Synthesis, biological assessment, and docking study of new pyrazolo[1,5-*a*]pyrimidine derivatives with potential anticancer activity

1. Experimental

1.1. Chemistry

All of the chemicals and solvents were commercially available, supplied from Aldrich (Germany), Merk (Germany), Loba Chemie (India) and used without any further purification. Melting points were measured in open capillary tubes using Griffin apparatus and were uncorrected apparatus. Elemental Microanalysis was carried out at the Regional Center for Mycology and Biotechnology, Al-Azhar University. The monitoring of the progress of all reactions and homogeneity of the synthesized compounds was carried out by thin layer chromatography (TLC) using Silica gel-precoated aluminum sheets (type 60, F 254 0.2mm thickness, Merck, Darmstadt, Germany). Visualization of compound spots on TLC and follow up were made using UV lamp at λ254 nanometer. Infrared IR spectra were recorded using potassium bromide disc technique on a Schimadzu 435 IR spectrophotometer at October 6 University. Mass Spectra were recorded using Shimadzu Gas Chromatograph Mass spectrometer-Qp 2010 plus (Japan) at the Regional Center for Mycology and Biotechnology, Al-Azhar University. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian-Mercury 400 (^{1}H, 400 MHz; ^{13}C, 400 MHz) spectrometer at the DNA Research Center, Faculty of Science, Zagazig University and Bruker high-performance digital FT-NMR spectrometer AVANCE III 400 MHz (Bruker Corporation, Germany) at the Microanalytical Unit, Faculty of Pharmacy, Cairo University using DMSO-*d*6 as a solvent. δ Parts per million (ppm) are the chemical shifts quotations downfielding from tetramethylsilane (TMS) as an internal standard. All chemical shifts were reported in ppm, the coupling constants (*J*) were expressed in hertz (Hz) and signals (multiplicities) were described as singlet (s), doublet (d), triplet (t), quartet (q) as well as multiplet (m).

1.2. Biology

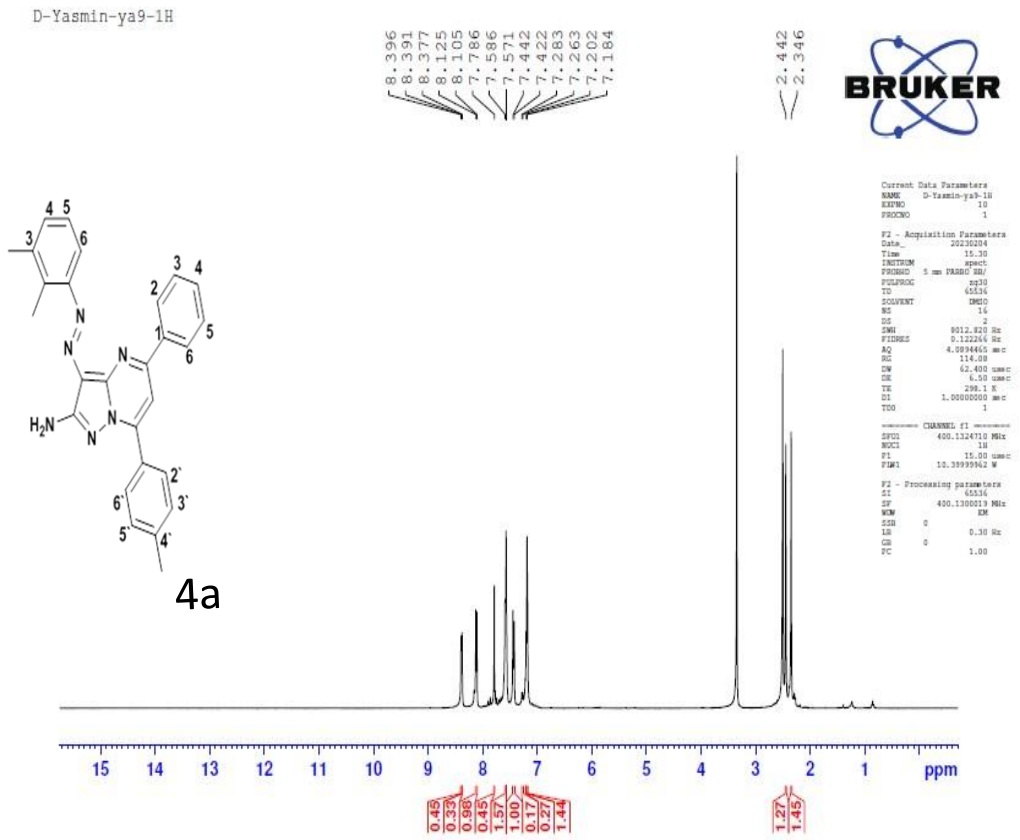
MCF-7 (breast), HepG2 (liver) and A549 (lung) cancer cell lines were grown as monolayer and routinely maintained in RPMI-1640 medium supplemented with 5% heat inactivated FBS, 2 mM glutamine and antibiotics (penicillin 100 U/ml, streptomycin 100 mg/ml) at 37° C in a humidified atmosphere containing 5% CO_2. Exponentially growing cells were obtained by plating 1.5 x105 cells/ml, followed by 24 h of incubation. The effect of the vehicle solvent DMSO on the growth of these cell lines was evaluated in all the experiments by exposing untreated control cells to the maximum concentration (0.5%) of DMSO used in each assay. The activity of the compounds was evaluated using sulforhodamine B dye where cells were treated with five different serial dilutions (up to 150 mM) of the compounds for 48 h. Doxorubicin was used as a positive control and tested in the same manner. The cytotoxicity of the compounds were then determined using the SRB method as previously described by Skehan *et al* [1]. The IC_{50} values were calculated according to the equation for Boltzman sigmoidal concentration response curve using the nonlinear regression fitting models (Graph Pad, Prism Version 5).

