-	906 ± 6.387

SEM[a] is the standard error of the mean, Acarbose is standard inhibitor for α-glucosidase

Figure-1:

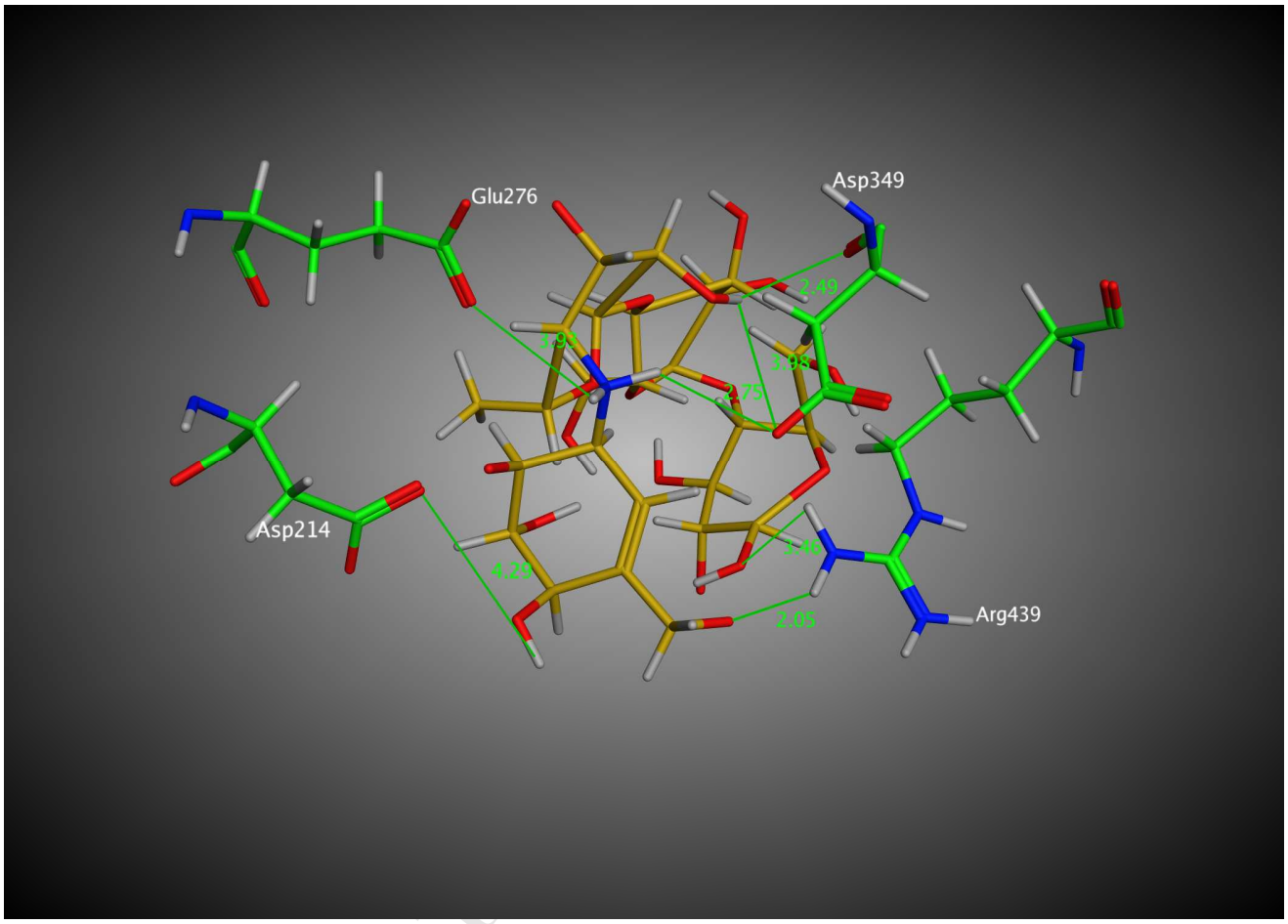


Figure-2:

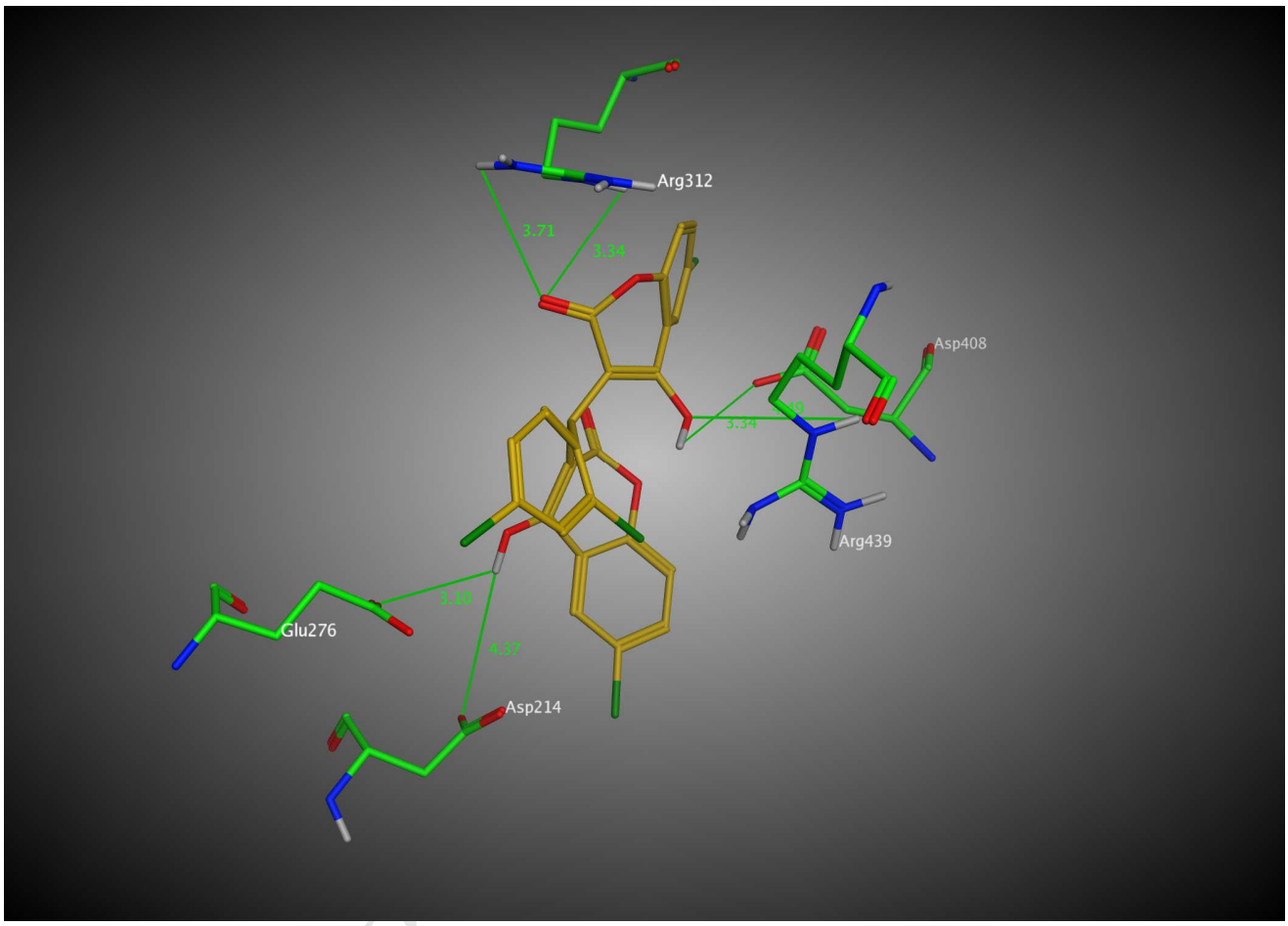


Figure-3:

