Biological Evaluation of some Novel 1,3-Bis substituted-2-isopropylamidines by In-Silico Molecular Dynamics and Simulation Studies

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Experimental Section

General Information:

All the reagents were purchased from commercial suppliers and used without further purification. Solvents were dried according to the standard procedures. All the reactions were monitored by thin layer chromatography (TLC) using standard TLC silica gel plates and visualized with UV light. Column chromatography was performed using Merck silica gel (100−200 mesh). Nuclear magnetic resonance spectra were recorded on Brucker (400 MHz) ultra shield plus and Jeol (600 MHz) ECZ 600R FT-NMR spectrometer with $CDCl_3$/ DMSO-d_6 as solvent. Chemical shifts are reported in δ (ppm) using residual solvent protons as the internal standard (δ 7.26 for $CDCl_3$ and δ 2.50 for DMSO-d_6, in ^{1}H NMR). Coupling constants are reported as J values in Hertz (Hz). Splitting patterns are designated as s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublet), dt (doublet of triplet), td (triplet of doublet), m (multiplet), and br (broad). Infrared spectra of neat samples were recorded in ATR (attenuated total reflectance) mode using a FT-IR instrument (Agilent technologies), and LCMS spectra on a 6538 UYHD accurate mass Q-TOF LC/MS spectrometer through electro spray ionization (ESI) mode. Elemental analysis was recorded using a Brucker Elemental analyser. Melting points were recorded using an electro thermal capillary melting point apparatus and remains uncorrected.

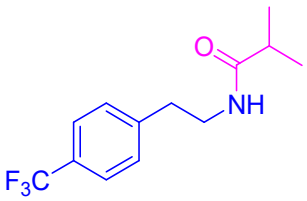
Molecular docking:

All the synthesized ligands **5a-e** were drawn, analyzed and converted to SDF format using ChemDraw Ultra Suite 12.0. Energy minimization of all the ligands were performed before proceeding to molecular docking. Based on the literature survey, the p38-α, cyclin dependent kinase-2 and vegfr-2 were selected as the cancer targets, HIV1 protease as HIV target, PBP2a for multi drug resistance in *Staphylococcus aureus*, main protease for SARS-COV-2, acetylcholinesterase and butylcholinesterases as targets for Alzheimers. The X-ray crystal structures of all the target proteins (PBD ID: 6YJC, 3PXQ, 3VHE, 1B6M, 4CJN, 6WTT, 7BGC and 7xn1) were retrieved from Protein Data Bank. The water molecules, hetero atoms and ligands were removed from the protein structure and analyzed for polar hydrogen atoms followed by the addition of Kollman charges using Autodock Tools.[1]

Protein-ligand docking studies were carried out using Autodock Vina.[2] Different orientations of the ligands were searched and ranked based on the lowest binding energy. The best conformationwith the least binding energy was analyzed by PyMol 2.5.[3]

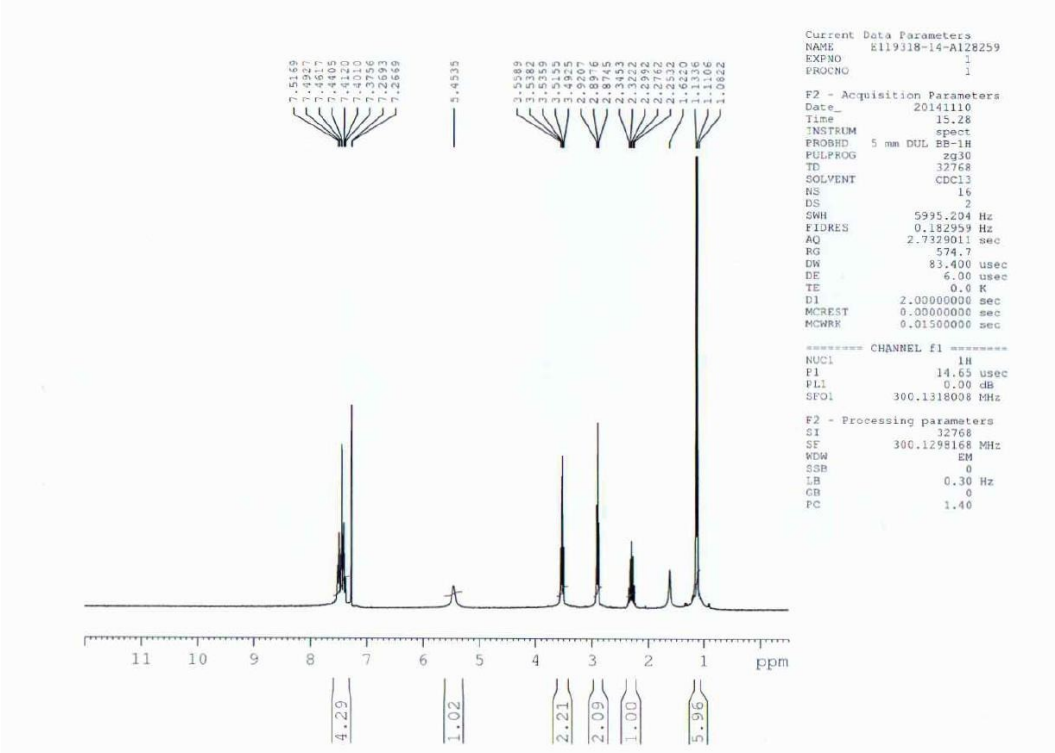
Molecular Dynamics Simulation:

Molecular dynamics simulation was performed using GROMACS,[4] for analysis of stability of the complex and to evaluate the changes in protein of interest after ligand binding. The protein-ligand complex with the best binding affinity was chosen for MD Simulations. CHARMM27 forcefield was used for simulations and energy minimizations of cdk2 protein was performed, with steepest algorithm. After the generation of topology files for protein and ligand, the complex was solvated using TIP3P water model in a dodecahedron box, followed by addition of Na^+ and Cl^- ions. 1Å buffer region was maintained between protein and box walls. MD simulations were performed at 300K temperature for 100ns, post equilibration in initial simulations, in two phases viz., NPT and NVT. Post simulations, trajectory was analyzed. MD Simulations movie was created using UCSF Chimera[5] and MMPBSA analysis was performed.

