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Design, synthesis and biological evaluation of small molecules as potent glucosidase inhibitors

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Abstract. Herein we have reported design, synthesis and *in vitro* biological evaluation of a library of bicyclic lactams that led to the discovery of compounds **6** and **7** as a novel class of α -glucosidase inhibitors. They inhibited α -glucosidase (yeast origin) in a mixed type of inhibition with an IC₅₀ of ~150 nM. Molecular docking studies further substantiated screening results. Interestingly phenotypic screening of this library against the human malaria parasite revealed **7** as a potent antiplasmodial agent.

1. Introduction.

Diabetes mellitus is one of the most common non-communicable diseases of the globe.¹⁻² The prevalence of diabetes is estimated to double by 2030 with 69% increase in developing countries and 20% increase among adults in developed countries. Despite phenomenal progress in medical science, diabetes continues to be a major killer.³

Diabetes is a disease characterized by chronic hyperglycemia that leads to development of macro and micro vascular complication.⁴ Hence, one of the therapeutic approaches in type 2 diabetes is to reduce the demand for insulin by lowering the corresponding postprandial hyperglycemic levels via inhibition of enzymes in the digestive organs such as the α -glucosidases.⁵ Research has

shown that inhibition of α -glucosidase enzyme located at the intestinal brush border of the intestine may play a role in the lowering of postprandial hyperglycemia.⁵

To date, the only reported inhibitors of glycosidase enzymes are either complex natural products, like Validoxylamine A, MDL 25637 (α -homonojirimycin-7-*O*- β -D-glucopyranoside), trehazolin, acarbose, nojirimycin, castanospermineetc., or synthetic analogs like DNJ, N-butyl-DNJ, DANA and others.⁶ Iminosugars and thiosugar derivatives are also one of the most promising glycosidase inhibitors.⁷ However there are not very many non-sugar synthetic compounds as potent glycosidase inhibitors.⁸⁻¹⁵ Hence, discovery of such molecules will have a niche appeal and applicability in the pharmaceutical world.

Herein we report our investigation involving identification of a small molecule inhibitor of α -glucosidase *via* routine glucosidase profiling of small molecule libraries unbiased towards any specific targets. This exercise resulted in generating a potential hit compound **1** possessing bicyclic lactam motif. Design and synthesis of a focused library of bicyclic lactams around this motif with diverse functionalities followed by *in vitro* screening against yeast α -glucosidase enzyme generated **6** and **7** as most potent compounds with IC₅₀ ~150 nM. Reaction kinetics suggested that they are mixed inhibitors.

In addition phenotypic screening against 3D7 strain of Plasmodium falciparum revealed decent antiplasmodial activity of **7**.

2. Results and discussion

2.1 Initial screening,

To begin with we subjected our in-house compound collection, unbiased towards any specific targets in a routine glycosidase panel profiling. The exercise resulted in generating **1** as an interesting hit molecule with IC₅₀ of 2 μ M against yeast α -glucosidase. **1** was obtained by the dihydroxylation of the previously reported cyclic ene-lactam **2**.¹⁶

2.2 Library design and synthesis

In a bid to generate the library from **1** we envisioned **8**, the diastereomer of **1**, as a library member followed by 6-membered bicyclic lactams **9** and **10** as the higher homologs of **1**. Additionally, we choose **3** and **5** where oxygen is located differently than in **1**. Also included in

the library are the dehydroxylated analog **6** and the unsaturated analogs **7** and **2** (the immediate precursor of **1**). We further envisioned that epoxide analogs **4** and **11** will streamline the SAR, hence they were synthesized too (fig. 4). In a bid to diversify the aromatic moiety of the bicyclic lactams, benzyl and indoline substituted bicyclic lactams **12** and **13** were incorporated. Finally we envisioned a set of dimeric bicyclic lactams **14-17** in our library. By virtue of possessing diverse biological activity the dimeric compounds have lately gained lot of importance in the pharmaceutical world and that prompted their inclusion in this focused library (figure 1).

(Figure 1)

Synthesis of compounds **3**, **4**, **5** and **10** were accomplished following literature procedures.¹⁷⁻¹⁹ Compound **2** was obtained from bicyclic lactam **18**, by phenyl selenium bromide mediated addition-reduction followed by oxidation of the arylselenium intermediate with hydrogen peroxide. **18** in turn was obtained by condensing *S*-pyroglutaminol and benzaldehyde with catalytic *p*-toluenesulfonic acid (PTSA) in refluxing toluene. Dihydroxylation of **2** in presence of *N*-methylmorpholine-N-Oxide and osmium tetroxide in 1:1 acetone: water afforded **1**. In a different reaction, epoxidation of **2** with meta-perbenzoic acid (mCPBA) resulted in **4**. In a similar effort compound **6** (synthesized from *R*-pyroglutaminol and benzaldehyde) was converted to **7**. And a similar dihydroxylation of **7** yielded **8**. Epoxidation of **7** provided **11**. Simple condensation of *S*-phenylalaninol and *S*-Tryptophanol with 2-(2-nitrophenyl)-4-oxopentanoic acid in toluene in catalytic *p*-toluene sulfonic acid furnished **12** and **13**. Finally oxidative homoenolate coupling of bicyclic lactams **5**, **18**, **19** and **20** in presence of lithium bis(trimethylsilyl)amide (2.1 eq.) and 2.1 eq. of the oxidant iodine (I_2) afforded **14-17**. The synthesis of the library was designed in a way where each member of the library can be obtained in minimum number of steps and with high steps/molecule ratio (~1.7), thereby making the library generation more facile (figure 2).

(Figure 2)

2.3 In vitro assay against α -glucosidase (yeast origin)

We screened all the compounds against α -glucosidase enzyme of yeast origin along with acarbose, a known α -glucosidase inhibitor, as the positive control. The percentage inhibition on the α -glucosidase was measured at a concentration of 10 μ M to 50 μ M

(refer *SI* for experimental details). To assess the potency of our compounds, the inhibitory activities were plotted against the concentrations using non-linear regression approach (sigmoid curves) (cf. *SI*) from which their IC₅₀ values were computed (Table 1). Among the compounds screened, **6** and **7** exhibited strong inhibition against α -glucosidase with IC₅₀s of 0.17 and 0.15 μ M respectively, faring much better than acarbose control (900nM). Compounds **2** and **8** also bind effectively with IC₅₀ ranging between 0.19 & 0.21 μ M. To further probe the inhibitory effect of our compounds, we obtained their dose responses against α -glucosidase. The results showed that the activity of α -glucosidase was reduced by majority of our compounds (**2**, **6**, **7** and **8**) in a dose-reliant fashion thereby indicating their strong affinity towards the enzyme (Figure 3).

(Table 1)

(Figure 3)

2.4 Molecular Docking and modelling

With the IC₅₀ values in hand, we wanted to understand the binding interactions of the initial compound **1** and most active compounds **6** and **7** with α -glucosidase. This would enable us to design more active analogues of **1**. Even though the X-ray crystallographic structure of α -glucosidase has been reported for some bacteria, the three dimensional structural information is not available for the yeast α -glucosidase enzyme. Hence we constructed the 3D structure of α -glucosidase by homology modeling. The sequence of α -glucosidase was downloaded from Uniprot (ID: P10253). BLASTP ® against protein data bank was performed in order to identify the template for sequence alignment. Human Maltase-Glucoamylase (PDB ID: 2QLY, 3L4T) were showing good similarity to our query sequence.²⁰ Residues starting from 89 are aligning to these PDB and showing 44% identity. These two protein structures were therefore selected to model α -glucosidase using homology modelling. The homology model of α -glucosidase was built using Modeller v9.14. Three models were generated using Modeller v9.14 and the model having best DOPE score is selected for further studies.²¹ The energy of the model was minimized using MOE energy minimization algorithm using Force Field MMFF94x. The stereochemical quality of this model was validated by the Ramachandran plot using the PROCHECK program. 98.1% residues are in the favored and allowed regions, only 1.9% are outliers and no active site residue lie in this outlier region. Further the model is also validated using verify 3D program (figure 3).

(Figure 4)

Molecular modelling of **1** revealed that it was bound deeply into the binding cavity of α -glucosidase and showed interaction with the residues Lys 609, Asp 724, Leu 613, Gln 688, Ile 726, Ala 610, Thr 725, Gln 575, Phe 579, Val 728 & Asp 686 residues. **1** was found to form a hydrogen bond with the δ -amino moiety of Lys 609 and carbonyl group of amide moiety of Asp 724 (figure 2).

(Figure 5)

Next both compounds **6** and **7** were docked against the protein (figure 6 and 7). **6** and **7** bind to the active site residues of modelled α -glucosidase protein by forming one hydrogen bond and few other non-bonded interactions. For **6**, the hydrogen bond is formed with Asp 686 residue while His 729, Asn 727, Val 728, Thr 725, Pro 722, Ile 726, Gln 688, Leu 719, Pro 720 residues shared non-bonded interactions. Similarly **7** binds to the active site residues of modelled α -glucosidase protein by the hydrogen bond formed with Asp 686 residue while Thr 725, Gln 688, His 729, Pro 722, Ile 726, Val 728, Asn 727, Leu 719, Pro 720 residues shared non-bonded interactions. **6** and **7** showed similar binding with the receptor but the energy of the docked model is different. **1** showed stronger binding as compared to **6** and **7** but the stability of docked model is better in case of **6** and **7**. This may be the reason why **6** and **7** demonstrated better activity than **1** (Figures 6a and b).

(Figure 6a)**(Figure 6b)****2.5 Reaction kinetics study**

Enzyme kinetic assays were conducted with the most active compounds *viz.* **6** and **7** to understand their mode of inhibition to α -glucosidase. This was determined by employing primary (Lineweaver-Burke) and secondary plots of inhibition kinetics (Figures 7 [a] and [b]) for the compounds. As revealed from the Lineweaver-Burke plot, the data points of **6** and **7** intersected in the second quadrant thereby indicating a mixed type of inhibition.

The mixed inhibition is an intermediate of the competitive and uncompetitive inhibition and can be represented as shown in the equation below (eq. 1).

(Equation 1)

As mixed inhibitors, **6** and **7** were able to bind to either the free α -glucosidase (E) or the α -glucosidase-substrate (pNPG) complex (E+S). The two inhibition constants in the mixed inhibition, K_{i_a} and K_{i_b} signified the equilibrium constant of dissociation of the $I + E$ (inhibitor-enzyme) complex and the $I + ES$ (inhibitor-bound enzyme substrate) complex. These values for **6** and **7** were computed from the secondary plots of the slope and Y-intercept from the Lineweaver-Burke plot versus inhibitor concentration (refer to SI). K_{i_a} and K_{i_b} of **6** were determined to be $11.2\mu M$ & $17.5\mu M$ and those for **7** were $22.6\mu M$ & $33.7\mu M$ respectively. These constants show that both the compounds have strong potency to bind to the free enzyme.

(Figure 7)

2.5 Phenotypic screening against malaria phenotype (3D7).

Since α -glucosidase inhibitors possess versatile mode of action against multiple therapeutic areas (*viz.* cancer, viral infection, malaria and etc.) other than diabetes we subjected the library of compounds to a phenotypic screening against the malaria parasite, Compound **7** showed remarkable inhibition against *3D7* strain of *Plasmodium Falciparum*). The parasites were treated at the ring stage and the percent parasitemia was calculated at the trophozoite stage of the second cycle (i.e. after 72 hours of incubation). The percent inhibition of invasion was calculated *w.r.t* the untreated control and nearly 65% inhibition was observed in presence of **7** (figure 8).

(Figure 8)

The fact that the same compound **7** showed activity against α -glucosidase and *3D7* strain of malaria parasite forced us to rethink about any connection between α -glucosidase and malaria parasite life cycle. However this is a preliminary result. Perhaps there is a plausible connection between the two but no direct evidence for such an observation is evident yet. Much exhaustive investigation is required to connect diabetes and malaria. It is an interesting avenue to explore for

the medicinal chemists. Further work on this is ongoing in our lab.

3. Conclusion

Herein we have discussed design and synthesis of a focused library of compounds based on bicyclic lactam **1**. It was identified from a random unbiased screening of our in-house library. *In vitro* screening of the library against yeast α -glucosidase generated two novel inhibitors **6** and **7**, with IC₅₀ in the range of 150 – 200 nM. Reaction kinetics indicated that both of them have mixed type inhibition against α -glucosidase. Molecular docking of **6** and **7** against the homology model of α -glucosidase rationalized their activity. Interestingly, phenotypic screening of the library yielded **7** as a potent inhibitor of 3D-7 strain of Malaria Parasite Plasmodium *Falciparum*. Both antiplasmodial and anti α -glucosidase property of the **7**, opens a possibility of development of this compound as a potential treatment option for both the diseases.

4. Materials and methods

General Experimental

Reagents were obtained from Aldrich Chemical (www.sigma-aldrich.com) or Acros Organics (www.fishersci.com) and used without further purification. Optima grade solvents were obtained from Fisher Scientific (www.fishersci.com), degassed with Argon, and purified on a solvent drying system as described, unless otherwise indicated. Lithium hexamethyl disilylamide (LHMDS) was obtained from Aldrich in SureSeal bottles. Phenyl selenium bromide was obtained from Acros. Compounds **6** and **3-5, 9-11** and **18-20** are prepared from the literature procedure.¹⁷⁻¹⁹ The single crystal X-Ray of **11a** (the amine compound from **11**), confirms the relative configuration of **11**. All reactions were performed in flame-dried glassware under positive Argon pressure with magnetic stirring unless otherwise noted. Rubber septa and syringes were used for the transfer of liquid reagents and solutions. Syringes were purged with argon prior to use. TLC was performed on 0.25 mm E. Merck silica gel 60 F254 plates and visualized under UV light (254 nm) or by staining with potassium permanganate (KMnO₄), phosphomolybdic acid (PMA), iodine (I₂), or *p*-anisaldehyde. Silica flash chromatography was performed on E. Merck 230-400 mesh silica gel 60. IR spectra were recorded on a Perkin Elmer Spectrum 100 FTIR spectrometer with peaks reported in cm⁻¹. NMR spectra were recorded on Varian

400MR and Varian 300 instruments at 24°C in CDCl₃ unless otherwise indicated. Spectra were processed using VNMR J 3.2 software, and chemical shifts are expressed in ppm relative to TMS (¹H, 0 ppm) or solvent signals: CDCl₃ (¹H, 7.26; ¹³C, 77.0 ppm); coupling constants are expressed in Hz. Mass spectra were obtained at the Shiv Nadar University Analytical Facility on an Agilent 6540QTOF by electrospray (ESI) ionization.

(3R,7aS)-3-phenyl-1,7a-dihydropyrrolo[1,2-c]oxazol-5(3H)-one (2).

To the solution of bicyclic lactam **18** (900mg, 1eq) in THF (9mL) was added 1M solution of LiHMDS in THF (8.8mL, 2eq) at -78°C, pale yellow solution was allowed to stir 30 min. After that solution of PhSeBr (1.15g, 1.1 eq.) in THF was added drop-wise over 10 minutes. Reaction mixture was further stirred for 30 min at -78°C. Reaction progress was monitored by TLC. Reaction mixture was quenched by adding saturated aqueous NH₄Cl solution, and then warmed to rt and extracted in diethyl ether (50 mL*3). The organic extracts were separated and concentrated to afford phenyl selenylated product.

Phenyl selenylated product dissolved in dichloromethane (40 mL) and treated with 30% H₂O₂ (6 eq.) at 0°C. The resulting heterogeneous mixture was allowed to warm to rt while stirring vigorously for 16 h. The aqueous phase was extracted with CH₂Cl₂, and the organic extracts were combined and washed successively with 1N HCl and saturated aqueous NaHCO₃. The organic phase was dried (Na₂SO₄) and concentrated under reduced pressure to afford a crude compound, which further purified by column chromatography (30% EA in *n*-Hexane) to afford alpha beta unsaturated compound **2** (600mg). ¹H NMR (300 MHz, DMSO-D₆): δ 7.57-7.35 (m, 7H), 6.18-6.17 (d, *J* = 5.2 Hz, 1H), 5.95 (s, 1H), 4.78-4.74 (t, *J*₁ = 7.6 Hz, *J*₂ = 15.6 Hz, 1H), 4.27-4.24 (t, *J*₁ = 7.2 Hz, *J*₂ = 15.2 Hz, 1H), 3.34-3.30 (m, 1H). ¹³C NMR (75 MHz, DMSO-D₆) 176.95, 147.82, 138.51, 129.17, 128.61, 128.42, 126.13, 87.37, 68.04, 65.11 ppm. AQUITY UPLC (MS ESI): [M + H]⁺ calcd for C₁₂H₁₁NO₂ 202.22, found 202.10

(3R,6R,7R,7aR)-6,7-dihydroxy-3-phenyltetrahydropyrrolo[1,2-c]oxazol-5(3H)-one (1).