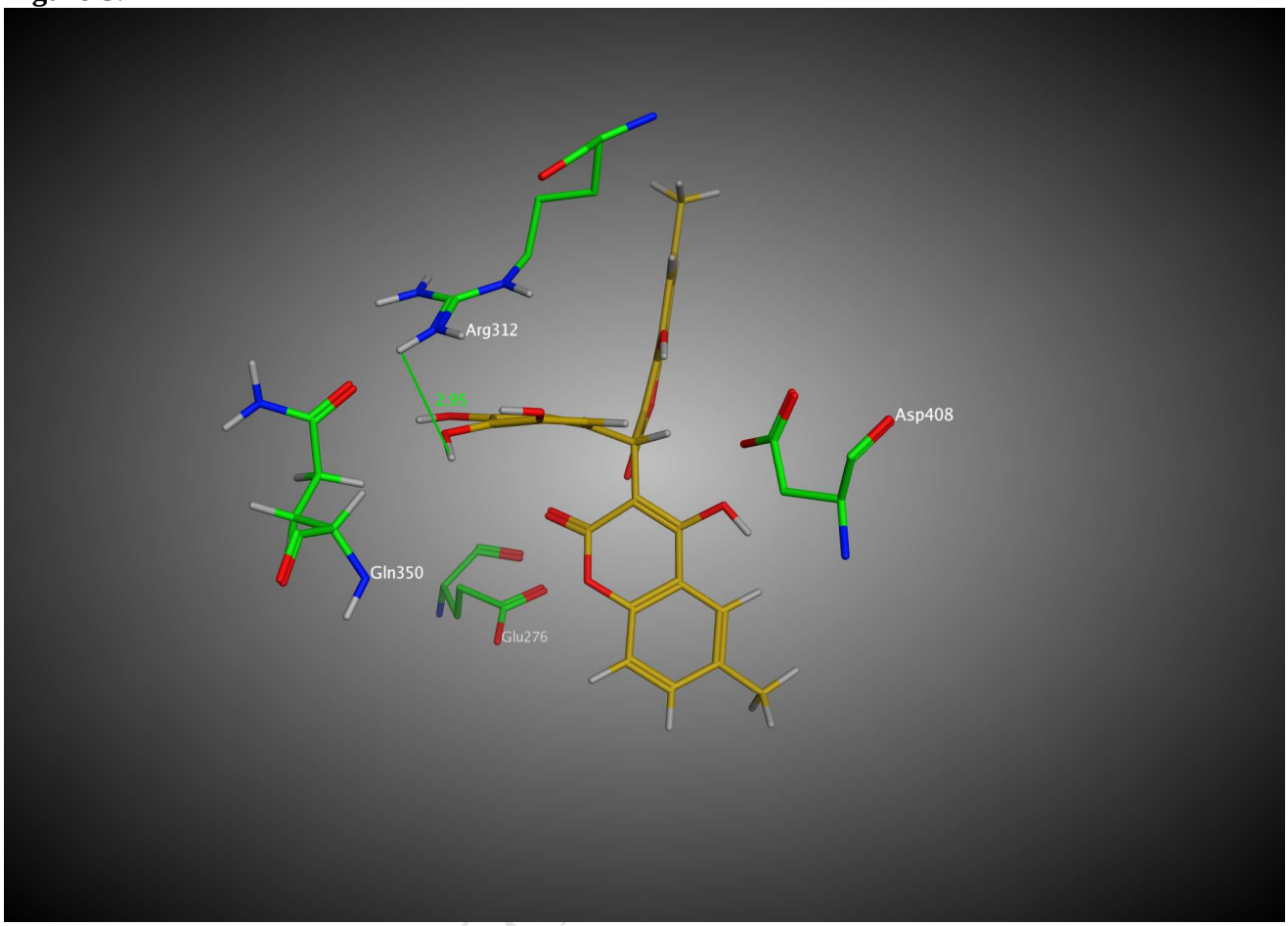


Figure-4:

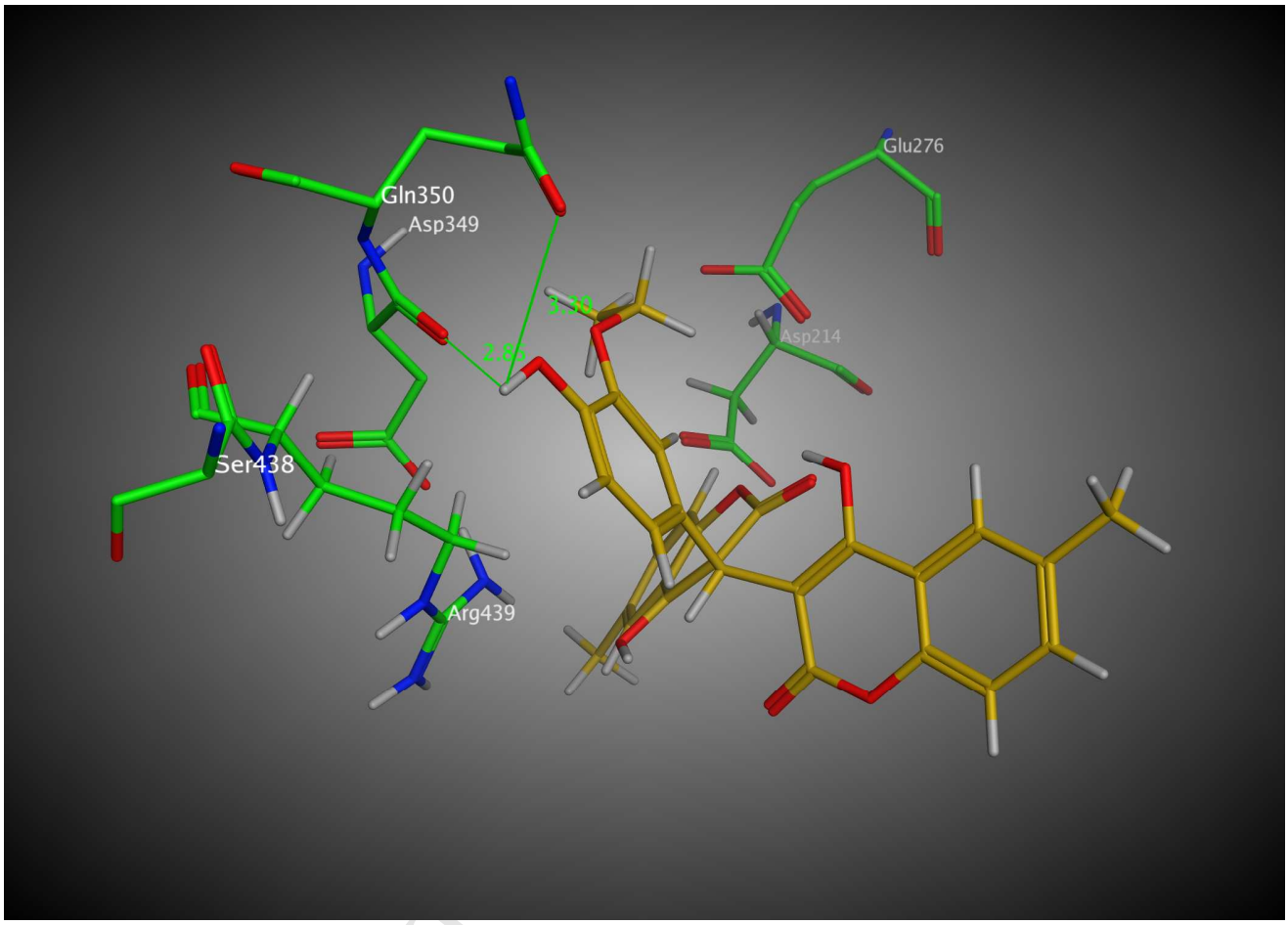
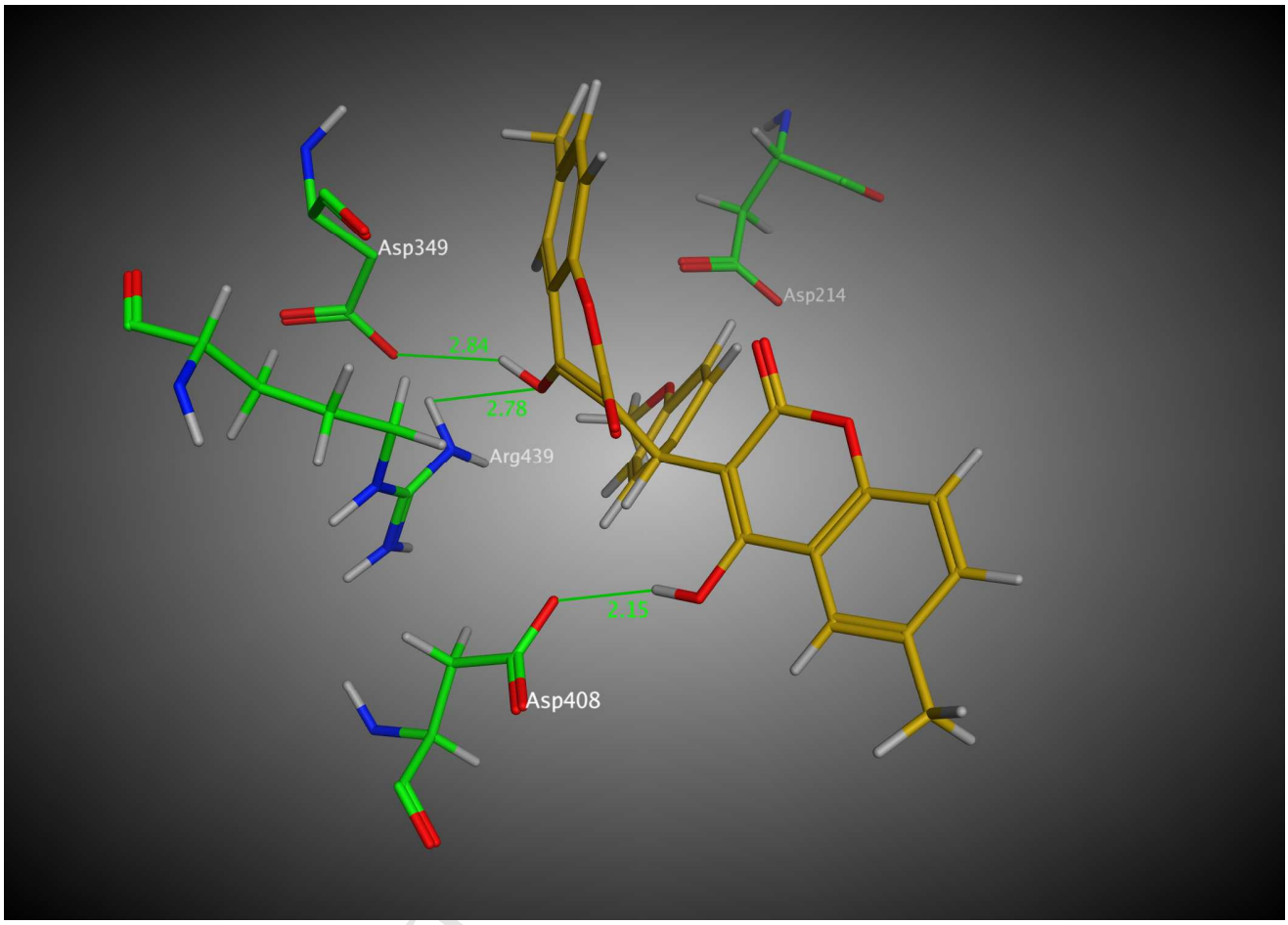


Figure-5:

