Synthesis of Azole-Linked s-Triazine-Isatin Molecular Architectures as Nanoscale DNA-Targeting Agents: A Combined Spectroscopic and Computational Study

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1. Substrates and Reagents

All required reagents were supplied by Sigma Aldrich, and Alfa Aesar, and were used without further purification unless otherwise specified. The reactions were performed using standard laboratory equipment. All air-sensitive reactions were carried out under a nitrogen atmosphere using oven-dried glassware. Low temperatures were achieved using an ice/water bath (0 ºC). All dried solvents were stored over molecular sieves (4A°).

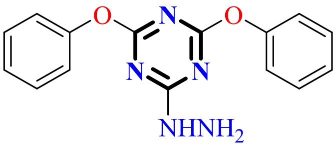
2. Instrumentation

All reactions were stirred using a stirrer plate, a magnetic stirrer bar and heating, if necessary, over a hotplate with a temperature probe control method. Büchi rotavapor was used for evaporation at lower pressures. To monitor the progress of reaction, Thin Layer Chromatography (TLC) was performed by using pre-coated silica plates (Kieselgel-60 F_{254}, Merck, Germany). Melting points were determined in open capillaries using StuartTM (SMP3) melting point apparatus and were uncorrected. FTIR spectra were recorded on Thermo Scientific Nicolet iS10 and Shimadzu IR Affinity-1 Spectrometer to confirm the presence of different functional groups in the target molecules in the range of 4000-400 cm^{-1}. ^1H NMR and ^{13}C NMR spectra were recorded on Bruker DPX 300 MHz apparatus were referenced to the residual proton solvent peak $CDCl_3$, δ 7.26 ppm (1H: H_2O, δ 1.56 ppm); DMSO-d_6 *,* δ 2.54 ppm (1H: H_2O, δ 3.33 ppm); $CO(CD_3)_2$-d_6, δ 2.09 ppm (1H: H_2O, δ 2.84 ppm) and solvent ^{13}C signal $CDCl_3$, δ 77.2 ppm, DMSO-d_6 , δ 39.5 ppm, $CO(CD_3)_2$-d_6 CO, 205.87 ppm. Chemical shifts δ were reported in ppm downfield of tetramethylsilane (δ = 0 ppm) and coupling constants (*J*) in Hertz.

3. Synthesis of 2-hydrazinyl-4,6-diphenoxy-1,3,5-triazine derivatives (3)

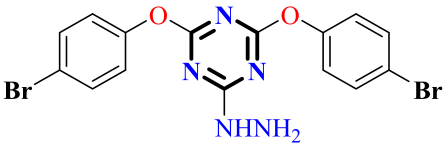
The synthesis of 2-hydrazinyl-4,6-diphenoxy-1,3,5-triazine derivatives **3** was carried out according to a previously reported procedure.[74] For the preparation of the intermediate 2-chloro-4,6-diphenoxy-1,3,5-triazine derivatives **2**, a solution of trichlorotriazine **1** (5.42 mmol, 1 eq.) in dry tetrahydrofuran (THF) was prepared. Substituted phenols (10.85 mmol, 2 eq.) and anhydrous potassium carbonate (16.27 mmol, 3 eq.) were added as nucleophiles and base, respectively. The reaction mixture was stirred at room temperature for 72 hours, and the progress was monitored via thin-layer chromatography (TLC). Upon completion, the reaction mixture was poured into ice-cold water, resulting in the formation of white precipitates **2**, which were subsequently filtered, washed with water, and dried under vacuum. The resulting 2-chloro-4,6-diphenoxy-1,3,5-triazines **2** were then refluxed with hydrazine monohydrate in chloroform to afford the target compounds, 2-hydrazinyl-4,6-diphenoxy-1,3,5-triazine derivatives **3** in good yield.

2-Hydrazinyl-4,6-diphenoxy-1,3,5-triazine (3a)



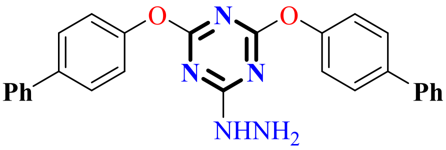
White solid: Yield: 65 %; Melting Point: 106-108 °C; R*f*: 0.5(n-hexane: ethyl acetate, 1:1).

2 2,4-Bis(4-bromophenoxy)-6-hydrazinyl-1,3,5-triazine (3b)



White solid: Yield: 65 %; Melting Point: 183-185 °C; R*f*: 0.45 (n-hexane: ethyl acetate, 3: 7).

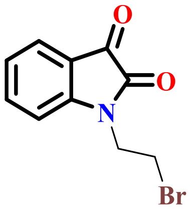
2,4-Bis(4-phenylphenoxy)-6-hydrazinyl-1,3,5-triazine (3c)



White solid: Yield: 58%; MeltingPoint:172-173 °C; R*f*: 0.49 (*n*-hexane: ethyl acetate, 3: 7).

4. Synthesis of 1-(2-Bromoethyl) Isatin (5)

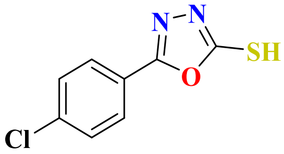
The synthesis of 1-(2-bromoethyl)isatin **5** was carried out following a previously reported method with slight modifications.[75] A mixture of isatin **4** and anhydrous potassium carbonate was stirred in dimethylformamide (DMF), and this suspension was gradually added dropwise to a solution of 1,2-dibromoethane (4eq) in DMF under continuous stirring. The reaction mixture was stirred at room temperature for 12 hours. Upon completion, as monitored by thin-layer chromatography (TLC), the mixture was poured into ice-cold water, resulting in the formation of an orange-red solid. The product was isolated by filtration, washed with water, and dried. The identity and purity of the product were confirmed by comparison of its physical data with those reported in the literature.



Orange red crystals; Yield: 85%; Melting Point: 126-128 (Lit. 126-128)[75] °C; R_f: 0.56 (*n*-hexane: ethyl acetate, 7:3).

5. Synthesis of 5-(4-chlorophenyl)-1,3,4-oxadiazole-2-thiol (6c)

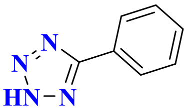
The intermediate 5-(4-chlorophenyl)-1,3,4-oxadiazole-2-thiol **6c** was synthesized following a previously reported protocol.[76] The 4-chlorobenzoic acid was esterified by dissolving in ethanol, followed by the addition of a few drops of concentrated sulfuric acid as a catalyst. The reaction mixture was refluxed for 4 hrs, and the resulting ester was extracted using ethyl acetate. Subsequently, ethyl 4-chlorobenzoate (7.21 mmol) was dissolved in ethanol (20 mL) and added dropwise to hydrazine hydrate (36.06 mmol) in a 100 mL round-bottom flask maintained at 78 °C. The reaction progress was monitored by thin-layer chromatography (TLC). After 12 hours of reflux and subsequent cooling, white precipitates of the corresponding acid hydrazide were formed, filtered, and washed thoroughly with water. The obtained acid hydrazide (3.80 mmol) was then stirred in ethanol in the presence of potassium hydroxide (5.74 mmol) until a clear solution was obtained. Carbon disulfide (7.60 mmol) was added dropwise under constant stirring, and the reaction mixture was refluxed for 15 hours. After completion, the reaction mixture was concentrated, cooled, and acidified using dilute hydrochloric acid. The resulting solid was filtered, washed with water, and recrystallized from ethanol to afford the pure desired product **6c**.



Yellow solid; Yield: 80%; Melting Point: 172-175 (Lit. 171-175)[77] °C; R_f: 0.46 (*n*-hexane: ethyl acetate, 7:3).

6. Synthesis of 5-Phenyl-1*H*-tetrazole (6d)

The intermediate 5-phenyl-1*H*-tetrazole **6d** was synthesized following a previously reported protocol.[78] A mixture of benzonitrile (10 mmol), sodium azide (10 mmol), and ammonium chloride (10 mmol) was dissolved in dimethylformamide (DMF) and heated at 125 °C in an oil bath for 7 hours. The reaction progress was monitored periodically using thin-layer chromatography (TLC). Upon completion, the reaction mixture was cooled to room temperature and carefully acidified to pH 2 using concentrated hydrochloric acid, followed by further cooling in an ice-water bath. The resulting white precipitate was collected by filtration, washed thoroughly with water, and dried to afford the pure product.

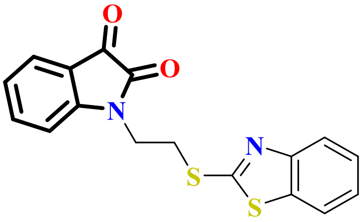


White solid, Yield: 81 % Melting Point: 215-220 (Lit. 215-216)[78] °C; R_f^*: 0.3 (chloroform: methanol, 8:2).