Compound **2** (200 mg, 1 eq.) and 50% solution of acetone in water (4mL) purged with nitrogen and then it was charged with 50% aqueous N-methylmorpholine-N-oxide (NMO) (0.4mL) and 2% solution of OsO₄ in butanol (0.4mL) at 25°C. Reaction mixture was stirred for 16h at rt. Once TLC indicates complete consumption of starting material

the reaction mixture was quenched with aq. solution of Na₂SO₃. It was extracted with ethyl acetate (4 X 25mL), Organic layer dried over sodium sulphate and distilled off on rotary evaporator to afford white solid compound **1** (150mg). ¹H NMR (300 MHz, DMSO-D₆): δ 7.40-7.34 (m, 5H), 6.08 (s, 1H), 5.88-5.87 (m, 1H), 5.52-5.38 (m, 1H), 4.27-4.22 (m, 1H), 4.08-3.96 (m, 3H), 3.95-3.66 (m, 1H). ¹³C NMR (75 MHz, DMSO-D₆) 174.78, 137.22, 128.92, 128.58, 125.99, 110.01, 87.00, 74.23, 71.00, 69.24, 65.07 ppm. AQUITY UPLC (MS ESI): [M + H]⁺ calcd for C₁₂H₁₃NO₄ 236.24, found 236.14. $[\alpha]^{20}_D$ +46.3 (c 1, CH₃OH)

(3S,7aR)-3-phenyl-1,7a-dihydropyrrolo[1,2-c]oxazol-5(3H)-one (7).

Reaction procedure is same as **2**, Reaction done on 800 mg scale from **8** to afford 500mg of desired compound (**7**). ¹H NMR (300 MHz, DMSO-D₆): δ 7.61-7.59 (d, J = 8Hz, 2H), 7.41-7.28 (m, 3H), 7.26-7.19 (m, 1H), 6.19- 6.17 (m, 2H), 4.65-4.61 (t, J₁ = 7.2 Hz, J₂ = 15.2 Hz, 1H), 4.29-4.21 (dd, J₁ = 7.6 Hz, J₂ = 17.2 Hz, 1H), 3.45-3.41 (t, J₁ = 8.4 Hz, J₂ = 16.8 Hz, 1H). ¹³C NMR (75 MHz, DMSO-D₆) 176.94, 150.59, 139.07, 128.54, 128.33, 127.75, 126.39, 86.95, 67.88, 65.27 ppm. AQUITY UPLC (MS ESI): [M + H]⁺ calcd for C₁₂H₁₁NO₂ 202.22, found 202.05

(3S,6S,7S,7aS)-6,7-dihydroxy-3-phenyltetrahydropyrrolo[1,2-c]oxazol-5(3H)-one (8).

Reaction procedure is same as **1**, reaction done on 230mg scale to afford 220mg of **8** to generate desired compound as white solid. ¹H NMR (300 MHz, DMSO-D₆): δ 7.38-7.37 (m, 5H), 6.08 (s, 1H), 6.04-5.89 (m, 1H), 4.52-4.49 (m, 1H), 4.08-3.69 (m, 2H), 3.67-3.33 (m, 1H). ¹³C NMR (75 MHz, DMSO-D₆) 175.55, 175.24, 138.94, 138.68, 128.58, 128.45, 128.37, 126.13, 86.29, 86.20, 74.82, 73.78, 71.01, 70.03, 69.22, 64.40, 64.18, 58.81ppm. AQUITY UPLC (MS ESI): [M + H]⁺ calcd for C₁₂H₁₃NO₄ 236.24, found 236.0. $[\alpha]^{20}_D$ -40.3 (c 1, CH₃OH)

(3S,6R,7aR)-3-benzyl-7a-methyl-6-(2-nitrophenyl)tetrahydropyrrolo[2,1-b]oxazol-5(6H)-one (12).

2-(2-nitrophenyl)-4-oxopentanoic acid (12 g, 50.6 mmol), (S)-phenyl alaninol (7.3 g, 52 mmol) and PTSA (12 mg, cat) were dissolved in toluene (120 mL). The flask was equipped with a Dean-Stark trap, and the solution was heated to reflux. After 16 h the solution was cooled, washed with saturated aqueous NaHCO₃, dried, and concentrated. Purification by flash column chromatography on silica gel (hexane/EtOAc, 1:1) generated

the desired compound which was triturated with diethyl ether to afford **12** (7.4 g, 41.5%) as white solid. ¹H NMR (300 MHz, DMSO-D₆): δ 7.98-7.95 (d, J = 8.1 Hz, 1H), 7.59-7.36 (m, 3H), 7.32-7.22 (m, 6H), 7.15-7.02 (m, 1H), 4.68-4.48 (m, 1H), 4.40-4.28 (m, 1H), 4.25-4.16 (t, J_1 = 8.7 Hz, J_2 = 18Hz, 1H) , 4.01-3.89 (m, 1H), 3.15-3.11(m, 1H), 2.88-2.72 (m, 2H), 2.49-2.41 (t, J = 12Hz, 1H), 1.55 (s, 3H). ¹³C NMR (75 MHz, DMSO-D₆) 175.28, 149.58, 136.93, 133.24, 132.23, 131.44, 129.62, 129.32, 128.57, 128.35, 126.76, 126.54, 125.46, 125.15, 124.26, 122.67, 122.57, 120.47, 120.25, 119.95, 111.78, 111.38, 97.18, 55.89, 47.98, 44.36, 39.99, 27.72, 25.02 ppm. AQUITY UPLC (MS ESI): [M + H]⁺ calcd for C₁₂H₁₃NO₄ 353.39, found 353.30. $[\alpha]^{20}_D$ +21.5 (c 1, CH₃OH)

(3*R*,6*S*,7*aS*)-3-((1*H*-indol-3-yl)methyl)-7*a*-methyl-6-(2-nitrophenyl)tetrahydropyrrolo[2,1-*b*]oxazol-5(6*H*)-one (**13**).

2-(2-nitrophenyl)-4-oxopentanoic acid (6 g, 25 mmol), (S)-tryptophanol (4.94 g, 26 mmol) and PTSA (12 mg, cat) were dissolved in toluene (120 mL). The flask was equipped with a Dean-Stark trap, and the solution was heated to reflux. After 16 h the solution was cooled, washed with saturated aqueous NaHCO₃, dried, and concentrated. Purification by flash column chromatography on silica gel (hexane/EtOAc, 1:1) generated the desired compound which was triturated with diethyl ether to afford **13** (3.4 g, 35%) as colourless solid. ¹H NMR (300 MHz, DMSO-D₆): δ 10.90 (s, 1H), 7.59-7.56 (m, 1H), 7.37-7.35 (d, J = 8Hz, 1H), 7.29-7.28 (d, J = 1.6Hz, 1H), 7.17-7.13 (m, 1H), 7.10-7.06 (m, 1H), 7.01-6.98 (t, J = 1.2Hz, 1H), 4.92 (s, 2H), 4.34-4.31 (m, 2H), 4.20-4.11 (m, 1H), 3.93-3.89 (m, 1H), 3.17-3.08 (m, 1H), 2.96-2.91 (m, 1H), 2.10-2.04 (m, 1H), 1.51 (s, 3H). AQUITY UPLC (MS ESI): [M + H]⁺ calcd for C₂₂H₂₄N₃O₄ 392.42, found 392.78. $[\alpha]^{20}_D$ +11.3 (c 1, CH₃OH)

Procedure for the synthesis of dimeric bicyclic lactam **14**:

(3*aR*,3'*aR*,4*aS*,4'*aS*,9*bR*,9'*bR*)-3*a*,3'*a*-dimethyl-3,3*a*,3',3'*a*,4*a*,4'*a*,5,5'-octahydro-1*H*,1'*H*-[2,2'-biindeno[1,2-*d*]pyrrolo[2,1-*b*]oxazole]-1,1'(2*H*,2'*H*,9*bH*,9'*bH*)-dione (**14**).

To a solution of bicyclic lactam **19** (10.0 g, 43.6 mmole) in THF (100 ml) was added LiHMDS (1.0 M solution in THF, 47.97 ml, 47.97 mmole) at -78°C in a drop wise manner. The reaction mixture was stirred for 30 min at that temperature. A solution of

iodine (11.07g, 43.6 mmole) in THF(10 ml) was added to the reaction mixture in drop wise manner over a period of 30 min. The reaction mixture was further stirred for 2 hours at -78°C and the warmed upto room temperature. Reaction progress was monitored by thin layer chromatography [eluent: 30% EA in *n*-hexane]. After completion of the reaction, the reaction mixture was cooled to -78°C and quenched by saturated ammonium chloride solution. The crude product was extracted in ethyl acetate (200 ml*2). Organic layer was washed with aqueous sodium thiosulphate. 5H₂O solution (250 ml), water (250 ml) and brine solution (250 ml). And then dried over sodium sulphate and distilled off on a rotary evaporator to afford the crude solid which was further purified by column chromatography (silica:100-200 mesh, eluent: 16% ethyl acetate in *n*-hexane) to afford off white solid compound (9.5 g, 47.7%). ¹H NMR (300 MHz, DMSO-D₆): δ 7.41-7.31 (t, *J* = 8Hz, 1H), 7.29-7.23 (m, 4H), 5.68-5.66 (d, *J* = 8Hz, 1H), 4.92-4.89 (m, 1H), 3.49-3.45 (m, 1H), 3.28-3.14 (m, 2H), 2.33-2.28 (m, 1H), 2.11-1.60 (m, 1H), 1.03 (s, 3H). ¹³C NMR (75 MHz, DMSO-D₆) 179.2, 131.2, 130.4, 130.2, 126.6, 125.8, 72.1, 58.5, 52.3, 46.4, 42.8, 21.2 ppm. AQUITY UPLC (MS ESI): [M + H]⁺ calcd for C₂₈H₂₈N₂O₄ 457.53, found 457.2. $[\alpha]^{20}_D$ +34.8 (c 1, CH₃OH).

(3R,3'R)-3,3'-diphenyloctahydro-[6,6'-bipyrrolo[2,1-b]oxazole]-5,5'(6H,6'H)-dione (**15**).

The above procedure was followed with bicyclic lactam **5** (0.5g, 2.46mmol), 1M LiHMDS (2.75mL), I₂ (0.63g, 2.46 mmol) and THF (10mL). ¹H NMR (300 MHz, CDCl₃): δ 7.38-7.28 (m, 10H), 5.31-5.30 (m, 2H), 5.29-5.08 (m, 2H), 4.60-4.56 (m, 2H), 3.87-3.83 (m, 2H), 3.64-3.59 (m, 1H), 2.68-2.60 (m, 2H), 1.99-1.92 (m, 2H). ¹³C NMR (75 MHz, CDCl₃) 181.2, 128.2, 126.4, 126.3, 124.2, 67.3, 62.8, 61.9, 60.4, 42.1, 40.1, 28.1, 24.2 ppm. AQUITY UPLC (MS ESI): [M + H]⁺ calcd for C₂₄H₂₄N₂O₄ 405.46, found 405.0.

(3R,3'R,7aR,7'aR)-7a,7'a-dimethyl-3,3'-diphenyloctahydro-[6,6'-bipyrrolo[2,1-b]oxazole]-5,5'(6H,6'H)-dione (**16**)

The above procedure was followed with bicyclic lactam **20** (0.5g, 2.30mmol), 1M LiHMDS (2.75mL), I₂ (0.6g, 2.30 mmol) and THF (10mL). ¹H NMR (300 MHz, CDCl₃):

δ 7.37-7.33 (t, J = 8Hz, 2H), 7.29-7.24 (m, 2H), 5.14-5.11 (t, J = 8Hz, 15.2Hz, 1H), 4.62-4.57 (t, J = 8.4 Hz, 16.8Hz, 1H), 4.11-4.07 (t, J = 7.2 Hz, 16Hz, 1H), 3.70-3.66 (t, J = 8.8 Hz, 17.6Hz, 1H), 2.45-2.40 (m, 1H), 2.21-2.15 (m, 1H), 1.49 (s, 3H). ^{13}C NMR (75 MHz, CDCl_3). AQUITY UPLC (MS ESI): [M + H]⁺ calcd for $\text{C}_{26}\text{H}_{28}\text{N}_2\text{O}_4$ 433.5, found 433.0.

(3S,3'S)-3,3'-diphenyloctahydro-[6,6'-bipyrrolo[1,2-c]oxazole]-5,5'(3H,3'H)-dione (**17**)

The above procedure was followed with bicyclic lactam **18** (0.5g, 2.46mmol), 1M LiHMDS (2.75mL), I_2 (0.63g, 2.46 mmol) and THF (10mL). ^1H NMR (300 MHz, CDCl_3): δ 7.50-7.26 (m, 10H), 6.36 (s, 2H), 4.39-4.36 (m, 2H), 4.19-4.16 (m, 2H), 3.41-3.40 (m, 4H), 3.39-3.25 (m, 2H). ^{13}C NMR (75 MHz, CDCl_3). AQUITY UPLC (MS ESI): [M + H]⁺ calcd for $\text{C}_{26}\text{H}_{28}\text{N}_2\text{O}_4$ 405.46, found 405.1.

In vitro assay of α -Glucosidase inhibitory activity

The inhibitory potency of the compounds against α -glucosidase activity was assessed in 96-well plates using PNPG (4-nitrophenyl α -D-glucopyranoside) as a substrate according to the procedure reported by Ferreres et al.²² Prior to screen, all the test compounds were solubilized in a suitable solvent, Dimethylsulfoxide (DMSO) and eventually diluted to attain the desired concentration. Briefly, each well was comprised of 100 μL of substrate (2mM, PNPG dissolved in 2mM phosphate buffer at a pH of 7.2) and different test concentrations (10-100 μM). Then the final volume of the reaction mixture was made up to 200 μl with 2mM phosphate buffer (pH 7.2). The hydrolytic reaction was commenced by the addition of α -glucosidase enzyme (0.5 IU/mL) (obtained from Sigma Aldrich, Bangalore) and the plates were incubated at 37°C for 15 min. The reaction was terminated by the addition of 50 μL of 2N Na_2CO_3 solution. The absorbance was measured spectrophotometrically at 400 nm (Epoch reader; version 2.00.18). The decrease/increase in absorbance (ΔA) was compared with that of control (buffer instead of test compound) to compute the inhibitory profile of enzyme. The data used for the determination of IC₅₀ concentrations were fitted by non-linear regression fitting and the variance analysis was carried out by using MINITAB 15 software (trail version). The concentration of inhibition required for 50% of α -glucosidase activity under the assay conditions was defined as the IC₅₀ value. The half maximal inhibitory (IC₅₀) concentrations were determined from two independent

assays, performed in duplicate. Acarbose, an eminent α -glucosidase inhibitor, was employed as a positive control.

$$\text{Inhibition (\%)} = (\Delta A_{control} - \Delta A_{sample}) / \Delta A_{control} \times 100\%$$

One unit (IU) is defined as the amount of enzyme (α -glucosidase) which produces 1 μmol of PNP (*p*-nitro phenol) per min at 37°C and pH 7.2 under the conditions described above.

Inhibition-kinetic studies

The kinetic mode of inhibition of selected active compounds against α -glucosidase was determined as said above by preparing a series of test solutions in which the concentration of the substrate (PNPG) was varied in the presence of different concentrations of the inhibitors (10 μM -100 μM). The mode of inhibition (i.e. competitive, non-competitive, uncompetitive or mixed-type) of the test compounds was evaluated on the basis of the inhibitory effects on K_m (dissociation constant) and V_{max} (maximum reaction velocity) of the enzyme.²³ This was determined using the primary (Lineweaver-Burke plot) plot, which is the double reciprocal plot of enzyme reaction velocity (V) versus substrate (pNPG) concentration (1/V versus 1/[pNPG]). Analysis of the same data by secondary plots of slope versus [Inhibitor] and Y-intercept versus [Inhibitor] were also performed. The Lineweaver-Burke equation follows as,

$$\frac{1}{v} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} \times \frac{1}{[S]}$$

Malaria-growth inhibition assays (GIA):

P. falciparum culture 3D7 was maintained in vitro at pH 7.4 in plastic petri-dishes using human O⁺ erythrocytes, at 4% hematocrit, in RPMI-HEPES medium supplemented with 5% Albumax, 50 $\mu\text{g}/\text{ml}$ hypoxanthine, 25 mM NaHCO₃ and 20 $\mu\text{g}/\text{ml}$ gentamicin maintained in an atmosphere of 1% O₂, 4% CO₂, and 95% N₂ at 37°C, as previously described (Ref).²⁴ Parasite cultures were synchronized using the sorbitol treatment. The GIA was put at ring stage at 0.3% parasitemia and 2% hematocrit in 96-well plates that were incubated in a sealed, humidified, gassed box. The assay plate was read after 72 hours i.e., at the trophozoite stage of the second cycle of invasion. 10 compounds were tested at two different concentrations of 10 μM and 50 μM in the GIAs. Parasitemia was determined using flow cytometry. For flow cytometry, 100 μl of 10- $\mu\text{g}/\text{ml}$ ethidium bromide in phosphate-buffered saline (PBS; pH 7.3) was added to each well and incubated for 1 h in dark at room temperature. After centrifugation, the supernatant was discarded, cells were washed with PBS and re-suspended in 200 $\mu\text{l}/\text{well}$ of PBS, and the samples

were processed using a FACS Calibar flow cytometer (Becton Dickinson, Franklin Lakes, NJ). Parasitemia was evaluated using FlowJo software (Tree Star, Inc., Ashland, OR) by first gating for intact erythrocytes by side scatter and forward scatter parameters and subsequently determining the proportion of ethidium bromide-positive cells indicating the percent parasitemia. All compounds were tested in duplicate.

