

SUPPLEMENTARY INFORMATION

Hydrazone analogs as DNA gyrase inhibitors and antioxidant agents: structure-activity relationship and pharmacophore modeling

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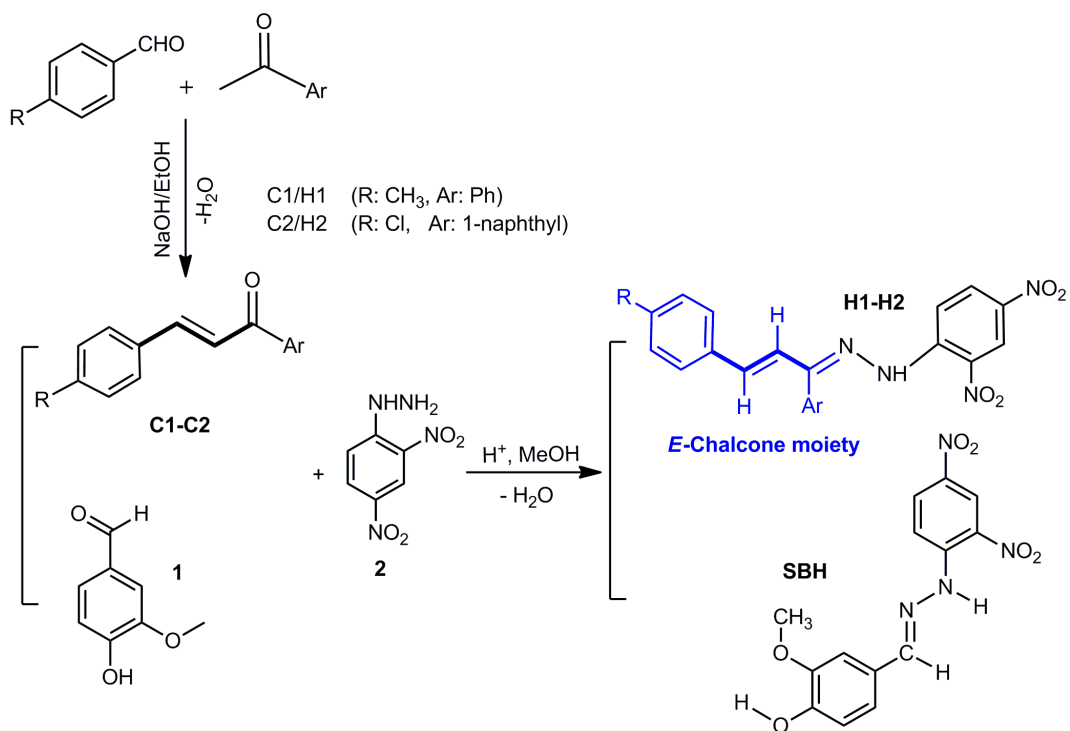
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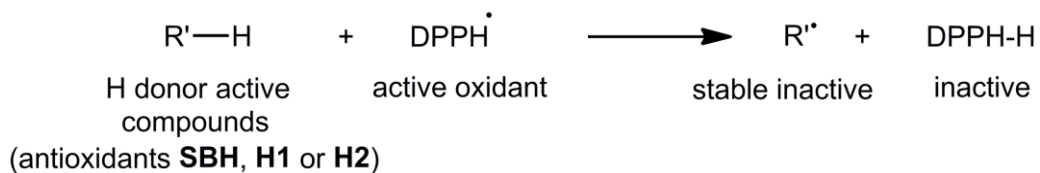
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Scheme S1. Synthesis of SBH, H1 and H2 hydrazone analogs.



Scheme S2. Free radical DPPH[•] scavenging.

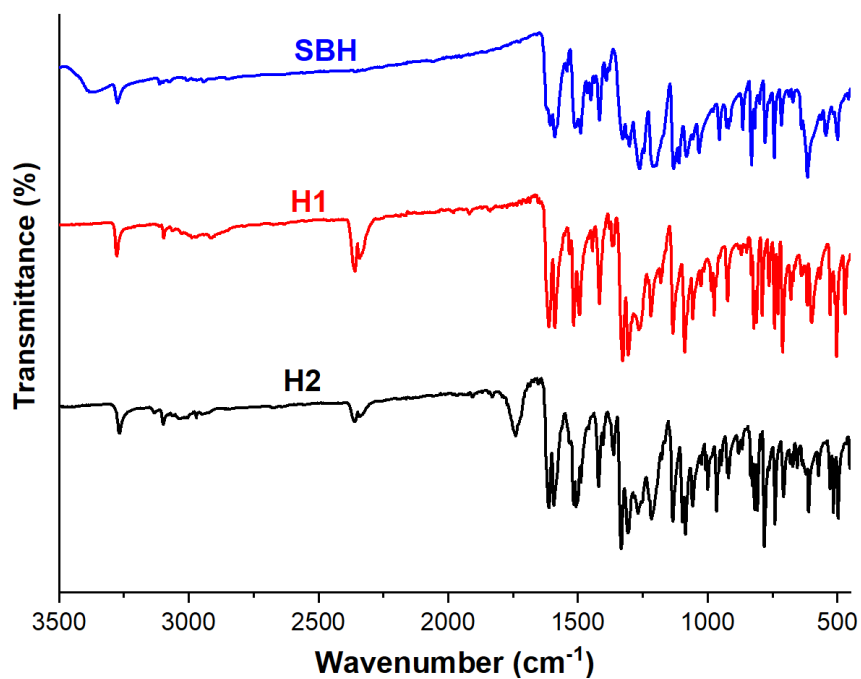


Figure S1. Infrared spectra of SBH, H1 and H2 synthesized hydrazones.