7. Synthesis of Azole-pendant Isatins (7a-d)

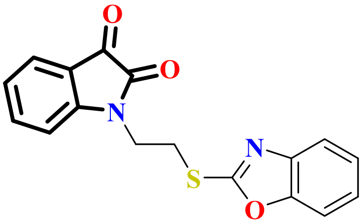
For the synthesis of azole-pendant isatins **7a-d**, a solution of the respective azole derivatives **6a-d** (2 mmol) and potassium carbonate (6 mmol, 3 eq.) in dimethylformamide (DMF, 4 mL) was prepared. To this solution, 1-(2-bromoethyl)isatin **5** (2 mmol) was added, and the reaction mixture was stirred at 70-80 °C in an oil bath for 24 hours. The progress of the reaction was monitored using thin-layer chromatography (TLC). Upon completion, the reaction mixture was poured into ice-cold water, resulting in the precipitation of an orange-red solid. The precipitate was collected by filtration, washed with water, and dried. The crude product was recrystallized from ethanol to afford the pure azole-pendant isatin **7a-d**.

1-(2-(Benzo[d]thiazol-2-ylthio)ethyl)indoline-2,3-dione (7a)



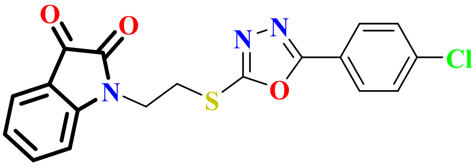
Bright orange; Yield: 80 %; melting point: 152-154 °C; R_f: 0.42 (ethyl acetate: *n*-hexane, 1:1)

1-(2-(Benzo[d]oxazol-2-ylthio)ethyl)indoline-2,3-dione (7b)



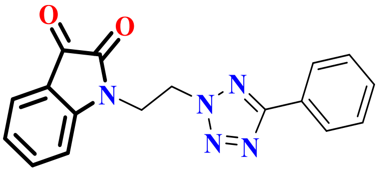
Bright orange; Yield: 75%; Melting Point: 156-157 °C; R_f: 0.46 (ethyl acetate: *n*-hexane, 1:1)

1-(2-((5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)ethyl)indoline-2,3-dione (7c)



Bright orange; Yield: 78 %; Melting Point: 150-155 °C; R_f: 0.40 (ethyl acetate: *n*-hexane, 1:1)

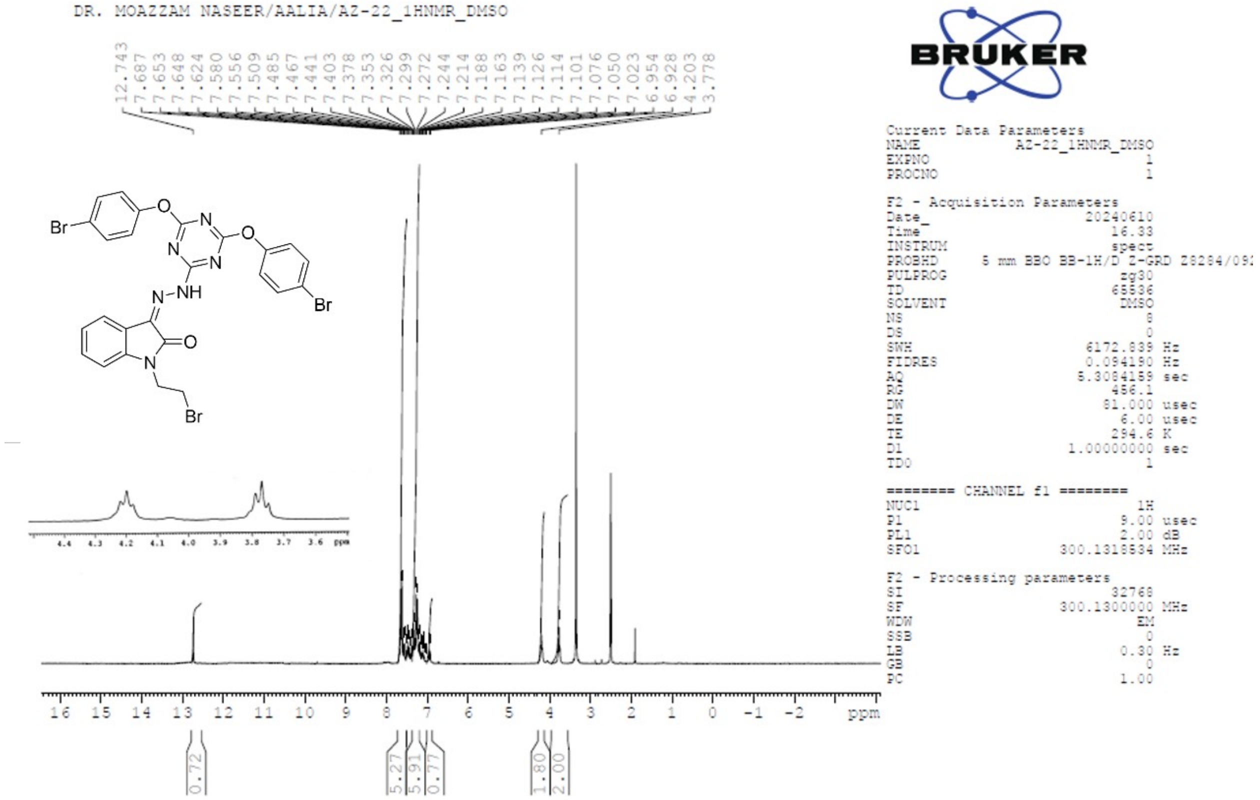
1-(2-(5-Phenyl-2*H*-tetrazol-2-yl)ethyl)indoline-2,3-dione (7d)



Bright orange; Yield: 74 %; Melting Point: 150-155 °C; R_f: 0.48 (ethyl acetate: *n*-hexane, 1:1)

8. Copies of NMR Spectras

^{1}H NMR spectrum of compound 8



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SOLVENT                     DMSO
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FIDRES                  0.094190 Hz
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DE                          6.00 usec
TE                         294.6 K
D1                    1.00000000 sec
TD0                            1

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PL1                         2.00 dB
SFO1                 300.1318534 MHz

F2 - Processing parameters
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SSB                            0
LB                          0.30 Hz
GB                             0
PC                          1.00
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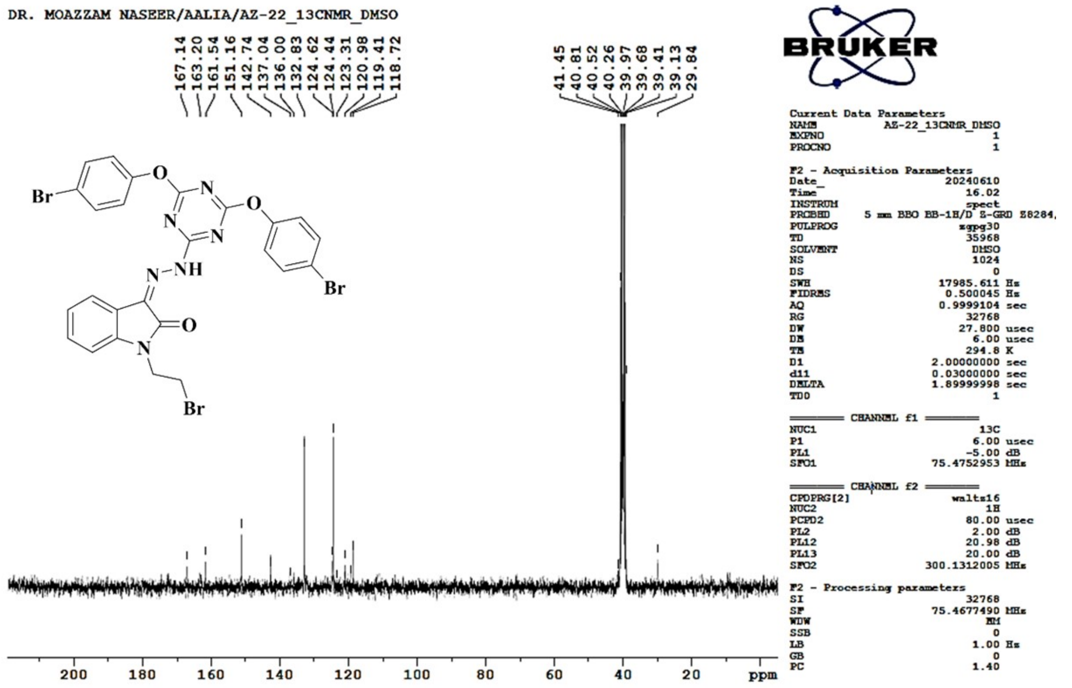
^{13}C NMR spectrum of compound 8



