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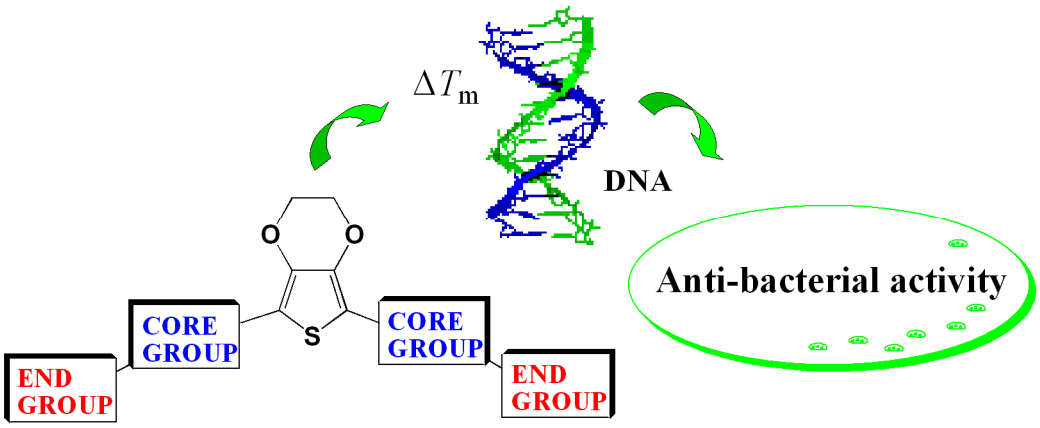

Highlights

9 new amidine derivatives of 3,4-ethylenedioxythiophene were synthesized.

27 amidines were tested for their antibacterial activities.

Bis-phenyl derivatives show highest activity against sensitive and resistant strains.

Bis-benzimidazole derivative had the best spectrum of activity and DNA binding.

Synthesis and structure-activity relationship of amidine derivatives of 3,4-ethylendioxythiophene as novel antibacterial agents

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ABSTRACT

Current antibacterial chemotherapeutics are facing an alarming increase in bacterial resistance pressuring the search for novel agents that would expand the available therapeutic arsenal against resistant bacterial pathogens. In line with these efforts, a series of 9 amidine derivatives of 3,4-ethylenedioxythiophene were synthesized and, together with 18 previously synthesized analogs, evaluated for their relative DNA binding affinity, *in vitro* antibacterial activities and preliminary *in vitro* safety profile. Encouraging antibacterial activity of several subclasses of tested amidine derivatives against Gram-positive (including resistant MRSA, MRSE, VRE strains) and Gram-negative bacterial strains was observed. The bis-phenyl derivatives were the most antibacterially active, while compound **19** from bis-benzimidazole class exhibited the widest spectrum of activity (with MIC of 4, 2, 0.5 and ≤0.25 μg/ml against laboratory strains of *S. aureus, S. pneumoniae, S. pyogenes, M. catarrhalis,* respectively and 4-32 μg/ml against clinical isolates of sensitive and resistant *S. auresu, S. epidermidis and E. faecium*) and also demonstrated the strongest DNA binding affinity (ΔT_m of 15.4º C). Asymmetrically designed compounds and carboxamide-amidines were, in general, less active. Molecular docking indicated that the shape of the 3,4-ethylenedioxythiophene derivatives and their ability to form multiple electrostatic and hydrogen bonds with DNA, corresponds to the binding modes of other minor-groove binders. Herein reported results encourage further investigation of this class of compounds as novel antibacterial DNA binding agents.

Key words: thiophene, amidine, minor groove binders, minimum inhibitory concentration, antibacterial activity, DNA binding

Non-standard abbreviations: Cpd – compound, Ar – aromate, MGB – minor groove binder

1. INTRODUCTION

Infectious diseases remain a major health problem worldwide and continue to challenge both medical and pharmaceutical communities. Bacterial pathogens causing infections in the hospital and community settings are continuously developing resistance to the existing antibacterial therapeutics [1-5]. In particular, increasing drug resistance among Gram-positive bacterial pathogens, including methicilline-resistant *Staphyloccocus aureus* (MRSA) and vancomycin-resistant enterococci (VRE), represent a significant health issue [6]. As pathogens mutate, continued success in treating infectious diseases requires a steady stream of new antimicrobial agents to which existing bacteria have not developed resistance. Also, as the majority of the high level bacterial resistance mechanisms affect antibiotics with related chemical structures, it is considered that the new antibacterial agents should possess chemical characteristics which are clearly different from those of already known agents.

