Supplementary Material

Evaluation of the Test Compounds Using Lipinski's Rule

Drug likeliness of the compounds is evaluated based on Lipinski's rule. This rule describes the important pharmacokinetic parameters (ADME) on the basis of molecular properties of the drug.

Lipinski explained the following basic criteria in his rule.

- Hydrogen bond donors should not be more than 5 (like OH & NH)
- Hydrogen bond acceptors should not be more than 10 (electronegative atoms like O & N)
- The molecular weight should be less than 500 daltons
- The partition coefficient (logP) should not be greater than 5

Characterization of Compounds

2-[(4-hydrazinylidenepiperidin-1-yl)methyl]-1H-benzimidazole (**PB3**)

^{1}H-NMR (DMSO-d_6) δ: 1.43 (t, 4H, J=6.8Hz, H_3&H_5 piperidine), 2.45 (t, 4H, J=6.8Hz, H_2&H_6 piperidine), 3.57 (s, 2H, -N-C$\underline{H_2}$), 5.95 (s, 2H, =N-N$\underline{H_2}$); 7.23 (d, 2H, J=8.4 Hz, H_5&H_6 benzimidazole), 7.59 (d, 2H, J=8.4 Hz, H_4&H_7 benzimidazole), 12.19 (s, 1H, NH benzimidazole). ^{13}C-NMR (DMSO-d_6) δ: 23.51, 53.71 (4C, piperidine), 55.54 (CH_2), 114.96, 122.93, 138.75 (6C, Ar), 141.71 (imidazole), 161.58 (C=N). IR (KBr) cm^{-1}: 3314, 3247 (NH_2, NH), 3057, 2975, 2884 (CH), 1585 (C=N). MS m/z: 243 $(M)^+$. Anal. calcd for $C_{13}H_{17}N_5$: C, 64.17; H, 7.04; N, 28.78. Found: C, 63.87, H, 6.65; N, 29.11.N-[1-(1H-benzimidazol-2-ylmethyl)piperidin-4-ylidene]-4-fluoroaniline (**PB6**)

^{1}H-NMR (DMSO-d_6) δ: 1.45 (t, 4H, J=6.8 Hz, H_3&H_5 piperidine), 2.40 (t, 4H, J=6.8 Hz H_2&H_6 piperidine), 3.60 (s, 2H, -N-C$\underline{H_2}$), 7.01-7.60 (m, 8H, Ar-H), 12.15 (s, 1H, NH benzimidazole). ^{13}C-NMR (DMSO-d_6) δ: 25.25, 53.07 (4C, piperidine), 55.37 (CH_2), 115.55, 123.07, 138.33, (6C, Ar), 116.90 (d, 2C, J=23.3Hz, C3&C5 flurophenyl), 130.01 (d, 2C, J=8.5Hz, C2&C6 flurophenyl), 144.57 (1C, C1 flurophenyl), 161.45 (d, 1C, J=250.7Hz, C4 flurophenyl), 141.56 (imidazole), 187.79 (C=N). IR (KBr) cm^{-1}: 3253 (NH), 3059, 2974, 2884 (CH), 1639 (C=N). MS m/z: 322 $(M)^+$. Anal. calcd for $C_{19}H_{19}FN_4$: C, 70.79; H, 5.94; N, 17.38. Found: C, 71.04, H, 5.63; N, 17.69.

N-[1-(1H-benzimidazol-2-ylmethyl)piperidin-4-ylidene]-3-chloro-2-methylaniline (**PB10**)

^{1}H-NMR (DMSO-d_6) δ: 1.46 (t, 4H, J=6.8 Hz, H_3&H_5 piperidine), 2.37 (s, 3H, CH_3); 2.43 (t, 4H, J=6.8 Hz, H_2&H_6 piperidine), 3.65 (s, 2H, -N-C$\underline{H_2}$), 6.96-7.51 (m, 7H, Ar-H), 12.20 (s, 1H, NH benzimidazole). ^{13}C-NMR (DMSO-d_6) δ: 24.47, 53.34 (4C, piperidine), 55.34 (CH_2), 115.54, 120.36, 123.05, 126.47, 127.24, 128.32, 135.65, 139.11, 148.37 (12C, Ar), 141.58 (imidazole), 188.06 (C=N). IR (KBr) cm^{-1}: 3233 (NH), 3036, 2914, (CH), 1617 (C=N). MS m/z: 352 $(M)^+$. Anal. calcd for $C_{20}H_{21}ClN_4$: C, 68.08; H, 6.00; N, 15.88. Found: C, 67.92, H, 5.60; N, 16.25.

