**Ligand and structure based *in silico* studies to identify**

**kinesin spindle protein (KSP) inhibitors as potential anticancer agents**

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**Supplementary information**

**Table S1** Test set employed in the validation

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **S. No** | **Compd Id** | **Structure** | **IC50 (nM)** | **Reference** | **Category** |
| 1 | 24 |  | 0.4 | (Garbaccio et al., 2007) | Active |
| 2 | 28 |  | 0.5 | (Garbaccio et al., 2007) | Active |
| 3 | 48 |  | 0.82 | (Coleman et al., 2007) | Active |
| 4 | 11 |  | 0.9 | (Coleman et al., 2007) | Active |
| 5 | 19 |  | 1 | (Coleman et al., 2007) | Active |
| 6 | 26 |  | 1 | (Garbaccio et al., 2007) | Active |
| 7 | 15 |  | 1.2 | (Garbaccio et al., 2006) | Active |
| 8 | 12 |  | 1.4 | (Coleman et al., 2007) | Active |
| 9 | 55 |  | 1.6 | (Garbaccio et al., 2007) | Active |
| 10 | 14 |  | 1.8 | (Coleman et al., 2007) | Active |
| 11 | 17 |  | 1.8 | (Coleman et al., 2007) | Active |
| 12 | 21 |  | 2 | (Coleman et al., 2007) | Active |
| 13 | 62 |  | 2 | (Theoclitou et al., 2011) | Active |
| 14 | SCH |  | 2 | (Basso et al., 2010) | Active |
| 15 | 13 |  | 2.8 | (Coleman et al., 2007) | Active |
| 16 | 20 |  | 4 | (Coleman et al., 2007) | Active |
| 17 | 29 |  | 4.3 | (Garbaccio et al., 2007) | Active |
| 18 | 64 |  | 5 | (Theoclitou et al., 2011) | Active |
| 19 | 14 |  | 5 | (Cox et al., 2008) | Active |
| 20 | 13 |  | 5.1 | (Cox et al., 2008) | Active |
| 21 | 15 |  | 5.9 | (Cox et al., 2008) | Active |
| 22 | 16 |  | 6.5 | (Garbaccio et al., 2006) | Active |
| 23 | Litronesib |  | 7 | (Verschraegen et al., 2014) | Active |
| 24 | Pyrrolotriazine |  | 7 | (Kim et al., 2006) | Active |
| 25 | 12 |  | 7.4 | (Cox et al., 2008) | Active |
| 26 | 56 |  | 8 | (Theoclitou et al., 2011) | Active |
| 27 | EMD |  | 8 | (Schiemann et al., 2010) | Active |
| 28 | 10A |  | 8 | (Cox et al., 2006) | Active |
| 29 | 3 |  | 10 | (Theoclitou et al., 2011) | Active |
| 30 | Ispinesib |  | 10 | (Davis, Sarkar, Hussain, Li, & Sarkar, 2006) | Active |
| 31 | Quinazoline |  | 11 | (Jiang, Yang, Wu, Guo, & You, 2011b) | Active |
| 32 | CK |  | 12 | (Sakowicz et al., 2004) | Active |
| 33 | 54 |  | 13 | (Theoclitou et al., 2011) | Active |
| 34 | 63 |  | 16 | (Theoclitou et al., 2011) | Active |
| 35 | 38 |  | 20 | (Theoclitou et al., 2011) | Active |
| 36 | 5 |  | 20 | (Theoclitou et al., 2011) | Active |
| 37 | 55 |  | 21 | (Theoclitou et al., 2011) | Moderately  active |
| 38 | 10C |  | 26 | (Cox et al., 2006) | Moderately  active |
| 39 | CPUYJ |  | 40 | (Jiang, Yang, Wu, Guo, & You, 2011a) | Moderately  active |
| 40 | BMCL |  | 44 | (Yamamoto et al., 2014) | Moderately  active |
| 41 | 8H |  | 44 | (Cox et al., 2006) | Moderately  active |
| 42 | JMC |  | 50 | (Theoclitou et al., 2011) | Moderately  active |
| 43 | 10B |  | 55 | (Cox et al., 2006) | Moderately  active |
| 44 | 26 |  | 60 | (Kim et al., 2006) | Moderately  active |
| 45 | 28 |  | 60 | (Kim et al., 2006) | Moderately  active |
| 46 | 18 |  | 60 | (Kim et al., 2006) | Moderately  active |
| 47 | Pyrrolotriazine analog |  | 60 | (Kim et al., 2006) | Moderately  active |
| 48 | 8I |  | 67 | (Cox et al., 2006) | Moderately  active |
| 49 | 7 |  | 70 | (Kim et al., 2006) | Moderately  active |
| 50 | 20 |  | 80 | (Kim et al., 2006) | Moderately  active |
| 51 | 58 |  | 85 | (Theoclitou et al., 2011) | Moderately  active |
| 52 | 10D |  | 85 | (Cox et al., 2006) | Moderately  active |
| 53 | ARMC |  | 90 | (Hotha et al., 2003) | Moderately  active |
| 54 | 7 |  | 100 | (Theoclitou et al., 2011) | Weakly active |

**Table S2** CDOCKER energy and molecular interactions of bound ligand, training set ligands and top-ranked hits from each database