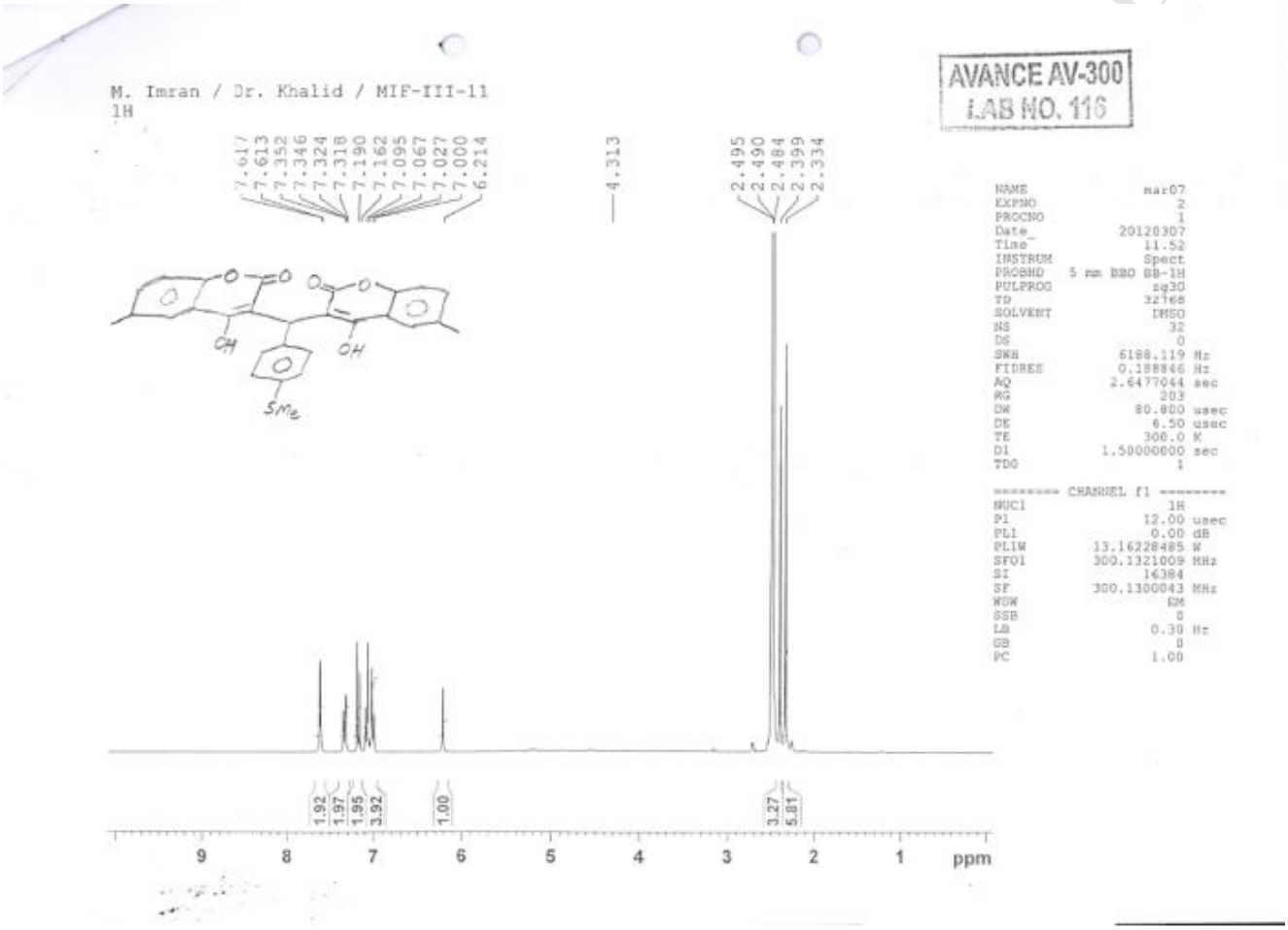


Highlights:

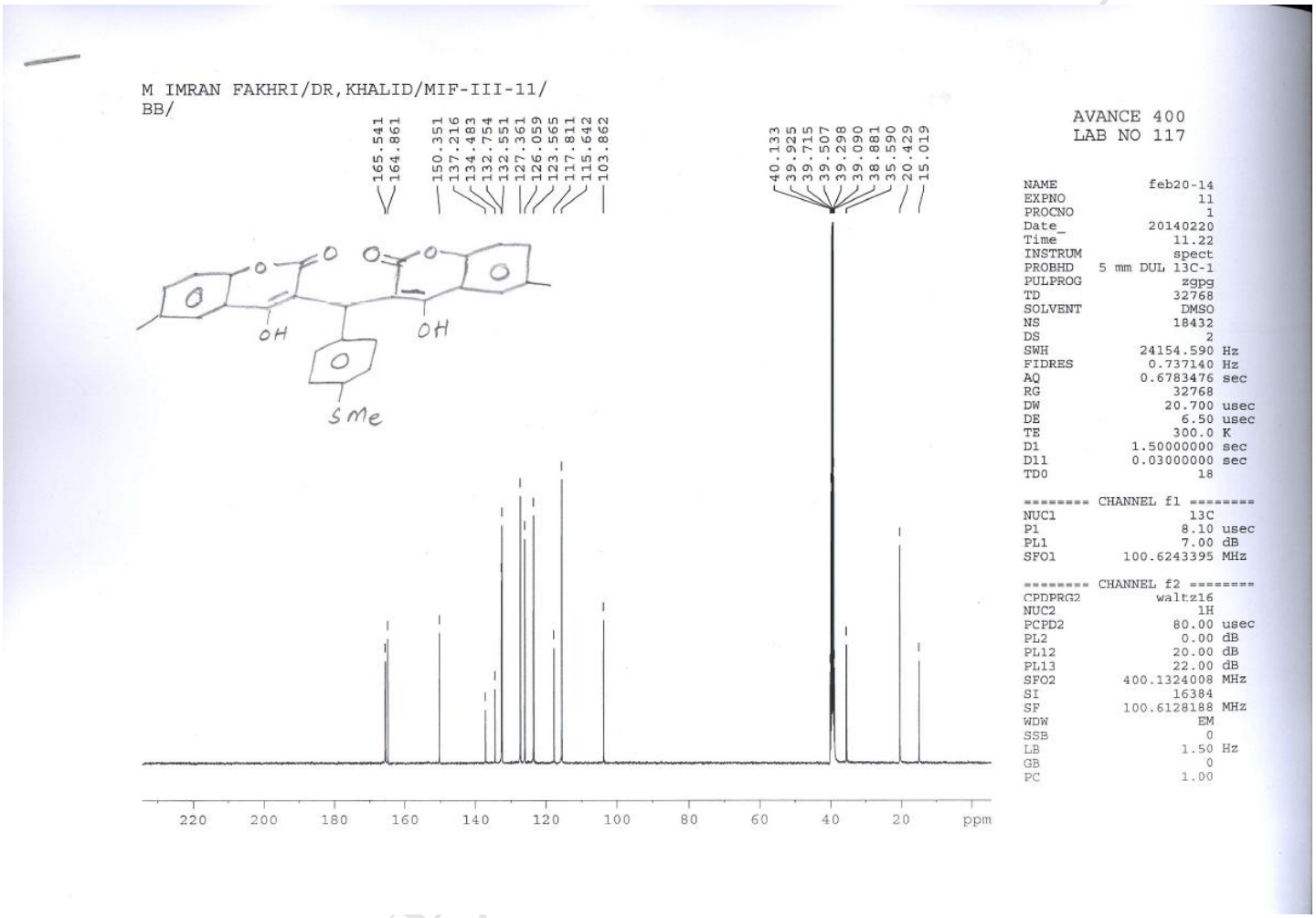
- Synthesis of biscoumarion derivatives
- *In vitro* α-glucosidase inhibitory activity
- Identification of a novel class of α-glucosidase inhibitors
- Molecular Docking studies
- All compounds found to be potent