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SOLVENT                     DMSO
NS                          1024
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SWH                    17985.611 Hz
FIDRES                  0.500045 Hz
AQ                     0.9999104 sec
RG                         32768
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DE                          6.00 usec
TE                         294.8 K
D1                    2.00000000 sec
d11                   0.03000000 sec
DELTA                 1.89999998 sec
TD0                            1

======== CHANNEL f1 ========
NUC1                         13C
P1                          6.00 usec
PL1                        -5.00 dB
SFO1                  75.4752953 MHz

======== CHANNEL f2 ========
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PCPD2                      80.00 usec
PL2                         2.00 dB
PL12                       20.98 dB
PL13                       20.00 dB
SFO2                 300.1312005 MHz

F2 - Processing parameters
SI                         32768
SF                    75.4677490 MHz
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SSB                            0
LB                          1.00 Hz
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PC                          1.40
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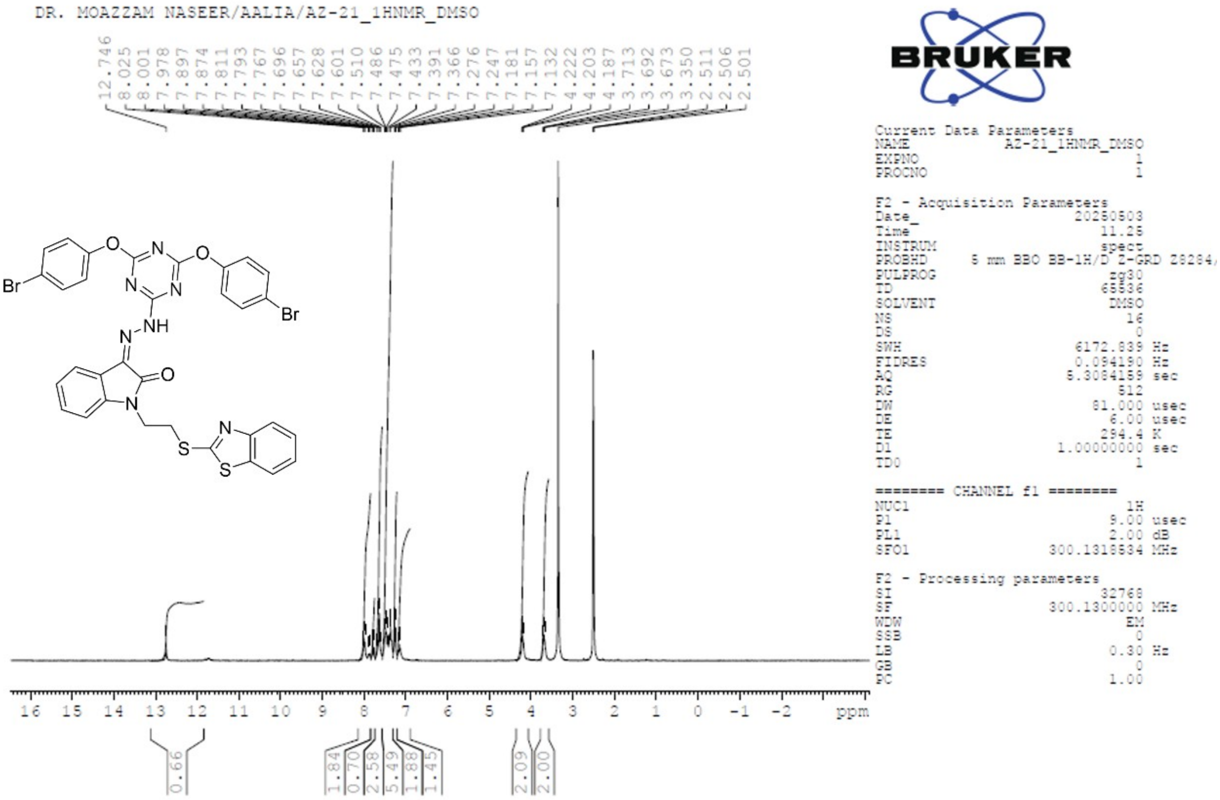
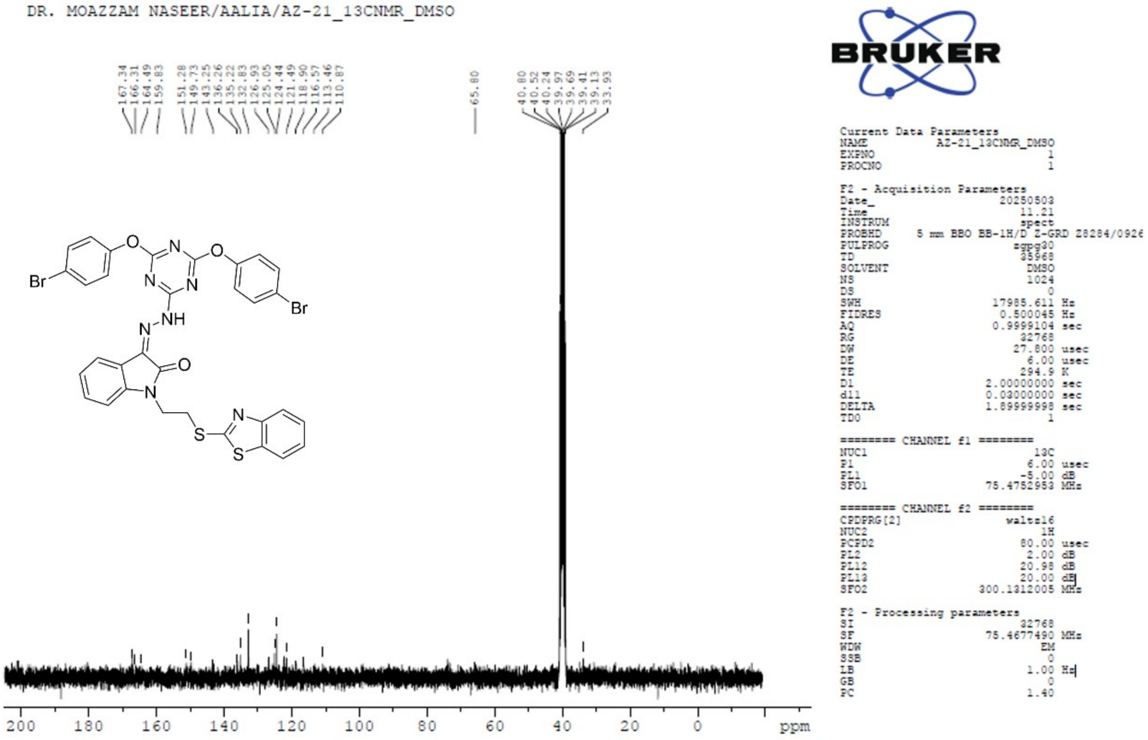
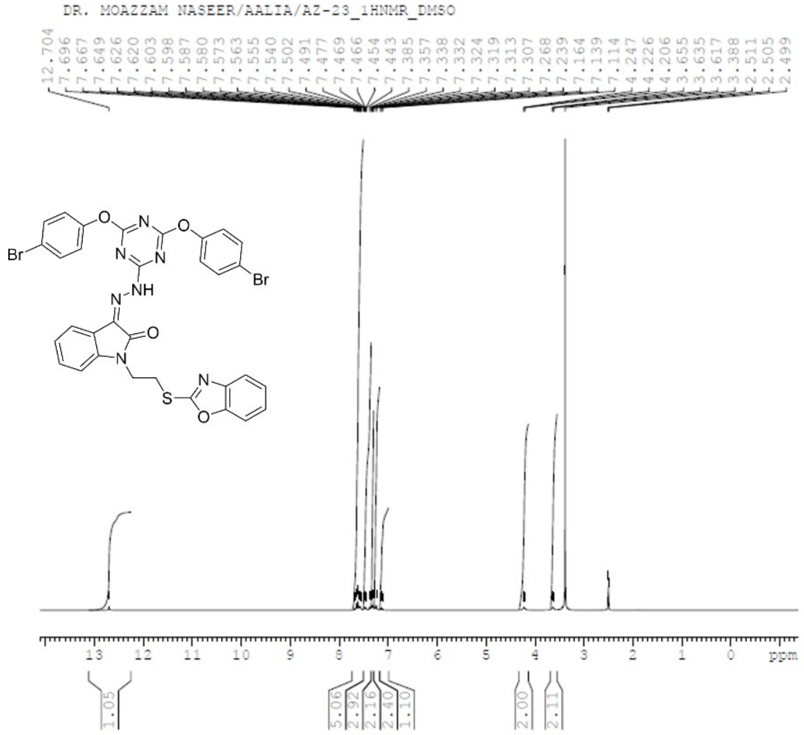
^{1}H NMR spectrum of compound 9a