N-[1-(1H-benzimidazol-2-ylmethyl)piperidin-4-ylidene]-2,4-dichloroaniline (**PB11**)

^{1}H-NMR (DMSO-d_6) δ: 1.44 (t, 4H, J=6.8 Hz, H_3&H_5 piperidine), 2.41 (t, 4H, J=6.8 Hz, H_2&H_6 piperidine), 3.62 (s, 2H, -N-C$\underline{H_2}$), 6.76-7.47 (m, 7H, Ar-H), 12.18 (s, 1H, NH benzimidazole). ^{13}C-NMR (DMSO-d_6) δ: 24.51, 53.59 (4C, piperidine), 55.34 (CH_2), 115.04, 123.07, 125.32, 128.04, 129.56, 131.84, 134.11, 137.58, 139.35 (12C, Ar), 141.77 (imidazole), 187.82 (C=N). IR (KBr) cm^{-1}: 3227 (NH), 3073, 2954, 2893 (CH), 1604 (C=N). MS m/z: 373 $(M)^+$. Anal. calcd. for $C_{19}H_{18}Cl_2N_4$: C, 61.13; H, 4.86; N, 15.01. Found: C, 60.80, H, 4.51; N, 14.70.

N-[1-(1H-benzimidazol-2-ylmethyl)piperidin-4-ylidene]-2,4-difluoroaniline (**PB12**)

^{1}H-NMR (DMSO-d_6) δ: 1.40 (t, 4H, J=6.8 Hz, H_3&H_5 piperidine), 2.39 (t, 4H, J=6.8 Hz, H_2&H_6 piperidine), 3.66 (s, 2H, -N-C$\underline{H_2}$), 6.72-7.52 (m, 7H, Ar-H), 12.17 (s, 1H, NH benzimidazole). ^{13}C-NMR (DMSO-d_6) δ: 25.64, 53.18 (4C, piperidine), 55.47 (CH_2), 115.39, 123.15, 139.02, (6C, Ar), 106.06 (t, 1C, J=50.9 Hz, C3 diflurophenyl), 112.54 (dd, 1C, J=27.5, 5.4 Hz, C5 diflurophenyl), 125.77 (dd, 1C, J=13.1, 3.9 Hz, C6 diflurophenyl), 131.51 (dd, 1C, J=9.4, 4.5 Hz, C1 diflurophenyl), 155.24 (dd, 1C, J=267.6, 14.6 Hz, C2 diflurophenyl), 163.06 (dd, 1C, J=259.9, 14.6 Hz, C4 diflurophenyl), 141.43 (imidazole), 187.96 (C=N). IR (KBr) cm^{-1} 3238 (NH), 3027, 2966, 2894 (CH), 1628 (C=N). MS m/z: 340 $(M)^+$. Anal. calcd for $C_{19}H_{18}F_2N_4$: C, 67.05; H, 5.33; N, 16.46. Found: C, 66.77, H, 5.54; N, 16.11.

N-[1-(1H-benzimidazol-2-ylmethyl)piperidin-4-ylidene]aniline (**PB14**)

^{1}H-NMR (DMSO-d_6) δ: 1.44 (t, 4H, J=6.8 Hz, H_3&H_5 piperidine), 2.44 (t, 4H, J=6.8 Hz, H_2&H_6 piperidine), 3.65 (s, 2H, -N-C$\underline{H_2}$), 7.07-7.52 (m, 9H, Ar-H), 12.21 (s, 1H, NH benzimidazole). ^{13}C-NMR (DMSO-d_6) δ: 24.96, 53.68 (4C, piperidine), 55.54

(CH_2), 115.34, 122.47, 123.11, 127.49, 130.21, 138.75, 149.17 (12C, Ar), 141.27 (imidazole), 188.20 (C=N). IR (KBr) cm^{-1}: 3219 (NH), 3044, 2980, 2881 (CH), 1587 (C=N). MS m/z: 304 $(M)^+$. Anal. calcd for $C_{19}H_{20}N_4$: C, 74.97; H, 6.62; N, 18.41. Found: C, 74.71, H, 6.36; N, 18.20.N-[1-(1H-benzimidazol-2-ylmethyl)piperidin-4-ylidene]-4-chloroaniline (**PB15**)

