

Supplementary Material

Biological evaluation and molecular modelling study of thiosemicarbazide derivatives as bacterial type IIA topoisomerases inhibitors

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1. Chemistry	S2
2. Computational proceduresS3
3. Fig. S1S4
4. Fig. S2S5
5. Table S1S7
6. ReferencesS7

1. Chemistry

All commercial reactants and solvents were purchased from either Sigma-Aldrich or Lancaster with the highest purity and used without further purification. The melting points were determined on a Fischer-Johns block and are uncorrected. Elemental analyses were determined by a AMZ-CHX elemental analyzer and are within $\pm 0.4\%$ of the theoretical values. IR spectra were recorded in KBr using a Specord IR-75 spectrophotometer. $^1\text{H-NMR}$ spectra were recorded on a Bruker Avance (300 MHz) spectrometer. Analytical thin layer chromatography was performed with Merck 60F₂₅₄ silica gel plates and visualized by UV irradiation (254 nm).

Physicochemical characterizations of compounds **3-6**, **8**, and **11** were presented in our previous contributions (1,2,3). The structure of **9** (CAS number 448929-00-8) is known, however there is no reference reporting its use or preparation or physicochemical characterization, therefore its data has been included into the manuscript.

Procedure for synthesis 4-benzoylthiosemicarbazides 7, 9, and 10

A reaction mixture of carboxylic acid hydrazide (0.01 mole) and benzoyl isothiocyanate (0.01 mole) was heated in an oil bath at 70-80 °C and progress of reaction was monitored by thin layer chromatography. After 10-12 h, the reaction was completed and the crude reaction mixture was washed with diethyl ether and crystallized from ethanol.

4-benzoyl-1-(2-methyl-furan-3-ylcarbonyl)thiosemicarbazide 7

Yield: 92 %. Mp. 152-4 °C. $^1\text{H NMR}$ (DMSO- d_6) δ (ppm): 2.55 (s, 3H, CH₃, $J= 6.9$ Hz); 6.95-6.96 (d, 1H, CH_{furan}); 7.52-7.57 (m, 2H, 2 \times CH_{arom}); 7.59-7.60 (d, 1H, CH_{furan}); 7.64-7.70 (m, 1H, CH_{arom}); 7.97-7.99 (m, 2H, 2 \times CH_{arom}); 10.68, 11.74, 12.25 (3s, 3H, 3 \times NH). IR (KBr, ν , cm^{-1}): 3427, 3218, 3087, 1675, 1605, 1569, 1485, 1305, 1196, 742. Anal. C₁₄H₁₃N₃O₃S (C, H, N).

4-benzoyl-1-(thiophen-2-ylcarbonyl)thiosemicarbazide **9**

Yield: 95 %. Mp. 173-5 °C. ¹H NMR (DMSO-d₆) δ (ppm): 7.21-7.24 (dd, 1H, CH_{thiophene}); 7.52-8.03 (m, 7H, 5×CH_{arom} & 2×CH_{thiophene}); 11.14, 11.76, 12.00 (3s, 3H, 3×NH). IR (KBr, ν, cm⁻¹): 3270, 3064, 1651, 1598, 1580, 1526, 1435, 1240, 801, 771, 709. Anal. C₁₃H₁₁N₃O₂S₂ (C, H, N).

4-benzoyl-1-(3,3-diethoxypropionyl)thiosemicarbazide **10**

Yield: 88 %. Mp. 134-6 °C. ¹H NMR (DMSO-d₆) δ (ppm): 1.15-1.20 (t, 6H, 2×CH₃); 3.51-3.71 (m, 4H, 2×CH₂); 4.92-4.96 (t, 3H, CH); 7.55-7.62 (m, 2H, 2×CH_{arom}); 7.69-7.72 (m, 1H, CH_{arom}); 7.99-8.03 (m, 2H, 2×CH_{arom}); 11.05, 11.75, 12.75 (3s, 3H, 3×NH). IR (KBr, ν, cm⁻¹): 3263, 3060, 1655, 1581, 1510, 1430, 1228. Anal. C₁₅H₂₁N₃O₄S (C, H, N).