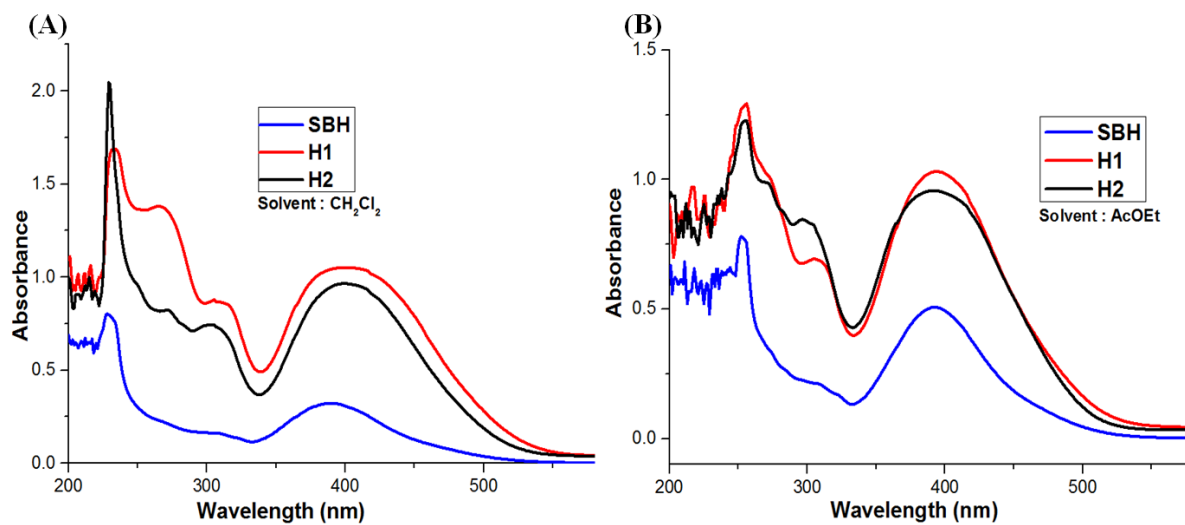


Figure S2. UV-Visible spectra of SBH, H1 and H2 (A) in CH_2Cl_2 (0.5×10^{-4} M); (B) in AcOEt (0.5×10^{-4} M).

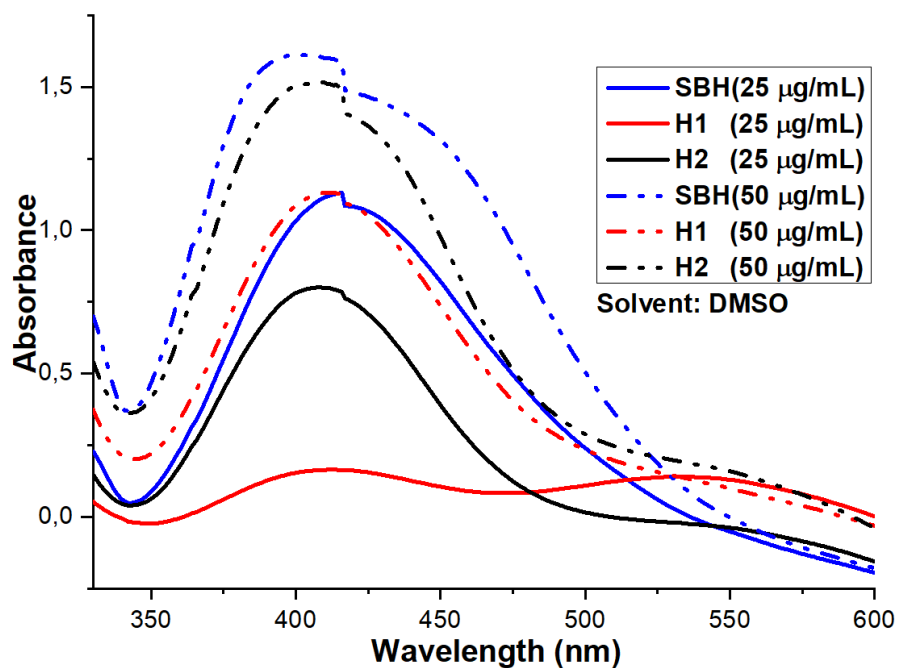


Figure S3. Absorption bands attributed to the electronic (D- π -A) transition of SBH, H1 and H2.

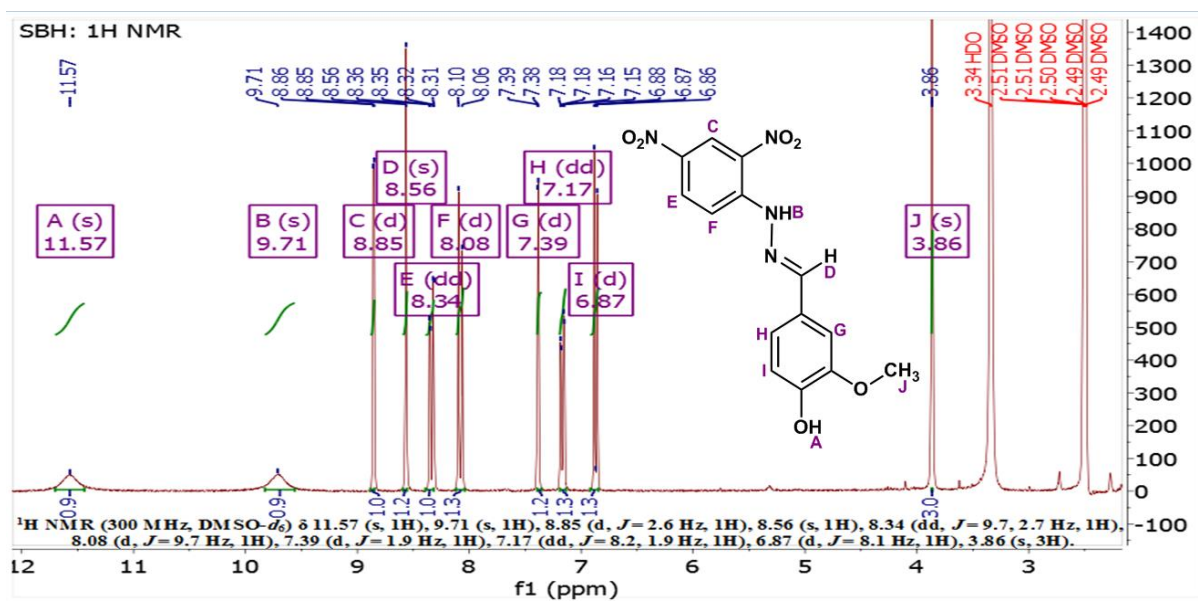


Figure S4. ^1H NMR spectrum of the synthesized hydrazone SBH in $\text{DMSO}-d_6$.

