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Laboratory note

Synthesis and structure–activity relationship studies of 4-arylthiosemicarbazides as topoisomerase IV inhibitors with Gram-positive antibacterial activity. Search for molecular basis of antibacterial activity of thiosemicarbazides

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ABSTRACT

1-(indol-2-carbonyl)-4-(4-nitrophenyl)-thiosemicarbazide was synthesized and antibacterial and type IIA topoisomerases (DNA gyrase and topoisomerase IV) activity was evaluated. It was found that it shows activity against Gram-positive bacteria with MICs of 50 μg/mL and inhibitory action against topoisomerase IV with an IC_{50} of 14 μM. Although modification of its structure resulted in molecules with a lower biological profile, our observations strongly implicate that thiosemicarbazide derivatives participate in at least two different mechanisms of antibacterial activity; one is connected with the inhibition of topoisomerase IV, while the nature of the other cannot be elucidated from the limited data collected thus far. The differences in bioactivity further investigated by the molecular modeling approach and docking studies suggest that inhibitory activity of 4-arylthiosemicarbazides is connected with electronic structure rather than the geometry of the molecule.

1. Introduction

Increasing antibiotic resistance of bacterial pathogens has been observed for many years, both in Gram-negative and Gram-positive bacteria, concerning the isolates from hospitalized and ambulatory patients. [1–5] Therefore, significant efforts have been made by many research groups to improve the potency and antibacterial spectrum of existing drugs. [5–9] A major goal, however, is that of finding new chemical entries rather than continuing the search for new members of already defined chemical classes. [10–13]

Attractive targets in the search for new antibiotics are well-studied bacterial type IIA topoisomerases DNA gyrase and topoisomerase IV, two highly homologous enzymes that play essential roles in bacterial DNA replication, chromosome segregation, and DNA compaction. [14–21] These enzymes act by transporting one segment of the DNA duplex through transient protein-linked double-strand breaks in another DNA segment. This ATP-dependent strand-passing reaction plays an essential role in modulating DNA supercoiling, knotting, and catenation. DNA gyrase and topoisomerase IV are structurally and mechanistically related but have acquired distinct characteristics during evolution. DNA gyrase facilitates DNA unwinding at replication forks, and topoisomerase IV has a specialized function in mediating the decatenation of interlocked daughter chromosomes. Both DNA gyrase and topoisomerase IV are heterotetramers consisting of two A and two B subunits (A_2B_2). The A subunit contains the active-site tyrosine involved in the DNA breakage–reunion activity while the B subunit catalyzes ATP hydrolysis. [19,22]

Recently, [23] in the search for novel bacterial topoisomerase inhibitors, we have reported that two of our compounds, 4-benzoyl-1-(indol-2-yl)-carbonylthiosemicarbazide (compound **1p**, Fig. 1), and 4-benzoyl-1-(4-methyl-imidazol-5-yl)-carbonylthiosemicarbazide (compound **5p**, Fig. 1) represent a new class of topoisomerase IV inhibitors. Based on the DFT and docking studies it was proposed that the inhibitory activity of 4-benzoylthiosemicarbazides is strongly connected with the geometry of the molecule. In order to expand our initial disclosure with further details on the structural features required for bioactivity of thiosemicarbazide derivatives, new analogs with 4-arylthiosemicarbazide skeleton were synthesized and tested for their antibacterial and topoisomerases (DNA gyrase and

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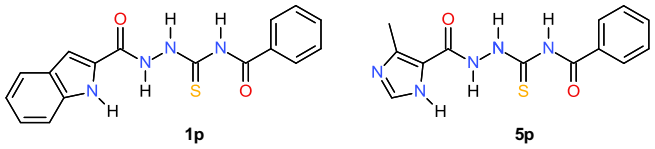


Fig. 1. Structures of bacterial topoisomerase IV inhibitors with 4-benzoylthiosemicarbazide skeleton.

topoisomerase IV) inhibitory activities. Thus, the present study reports the synthesis, the biological profile, and the structure–activity relationship (SAR) evaluation of three series with 4-nitrophenyl-thiosemicarbazide (series **o**), indole–thiosemicarbazide (series **1**), and thiadiazole–thiosemicarbazide (series **2**) core. In SAR studies, biological properties of these molecules were compared with several theoretical parameters frequently used in SAR considerations. Since the geometry of the molecule may be important for a topoisomerase IV inhibitory action, conformational studies were carried out. In addition, the distribution of the frontier orbitals HOMO and LUMO as well as the electrostatic potentials for geometries that resulted from docking studies were analyzed as these features may be related to the interaction of the molecule with the target receptor.

2. Results and discussion

2.1. Chemistry

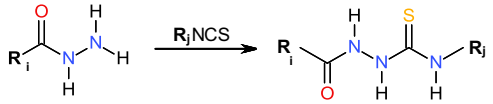
The title 4-arylthiosemicarbazides were synthesized based on known procedure [24–27] in the reaction of related heterocarboxylic acid hydrazide with phenyl isothiocyanate that contained electron-rich or electron-poor substituent (Scheme 1). In this way, twenty compounds with the general formula R_i–(C=O)–NH–NH-(C=S)–NH–R_j were obtained. Substituents R_i (i = 1, 2, 3 …) at the carbon atom and R_j (j = a, b, c …) at the nitrogen atom that were used in this study are listed in Table 1. A combination of the number "i" and the letter "j" allows for the unique identification of a compound, e.g., **1o** corresponds to 1-(indol-2-carbonyl)-4-(4-nitrophenyl)-thiosemicarbazide, and is used in the following sections.

2.2. Biological evaluation

As starting compound for our study, 1-(indol-2-carbonyl)-4-(4-nitrophenyl)-thiosemicarbazide **1o**, new analog of previously described **1p**, [23] was synthesized and antibacterial and type IIA topoisomerases (DNA gyrase and topoisomerase IV) activity evaluated. It was found that it shows activity against Gram-positive bacteria with MICs of 50 μg/mL (see Table 2) and inhibitory activity against topoisomerase IV with an IC_{50} of 14 μM. To confirm that 4-nitrophenylthiosemicarbazide scaffold can be developed into an efficient Gram-positive antibacterials targeting topoisomerase IV, three new analogs **2o**–**4o** with five-membered heterocyclic ring were synthesized. Based on our previous experiments it was expected that replacement of the indole with a smaller sized heterocyclic ring would result in improved antibacterial activity. Surprisingly, among tested compounds only **2o** was found to be active against Gram-positive stains. However, except for *Micrococcus luteus* ATCC 10240, it was ~2-fold weaker in activity as compared to **1o**. Moreover, it had not shown inhibitory action against both DNA gyrase and topoisomease IV. Based on these results it was proposed that either 4-nitrophenylthiosemicarbazide scaffold is not the key functionality required for antibacterial and topoisomerase IV inhibitory activity or physicochemical properties of molecule have very pronounced influence on its bioactivity. In hope to provide deeper insight into molecular basis of antibacterial activity of thiosemicarbazide derivatives, two series with indole–thiosemicarbazide **1** and thiadiazole–thiosemicarbazide **2** skeleton were synthesized and their biological potency was evaluated. As can be seen from the results collected in Table 2, for series of 4-aryl-1-(indol-2-yl)-carbonylthiosemicarbazides **1** substitution of the *para*-position of phenyl ring with an electron withdrawing fluoro group, exemplified by **1h**, resulted in 2-fold increase in activity against *M. luteus* ATCC 10240 as compared to the lead **1o**. Its antibacterial activity was also observed against the other Gram-positive strains tested, however with 2-fold higher MICs than that of **1o**. Addition of a second fluoro group in the *ortho*-position, exemplified by **1i**, decreased slightly the activity in comparison to **1h**. Substitution of *ortho*-position of the phenyl ring with fluoro **1f** or bromo **1k** groups was not well tolerated; the molecules were found to be 2–4-fold less active than that of **1o**. Subsequently, the antibacterial potency of 4-aryl-1-(4-methyl-thiadiazol-5-yl)-carbonylthiosemicarbazides **2** was tested. Compounds with an electron donating *para*-methoxy **2d** and *ortho*-methyl **2b** groups were inactive. Among molecules with an electron withdrawing substituents, *para*-chloro **2m** showed the highest antibacterial potency, MIC range 50–100 μg/mL. Biological activity diminishes regardless of whether chlorine atom in **2m** was replaced by less hydrophobic fluorine **2h** or more hydrophobic bromine **2l**. Addition of a second chloro group in *ortho*-position, exemplified by **2n**, led to ~2-fold decrease in activity. The same trend was observed when antibacterial activities of **2h** and **2i** were analyzed; *para*-fluoro **2h** had antibacterial potency with MIC range 100–400 μg/mL while 2,4-difluoro **2i** was inactive. These observations together seem to indicate that *ortho*-substitution is not well tolerated in this chemical series. This hypothesis is supported by the antibacterial results for **2j** and **2k**.