1.3. Docking

Molecular Operating Environment (MOE) software model 2019.01 version was used for performing the molecular docking studies. The X-ray crystal structure of VEGFR-2 in a complex with sorafenib was downloaded from https://www.rcsb.org/ (PDB ID: 4ASD). The protein-ligand complex bought from the protein data bank was organized for docking: (1) chain B of the protein along with co-crystallized water molecules were deleted; (2) the enzyme was 3D protonated and (3) sorafenib was redocked into the active site for validation of the docking procedure. Scoring was performed using the Alpha triangle placement technique and London dG. Thirty of the most stable docking poses for ligand were retained with the best scoring conformation was done. The validation results confirmed a near ideal alignment with the original ligand and displayed the same binding interactions as obtained from the X-ray crystallography pdb document. Moreover **4d** compound was drawn on the MOE. A Hamiltonian-Force Field-MMFF94x was used to reduce structure energy and it was docked into the active site of enzyme. The most stable ten conformers for compound have been retained.

REFERENCES

1. Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, et al. New colorimetric cytotoxicity assay for anticancer-drug screening. J. Natl. Cancer Inst. 1990; 82: 1107-1112.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7.283
7.263
7.202
7.184
2.442
2.346
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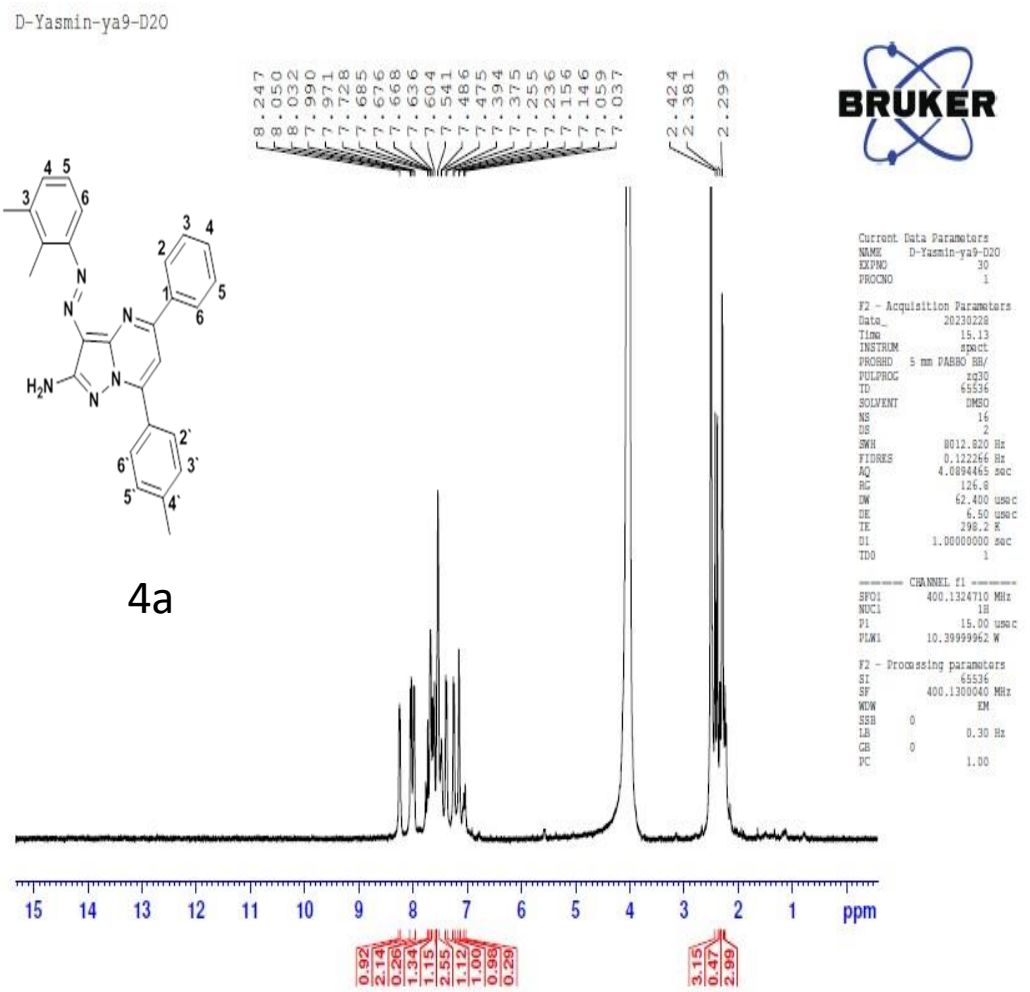
1.27 1.45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7.636
7.604
7.541
7.486
7.475
7.394
7.375
7.255
7.236
7.156
7.146
7.059
7.037
2.424
2.381
2.299
BRUKER
4a
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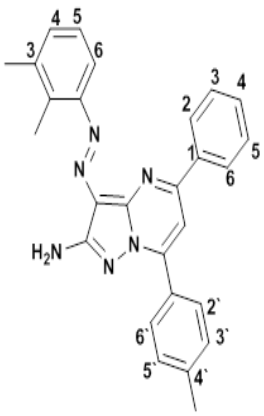
3.15 0.47 2.99