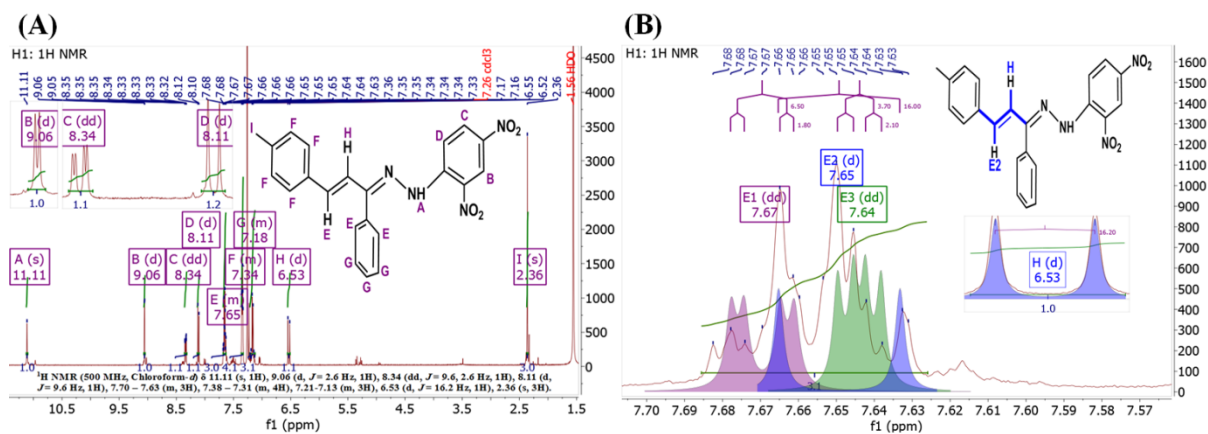


Figure S5. (A) ^1H NMR spectrum of H1 hydrazone in CDCl_3 (B) Simulated ^1H NMR spectrum of H1 hydrazone.

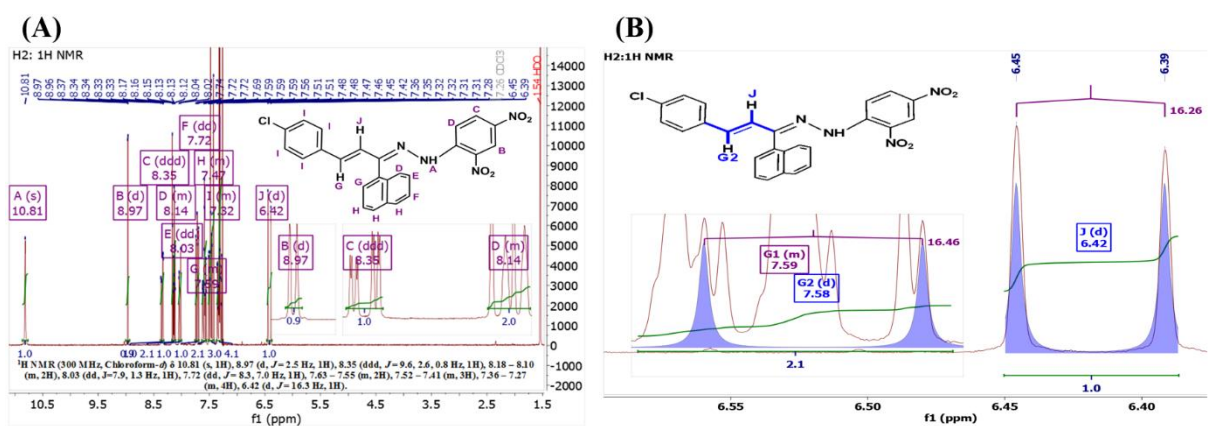
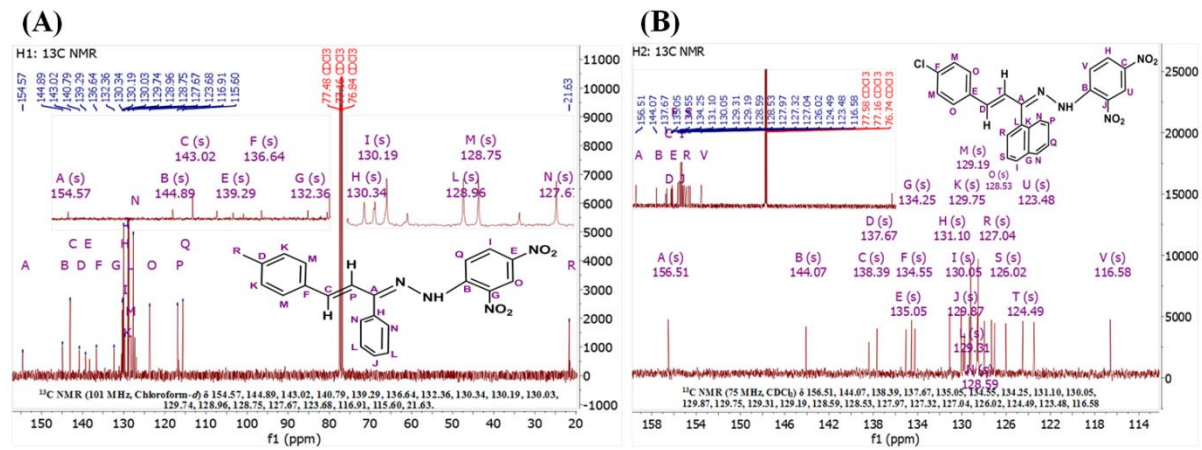
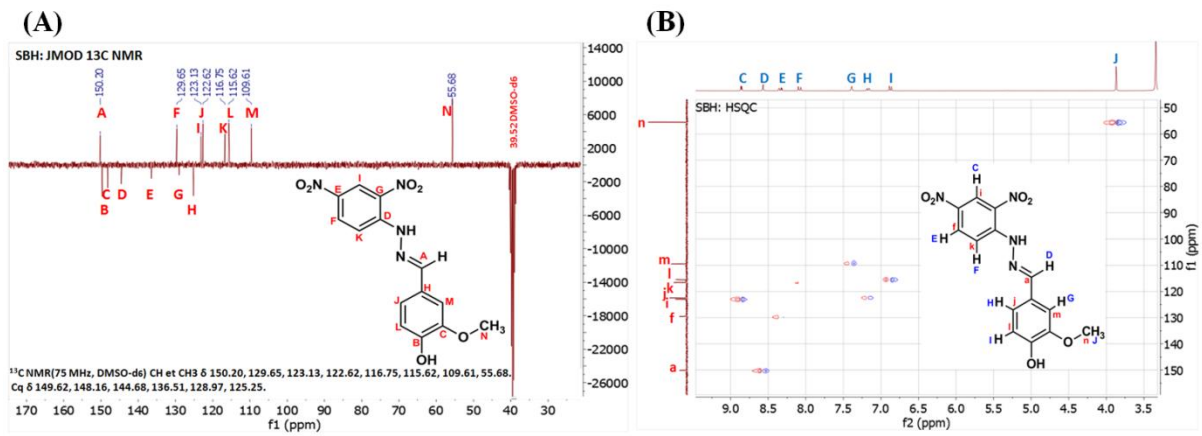