Presented above results of antibacterial assay suggest that the position and the identity of individual substitutions are important parameter influencing the activity of studied 4-arylthiosemicarbazides. *Para*-substitution seemed to be more important to increase the antibacterial activity than substitution at the *ortho*-position of the phenyl ring. Substitution of the phenyl ring by an electron withdrawing groups seemed to be better than that by an electron donating substituents. In order to clarify the effect of steric and electronic properties of substituents on antibacterial potency of tested thiosemicarbazides, some parameters such as the bulk parameter based on molar refraction MR and electronic parameters F and R were taken from the literature [28] and correlated with biological activity, see Table 3. Experiments have shown [29,30] that a parameter representing the volume of the substituents on each compound, relative to other members of the same series, may often be linearly correlated with the biological response. However, lack of relationship between antibacterial activity of series **1** and **2** and the bulk parameter MR or positionally weighted parameters F and R were observed. Thus, physicochemical properties of substituents are only secondary parameters for antibacterial activity of title 4-arylthiosemicarbazides.

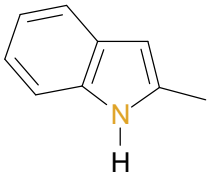
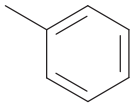
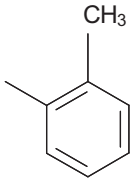
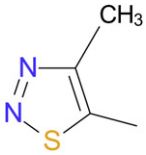
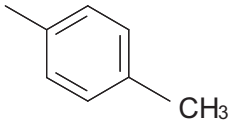
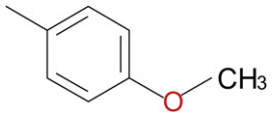
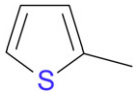
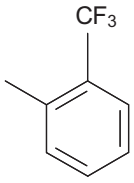
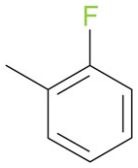
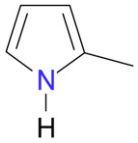
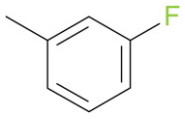
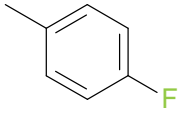
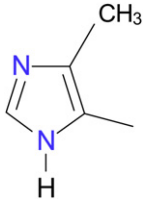
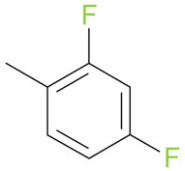
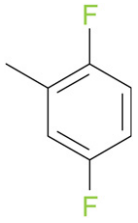
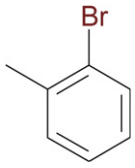
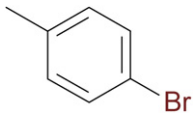
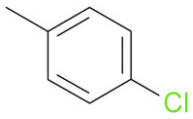
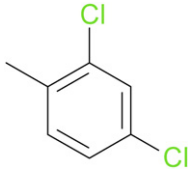
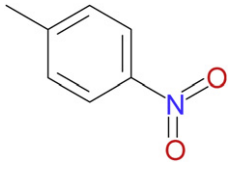
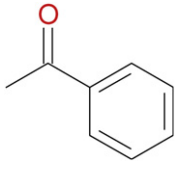
Considering the results of the *in vitro* antibacterial assay, we have conducted the DNA gyrase and topoisomerase IV inhibition tests for **1i** which showed high therapeutic potential against *M. luteus* ATCC 10240 with MIC of 25 μg/mL. Among series **2** we have selected the most active **2m**, two less active **2h** and **2l**, and, in order to exclude permeability barrier, efflux, *in vivo* affinity, [31] two



R_i = 1-5; R_j = a-o (for symbols used to identify studied compounds see Table 1)

Scheme 1.

Table 1
Symbols used to identify studied compounds using substituents at thiosemicarbazide moiety.

1		**a**		**b**	
2		**c**		**d**	
3		**e**		**f**	
4		**g**		**h**	
5		**i**		**j**	
k		**l**		**m**	
n		**o**		**p**	

inactive **2i** and **2k**. Unfortunately, among tested compounds only **1i** and **2l** were found as weak topoisomerase IV inhibitor with IC_{50} of 295 μM and 403 μM, respectively. Based on these findings we can support our previously conclusion that thiosemicarbazides participate in at least two different mechanisms of antibacterial activity; one is connected with inhibition of topoisomerase IV, while the nature of the other cannot be elucidated from the limited data collected thus far.

2.3. Computational part

Initially we have calculated several parameters that are frequently used in SAR studies to identify their role in modulating the biological profile of title 4-arylthiosemicarbazides. As known, lipophilicity of molecules has an important effect on their biological activity. For the compounds, however, no close correlation between lipophilicity, expressed as clogP, and biological activity was found.

Table 2
In vitro antibacterial activity (MIC [μg/mL]) of thiosemicarbazides.

Compound	*S. aureus*	*S. epidermidis*	*M. luteus*	*B. subtilis*	*B. cereus*
1o	50[a,b,c,d]	50[e]	50[f,g]	50[h]	50[i]
2o	100[a,b,c] 200[d]	100[e]	100[f] 50[g]	100[h]	100[i]
1f	200[a,c,d] 100[b]	200[e]	100[f,g]	100[h]	200[i]
1h	100[a,b,c,d]	100[e]	50[f] 25[g]	100[h]	100[i]
1i	100[a,b,c] 200[d]	100[e]	100[f] 25[g]	100[h]	100[i]
1k	200[a,c] 100[b,d]	200[e]	100[f,g]	200[h]	200[i]
2a	400[a,b,c,d]	400[e]	100[f] 200[g]	400[h]	400[i]
2e	100[a] 50[b] 400[c] 200[d]	200[e]	200[f,g]	200[h]	400[i]
2g	200[a,b] 400[c,d]	400[e]	200[f] 100[g]	400[h]	400[i]
2h	400[a,c,d] 200[b]	200[e]	100[f,g]	200[h]	200[i]
2j	400[a,c,d] 200[b]	400[e]	100[f] 200[g]	200[h]	400[i]
2l	200[a,c,d] 100[b]	200[e]	50[f] 100[g]	100[h]	100[i]
2m	100[a,c] 50[b,d]	50[e]	100[f] 50[g]	50[h]	50[i]
2n	100[a,b,c,d]	100[e]	100[f,g]	100[h]	100[i]
Ciprofloxacin	0.5[a,b,c,d]	0.5[e]	4[f] 2[g]	<0.125[h]	1[i]

Strains:
[a] NCTC 4163.
[b] ATCC 25923.
[c] ATCC 6538.
[d] ATCC 29213.
[e] ATCC 12228.
[f] ATCC 9341.
[g] ATCC 10240.
[h] ATCC 6633.
[i] ATCC 11778.

As can be seen from results collected in Table 3, **2j**, **2k**, and **2m** have similar level of lipophilicity but considerably different level of antibacterial activity. In the series of 4-nitrophenylthiosemicarbazides **o** two antibacterials **1o** and **2o** have clogP 3.10 and 0.44, respectively while **3o** with clogP 0.92, **4o** with clogP 0.40, and **5o** with clogP −3.51 were inactive. Moreover, **1o** exhibited a strong inhibitory action against topoisomerase IV while **1i**, **2m**, **2k**, and **2l**, with comparable level of lipophilicity to **1o**, were slightly active or inactive. No correlation was also observed between the positionally weighted distributive parameter π, which is considered the parameter of choice for correlating both binding to biological macromolecules and transport through a biological system, [32–35] and biological activity of studied thiosemicarbazides. On the other hand, in analyzing the physicochemical parameters presented in Table 3 there are some SAR trends that were evident. As can be seen, the most active **1o** presents the highest values of surface area (SA), volume (V), core–core interaction (I_{C-C}), and heat of formation (HF). Moreover, it possesses higher values of refractivity (R_f) and polarizabilitiy (α) and lower values of total energy (E_T), isolated atomic energy (E_{IA}), and electronic energy (E_E) than the other compounds. In this way it can be proposed that both structural and electronic properties of 4-arylthiosemicarbazides are important for their biological potency while lipophilicity is only a secondary parameter.

