J Mol Model (2011) 17:2297–2303
DOI 10.1007/s00894-010-0889-z

ORIGINAL PAPER

Biological and docking studies of topoisomerase IV inhibition by thiosemicarbazides

Agata Siwek · Paweł Stączek · Monika Wujec · Joanna Stefańska · Urszula Kosikowska · Anna Malm · Stefan Jankowski · Piotr Paneth

Received: 26 September 2010 / Accepted: 21 October 2010 / Published online: 16 November 2010

Abstract 4-Benzoyl-1-(4-methyl-imidazol-5-yl)-carbonylthiosemicarbazide (**1**) was synthesized, and its antibacterial and type IIA topoisomerase (DNA gyrase and topoisomerase IV) activity evaluated. (**1**) was found to have high therapeutic potential against opportunistic Gram-positive bacteria, and inhibitory activity against topoisomerase IV (IC_{50}=90 μM) but not against DNA gyrase. An increase in activity against topoisomerase IV (IC_{50}=14 μM) was observed when the imidazole moiety of (**1**) was replaced with the indole group in 4-benzoyl-1-(indol-2-yl)-carbonylthiosemicarbazide (**2**). However, (**2**) showed only weak antibacterial activity. Although the results of the bacterial type IIA topoisomerases inhibition study did not parallel antibacterial activities, our observations strongly imply that a 4-benzoylthiosemicarbazide scaffold can be developed into an efficient Gram-positive antibacterial targeting topoisomerase IV. The difference in activity against type IIA topoisomerases between (**1**) and (**2**) was further investigated by docking studies, which suggested that these compounds target the ATP binding pocket.

Keywords Thiosemicarbazide derivative · Antibacterial activity · Bacterial topoisomerase IV · Enzyme inhibitor · Docking

A. Siwek (✉) · M. Wujec
Department of Organic Chemistry, Faculty of Pharmacy,
Medical University,
Staszica 6,
20-081, Lublin, Poland
e-mail: agata.siwek@am.lublin.pl

P. Stączek
Department of Genetics of Microorganisms, University of Lodz,
Banacha 12/16,
90-237, Lodz, Poland

J. Stefańska
Department of Pharmaceutical Microbiology, Medical University,
Oczki 3,
02-007, Warsaw, Poland

U. Kosikowska · A. Malm
Department of Pharmaceutical Microbiology,
Faculty of Pharmacy, Medical University,
Chodzki 1,
20-093, Lublin, Poland

S. Jankowski
Institute of Organic Chemistry, Technical University of Lodz,
Zeromskiego 116,
90-924, Lodz, Poland

P. Paneth
Institute of Applied Radiation Chemistry,
Technical University of Lodz,
Zeromskiego 116,
90-924, Lodz, Poland

Introduction

Attractive targets in the search for new antibiotics are the well-studied bacterial topoisomerases [1 (and references therein), 2–4]. Bacterial topoisomerases have been a focus of antibacterial research since the discovery of *Escherichia coli* topoisomerase II (named DNA gyrase) and its involvement in the mechanism of action of nalidixic acid. At present, four main classes of bacterial topoisomerases, designated I through IV, are known. Bacterial topoisomerases I and III are type IA topoisomerases, which relax or decatenate DNA by formation and bridging of a single-stranded DNA gap through which a second, intact DNA strand is passed, followed by resealing of a gap. Bacterial topoisomerases II and IV are type IIA topoisomerases, which also bridge the DNA gap they form but in this case

both strands are cut and a DNA duplex is passed before the enzyme reseals a gap [5]. Depending on the reaction conditions and substrates, topoisomerase II can relax negative and positive supercoils, decatenate or unknot DNA, but it primarily introduces negative supercoils, a function essential for bacterial DNA metabolism. It is composed of two heterodimeric subunits, GyrA comprising the DNA binding, cleavage and re-ligation activity, and GyrB comprising the ATPase activity. Topoisomerase IV, which also reveals relaxation activity towards negatively and positively supercoiled DNA molecules, acts primarily to decatenate interlinked DNA molecules before cell division and is a heterotetramer consisting of subunits *ParC* and *ParE*, which share homology and mechanistic similarity to these of gyrase [6].