Representative ^{1}HNMR, ^{13}CNMR and EI MS spectrum

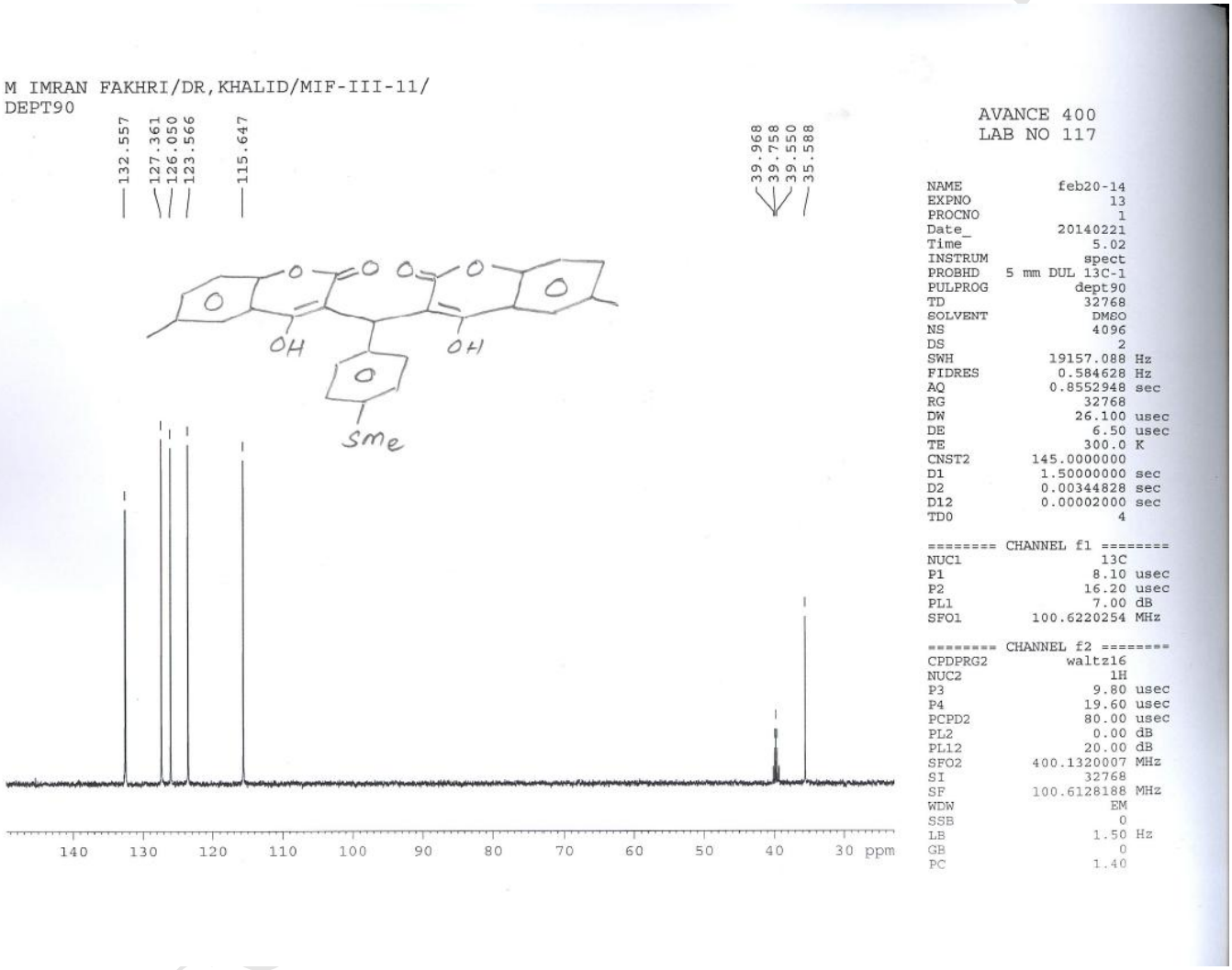
^{1}HNMR of Compound 3



^{13}CNMR of Compound 3



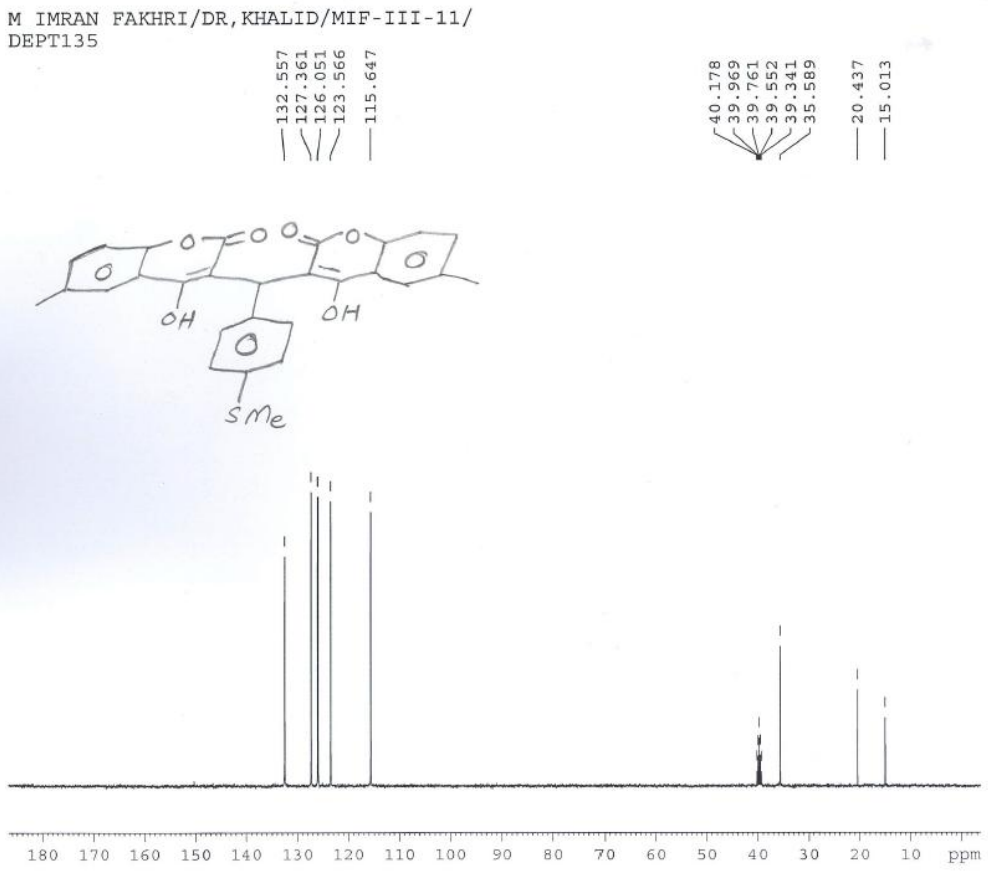
^{13}CNMR (90) of Compound 3



AVANCE 400
LAB NO 117

NAME	feb20-14	
EXPNO	13	
PROCNO	1	
Date_	20140221	
Time	5.02	
INSTRUM	spect	
PROBHD	5 mm DUL 13C-1	
PULPROG	dept90	
TD	32768	
SOLVENT	DMSO	
NS	4096	
DS	2	
SWH	19157.088	Hz
FIDRES	0.584628	Hz
AQ	0.8552948	sec
RG	32768	
DW	26.100	usec
DE	6.50	usec
TE	300.0	K
CNST2	145.0000000	
D1	1.50000000	sec
D2	0.00344828	sec
D12	0.00002000	sec
TD0	4	
======== CHANNEL f1 ========		
NUC1	13C	
P1	8.10	usec
P2	16.20	usec
PL1	7.00	dB
SFO1	100.6220254	MHz
======== CHANNEL f2 ========		
CPDPRG2	waltz16	
NUC2	1H	
P3	9.80	usec
P4	19.60	usec
PCPD2	80.00	usec
PL2	0.00	dB
PL12	20.00	dB
SFO2	400.1320007	MHz
SI	32768	
SF	100.6128188	MHz
WDW	EM	
SSB	0	
LB	1.50	Hz
GB	0	
PC	1.40	