As it was mentioned in the introduction, in our previous paper [23] we have proposed that a very important factor influencing the inhibitory effect of 4-benzoylthiosemicarbazides on topoisomerase IV activity is the geometry of the molecule. It was observed that the geometries of biologically active inhibitors **1p** and **5p** were almost similar and considerably different from the structure of inactive **ap**. Thus, in the search for the steric motif that may be responsible for the inhibitory action of 4-arylthiosemicarbazides, which differ from 4-benzoylthiosemicarbazides in the "length" of the molecule, conformational analysis of thiosemicarbazides from series **o**, **1**, and **2** has been performed. The 4-arylthiosemicarbazides skeleton has 6 rotatable single bonds thus huge conformational space that cannot be searched systematically at either *ab initio* or DFT levels should be sampled in order to find the most stable conformations. We have therefore carried out extensive conformational searches at the molecular mechanics level with OPLS force field implemented in HyperChem which gives very good results for geometries and the energetics of this class of compounds. [36] The most stable structures obtained were subsequently optimized to the closest local minimum at the semiempirical level using RM1 parametrization, which has been shown to perform very well for *s*-triazoles, cyclic derivatives of thiosemicarbazides. [37] As illustrated in Fig. 2, the most stable conformations of inhibitors **1o**, **1i**, **2l** are similar. Confusingly, the superposition of the conformers of active **1o** and inactive **2o** also revealed only minor deviations while structures of active **2l** and inactive **2k** are almost superimposable. This may imply that the binding of 4-arylthiosemicarbazides by the receptor depends on their electronic rather than structural properties. It was reasonable to suppose that the electrostatic interaction of thiosemicarbazides with the active site of topoisomerase IV could be explained in terms of the frontier molecular orbitals maps. [38] As expected, the molecular modeling analysis of frontier molecular orbitals revealed that the most active **1o** differs from the others in low distribution of both HOMO and LUMO around the sulfur atom, see Fig. 3, suggesting the importance of these orbitals for thiosemicarbazide-topoisomerase IV complex formation.

The thiosemicarbazide–receptor complexes were further analyzed by means of AutoDock Vina docking program using the same as the previous [23] model of the ATP binding site of *Thermus thermophilus* gyrase B (PDB 1KIJ) complexed with novobiocin as template. Default docking parameters and flexible space of 24 × 24 × 24 Å [3] were validated by re-docking native ligand which docked exactly in the position present in the crystal structure with affinity of −11.0 kcal/mol. Subsequently, the binding affinity of title thiosemicarbazides was evaluated. The overall good correlation between the bioactivity of studied compounds and the binding affinities predicted by AutoDock Vina was found as indicated in Table 4. Although all molecules, with "length" very similar to that of novobiocin, were found to be well fitted in the binding pocket of the topoisomerase (see Fig. 4) our bioassay protocol indicated that only **1o** has a significant inhibitory action most probably due to its ability to form strong hydrogen bond with Glu49 as well as a large electrostatic contribution to ligand–receptor binding. As can be seen from Fig. 5, all studied compounds are characterized by a charge distribution substantially different than that of novobiocin. The electrostatics of **1o** shows negative charge around carbonyl oxygen larger than in other compounds and resembling high charge density of the amid part of novobiocin as well as a large positive charge on the indole N–H proton resembling that of hydroxyl of novobiocin. Finally, geometries of the molecules in the active site of topoisomerase were compared with those optimized without the presence of the enzyme. No correlation between the strain energy defined as the difference of heats of formation of the structure fully optimized and the structure in the active site and the bioactivity defined by IC_{50} was observed.

Table 3
SAR parameters[a] of studied thiosemicarbazides.

Comp	MR	R	π	V	α	HOMO	HLG	χ	E_B	E_E	HF
	F	clogP	SA	R_f	μ	LUMO	η	E_T	E_{IA}	I_{C-C}	
1o	6.0	0.16	0.22	918.58	38.26	−9.06	−7.82	5.15	−3240.93	−723,604.30	980.71
	1.11	3.10	434.71	97.43	3.38	−1.24	3.91	−100,864.51	−97623.58	622,739.80	
2o	6.0	0.16	0.22	817.84	33.66	−9.33	−7.66	5.50	−2567.62	−629,588.74	822.67
	1.11	0.44	388.93	88.49	3.05	−1.67	3.83	−94,561.36	−91,993.74	535,027.38	
3o	6.0	0.16	0.22	804.79	33.24	−9.14	−7.93	5.18	−2365.93	−571,316.25	969.25
	1.11	0.92	395.83	84.80	4.77	−1.21	3.97	−87,810.35	−85,444.42	483,505.90	
4o	6.0	0.16	0.22	788.94	30.99	−9.20	−7.90	5.25	−2669.23	−580,415.93	764.65
	1.11	0.40	376.11	80.60	4.70	−1.30	3.95	−88,622.65	−85,953.42	491,793.28	
5o	6.0	0.16	0.22	820.94	31.99	−9.34	−7.92	5.38	−2899.19	−633,407.46	699.80
	1.11	−3.51	397.32	85.72	5.43	−1.42	3.96	−93,783.14	−90,883.95	539,624.32	
1f	−0.4	−0.29	0.00	876.73	36.33	−8.92	−8.27	4.79	−3274.04	−634,705.81	734.37
	0.88	3.29	389.26	90.32	6.32	−0.65	4.14	−92,772.84	−89,498.80	541,932.98	
1h	−0.4	−0.34	0.15	867.61	36.33	−8.92	−8.29	4.78	−3274.00	−633,619.74	734.41
	0.71	3.29	390.51	90.32	5.02	−0.63	4.15	92,772.80	−89,498.80	540,846.94	
1i	−0.8	–	–	874.24	36.24	−8.97	−8.26	4.84	3338.13	−695,294.52	637.07
	–	3.43	398.37	90.54	5.34	−0.71	4.13	−103,696.24	−100,358.11	591,598.28	
1k	7.6	−0.15	0.84	915.69	39.04	−8.87	−8.23	4.76	−3276.79	−626,521.90	739.47
	0.91	3.94	413.37	97.73	6.43	−0.64	4.12	−89,778.19	−86,501.40	536,743.71	
2a	–	–	–	751.36	31.82	−8.93	−7.49	5.19	−2534.37	−493,417.47	675.90
	–	2.42	328.39	81.67	3.88	−1.44	3.75	−75,544.02	−73,009.65	417,873.45	
2b	4.7	−0.12	0.49	793.16	33.65	−8.91	−7.48	5.17	−2783.28	−541,813.27	702.08
	−0.07	2.89	350.50	86.71	4.04	−1.43	3.74	−79,104.68	−76,321.39	462,708.59	
2d	6.5	−0.50	−0.12	836.30	34.29	−8.95	−7.64	5.13	−2784.72	−589,644.89	760.21
	0.41	2.17	391.57	88.13	5.30	−1.31	3.82	−86,395.69	−83,610.98	503,249.20	
2e	4.00	0.16	1.04	818.91	33.38	−9.15	−7.53	5.39	−2993.24	−728,866.77	392.49
	0.79	3.30	358.28	87.64	4.39	−1.62	3.77	−111,892.56	−108,899.32	616,974.21	
2g	−0.40	−0.12	0.22	767.27	31.73	−9.08	−7.59	5.29	−2600.92	−550,565.85	576.14
	0.69	2.56	337.81	81.88	4.47	−1.49	3.80	−86,469.88	−83,868.96	464,095.96	
2h	−0.40	−0.34	0.15	770.62	31.73	−9.15	−7.75	5.23	−2602.38	−546,963.43	574.68
	0.71	2.56	345.56	81.88	2.83	−1.40	3.88	−86,471.34	−83,868.96	460,492.09	
2i	−0.80	–	–	765.86	31.63	−9.11	−7.55	5.34	−2663.70	−606,441.76	480.14
	–	2.70	350.93	82.10	2.27	−1.56	3.78	−97,391.97	−94,728.27	509,049.79	
2j	−0.80	–	–	774.89	31.63	−9.12	−7.53	5.36	−2664.94	−608,541.73	478.91
	–	2.70	347.64	82.10	3.83	−1.59	3.77	−97,393.21	−94,728.27	511,148.52	
2k	7.60	−0.15	0.84	811.43	34.44	−9.01	−7.47	5.28	−2603.90	−540,850.80	581.01
	0.91	3.21	367.37	89.29	4.09	−1.54	3.74	−83,475.46	−80,871.56	457,375.35	
2l	7.60	−0.18	1.19	816.94	34.44	−9.09	−7.57	5.31	−2604.02	−539,278.50	580.89
	0.73	3.21	374.23	89.29	3.11	−1.52	3.79	−83,475.58	−80,871.56	455,802.92	
2m	4.80	−0.16	0.73	791.75	33.75	−9.07	−7.57	5.29	−2739.16	−540,372.12	447.99
	0.69	2.94	368.45	86.47	2.72	−1.50	3.79	−84,069.27	−81,330.11	456,302.85	
2n	9.60	–	–	831.13	35.67	−9.15	−7.59	5.36	−2943.49	−592,273.66	220.55
	–	3.46	397.76	91.28	2.00	−1.56	3.80	−92,594.05	−89,650.56	499,679.61	

[a] Surfaces and refractivity in Å^2, volumes and polarizability in Å^3, moment dipoles in D, energies in kcal/mol.