2. Computational procedures

Conformational search, physicochemical parameters, and HOMO/LUMO maps were calculated using HyperChem8.0.1 (4). Extensive conformational searches were carried out using the molecular mechanics level with MM+ force field. The most stable structures obtained were subsequently optimized to the closest local minimum at the semiempirical level using RM1 parametrization. Convergence criteria were set to 0.1 and 0.01 kcal/mol/Å for MM+ and RM1 calculations, respectively. Electrostatic potentials were calculated and visualized using Gaussian 09 and GaussView 5 (5), respectively at the HF/6-31G level (6).

Docking studies: FlexX and HYDE docking algorithms (7) as implemented in LeadIT platform of BioSolveIT program (8) were used with default parameters. Model of the ATP binding site based on the structure deposited in the Protein Data Bank (9) under the PDB ID 3U2K (10) and model of the DNA binding pocket 4BUL under the PDB ID 4BUL (11) were employed. The receptor for FlexX was prepared using default setting; active sites were defined to include all residues within 6.5 Å radius of the native ligand, Maximum Allowed

Overlap Volume = 2.9 \AA^3 (i.e., hard docking), and hybrid Enthalpy and Entropy strategy. The Clash Factor was set to 0.6. For consistency across all structures screened water molecules were removed. The top ranked docking (with the highest *score*) poses from each docking run were used. The FlexX scoring function (12,13) is given by the general formula:

$$\text{score} = \text{match} + \text{lipo} + \text{ambig} + \text{clash} + \text{rot}$$

where *match* – component of main score for matched interacting groups (hydrogen, metal and specific aromatic interactions), *lipo* – lipophilic interactions term (for nonpolar atom pairs), *ambig* – lipophilic interactions (for polar-nonpolar atom pairs), *clash* – penalty for overlapping atoms of a ligand and a protein, and *rot* – weighted number of rotatable bonds were not used basing on our preliminary study (14).