Figure S6. (A) ^1H NMR spectrum of H2 hydrazone in CDCl_3 (B) Simulated ^1H NMR spectrum of H2 hydrazone.



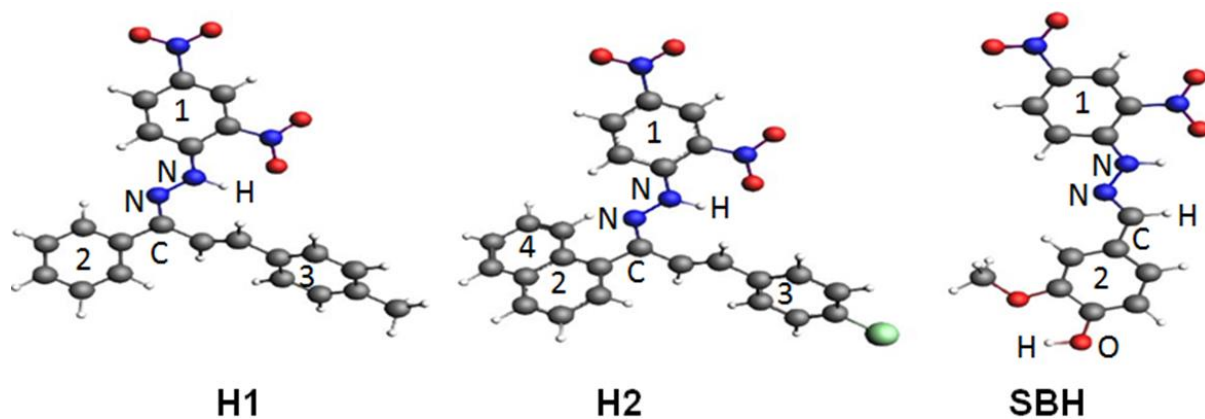


Figure S9. The lowest optimized structures of H1, H2 and SBH compounds. Atom colors: carbon (grey), oxygen (red), nitrogen (blue) chlorine (green) and hydrogen (white).

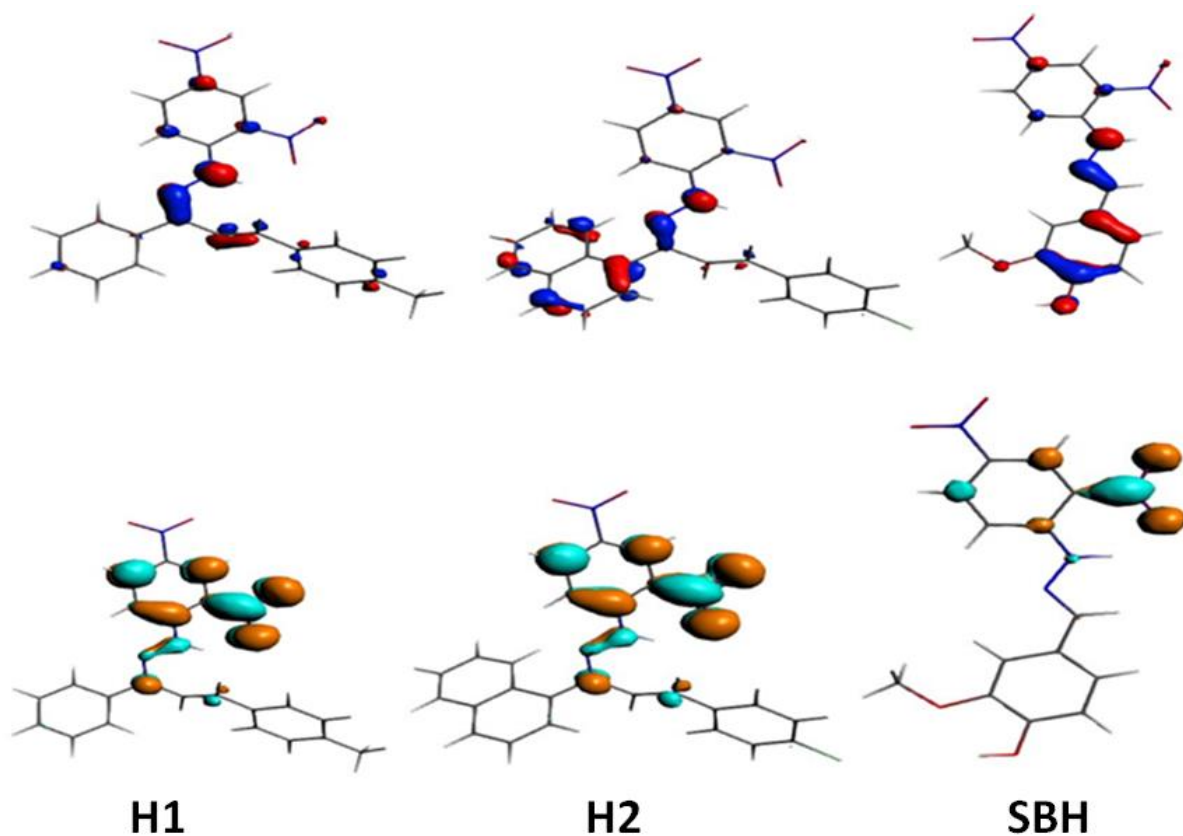


Figure S10. HOMOs (top) and LUMOs (bottom) for H1, H2 and SBH compounds. Contour values are of ± 0.06 (e/Bohr^3).