Most antibiotics in clinical use are designed in the way that their antibacterial activity is achieved through one of the five possible mechanisms of action: inhibition of cell wall synthesis, inhibition of protein synthesis, alteration of cell membranes, inhibition of enzymes involved in nucleic acid synthesis and exhibiting antimetabolite activity. The introduction of bacterial DNA as a therapeutic target has led to the design of novel classes of antibacterial drugs [7]. Targeting bacterial DNA with new antibacterial agents is a promising direction of research that could potentially result in the expansion of available arsenal to combat existing bacterial drug resistance. This approach requires that bacterial DNA is preferentially targeted over the human DNA, thus subsequently minimizing potential toxicity to host cells.

In addition, presence of the negatively charged phospholipids and other polyanionic groups in prokaryotic membranes and their different composition compared to eukaryotic membranes is used as a basis in the design of potential antibacterial peptide drugs [8-10]. Literature evidence for benzophenone-based tetraamide compounds also showed that positive charge is a necessary requirement for selective interaction with bacterial over mammalian membranes [11]. Thus, cationic compounds are strongly attracted by electrostatic interactions to bacterial over mammalian membranes, and this characteristic is used in drug design in order to increase selectivity of antibacterial agents.

During the last decade, a series of reports on new classes of compounds that bind to DNA and display potent antibacterial activity were published [12-16]. These DNA binding antibiotics are structurally based on natural products distamycin and netropsin. Mechanism of action of antibacterial agents derived from distamycin, as well as other minor groove binders

(MGBs), relies on inhibiting DNA function and RNA synthesis in bacteria [15] by binding to duplex DNA specifically in the minor groove. In general, MGBs are molecules of diverse structures composed of several subunits that have the ability to adopt a “crescent” shape allowing the MGB moiety to fit into the minor groove of DNA. Bis-amidine type of MGBs is known to have antibacterial, antiviral, antifungal and antiparasitic activities [12,17-19]. Intense research of bis-amidines as antibacterial drugs led to the synthesis of numerous compounds that exhibit broad-spectrum *in vitro* activity against Gram-positive and Gram-negative bacterial species [11,18,20-22]. With regard to the structural units’ arrangement MGBs can generally be divided on head-to-tail (distamycin) and head-to-head (compounds I and II) groups of compounds (Fig.1). Generally, diamidine MGBs of head-to-head type are antibacterially more potent than the head-to-tail compounds with MICs against sensitive *S. aureus* $\leq$ 0.5 μg/mL for compounds I and II and 6.25 and 50 μg/mL for compound III and distamycin, respectivelly [21,23,24].

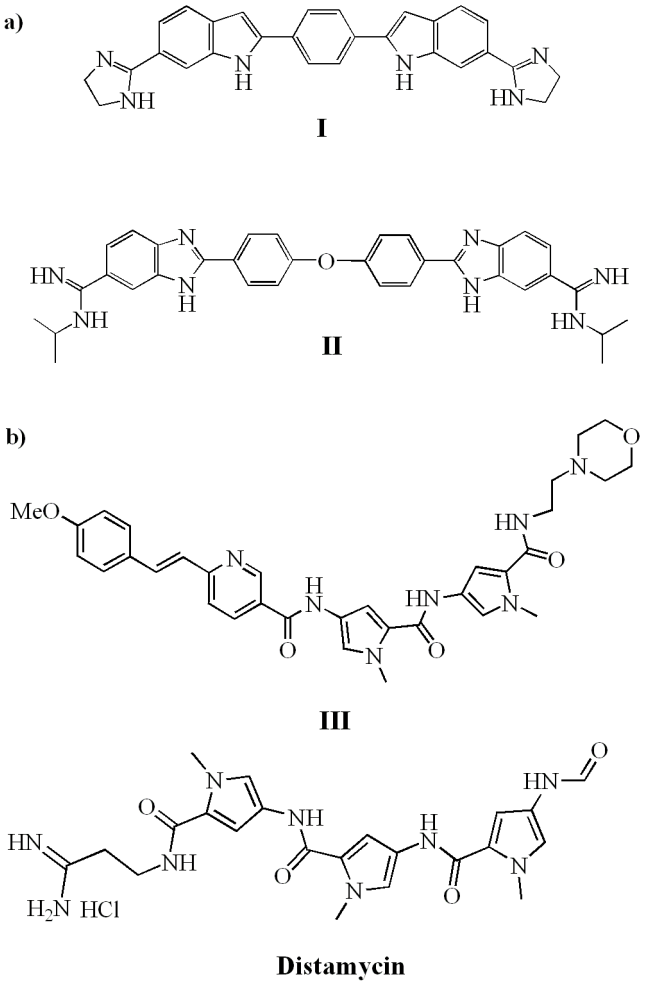


Figure 1. Structures of a) head-to-head and b) head-to-tail linked MGBs with demonstrated antibacterial activity. MICs against sensitive *S. aureus* are ≤ 0.5 μg/mL for compounds **I** and **II** and 6.25 and 50 μg/mL for compound **III** and distamycin, respectively [12,21,23,25].