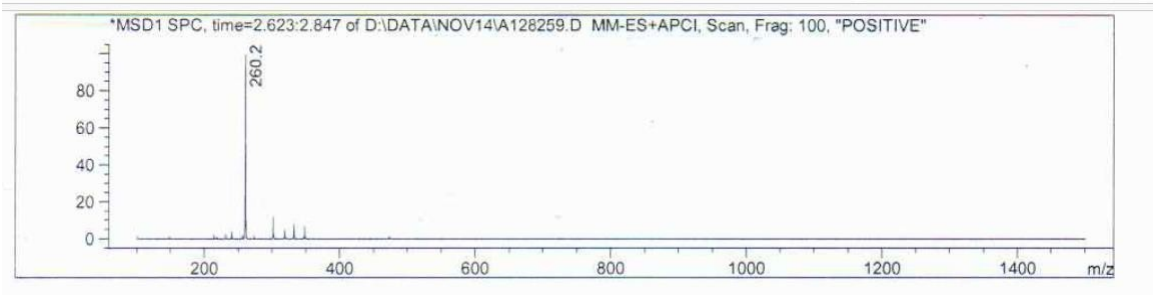


3a

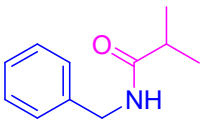
***N*-[2-(*p*-trifluoromethylphenyl)ethyl]-2-methylpropanamide (3a)**. Off White solid; yield 84%;142-144 °C; R_f 0.24 (4:6 EtOAc/hexane); IR (neat, cm^{-1}) 3442, 3327, 1750, 1676, 1297, 1170, 798; 1H NMR (400 MHz, $CDCl_3$) δ 7.52 – 7.38 (m, 4H), 5.45 (s, 1H), 3.54 (q, J = 5Hz, 2H), 2.89 (t, J = 9.2 Hz, 2H), 2.35 – 2.25 (m, 1H), 1.11 (s, 6H); 13C{1H} NMR (100 MHz, $CDCl_3$) δ 179.2, 130.6, 129.2, 128.1, 124.6, 122.7, 48.7, 39.2, 37.1, 23.2.; LCMS (ESI) *m/z*: $[M+H]^+$calcd., for $C_{13}H_{17}F_3NO$ 260; found 260. Elemental analysis calcd., C - 60.22%, H – 6.22, N – 5.40%; found C – 60.21%, H – 6.22%, N – 5.39%.