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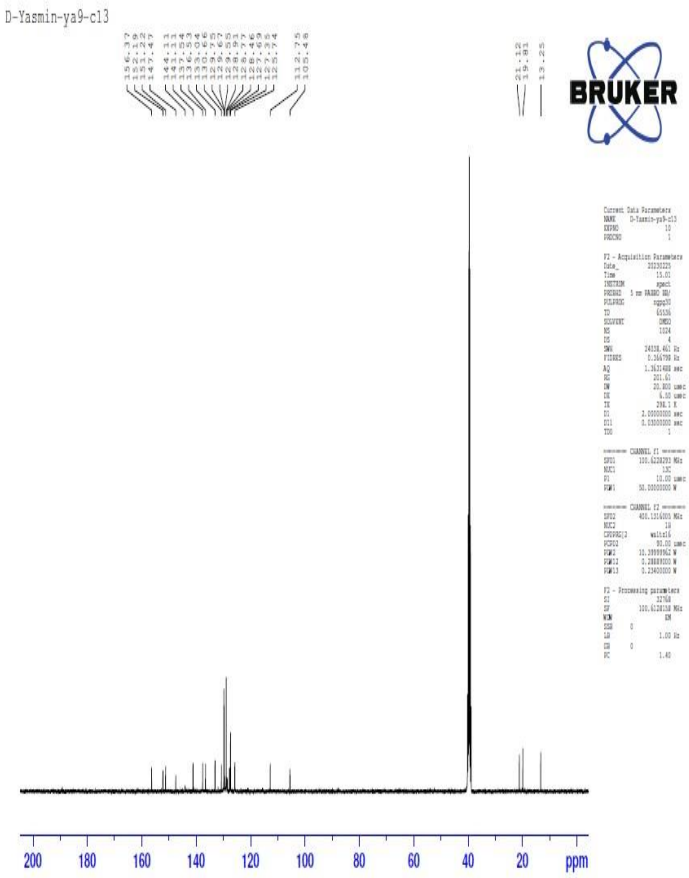
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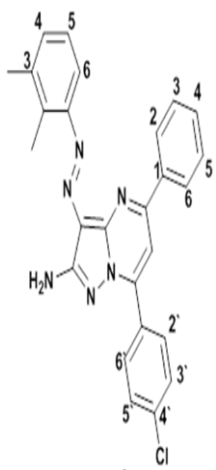
140
120
100
80
60
40
20
ppm



4b

D-Yasmin-ya1-1H
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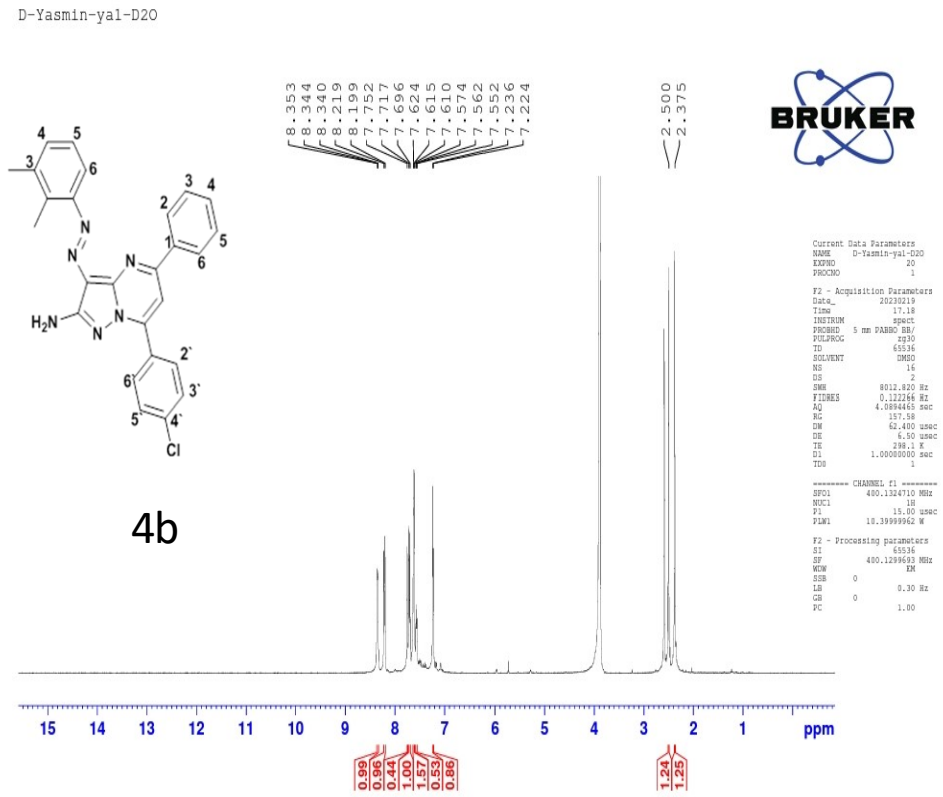
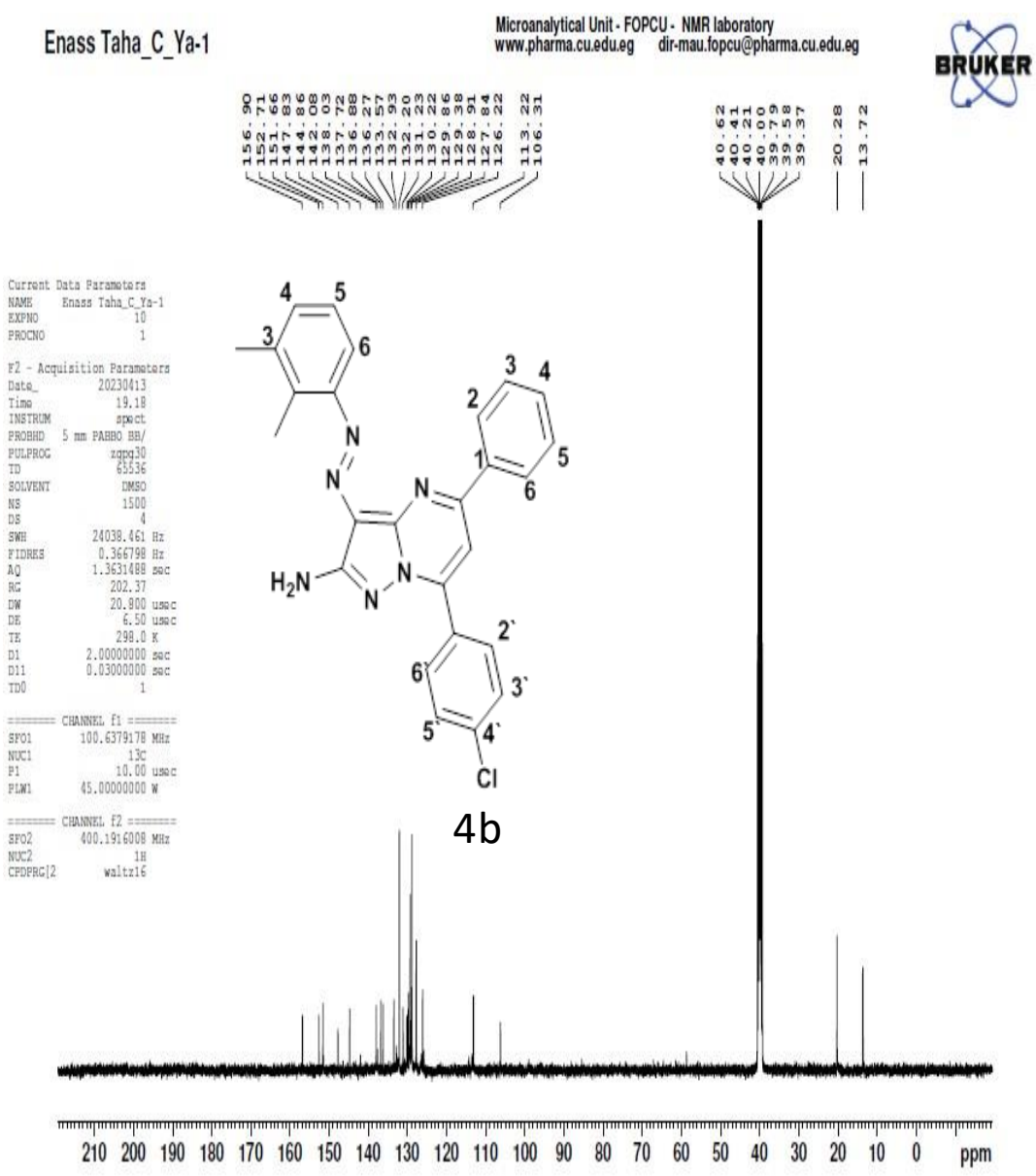
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm





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SOLVENT DMSO
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FIDRES 0.122266 Hz
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RG 157.58
DW 62.400 usec
DE 6.50 usec
TE 298.1 K
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PROCNO 1

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PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 1500
DS 4
SWH 24038.461 Hz
FIDRES 0.366798 Hz
AQ 1.3631488 sec
RG 202.37
DW 20.800 usec
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TE 298.0 K
D1 2.00000000 sec
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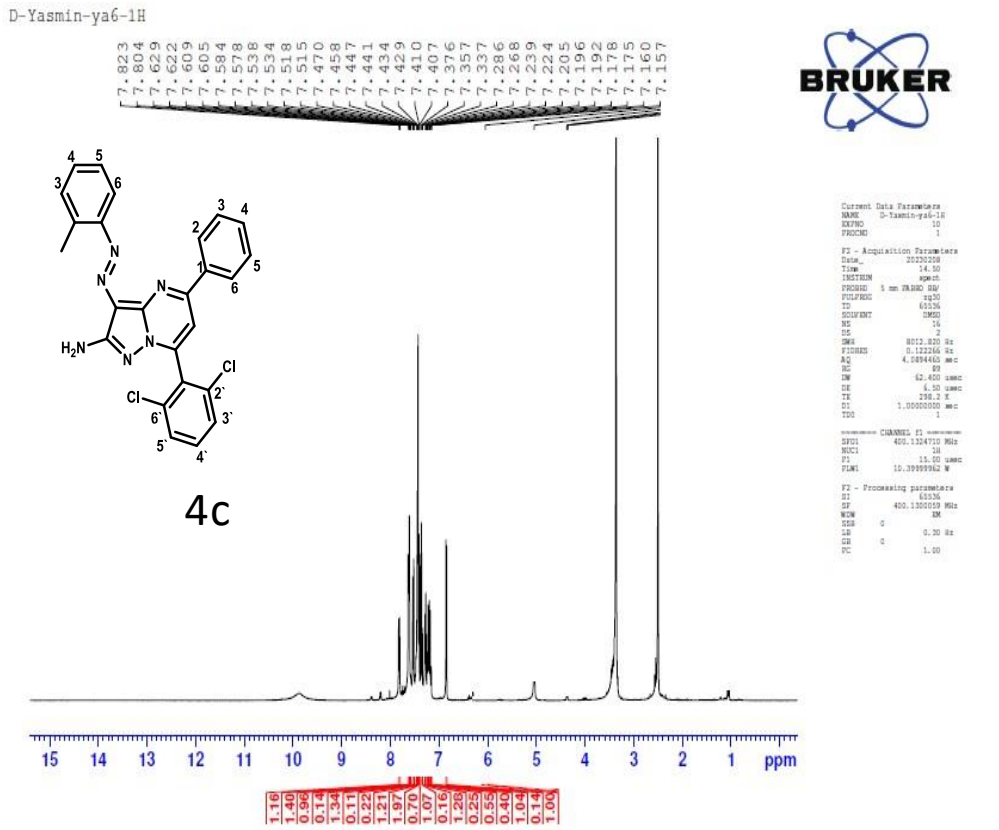