3. Conclusions

In this contribution we have synthesized twenty 4-arylthiosemicarbazides with six rotatable bonds to evaluate their antibacterial and inhibitory activity against type IIA topoisomerases DNA gyrase and topoisomerase IV and have correlated biological results with some molecular properties. The study revealed new lead 1-(indol-2-carbonyl)-(4-nitrophenyl)-thiosemicarbazide with significant action against topoisomerase IV. Although modification of its structure resulted in molecules with a lower biological profile, some indications for a molecular basis of antibacterial activity of thiosemicarbazides have been interred from SAR studies and can be summarized as follows: (i) the type and the position of substitution at phenyl ring are of great importance for antibacterial activity of 4-arylthiosemicarbazides; the most appropriate is substitution on the *para*-position by the electron withdrawing group, (ii) both 4-benzoylthiosemicarbazides and 4-arylthiosemicarbazides participate in at least two different mechanisms of antibacterial activity; one is connected with inhibition of topoisomerase IV, while the nature of the other cannot be elucidated from the limited data collected thus far, (iii) in contrast to 4-benzoylthiosemicarbazides, inhibitory activity of 4-arylthiosemicarbazides is connected with electronic structure rather than geometry of the molecule, (iv) the H-bond interactions seem to be the prevalent factors modulating inhibitory activity of both 4-benzoyl- and 4-arylthiosemicarbazides.

The above results allow for rational design of new thiosemicarbazide derivatives that should show higher affinities to ATP binding pocket of topoisomerase and thus with increased inhibitory activity.

4. Experimental section

4.1. Chemistry

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

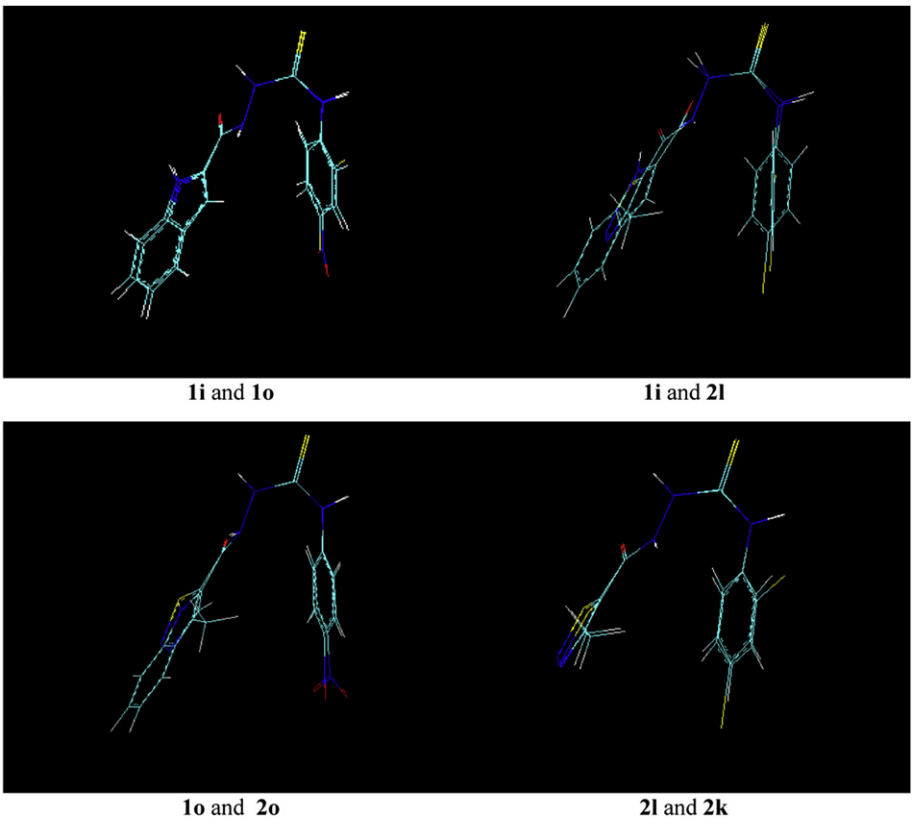


Fig. 2. Overlay of structures of the most active topoisomerase IV inhibitor **1o**, slightly active **1i** and **2l**, and inactive **2k** and **2o**.

4.1.1. General procedure for synthesis of 1-substituted-4-aryl-thiosemicarbazides

A reaction mixture of appropriate heterocarboxylic hydrazide (0.01 mol) and related isothiocyanate (0.01 mol) was heated in an oil bath at 80 °C and progress of reaction was monitored by thin layer chromatography. After 12 h, the reaction was completed and the crude reaction mixture was washed with diethyl ether and crystallized from ethanol.

Physicochemical characterization of **2a** and **2b** was presented previously. [39] Compound **3o** is commercially available.

4.1.1.1. 1-(Indol-2-carbonyl)-4-(4-nitrophenyl)-thiosemicarbazide ***1o***. Yield: (3.23 g, 91%). Mp: 230–2 °C. IR (ν, cm^{-1}) 3100 (NH), 1661 (C=O), 1625, 1577, 1510, 1491 (Ar–H), 1320 (C=S). ^{1}H-NMR (DMSO-d_6) δ_H 7.00–7.09 (m, 1H, CH), 7.14–7.26 (m, 2H, 2 × CH), 7.41–7.47 (m, 1H, CH), 7.65–7.68 (d, 1H, CH), 7.92–7.94 (d, 2H, 2 × CH), 8.20–8.23 (d, 2H, 2 × CH), 10.19, 10.23, 10.65, 11.60 (4s, 4H, 4 × NH). Anal. $C_{16}H_{13}N_5O_3S$ (C, H, N).

4.1.1.2. 1-(4-Methyl-thiadiazol-5-carbonyl)-4-(4-nitrophenyl)-thiosemicarbazide ***2o***. Yield: (3.01 g, 89%). Mp: 191–3 °C. IR (ν, cm^{-1}) 3225 (NH), 2925, 2850, 1455, 1358 (Aliph.), 1665 (C=O), 1610, 1580, 1512, 1479 (Ar–H), 1319 (C=S). ^{1}H-NMR (DMSO-d_6) δ_H 2.86 (s, 3H, CH_3), 7.85–7.90 (m, 2H, 2 × CH), 8.20–8.26 (m, 2H, 2 × CH), 10.29, 10.51, 10.97 (3s, 3H, 3 × NH). Anal. $C_{11}H_{10}N_6O_3S_2$ (C, H, N).

4.1.1.3. 4-(4-nitrophenyl)-1-(pyrrol-2-yl)-carbonylthiosemicarbazide ***4o***. Yield: (2.9 g, 95%). Mp: 230–32 °C. IR (ν, cm^{-1}) 3250 (NH), 1656 (C=O), 1620, 1579, 1521, 1487 (Ar–H), 1320 (C=S). ^{1}H-NMR (DMSO-d_6) δ_H 6.15 (s, H, CH), 6.96 (s, 2H, 2 × CH), 7.92 (d, 2H, 2 × CH), 8.18–8.21 (d, 2H, 2 × CH), 10.07, 10.17, 10.54, 11.69 (4s, 4H, 4 × NH). Anal. $C_{12}H_{11}N_5O_3S$ (C, H, N).

4.1.1.4. 4-(2-Fluorophenyl)-1-(indol-2-yl)-carbonylthiosemicarbazide ***1f***. Yield: (2.96 g, 90%). Mp: 210–12 °C. IR (ν, cm^{-1}) 3299, 3215 (NH), 3122, 1621, 1595, 1503, 1474, 744 (Ar–H), 1664 (C=O) 1314 (C=S). ^{1}H-NMR (DMSO-d_6) δ_H 7.02–7.07 (m, 1H, CH), 7.15–7.32 (m, 6H, 6 × CH), 7.43–7.46 (d, 1H, CH), 7.63–7.66 (d, 1H, CH), 9.69, 9.92, 10.62, 11.74 (4s, 4H, 4 × NH). Anal. $C_{16}H_{13}FN_4OS$ (C, H, N).