1H NMR of 3a

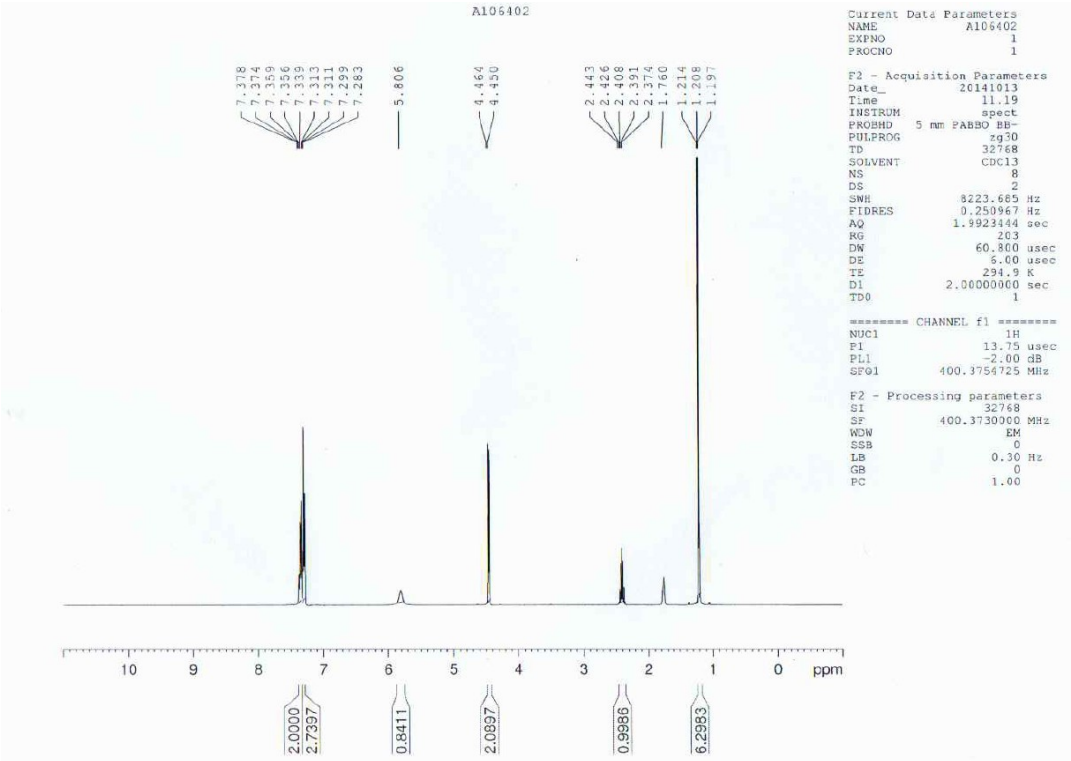


LS-MS of 3a

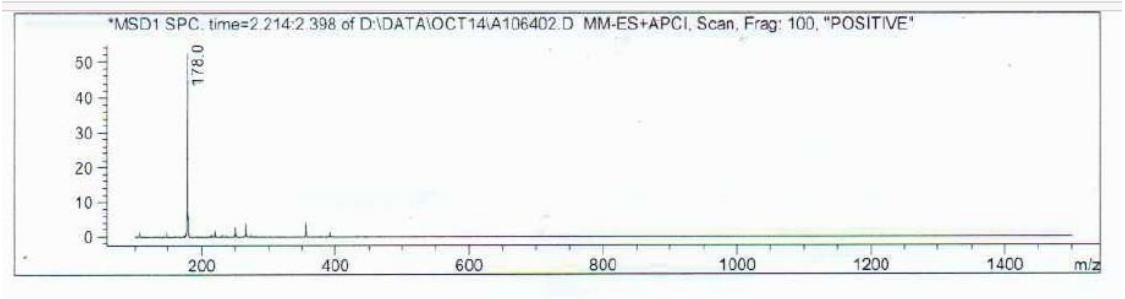


3b

***N*-methelenephenyl-2-methylpropanamide (3b)**. Off white solid; yield 92%; 125-127 °C; R_f 0.34 (4:6 EtOAc/hexane); IR (neat, cm^{-1}) 3423, 3397, 1719, 1701, 1282, 1011, 752; 1H NMR (400 MHz, $CDCl_3$) δ 7.37 (q, J = 7.4 Hz, 2H), 7.34 – 7.28 (m, 3H), 5.81 (s, 1H), 4.46 (d, J = 5.6 Hz, 2H), 2.41 (m,1H), 1.21 (s, 6H); 13C{1H} NMR (100 MHz, $CDCl_3$) δ 175.2, 142.1, 129.2, 126.2, 125.3, 49.7, 42.1, 27.5; LCMS (ESI) *m/z*: $[M+H]^+$calcd., for $C_{11}H_{16}NO$ 178.3; found 178. Elemental analysis calcd., C – 74.54%, H – 8.53, N – 7.90%; found C – 74.55%, H – 8.52%, N – 7.89%.



1H NMR of 3b



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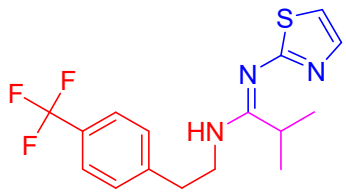
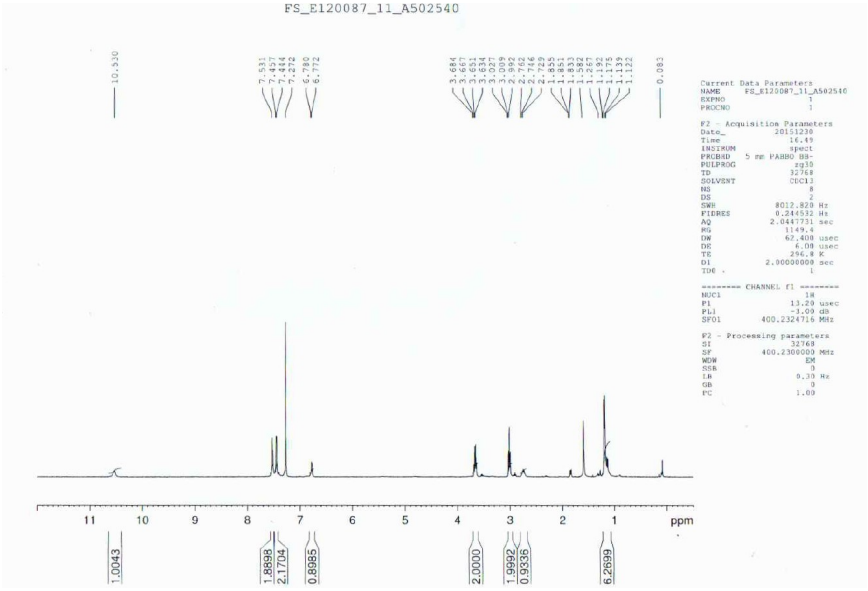
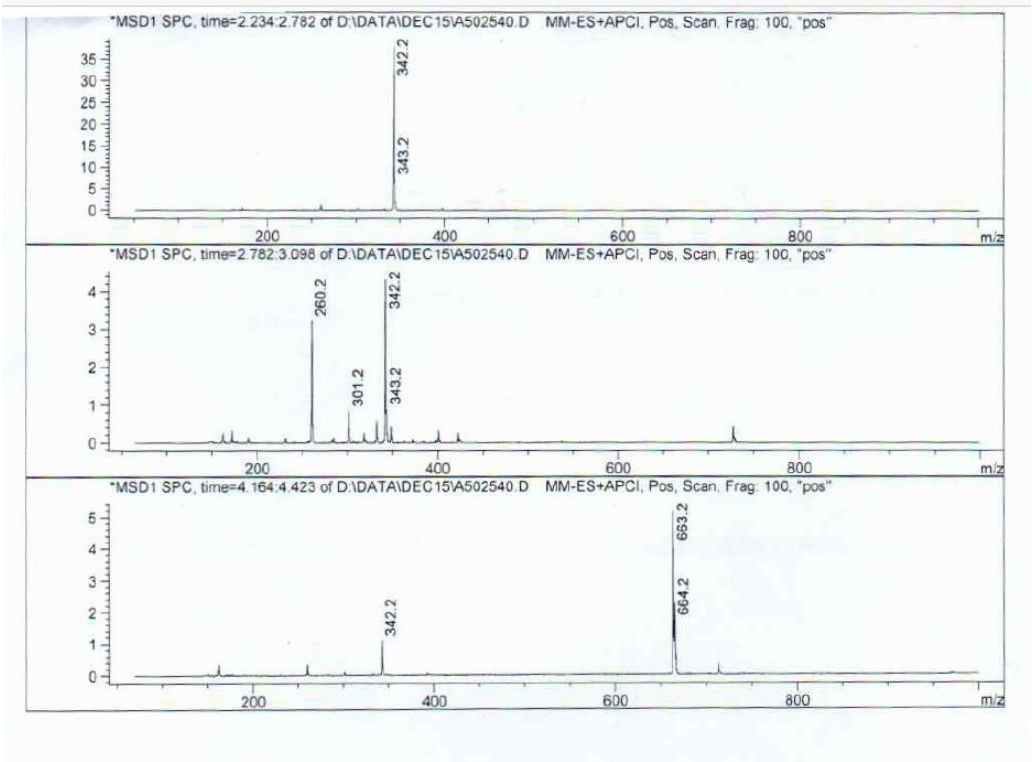