^{13}C NMR spectrum of compound 9a



^{1}H NMR spectrum of compound 9b



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PULPROG                       zg30
TD                           65536
SOLVENT                       DMSO
NS                              16
DS                               0
SWH                       6172.839 Hz
FIDRES                    0.094190 Hz
AQ                       5.3084159 sec
RG                             256
DW                          81.000 usec
DE                            6.00 usec
TE                           294.1 K
D1                      1.00000000 sec
TD0                              1

======== CHANNEL f1 ========
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P1                            9.00 usec
PL1                           2.00 dB
SFO1                   300.1318534 MHz

F2 - Processing parameters
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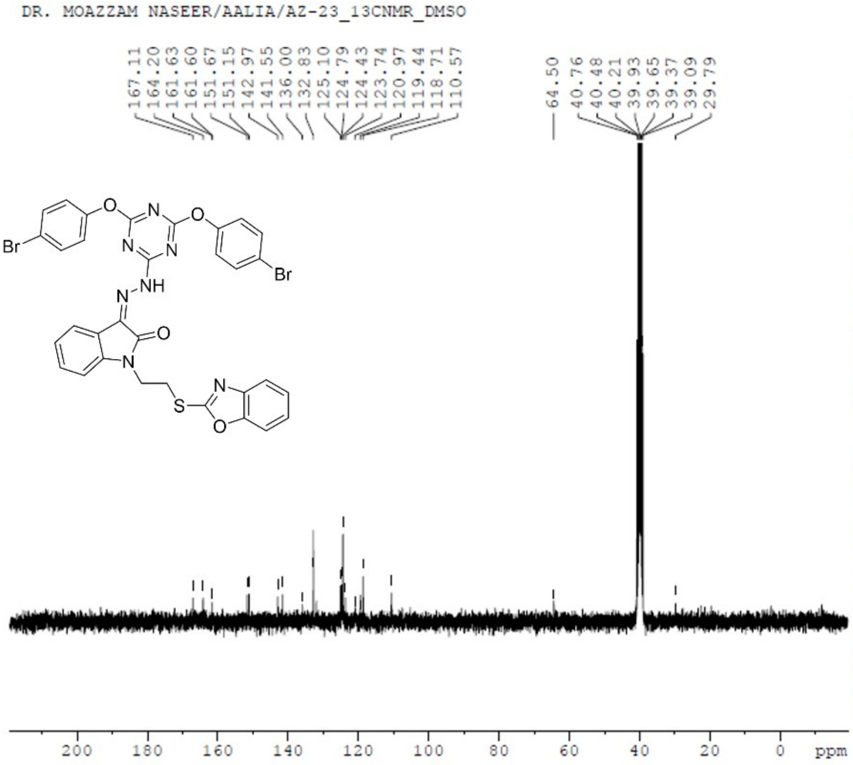
^{13}C NMR spectrum of compound 9b



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PULPROG                     zgpg30
TD                           35968
SOLVENT                       DMSO
NS                            1024
DS                               0
SWH                      17985.611 Hz
FIDRES                    0.500045 Hz
AQ                       0.9999104 sec
RG                           32768
DW                          27.800 usec
DE                            6.00 usec
TE                           294.8 K
D1                      2.00000000 sec
d11                     0.03000000 sec
DELTA                   1.89999998 sec
TD0                              1

======== CHANNEL f1 ========
NUC1                           13C
P1                            6.00 usec
PL1                          -5.00 dB
SFO1                    75.4752953 MHz

======== CHANNEL f2 ========
CPDPRG[2]                  waltz16
NUC2                            1H
PCPD2                        80.00 usec
PL2                           2.00 dB
PL12                         20.98 dB
PL13                         20.00 dB
SFO2                   300.1312005 MHz

F2 - Processing parameters
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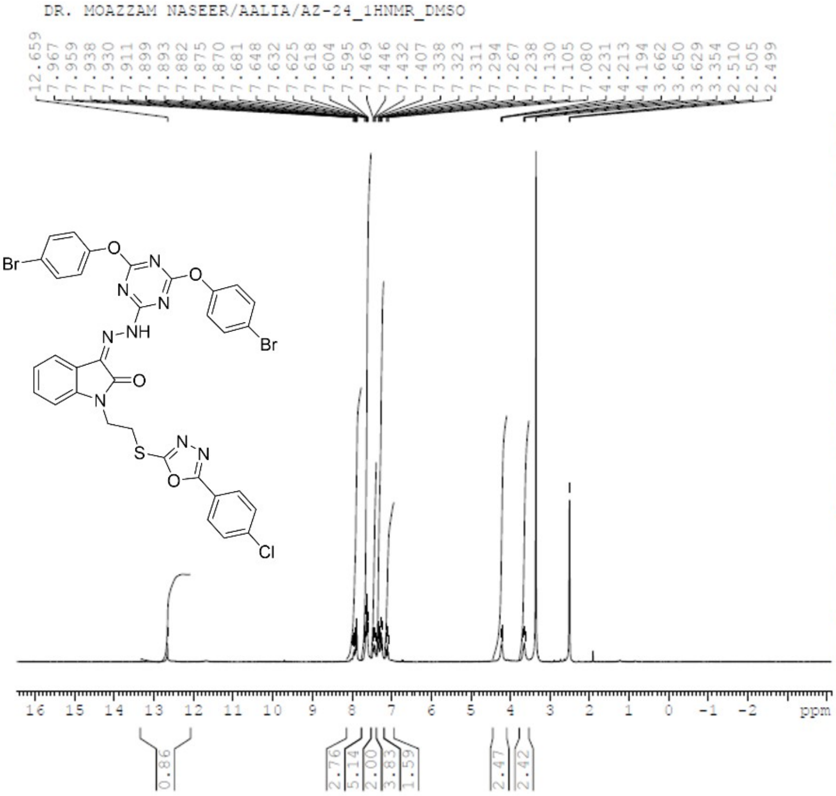
^{1}H NMR spectrum of compound 9c



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PROCNO                         1

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Time                       16.04
INSTRUM                    spect
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PULPROG                     zg30
TD                         65536
SOLVENT                     DMSO
NS                             8
DS                             0
SWH                     6172.839 Hz
FIDRES                  0.094190 Hz
AQ                     5.3084159 sec
RG                           512
DW                        81.000 usec
DE                          6.00 usec
TE                         294.7 K
D1                    1.00000000 sec
TD0                            1

======== CHANNEL f1 ========
NUC1                          1H
P1                          9.00 usec
PL1                         2.00 dB
SFO1                 300.1318534 MHz

F2 - Processing parameters
SI                         32768
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WDW                           EM
SSB                            0
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PC                          1.00
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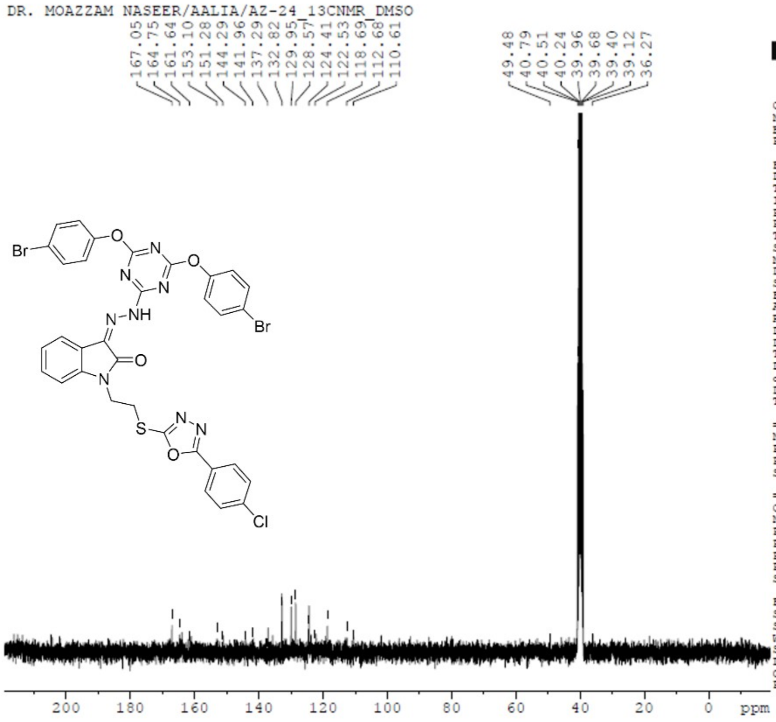
^{13}C NMR spectrum of compound 9c