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **S. No.** | **Ligands** | **CDOCKER energy** | **Interactions after molecular docking** | | **Interactions after molecular dynamics** | |
| **Type of interactions**  **(Distance in Å)** | **Key residues** | **Type of interactions**  **(Distance in Å)** | **Key residues** |
| 1 | SB-743921 (PDB ID: 4BXN) | -26.05 | -NH: H-bond (2.81);  -Cl: halogen (1.35) | Gly117 (O=C);  Ala218 (-NH) | ----------- | ----------- |
| 2 | SB-743921  (**1**) | -26.05 | -Cl: hydrophobic;  -CH3 of tolyl moiety: alkyl interactions | Leu214, Lys216;  Leu132, Ala133 | ring-C=O: H-bond (1.83);  -CH2: H-bond (2.50);  NH2:Salt bridge;  π-Orbitals: hydrophobic;  alkyl interactions | -NH:Arg104;  O=C:Glu103;  Asp115;  Ala203, Pro122,  Leu199, Leu125 and Val125 |
| 3 | **2** | -32.63 | -NH: H-bond (2.11);  -N:C-H bond (2.92);  -CH3:C-H bond (2.73);  π-Orbitals: hydrophobic;  π-alkyl interaction | Glu116 (O=C);  Glu118;  Glu116 (O=C);  Glu118;  Ala218 | **-----------** | **-----------** |
| 4 | ARQ-621 (**3**) | -33.68 | -NH2: H-bond (2.78 and 1.83);  -Methylene: C-H bond (2.76);  -Methylene: C-H bond (2.92);  π-Orbitals: hydrophobic;  alkyl interactions;  π-cation: electrostatic | Glu116 (O=C);  Glu116 (O=C);  Gly117;  Leu214;  Pro137 and Ala133;  Arg119 | **-----------** | **-----------** |
| 5 | AZD-4877 (**4**) | -33.47 | -NH: H-bond (1.90);  -Methylene: C-H bond (2.48);  -Methylene: C-H bond (2.80);  -Methylene: C-H bond (2.83);  π-Orbitals: hydrophobic;  alkyl interactions | Glu118 (O=C);  Glu118 (O=C);  Gly117;  Glu118 (O=C);  Ala218, Leu214 and Pro137;  Pro137 | **-----------** | **-----------** |
| 6 | MK-0731 (**5**) | -4.58 | OH:H bond (2.20)  -F: halogen (3.66, 2.77 and 3.05);  -CH3:C-H bond (2.46 and 2.99);  π-Orbitals: hydrophobic | Arg119;  Trp127,Glu118 and Gly117;  Gly117 and Glu116;  Ala218, Pro137 and Ala133 | **-----------** | **-----------** |
| 7 | Filanesib (**6**) | -33.78 | -F: halogen (2.79);  -OCH3:C-H bond (2.63 and 2.73);  -NCH3:C-H bond (2.59 and 2.90);  π-Orbitals: hydrophobic;  π-π stacking | Glu118 (O=C);  Glu116 (O=C);  Leu214;  Pro137 and Ala133;  Trp127 | **-----------** | **-----------** |
| 8 | MB-41570  (Maybridge) | -42.70 | -Ester CH2: C-H bond (2.99);  N-CH3: C-H bond(2.89);  π- sulphur;  vdW interactions;  alkyl interactions | Glu116;  Glu118;  Tyr211;  Ala133,  Gly117, Ala218, Leu214, Glu128, and Phe144;  Arg119 and Val210 | -Ester C=O: H bond (2.82);  N-CH3: C-H bond (2.59);  π- sulphur;  Piperazine CH2: C-H bond (2.90);  Ester methylene: C-H bond (2.73 & 2.65);  alkyl interactions | Glu103;  Lys192, Leu125,  Trp122;  Tyr196;  Pro122;  Glu103 and Trp112;  Arg104 and Val195 |
| 9 | CB-10358  (ChemBridge) | -39.19 | -OCH3: C-H bond (3.06);  Piperidine-CH2:C-H bond (2.85);  π- π stacking;  vdW interactions;  π-alkyl interactions; | Glu116;    Gly117  Tyr211;  Trp127, Glu128, and Ala133;  Leu214, Pro137,  and Ala218 | -CH2: C-H bond (2.84);  Piperidine-CH2: C-H bond (3.00);  -Ester CH3: C-H bond (2.45);  π- π stacking;  π-alkyl interactions | O=C of Glu103 ;    Gly102  Glu101;  Try196;  Ala203 and Leu199 |

**Table S3** Top-ranked complex from each database and their fit values

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **S. No.** | **Ligand ID** | **Structure** | **CDOCKER energy (kcal/mol)** | **Fit value** |
| 1 | CB-10358  (ChemBridge) |  | -39.19 | 3.12 |
| 2 | MB-41570  (Maybridge) |  | -42.70 | 3.28 |