5a

***N*-[2-(*p*-trifluoromethylphenyl)ethyl]-*N'*-(1,3-isothiazolyl)2-isopropylamidine (5a).** Yellow solid; yield 89%; 132-134 °C; R_f 0.43 (4:6 EtOAc/hexane); IR (neat, cm^{-1}) 3649, 2970, 1739, 1566, 1192, 1370, 829; 1H NMR (400 MHz, $CDCl_3$) δ 10.53(s, 1H), 7.54-7.53 (d, J = 5.2 Hz, 2H), 7.46-7.45 (d, J = 5.2 Hz, 2H), 6.78 – 6.77 (d, J = 3.2 Hz, 1H), 3.66 (q, J = 13.2 Hz, J = 6.8 Hz, 2H), 3.01 (t, J = 7.2 Hz, 2H), 2.75 (t, J = 6.4 Hz, 1H), 1.85 (t, J = 1.6 Hz, 1H), 1.19 – 1.12 (m, 6H) ; 13C{1H} NMR (100 MHz, $CDCl_3$) δ 171.0, 151.0, 140.3, 131.7, 130.1, 128.7, 122.3, 121.6, 109.2, 44.3, 35.2, 26.7, 20.1; LCMS (ESI) *m/z*: $[M+H]^+$calcd., for $C_{16}H_{19}F_3N_3S$ 342.1; found 342.2. Elemental analysis calcd., C – 56.29%, H – 5.31, N – 12.31%; found C – 56.30%, H – 5.31%, N – 12.30%.



1H NMR of 5a



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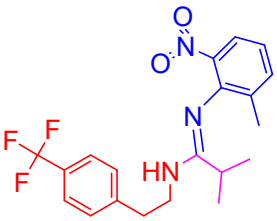
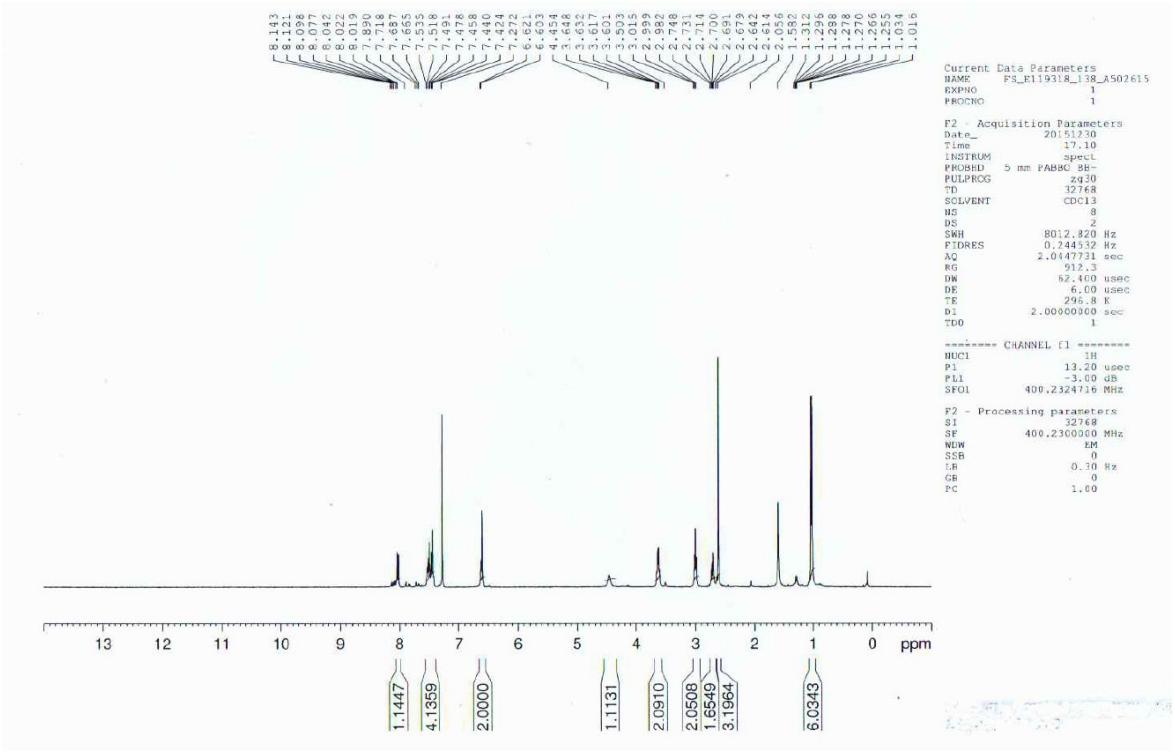