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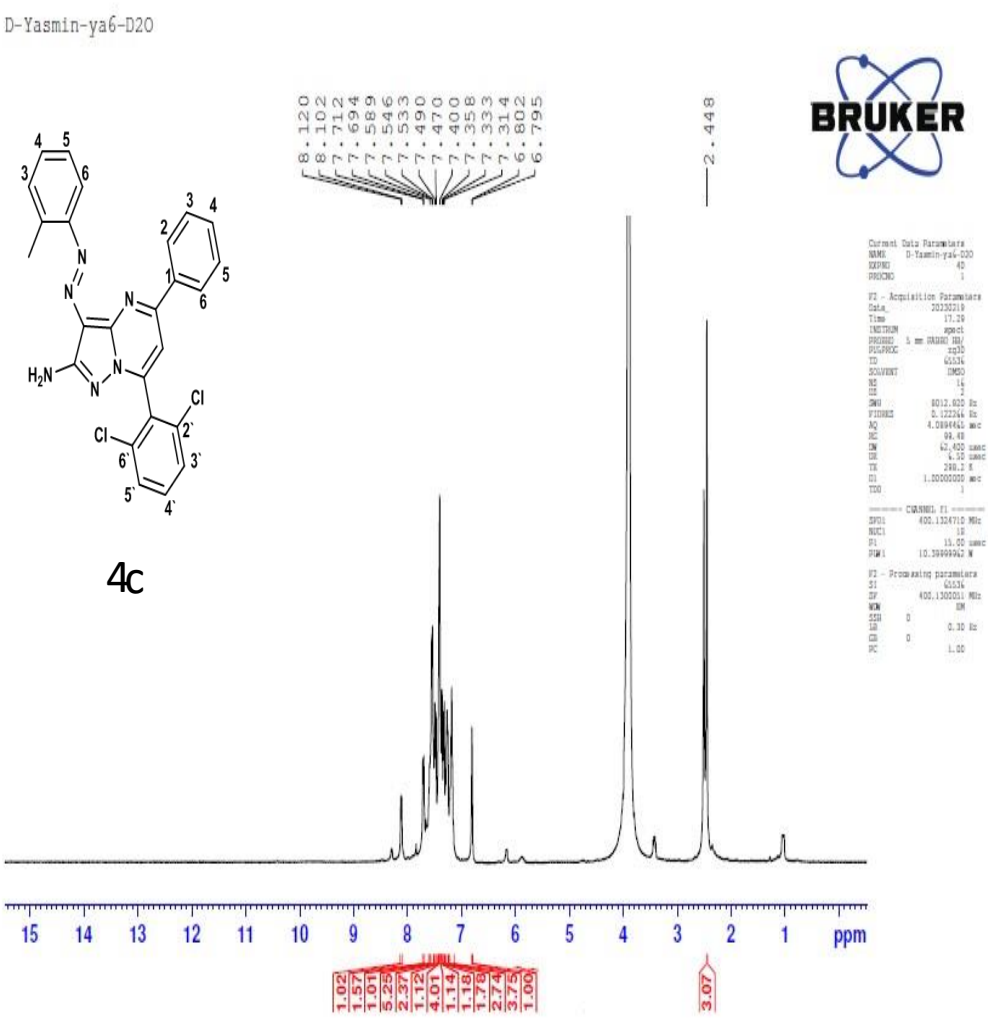
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4c
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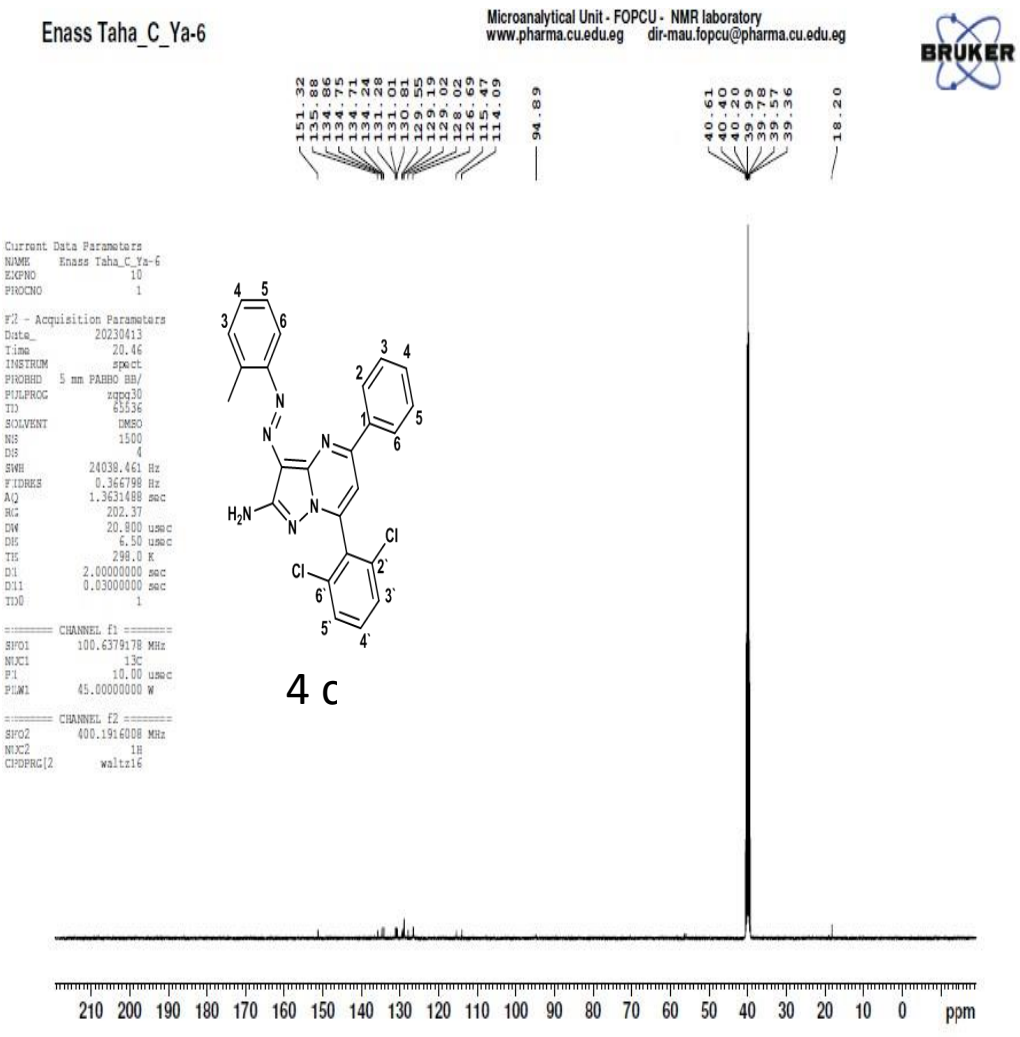
Enass Taha_C_Ya-6
Microanalytical Unit - FOPCU - NMR laboratory
www.pharma.cu.edu.eg dir-mau.fopcu@pharma.cu.edu.eg
BRUKER
151.32 135.88 134.76 134.71 134.24 131.28 131.01 130.81 129.55 129.19 129.02 128.02 126.69 115.47 114.09 94.89
40.61 40.40 40.20 39.99 39.78 39.57 39.36 18.20
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PROCNO 1
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PULPROG zgpg30