4.1.1.5. 4-(4-Fluorophenyl)-1-(indol-2-yl)-carbonylthiosemicarbazide ***1h***. Yield: (2.89 g, 88%). Mp: 198–200 °C. IR (ν, cm^{-1}) 3310, 3212 (NH), 1623, 1580, 1491, 1455 (Ar–H), 1664 (C=O), 1316 (C=S). ^{1}H-NMR (DMSO-d_6) δ_H 7.02–7.08 (m, 1H, CH), 7.13–7.23 (m, 4H, 4 × CH), 7.43–7.47 (m, 3H, 3 × CH), 7.64–7.66 (d, 1H, CH), 9.79, 9.88, 10.55, 11.73 (4s, 4H, 4 × NH). Anal. $C_{16}H_{13}FN_4OS$ (C, H, N).

4.1.1.6. 4-(2,4-Difluorophenyl)-1-(indol-2-yl)-carbonylthiosemicarbazide ***1i***. Yield: (2.94 g, 85%). Mp: 178–80 °C. IR (ν, cm^{-1}) 3319 (NH), 1630, 1589, 1499 (Ar–H), 1660 (C=O) 1322 (C=S). ^{1}H-NMR (DMSO-d_6) δ_H 7.03–7.09 (m, 2H, 2 × CH), 7.18–7.31 (m, 4H, 4 × CH), 7.44–7.47 (m, 1H, CH), 7.64–7.66 (d, 1H, CH), 9.66, 9.96, 10.63, 11.75 (4s, 4H, 4 × NH). Anal. $C_{16}H_{12}F_2N_4OS$ (C, H, N).

4.1.1.7. 4-(2-Bromophenyl)-1-(indol-2-yl)-carbonylthiosemicarbazide ***1k***. Yield: (3.43 g, 88%). Mp: 191–3 °C. IR (ν, cm^{-1}) 3305, 3212 (NH), 1618, 1579, 1501, 1470, (Ar–H), 1658 (C=O), 1327 (C=S). ^{1}H-NMR (DMSO-d_6) δ_H 7.02–7.08 (m, 2H, 2 × CH), 7.14–7.26 (m, 4H, 4 × CH), 7.34–7.46 (m, 2H, 2 × CH), 7.62 (d, 1H, CH), 9.64, 9.88, 10.60, 11.72 (4s, 4H, 4 × NH). Anal. $C_{16}H_{13}BrN_4OS$ (C, H, N).

4.1.1.8. 4-(4-Methoxyphenyl)-1-(4-methyl-thiadiazol-5-yl)-carbonylthiosemicarbazide ***2d***. Yield: (2.91 g, 90%). Mp: 118–20 °C. IR (ν,

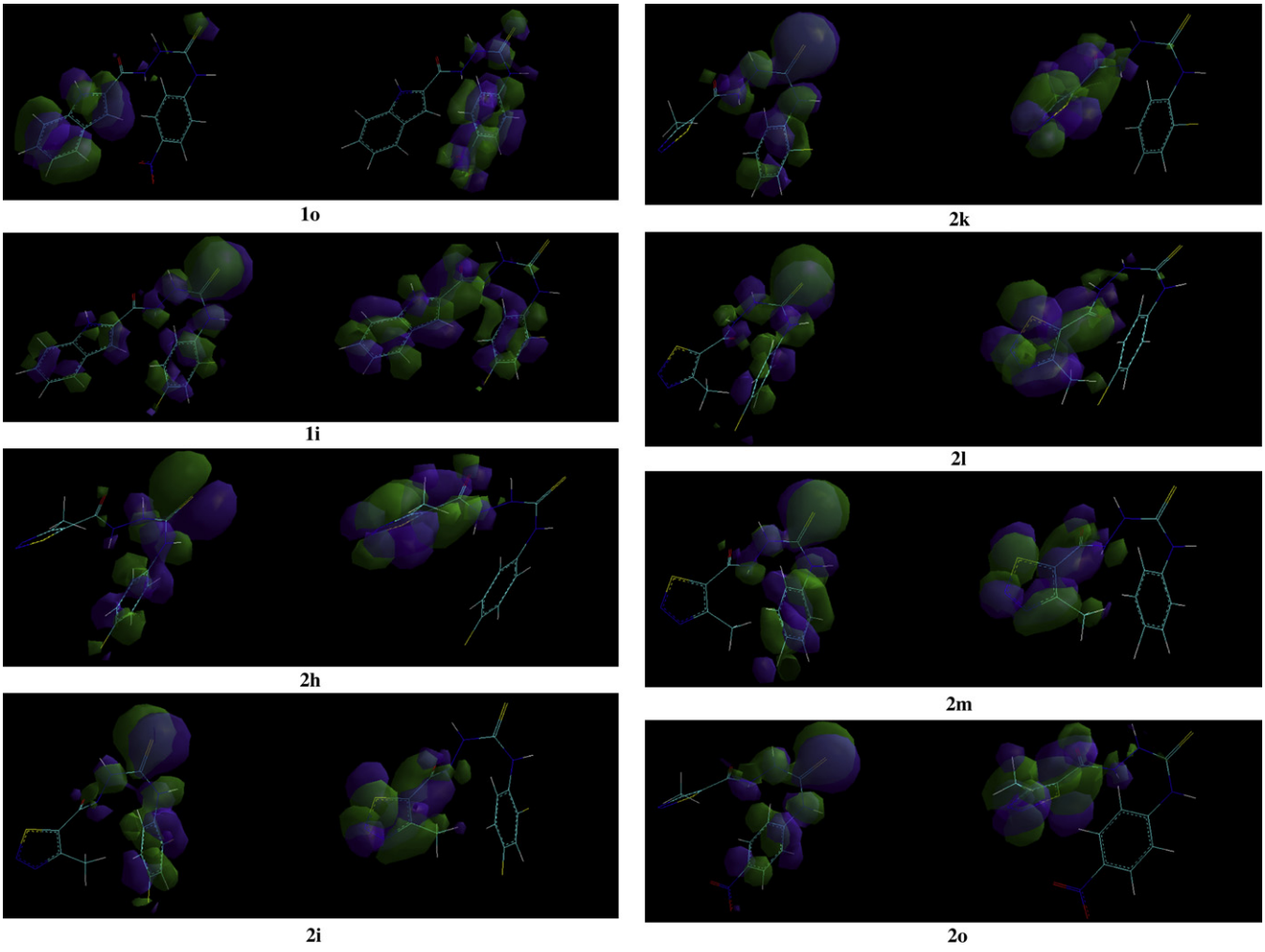


Fig. 3. HOMO (left) and LUMO (right) maps of the most active topoisomerase IV inhibitor **1o**, slightly active **1i** and **2l**, and inactive **2h**, **2i**, **2k**, **2m**, and **2o**.

cm^{-1}) 3300 (NH), 3100, 1590, 1509, 1463 (Ar–H), 2865, 1446 (Aliph.), 1675 (C=O), 1190 (C=S). ^{1}H-NMR (DMSO-d_6) δ_H 2.83 (s, 3H, CH_3), 3.75 (s, 3H, OCH_3), 6.88–6.94 (m, 2H, 2 × CH), 7.23–7.32 (m, 2H, 2 × CH), 9.76, 9.83, 10.80 (3s, 3H, 3 × NH). Anal. $C_{12}H_{13}N_5O_2S_2$ (C, H, N).

4.1.1.9. 4-(2-Trifluoromethylphenyl)-1-(4-methyl-thiadiazol-5-yl)-carbonylthiosemicarbazide ***2e****.* Yield: (3.29 g, 91%). Mp: 184–6 °C. IR (ν, cm^{-1}) 3318 (NH), 3112, 1587, 1519, 1468 (Ar–H), 3030, 1622, 1487 (Aliph.), 1668 (C=O), 1223 (C=S). ^{1}H-NMR (DMSO-d_6) δ_H 2.83 (s, 3H, CH_3), 7.47–7.60 (m, 2H, 2 × CH), 7.65–7.75 (m, 2H, 2 × CH), 9.78, 10.01, 10.97 (3s, 3H, 3 × NH). Anal. $C_{12}H_{10}F_3N_5OS_2$ (C, H, N).

4.1.1.10. 4-(3-Fluorophenyl)-1-(4-methyl-thiadiazol-5-yl)-carbonylthiosemicarbazide ***2g****.* Yield: (2.68 g, 86%). Mp: 154–6 °C. IR (ν, cm^{-1}) 3322 (NH), 3110, 1591, 1522, 1466 (Ar–H), 3033, 1629 (Aliph.), 1667 (C=O), 1235 (C=S). ^{1}H-NMR (DMSO-d_6) δ_H 2.85 (s, 3H,

Table 4
Binding free energy, H-bond contacts, and (RM1) heats of formation of the structures fully optimized and the structures of the best docking poses.