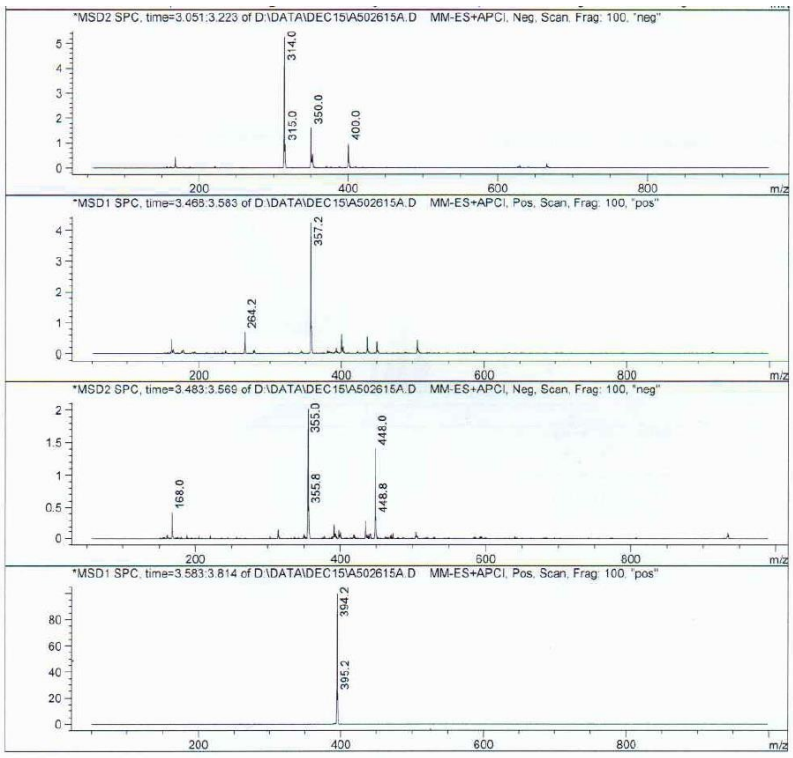
5b

***N*-[2-(*p*-trifluoromethylphenyl)ethyl]-*N'*-(2-methyl-6-nitrophenyl)-2-isopropylamidine (5b).**

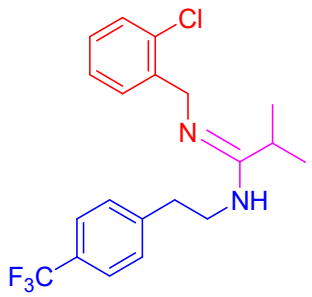
Yellow solid; yield 91%; 151-152 °C; Rf 0.57 (1:1 EtOAc/hexane); IR (neat, cm–1) 3423, 3122, 1574, 1492, 1321, 1012, 842.; 1H NMR (400 MHz, CDCl3) δ 8.14 – 8.01 (m, 1H), 7.54 - 7.42 (m, 4H), 6.61 (d, J = 8 Hz, 2H), 4.45 (s, 1H), 3.65 – 3.60 (m, 2H), 3.02 – 2.98 (m, 2H), 2.79 – 2.61 (m, 4H), 1.02 (s, 6H); 13C{1H} NMR (100 MHz, CDCl3) δ 147.6, 137.5, 134.2, 130.4, 129.7, 127.8, 125.2, 124.3, 122.7, 117.2, 113.1, 40.7, 35.2, 27.4, 20.1, 15.4.; LCMS (ESI) m/z: [M+H]+calcd., for C20H22F3N3O2 394.2; found 394.2. Elemental analysis calcd., C - 61.06%, H – 5.64%, N – 10.68%; found C – 61.07 %, H – 5.63%, N – 10.68%.