Most research into antibacterial topoisomerase inhibitors has focused on the type IIA topoisomerases. Topoisomerases II and IV are good antibacterial targets for several reasons: (1) they are proteins essential for bacterial viability that are involved in bacterial DNA replication, (2) they are essential components of all bacteria, (3) inhibition of their function in bacteria usually leads to a bactericidal (versus bacteriostatic) event, (4) there are distinct structural differences from their mammalian enzyme counterparts to allow for bacterial specificity, and (5) multiple target sites have been identified within the enzymes. To date, ten main chemical classes of bacterial topoisomerase II inhibitors are known [1]. Among them, quinolones are highly effective antimicrobial drugs. However, a significant limitation to the widespread use of quinolone inhibitors is the emergence of drug-resistant bacteria due to alterations in the DNA gyrase/topoisomerase IV or to a decrease in intracellular drug levels caused by changes in membrane permeability or overexpression of drug efflux pumps [7, 8]. In search of novel gyrase inhibitors, Ostrov et al. employed virtual screening of a small molecule library [2]. A chemical ligand database containing approximately 140,000 small molecules (molecular weight <500) was molecularly docked onto an unexplored binding pocket of GyrA and the ATP binding pocked of GyrB. This approach identified several novel chemotypes, including imidazole, identifying inhibition of DNA gyrase supercoiling as a potential starting point for further structural optimization. Promising results for imidazoles as potent topoisomerase II inhibitors were also described by Oblak et al. [3]. These latter authors employed the concept of fragment-based drug design to search for novel DNA gyrase inhibitors. Using the tools of virtual screening and NMR spectroscopy, they identified the binding of two low-molecular weight fragments (benzimidazole and indolinone) to the 24 kDa N-terminal fragment of GyrB. In silico optimization of these structures indicated that the imidazole ring may be essential for the antigyrase activity of indolinones. Moreover, it was found that the imidazole ring is likely to be located in the hydrophobic part of the active site, most probably in the vicinity of residues Ile78, Pro79, and Ile94. The selected compound, possessing indolinone and imidazole moieties in one frame, was studied further and shown to bind to the ATP-binding domain of GyrB. Molecular modeling of the GyrB24-indolinonimidazole complex demonstrated the binding of the imidazole moiety to the hydrophobic area formed by Gly77, Ile78, Pro79, and Ile94 [4]. However, studies on a 43 kDa fragment of GyrB from *E. coli* indicated that the ATP-binding pocket comprises residues from two GyrB subunits that form a dimer [9]. The antibacterial activity of novel and potent DNA gyrase inhibitors with an azole ring was also described by Tanitame et al. [10]. Although no docking studies in the binding site of DNA gyrase were carried out, a good correlation was found between the minimum inhibitory concentration (MIC) values of imidazole against *Staphylococcus aureus* or *E. coli* and IC_{50} values against topoisomerases II and IV. It can thus be concluded that the imidazole might be a potential starting point for further structural optimization in developing novel gyrase inhibitors.

As part of our program to discover new antimicrobials in this class of thiosemicarbazides, based on the above results and recent reports on the antibacterial activity of benzoylthioureas [11, 12], we speculated that a thiosemicarbazide skeleton having both imidazole and benzoyl moieties may possess good antibacterial activity via inhibition of bacterial topoisomerase. In this context, 4-benzoyl-1-(4-methyl-imidazol-5-yl)-carbonylthiosemicarbazide (**1**) was synthesized and evaluated for its antibacterial and type IIA topoisomerase inhibitory activities. It was found that the compound has good activity against Gram-positive opportunistic bacteria and exhibits inhibitory activity against topoisomerase IV. In order to confirm the role of the structural elements responsible for its biological potency, a known 1,4-dibenzoylthiosemicarbazide (**3**) [11] as well as a newly synthesized 4-benzoyl-(indol-2-yl)-carbonylthiosemicarbazide (**2**) were tested for their antibacterial and topoisomerase inhibitory activities in the hope of providing insights into the rational synthesis of thiosemicarbazides with improved antimicrobial properties. Since we first documented the inhibitory properties against bacterial topoisomerase of this class of compounds [13], other communications confirming our observations have appeared in literature; Farag and El-Tayeb [14] constructed a protein–ligand complex based on the X-ray structure of topoisomerase II gyrase B with its inhibitor, Clorobicin, bound in the active site (structure available through the RCSB Protein Data Bank). The ICM score values and hydrogen bonds formed with the surrounding amino acids showed good agreement with predicted binding affinities obtained by molecular docking studies as verified by antimicrobial screening, where furobenzopyranone-/pyranobenzopyranone-thiosemicarba-

zides were the most active compounds against *Bacillus subtilis*, *S. aureus*, and *E. coli*. Pyranobenzopyranone-thiosemicarbazide had good affinity with the receptor and formed six hydrogen bonds with Asp73 and two bonds with Thr165; furobenzopyranone-thiosemicarbazide had an ICM score value of −85.66, formed one hydrogen bond with Asp73, and three with Thr165. All hydrogen bounds were formed between a proton of the thiosemicarbazide skeleton and the oxygen atom of Asp73 or Thr165. It was concluded that hydrogen bond formation with amino acid residues Asp73 and Thr165 may be responsible for the antibacterial activity in the case of the Clorobiocin. Herein we present findings from inhibitory activity and docking studies that contradict conclusions reached by Farag and El-Tayeb.