^{1}H-NMR (DMSO-d_6) δ: 1.38 (t, 4H, J=6.8 Hz, H_3&H_5 piperidine), 2.39 (t, 4H, J=6.8 Hz, H_2&H_6 piperidine), 3.60 (s, 2H, -N-C$\underline{H_2}$), 7.22-7.56 (m, 8H, Ar-H), 12.16 (s, 1H, NH benzimidazole). ^{13}C-NMR (DMSO-d_6) δ: 24.36, 53.04 (4C, piperidine), 55.13 (CH_2), 115.37, 123.07, 123.91, 130.24, 132.89, 138. 86, 147.37 (12C, Ar), 141.26 (imidazole), 187.79 (C=N). IR (KBr) cm^{-1}: 3243 (NH), 3064, 2914, 2884 (CH), 1621 (C=N). MS m/z: 338 $(M)^+$. Anal. calcd for $C_{19}H_{19}ClN_4$: C, 67.35; H, 5.65; N, 16.54. Found: C, 67.73, H, 5.94; N, 16.24.

N-[1-(1H-benzimidazol-2-ylmethyl)piperidin-4-ylidene]-2,4-dinitroaniline (**PB17**)

^{1}H-NMR (DMSO-d_6) δ: 1.47 (t, 4H, J=6.8 Hz, H_3&H_5 piperidine), 2.45 (t, 4H, J=6.8 Hz, H_2&H_6 piperidine), 3.63 (s, 2H, -N-C$\underline{H_2}$), 7.26-8.67 (m, 7H, Ar-H), 12.14 (s, 1H, NH benzimidazole). ^{13}C-NMR (DMSO-d_6) δ: 25.07, 53.60 (4C, piperidine), 55.44 (CH_2), 115.35, 121.47, 123.17, 124.19, 131. 66, 139.04, 144.18, 147.43, 148.40 (12C, Ar), 141.64 (imidazole), 187.96 (C=N). IR (KBr) cm^{-1}: 3249 (NH), 3029, 2964, 2875 (CH), 1590 (C=N). MS m/z 394 $[M]^+$. Anal. calcd for $C_{19}H_{18}N_6O_4$: C, 57.86; H, 4.60; N, 21.31. Found: C, 58.17, H, 4.84; N, 20.97.

1-(1H-benzimidazol-2-ylmethyl)-N-methoxypiperidin-4-imine (**PB19**)

^{1}H-NMR (DMSO-d_6) δ: 1.40 (t, 4H, J=6.8 Hz, H_3&H_5 piperidine), 2.44 (t, 4H, J=6.8 Hz, H_2&H_6 piperidine), 3.58 (s, 2H, -N-CH_2), 4.04 (s, 3H, OCH_3), 7.20 (d, 2H, H_5&H_6 benzimidazole), 7.44 (d, 2H, H_4&H_7 benzimidazole), 12.22 (s, 1H, NH benzimidazole). ^{13}C-NMR (DMSO-d_6) δ: 27.04, 52.15 (4C, piperidine), 55.57 (CH_2), 62.04 (OCH_3), 115.18, 123.37, 140.15 (6C, Ar), 141.80 (imidazole), 160.37 (C=N). IR (KBr) cm^{-1}: 3254 (NH), 3036, 2987, 2890 (CH), 1620 (C=N). MS m/z: 258 $(M)^+$. Anal. calcd for $C_{14}H_{18}N_4O$: C, 65.09; H, 7.02; N, 21.69. Found: C, 65.39, H, 7.12; N, 22.03.

Docking Methodology

Ligand preparation

LigPrep is Schrödinger software that generates 3D structures from 2D representation. LigPrep also assigns an appropriate bond order with correct configuration and creates many numbers of structures with various tautomers, stereoisomers, ring conformations and ionization states from each structure. Subsequently optimizations of these structures are carried out by using OPLS-2005 using a default setting in the LigPrep.