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SOLVENT DMSO
NS 1500
DS 4
SWH 24038.461 Hz
FIDRES 0.366798 Hz
AQ 1.3631488 sec
RG 202.37
DW 20.800 usec
DE 6.50 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 100.6379178 MHz
NUC1 13C
P1 10.00 usec
PLW1 45.00000000 W
======== CHANNEL f2 ========
SFO2 400.1916008 MHz
NUC2 1H
CPDPRG[2 waltz16
4 c
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm
D-Yasmin-ya7-1H
7.794 7.791 7.774 7.764 7.745 7.654 7.651 7.634 7.590 7.585 7.547 7.544 7.528 7.524 7.510 7.494 7.474 7.469 7.452 7.437 7.434 7.418 7.415 7.386 7.366 7.346 7.302 7.285 7.267 7.263 7.175 7.155 3.907
BRUKER
4 d
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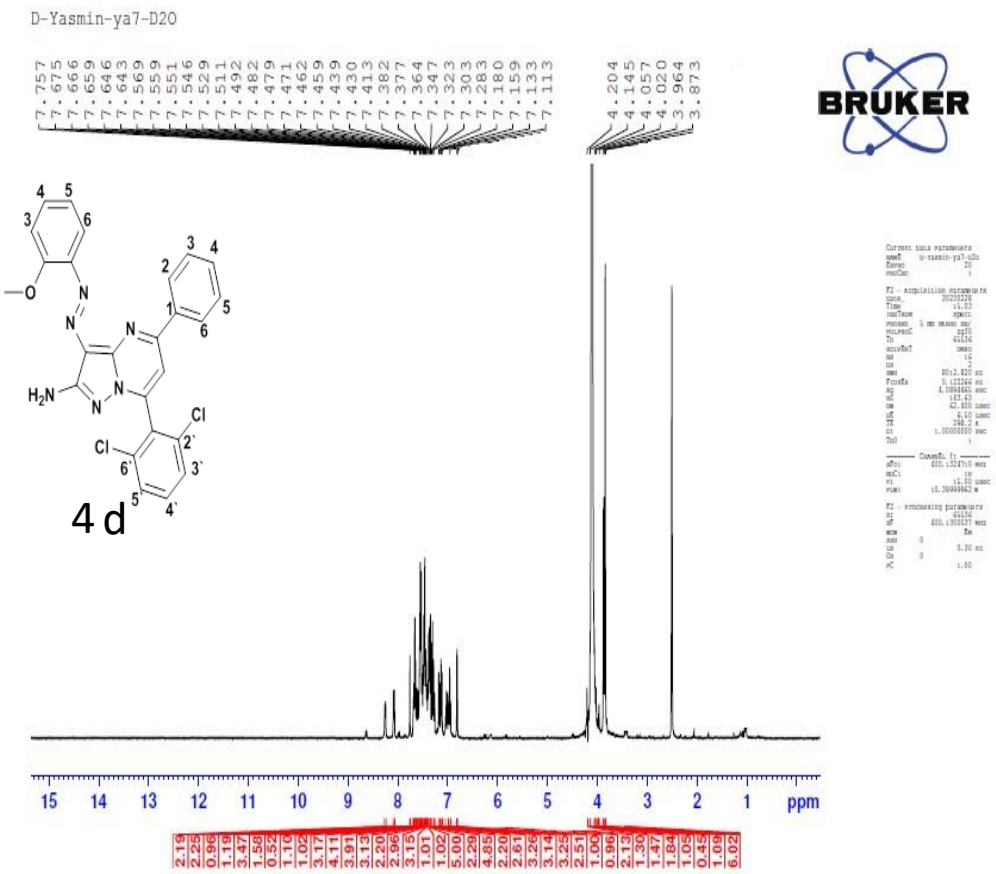