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PROCNO                          1

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PULPROG                    zgpg30
TD                          35968
SOLVENT                      DMSO
NS                            883
DS                              0
SWH                     17985.611 Hz
FIDRES                   0.500045 Hz
AQ                      0.9999104 sec
RG                          32768
DW                         27.800 usec
DE                           6.00 usec
TE                          294.9 K
D1                     2.00000000 sec
d11                    0.03000000 sec
DELTA                  1.89999998 sec
TD0                             1

======== CHANNEL f1 ========
NUC1                          13C
P1                           6.00 usec
PL1                         -5.00 dB
SFO1                   75.4752953 MHz

======== CHANNEL f2 ========
CPDPRG[2]                 waltz16
NUC2                           1H
PCPD2                       80.00 usec
PL2                          2.00 dB
PL12                        20.98 dB
PL13                        20.00 dB
SFO2                  300.1312005 MHz

F2 - Processing parameters
SI                          32768
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SSB                             0
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PC                           1.40
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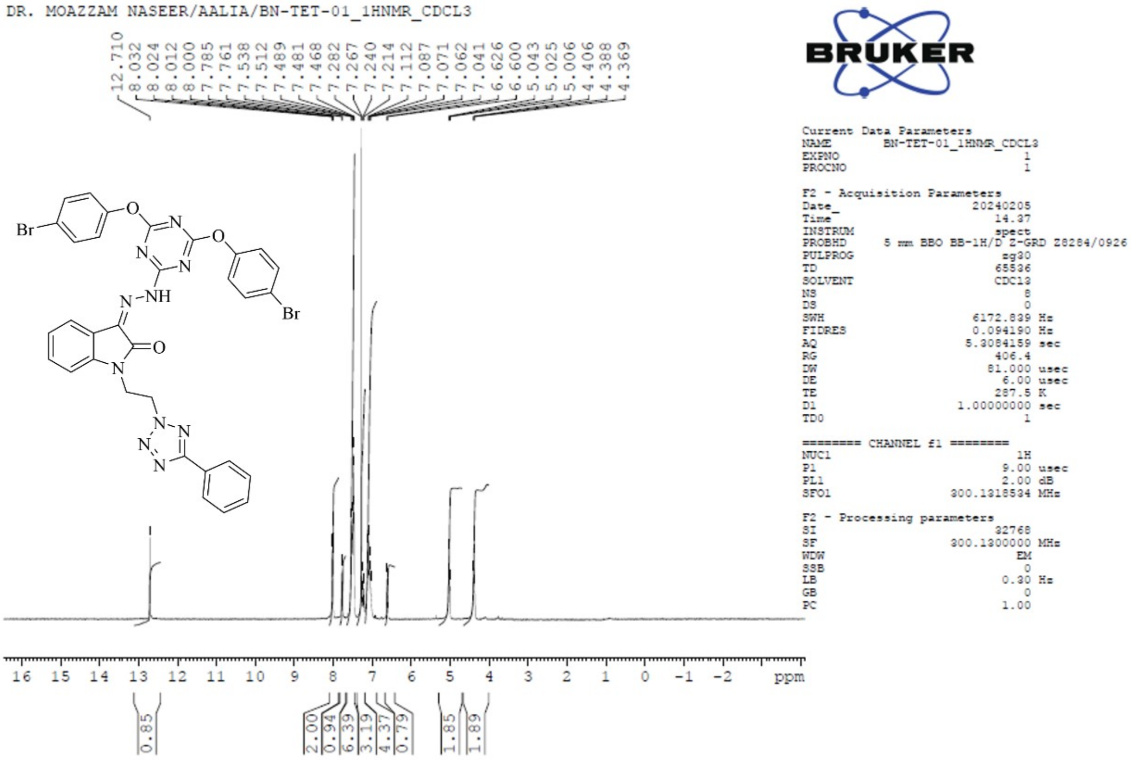
^{1}H NMR spectrum of compound 9d



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DR. MOAZZAM NASEER/AALIA/BN-TET-01_1HNMR_CDCL3

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PROCNO                          1

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Time                        14.37
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PULPROG                      zg30
TD                          65536
SOLVENT                     CDCl3
NS                              8
DS                              0
SWH                      6172.839 Hz
FIDRES                   0.094190 Hz
AQ                      5.3084159 sec
RG                          406.4
DW                         81.000 usec
DE                           6.00 usec
TE                          287.5 K
D1                     1.00000000 sec
TD0                             1

======== CHANNEL f1 ========
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P1                           9.00 usec
PL1                          2.00 dB
SFO1                  300.1318534 MHz

F2 - Processing parameters
SI                          32768
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LB                           0.30 Hz
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PC                           1.00
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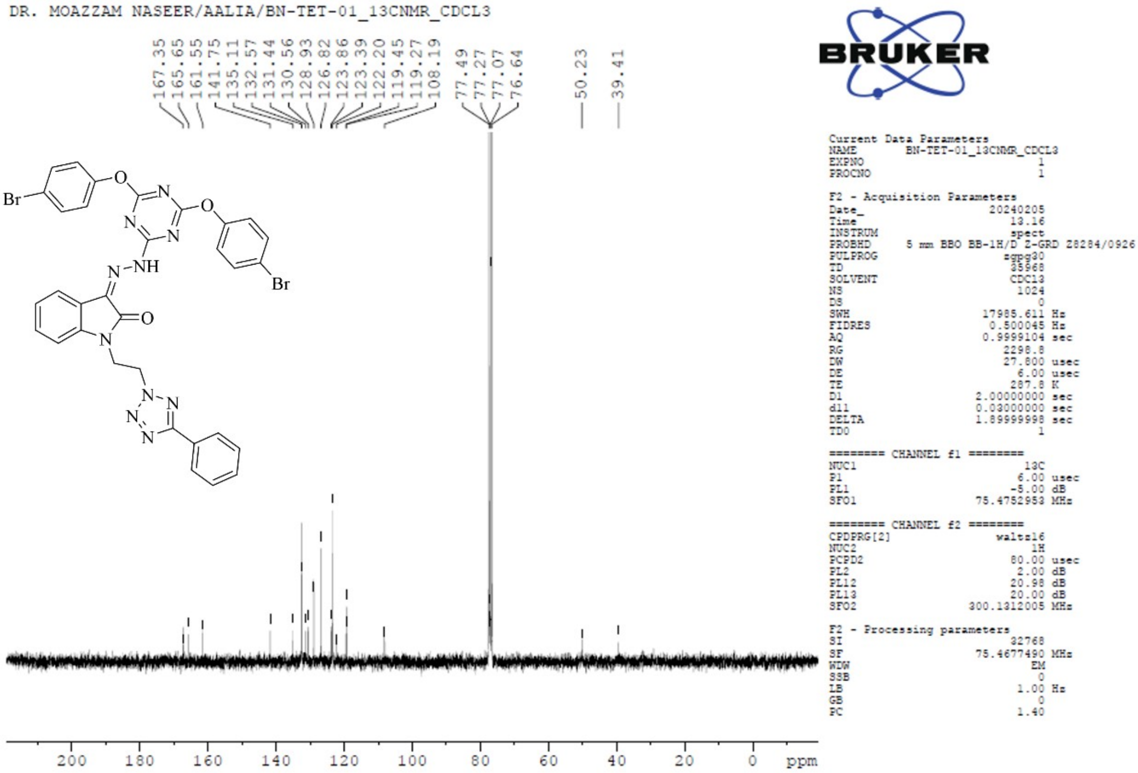
^{13}C NMR spectrum of compound 9d



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DR. MOAZZAM NASEER/AALIA/BN-TET-01_13CNMR_CDCL3

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PROCNO                          1

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PULPROG                    zgpg30
TD                          35968
SOLVENT                     CDCl3
NS                           1024
DS                              0
SWH                     17985.611 Hz
FIDRES                   0.500045 Hz
AQ                      0.9999104 sec
RG                         2298.8
DW                         27.800 usec
DE                           6.00 usec
TE                          287.8 K
D1                     2.00000000 sec
d11                    0.03000000 sec
DELTA                  1.89999998 sec
TD0                             1

======== CHANNEL f1 ========
NUC1                          13C
P1                           6.00 usec
PL1                         -5.00 dB
SFO1                   75.4752953 MHz

======== CHANNEL f2 ========
CPDPRG[2]                 waltz16
NUC2                           1H
PCPD2                       80.00 usec
PL2                          2.00 dB
PL12                        20.98 dB
PL13                        20.00 dB
SFO2                  300.1312005 MHz