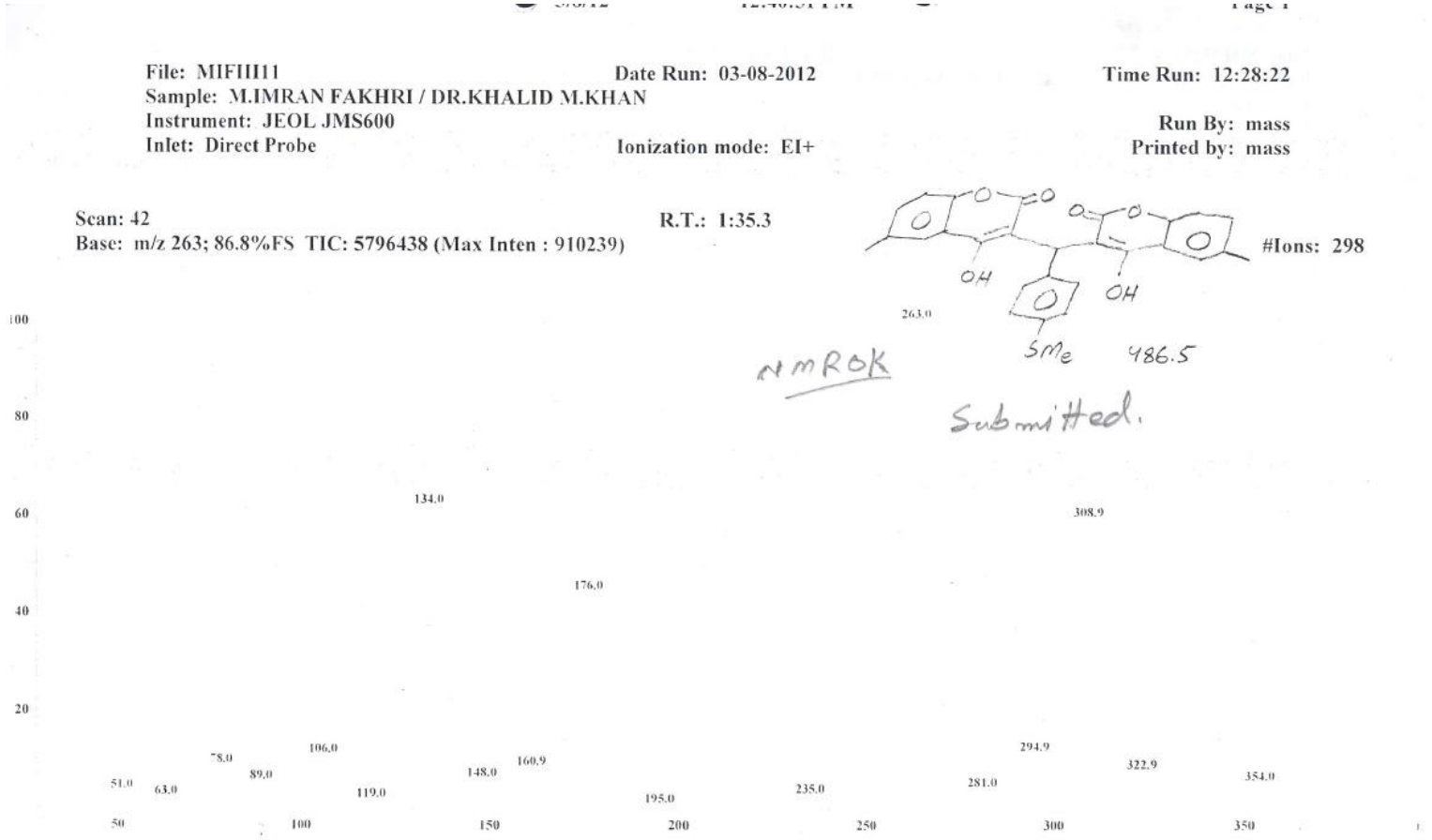
[13] CNMR (135) of Compound 3



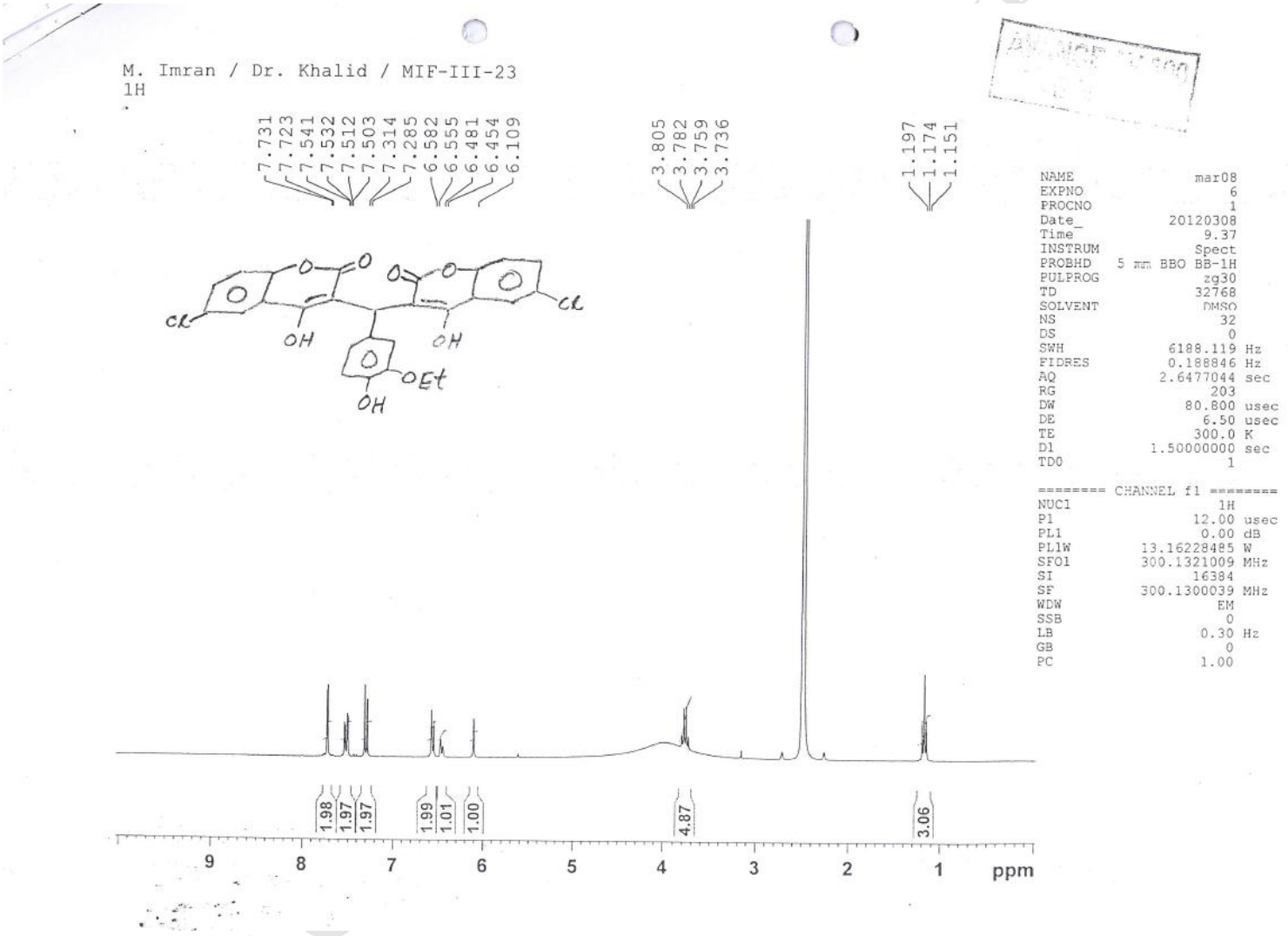
AVANCE 400
LAB NO 117

NAME	feb20-14
EXPNO	12
PROCNO	1
Date_	20140220
Time	22.53
INSTRUM	spect
PROBHD	5 mm DUL 13C-1
PULPROG	dept135
TD	32768
SOLVENT	DMSO
NS	9216
DS	2
SWH	19157.088 Hz
FIDRES	0.584628 Hz
AQ	0.8552948 sec
RG	32768
DW	26.100 usec
DE	6.50 usec
TE	300.0 K
CNST2	145.0000000
D1	1.50000000 sec
D2	0.00344828 sec
D12	0.00002000 sec
TD0	9
======== CHANNEL f1 ========	
NUC1	13C
P1	8.10 usec
P2	16.20 usec
PL1	7.00 dB
SFO1	100.6220254 MHz
======== CHANNEL f2 ========	
CPDPRG2	waltz16
NUC2	1H
P3	9.80 usec
P4	19.60 usec
PCPD2	80.00 usec
PL2	0.00 dB
PL12	20.00 dB
SFO2	400.1320007 MHz
SI	32768
SF	100.6128188 MHz
WDW	EM
SSB	0
LB	1.50 Hz
GB	0
PC	1.40