These findings prompted our investigation of diamidine derivatives of 3,4-ethylenedioxythiophene, synthesized in our laboratory, as potential antibacterial agents [26-28]. Here we report the antibacterial activities of compounds with 3,4-ethylenedioxythiophene central unit combined with benzimidazole, phenyl, carboxamidophenyl and amidine building blocks employed as already known pharmacophores.

3,4-ethylenedioxythiophene as a central unit in derivatives presented here was selected to explore the chemical space around the already known thiophene central linker employed in some other antimicrobially active MGBs [29,30]. Also, this moiety is considered chemically very stable and robust. Specifically, 3,4-ethylenedioxythiophene is commonly used in conducting polymers where its two electron-donating oxygen atoms adjacent to the thiophene ring stabilize the positive charges generated in oxidized polymer [31,32]. We assumed that

such electron donor capacity of 3,4-ethylenedioxythiophene will additionally stabilize amidine end groups thus contributing to the overall stability of the studied molecules.

Benzimidazole ring is an important heterocyclic pharmacophore in drug discovery and the compounds carrying different substituents on benzimidazole structure are associated with a wide range of biological activities, including antibacterial properties [18,20,22,33]. A number of dicationically substituted bis-benzimidazoles originally developed as DNA binding agents have shown antibacterial activity [19,25,34,35]. In addition, compounds with benzimidazol-phenyl moiety separated by different linkers exhibit high activity against drug–resistant Gram-positive bacteria without cytotoxicity in their therapeutic concentrations [36]. Extensive biological studies of benzimidazoles and phenyl derivatives have confirmed that presence of these structural units was crucial for antibacterial activity of compounds [18,19,34,35]. Several bis-benzimidazoles have shown significant antibacterial activities against drug-resistant bacteria (methicillin-resistant Staphylococcus *aureus* – MRSA and methicillin resistant *S. epidermidis* – MRSE and vancomycin resistant enterococci – VRE strains) [19,37]. In addition, diamidine derivatives of bis-indoles, as structural analogues of benzimidazoles, were shown to exhibit *in vitro* activity against a broad spectrum of Gram-negative and Gram-positive pathogens such as *S. aureus* (including MRSA) and *E. faecalis* (including VRE) which additionally supports the use of mono- and bis-benzimidazole units in 3,4-ethylenedioxythiophene series [21,24].

Furthermore, bis-phenyl derivatives were synthesized in order to explore the influence of aromatic phenyl unit on the compound DNA binding and antibacterial activity. Previous reports by other research groups have suggested that planar and hydrophobic phenyl building blocks contribute to increased antibacterial activity, especially against MRSA and VRE strains [25]. Also, it is widely recognised that the introduction of phenyl group increases lipophilicity. Literature data indicate that increased lipophilicity in some distamycin derivatives was associated with improved DNA binding and higher activity against gram positive bacteria [25,38].

In our compound design diamidines represent the main pharmacologically relevant structural feature. Previously reported aromatic diamidines achieve their biological activities through the interaction with DNA, acting as MGBs [39]. It is therefore generally accepted that MGBs must have cationic end groups needed for electrostatic interactions with negatively charged sugar-phosphate backbone of DNA [40,41]. In addition, acyclic and cyclic amidine terminal groups with multiple positive charges show increased accumulation within cell nuclei probably as a result of stronger DNA binding affinity [42].

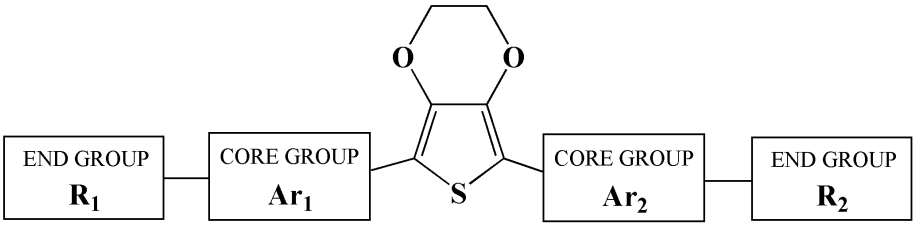
The present report describes the synthesis of nine new amidine derivatives (**9**–**17**) and evaluation of antibacterial activity of 27 amidine derivatives from 3,4-ethylenedioxythiophene class (including 18 previously synthesized analogues). The structure-activity relationship of their antibacterial potencies and DNA binding affinities is examined.