1H NMR of 5b



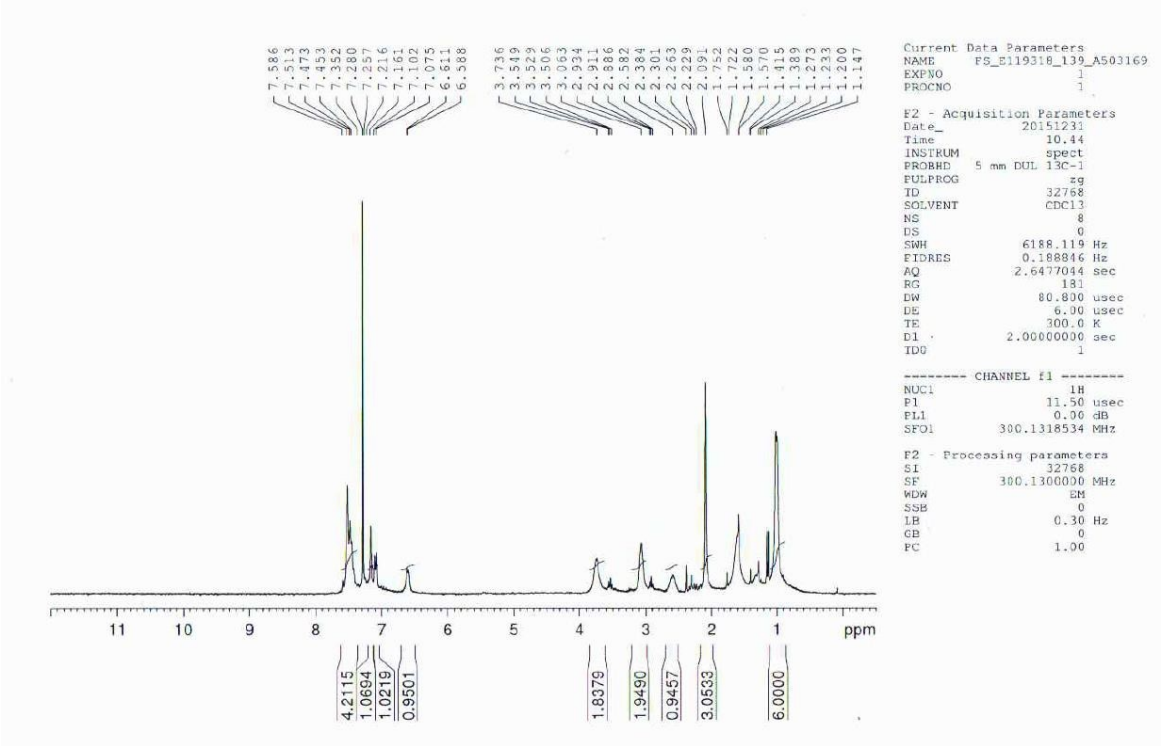
LS-MS of 5b



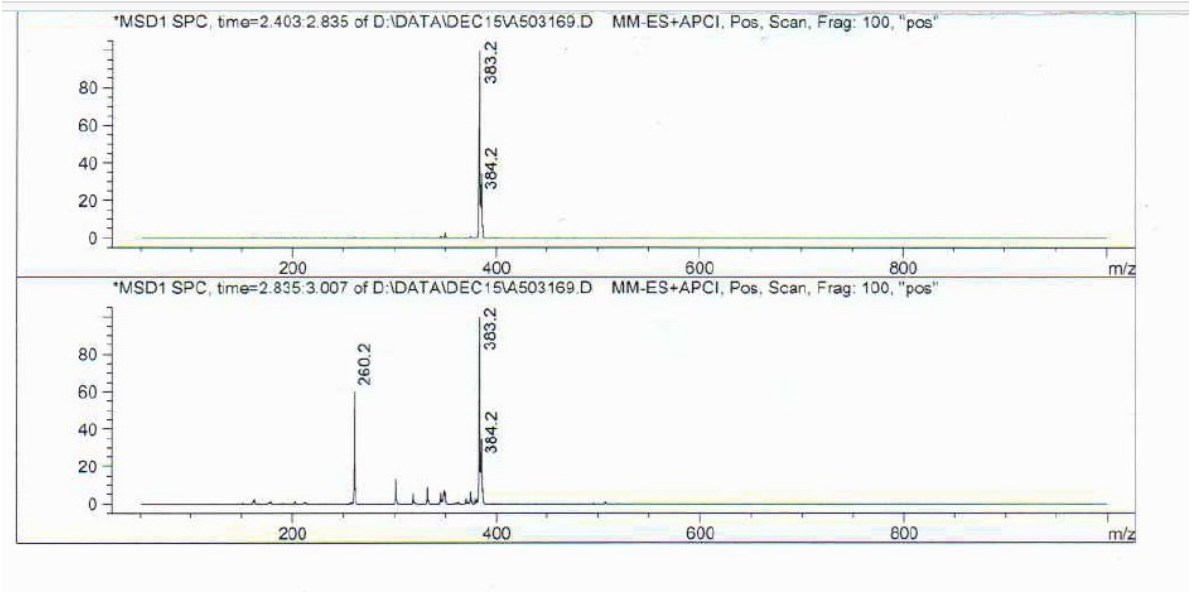
5c

***N*-[2-(*p*-trifluoromethylphenyl)ethyl]-*N′*-(2-chlorobenzyl)-2-isopropylamidine (5c)**

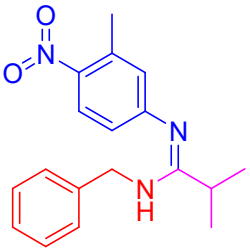
Orange solid; yield 87%; 121-123 °C; R_f 0.23 (2:8 EtOAc/hexane); IR (neat, cm^{-1}) 3416, 3097, 1432, 1221, 984.; 1H NMR (400 MHz, $CDCl_3$) δ 7.59 – 7.45 (m, 4H), 7.16 (s, 1H), 7.09 (d, J = 10.8 Hz, 1H), 6.59 (d, J = 9.2 Hz, 1H), 3.74 (s, 2H), 3.06 (s, 2H), 2.58 (s, 1H), 2.09 (s, 3H), 1.01(s, 6H).; 13C{1H} NMR (100 MHz, $CDCl_3$) δ 148.7, 139.2, 132.1, 130.6, 130.1, 128.7, 128.5, 128.3, 127.9, 126.4, 125.1, 123.5, 43.6, 43.2, 37.2, 32.2, 31.1.; LCMS (ESI) *m/z*: $[M+H]^+$calcd., for $C_{20}H_{22}ClF_3N_2$ 383.9; found 383.2. Elemental analysis calcd., C – 62.74%, H – 5.79%, N –7.32%; found C – 62.73 %, H – 5.79%, N – 7.33%.