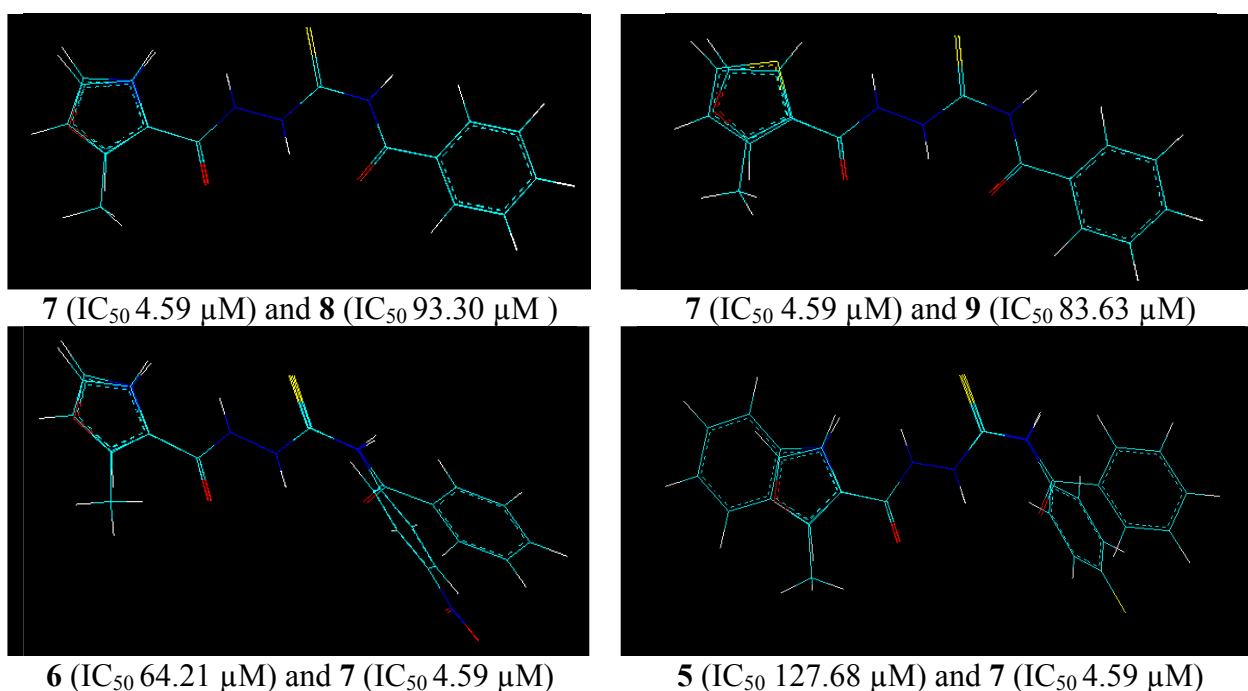
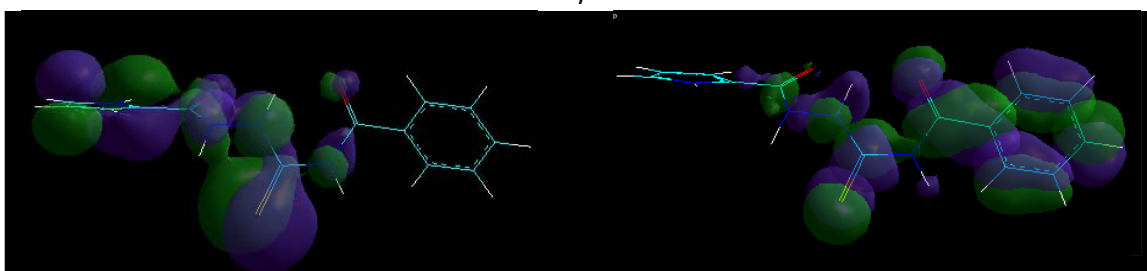
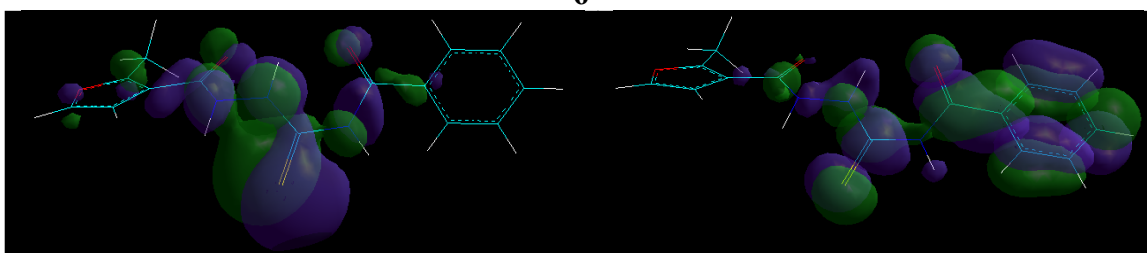
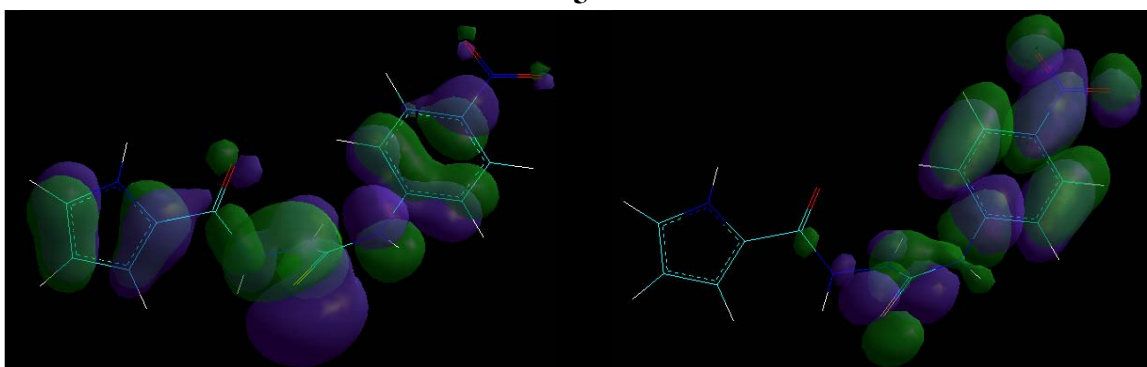