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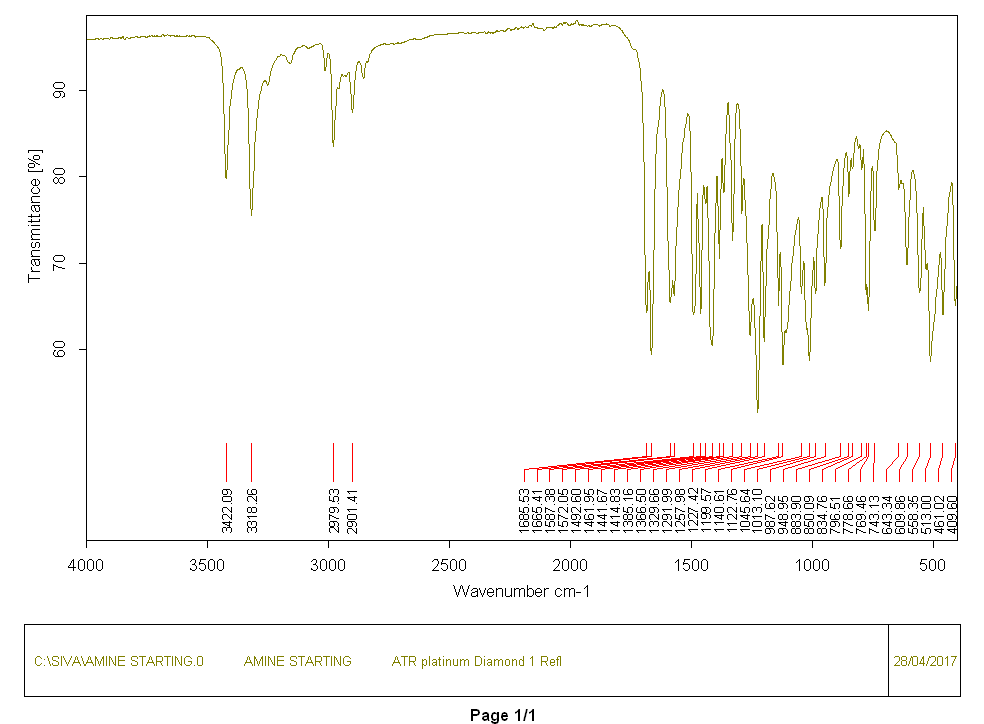
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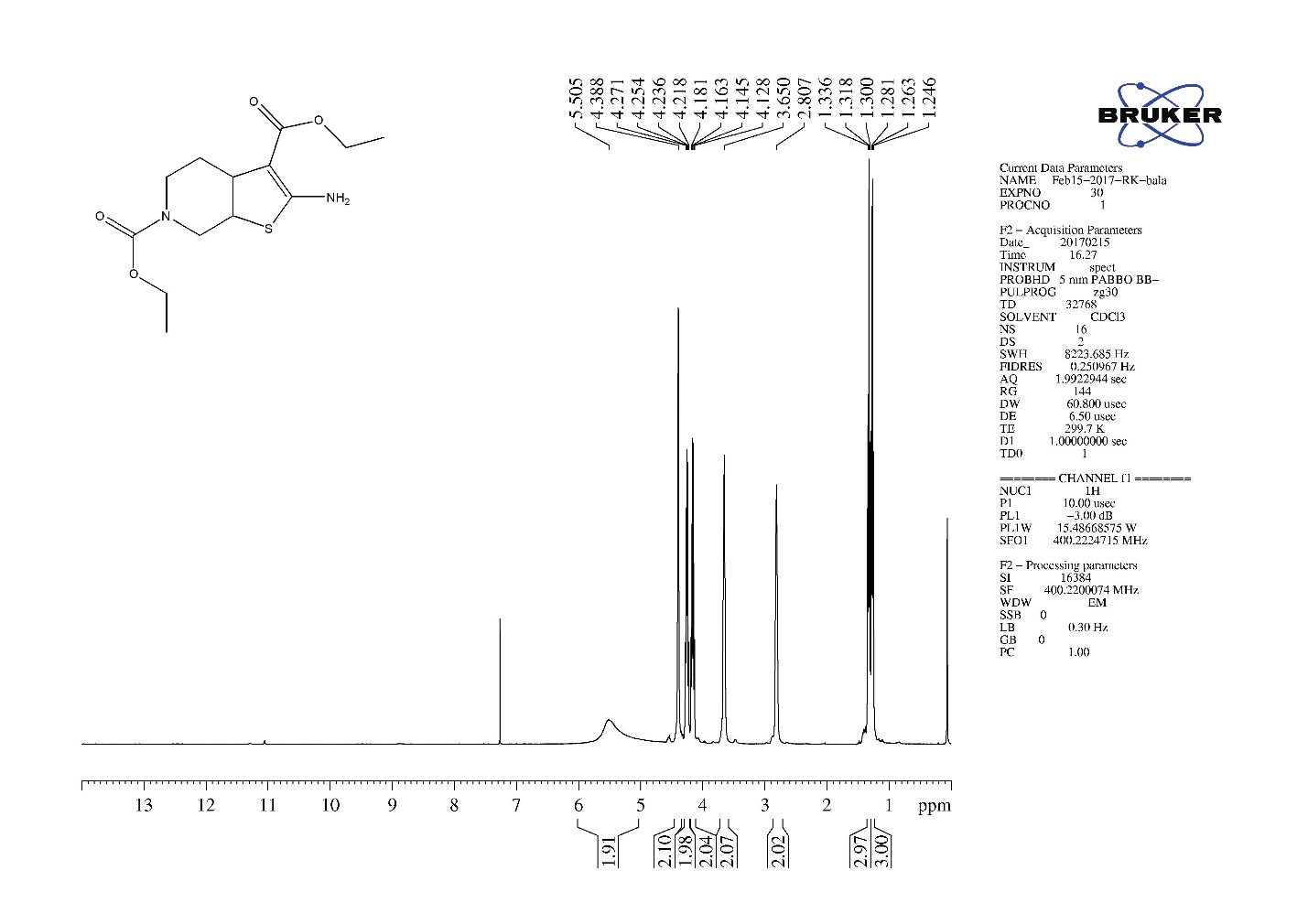
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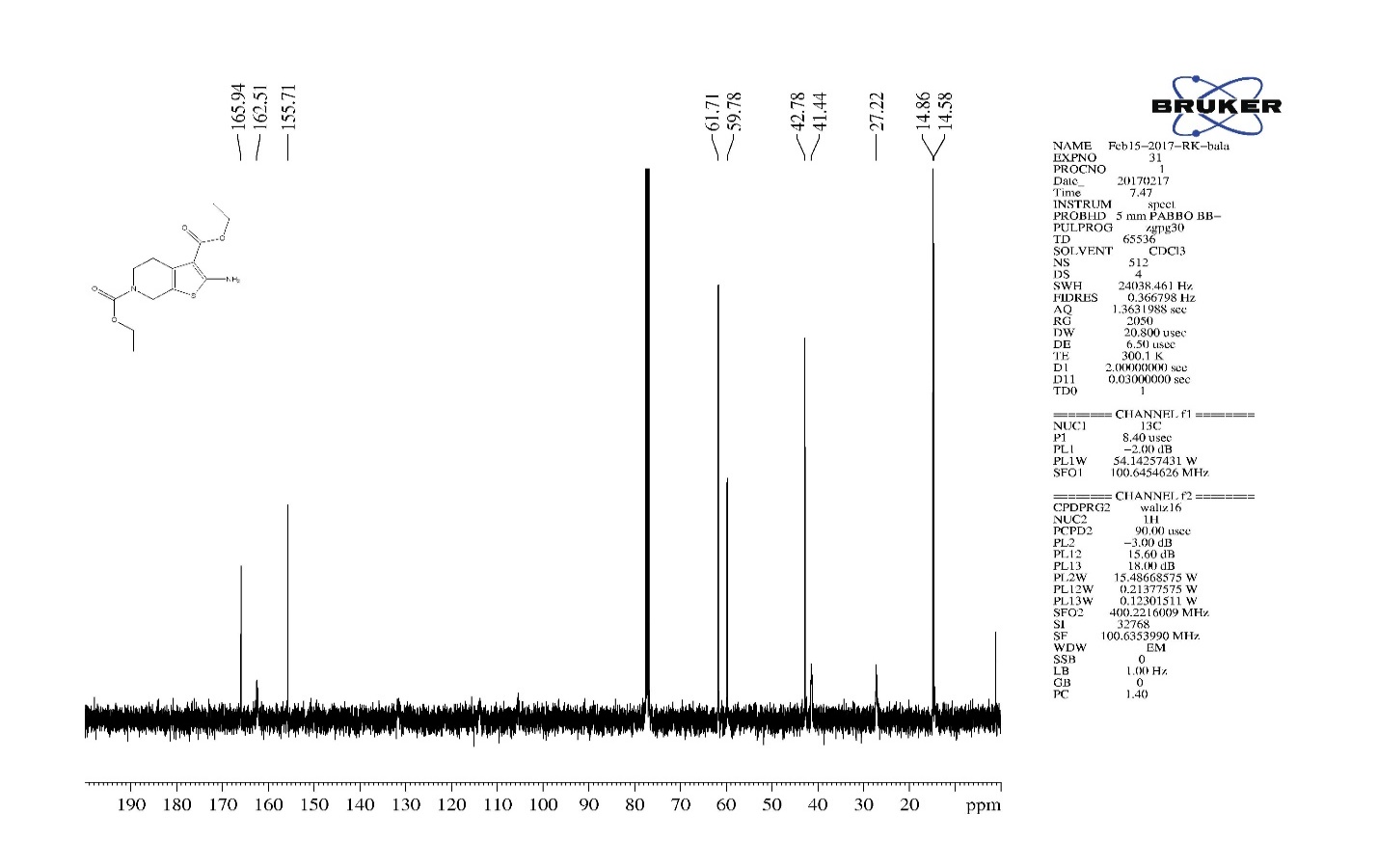
**Spectral characterization of the hits (Maybridge and ChemBridge)**