5. Acknowledgement

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7. Figure Captions

Figure 1. The library of bicyclic lactams designed around **1**

Figure 2. The synthetic scheme to access the small molecules depicted in the design (figure 1)

Table. 1. The *in vitro* activity of the library of pyrrolidines against yeast α -glucosidase

Figure 3. Inhibition of α -glucosidase enzyme activity as a function of inhibitors (from 10 μM to 50 μM) with Acarbose, as a reference standard

Figure 4. Ramachandran plot of the modelled α -glucosidase enzyme

Figure 5. Predicted binding mode of **1** in modelled α -glucosidase enzyme

Figure 6a. Predicted binding mode of **6** in modelled α -glucosidase enzyme

Figure 6b. Predicted binding mode of **7** in modelled α -glucosidase enzyme

Equation 1. Mixed inhibition

Figure 7. Double-reciprocal (Lineweaver-Burke) plot of the inhibition kinetics of α -glucosidase by compound **6** (**A**) and **7** (**B**); α -Glucosidase (activity 0.5IU/mL) was subjected with compound **6** & **7** at 37°C for 10min, followed by varying concentrations of 4-nitrophenyl α -D-glucopyranoside (PNPG). IU: Defined as the amount of enzyme (α -glucosidase) which produces 1 μmol of PNP (p-nitrophenol) per minute at 37°C and pH under the conditions described in *SI*.

Figure 8. Malaria parasite growth inhibition assay

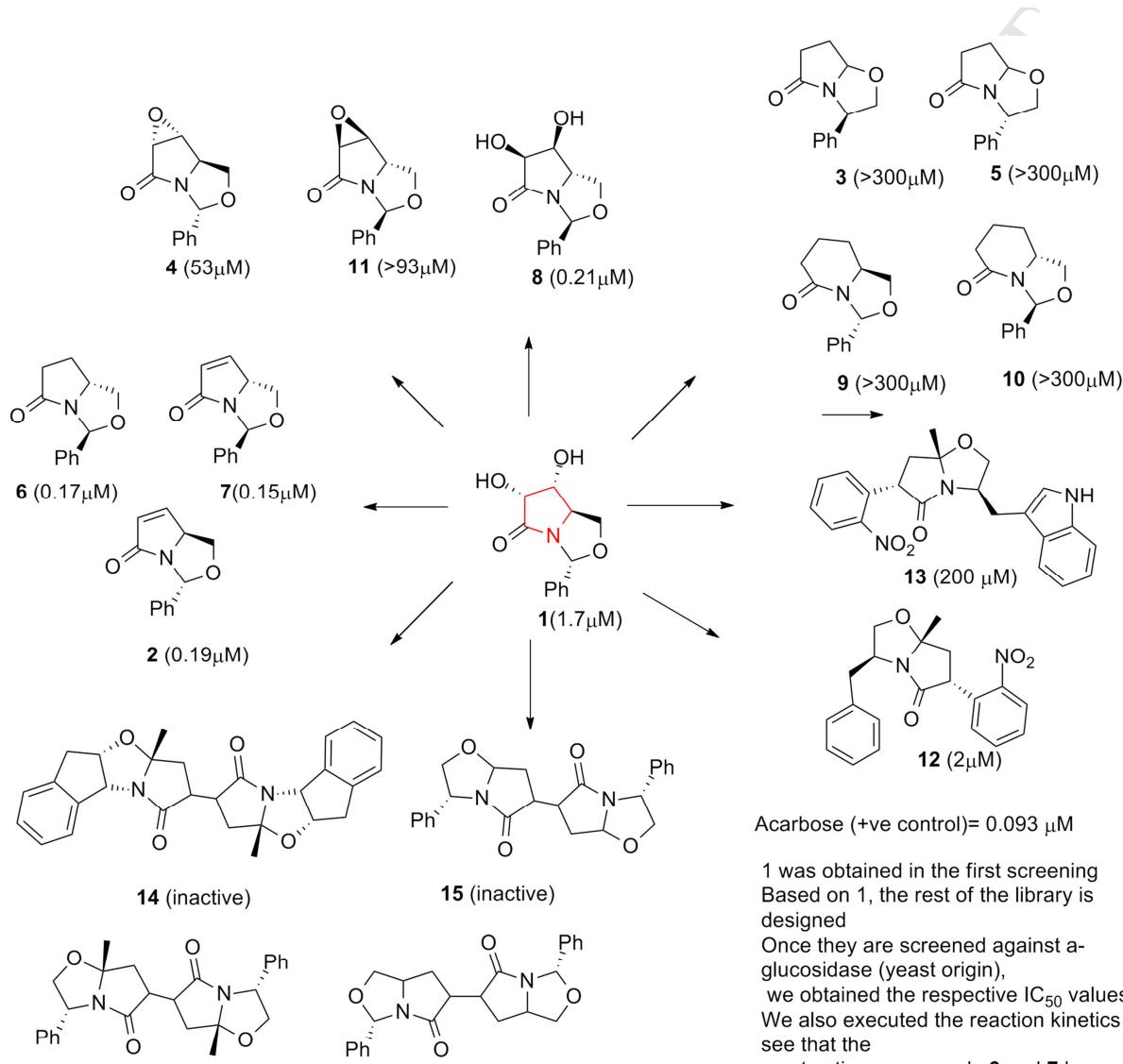


Figure 1. The library of bicyclic lactams designed around **1**

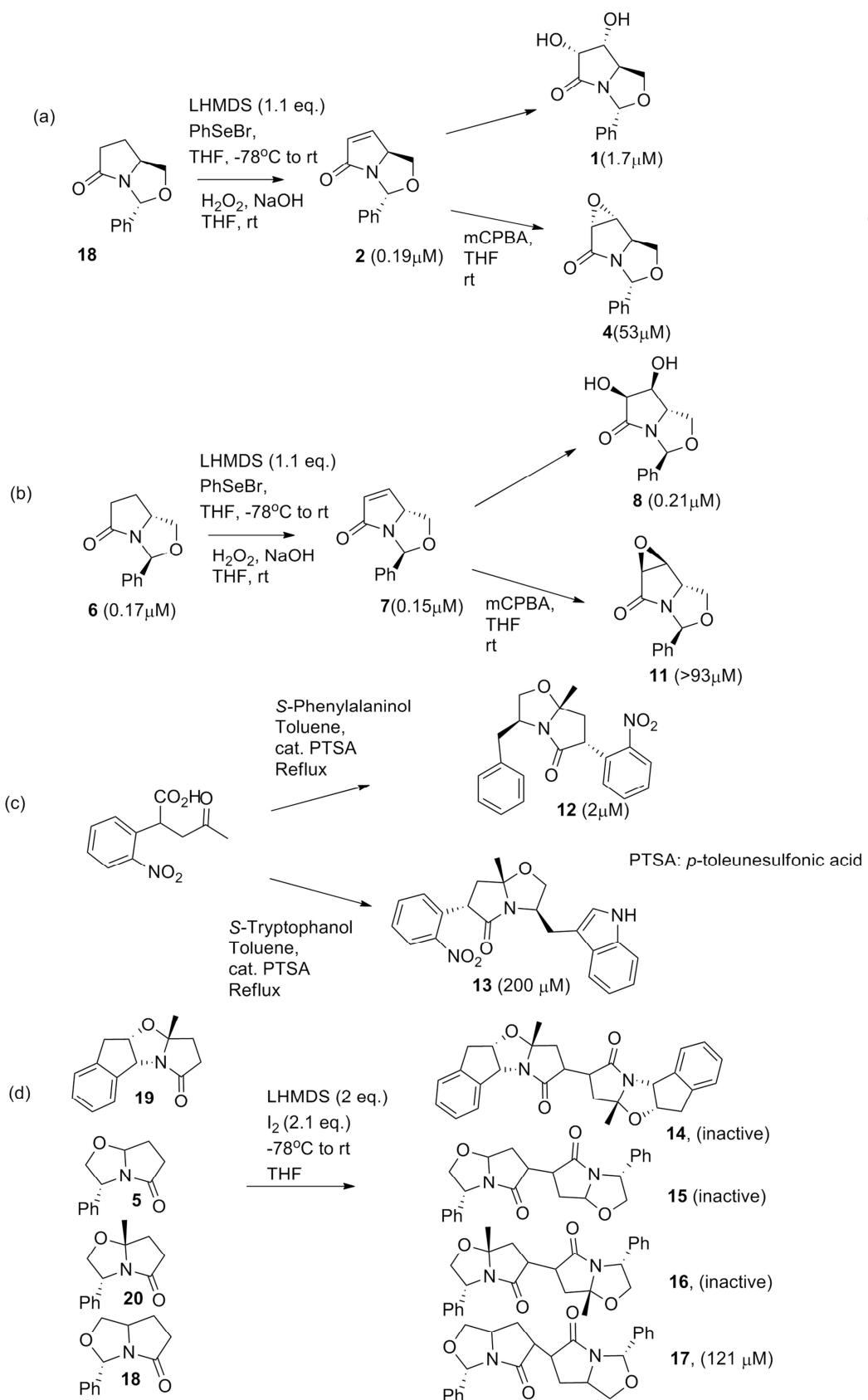


Figure 2. The synthetic scheme to access the small molecules depicted in the design (figure 1)**Table 1.** The *in vitro* activity of the library of pyrrolidines against yeast α -glucosidase

Entry	Compound	α -glucosidase (yeast) ^a IC ₅₀ (μ mol)
1	2	0.19
2	3^c	-
3	4	53
4	5^c	-
5	6	0.17
6	7	0.15
7	8	0.21
8	9^c	-
9	1	1.70
10	10^c	-
11	11	95
13	12	-
14	13	213
15	14	-
16	15	-
17	16	-
18	17	121
19	Acarbose	0.093 ^b

^aConcentration of compound that reduced enzyme activity by 50% in an Activity
(The values furnished were the means of two independents)

^b The concentration was observed in our laboratory assay method

^c> 50 \square 50 concentration was observed in our laboratory

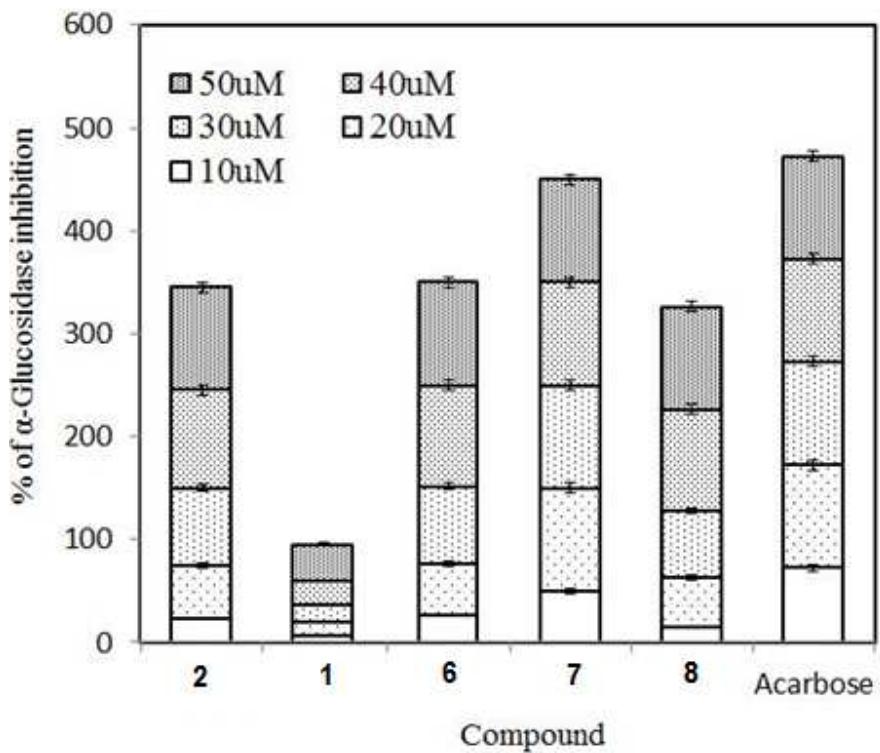


Figure 3. Inhibition of α -glucosidase enzyme activity as a function of inhibitors (from 10 μ M to 50 μ M) with Acarbose, as a reference standard

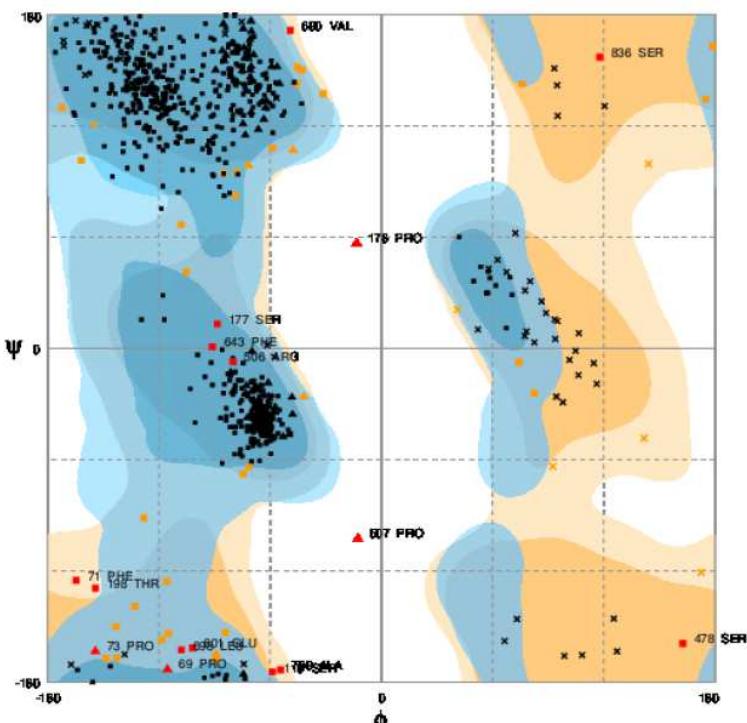


Figure 4. Ramachandran Plot of our α -glucosidase homology model

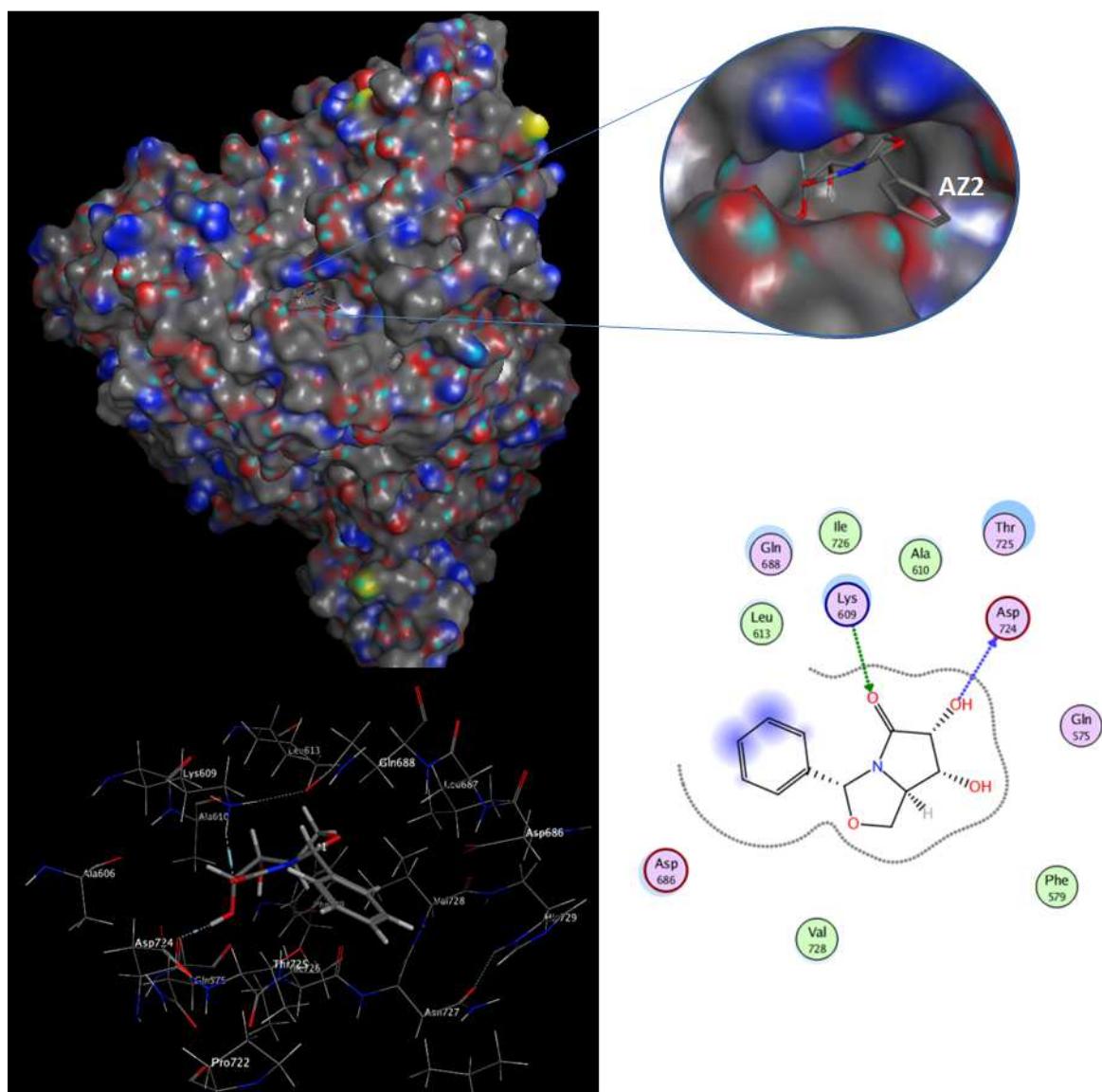


Figure 5. Predicted binding mode of **1** in modeled protein in a 3D representation along with 2D representation