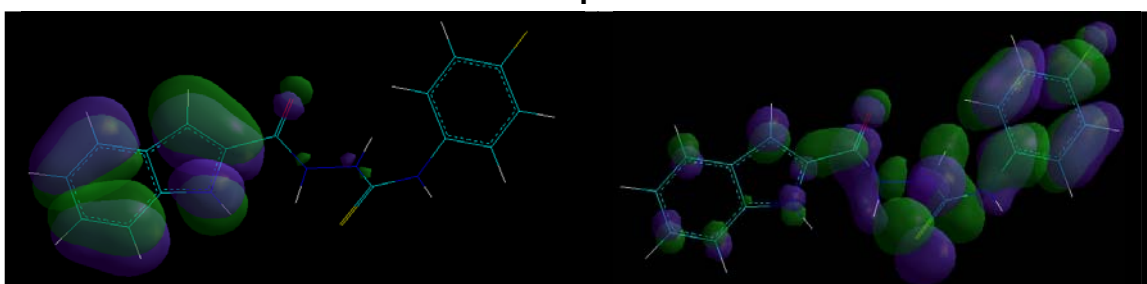
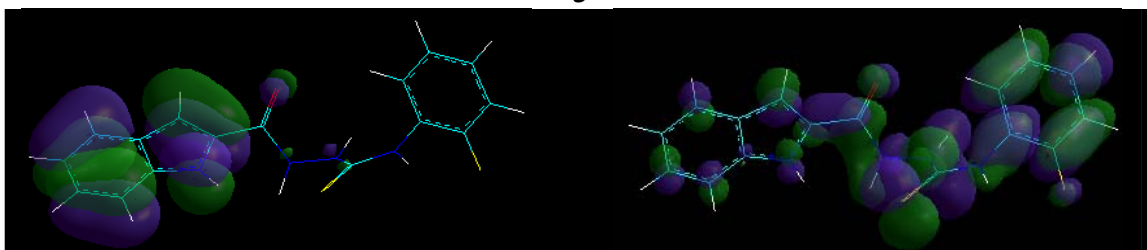
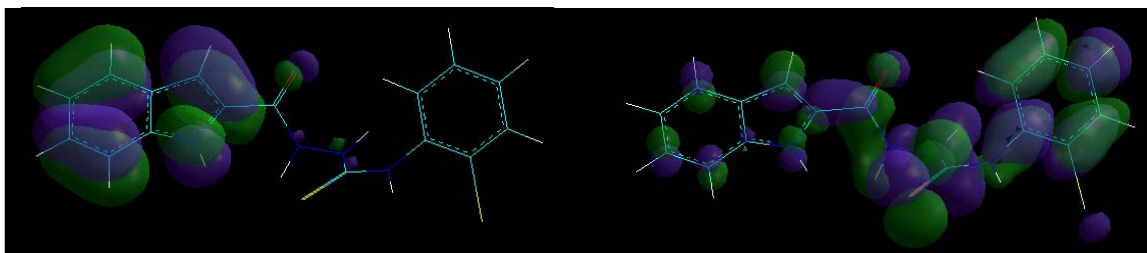