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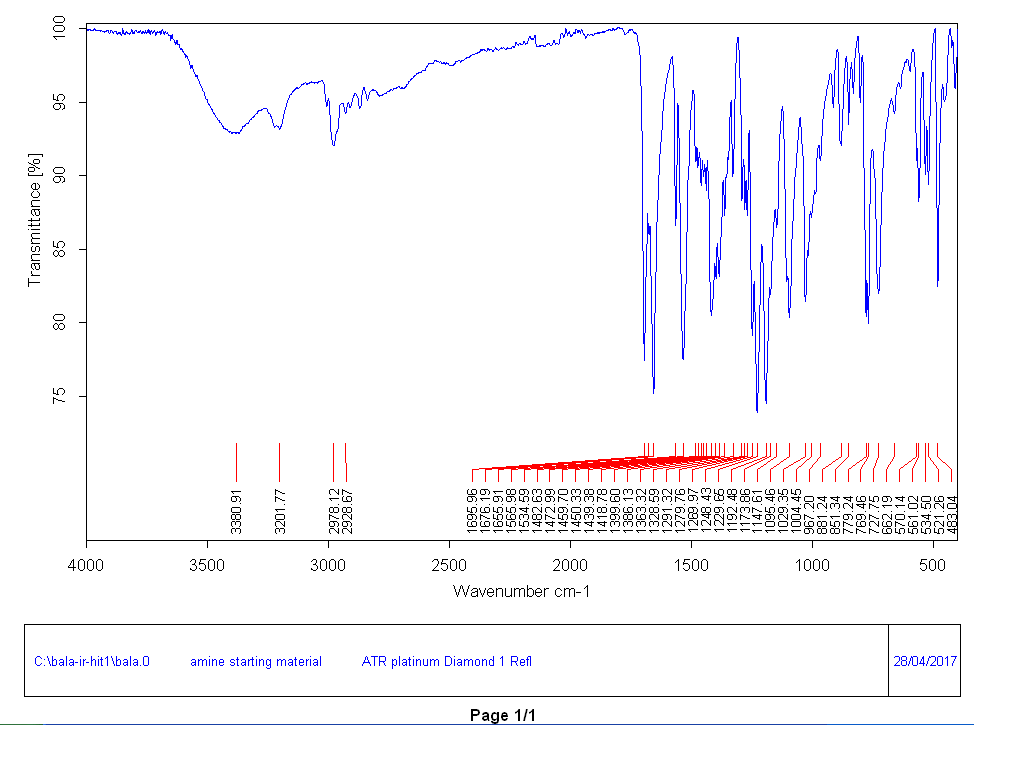
**Figure S1** FT-IR spectrum of starting compound **3**

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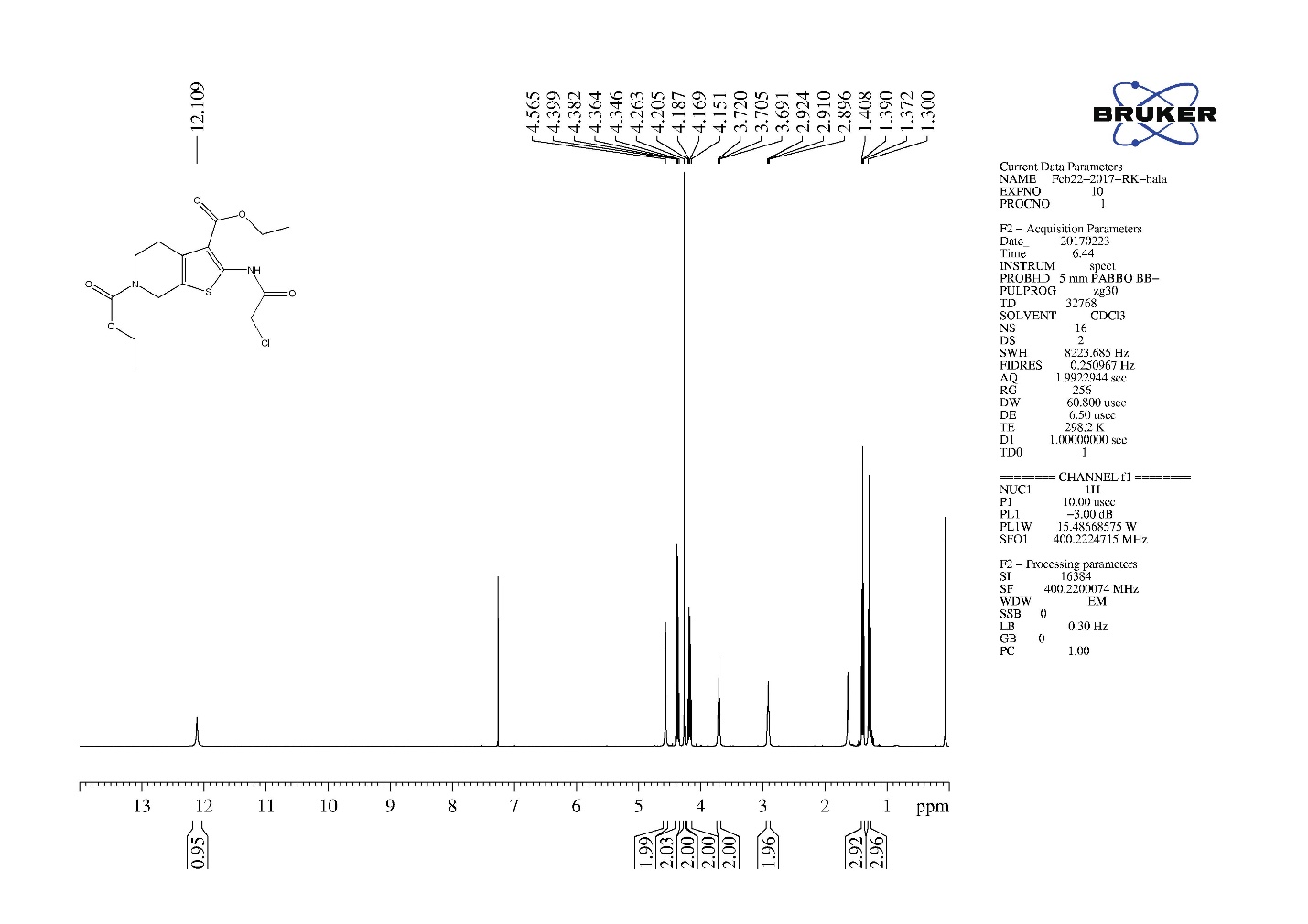
**Figure S2** 1H NMR of compound **3**