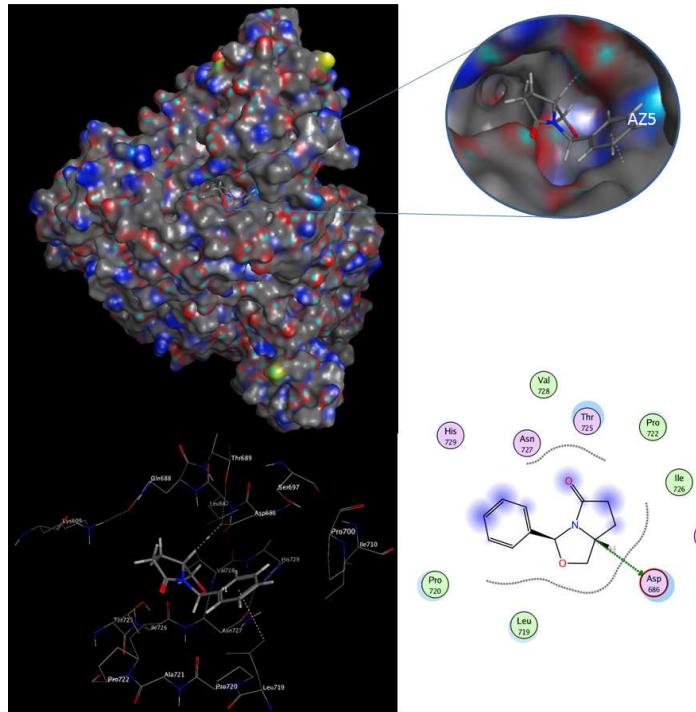


Figure 6a. Predicted binding mode of **6** in modelled protein

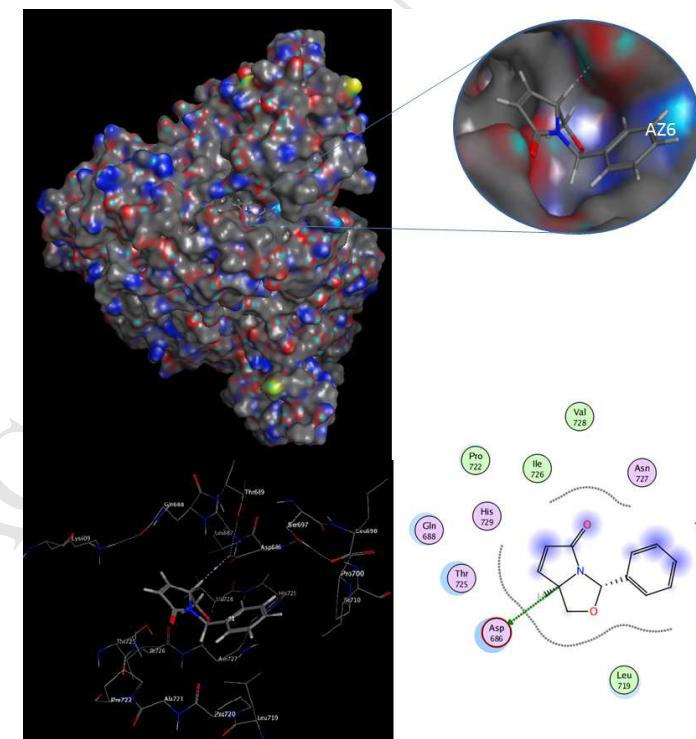


Figure 6b. Predicted binding mode of **7** in modelled protein

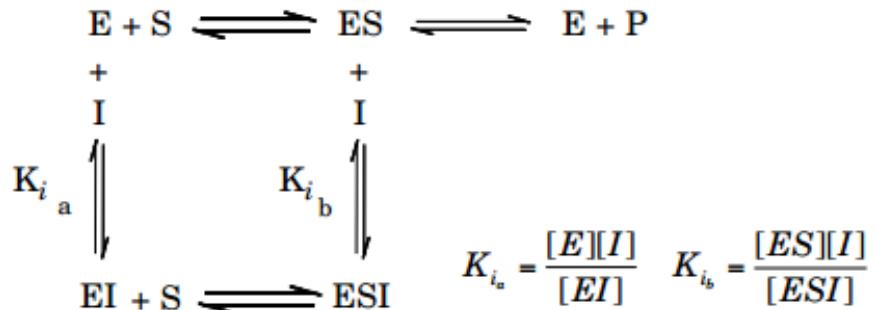
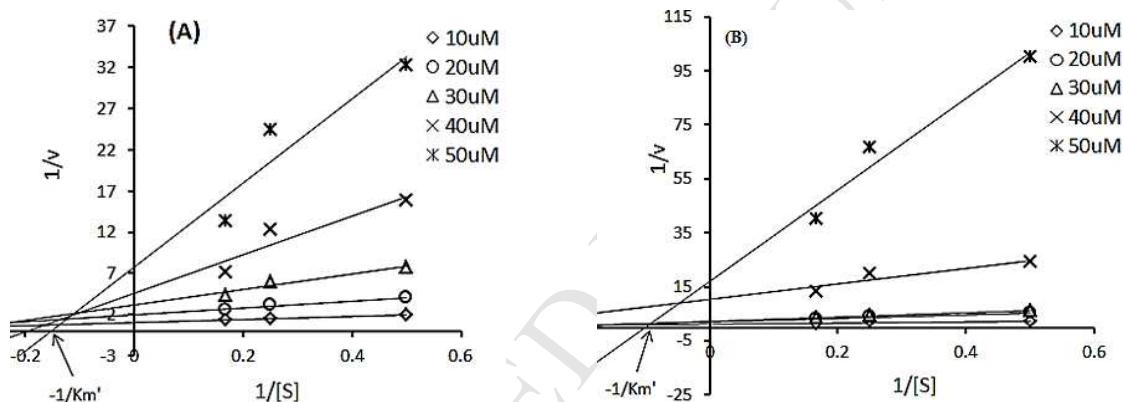
**Equation 1.** Mixed inhibition

Figure 7. Double-reciprocal (Lineweaver-Burke) plot of the inhibition kinetics of α -glucosidase by compound **6** (**A**) and **7** (**B**); α -Glucosidase (activity 0.5IU/mL) was subjected with compound **6** & **7** at 37°C for 10min, followed by varying concentrations of 4-nitrophenyl α -D-glucopyranoside (PNPG). IU: Defined as the amount of enzyme (α -glucosidase) which produces 1 μ mol of PNP (p-nitrophenol) per minute at 37°C and pH under the conditions described in *SI*.

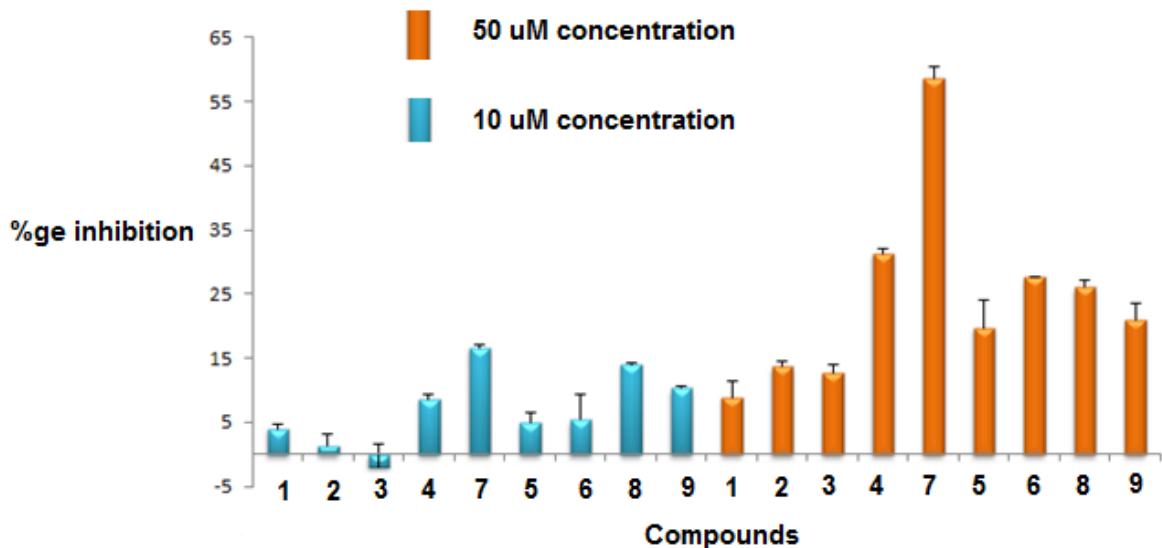
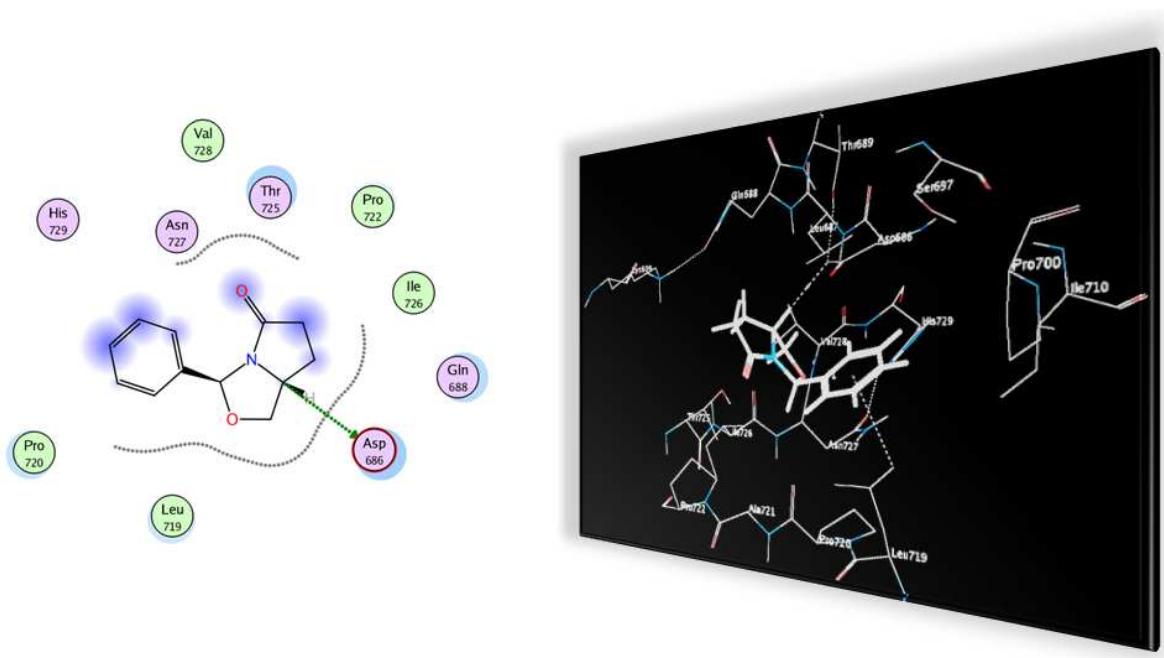


Figure 8. Malaria parasite growth inhibition assay

Graphical Abstract

A bicyclic lactam has been discovered as a novel mixed inhibitor of α -glucosidase (yeast) enzyme that also inhibits 3D-7 strain of *Plasmodium Falciparum*.

Highlights

- We executed virtual screening of a library against a homology model of α -glucosidase.
- We designed and synthesized a molecular library.
- *In vitro* screening identified two compounds as potent α -glucosidase inhibitors
- Reaction kinetics suggested mixed type inhibition.
- Phenotypic screening of one of the compounds also indicated antimalarial properties

Design, synthesis and biological evaluation of small molecules as potent glycosidase inhibitors and antimalarials

Analysis of novel compounds

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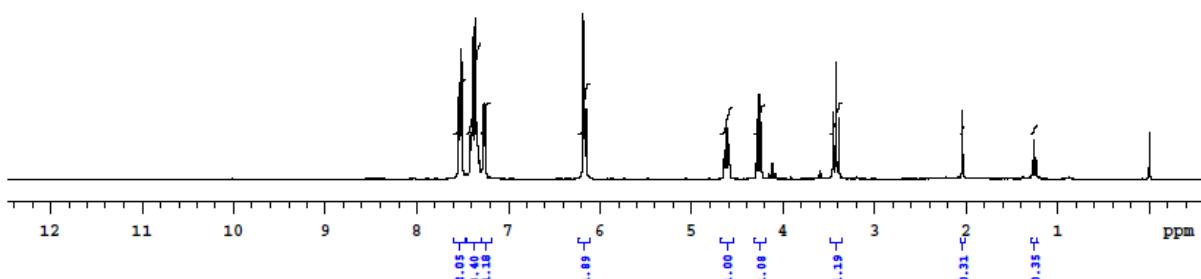
³ Department of Life Science, Shiv Nadar University, PO Shiv Nadar University, Uttar Pradesh, India

⁴ International Centre for Genomics Engineering and Biotechnology, New Delhi, India

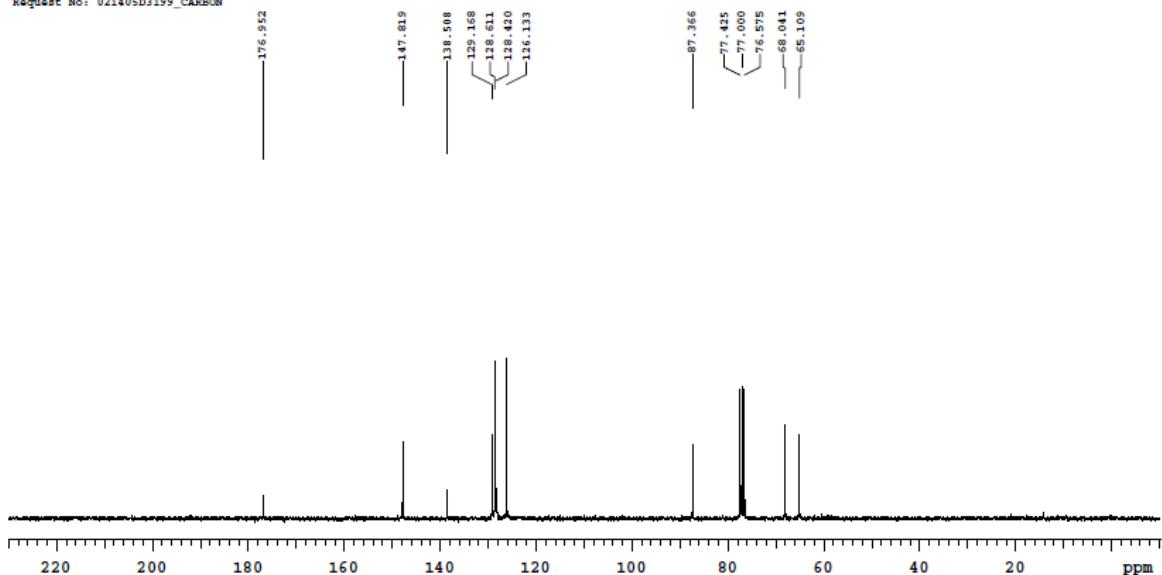
⁵ Department of Chemistry, Shiv Nadar University, PO Shiv Nadar University, Uttar Pradesh, India