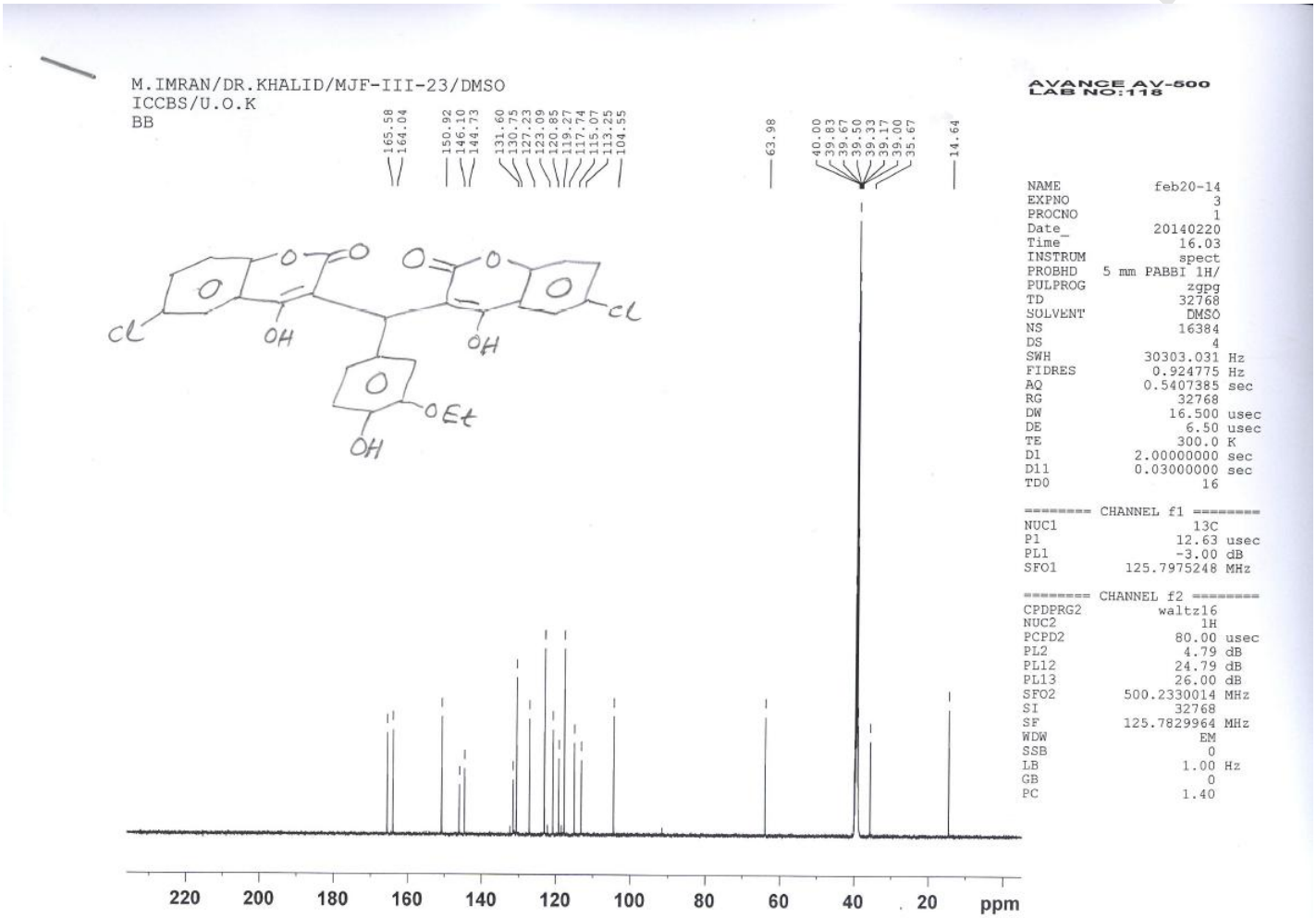
EI MS Spectra of Compound 3



[1]HNMR of Compound 9



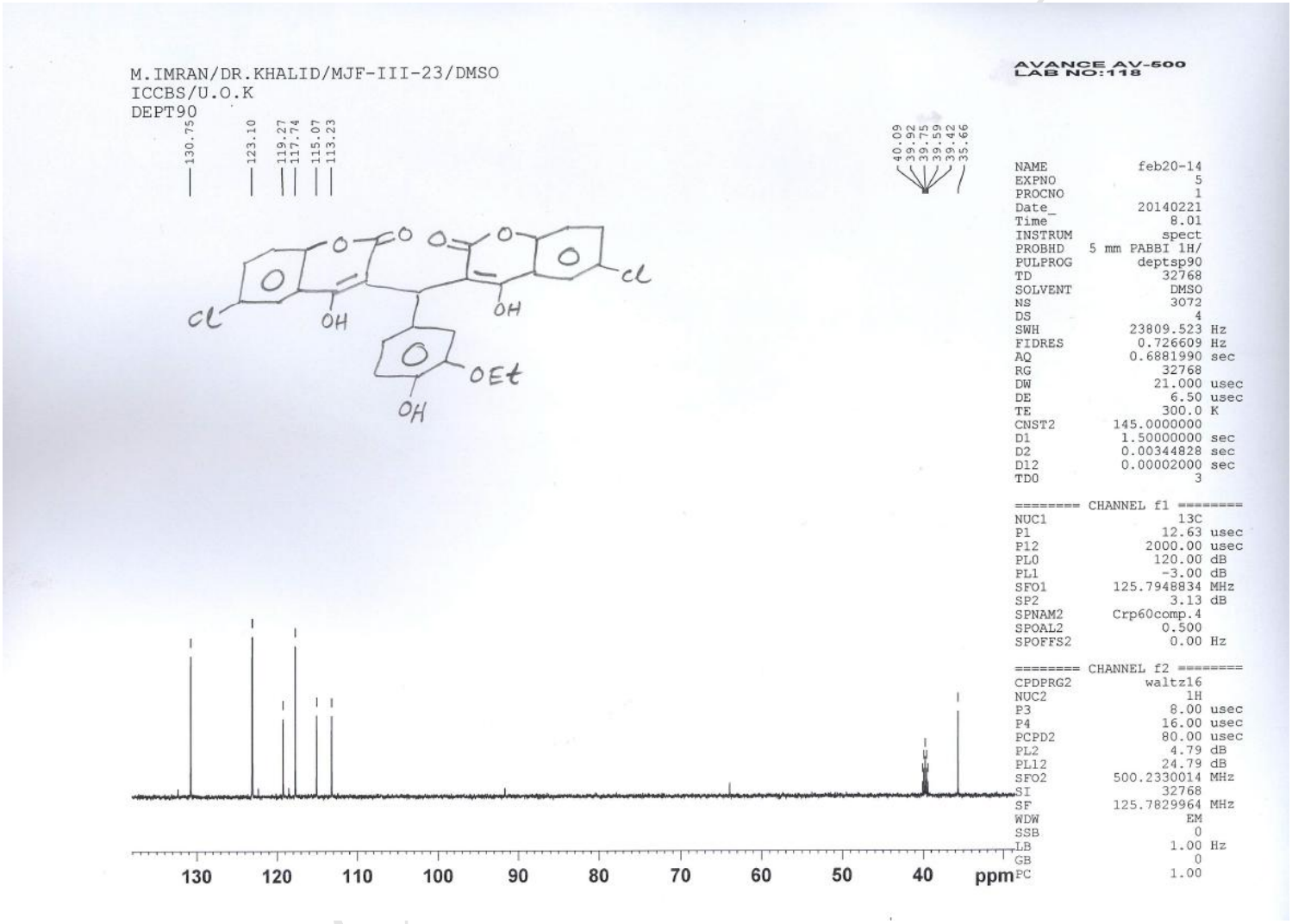
[13] CNMR of Compound 9



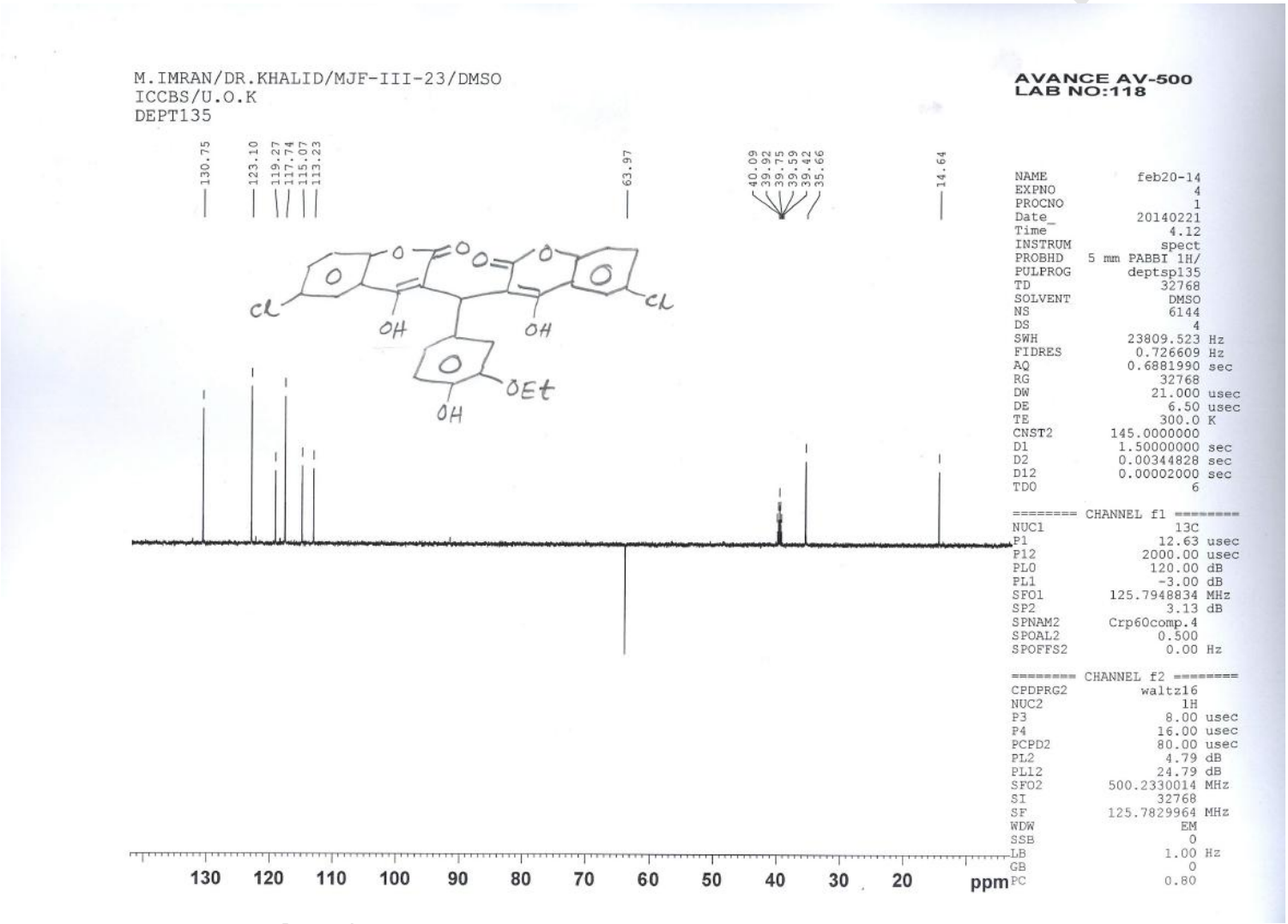
AVANCE AV-500
LAB NO:118

NAME	feb20-14
EXPNO	3
PROCNO	1
Date_	20140220
Time	16.03
INSTRUM	spect
PROBHD	5 mm PABBI 1H/
PULPROG	zgpg
TD	32768
SOLVENT	DMSO
NS	16384
DS	4
SWH	30303.031 Hz
FIDRES	0.924775 Hz
AQ	0.5407385 sec
RG	32768
DW	16.500 usec
DE	6.50 usec
TE	300.0 K
D1	2.00000000 sec
D11	0.03000000 sec
TD0	16
======== CHANNEL f1 ========	
NUC1	13C
P1	12.63 usec
PL1	-3.00 dB
SFO1	125.7975248 MHz
======== CHANNEL f2 ========	
CPDPRG2	waltz16
NUC2	1H
PCPD2	80.00 usec
PL2	4.79 dB
PL12	24.79 dB
PL13	26.00 dB
SFO2	500.2330014 MHz
SI	32768
SF	125.7829964 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40