2. RESULTS AND DISCUSSION

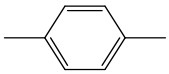
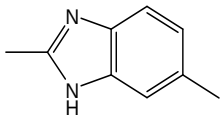
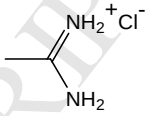
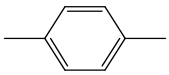
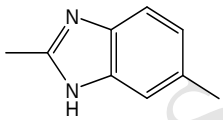
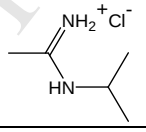
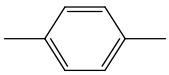
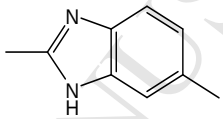
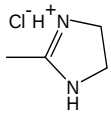
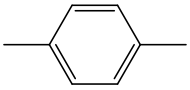
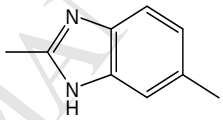
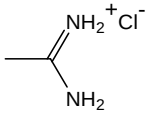
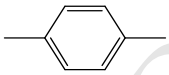
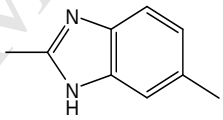
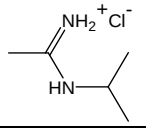
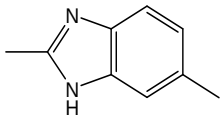
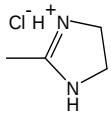
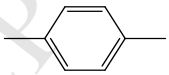
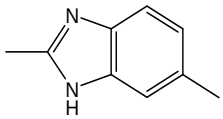
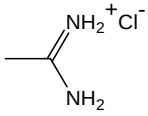
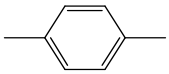
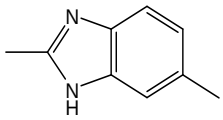
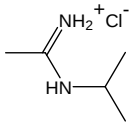
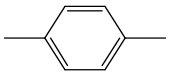
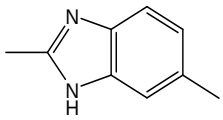
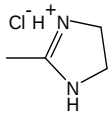
2.1. Chemistry

The synthesis and characterization of compounds **18**–**35** have been described in our previous reports [26-28]. In this study we extended our investigations towards evaluation of the antibacterial activity. The preliminary testing of derivatives **18-35** demonstrated promising *in vitro* antibacterial activity (Table 2). Based on the obtained results, our studies were further expanded towards the synthesis of new amidine analogues. Thus, we designed and prepared novel asymmetrical compounds (**9**–**17**) to investigate the structural features of this subclass contributing to the biological activity.

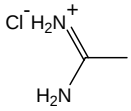
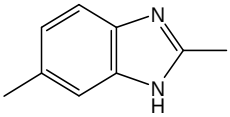
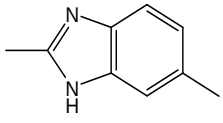
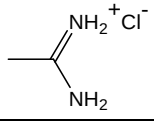
Table 1. Structures of tested compounds illustrated by their major building blocks: 3,4-ethylenedioxythiophene central unit, core groups (Ar_1 and Ar_2) and end groups (R_1 and R_2). **A**. Structures of newly synthesized compounds. **B**. Structures of previously synthesized compounds [26-28].

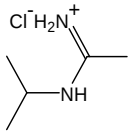
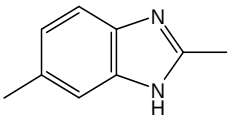
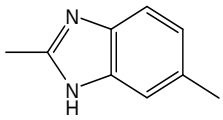
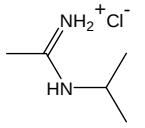
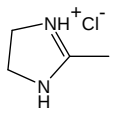
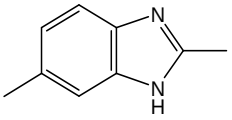
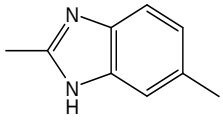
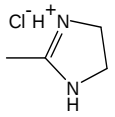
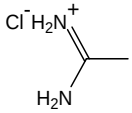
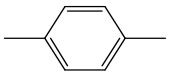
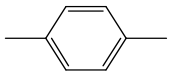
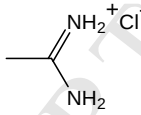
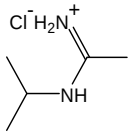
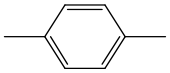
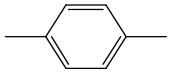
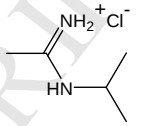
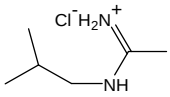
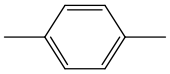
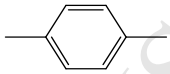
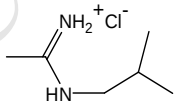
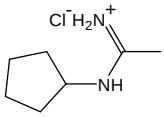
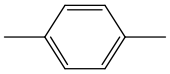
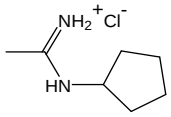
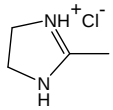
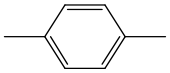
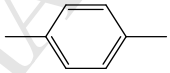
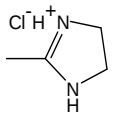
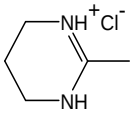
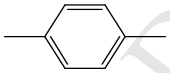
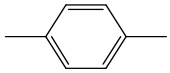
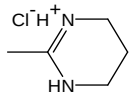
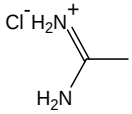
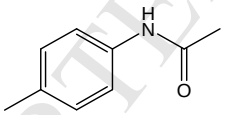
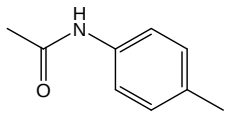
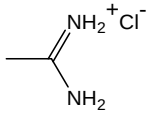
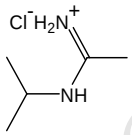
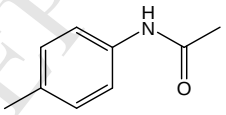
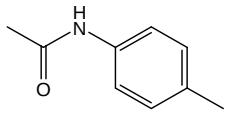
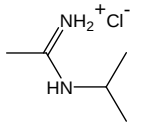