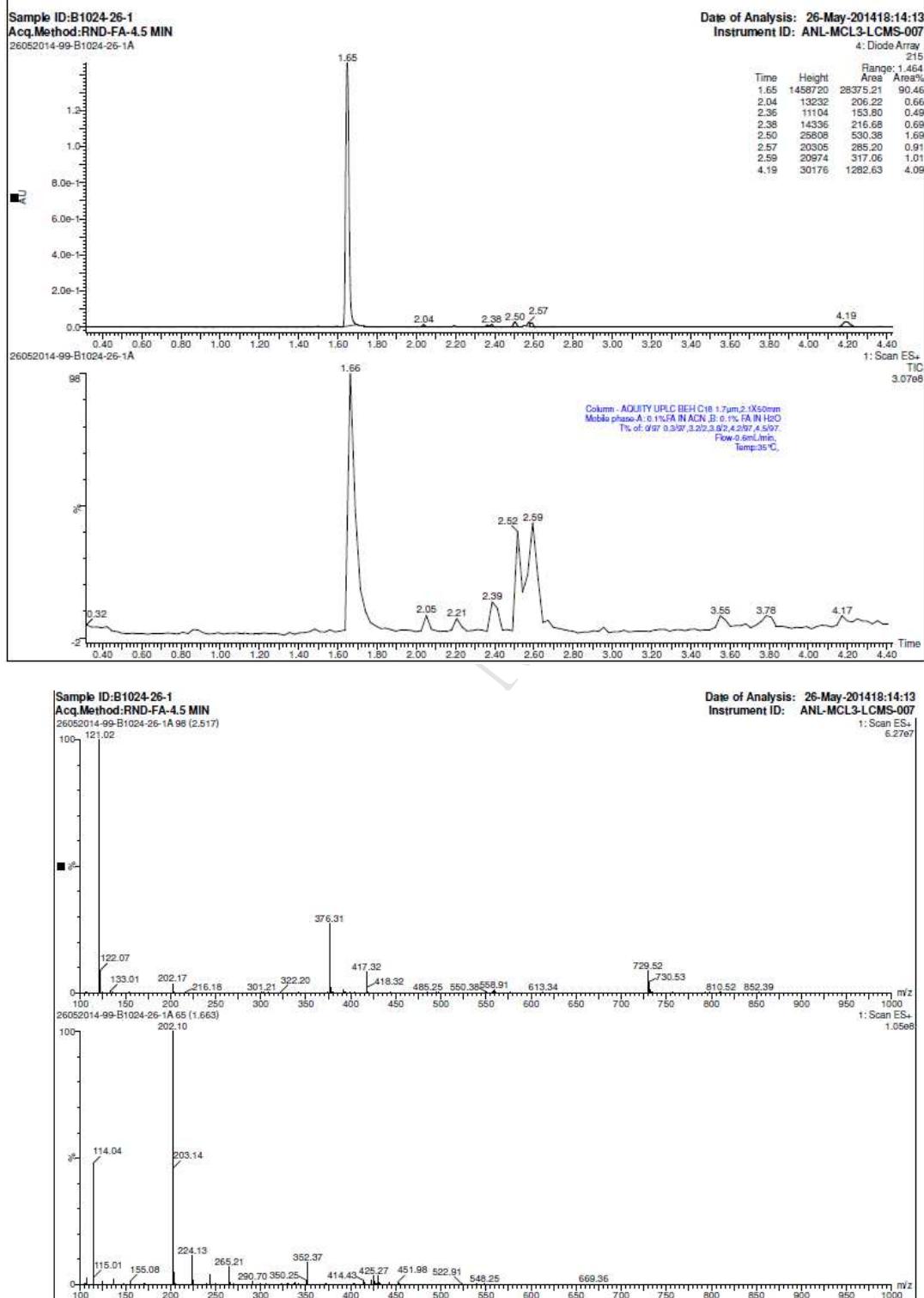
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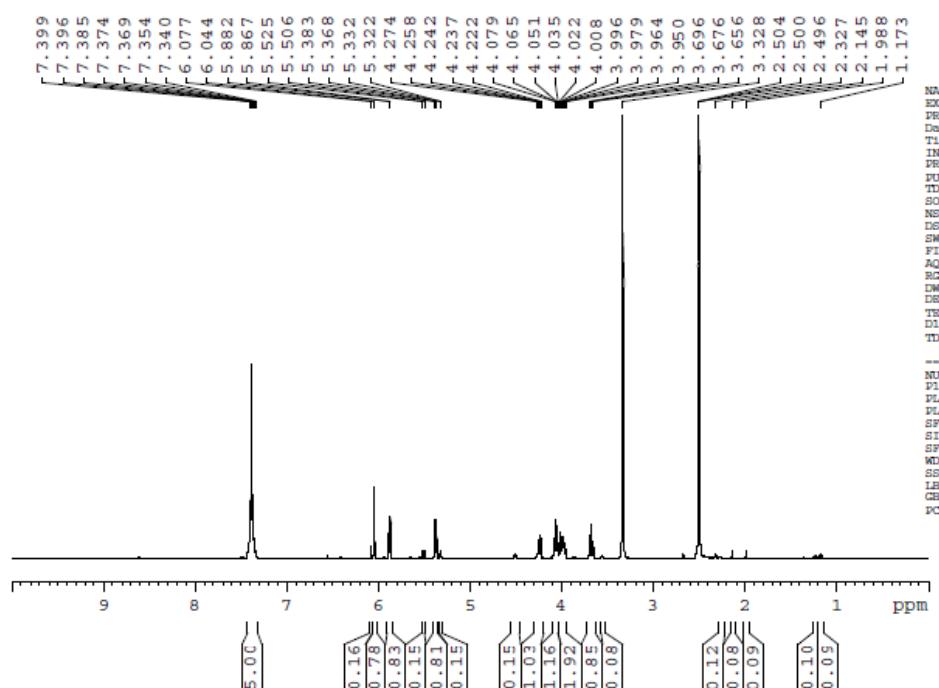


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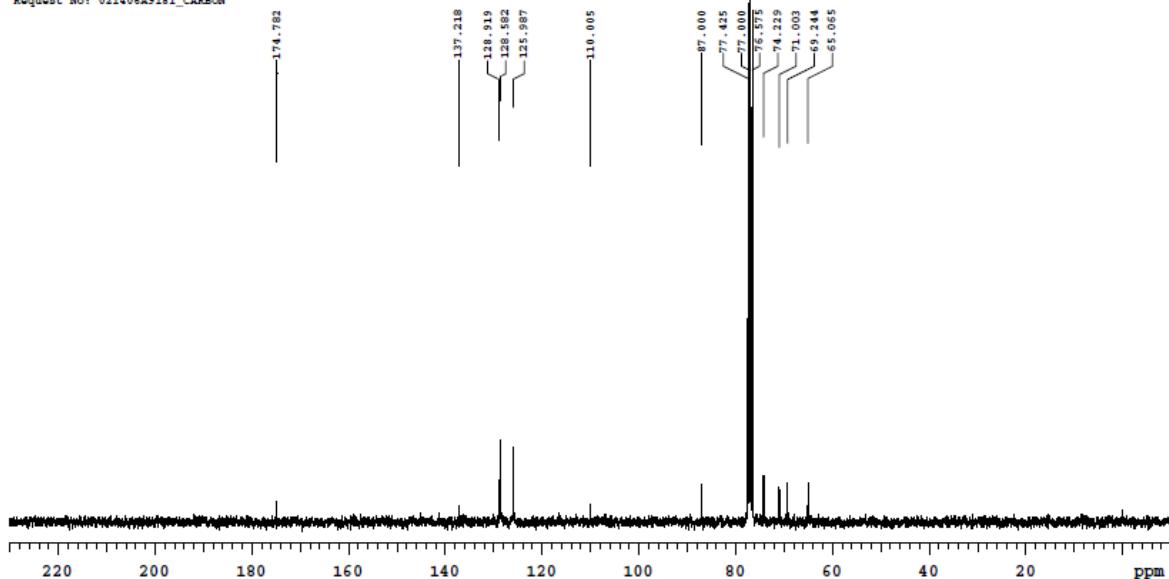


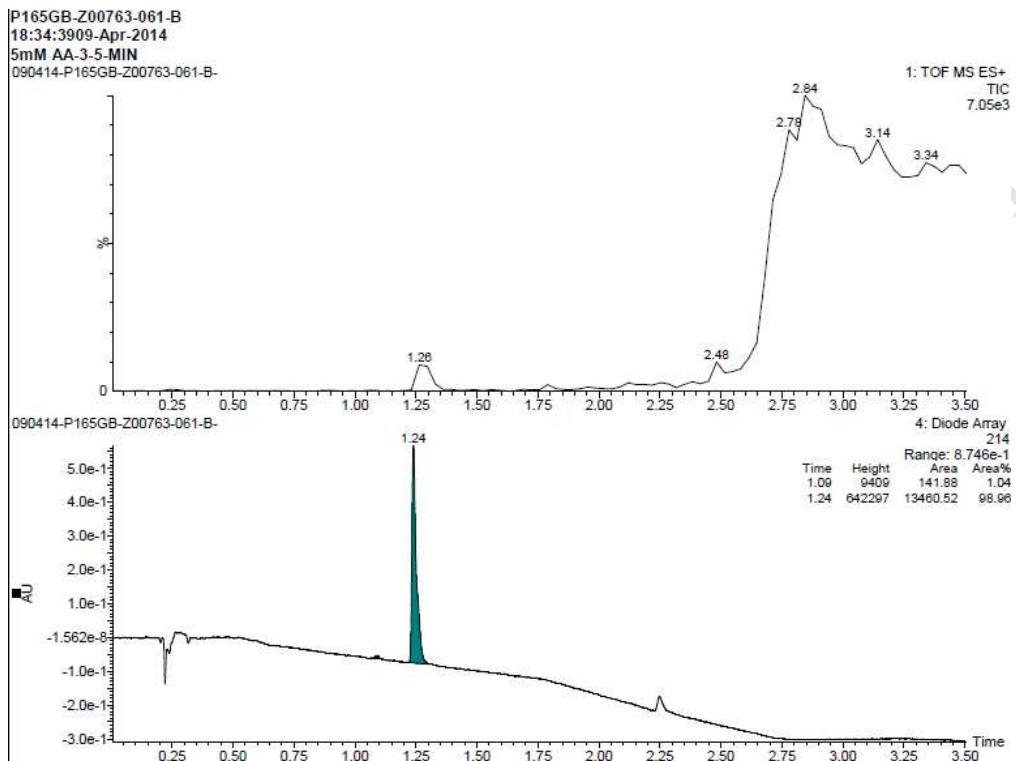


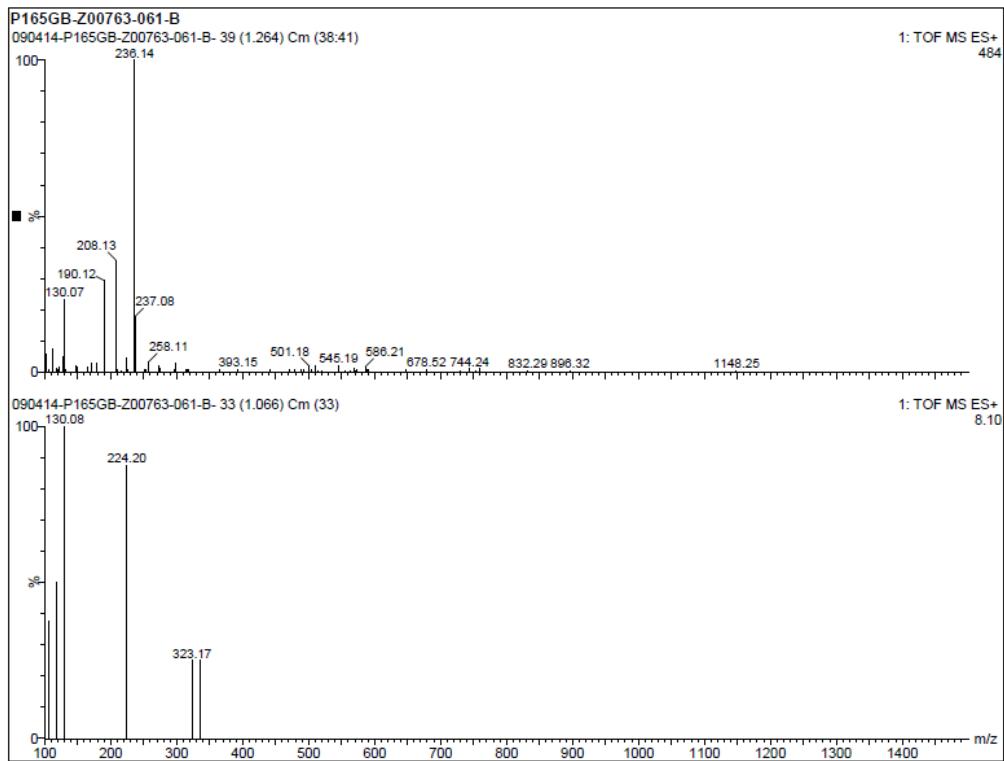
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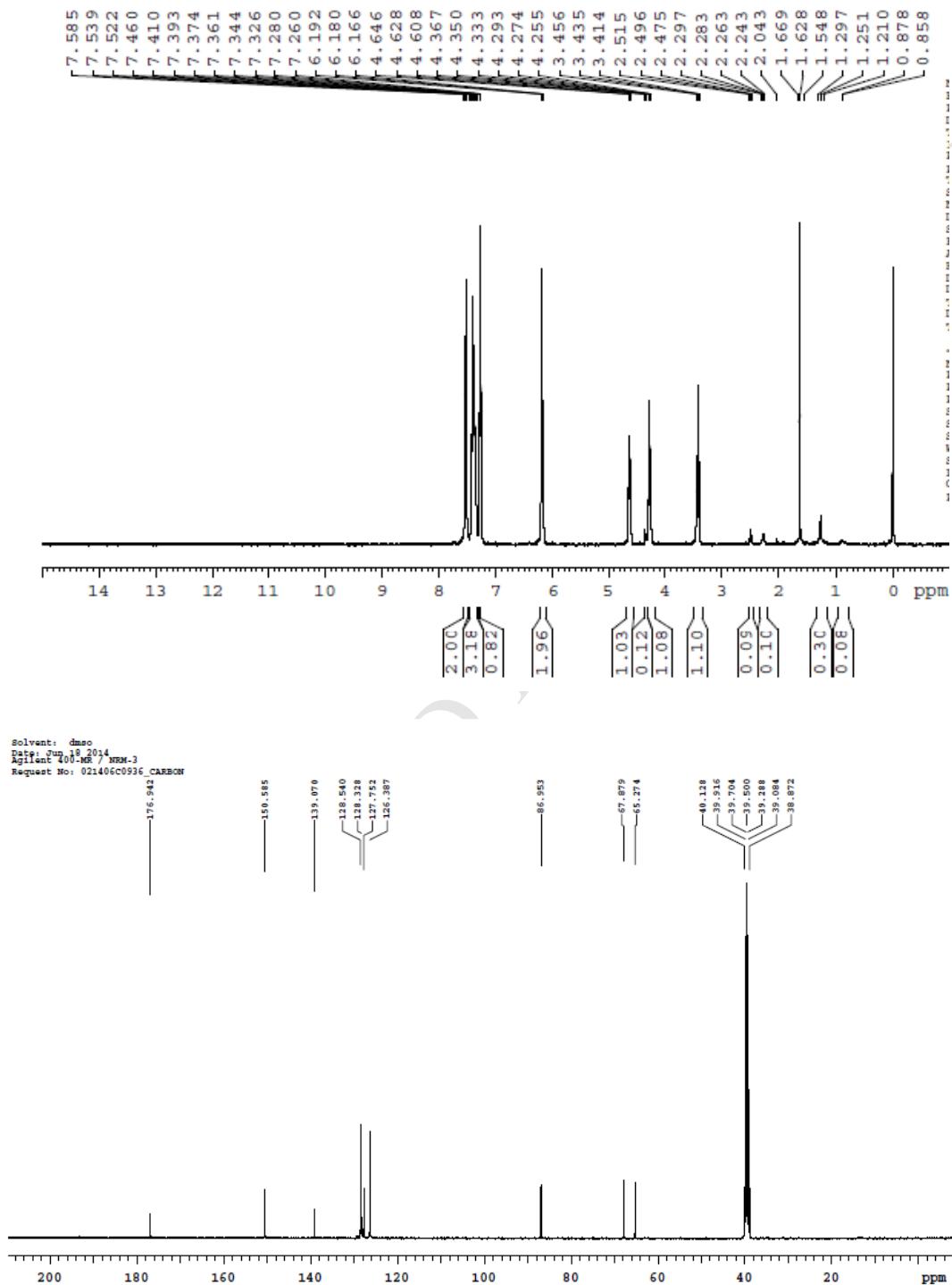
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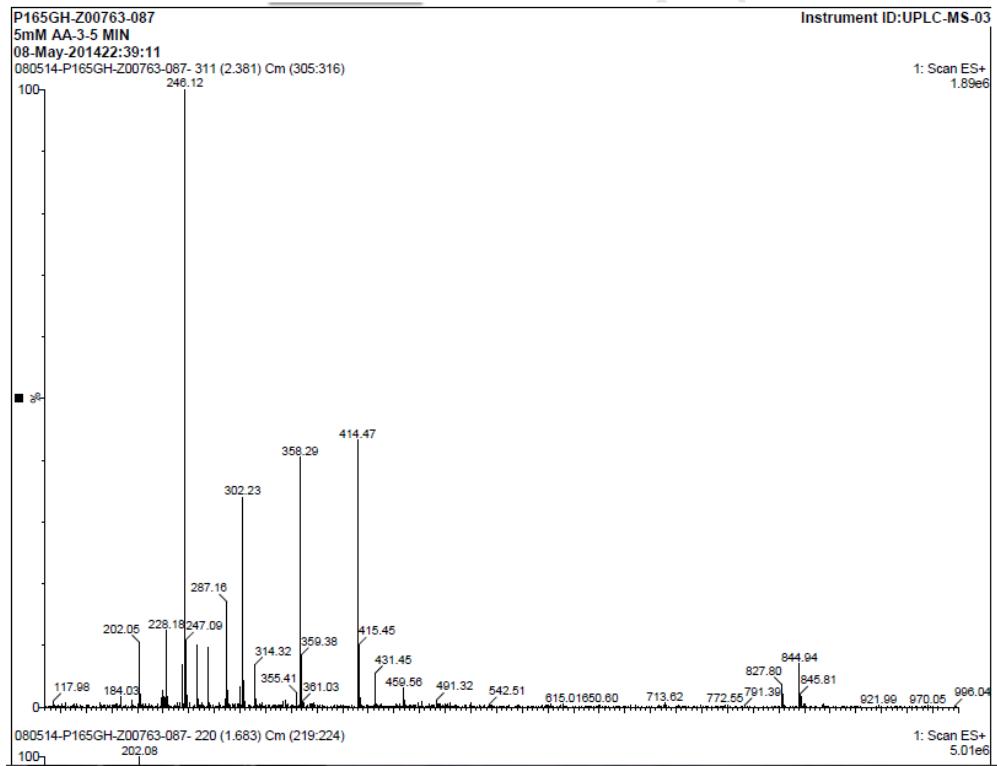
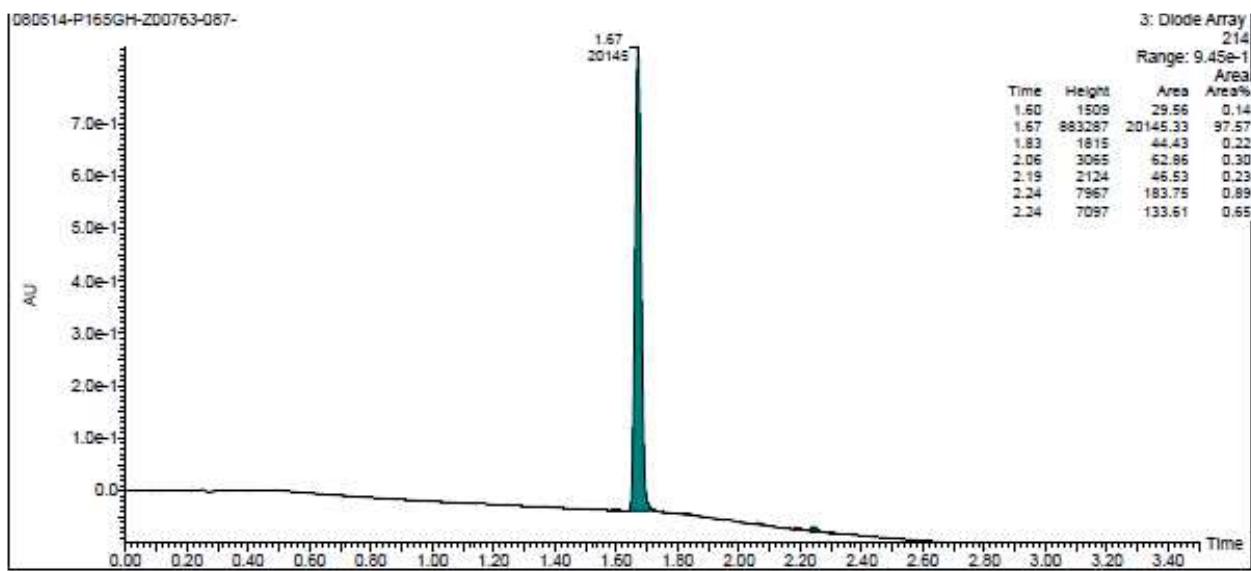