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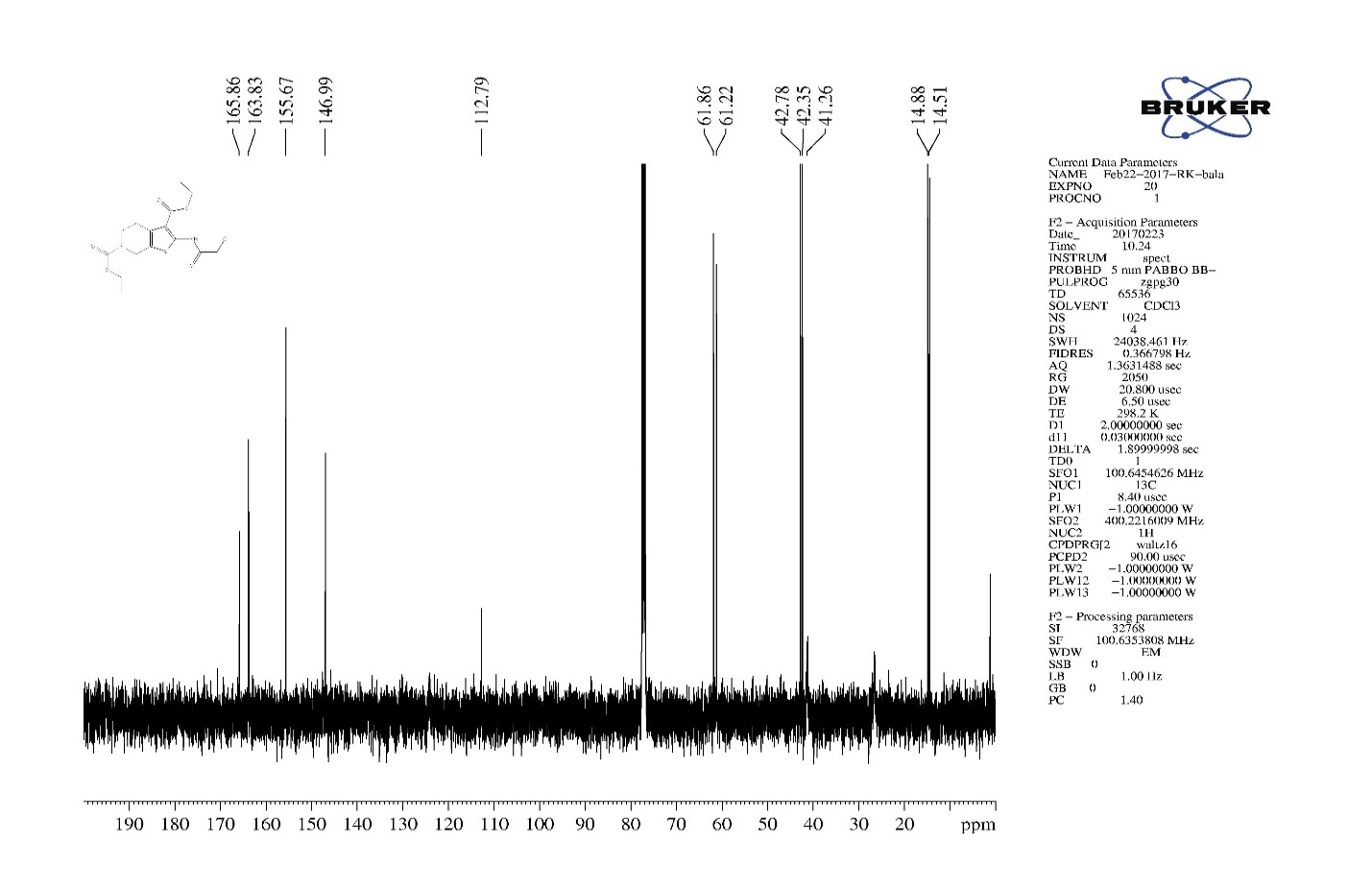
**Figure S3** 13C NMR of compound **3**