Fig. S1. Overlay of the structures of compounds with inhibitory potency against *S. aureus* DNA gyrase



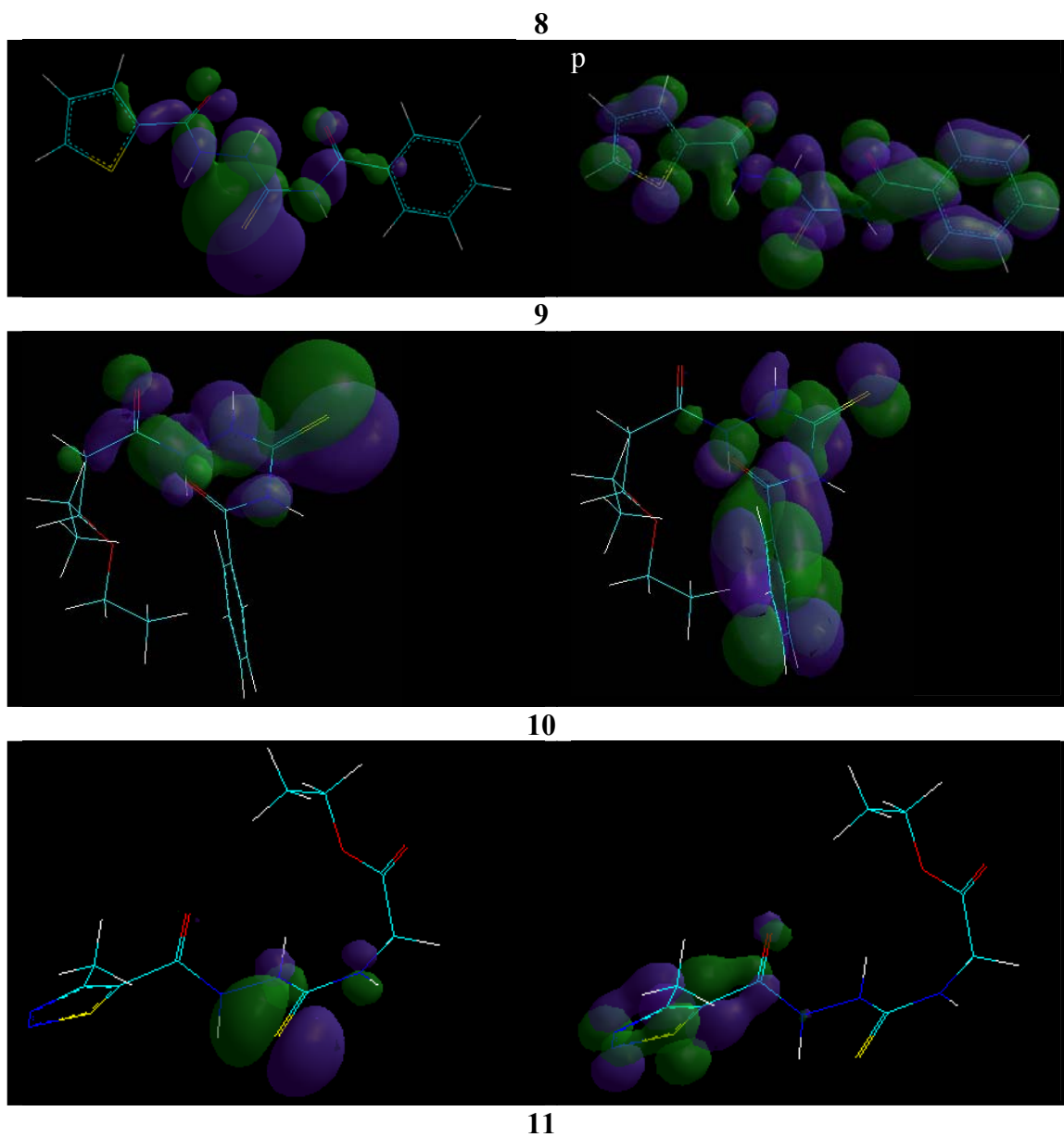


Fig. S2. HOMO (left) and LUMO (right) maps of thiosemicarbazide derivatives **3-11**

Table S1. SAR parameters^a of thiosemicarbazide derivatives **3-11**

Cmpd.	clogP	HE	SA	V	Rf	α	μ	HOMO	LUMO	HLG	HF
3	3.94	-13.80	562.14	934.56	97.73	39.04	2.81	-8.75	-0.68	-8.07	738.85
4	3.29	-14.01	536.12	885.25	90.32	36.33	3.00	-8.74	-0.67	-8.07	734.35
5	3.29	-14.34	533.62	884.36	90.32	36.33	1.92	-8.75	-0.69	-8.06	733.57
6	-1.53	-20.38	504.49	812.59	80.10	30.99	3.09	-9.28	-1.34	-7.94	982.03
7	2.32	0.33	524.05	852.32	81.57	31.70	1.96	-9.07	-0.57	-8.50	869.42
8	1.89	-15.40	515.35	817.77	78.55	31.07	3.25	-9.07	-0.61	-8.46	720.25
9	2.41	-13.15	510.34	826.14	82.74	33.32	2.08	-9.16	-0.64	-8.52	925.48
10	1.97	-9.35	543.68	941.87	90.17	35.69	8.08	-8.72	-0.42	-8.30	857.61
11	0.52	-13.91	476.58	852.22	77.20	30.22	4.53	-9.32	-1.16	-8.16	604.86

^asurfaces and refractivity in Å², volumes and polarizability in Å³, moment dipoles in D, energies in kcal/mol

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