A

Cpd	R_1	Ar_1	Ar_2	R_2
9	NC–			
10	NC–			
11	NC–			
12	O_2N–			
13	O_2N–			
14	O_2N–			
15	$Cl^-H_3^+N$–			
16	$Cl^-H_3^+N$–			
17	$Cl^-H_3^+N$–			

B

Cpd	R_1	Ar_1	Ar_2	R_2
18[a]				

19[a]				
20[a]				
21[b]				
22[b]				
23[b]				
24[b]				
25[b]				
26[b]				
27[c]				
28[c]				
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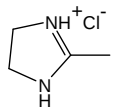
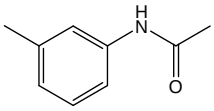
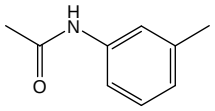
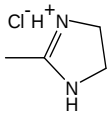
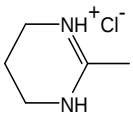
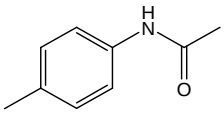
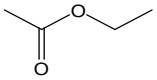
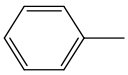
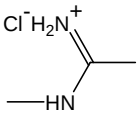
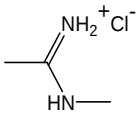
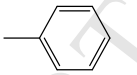
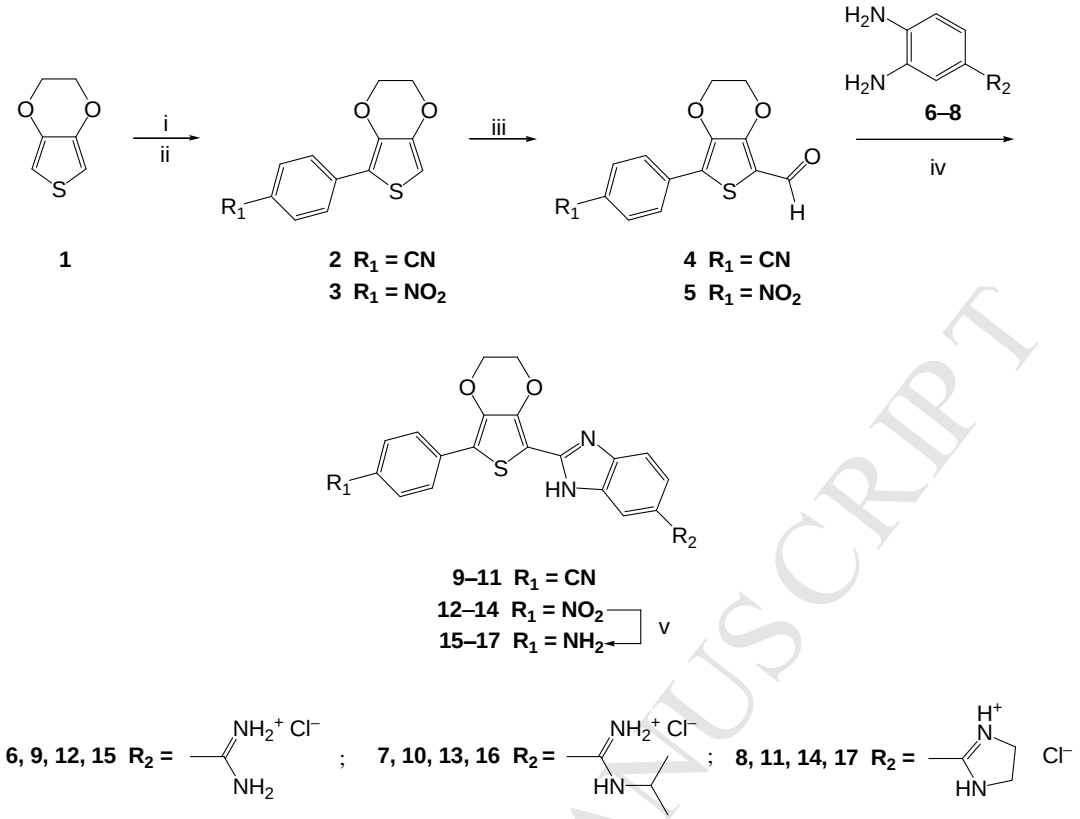
33[c]				
34[c]				-
35[b]				