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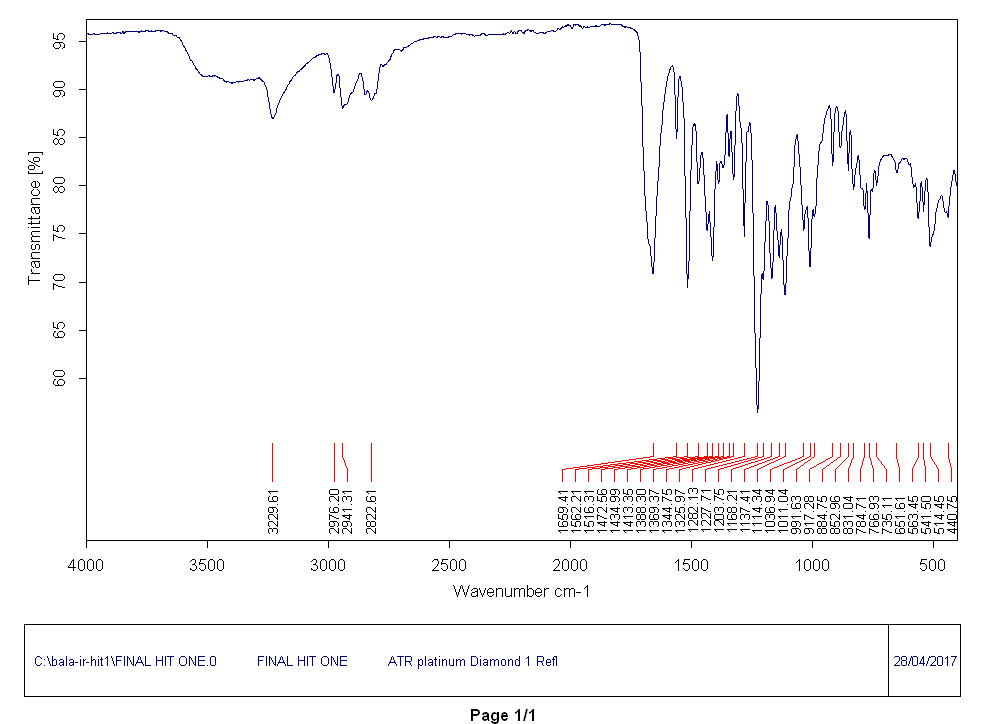
**Figure S4** FT-IR spectrum of compound **4**

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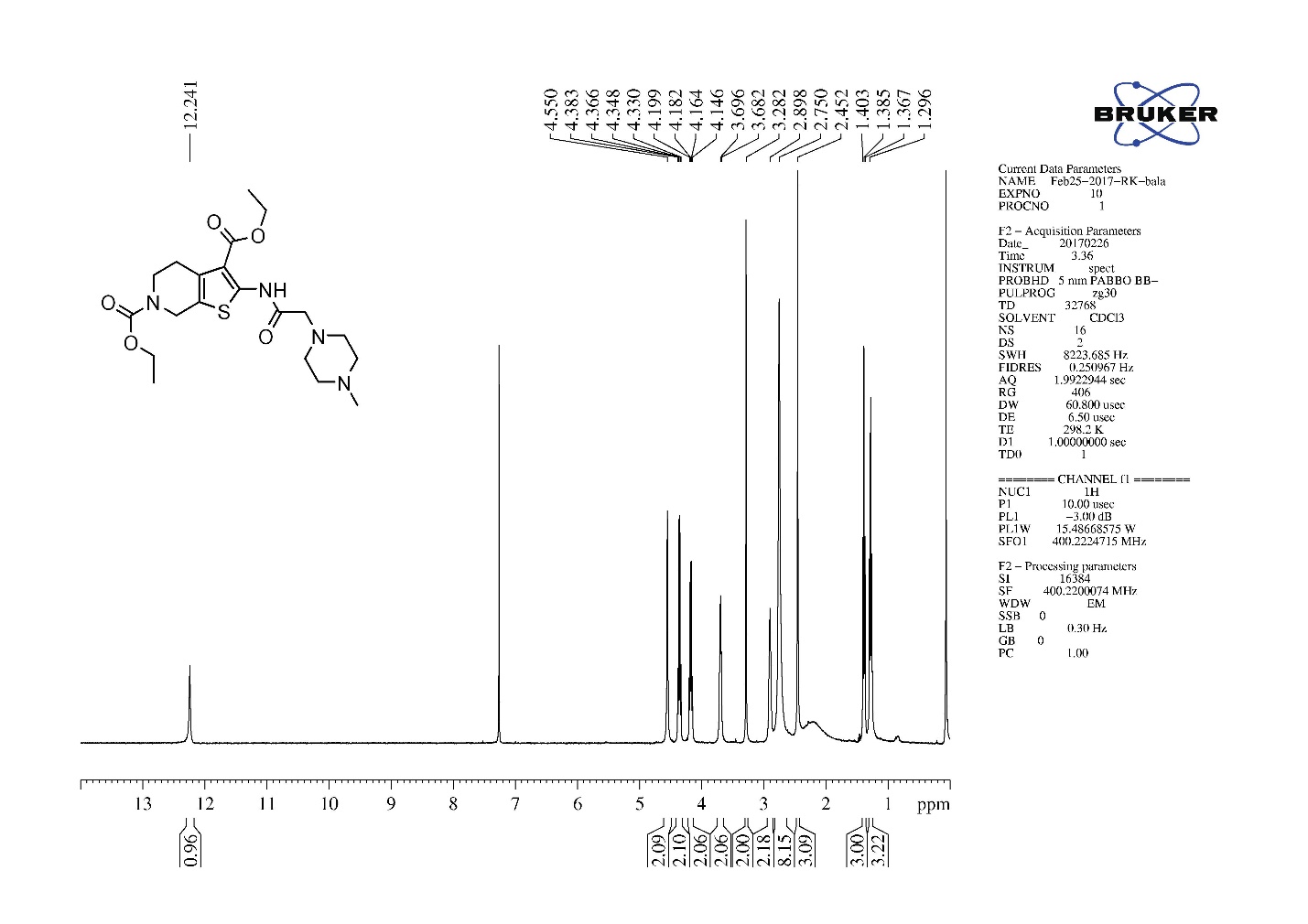
**Figure S5** 1H NMR of compound **4**