Experimental methods

Chemistry

All commercial reactants and solvents were purchased from either Sigma-Aldrich (St. Louis, MO) or Lancaster Synthesis (Windham, NH) and were of 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 (within ±0.4 % of the theoretical values). ^{1} H- and ^{13} C-NMR spectra were recorded on a Bruker Avance II Plus (700 MHz) spectrometer (http://www.bruker-biospin.com/). All spectra were recorded at 25 °C in DMSO-d_6 using the solvent methyl group signal as the internal standard (δ_H 2.50 or δ_C 40.0, respectively). Analytical thin layer chromatography (TLC) was performed with Merck (Darmstadt, Germany) 60 F_{254} silica gel plates and visualized by UV irradiation (254 nm).

4-benzoyl-1-(4-methyl-imidazol-5-yl)-carbonylthiosemicarbazide (**1**)

A reaction mixture of 4-methyl-imidazol-5-carboxylic acid hydrazide (1.40 g, 0.01 mol) and benzoyl isothiocyanate (1.63 g, 0.01 mol) was heated in an oil bath at 80°C. Progress of the reaction was monitored by TLC. After 12 h, the reaction was completed, the crude reaction mixture was washed with diethyl ether and crystallized from ethanol to give the product (2.67 g, 88%). Mp: 226–228 °C. ^{1} H-NMR (700 MHz, DMSO-d_6) δ_H 2.48 (s, 3 H, CH_3), 7.53–7.56 (m, 2 H, $Ar_{3,5}$), 7.65–7.69 (m, 1 H, Ar_4), 7.68 (s, 1 H, 2-imidazole), 7.97-8.00 (m, 2 H, $Ar_{2,6}$), 10.49 [br s, 1 H, C(O)-N*H*-NH-C(S)], 11.88 [br s, 1 H, C(S)-N*H*-C(O)], 12.55 (br s, 1 H, N*H*-imidazole), 12.67 [br s, 1 H, C(O)-NH-N*H*-C(S)]. ^{13} C-NMR (176 MHz, DMSO-d_6) δ_C 10.76 (CH_3). 127.62 (4-imidazole), 128.93 ($Ar_{2,6}$), 129.19 ($Ar_{3,5}$), 132.27 (Ar_1), 133.37 (5-imidazole), 133.64 (Ar_4), 134.98 (2-imidazole), 159.36 (C=O), 168.95 (C=O, benzamide), 172.91 (C=S). Anal. Calc. for $C_{13}H_{13}N_5O_2S$: C, 51.47; H, 4.32; N, 23.09. Found: C, 51.33; H, 4.51; N, 22.83.

4-benzoyl-(indol-2-yl)-carbonylthiosemicarbazide (**2**)

Compound (**2**) (3.06 g, 86%) was prepared from indol-2-carboxylic acid hydrazide (1.75 g, 0.01 mol) and benzoyl isothiocyanate (1.63 g, 0.01 mol) in the same manner as described for (**1**). Mp: 254–256 °C. ^{1} H-NMR (700 MHz, DMSO-d_6) δ_H 7.07 (t, 1 H, *J*=7.6, 8.3 Hz, 6-indole), 7.22 (dd, 1 H, *J*=7.6, 7.9 Hz, 5-indole), 7.33 (s, 1 H, 3-indole), 7.46 (d, 1 H, *J*=8.3 Hz, 7-indole), 7.54 (dd, 2 H, *J*=7.3, 7.8 Hz, $Ar_{3,5}$), 7.66 (t, 1 H, *J*=7.8 Hz, Ar_4), 7.67 (d, 1 H, *J*=7.9 Hz, 4-indole), 7.99 (d, 2 H, *J*=7.3 Hz, $Ar_{2,6}$), 11.10 [s, 1 H, C(O)N*H*-NHC(S)], 11.77 [s, 1 H, C(S)-N*H*-C(O)], 11.79 (s, 1 H, N*H*-indole), 12.34 [s, 1 H, C(O)NH-N*H*C(S)]. ^{13} C-NMR (176 MHz, DMSO-d_6) δ_C 104.50 (3-indole), 112.59 (4-indole), 120.20 (5-indole), 122.01 (4-indole), 124.12 (5-indole), 127.10 (8-indole), 128.66 ($Ar_{3,5}$), 128.95 ($Ar_{2,6}$), 129.14 (2-indole), 132.08 (Ar_1), 133.36 (Ar_4), 136.96 (9-indole), 159.49 (indol-2-carbonyl), 167.90 (C=O, benzamide), 181.07 (C=S). Anal. Calc. for $C_{17}H_{14}N_4O_2S$: C, 60.34; H, 4.17; N, 16.56. Found: C, 60.46; H, 4.19; N, 16.67.