Protein preparation

The protein used in the present study is INHa (PDB.i.d - 1ZID). Maestro software package (Mastro, Schrödinger) was used to prepare the Protein structures and protein structure alignment module in Prime (Prime, Schrödinger) was used to align the protein structures. Formal charges and bond orders has been added for hydrogens and heterogroups and to all atoms. The χ2 dihedral angle of Asn,His residues and χ3 dihedral angle of Gln residue was inspected visually for accuracy. Hydrogen bonding was maximized if necessary, by 180°rotation and also by the manual selection of

proper His tautomer. Water molecules present in all the sites excluding the active site have been removed from the protein complex. A brief relaxation has been carried out on the structure using the option "Refinement Only" in Maestro. This procedure consisting of two steps which is optimization of hydroxyl and thiol torsions in the first step and In the second step the steric clashes in the original structures(PDB) has been alleviated using OPLS-2005 force field Impref inorder to get all-atom constrained minimization (0.30 Å)

Grid generation and docking

Default box size setting in Glide was used for grid generation. Hydrogen bond constraints were not applied and partial atomic charge of < 0.25 by 1.0 for the scaling of van der waals radii of protein atoms. Non-planar conformation was penalized for amide bonds and flips of 5- and 6-membered rings were allowed. Partial atomic charge less than 0.15 was scaled by 0.8 for van der waals radii of ligand atoms. The prepared ligands were docked against the proteins. "Extra precision" (XP) mode of Glide was used to perform all docking calculations. To locate the ligand in the active-site of the receptor, hierarchical series of filters were used by Glide. The poses that pass the initial filters test enters the final stage in which the minimization and evaluation of a grid approximation to the OPLS-AA nonbonded ligand–receptor interaction energies were involved. Final scoring was performed on the energy-minimized poses. Schrödinger's proprietary Glide Score (G Score) scoring function was used for rescoring of the energy minimised poses. All docking computations were carried out with the Linux OS (Red Hat Enterprise WS 5.0).

Cytotoxicity assay

Cultures of Vero cell lines (African green monkey kidney cells) were procured from National Centre for Cell Sciences (NCCS), Pune, India. The cells were cultured and supplemented with amphotericin-B (5 μg/mL), penicillin (100 IU/mL),10% heat inactivated Fetal bovine serum (FBS) and streptomycin (100 μg/mL) in Dulbecco's modified essential medium (DMEM) maintaining 37°C and 5% CO_2 atmosphere until confluent. Trypsinization of the cells was carried out using TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). 25 cm^2flat bottles were used to grow the cultures and 96 well plates were used to perform the studies.

Viability of the cells were assessed on the basis of the conversion of MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] into a purple coloured product, which was recorded at 540nm.

The percentage growth calculation is as depicted below:

$$\%\ \text{growth inhibition} = \frac{\text{control absorbance} - \text{test absorbance}}{\text{control absorbance}} \times 100$$

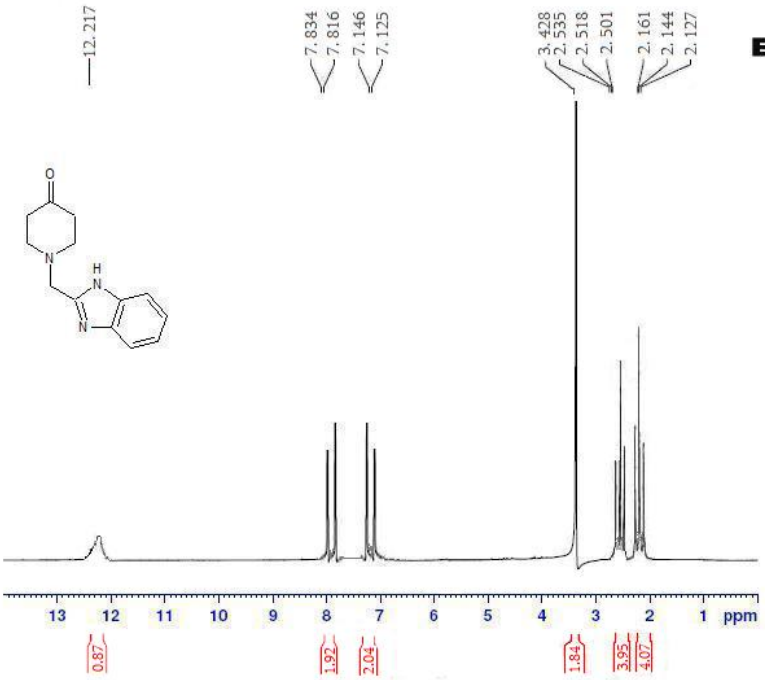
IC_{50} (compound concentration that reduces the number of cells by 50%) values were calculated[1] and selectivity index (SI) was obtained from the formula as given below:

$$\text{SI} = \frac{\text{IC50}}{\text{MIC}}$$

SI value of more than 10 is considered to be nontoxic for the *in-vitro* antitubercular evaluation.[2,3]