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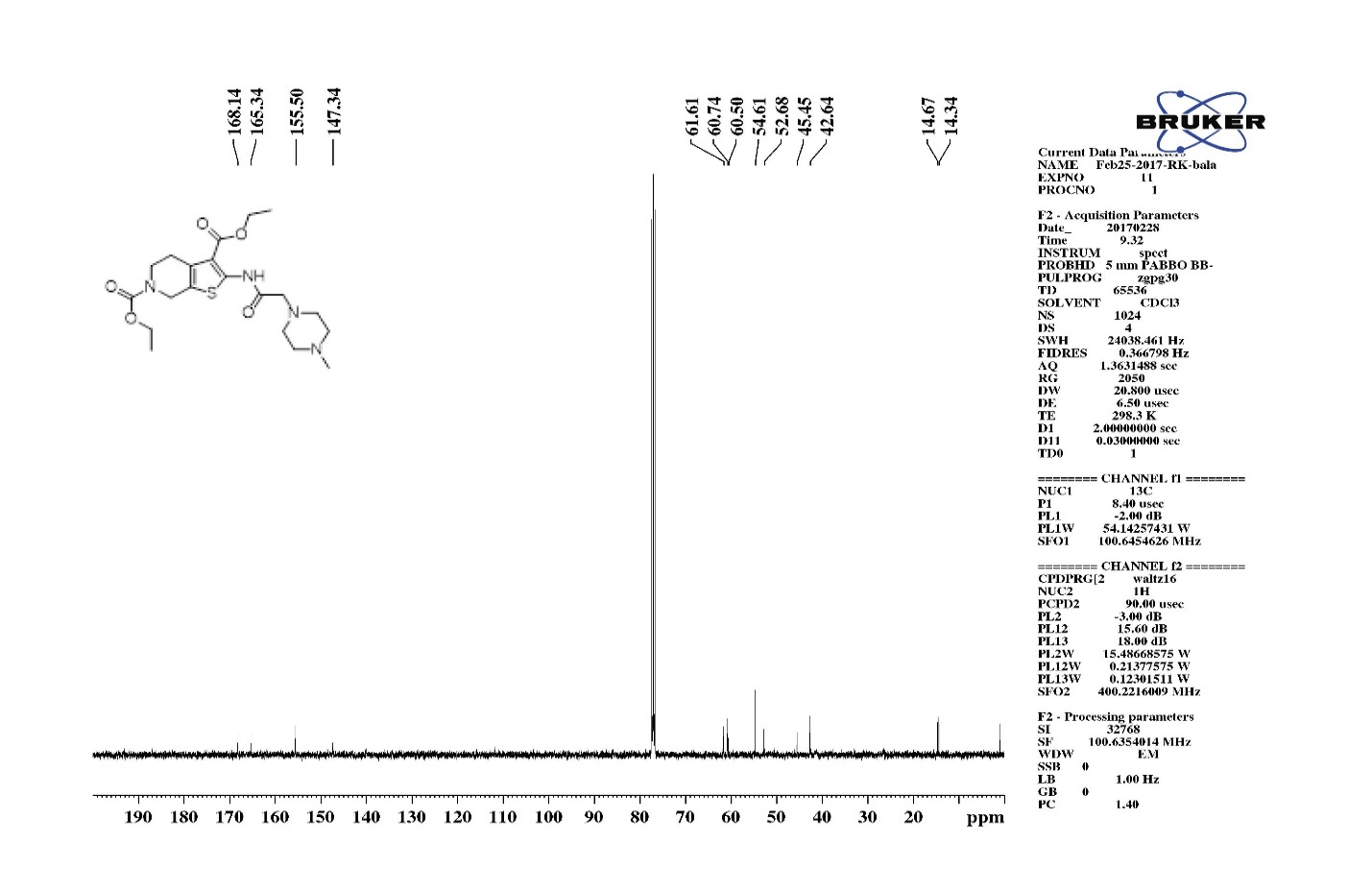
**Figure S6** 13C NMR of compound **4**

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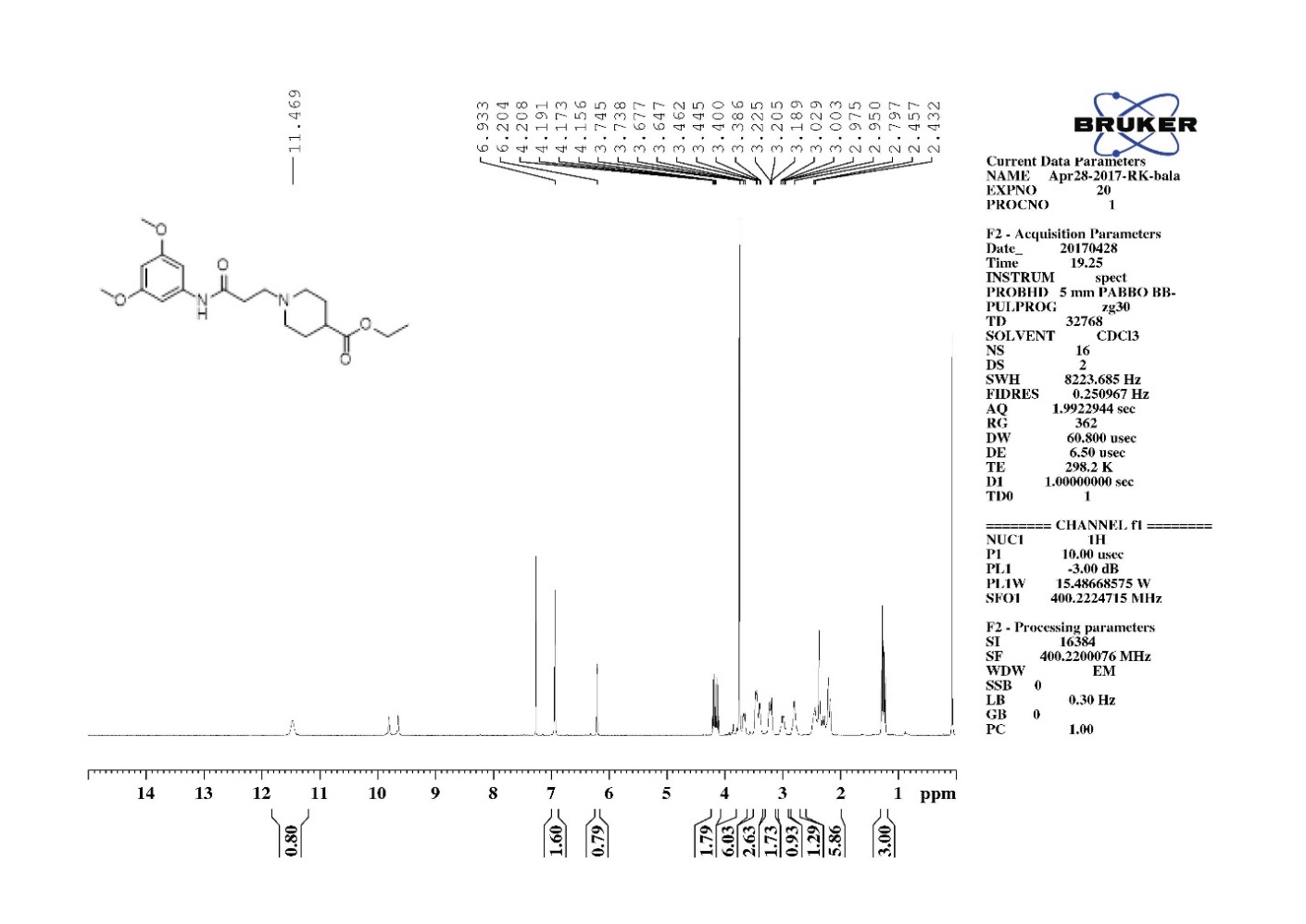
**Figure S7** FT-IR spectrum of compound **5 (Maybridge hit)**