Antimicrobial assay

Microorganisms used in this study were as follows: *S. aureus* ATCC 25923, *S. aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *B. subtilis* ATCC 6633, *Bacillus cereus* ATCC 11778, *B. cereus* ATCC 10876, *Micrococcus luteus* ATCC 9341, *M. luteus* ATCC 10240 (as representative examples of Gram-positive bacteria) and *E. coli* ATCC 10538, *E. coli* ATCC 25922, *E. coli* NCTC 8196, *Proteus vulgaris* NCTC 4635, *Pseudomonas aeruginosa* ATCC 15442, *P. aeruginosa* NCTC 6749, *P. aeruginosa* ATCC 27853, *P. aeruginosa* ATCC 9027, *Klebsiella pneumoniae* ATCC 13883, *Proteus mirabilis* ATCC 12453, *Bordetella bronchiseptica* ATCC 4617 (as representative examples of Gram-negative bacteria).

Preliminary antibacterial potency of (**1**) and (**2**) against a panel of Gram-positive and Gram-negative bacteria was screened on the basis of growth inhibition zones (GIZ) utilizing the agar well diffusion method. Next, MICs were determined using the broth microdilution technique [for (**1**)] or the agar dilution method [for (**2**)]. The MIC was defined as the lowest concentration of the compound preventing growth of the tested microorganism. In all methods, the recommended Mueller-Hinton medium was used—agar or broth, respectively [15, 16].

Agar well diffusion method

The agar well diffusion method was used to determine the preliminary activity of compound (**1**). Sterile swabs were used to spread microbial suspensions (0.5 McFarland inoculum diluted 1:100 in Mueller-Hinton broth) onto the medium surface, then the compound solution (at a concentration of 5,000 μg/mL) was introduced into the wells (80 μL per well separately). Wells (8-mm diameter) were made in the agar with a sterile cork-borer. The plates were preincubated at room temperature for 1.5 h to allow diffusion of the solution into the medium and then incubated at 37 °C for 18 h.

Disc diffusion method

The disc diffusion method was used to determine the preliminary activity of (**2**). Sterile filter paper discs (9 mm diameter) were dipped in compound solution to load 400 μg of a compound per disc. Dry discs were placed on the surface of Mueller–Hinton II agar medium. Diameter of the growth inhibition zone was read after 18 h of incubation at 35°C.

Broth microdilution method

The broth microdilution method used to determine MICs for (**1**) was performed in 96-well microplates with Mueller-Hinton broth containing from 1.95 to 1,000 μg/mL of (**1**). A 20 μL aliquot of each bacterial 0.5 McFarland suspension was added to Mueller-Hinton broth per each well; total volume was 200 μL. After incubation (37°C for 18 h), optical density (OD_{600}) measurements were determined for bacterial cultures in the presence and absence of the tested compounds. Cefuroxim (at 0.06 to 500 μg/mL) was used as a control antimicrobial. The MIC results were repeated three times.

Agar dilution method

The agar dilution method was used to determine MICs for (**2**). For MIC determination, the compound was dissolved in DMSO. Concentrations of the agent tested in solid medium ranged from 6.25 to 400 μg/mL. The final inoculum of all organisms studied was 10^4 colony forming units per mL (CFU/mL). MICs were read off after 18 h of incubation at 35 °C. Ciprofloxacin (5 μg per disc) was used as a control antimicrobial. The MIC results were repeated three times.

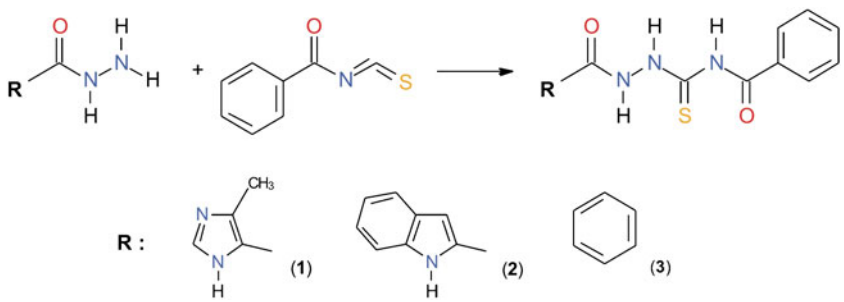
Inhibition of bacterial type IIA topoisomerases

Supercoiling assay

The assays was performed using *S. aureus* gyrase supercoiling assay kits (Inspiralis, http://www.inspiralis.com). Briefly, supercoiled pBR322 plasmid DNA (0.5 μg) was incubated with 1 U 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, 0.5 mg/ml bromophenol blue) and chloroform/isoamyl 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.