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7.666
7.659
7.646
7.643
7.569
7.559
7.551
7.546
7.529
7.511
7.492
7.482
7.479
7.471
7.462
7.459
7.439
7.430
7.413
7.382
7.377
7.364
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7.303
7.283
7.180
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7.133
7.113
4.204
4.145
4.057
4.020
3.964
3.873
BRUKER
4 d
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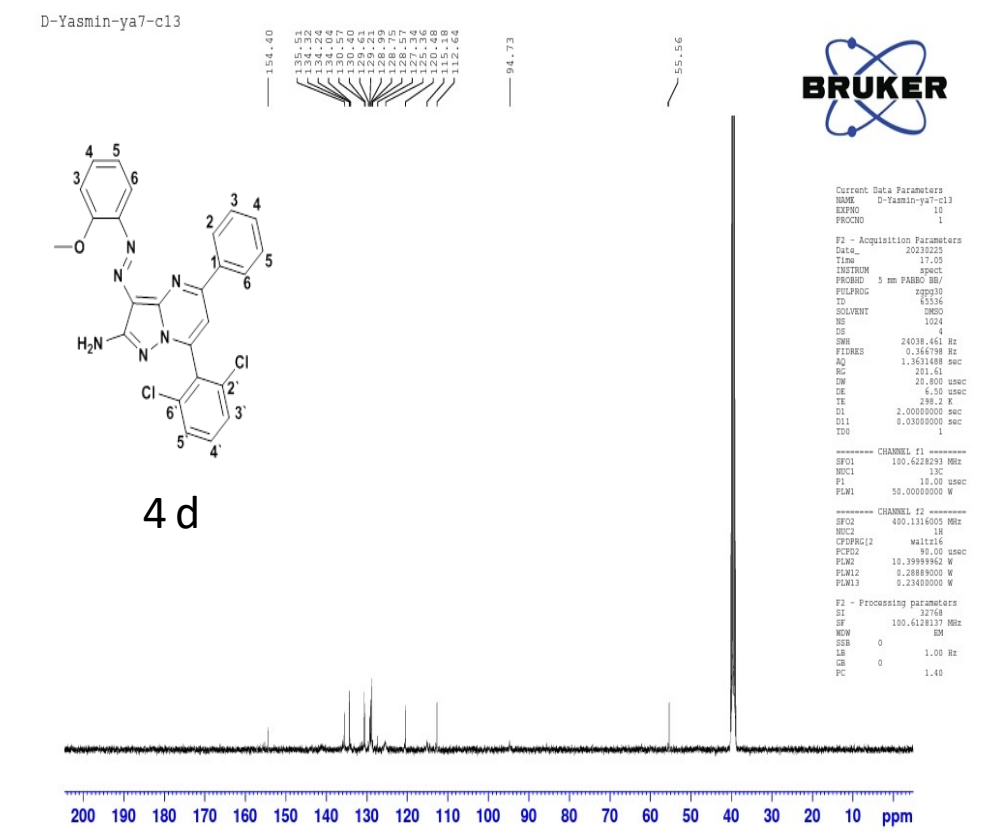
2.19 2.25 0.96 1.19 3.47 1.58 0.52 1.10 1.02 3.17 4.11 3.91 3.13 2.20 2.96 3.15 1.01 1.02 5.00 2.29 4.85 2.20 2.61 3.26 3.14 3.25 2.51 1.00 0.96 2.13 1.30 1.47 1.84 1.05 0.45 1.09 6.02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134.24