References

1. Cheng Y, Prusoff WH. Relationship between the inhibition constant (K1) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction. *Biochem Pharmacol.* 1973;22(23):3099–3108.
2. Luo X, Pires D, Aínsa JA, et al. Zanthoxylumcapense constituents with antimycobacterial activity against Mycobacterium tuberculosis in vitro and ex vivo within human macrophages. *J Ethnopharmacol.* 2013;146(1):417–422.
3. Protopopova M, Hanrahan C, Nikonenko B, et al. Identification of a new antitubercular drug candidate, SQ109, from a combinatorial library of 1,2-ethylenediamines. *J Anitimicrob Chemother*. 2005;56(5):968-974



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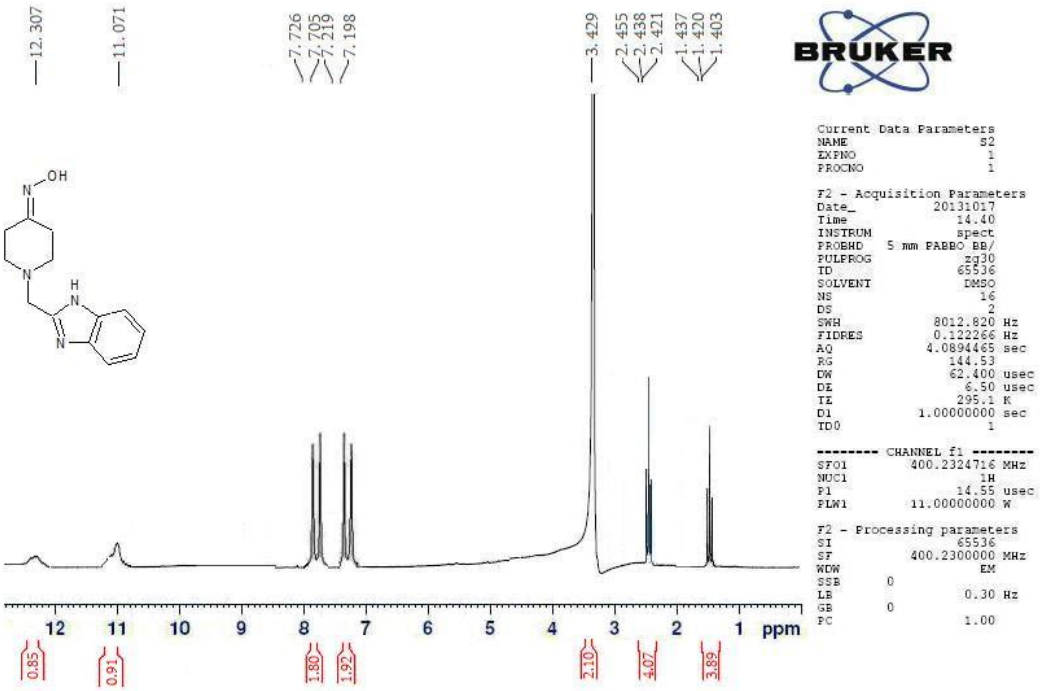