F2 - Processing parameters
SI                          32768
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GB                              0
PC                           1.40
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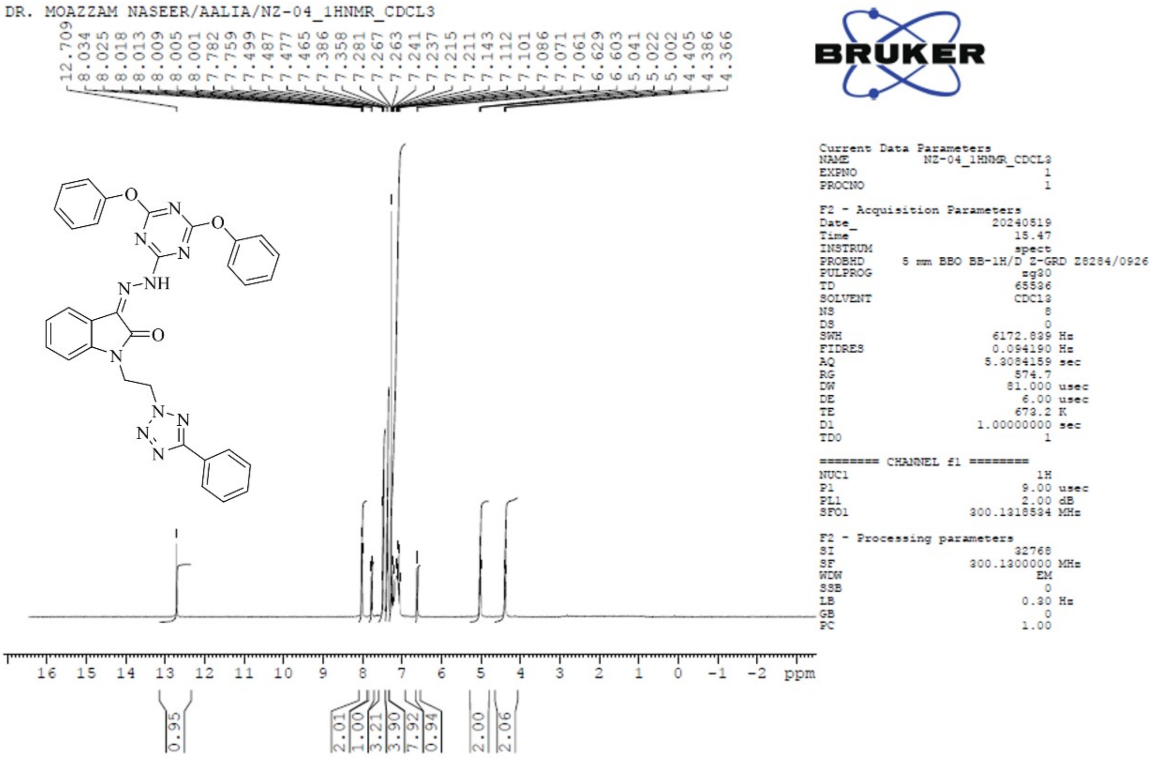
^{1}H NMR spectrum of compound 9e



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PROCNO                        1

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PULPROG                    zg30
TD                        65536
SOLVENT                   CDCl3
NS                            8
DS                            0
SWH                    6172.839 Hz
FIDRES                 0.094190 Hz
AQ                    5.3084159 sec
RG                        574.7
DW                       81.000 usec
DE                         6.00 usec
TE                        673.2 K
D1                   1.00000000 sec
TD0                           1

======== CHANNEL f1 ========
NUC1                         1H
P1                         9.00 usec
PL1                        2.00 dB
SFO1                300.1318534 MHz

F2 - Processing parameters
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SF                  300.1300000 MHz
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LB                         0.30 Hz
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PC                         1.00
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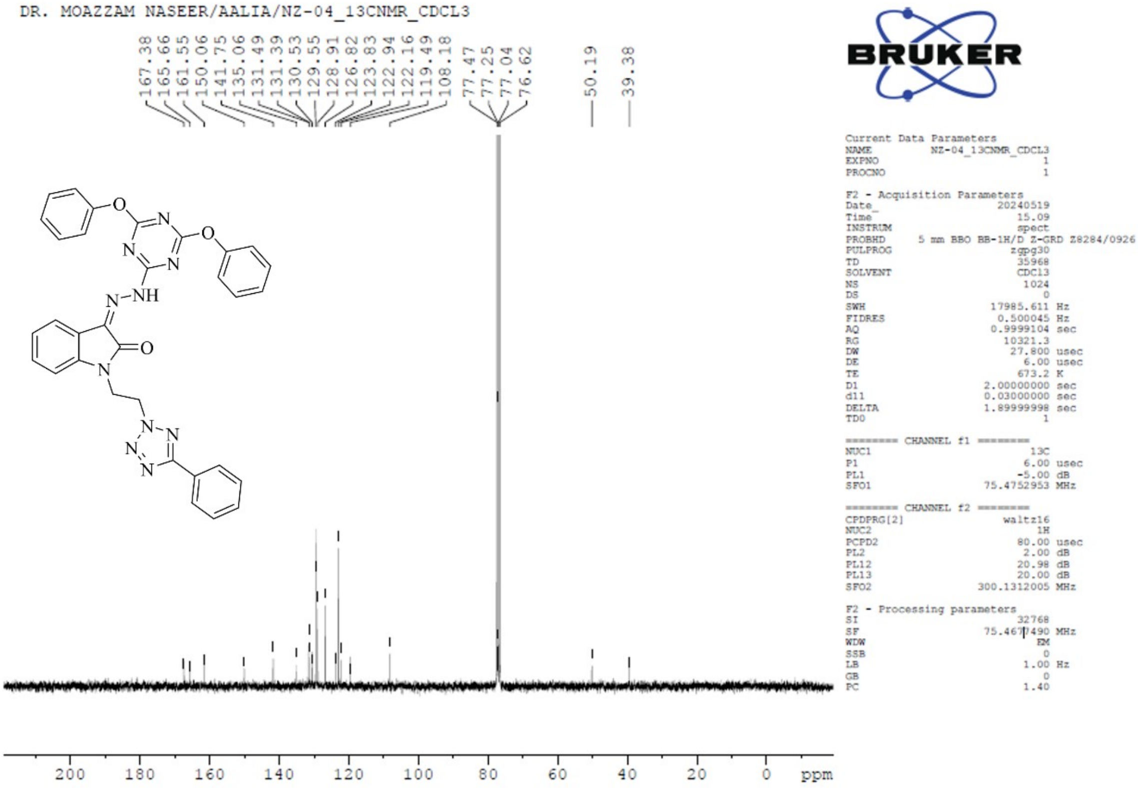
^{13}C NMR spectrum of compound 9e



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PROCNO                         1

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PULPROG                   zgpg30
TD                         35968
SOLVENT                    CDCl3
NS                          1024
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RG                       10321.3
DW                        27.800 usec
DE                          6.00 usec
TE                         673.2 K
D1                    2.00000000 sec
d11                   0.03000000 sec
DELTA                 1.89999998 sec
TD0                            1

======== CHANNEL f1 ========
NUC1                         13C
P1                          6.00 usec
PL1                        -5.00 dB
SFO1                  75.4752953 MHz