Compd.	⊿Gb (kcal/mol)	Hydrogen bonds between atoms of compounds and amino acids		$RM1_{opt}$ (kcal/mol)	$RM1_{bind}$ (kcal/mol)	⊿RM1 (kcal/mol)
		Atom of compound	Amino acid			
1i	−9.4	–	–	637.0706	693.6096	56.5
1o	−8.6	NHNHC(]S)	Glu49	980.7148	1017.589	36.9
2h	−7.8	–	–	574.6769	600.9854	26.3
2i	−7.8	–	–	480.1422	507.1369	27.0
2k	−7.8	–	–	581.0132	617.0674	36.1
2m	−7.7	–	–	447.9944	458.6842	10.7
2o	−7.6	–	–	822.6685	843.5225	20.9
2l	−7.5	–	–	580.8924	606.3458	25.5

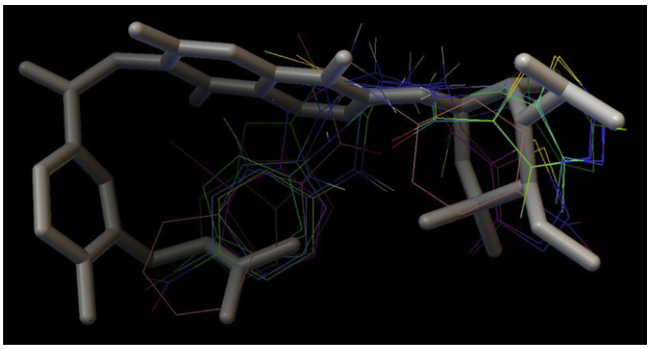


Fig. 4. Superimposition of the native ligand, novobiocin (rendered as tubes) and the best conformations of **1o**, **1i**, **2l**, **2h**, **2i**, **2k**, **2m**, and **2o** docked to the ATP binding pocket of gyrase B (1KIJ).

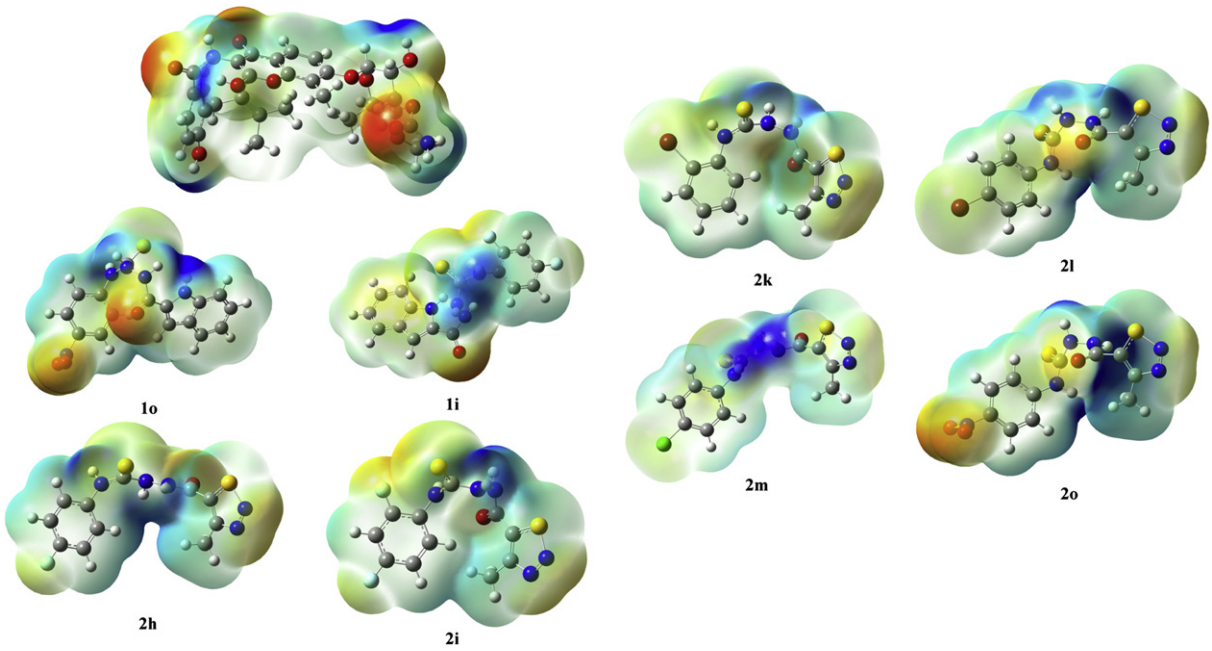


Fig. 5. Comparison of the electrostatic potential surfaces of novobiocin (top) and geometries of thiosemicarbazides that resulted from docking studies.

CH_3), 7.46–7.66 (m, 2H, 2 × CH), 7.69–7.72 (m, 2H, 2 × CH), 9.78, 10.06, 10.99 (3s, 3H, 3 × NH). Anal. $C_{11}H_{10}FN_5OS_2$ (C, H, N).

4.1.1.11. 4-(4-Fluorophenyl)-1-(4-methyl-thiadiazol-5-yl)-carbonylthiosemicarbazide ***2h***. Yield: (2.74 g, 88%). Mp: 155–7 °C. IR (ν, cm^{-1}) 3335 (NH), 3088, 1589, 1509, 1466 (Ar–H), 3055, 1632, 1479 (Aliph.), 1670 (C=O), 1239 (C=S). ^{1}H-NMR (DMSO-d_6) δ_H 2.83 (s, 3H, CH_3), 7.14–7.23 (m, 2H, 2 × CH), 7.39–7.43 (m, 2H, 2 × CH), 9.91, 10.35, 10.85 (3s, 3H, 3 × NH). Anal. $C_{11}H_{10}FN_5OS_2$ (C, H, N).

4.1.1.12. 4-(2,4-Difluorophenyl)-1-(4-methyl-thiadiazol-5-yl)-carbonylthiosemicarbazide ***2i***. Yield: (2.83 g, 86%). Mp: 148–50 °C. IR (ν, cm^{-1}) 3323 (NH), 3080, 1592, 1511, 1455 (Ar–H), 3015, 1630 (Aliph.), 1666 (C=O), 1243 (C=S). ^{1}H-NMR (DMSO-d_6) δ_H 2.84 (s, 3H, CH_3), 7.07–7.13 (m, 1H, CH), 7.29–7.36 (m, 2H, 2 × CH), 9.74, 10.09, 10.93 (3s, 3H, 3 × NH). Anal. $C_{11}H_9F_2N_5OS_2$ (C, H, N).

4.1.1.13. 4-(2,5-Difluorophenyl)-1-(4-methyl-thiadiazol-5-yl)-carbonylthiosemicarbazide ***2j***. Yield: (2.90 g, 88%). Mp: 156–8 °C. IR (ν, cm^{-1}) 3300 (NH), 3079, 1591, 1518, 1460 (Ar–H), 3010, 1633 (Aliph.), 1669 (C=O), 1250 (C=S). ^{1}H-NMR (DMSO-d_6) δ_H 2.68 (s, 3H, CH_3), 7.08–7.29 (m, 3H, 3 × CH), 9.75, 10.10, 10.88 (3s, 3H, 3 × NH). Anal. $C_{11}H_9F_2N_5OS_2$ (C, H, N).

4.1.1.14. 4-(2-Bromophenyl)-1-(4-methyl-thiadiazol-5-yl)-carbonylthiosemicarbazide ***2k***. Yield: (3.54 g, 91%). Mp: 107–9 °C. IR (ν, cm^{-1}) 3357 (NH), 3056, 1622, 1580, 1493 (Ar–H), 2966, 1460, 1391 (Aliph.), 1669 (C=O), 1242 (C=S). ^{1}H-NMR (DMSO-d_6) δ_H 2.83 (s, 3H, CH_3), 7.20–7.26 (m, 2H, 2 × CH), 7.36–7.43 (m, 2H, 2 × CH), 9.81, 9.98, 10.91 (3s, 3H, 3 × NH). Anal. $C_{11}H_{10}BrN_5OS_2$ (C, H, N).

4.1.1.15. 4-(4-Bromophenyl)-1-(4-methyl-thiadiazol-5-yl)-carbonylthiosemicarbazide ***2l***. Yield: (3.05 g, 82%). Mp: 181–3 °C. IR (ν, cm^{-1}) 3269 (NH), 3078, 1585, 1491, 1455 (Ar–H), 2962, 2870, 1466, 1365 (Aliph.), 1666 (C=O), 1240 (C=S). ^{1}H-NMR (DMSO-d_6) δ_H 2.84 (s, 3H, CH_3), 7.42–7.43 (m, 2H, 2 × CH), 7.51–7.56 (m, 2H, 2 × CH), 9.98, 10.33, 10.87 (3s, 3H, 3 × NH). Anal. $C_{11}H_{10}BrN_5OS_2$ (C, H, N).