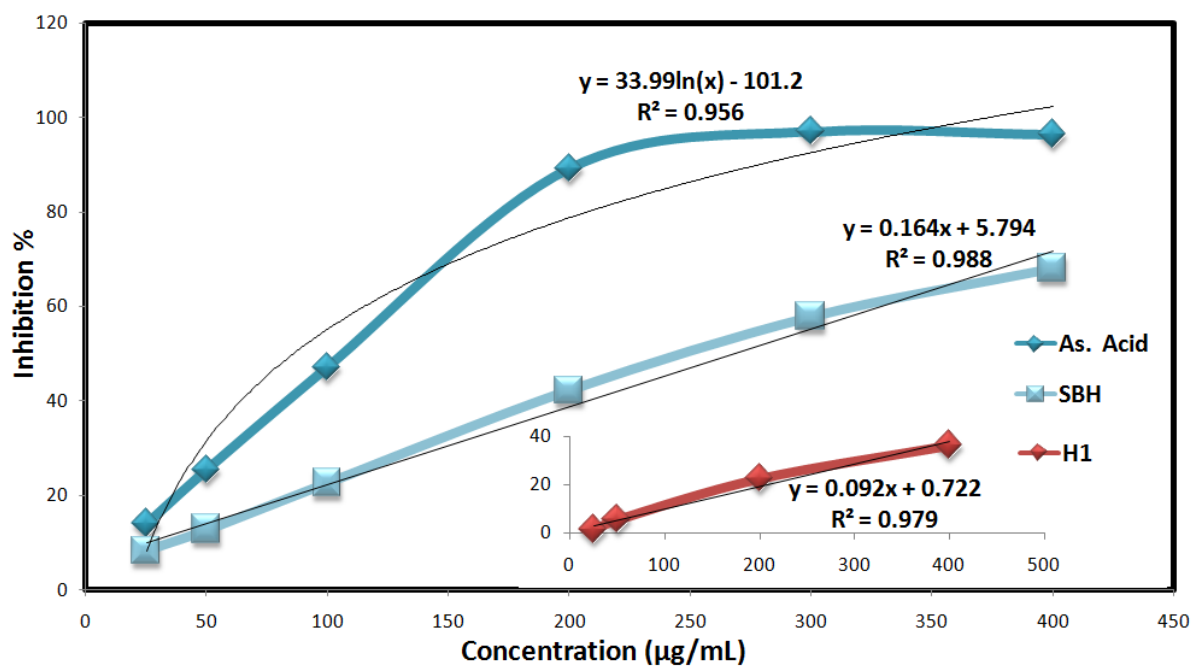


Figure S11. Antiradical activity of SBH, H1 hydrazones and standard ascorbic acid after 60 mn.

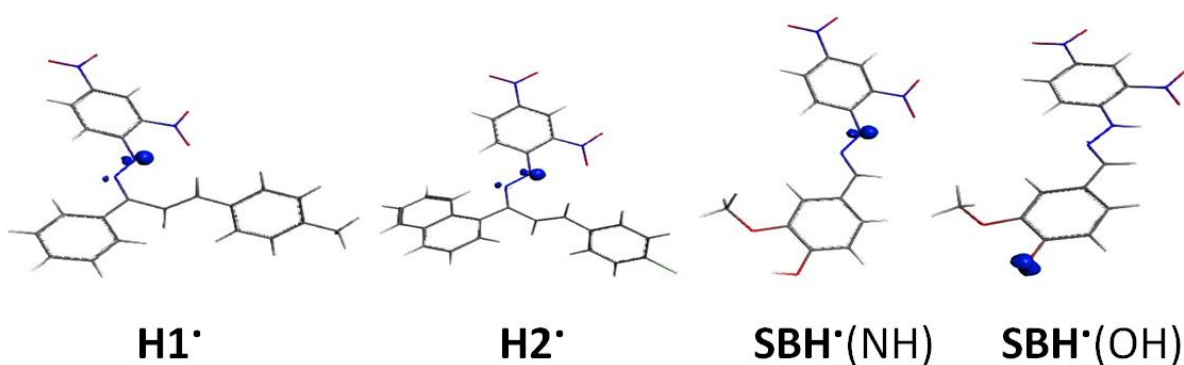


Figure S12. Spin density plots of H1, H2, SBH (NH) and SBH (OH) radicals.