^{13}CNMR (90) of Compound 9



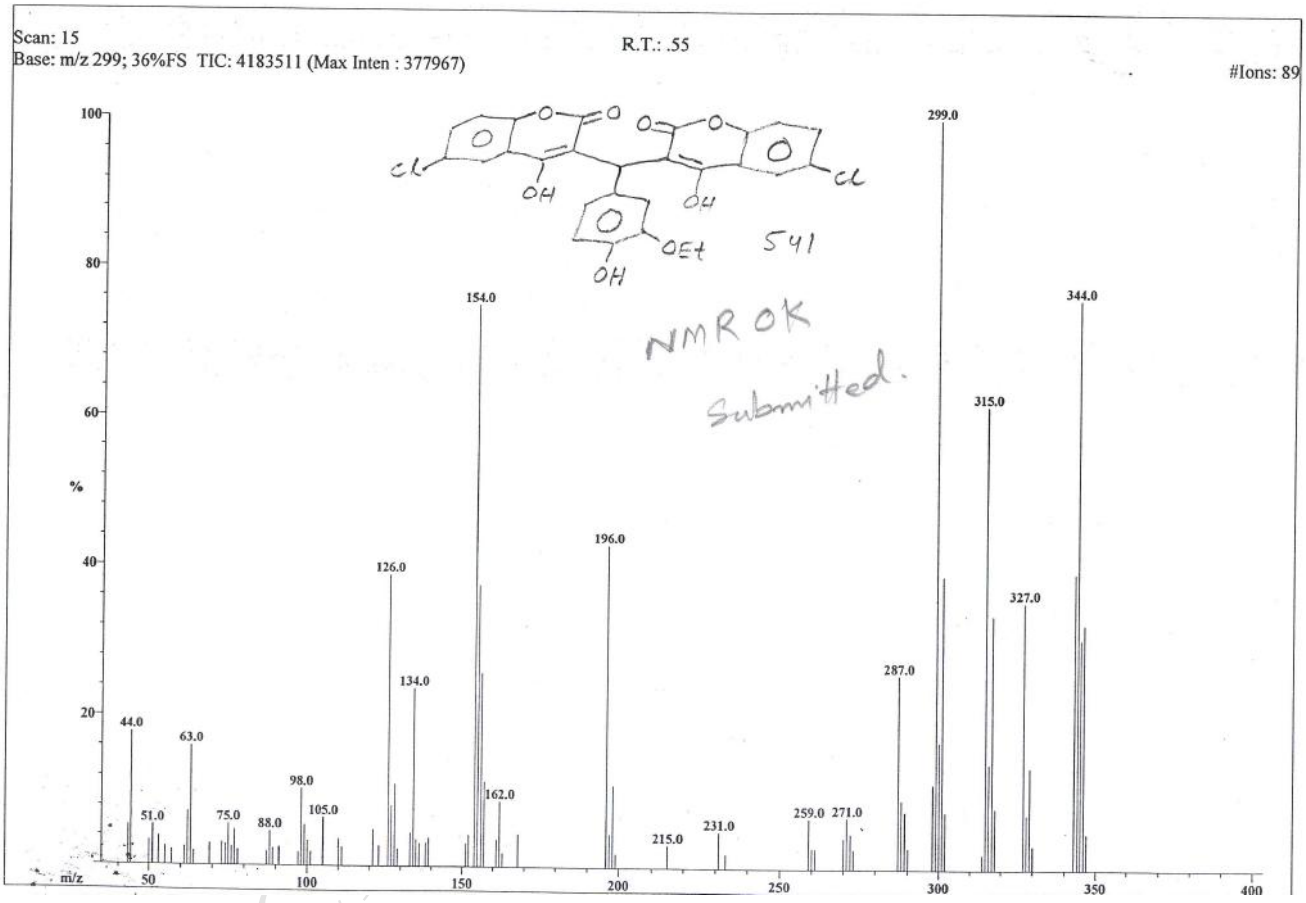
^{13} CNMR (135) of Compound 9



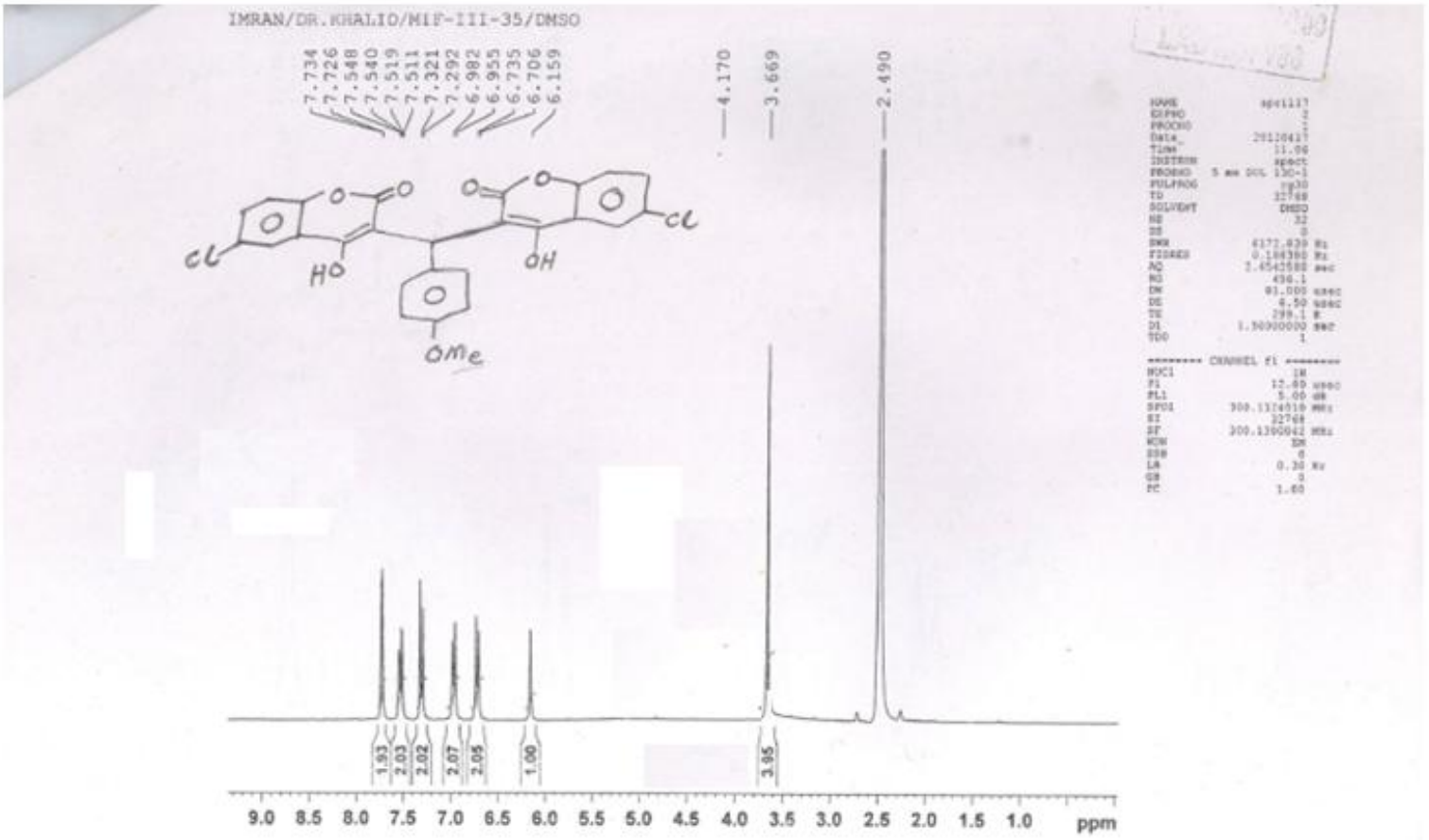
EI MS Spectra of Compound 9

File: MIF-III-23 Date Run: 03-09-2012 (Time Run: 11:04:49)
Sample: M IMRAN FAKHRI/DR. KHALID M KHAN
Instrument: JEOL MSRoute
Inlet: Direct Probe Ionization mode: EI+

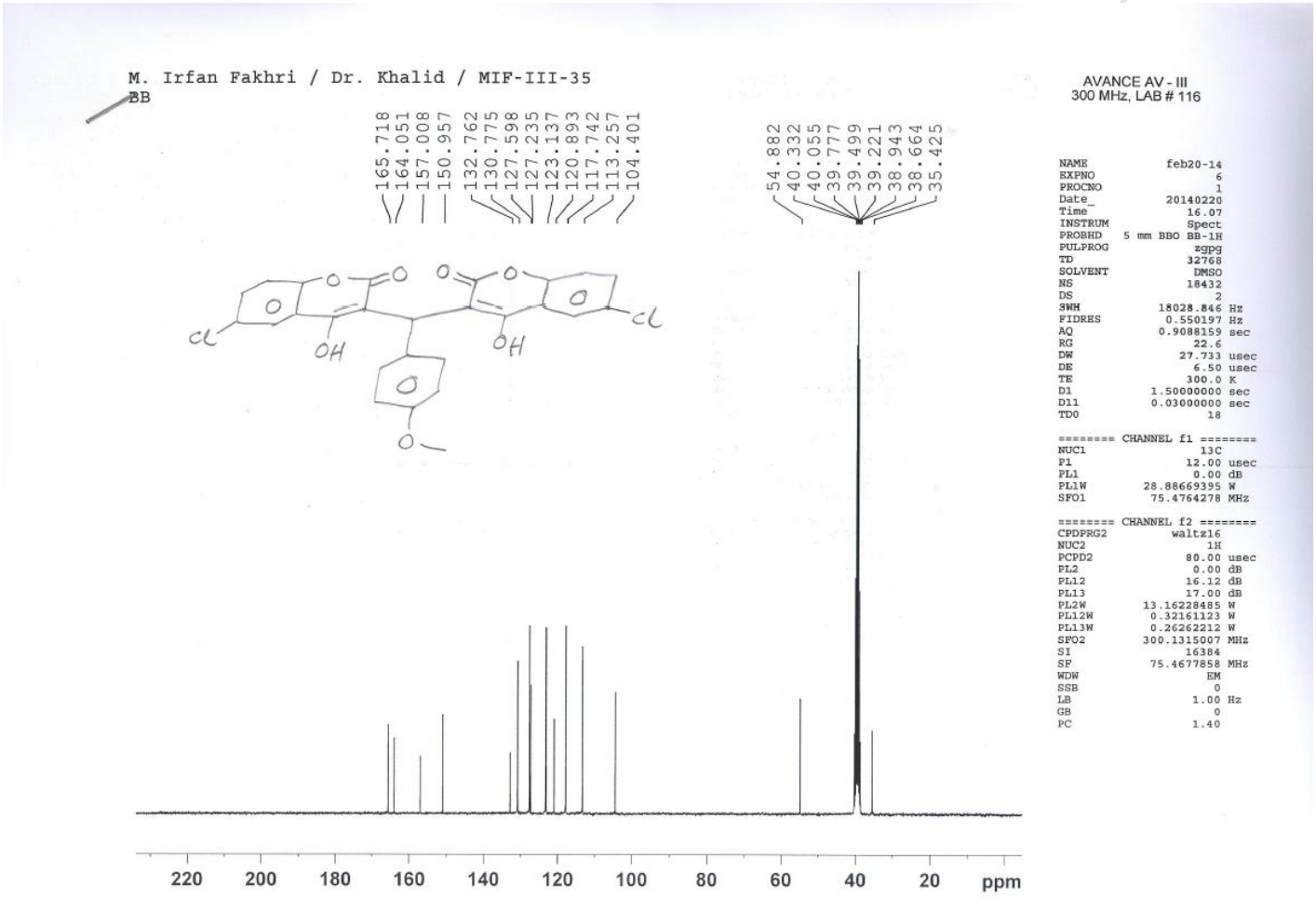
Scan: 15 R.T.: .55
Base: m/z 299; 36%FS TIC: 4183511 (Max Inten : 377967) #Ions: 89