Decatenation assay

The decatenation assay was performed using *S. aureus* topoisomerase IV decatenation kits (Inspiralis). Interlinked kDNA substrate (0.5 μg) was incubated with 1 U 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/isoamyl 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. Concentrations of inhibitor that prevented 50% of the kinetoplast DNA from being converted into decatenated minicircles (IC_{50} values) were determined by plotting the results obtained



Scheme 1 Synthesis of 4-benzoyl-1-(4-methyl-imidazol-5-yl)-carbonylthiosemicarbazide (**1**) and 4-benzoyl-1-(indol-2-yl)-carbonylthiosemicarbazide (**2**)

Table 1 In vitro antimicrobial activity of (**1**), (**2**), (**3**), and reference antibiotics expressed as the growth inhibition zone (GIZ, mm) and minimal inhibitory concentration (MIC, μg/ml)

Compound	*Staphylococcus aureus*		*Staphylococcus epidermidis*		*Micrococcus luteus*		*Bacillus subtilis*		*Bacillus cereus*	
	GIZ	MIC	GIZ	MIC	GIZ	MIC	GIZ	MIC	GIZ	MIC
(**1**)	34[a] 18[b]	500[a] 1,000[b]	24[c]	3.91[c]	50[d]	0.98[d]	21[e]	7.81[e]	8[f]	62.5[f]
(**2**)	18[a] 14[a]	100[a] 100[b]	14[c]	50[c]	13[d] 13 [g]	50[d] 50 [g]	17[e]	50[e]	14 [h]	50 [h]
(**3**)		3.1[i]	nt [j]			3.1[d]		6.3[e]	nt	
Cefuroxim	0.49[a]	0.98[b]		0.98[c]		0.98[d]		125[e]		31.25[f]
Ciprofloxacin		0.5[b] 0.5[a]		0.5[c]		2[d] 4 [g]		<0.125[e]		1[f]

Strains ATCC: [a] 25923, [b] 6538, [c] 12228, [d] 10240, [e] 6633, [f] 10876, [g] 9341, [h] 11778, [i] 6638, [j] not tested

from the densytometric analyses of the gel images using Quantity One software (BioRad; http://www.bio-rad.com).

Computational details

The assignment of NMR shifts (see Chemistry) was confirmed by theoretical analysis; structures were optimized using the B3PW91 functional expressed in the 6-31+G(d) basis set as implemented in Gaussian03. Theoretical NMR shifts were then obtained using the same DFT functional with 6-311++G(2df,p) basis set. Default thresholds were used in all calculations.

Flexible ligand–rigid receptor docking was performed using the CAChe Worksystem 7.5 (Fujitsu; http://www.cache.fujitsu.com) using a genetic algorithm (GA) [17] and potential of mean force (PMF) [18] employing Amber van der Waals parameters and 6–12 Lennard-Jones potential [19]. The PMF potential, which implicitly includes solvent and entropic effects, has been computed on a grid with spacing of 0.3 Å in a box with edges 20 × 20 × 20Å secluding the receptor. The GA settings included a population of 50 chromosomes and 3,000 generations. Two small molecular compounds were docked to two topoisomerases, PDB id 1KIJ and 3LTN, representing ATP- and DNA-binding sites, respectively. Missing hydrogen atoms in the receptor structures were added and protonation of titratable amino acids was assigned at physiological pH.

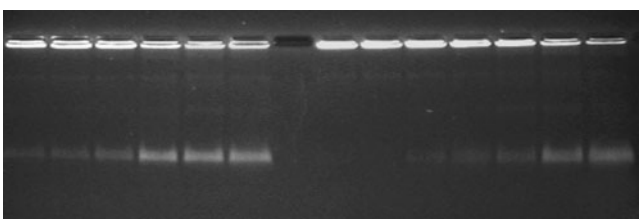


Fig. 1 Assay results of topoisomerase IV with (**1**) (*left*) and (**2**) (*right*)

Results and discussion

The synthesis of the title compounds was based on a published procedure (Scheme 1) [20–23]. In short, 4-methyl-imidazol-5-carboxylic acid hydrazide, indol-2-carboxylic acid hydrazide, or benzoic acid hydrazide were reacted with benzoyl isothiocyanate, giving (4-methyl-imidazol-5-yl)-carbonylthiosemicarbazide (**1**), 4-benzoyl-1-(indol-2-yl)-carbonylthiosemicarbazide (**2**), and 1,4-dibenzoylthiosemicarbazide (**3**), respectively.