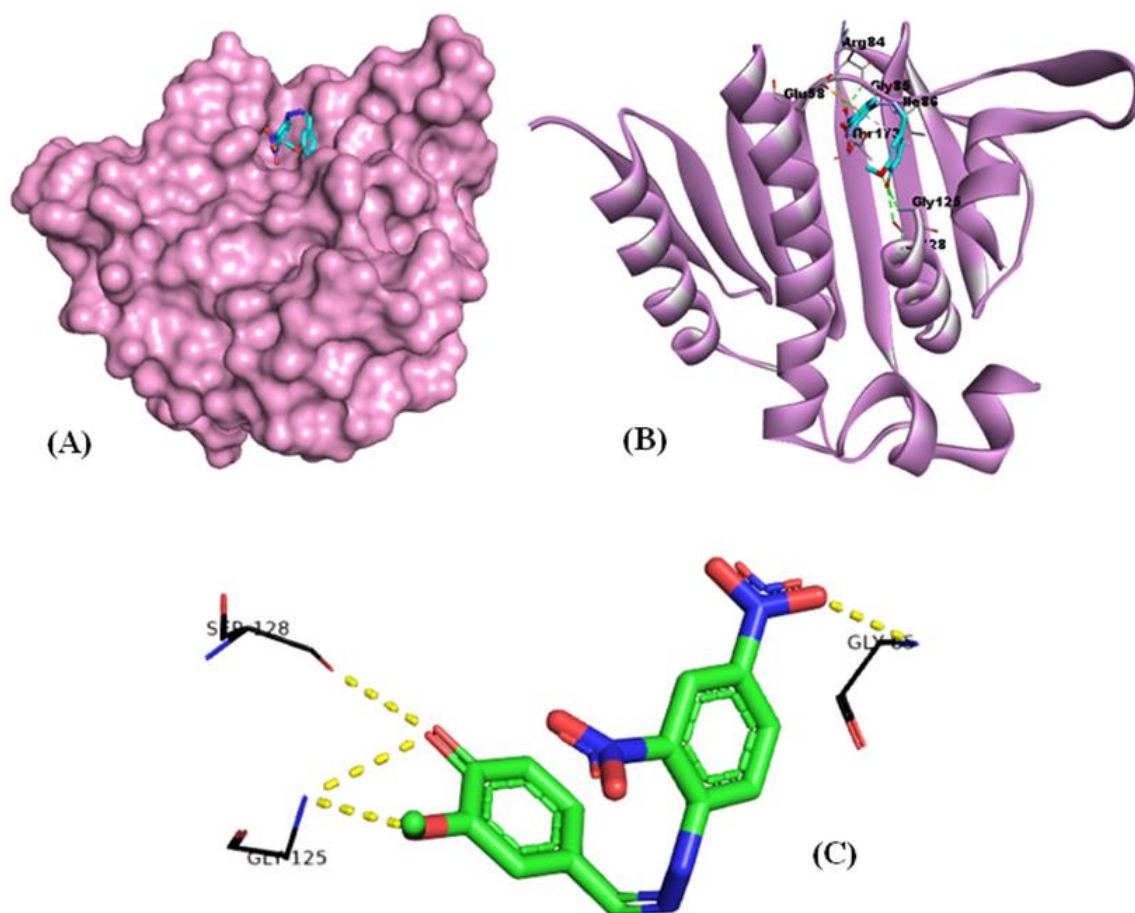


Figure S13. Predicted binding mode for SBH with gyrase. (A) View of the binding cavity, showing SBH as cyan sticks, bound to the surface of gyrase (4URO). (B) Profile view of SBH as cyan sticks bound in the gorge pocket of gyrase. Key residues for the binding of SBH are shown as green sticks. (C) 3D schematic diagram of docking model of SBH showing the hydrogen bonds formed with the gyrase active site.

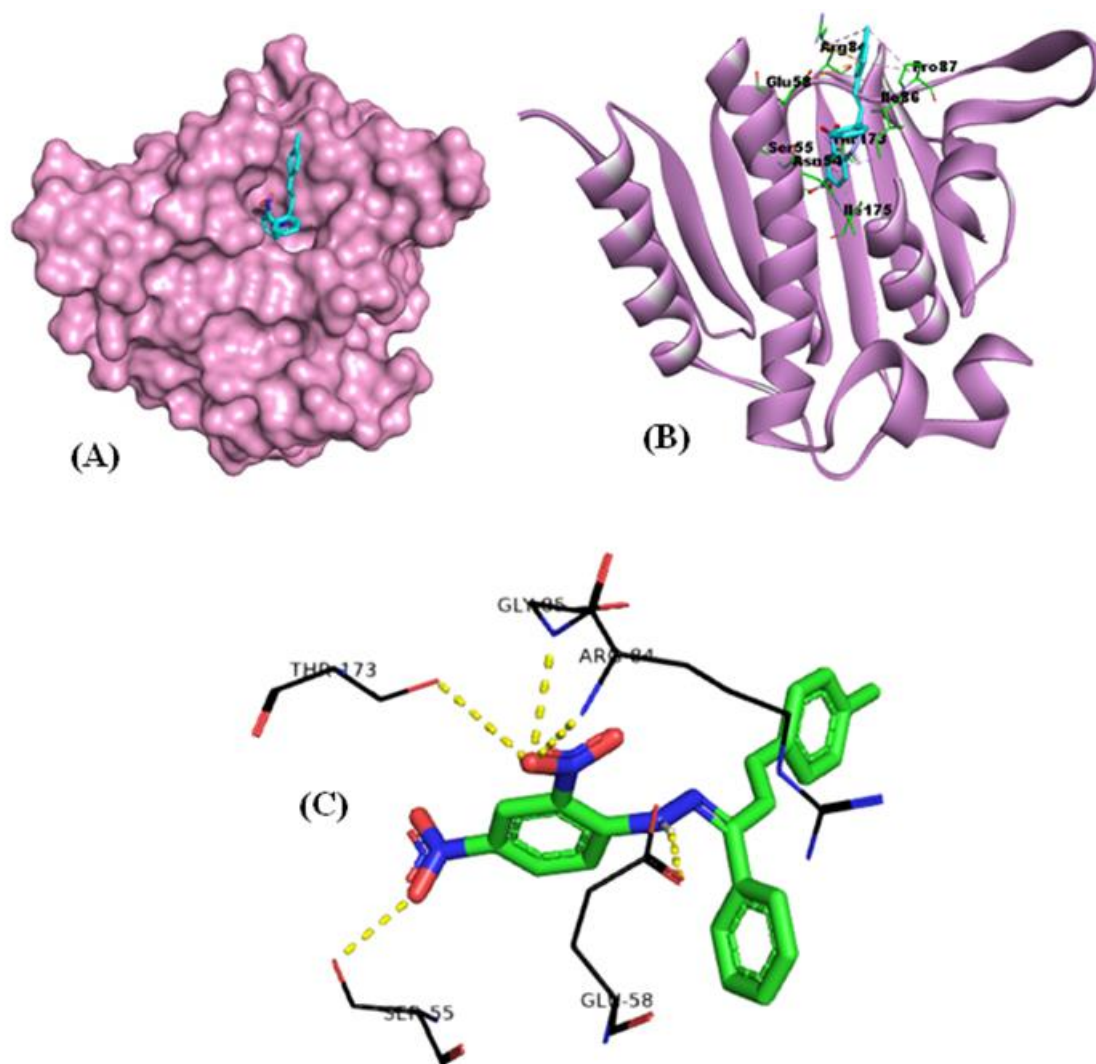


Figure S14. Predicted binding mode for H1 with gyrase. (A) View of the binding cavity, showing H1 as cyan sticks, bound to the surface of gyrase (4URO). (B) Profile view of H1 as cyan sticks bound in the gorge pocket of gyrase. Key residues for the binding of H1 are shown as green sticks. (C) 3D schematic diagram of docking model of H1 showing the hydrogen bonds formed with the gyrase active site.