Synthesis presented in: [a] Ref. [26], [b] Ref. [27], [c] Ref. [28].

The synthesis of novel asymmetrical benzimidazoles **9**–**17** outlined in Scheme 1 was achieved employing the similar approach developed to make the symmetrical bis-phenyl [27] or bis-benzimidazole [26] analogues. The synthesis started with Still cross-coupling reactions between 3,4-ethylenedioxythiophene (**1**) and 4-bromobenzonitrile or 1-bromo-4-nitrobenzene to give the 2-aryl-3,4-ethylenedioxythiophene derivatives **2** or **3** in moderate yields. Compounds **2** and **3** were converted to the aldehydes by reacting with $POCl_3$. The target benzimidazoles **9**–**17** were prepared by oxidative coupling of aldehydes **4** or **5** with the appropriate 3,4-diaminobenzamidines (**6**–**8**) in 60–70 % yields. Catalytic hydrogenation of nitro derivatives **12**–**14** gave corresponding amines **15**–**17** in good yields [43].



Scheme 1. Reagents: i) 1. n-BuLi, THF; 2. Bu_3SnCl; ii) $Pd(PPh_3)_4$, 4-bromobenzonitrile or 1-bromo-4-nitrobenzene, THF; iii) $POCl_3$, DMF; iv) 3,4-diaminobenzimidamide hydrochloride (**6**), 3,4-diamino-*N*-isopropylbenzimidamide hydrochloride (**7**), 4-(4,5-dihydro-1*H*-imidazol-2-yl)benzene-1,2-diamine hydrochloride (**8**), 1,4-benzoquinone, C_2H_5OH; v) H_2, 10 % Pd/C, CH_3OH.

Different classes of amidine based minor groove binders exhibited activity against Gram-positive bacteria while the related QSAR studies revealed benzoxazole, benzimidazole and benzothiazole as necessary functionalities for adequate antibacterial activity [44-47]. For that reason, we decided to use these pharmacophores in the design and synthesis of here reported antibacterial compounds. Besides, terminal aliphatic amines are often employed in the synthesis of biologically active compounds, due to their basic properties and strong protonation at the physiological pH contributing to ionic interactions and hydrogen bonding within groove [12]. In addition, compound curvature is an important factor for complex formation and strong binding in the minor groove. The diamidines as well as other MGBs either match the curvature of the DNA minor groove in their solution state or are able to adopt a low energy conformation to complement the DNA groove shape in the complex [48]. Taking into account all mentioned, biological activity can be seen as a net result of van der

Waals contacts, hydrogen bonding, and electrostatic interactions between investigated compounds and DNA [12,49].

In order to explore the influence of structural features on antibacterial activity and DNA binding affinity of here reported compounds all structural units (Ar_1, Ar_2, R_1 and R_2, Table 1) except the linker (3,4-ethylenedioxythiophene) were modified. Previously conducted research of influence of central linker on antibacterial activity of MGBs, including benzimidazoles and phenyl core containing molecules, revealed that central linker is very important for achieving potent antibacterial activity [19,25]. In this study we have chosen a rigid 3,4-ethylenedioxythiophene linker to favour interactions within groove. We hypothesized that the cumulative effects of steric factors and van der Walls contacts within groove, the linker size and potential for hydrogen bonds formation as well as nonbonding interactions of the rest of structure, will additionally contribute to DNA binding and antibacterial activity as compared with often used small, cyclic aromatic linkers, such as phenyl, pyrimidine, single atoms or aliphatic chains.