Compounds (**1**) and (**2**) were tested against a panel of Gram-positive and Gram-negative bacteria and the in vitro results of antibacterial assays are reported in Table 1 as (a) growth inhibition zones (GIZs) and (b) MICs. MICs against different microorganisms obtained for (**1**) and (**2**) are compared with MICs of a compound from the same class, (**3**), previously reported in the literature [11]. and control antibiotics.

As can be seen from the results presented in Table 1, 4-benzoyl-1-(4-methyl-imidazol-5-yl)-carbonylthiosemicarbazide (**1**) showed high therapeutic potential against Gram-positive bacteria, except for *M. luteus* strains, while it was inactive against Gram-negative rods. The compound has the same (MIC=0.98 μg/mL) or 16-fold higher (MIC= 7.81 μg/mL) potency against *M. luteus* and *B. subtilis*, respectively, than the control antibiotic cefuroxime. Its

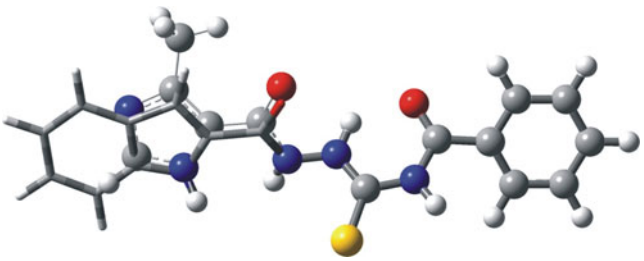


Fig. 2 Overlay of structures of (**1**) (*balls and sticks*) and (**2**) (*tubes*) optimized at the density functional theory (DFT) level of theory

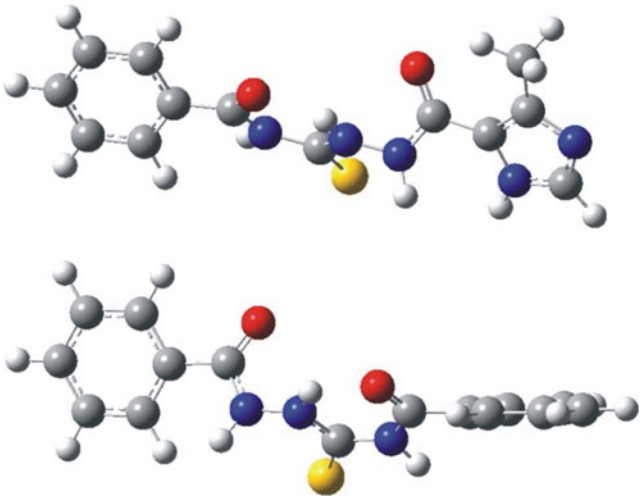


Fig. 3 Comparison of molecular shapes of (**1**) (*top*) and (**3**) (*bottom*) optimized at the DFT level of theory

strong antibacterial potency is also observed against *S. epidermidis*, with MIC=3.91 μg/mL. Additionally, bacterial type IIA topoisomerases inhibition results showed that (**1**) is a potential topoisomerase IV (topo IV) inhibitor with an IC_{50} of 90 μM while it is inactive against DNA gyrase (Fig. 1). These findings spurred us on to concentrate our studies on finding the molecular basis of the antibacterial activity of (**1**). We designed and synthesized a new analog, 4-benzoyl-1-(indol-2-yl)-carbonylthiosemicarbazide (**2**); compounds (**1**) and (**2**) have quite similar structures as confirmed by density functional theory (DFT) calculations but differ in the "length" of the molecule (see Fig. 2). Next, we conducted DNA gyrase and topoisomerase IV inhibition tests for (**2**) and found it to be an active inhibitor of topo IV with an IC_{50} of 14 μM (see Fig. 1). Based on this result, we expected that a more potent inhibitor of topo IV will demonstrate stronger antibacterial activity [24]. Surprisingly, compound (**2**), except for *M. luteus* strains, showed less potent antibacterial activity than that of (**1**). These results might suggest that (a) although the IC_{50} for (**1**) is six-fold higher than the IC_{50} for (**2**) (90 and 14 μM, respectively), the imidazole moiety allows good permeability through the bacterial cell membrane, and (b) a substituent at the 1-position of thiosemicarbazide is essential for interaction with the enzyme. This latter argument, however, appears contrary to that of Farag and El-Tayeb [14], who postulated strong interaction between protons of the thiosemicarbazide skeleton and oxygen atoms of the amino acid residues in peptide chains; thus, if the 4-benzoylthiosemicarbazide skeleton, especially protons from the NH–NH–C(=S)–NH fragment, play an important role in the H-bond interaction with topoisomerase, compound (**3**) should exhibit inhibitory activity. 1,4-Dibenzoylthiosemicarbazide (**3**), however, showed no inhibitory action against either DNA gyrase or