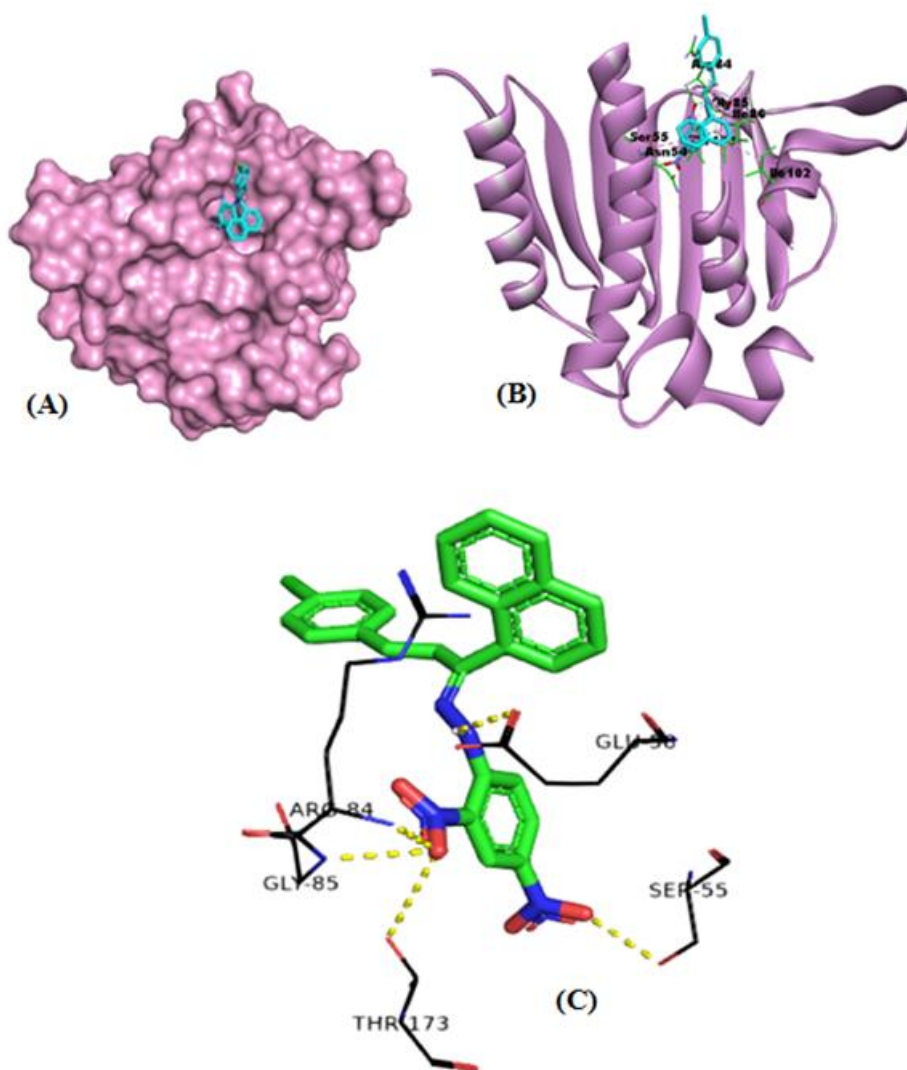


Figure S15. Predicted binding mode for H2 with gyrase. (A) View of the binding cavity, showing H2 as cyan sticks, bound to the surface of gyrase (4URO). (B) Profile view of H1 as cyan sticks bound in the gorge pocket of gyrase. Key residues for the binding of H1 are shown as green sticks. (C) 3D schematic diagram of docking model of H2 showing the hydrogen bonds formed with the gyrase active site.

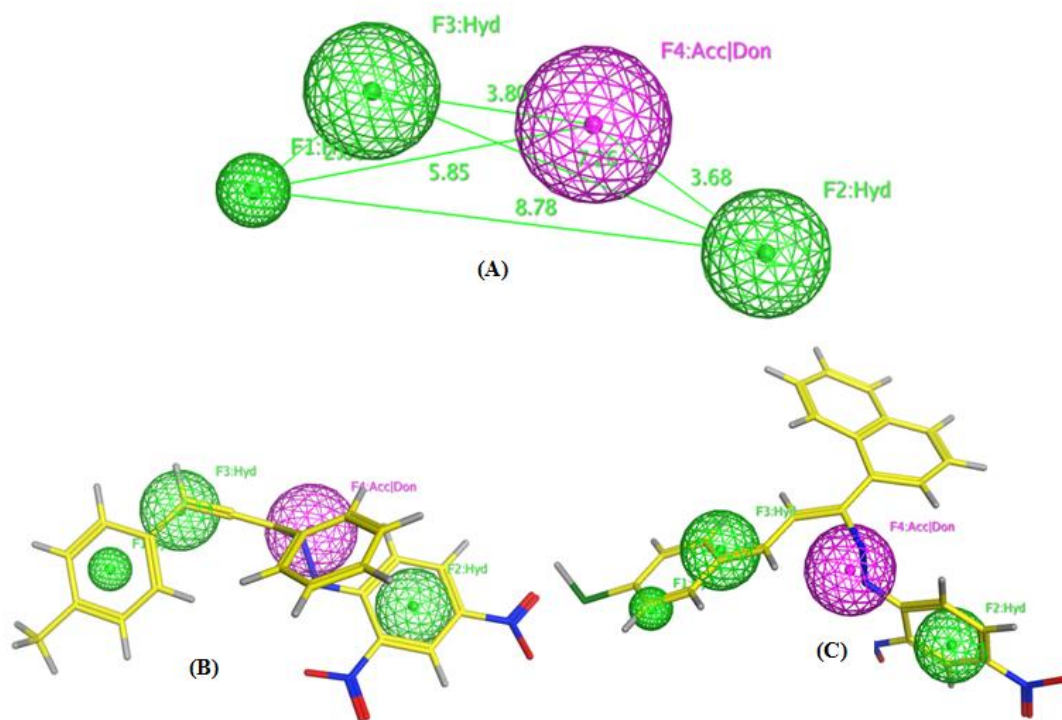


Figure S16. Pharmacophore model and its mapping to representative compounds. (A) The topological features of the model with distances in Angstrom: the magenta contour represents acceptor/donorH-bond (Acc/Don), green contour represents hydrophobic region (Hyd). (B) H1 mapped with the model. (C) H2 mapped with the model.