134.07
130.40
129.61
128.93
128.75
128.57
125.34
120.48
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112.64
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55.56
BRUKER
4 d
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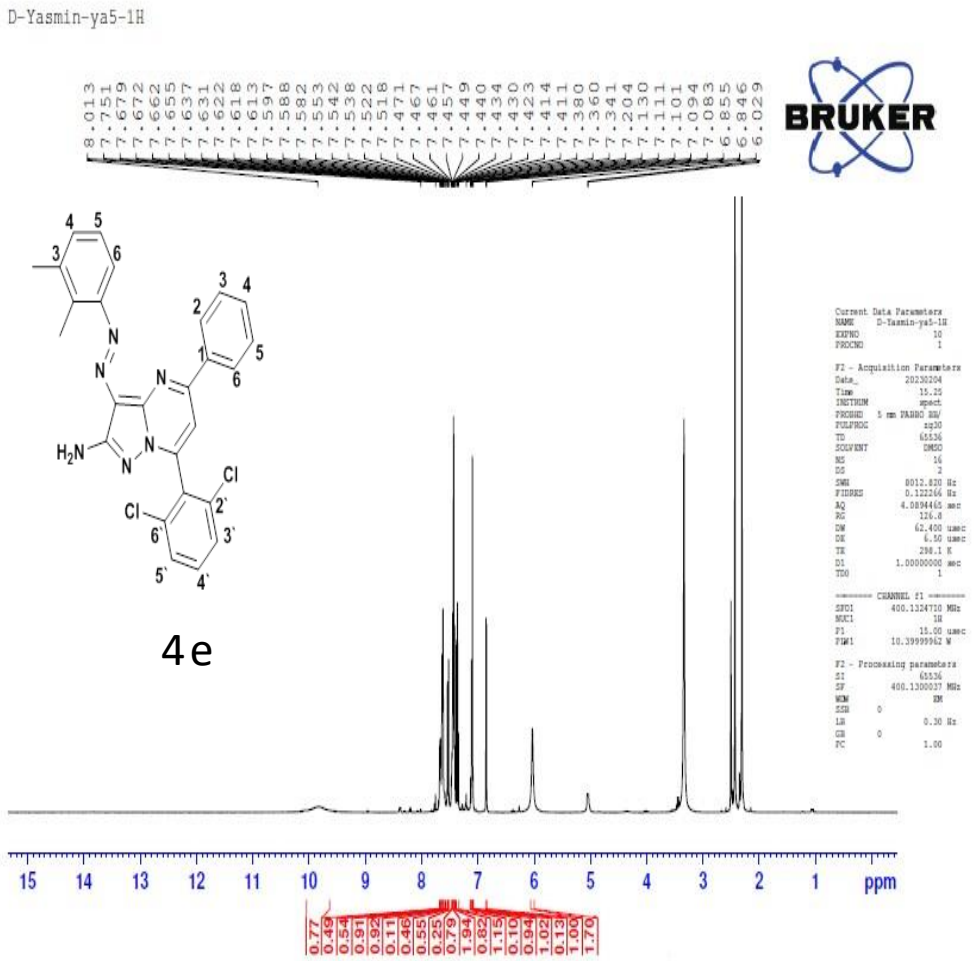
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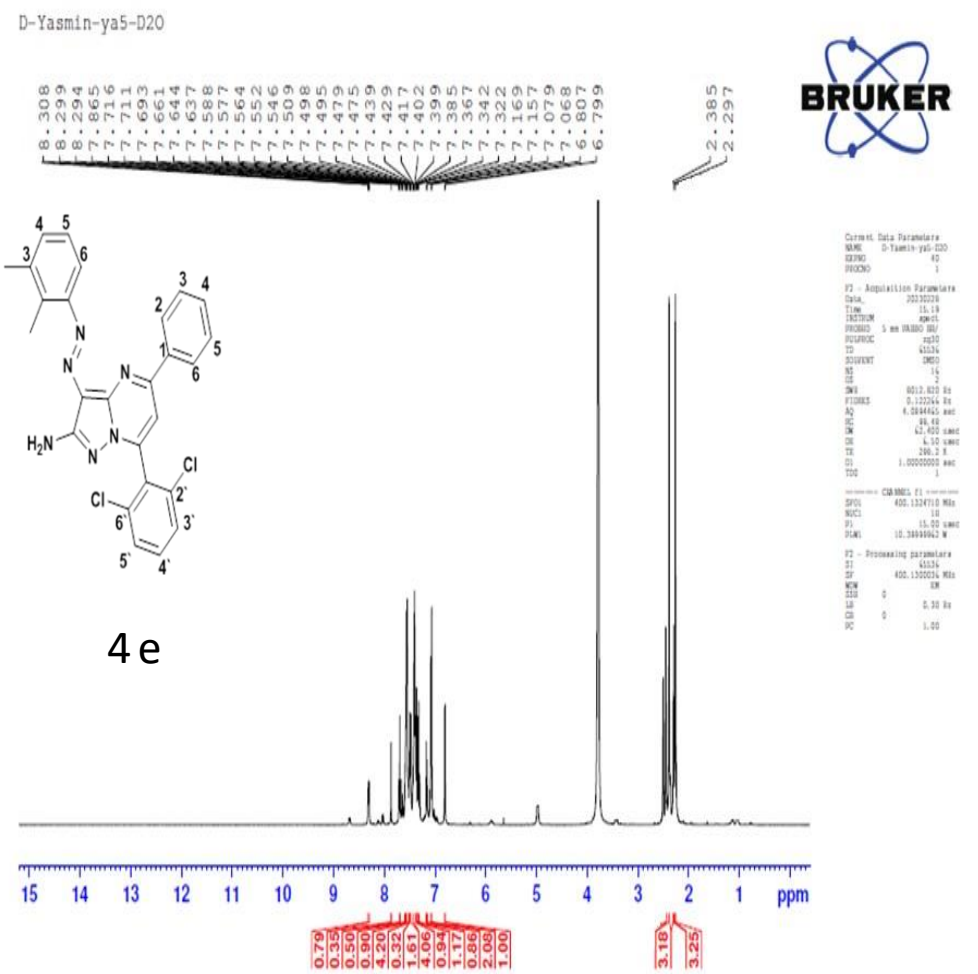
BRUKER
4e
ppm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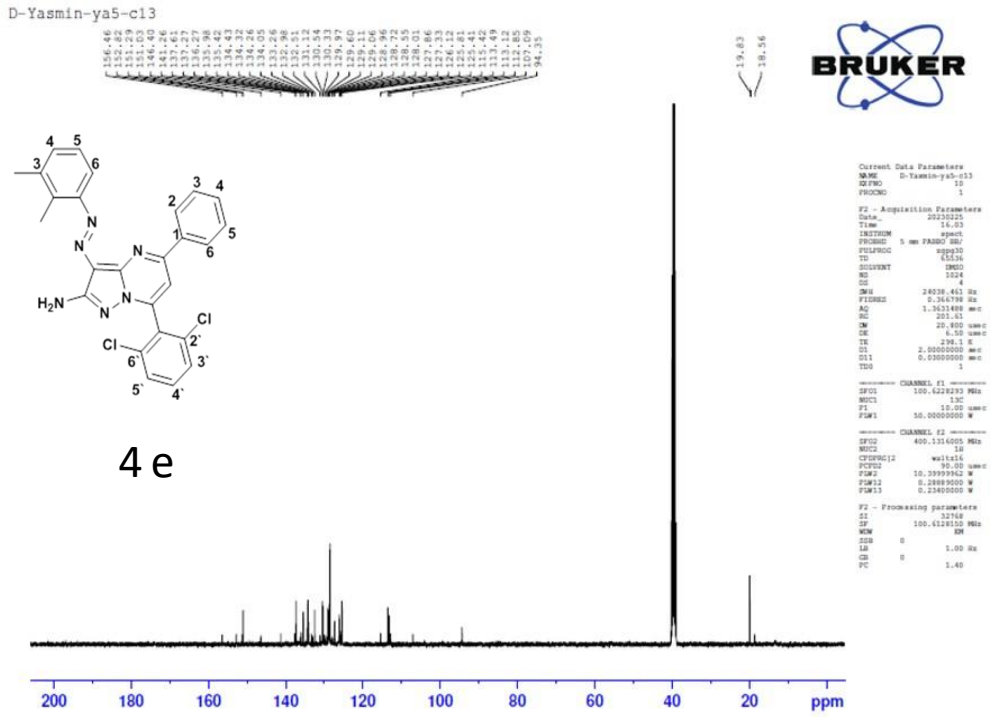
BRUKER
4e
ppm


D-Yasmin-ya5-c13
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4 e
200
180
160
140
120
100
80
60
40
20
ppm