======== CHANNEL f2 ========
CPDPRG[2]                 waltz16
NUC2                          1H
PCPD2                      80.00 usec
PL2                         2.00 dB
PL12                       20.98 dB
PL13                       20.00 dB
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F2 - Processing parameters
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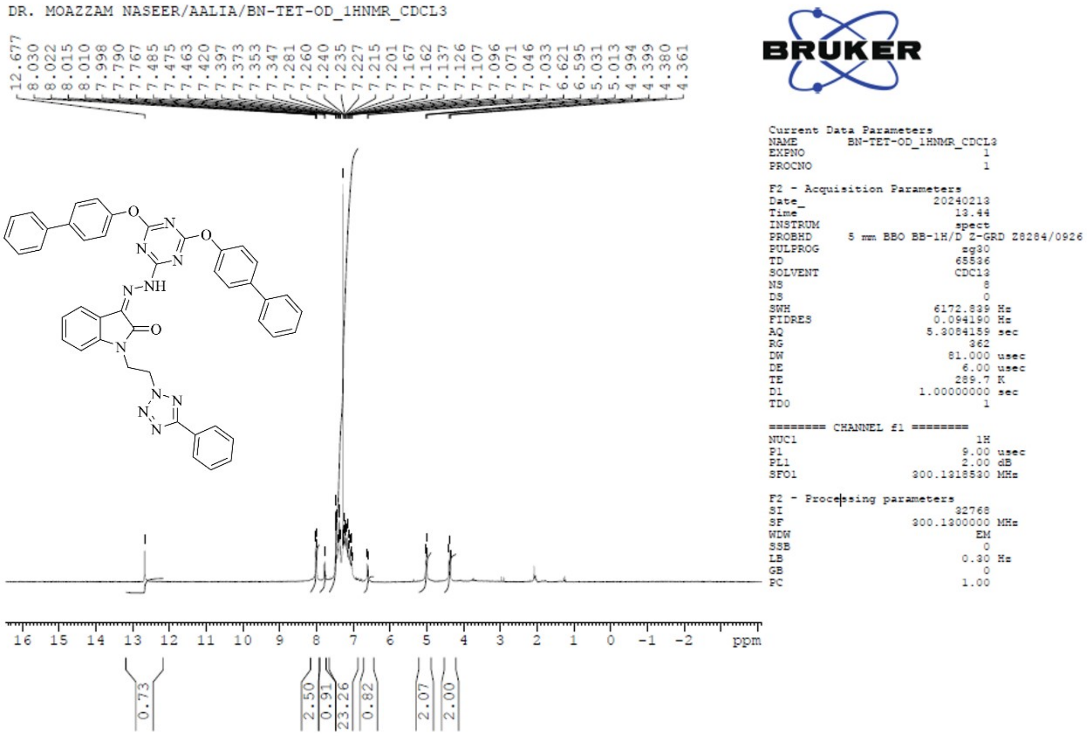
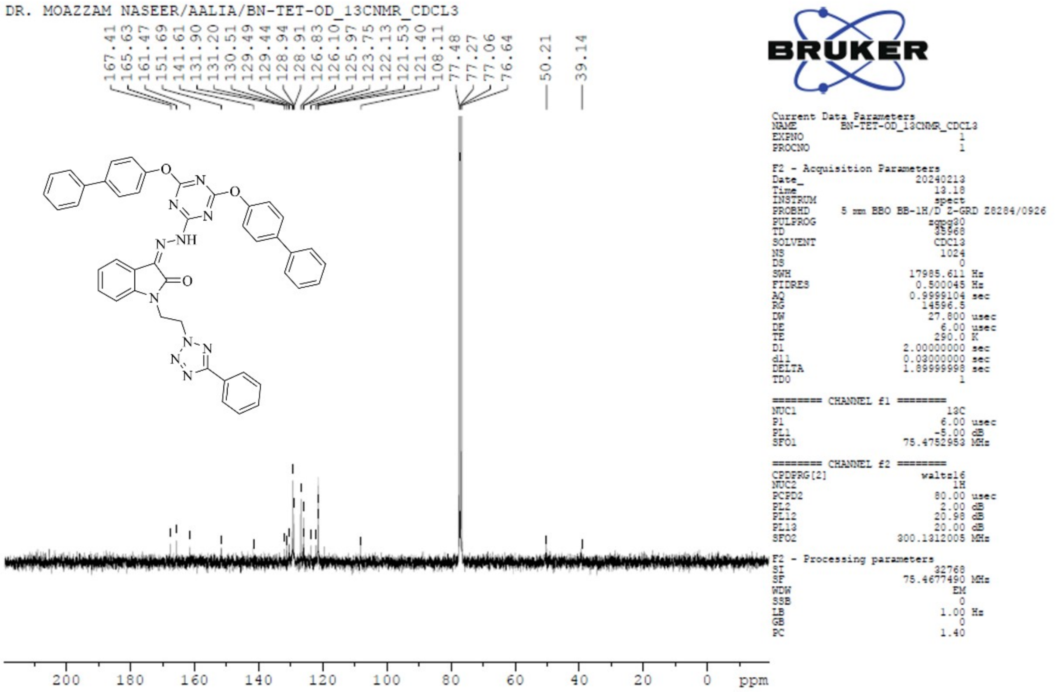
^{1}H NMR spectrum of compound 9f



^{13}C NMR spectrum of compound 9f



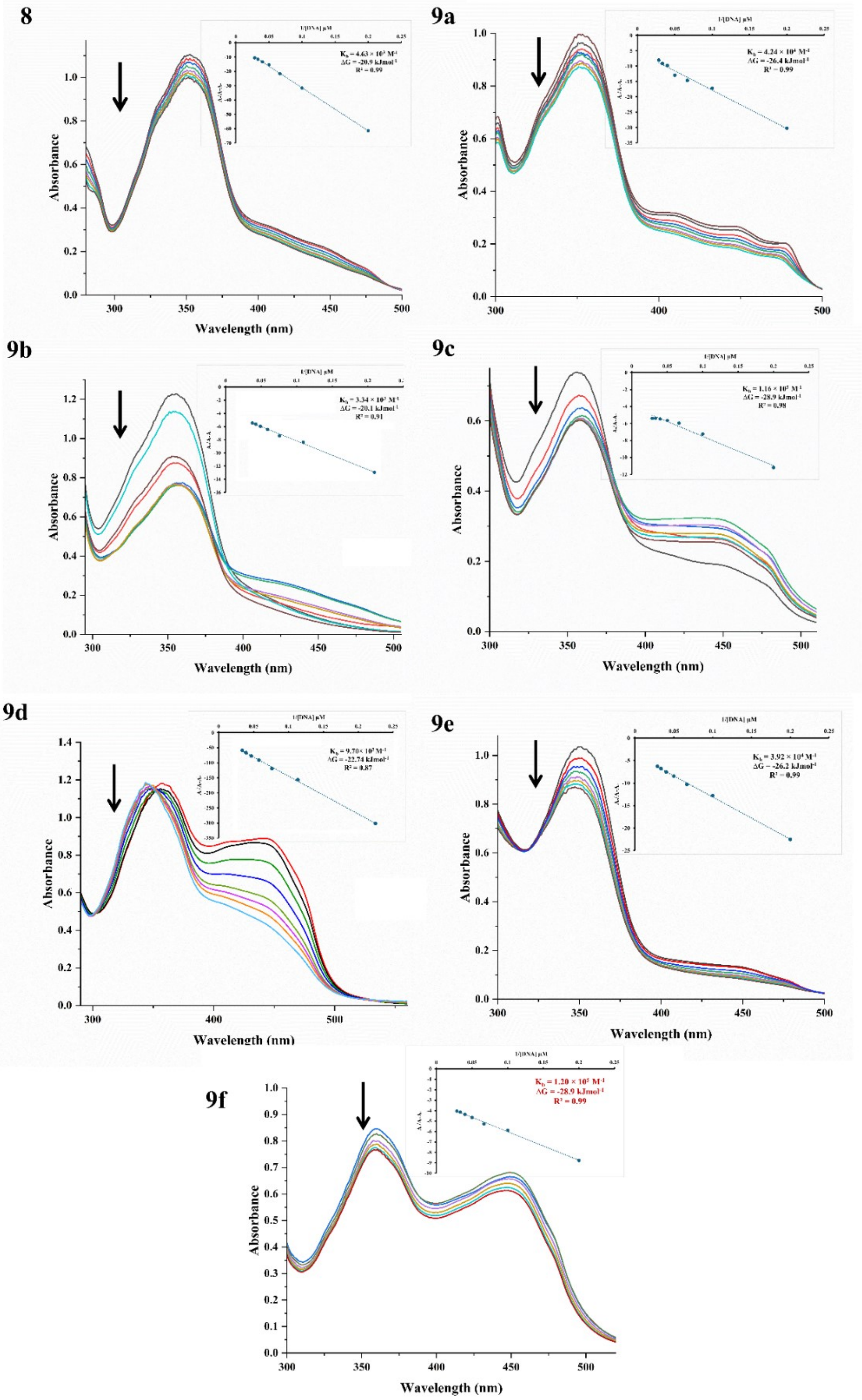


Figure S1: UV-Vis absorption spectra of compounds **8** & **9a-f** at pH 7.4 and room temperature, with and without varying SS-DNA concentrations (5–35 μM), demonstrating spectral shifts indicative of groove binding interactions. Inset: Plot of $A_o/(A-A_o)$ as a function of 1/[DNA].