[1]HNMR of Compound 18



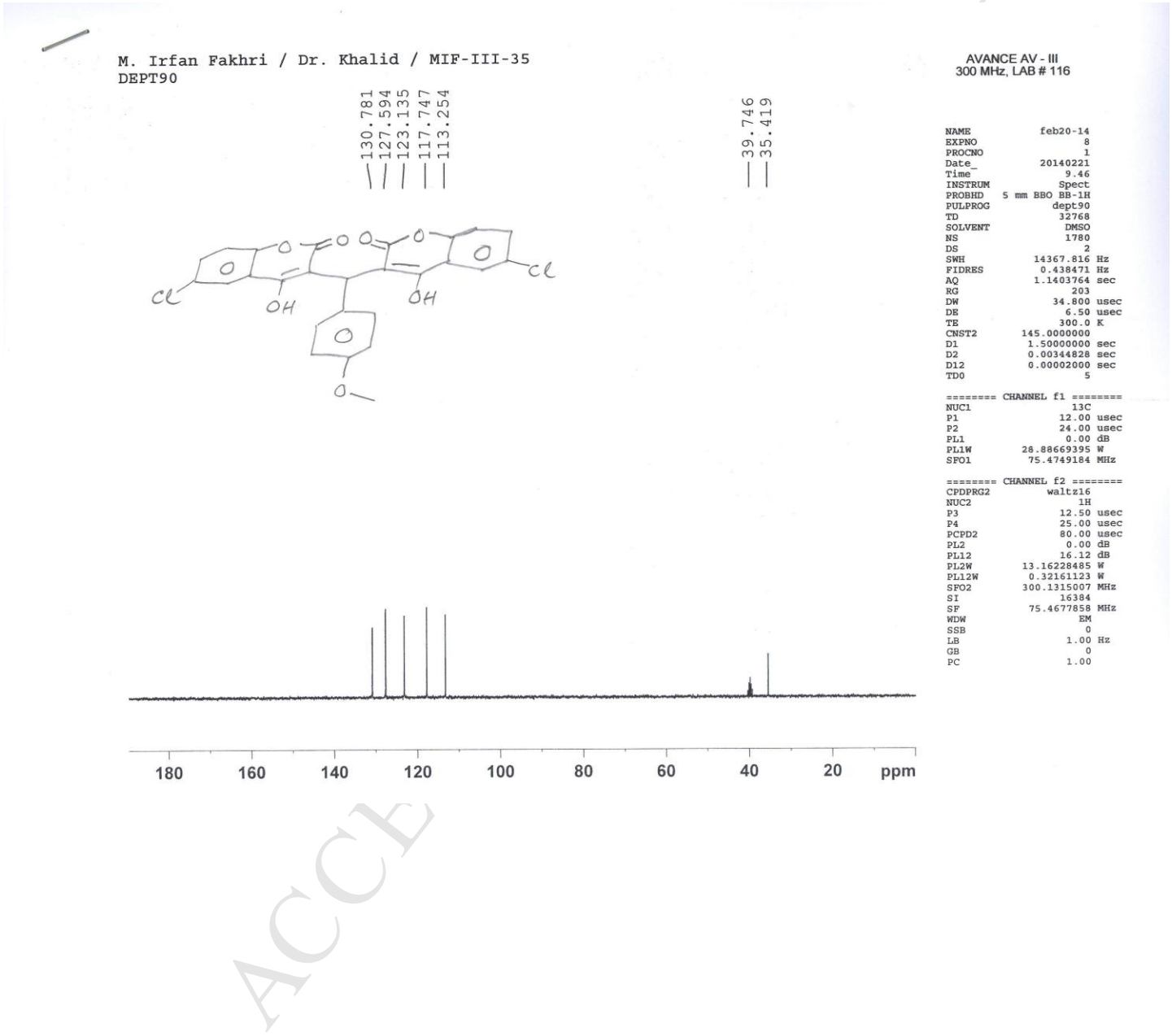
[13] CNMR of Compound 18



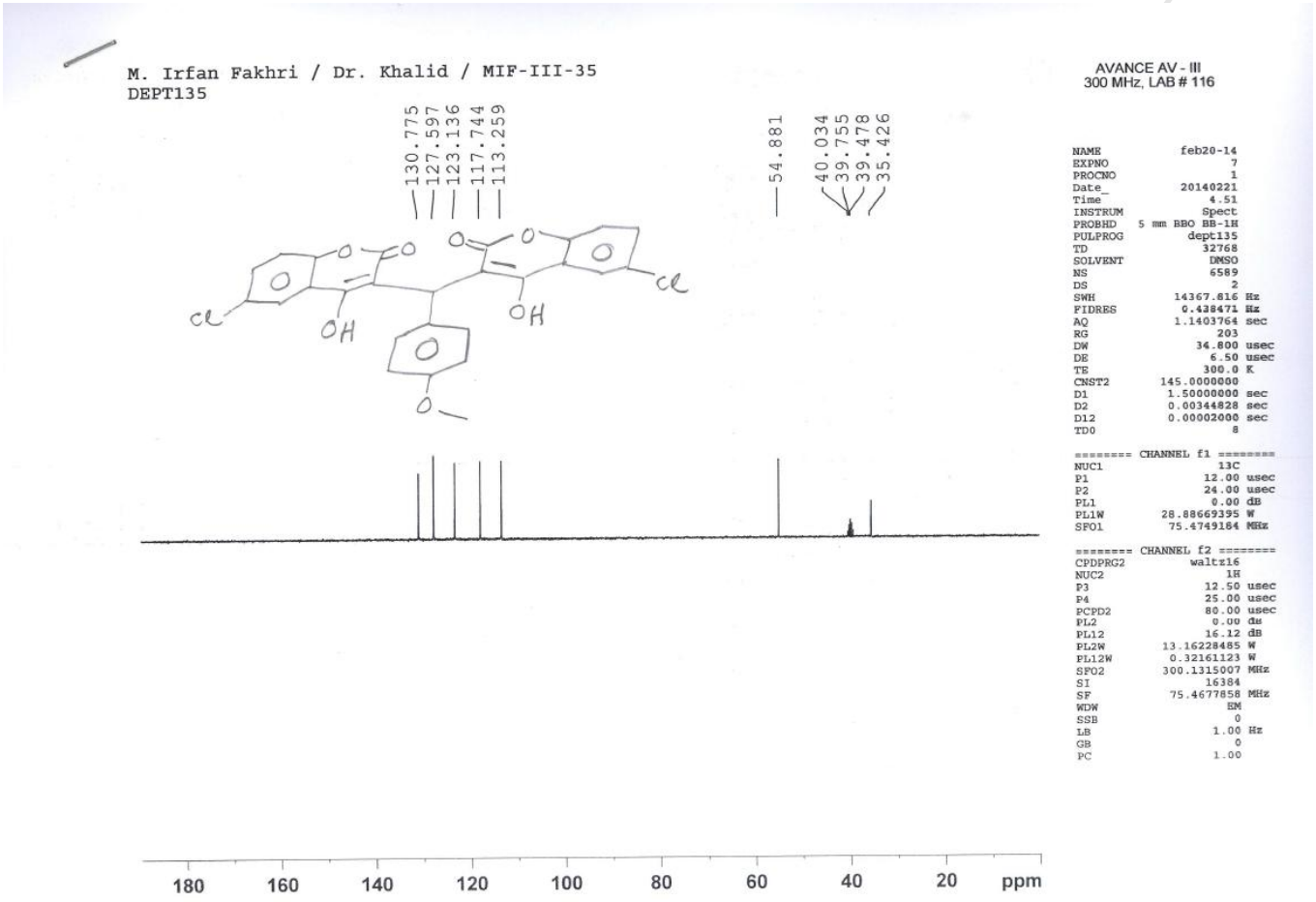
AVANCE AV - III
300 MHz, LAB # 116

NAME	feb20-14
EXPNO	6
PROCNO	1
Date_	20140220
Time	16.07
INSTRUM	Spect
PROBHD	5 mm BBO BB-1H
PULPROG	zgpg
TD	32768
SOLVENT	DMSO
NS	18432
DS	2
SWH	18028.846 Hz
FIDRES	0.550197 Hz
AQ	0.9088159 sec
RG	22.6
DW	27.733 usec
DE	6.50 usec
TE	300.0 K
D1	1.50000000 sec
D11	0.03000000 sec
TD0	18
======== CHANNEL f1 ========	
NUC1	13C
P1	12.00 usec
PL1	0.00 dB
PL1W	28.88669395 W
SFO1	75.4764278 MHz
======== CHANNEL f2 ========	
CPDPRG2	waltz16
NUC2	1H
PCPD2	80.00 usec
PL2	0.00 dB
PL12	16.12 dB
PL13	17.00 dB
PL2W	13.16228485 W
PL12W	0.32161123 W
PL13W	0.26262212 W
SFO2	300.1315007 MHz
SI	16384
SF	75.4677858 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40

^{13}CNMR (90) of Compound 18



^{13}CNMR (135) of Compound 18



AVANCE AV - III
300 MHz, LAB # 116

NAME	feb20-14
EXPNO	7
PROCNO	1
Date_	20140221
Time	4.51
INSTRUM	Spect
PROBHD	5 mm BBO BB-1H
PULPROG	dept135
TD	32768
SOLVENT	DMSO
NS	6589
DS	2
SWH	14367.816 Hz
FIDRES	0.438471 Hz
AQ	1.1403764 sec
RG	203
DW	34.800 usec
DE	6.50 usec
TE	300.0 K
CNST2	145.0000000
D1	1.5000000 sec
D2	0.00344828 sec
D12	0.00002000 sec
TD0	8
======== CHANNEL f1 ========	
NUC1	13C
P1	12.00 usec
P2	24.00 usec
PL1	0.00 dB
PL1W	28.88669395 W
SFO1	75.4749184 MHz
======== CHANNEL f2 ========	
CPDPRG2	waltz16
NUC2	1H
P3	12.50 usec
P4	25.00 usec
PCPD2	80.00 usec
PL2	0.00 dB
PL12	16.12 dB
PL2W	13.16228485 W
PL12W	0.32161123 W
SFO2	300.1315007 MHz
SI	16384
SF	75.4677858 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.00

EI MS Spectra of Compound 18