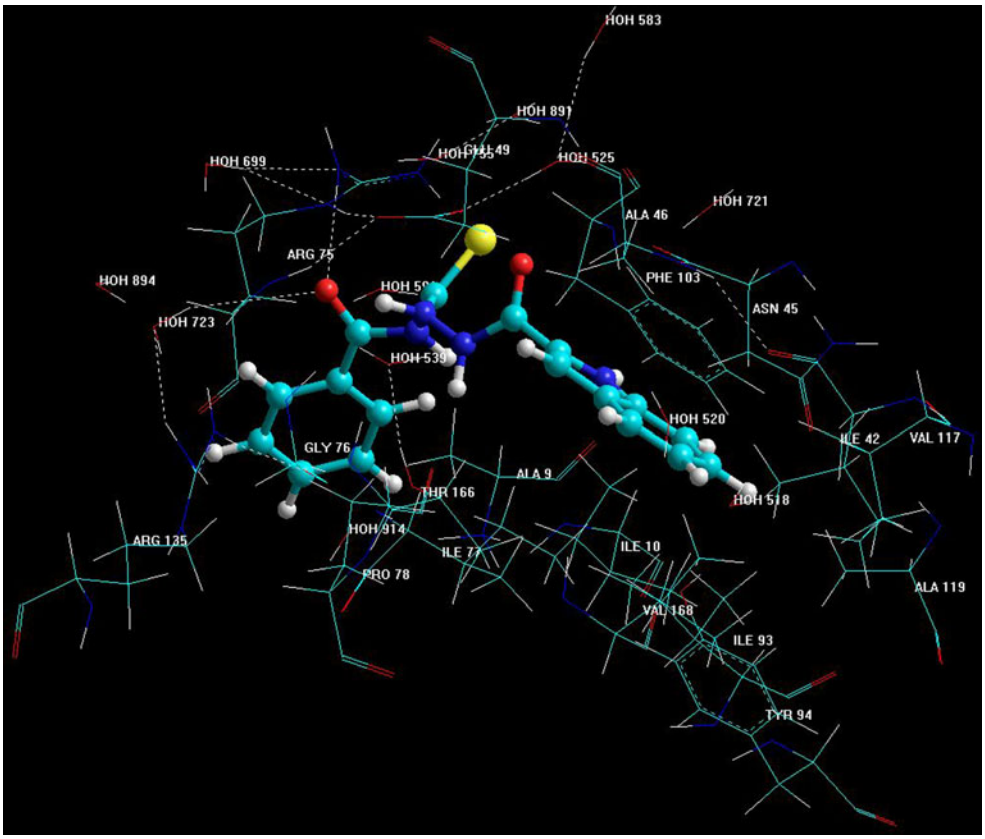


Fig. 4 Compound (**2**) docked to the ATP binding pocket of gyrase B (1KIJ)

topo IV, although its "length" is similar to that of (**1**) (15.6 and 14.5 Å, respectively). DFT-optimized structures of these two molecules exhibit some differences: both aromatic rings are located in the same plane in (**1**) while they are perpendicular in (**3**) (Fig. 3). Thus, the imidazole or indole moiety might be important for the inhibitory activity against topoisomerase IV, while low MIC values for (**3**) indicate that it exerts its effect by another mechanism. In the light of the above discussion it seems promising to search for active compounds among "long" molecules like (**2**), which should inhibit topo IV.

In our search for the origin of the difference in inhibitory activity of compounds (**1**) and (**2**), we performed docking studies of these two compounds to the DNA binding site using the topoisomerase IV-DNA cleavage complex from *Streptococcus pneumonie* (PDB 3LTN), and to the ATP binding site using the 43 K ATPase domain of *Thermus thermophilus* gyrase B (1KIJ) Gram-positive bacteria. These studies produced PMF scores of −263 and −243 kcal mol^{-1} for compounds (**1**) and (**2**), in the first case, and −437 and −454 kcal mol^{-1} in the second case, respectively. From this data, we conclude that the studied compounds have greater affinity toward the ATP binding site than to the DNA binding site. Consistent with experimental results, the docking calculations correctly assign the higher PMF score to compound (**2**) rather than compound (**1**). Figure 4 shows the best pose of compound (**2**) in the ATP binding pocket. Due to its relatively small volume, (**2**) forms only two hydrogen bonds—one to Arg75 and the other to a water molecule—while no hydrogen bonds are formed by compound (**1**).