Structural modifications of 3,4-ethylenedioxthiophene compounds have included aromatic cores Ar_1 and Ar_2 (phenyl-, benzimidazole-), end groups R_1 and R_2 (un-substituted amidine, alkyl substitute amidines, cyclic amidines, nitro-, amino- and cyano- substituents) and their combinations (Table 1). The influence of end group physical-chemical properties, mainly charge and hydrophobicity on overall biological activity was explored. It has been shown that enhancing the hydrophobicity of the cationic end groups could increase the binding affinity of the molecule to DNA and aid in drug transport and antibiotic activity [11]. In order to investigate the influence of hydrogen bonding capacity on DNA binding and biological activity two different aromatic scaffolds were introduced. Benzimidazole has the capacity to form hydrogen bonds with DNA through NH group, while phenyl group contributes to the stronger DNA binding through its hydrophobic interactions within the groove. Additionally, asymmetrical design was investigated since some asymmetrical MGBs exhibited high antimicrobial potencies [34].

Synthesized compounds are divided in eight structural groups: i) bis-phenyl- (**21**–**26**), ii) bis-benzimidazole- (**18**–**20**), iii) bis-carboxamidophenyl- (*para* **27**–**31**), iv) bis-carboxamidophenyl- (*meta* **32**,**33**), v) cyano-amidine (**9**–**11**), vi) nitro-amidine (**12**–**14**), vii) amino-amidine (**15**–**17**) and viii) miscellaneous derivatives (**34** and **35**). Compound **34** is derivative of compound **31** with Ar_2 and R_2 replaced by ethyl ester, while compound **35** is a derivative of compound **21** with the end groups and aromates inversely arranged.

2.2. UV-Vis spectroscopy

Since DNA binding studies are conducted by spectroscopic methods, the spectrometric behaviour of aqueous solutions of newly synthesised compounds was studied by UV-Vis spectroscopy. Linear dependence of UV-Vis spectra on the concentration of compounds **9**–**17** was in the range 1–5.5 × 10^{-5} mol dm^{-3} indicating the absence of intermolecular interactions in their aqueous solutions. The UV-Vis spectra of compounds **9**–**17** were determined to be temperature independent when measured in the 25–90 °C temperature range. Furthermore, upon cooling to 25 °C excellent reproducibility of spectra was observed. Aqueous solutions of all studied compounds were stable over several weeks at –18 °C.

2.3. Thermal denaturation experiments

Thermal melting enables the rapid qualitative evaluation of the relative binding affinities of compounds for polynucleotides [50]. The stability of the calf thymus DNA (ctDNA) double helix influences the DNA melting temperature (T_m), while the binding of compounds to DNA alters the T_m due to the interactions formed at the interface. Thus, the measurement of ΔT_m taking place after the formation of the small molecule-ctDNA complex indicates the strength of compound-DNA interaction. To investigate this characteristic, the changes in the T_m upon addition of amidines **9**–**17** to the ctDNA buffer solution were measured at r ([amidines]/[DNA in base pair]) = 0.1 (Table 2). The obtained results were compared to values for compounds **18**–**35** from previously published data set [26-28].

The ΔT_m values ranged from 0 (compounds **34** and **35** that do not bind to DNA) to 15.4 °C, with compound **19** having the highest ΔT_m. Previously conducted qualitative estimation of the DNA binding affinities of amidines showed that the compounds from bis-benzimidazole (**18**–**20**) and bis-phenyl (**21**–**26**) series had similar ΔT_m values (8.4–15.4 °C) [26,27], even though benzimidazoles have additional hydrogen bonding site available. This can be explained by the fact that even though hydrogen bonds established within the minor groove are one of the key factors for formation of stable compound-DNA complex, the number of hydrogen bonding sites on the molecule does not necessarily correlate with the binding strength, since not all of the bonds will be formed [42]. This assumption is further supported by the ΔT_m values of compounds from carboxamid-amidine series (**27**–**33**). Introduction of amid bond between linker and phenyl units in bis-phenyl series, although contributing additional hydrogen bonding sites, resulted in drastic decrease of ΔT_m values. This can be attributed to their inability to assume appropriate conformation within groove,

therefore failing to form maximum number of hydrogen bonds. Three newly synthesized series of phenyl-benzimidazole compounds (**9**–**11**, **12**–**14** and **15**–**17**), although having similar molecular geometry as those from bis-benzimidazole and bis-phenyl series, bind to DNA weakly (ΔT_m values of 1.1–3.8 °C). In addition, one amidino moiety was replaced with cyano-, nitro-, amino-end group and this substitution led to the decrease in compounds' DNA binding affinity. This is in accordance with the literature data claiming that basic nature and protonated state of amidino group in biological conditions strongly contributes to DNA binding [51].

2.4. Antibacterial activity and structure-activity relationship (SAR)

For the compounds from amidine series of 3,4-ethylenedioxythiophene, antibacterial activity against five gram-positive (*Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Staphylococcus epidermidis and Enterococcus faecium*) and three gram-negative (*Escherichia coli*, *Moraxella catarrhalis*, *Haemophilus influenzae*) bacterial species were determined. Activity against highly resistant strains of staphylococci (methicillin-resistant *S. aureus* – MRSA and methicillin resistant *S. epidermidis* – MRSE), as well as vancomycin resistant *E. faecium* (VRE) was also tested. Values for minimal inhibitory concentrations (MICs) are given in Tables 2 and 3.