Table S1. Selected geometrical and energetic parameters of the H1, H2 and SBH compounds obtained by B3LYP, HOMO-LUMO gaps are in eV. The bond distances are given in Å and angles in (°).

HOMO-LUMO gap (eV)	3.08	2.93	2.99
Bond length (Å)			
Average O-N (NO ₂)	1.243	1.269	1.249
N-N	1.360	1.357	1.368
N-H	0.916	0.954	0.942
N-C ring 1	1.357	1.360	1.351
N-C (hydrazone)	1.297	1.297	1.287
Average C-C ring 1	1.402	1.401	1.400
Average C-C ring 2	1.395	1.406	1.396
Average C-C ring 3	1.396	1.394	-
Natural populations (NPA)			
Average N (NO ₂)	+0.54	+0.55	+0.56
N (N=C)	-0.26	-0.27	-0.25
N (N-H)	-0.35	-0.37	-0.36
Average O	-0.40	-0.41	-0.42

Table S2. The molecular properties (HOMO and LUMO energies, HOMO-LUMO gap, Ionization potential (IP), Electron affinity (EA), Chemical potential and electrophilicity in eV and the dipole moment in Debye) calculated for the H1, H2 and SBH compounds obtained by B3LYP functional. BDEs are given in kcal/mol and spin densities are in parentheses.

Molecular property	H1	H2	SBH
E _{HOMO}	-6.321	-6.328	-6.275
E _{LUMO}	-3.244	-3.392	-3.282
HOMO-LUMO gap	3.08	2.97	2.99
Ionization potential (IP)	6.321	6.328	6.275
Electron affinity (EA)	3.244	3.392	3.282
Chemical hardness (η)	1.54	1.485	1.495
Chemical potential (μ)	-4.7825	-4.860	-4.7785
Electrophilicity (ω)	7.42	7.95	7.64
Dipolar moment	7.88	9.09	8.58
BDE and spin density			
N-H	66.3 (0.3775)	60.7 (0.3765)	62.7 (0.36)
O-H	-	-	59.3 (0.59)

Table S3. Zones of inhibition (mm) of the synthesized analogs (H1 and H2) and the antibiotic gentamicin in the disc diffusion test.

Samples		H1					H2					Gentamicin
Concentrations (mg/mL)		0.5	1	2	4	8	0.5	1	2	4	8	10 µg/mL
<i>E. coli</i>	Zones of inhibition (mm)	7	9	9	7	8	8	7	7	9	11	34
<i>S. aureus</i>		–	–	–	–	–	–	–	–	–	–	24
<i>P. aeruginosa</i>		–	–	–	–	–	–	–	–	–	–	30

Table S4. In vitro antioxidant activity of SBH and Standard after 60 mn.

Samples	Inhibition % at different concentrations (µg/mL)						IC ₅₀ (µg/mL)
	25	50	100	200	300	400	
SBH	8.24	12.76	22.61	42.20	57.80	68.08	269.55
As. Acid (Standard)	14.01	25.35	46.98	89.18	96.98	96.45	85.49

Table S5. Docking result of the co-crystallized, H1, H2, and SBH ligands within the active site of the gyrase enzyme.

	Binding energy (Kcal/mol)	Hydrogen interactions	Hydrophobic interactions	Van der Waals interactions	Electrostatic interactions
Novobiocin (co-crystallized)	-7.4	Asp81, Asn54, Arg144, Asp89, Ser128	Pro87, Ala98, Ile102, Arg84	Gln91, Gly85, Asp57, Thr173, Ser55, Gly125	Arg84, Glu58
SBH	-6.3	Gly85, Ser128, Gly125	-	Asp57, Asn54, Ser55, Asp81, Thr173, Glu58, Gly83, Pro87, Ile86, Ile102	-
H1	-7.7	Thr173, Ser55, Gly85, Glu58, Arg84	Ile86, Asn54, Ile175, Pro87	Gly125, Asp57, Ile102, Val174, Ile51, Val79, Asp81, Gly85, Arg144	Arg84, Glu58
H2	-7.5	Gly85, Thr173, Arg84, Ser55, Glu58	Ile102, Ile86, Asn54	Gly125, Pro87, Ile175, Ile51, Asp81, Glu58, Gly83, Gly172, Asp57	-

Table S6. Drug-likeness properties of the three hydrazone analogs SBH, H1, and H2.

	MW g/mol	LogP	Log S	HBA	HBD	TPSA (Å ²)	AMR	Lipinski	Ghose	Veber	Egan	Muegge
SBH	321.18	0.69	3.92	8	0	136.29	76.42	Yes	Yes	Yes	No	Yes
H1	402.40	2.71	5.88	5	1	115.49	120.26	Yes	No	Yes	Yes	No
H2	472.88	3.73	7.30	5	1	115.49	137.81	Yes	No	Yes	No	No

LogP: Lipophilicity; LogS: Water Solubility; HBA: Num. H-bond acceptors; HBD: Num. H-bond donors; TPSA: Topological polar surface area ; AMR: Atom Molar Refractivity

Table S7. ADMET properties of the three hydrazone analogs SBH, H1, and H2.

	BBB	Caco2	HIA	P-gp inhibitor	CYP _{1A2} inhibitor	CYP _{2C19} inhibitor	CYP _{2C9} inhibitor	CYP _{2D6} inhibitor	CYP _{3A4} inhibitor	Ames mutagenesis	Carcinogenicity	hERG_inhibition	H-HT
SBH	No	High	High	No	No	Yes	No	No	No	No	No	No	Yes
H1	No	Medium	Medium	No	Yes	Yes	Yes	No	No	No	No	No	Yes
H2	No	Medium	Medium	No	No	Yes	No	No	No	No	No	No	Yes

BBB: Blood-Brain Barrier; Caco2: Permeability assay; HIA: Human Intestinal Absorption; hERG: human Ether-a-go-go-Related Gene potassium channel; H-HT: Human Hepatotoxicity.