4.1.1.16. 4-(4-Chlorophenyl)-1-(4-methyl-thiadiazol-5-yl)-carbonylthiosemicarbazide ***2m***. Yield: (2.79 g, 85%). Mp: 185–7 °C. IR (ν, cm^{-1}) 3270 (NH), 3089, 1589, 1498, 1463 (Ar–H), 2964, 2888, 1366 (Aliph.), 1659 (C=O), 1241 (C=S). ^{1}H-NMR (DMSO-d_6) δ_H 2.84 (s, 3H, CH_3), 7.38–7.49 (m, 4H, 4 × CH), 9.98, 10.41, 10.87 (3s, 3H, 3 × NH). Anal. $C_{11}H_{10}ClN_5OS_2$ (C, H, N).

4.1.1.17. 4-(2,4-Dichlorophenyl)-1-(4-methyl-thiadiazol-5-yl)-carbonylthiosemicarbazide ***2n***. Yield: (3.26 g, 90%). Mp: 167–9 °C. IR (ν, cm^{-1}) 3290 (NH), 3079, 1577, 1500, 1468 (Ar–H), 2968, 2890, 1368 (Aliph.), 1667 (C=O), 1243 (C=S). ^{1}H-NMR (DMSO-d_6) δ_H 2.83 (s, 3H, CH_3), 7.40–7.48 (m, 4H, 4 × CH), 9.83, 10.26, 10.93 (3s, 3H, 3 × NH). Anal. $C_{11}H_9Cl_2N_5OS_2$ (C, H, N).

4.2. Antibacterial assay

The following microorganisms were used in this study: *Staphylococcus aureus* (ATCC 25923, ATCC 6538, ATCC 29213, NCTC 4163), *Staphylococcus epidermidis* ATCC 12228, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 11778, *M. luteus* (ATCC 9341, ATCC 10240), *Escherichia coli* (ATCC 10538, ATCC 25922, NCTC 8196), *Pemphigus vulgaris* NCTC 4635, *Pseudomonas aeruginosa* (ATCC 15442, ATCC 27853, NCTC 6749), and *Bordetella bronchiseptica* ATCC 4617.

Initially, antibacterial activity of thiosemicarbazides was screened on the basis of growth inhibition zone (giz) utilizing the disc diffusion method. For compounds showing the inhibitory effect on the growth of tested bacteria, monitored as an appearance of giz, the minimal inhibitory concentrations (MICs) were determined as the lowest concentration of the compound preventing growth of the tested microorganism using agar dilution method. [40,41]

4.3. Inhibition of bacterial type IIA topoisomerases

4.3.1. Supercoiling assay

The assays were performed using *S. aureus* Gyrase Supercoiling Assay Kits (Inspiralis). Briefly, supercoiled pBR322 plasmid DNA (0.5 μg) was incubated with 1 unit of gyrase, in the dedicated

supercoiling assay buffer supplied by the manufacturer, in the presence of varying concentrations of the test compounds. Reactions were carried out at 37 °C for 1 h and then terminated by the addition of equal volume of 2x STOP Buffer (40% sucrose, 100 mM Tris-Cl pH 7.5, 1 mM EDTA, and 0.5 mg/ml bromophenol blue) and chloroform/iso-amyl alcohol. Samples were vortexed, centrifuged and run through a 15 cm 1% agarose gel in TAE buffer (40 mM Tris-acetate, 2 mM EDTA) for 3 h at 50 V. Gels were stained with ethidium bromide and visualized under UV light.

4.3.2. Decatenation assay

The assay was performed using *S. aureus* topoisomerase IV decatenation kits (Inspiralis). Interlinked kDNA substrate (0.5 μg) was incubated with 1 unit of topoisomerase IV (Inspiralis), in the dedicated decatenation assay buffer supplied by the manufacturer, in the presence of varying concentrations of the test compounds. Reactions were carried out at 37 °C for 1 h and then terminated by the addition of equal volume of 2x STOP Buffer (40% sucrose, 100 mM Tris-Cl pH 7.5, 1 mM EDTA, 0.5 mg/ml bromophenol blue) and chloroform/iso-amyl alcohol. Samples were vortexed, centrifuged and run through a 15 cm 1% agarose gel in TAE buffer for 1.5 h at 80 V. Gels were stained with ethidium bromide and visualized under UV light. The concentrations of the inhibitor that prevented 50% of the kinetoplast DNA from being converted into decatenated minicircles (IC_{50} values) were determined by plotting the results obtained from the densytometric analyses of the gel images using Quantity One software (BioRad).

4.4. Computational details

Conformational search, physicochemical parameters, and HOMO/LUMO maps were calculated using HyperChem8.0.1 [42] Extensive conformational searches were carried out using the molecular mechanics level with OPLS 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^{-1} $Å^{-1}$ for OPLS and RM1 calculations, respectively. Electrostatic potentials were calculated for the geometries that resulted from docking using Gaussian 03 and GaussView 5 at the HF/6-31G level.

Abbreviations of physicochemical parameters collected in Table 3 are as follows: surface area (SA), volume (V), refractivity (R_f), polarizabilitiy (α), moment dipole (μ), the highest occupied molecular orbital energy (E_{HOMO}), the lowest unoccupied molecular orbital energy (E_{LUMO}), the difference between HOMO and LUMO energy levels (HLG), hardness (η) obtained from the equation $\eta = (E_{LUMO} - E_{HOMO})/2$, Mulliken electronegativity (χ) obtained from the equation $\chi = -(E_{HOMO} + E_{LUMO})/2$, total energy ($E_T$), binding energy ($E_B$), isolated atomic energy (E_{IA}), electronic energy (E_E), core–core interaction (I_{C-C}), heat of formation (HF) and HOMO/LUMO maps were calculated using HyperChem8.0.1 program at RM1 level of theory.

4.5. Automated docking setup

Flexible ligand–receptor docking was performed using Autodock Vina program [43] using the default settings. Model of the ATP binding site based on the structure deposited in the Protein Data Bank [44] under the PDB ID 1KIJ [45] was employed. Default docking parameters and flexible space of 24 × 24 × 24 Å [3] were validated by re-docking novobiocin which docked exactly in the position present in the crystal structure with affinity of −11.0 kcal/mol. Subsequently, eight small molecule compounds were docked using same docking parameters.