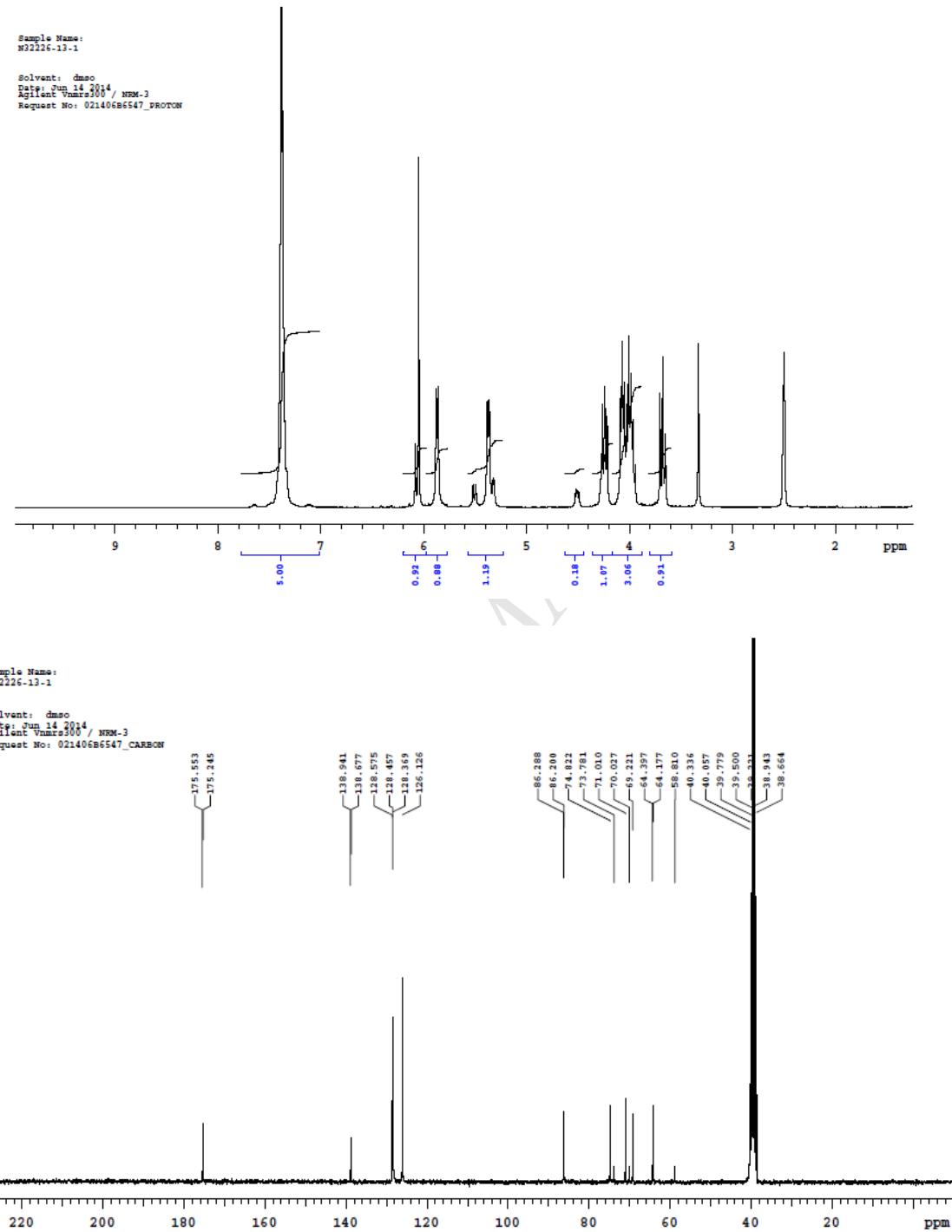


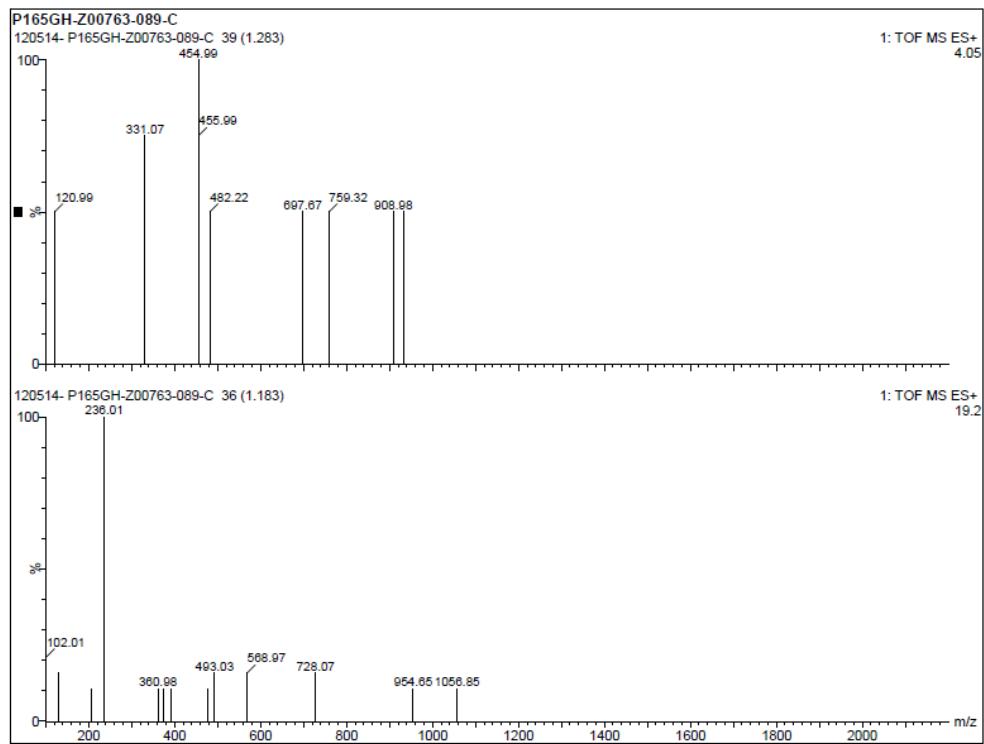
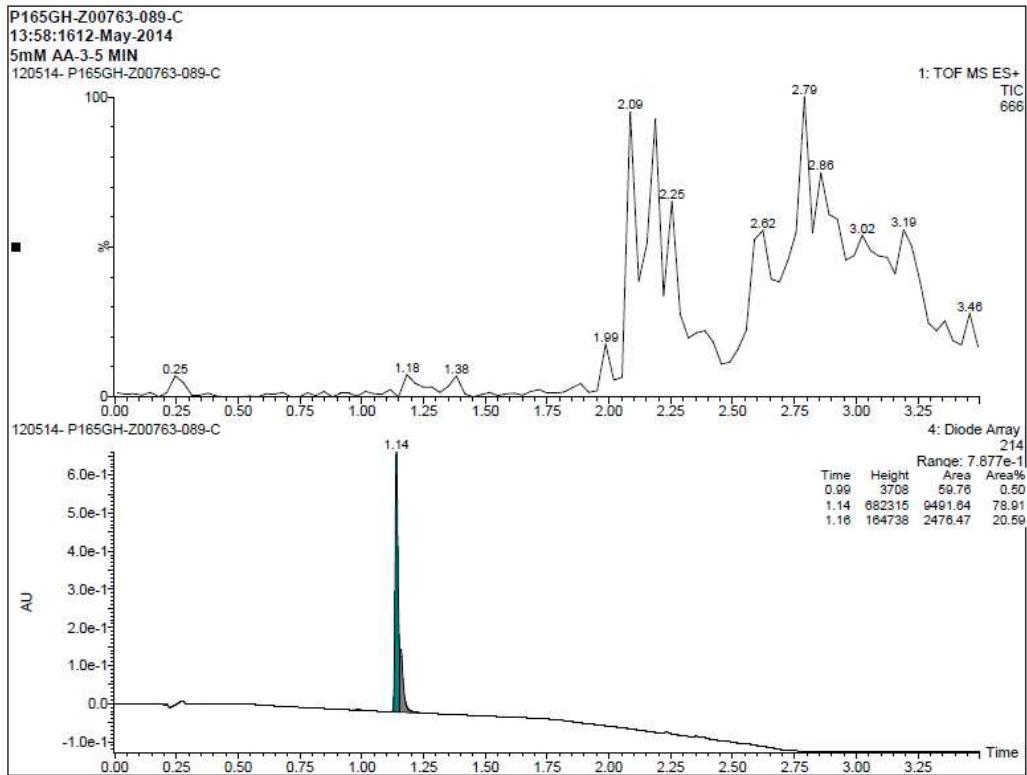
Compound 7