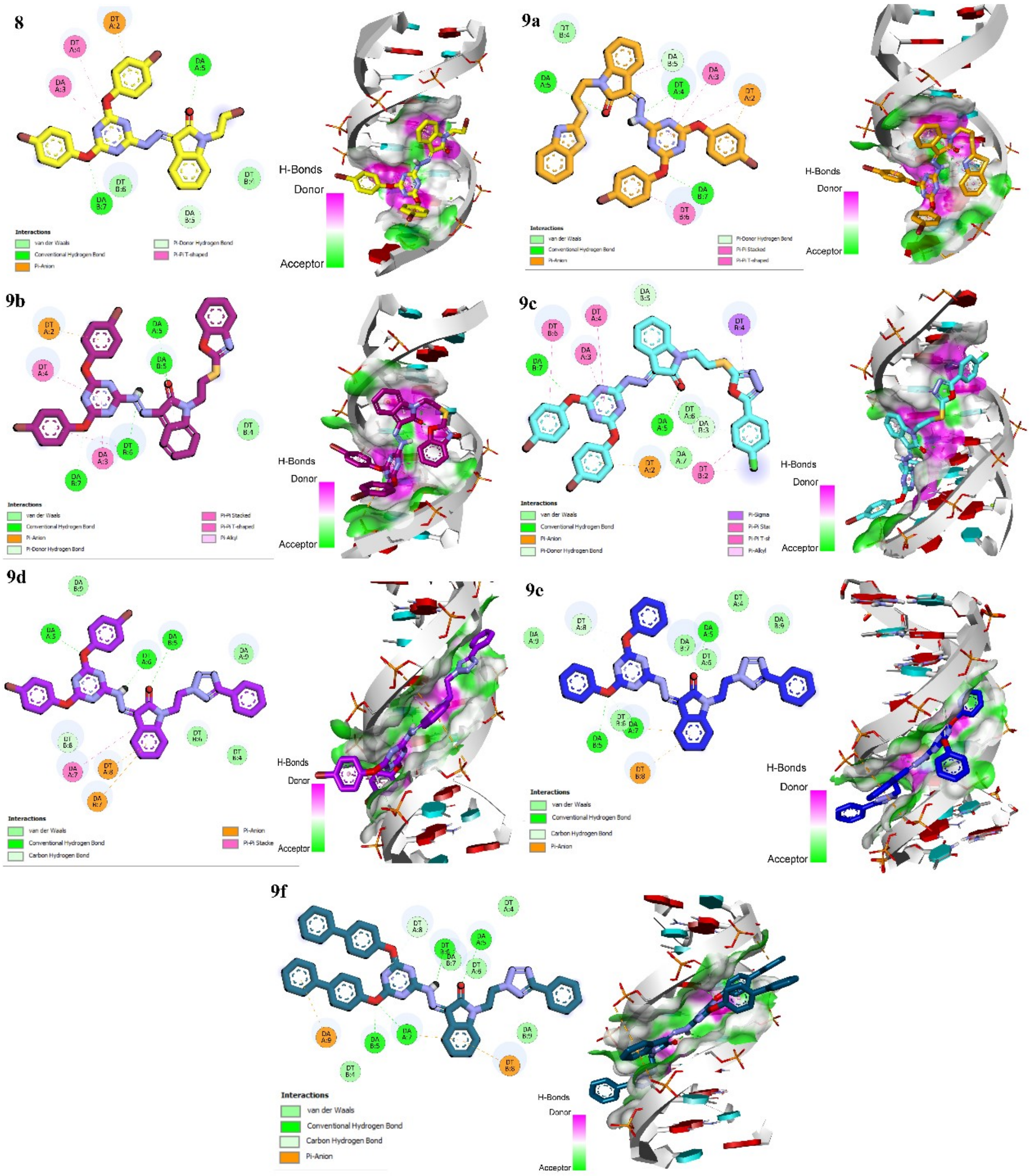


Figure S2: 2D & 3D illustrations of docked complexes of compounds **8 & 9a-f** with DNA (3EY0)

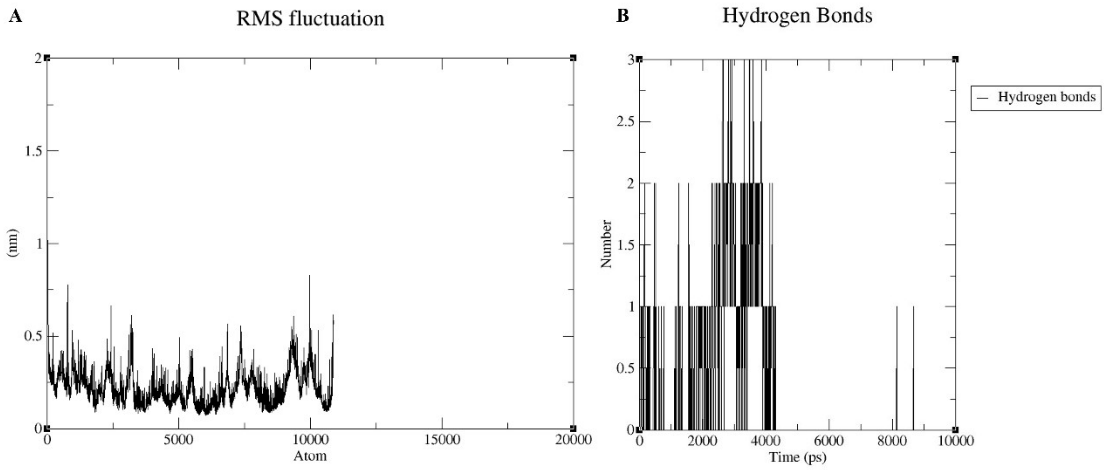


Figure S3: Flexibility and hydrogen bonding. (A) RMSF per residue, showing loop fluctuations up to 0.25–0.80 nm (B) Hydrogen bond analysis showing 2–3 persistent H-bonds.

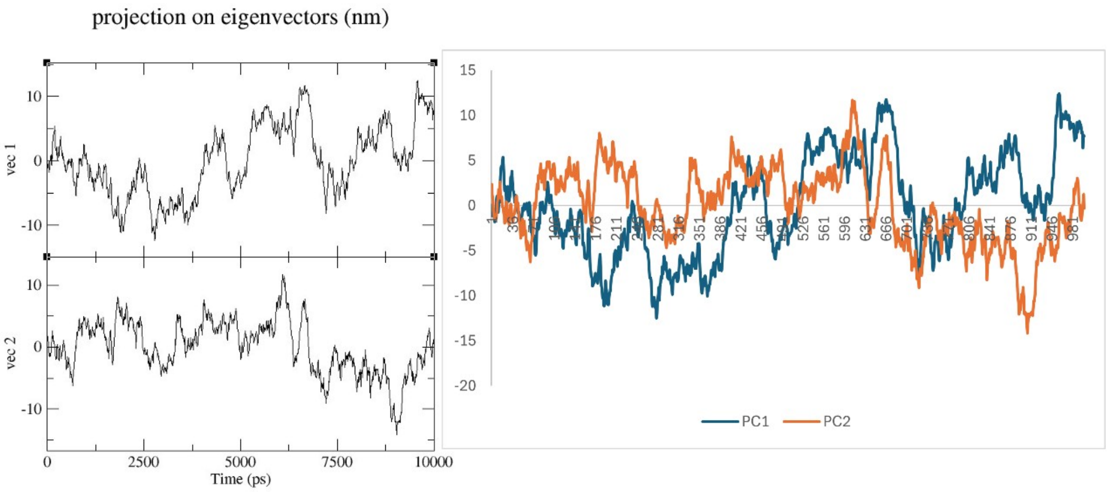


Figure S4: PCA scatter plots with PC1+PC2 accounting for 72% of variance.

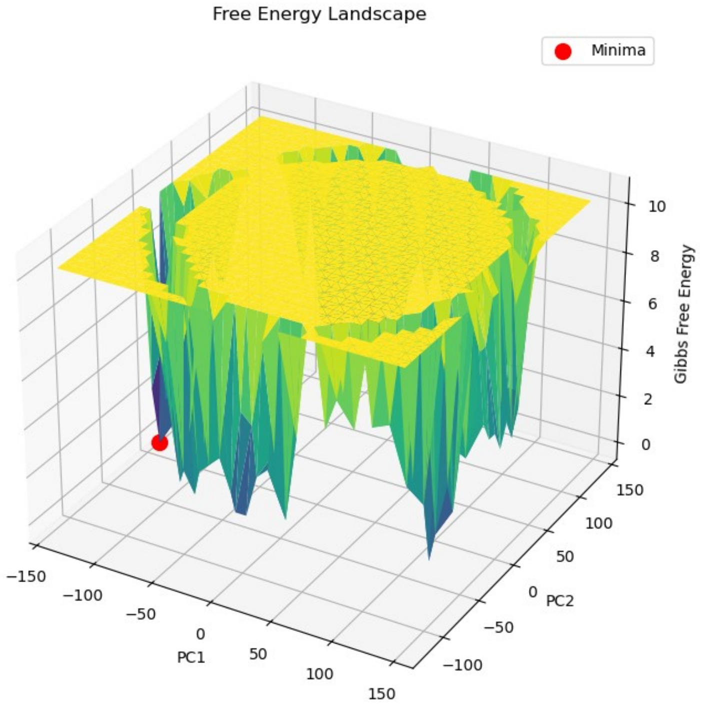


Figure S5: FEL along PC1 and PC2.