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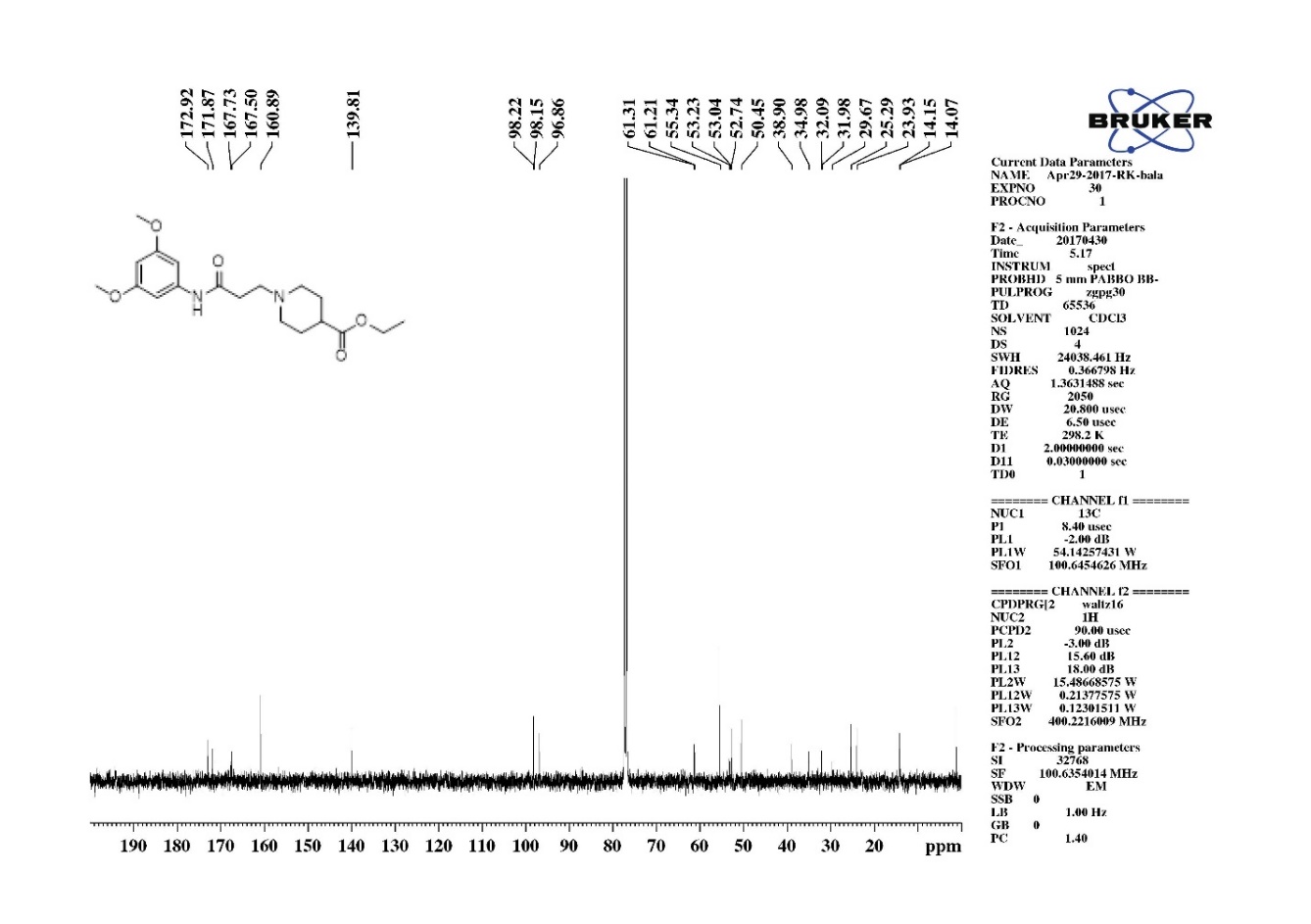
**Figure S8** 1H NMR of compound **5 (Maybridge hit)**

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**Figure S9** 13C NMR of compound **5 (Maybridge hit)**

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**Figure S10** 1H NMR spectrum of **ChemBridge hit**

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**Figure S11** 13C NMR spectrum of **ChemBridge hit**

**KSP ATPase inhibition profiles of Hits**

**(B)**



**(A)**



**Figure S12 Concentrations-response plots of Maybridge and ChemBridge hits for the basal and MT-stimulated human Eg5 ATPase activity. (A)** Inhibition of the basal Eg5 ATPase activity in the presence of increasing Maybridge hit (red dots) and ChemBridge hit (blue dots) concentrations. **(B)** Inhibition of the MT-stimulated Eg5 ATPase activities in the presence of increasing Maybridge hit (red dots) and ChemBridge hit (blue dots) concentrations. The results indicate that both the hits have no inhibition effect on neither the basal nor the MT-stimulated Eg5 ATPase activities. The data points were fitted using Kaleidagraph 4.0. All measurements were performed in triplicate.