Conclusions

Although results of our bacterial type IIA topoisomerase inhibition study did not parallel antibacterial activities, useful information regarding the source of antibacterial potency and topo IV inhibitory activity of thiosemicarbazides has been inferred from docking studies. These results indicate that the inhibitory activity against topo IV originates in the preferential binding of compound (**2**) in the ATP binding pocket. Further studies of other substituents at the thiosemicarbazide scaffold are necessary for finding derivatives with improved bioactivity. These studies, both experimental and theoretical, are underway in our laboratories.

Acknowledgments Access to supercomputing facilities at Interdisciplinary Centre for Mathematical and Computational Modeling, Poznań Supercomputing and Networking Center, and Cyfronet (Poland) is gratefully acknowledged.

References

1. Bradbury BJ, Pucci MJ (2008) Curr Opin Pharmacol 8:574–581
2. Ostrov DA, Hernández Prada JA, Corsino PE, Finton KA, Le N, Rowe TC (2007) Antimicrob Agents Chemother 51:3688–3698
3. Oblak M, Grdadolnik SG, Kotnik M, Jerala R, Filipič M, Šolmajer T (2005) Bioorg Med Chem Lett 15:5207–5210
4. Oblak M, Golič Grdadolnik S, Kotnik M, Poterszman A, Atkinson RA, Nierengarten H, Desplancq D, Moras D, Solmajer T (2006) Biochem Biophys Res Commun 349:1206–1213
5. Schoeffler AJ, Berger JM (2008) Q Rev Biophys 41:41–101
6. Dong KC, Berger JM (2007) Nature 450:1201–1205
7. Hooper DC (2002) Lancet Infect Dis 2:530–538
8. Coban AY, Ekinci B, Durupinar B (2004) Chemotherapy 50:22–26
9. Bellon S, Parsons JD, Wei Y, Hayakawa K, Swenson LL, Charifson PS, Lippke JA, Aldape R, Gross ChH (2004) Antimicrob Agents Chemother 48:1856–1864
10. Tanitame A, Oyamada Y, Ofuji K, Fujimoto M, Suzuki K, Ueda T, Terauchi H, Kawasaki M, Nagai K, Wachi M, Yamagishi J-i (2004) Bioorg Med Chem 12:5515–5524
11. Cunha S, Macedo FC Jr, Costa GAN, Rodrigues MT Jr, Verde RBV, de Souza Neta LC, Vencato I, Lariucci C, Sá FP (2007) Monatsh Chem 138:511–516
12. Zhong Z, Xing R, Liu S, Wang L, Cai S, Li P (2008) Carbohydr Res 343:566–570
13. Siwek A, Paneth P, Stączek P, Stefańska J (2010) Polish Patent Aplication No. P-390774
14. Farag NAH, El-Tayeb W (2010) Eur J Med Chem 45:317–325
15. Clinical and Laboratory Standards Institute (2006) Performance standards for antimicrobial disc susceptibility tests; document M2–A9. Clinical and Laboratory Standards Institute, Wayne, PA
16. Clinical and Laboratory Standards Institute (2006) Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; document M7-A7. Clinical and Laboratory Standards Institute, Wayne, PA
17. Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, Olson AJ (1998) J Comput Chem 19:1639–166
18. Muegge I, Martin Y (1999) J Med Chem 42:791–804
19. Cornell WD, Cieplak P, Bayly CI, Gould IR, Merz KM Jr, Ferguson DM, Spellmeyer DC, Fox T, Caldwell JW, Kollman PA (1995) J Am Chem Soc 117:5179–5197
20. Ezabadi IR, Camoutsis C, Zoumpoulakis P, Geronikaki A, Soković M, Glamoćilija J, Ćirić A (2008) Bioorg Med Chem 16:1150–1161
21. Kuş C, Ayhan-Kılcıgil G, Özbey S, Kaynak FB, Kaya M, Çoban T, Can-Eke B (2008) Bioorg Med Chem 16:4294–4303
22. Önkol T, Doğruer DS, Uzun L, Adak S, Özkan S, Şahin MFJ (2008) J Enzym Inhib Med Chem 23:277–284
23. Shams HZ, Mohareb RM, Helal MH, Mahmoud AE (2007) Phosphorus Sulfur Silicon Relat Elem 182:237–263
24. Wiles JA, Hashimoto A, Thanassi JA, Cheng J, Incarvito ChD, Deshpande M, Pucci MJ, Bradbury BJ (2006) J Med Chem 49:39–42