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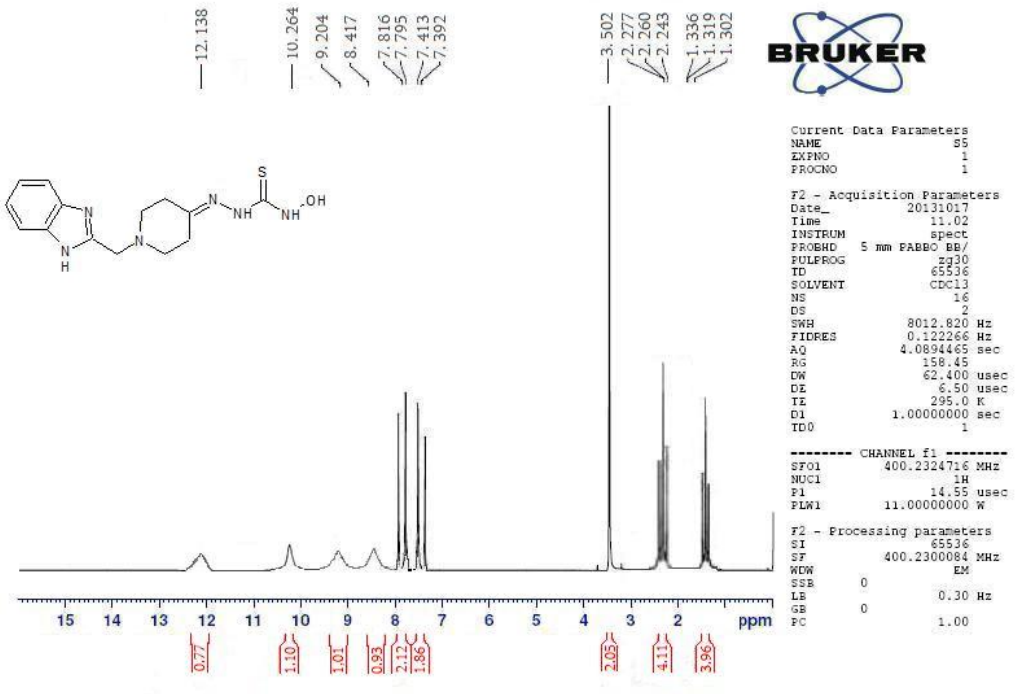
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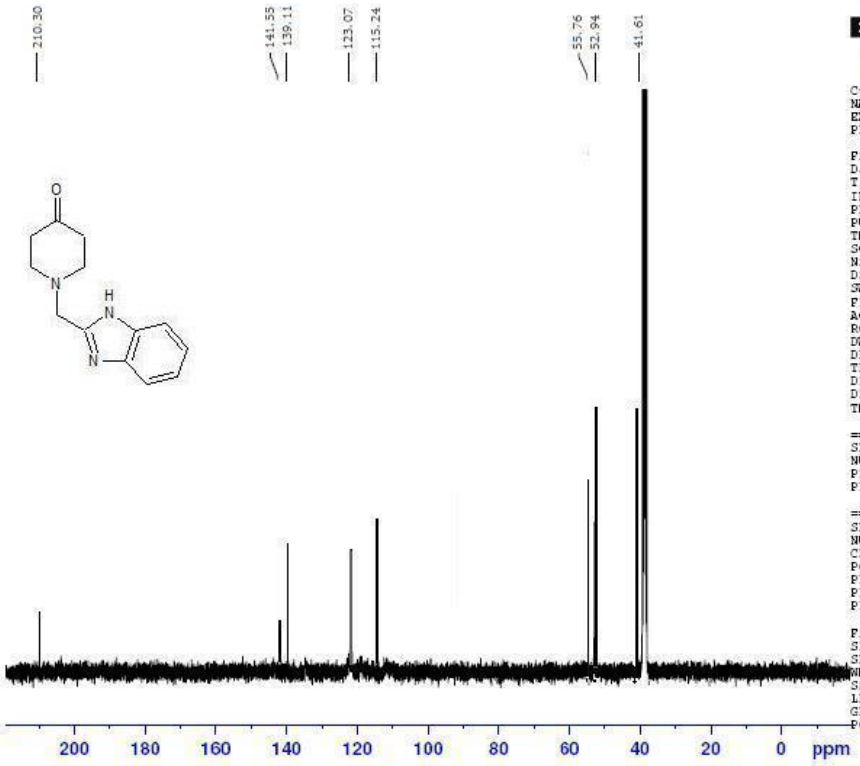