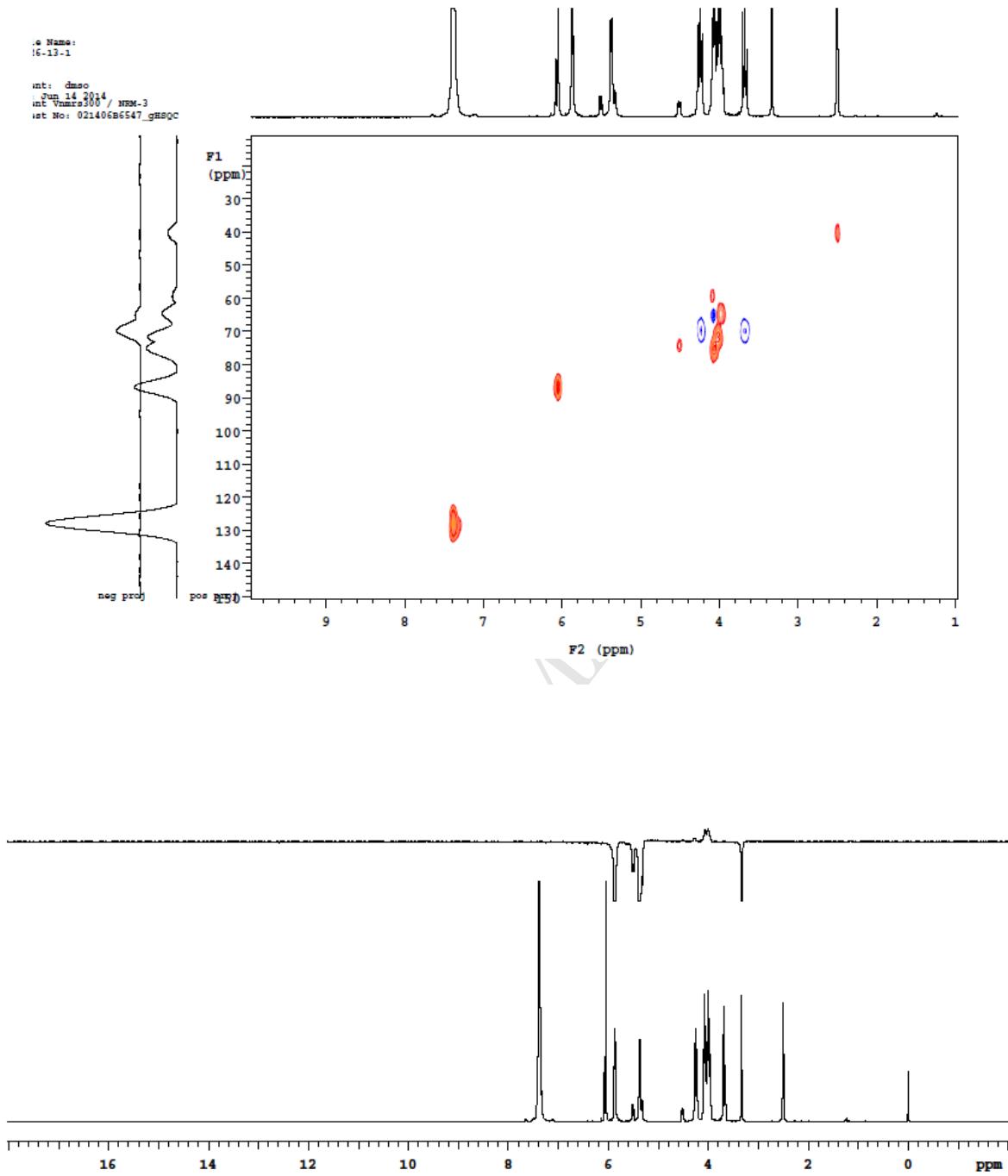




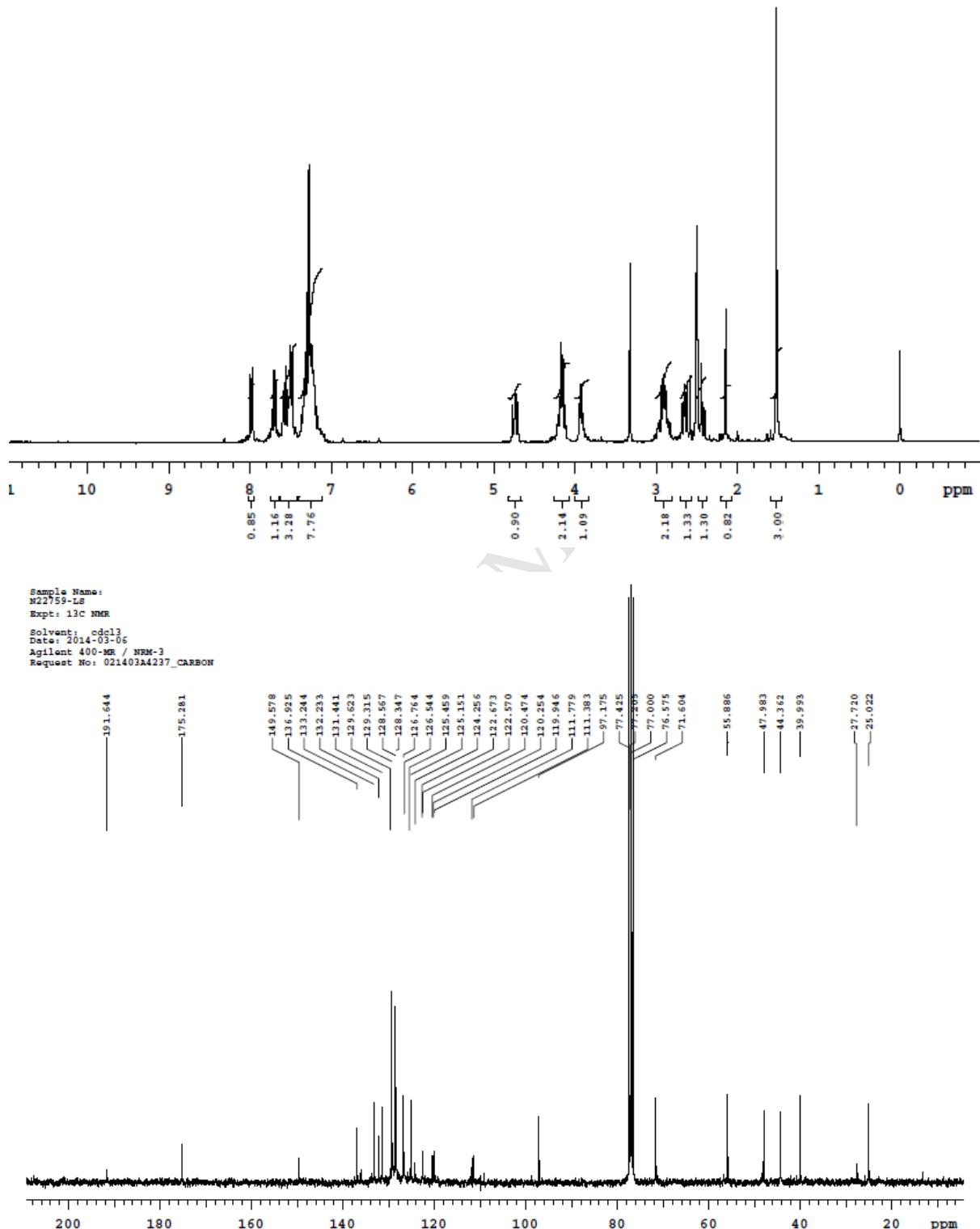
Compound 8

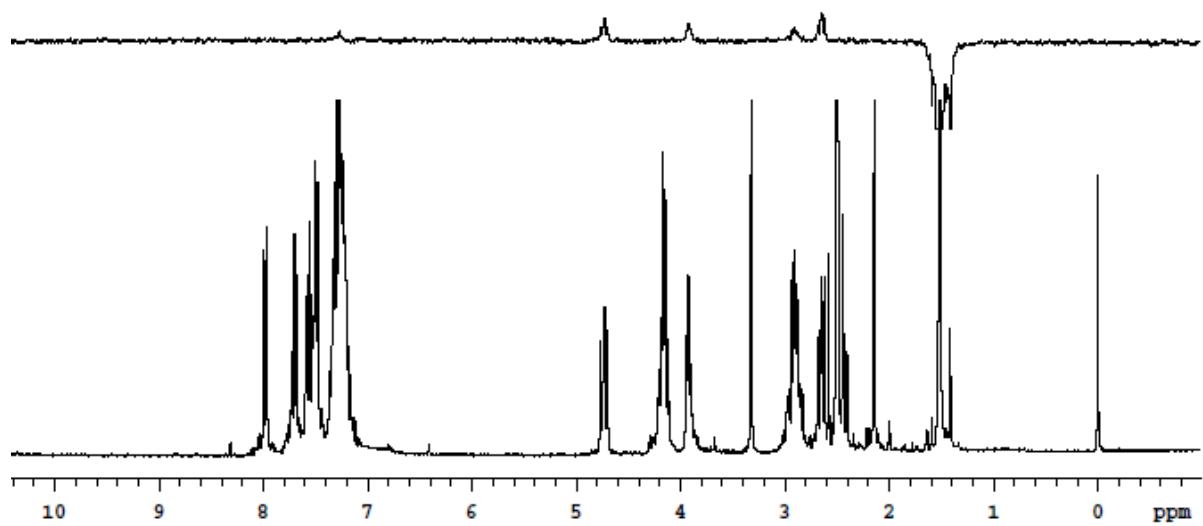




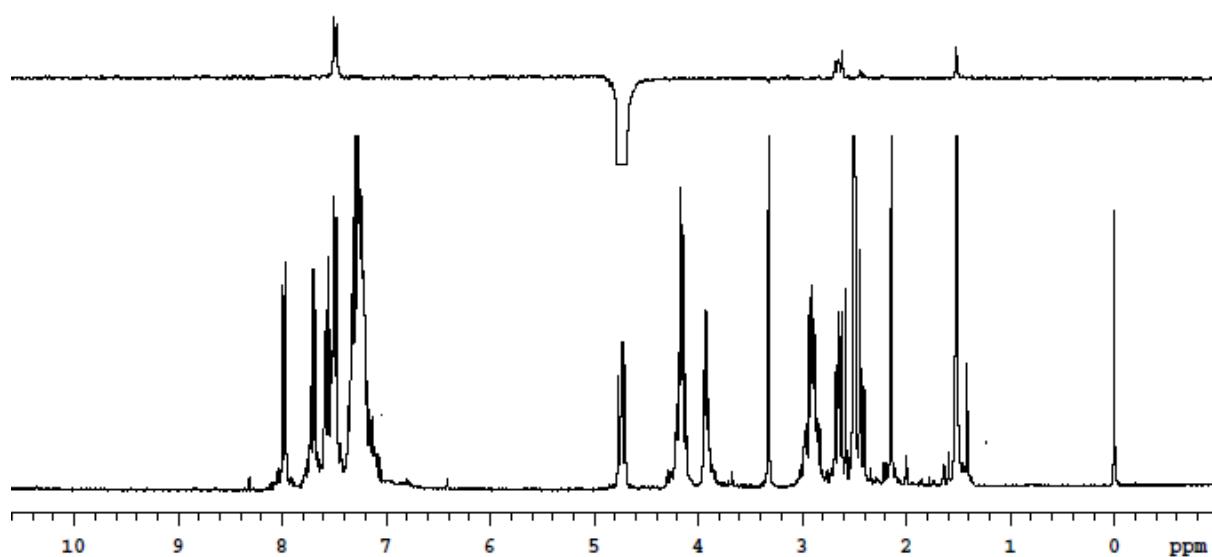


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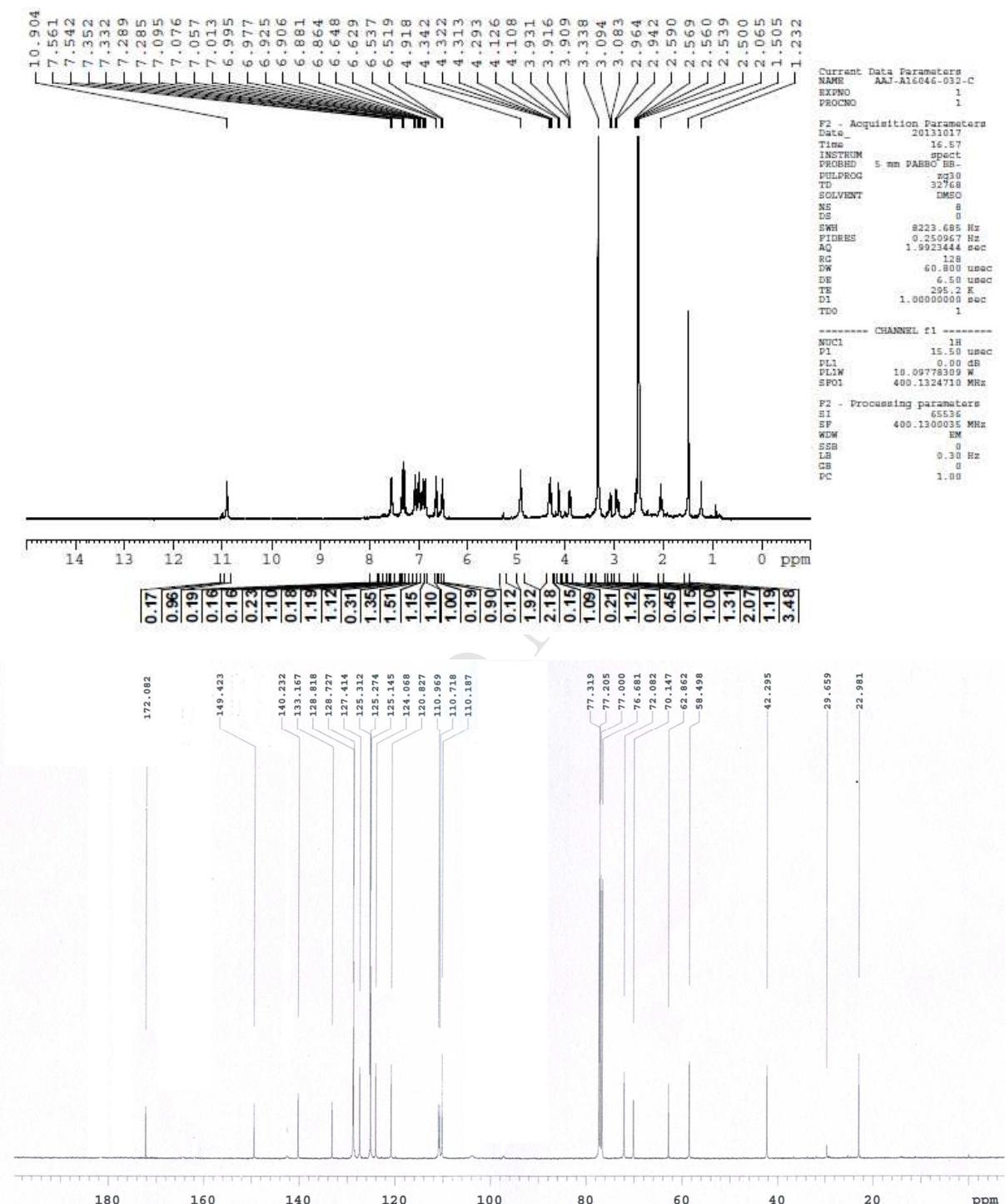


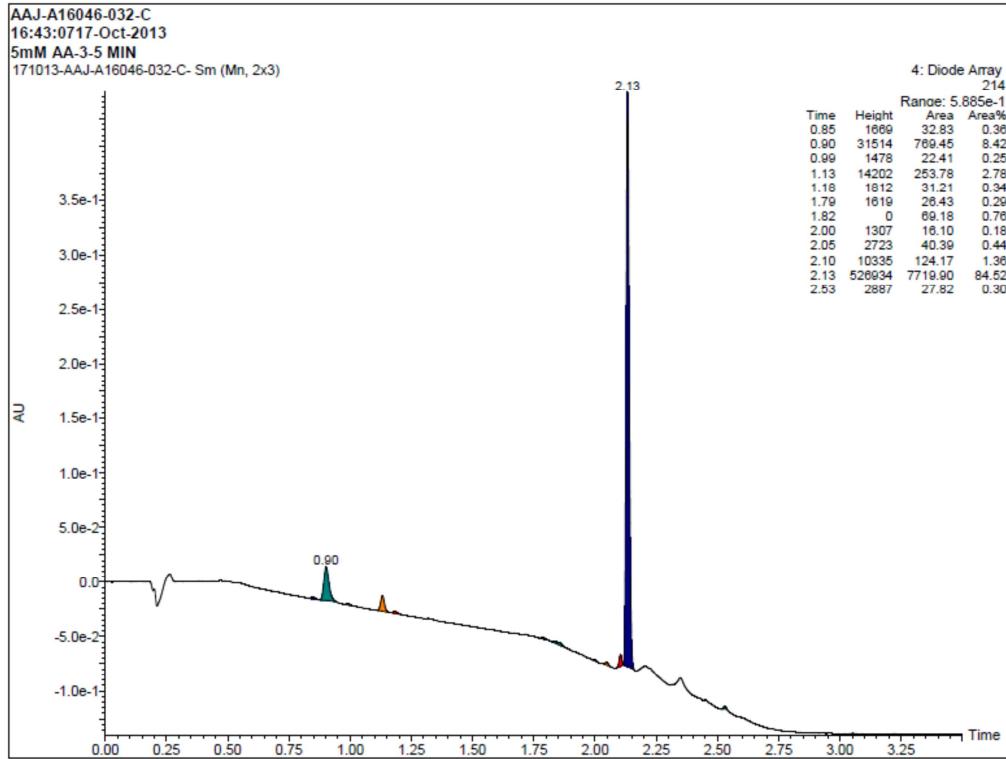


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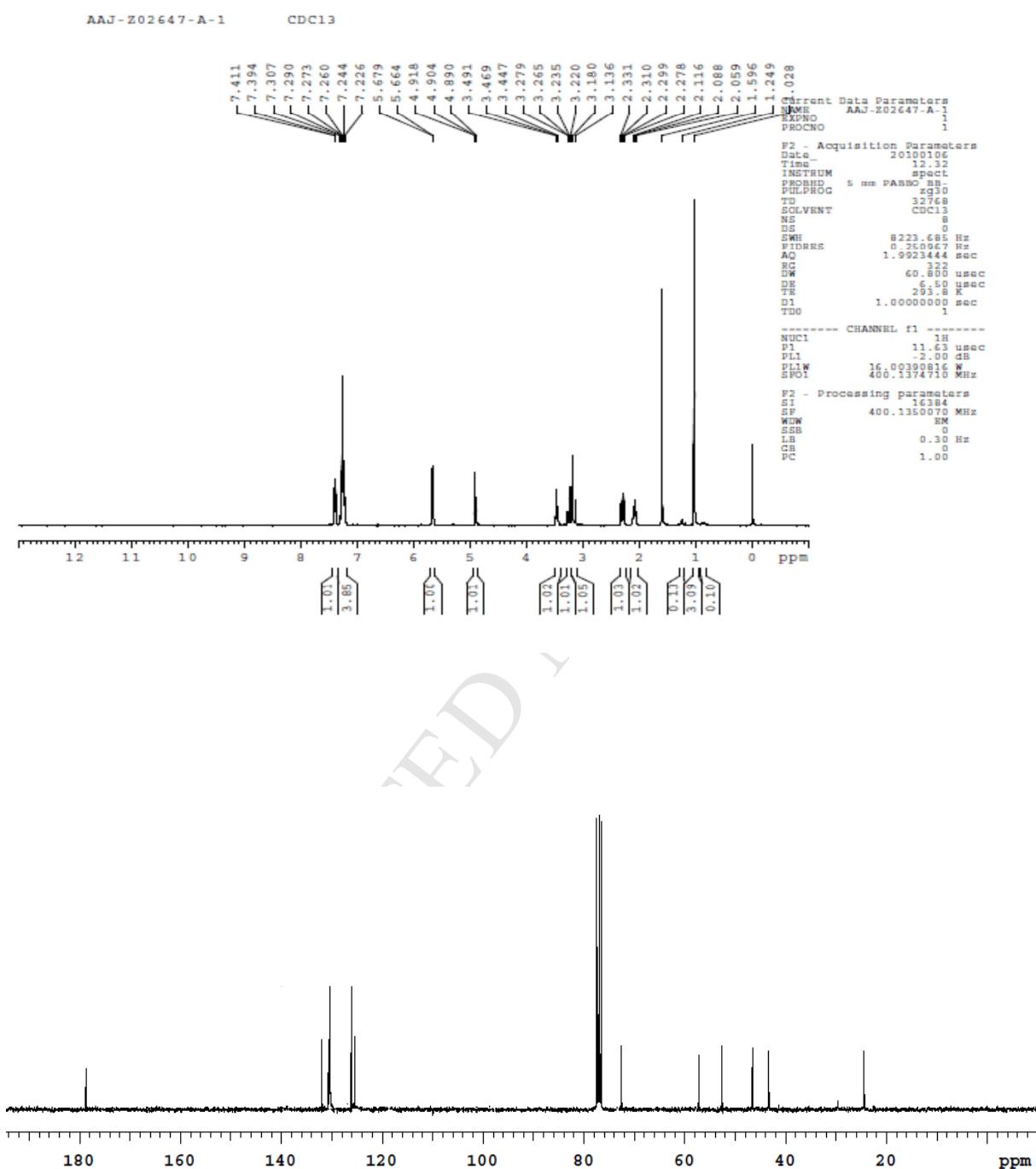