1H NMR of 5c

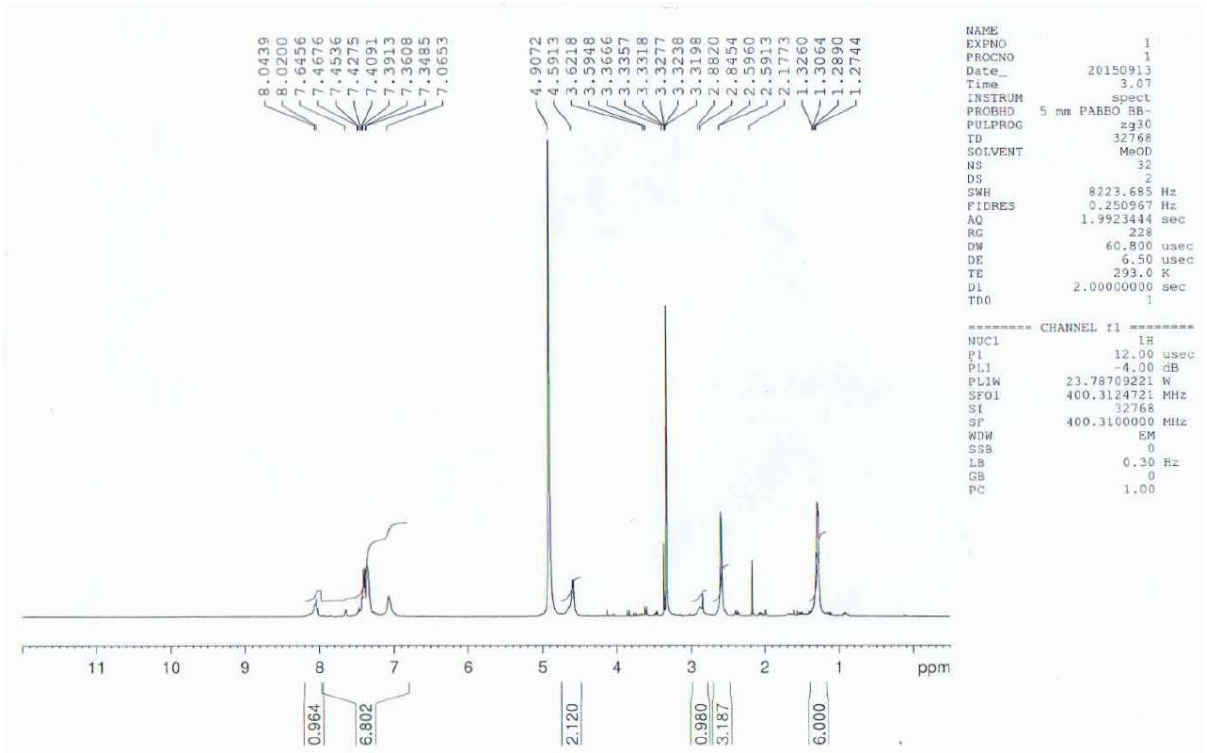


LS-MS of 5c

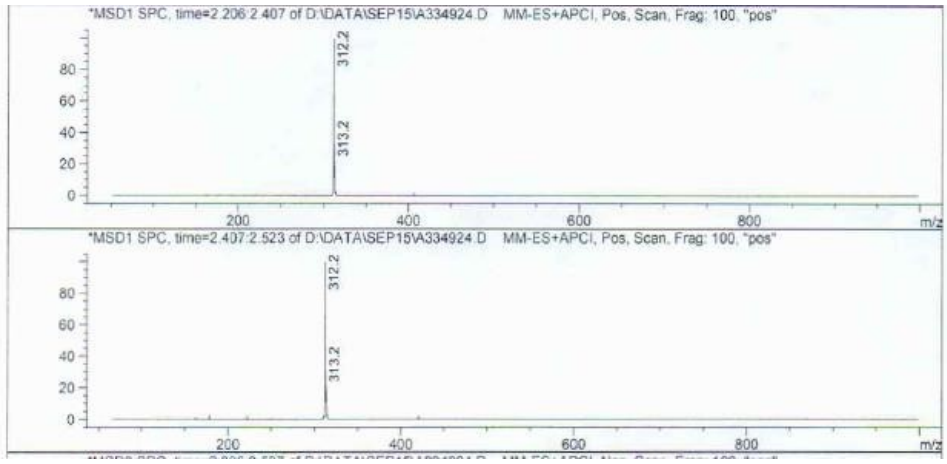


5d

***N*-[benzyl]-*N′*-(3-methyl-4-nitrophenyl)-2-isopropylamidine (5d)**. Orange solid; yield 87%; 132-134 °C; R_f 0.15 (4:6 EtOAc/hexane); IR (neat, cm^{-1}) 3642, 3170, 1712, 1523, 1234, 1112, 875; 1H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, J = 4 Hz, 1H), 7.47 - 7.35 (m, 7H), 4.59 (s, 1H), 2.88 (s, 1H), 2.59 (s, 3H), 1.32 – 1.27 (m, 6H) ; 13C{1H} NMR (100 MHz, $CDCl_3$) δ 149.4, 142.0, 138.2, 137.6, 128.4, 127.6, 127.2, 124.3, 122.1, 119.7, 116.3, 52.4, 33.2, 27.1, 20.1.; LCMS (ESI) *m/z*: $[M+H]^+$calcd., for $C_{18}H_{22}N_3O_2$ 312.4; found 312.2. Elemental analysis calcd., C – 69.43%, H – 6.80%, N – 13.49%; found C – 69.42 %, H – 6.81%, N – 13.48%.