References

[1] S.B. Levy, Multidrug resistance – a sign of the times, N. Eng. J. Med. 338 (1998) 1376–1378.
[2] W. Witte, Antibiotic resistance in gram-positive bacteria: epidemiological aspects, J. Antimicrob. Chem. 44 (1999) 1–9.
[3] R.F. Service, Antibiotics that resist resistance, Science 270 (1995) 724–727.
[4] J.A. Heinemann, R.G. Ankenbauer, C.F. Amábile-Cuevas, Do antibiotics maintain antibiotic resistance? Drug Discov. Today 5 (2000) 195–204.
[5] T. Komine, A. Kojima, Y. Asahina, T. Saito, H. Takano, T. Shibue, Y. Fukusa, Synthesis and structure–activity relationship studies of highly potent novel oxazolidinone antibacterials, J. Med. Chem. 51 (2008) 6558–6562.
[6] A.R. Renslo, G.W. Luehr, M.F. Gordeev, Recent developments in the identification of novel oxazolidinone antibacterial agents, Bioorg. Med. Chem. 14 (2006) 4227–4240.
[7] D.J. Bast, J.C.S. de Azavedo, Quinolone resistance: older concepts and newer developments, Curr. Infect. Dis. Rep. 3 (2001) 20–28.
[8] R.J. Kerns, M.J. Rybak, G.W. Kaatz, F. Vaka, R. Cha, R.G. Grucz, V.U. Diwadkar, Structural features of piperazinyl-linked ciprofloxacin dimmers required for activity against drug-resistant strains of *Staphylococcus aureus*, Bioorg. Med. Chem. Lett. 13 (2003) 2109–2112.
[9] M. Bukvić Krajačić, P. Novak, M. Dumić, M. Cindrić, H. Čipčić Paljetak, N. Kujundžić, Novel ureas and thioureas of 15-membered azalides with antibacterial activity against key respiratory pathogens, Eur. J. Med. Chem. 44 (2009) 3459–3470.
[10] L. Xu, A.K. Farthing, J.F. Dropinski, P.T. Meinke, Ch. McCallum, P.S. Leavitt, E.J. Hickey, L. Colwell, J. Barrett, K. Liu, Nocathiacin analogs: synthesis and antibacterial activity of novel water-soluble amides, Bioorg. Med. Chem. Lett. 19 (2009) 3531–3535.
[11] M.S. Kabir, K. Engelbrecht, R. Polanowski, S.M. Krueger, R. Ignasiak, M. Rott, W.R. Schwan, M.E. Stemper, K.D. Reed, D. Sherman, J.M. Cook, A. Monte, New classes of Gram-positive selective antibacterials: inhibitors of MRSA and surrogates of the causative agents of anthrax and tuberculosis, Bioorg. Med. Chem. Lett. 18 (2008) 5745–5749.
[12] Y. Hirokawa, H. Kinoshita, T. Tanaka, T. Nakamura, K. Fujimoto, S. Kashimoto, T. Kojima, S. Kato, Pleuromutilin derivatives having a purine ring. Part 1: new compounds with promising antibacterial activity against resistant Gram-positive pathogens, Bioorg. Med. Chem. Lett. 18 (2008) 3556–3561.
[13] G. Aridoss, S. Amirthaganesan, N.A. Kumar, J.T. Kim, K.T. Lim, S. Kabilan, Y.T. Jeong, A facile synthesis, antibacterial, and antitubercular studies of some piperidin-4-one and tetrahydropyridine derivatives, Bioorg. Med. Chem. Lett. 18 (2008) 6542–6548.
[14] B.J. Bradbury, M.J. Pucci, Recent advances in bacterial topoisomerase inhibitors, Curr. Opin. Pharmacol. 8 (2008) 574–581 and references therein.
[15] D.A. Ostrov, J.A. Hernández Prada, P.E. Corsino, K.A. Finton, N. Le, T.C. Rowe, Discovery of novel DNA gyrase inhibitors by high-throughput virtual screening, Antimicrob. Agents Chemother. 51 (2007) 3688–3698.
[16] M. Oblak, S.G. Grdadolnik, M. Kotnik, R. Jerala, M. Filipič, T. Šolmajer, In silico fragment-based discovery of indolin-2-one analogues as potent DNA gyrase inhibitors, Bioorg. Med. Chem. Lett. 15 (2005) 5207–5210.
[17] M. Oblak, S. Golič Grdadolnik, M. Kotnik, A. Poterszman, R.A. Atkinson, H. Nierengarten, D. Desplancq, D. Moras, T. Solmajer, Biophysical characterization of an indolinone inhibitor in the ATP-binding site of DNA gyrase, Biochem. Biophys. Res. Commun. 349 (2006) 1206–1213.
[18] C. Kuş, G. Ayhan-Kılcıgil, S. Özbey, F. Betül Kaynak, M. Kaya, T. Çoban, B. Can-Eke, DNA topoisomerases and their poisoning by anticancer and antibacterial drugs, Chem. Biol. 17 (2010) 421–433.
[19] J.J. Champoux, DNA topoisomerases: structure, function, and mechanism, Annu. Rev. Biochem. 70 (2001) 369–413.
[20] J.C. Wang, Cellular roles of DNA topoisomerases: a molecular perspective, Nat. Rev. Mol. Cell. Biol. 3 (2002) 430–440.
[21] A.M. Wilstermann, N. Osheroff, Stabilization of eukaryotic topoisomerase II-DNA cleavage complexes, Curr. Top. Med. Chem. 3 (2003) 321–338.
[22] C. Levine, H. Hiasa, K.J. Marians, DNA gyrase and topoisomerase IV: biochemical activities, physiological roles during chromosome replication, and drug sensitivities, Biochem. Biophys. Acta 1400 (1998) 29–43.
[23] A. Siwek, P. Stączek, M. Wujec, J. Stefańska, U. Kosikowska, A. Malm, S. Jankowski, P. Paneth, Biological and docking studies of topoisomerase IV inhibition by thiosemicarbazides, J. Mol. Model. 17 (2011) 2297–2303.
[24] I.R. Ezabadi, C. Camoutsis, P. Zoumpoulakis, A. Geronikaki, M. Soković, J. Glamočilija, A. Ćiri&cacute, Sulfonamide-1,2,4-triazole derivatives as antifungal and antibacterial agents: synthesis biological evaluation, lipophilicity, and conformational studies, Bioorg. Med. Chem. 16 (2008) 1150–1161.
[25] C. Kuş, G. Ayhan-Kılcıgil, S. Özbey, F.B. Kaynak, M. Kaya, T. Çoban, B. Can-Eke, Synthesis and antioxidant properties of novel *N*-methyl-1,3,4-thiadiazol-2-amine and 4-methyl-2*H*-1,2,4-triazole-3(4*H*)-thione derivatives of benzimidazole class, Bioorg. Med. Chem. 16 (2008) 4294–4303.
[26] T. Önkol, D.S. Doğruer, L. Uzun, S. Adak, S. Özkan, M.F.J. Şahin, J. Enzym. Inhib. Med. Chem. 23 (2008) 277–284.
[27] H.Z. Shams, R.M. Mohareb, M.H. Helal, A.E. Mahmoud, Phosphorus Sulfur 182 (2007) 237–263.
[28] F.E. Norrington, R.M. Hyde, S.G. Williams, R. Wootton, Physiochemical-activity relations in practice. 1. A rational and self-consistent data bank, J. Med. Chem. 18 (1975) 604–607.

[29] A. Cammarata, Quantitated structure–activity relationships, Annu. Rep. Med. Chem. 6 (1971) 245–253.
[30] E. Kutter, C. Hansch, Steric parameters in drug design. Monoamine oxidase inhibitors and antihistamines, J. Med. Chem. 12 (1969) 647–652.
[31] C. Oefner, A. Douangamath, A. D'Arcy, S. Häfeli, D. Mareque, A. Mac Sweeney, J. Padilla, S. Pierau, H. Schulz, M. Thormann, S. Wadman, G.E. Dale, The 1.15A crystal structure of the Staphylococcus aureus methionyl-aminopeptidase and complexes with triazole based inhibitors, J. Mol. Biol. 332 (2003) 13–21.
[32] T. Fujita, J. Iwasa, C. Hansch, A new substituent constant, π, derived from partition coefficients, J. Am. Chem. Soc. 86 (1964) 5175–5180.
[33] C. Hansch, W.J. Dunn, Linear relationship between lipophilic character and biological activity of drugs, J. Pharm. Sci. 61 (1972) 1–19.
[34] C. Hansch, J.M. Clayton, Lipophilic character and biological activity of drugs II: the parabolic case, J. Pharm. Sci. 62 (1973) 1–21.
[35] A.E. Bird, A.C. Marshall, Correlation of serum binding of penicillins with partition coefficients, Biochem. Pharmacol. 16 (1967) 2275–2290.
[36] A. Siwek, K. Świderek, S. Jankowski, Problems with molecular mechanics implementations on the example of 4-benzoyl-1-(4-methyl-imidazol-5-yl)-carbonylthiosemicarbazide, J. Mol. Model., online first doi: 10.1007/s00894-011-1117-1.
[37] A. Siwek, M. Wujec, J. Stefańska, P. Paneth, Antimicrobial properties of 4-aryl-3-(2-methyl-furan-3-yl)-Δ2-1,2,4-triazoline-5-thiones, Phosphorus Sulfur 184 (2009) 3149–3159.
[38] ÉC. Moreno Nascimento, J.B.L. Martins, Electronic structure and PCA analysis of covalent and non-covalent acetylcholinesterase inhibitors, J. Mol. Model. 17 (2011) 1371–1379.
[39] A. Siwek, M. Wujec, M. Dobosz, I. Wawrzycka-Gorczyca, Study of direction of cyclization of 1-azolil-4-aryl/alkyl-thiosemicarbazides, Heteroatom. Chem. 21 (2010) 521–532.
[40] Clinical and Laboratory Standards Institute, Performance Standards for Antimicrobial Disc Susceptibility Tests; Approved Standard M2–A9. Clinical and Laboratory Standards Institute, Wayne, PA, USA, 2006.
[41] Clinical and Laboratory Standards Institute, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard M7–A7. Clinical and Laboratory Standards Institute, Wayne, PA, USA, 2006.
[42] Hyperchem 8.0.3. HyperCube Inc., Gainsville, FL, USA, 2007.
[43] O. Trott, A.J. Olson, AutoDockVina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading, J. Comput. Chem. 31 (2010) 455–461.
[44] www.pdb.org and the following citation: H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov, P.E. Bourne, The protein data bank, Nucleic Acids Res. 28 (2000) 235–242.
[45] V. Lamour, L. Hoermann, J.M. Jeltsch, P. Oudet, D. Moras, An open conformation of the *Thermus thermophilus* gyrase B ATP-binding domain, J. Biol. Chem. 277 (2002) 18947–18953.