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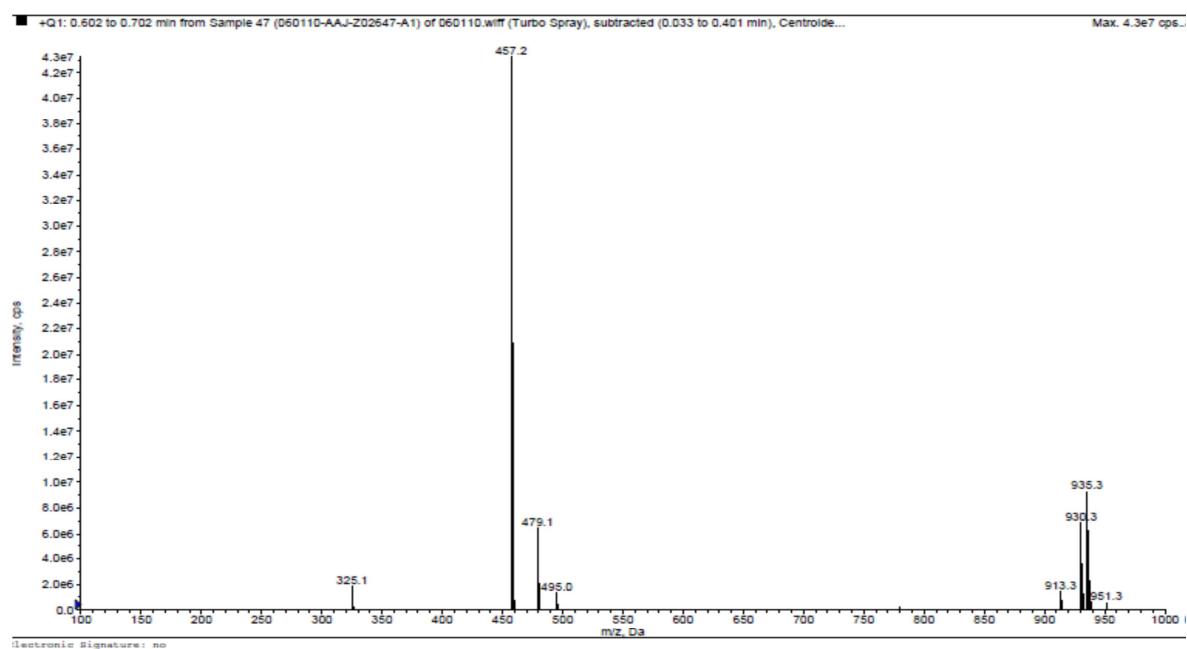




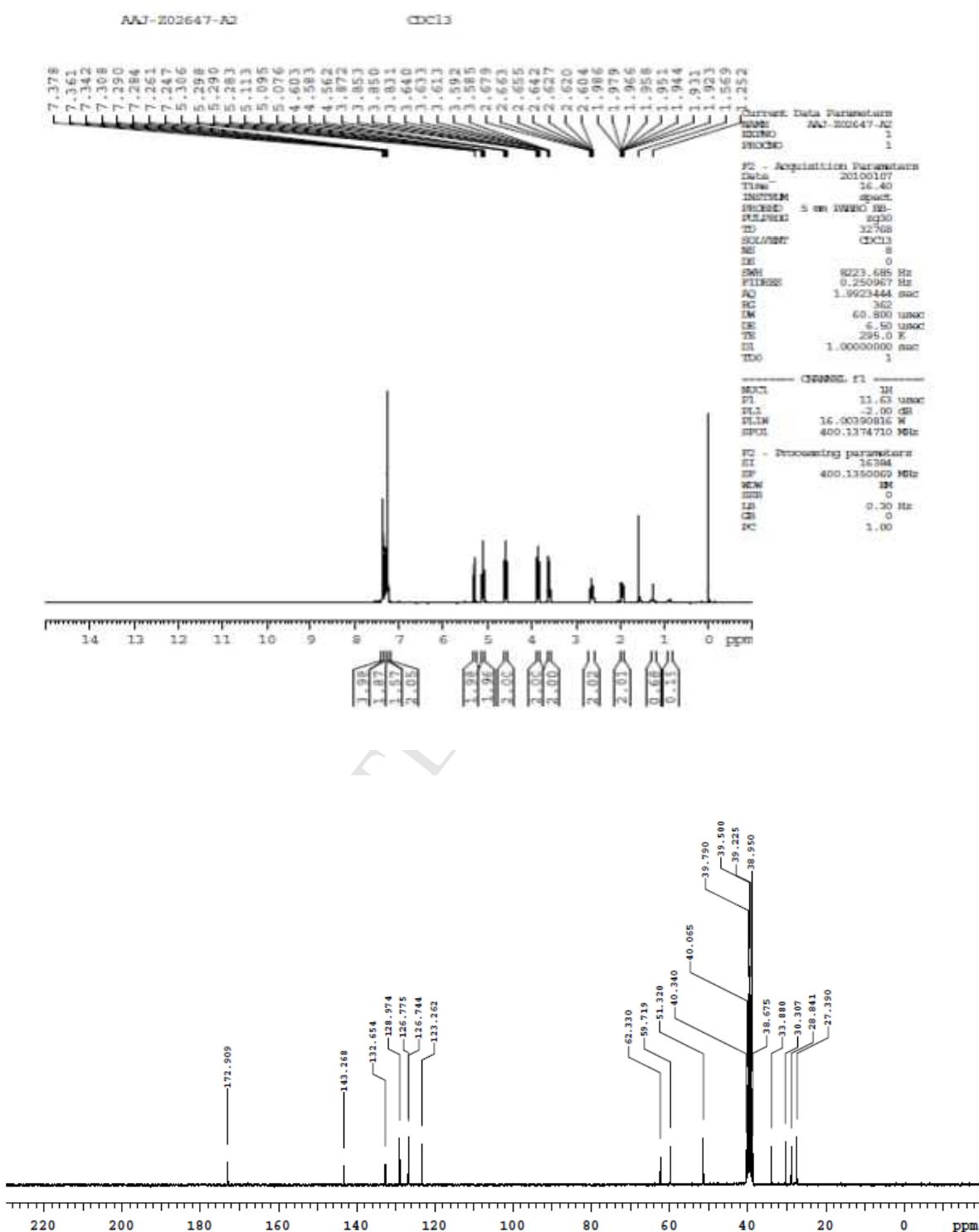
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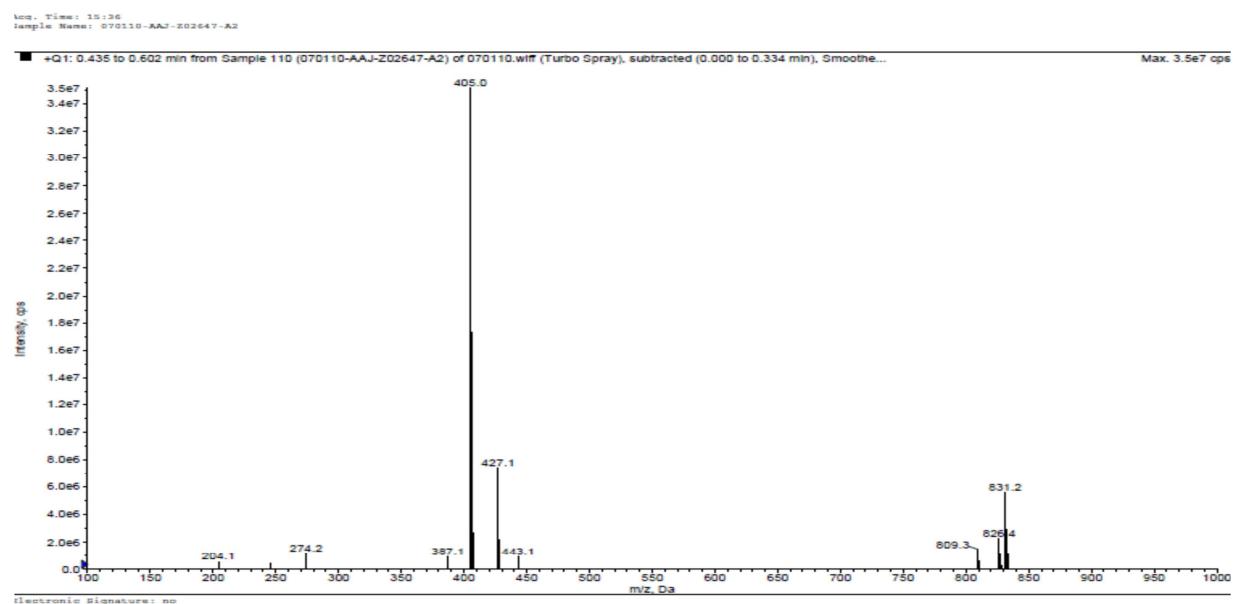


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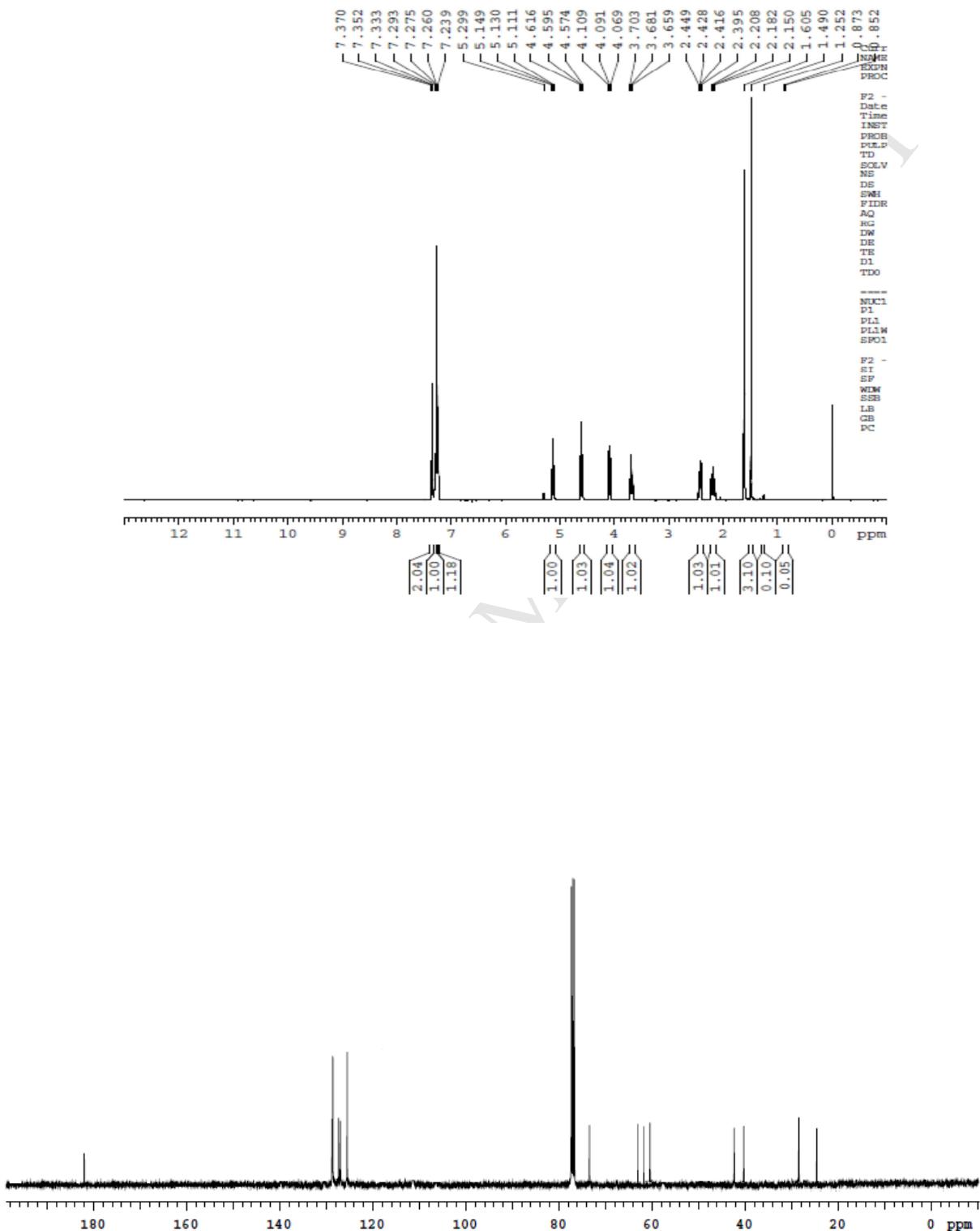


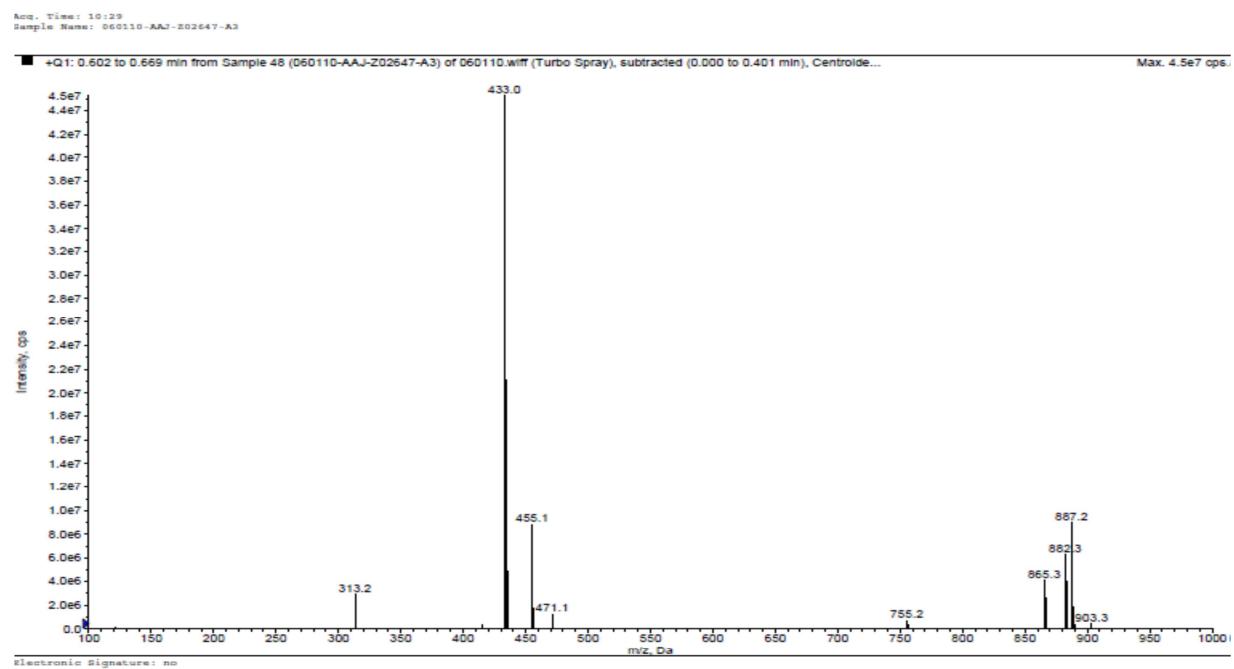
Compound 15





Compound 16





Compound 17