1H NMR of 5d



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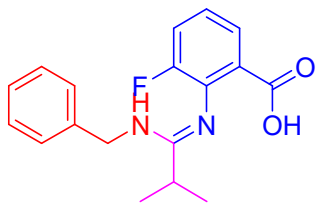
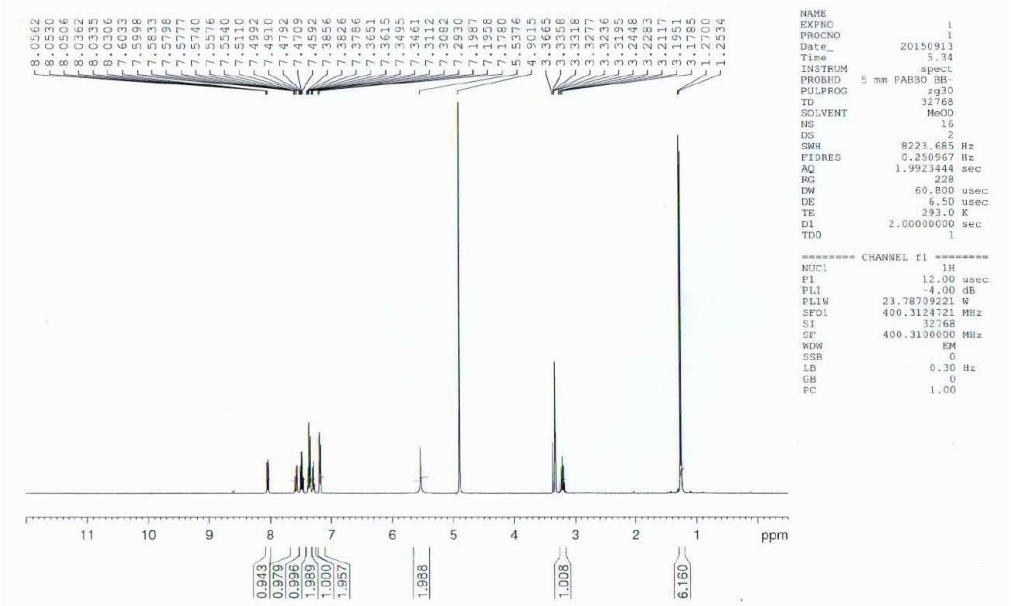


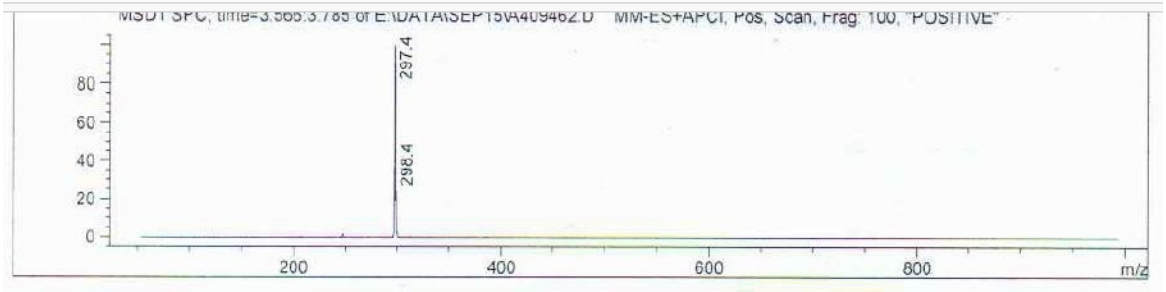
5e

***N*-(benzyl)-*N′*-(2-carboxy-6-fluoro)-2-isopropylamidine (5e)**. Pale orange solid; yield 85%; 147-149 °C; R_f 0.12 (1:1 EtOAc/hexane); IR (neat, cm^{-1}) 3529, 3310, 2812, 1757, 1573, 1187, 1215, 779; 1H NMR (400 MHz, $CDCl_3$) δ 8.05 – 8.03 (3, 1H), 7.60

- 7.18 (m, 7H), 5.54 (s, 2H),3.25 – 3.18 (m, 1H), 1.26 (, 6H); 13C{1H} NMR (100 MHz, $CDCl_3$) δ 167.4, 157.2, 152.8, 142.4, 139.2, 134.2, 131.2, 128.4, 127.8, 127.2, 127.1, 125.6, 112.7, 52.7, 33.1, 30.1; LCMS (ESI) *m/z*: $[M+H]^+$calcd., for $C_{18}H_{19}N_2O_2$ 297.3; found 297.0. Elemental analysis calcd., C – 68.77%, H – 6.09%, N – 8.91%; found C – 68.76 %, H – 6.10%, N – 8.90%.



1H NMR of 5e



LS-MS of 5e

References

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