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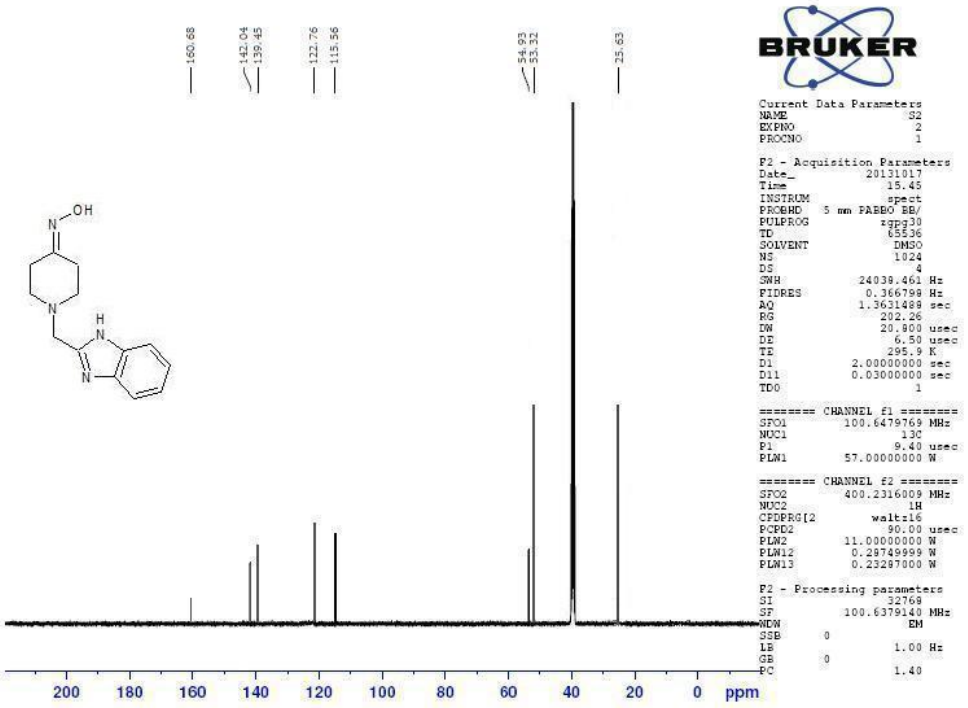

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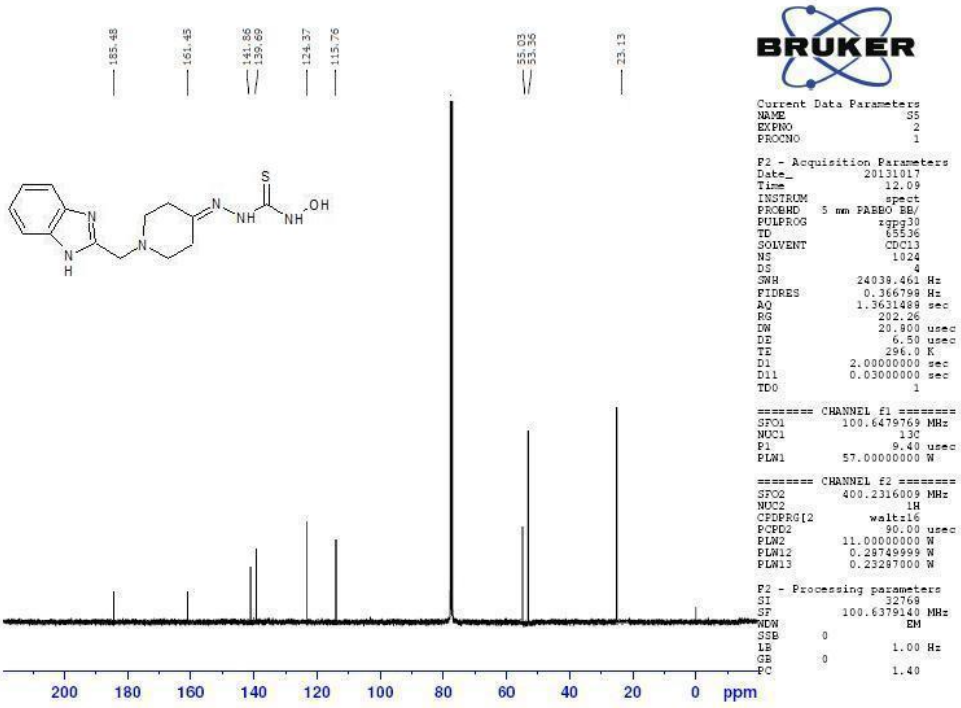

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