Table 2. Biological activities of compounds **9**–**35**: the influence of the compounds on the calf thymus DNA stability expressed as the change in the melting temperature (ΔT_m) and inhibition of the *in vitro* bacterial growth expressed as the MIC (minimal inhibitory concentration) values. ΔT_mvalues for compounds **18**–**35** are from [26-28].

		MIC (μg/ml)					
Cpd	**ΔT_m (° C)**	***S. aureus ATCC 13709***	***S. pneumoniae ATCC 49619***	***S. pyogenes ATCC 700294***	***M. catarrhalis ATCC 23246***	***E. coli ATCC25922***	***H. influenzae ATCC 49247***
9	3.8	> 128	> 128	> 128	32	> 128	> 128
10	2.2	4	64	4	0.5	> 128	> 128
11	1.1	4	16	4	0.5	128	> 128
12	2.2	> 128	> 128	> 128	32	> 128	> 128
13	3.1	32	> 128	> 128	1	> 128	> 128
14	2.3	> 128	> 128	> 128	32	> 128	> 128
15	2.0	64	> 128	> 128	2	> 128	> 128
16	1.8	32	> 128	16	2	> 128	> 128
17	1.1	> 128	> 128	4	1	> 128	> 128
18	14.4	128	> 128	4	2	> 128	> 128
19	15.4	4	2	0.5	≤ 0.25	> 128	> 128
20	11.9	> 128	> 128	16	64	> 128	> 128
21	12.2	4	> 128	0.5	≤ 0.25	32	128
22	8.4	8	128	2	0.5	32	64
23	9	4	64	2	≤ 0.25	16	32
24	12.3	2	64	0.5	≤ 0.25	8	64
25	10.8	16	64	2	2	128	> 128
26	11.8	16	> 128	4	1	32	32
27	1.3	8	> 128	4	8	64	> 128
28	1.6	> 128	> 128	> 128	> 128	> 128	> 128
29	2.1	> 128	> 128	> 128	> 128	> 128	> 128
30	1.2	64	> 128	4	16	>128	> 128
31	1.4	64	> 128	32	1	>128	> 128
32	0.8	128	> 128	32	2	> 128	> 128
33	1.8	64	128	16	32	> 128	> 128
34	0	> 128	> 128	> 128	64	> 128	> 128
35	0	> 128	> 128	> 128	> 128	> 128	> 128
azithromycin		1	0.25	0.125	0.06	8	2

Table 3. Antibacterial activity of selected active compounds against additional clinical strains of Gram-positive organisms.

	MIC (μg/ml)					
Compound	***S. aureus B0008 MRSA***	***S. aureus B0967 MRSA***	***S. epidermidis B0423 MSSE***	***S. epidermidis B0674 MRSE***	***E. faecium B0557 VSE***	***E. faecium B0085 VRE***
10	8	>128	32	64	128	32
11	4	128	16	16	32	16
12	>128	>128	>128	128	128	64
13	16	>128	128	128	128	64
15	128	>128	128	128	128	128
16	64	>128	64	128	128	128
17	>128	>128	>128	>128	>128	128
18	128	>128	128	64	32	32
19	16	32	16	16	4	8
20	128	128	128	128	32	32
21	8	64	8	4	2	16
22	16	128	4	64	32	>128
23	8	32	16	16	32	64
24	8	32	8	16	2	32
25	32	32	16	32	32	32
26	64	64	32	32	32	128
27	8	32	4	1	128	32
30	64	16	32	16	64	32
31	32	128	64	0.5	128	64
32	128	128	16	32	128	64
33	64	>128	32	32	128	64
oxacillin	>128	>128	≤0.25	>128	32	>128
ampicillin	128	128	≤0.25	64	0.5	64
vancomycin	1	2	2	1	0.25	>16
azithromycin	>128	>128	1	1	≤0.25	32

The results of antibacterial screening revealed that, in general, the bis-phenyl derivatives (**21**–**27**) were the most active compound subset with promising activity against vancomycin resistant *E. faecium* strain (16–64 μg/ml). This subclass also exhibited high binding affinity towards DNA (compounds **21**, **24**, **25, 26** all had ΔTm values >10 °C). Within the bis-phenyl series, compound **24** displayed the best overall antibacterial activity. The most potent effect of compound **24** was manifested against *E. coli* and *S. aureus* (MICs of 8 and 2 μg/ml, respectively) approaching the activity of standard antibiotic azithromycin. In addition, compound **24** showed marked activity toward *S. pyogenes* and *M. catarrhalis* (MICs of 0.5 and ≤0.25 μg/ml, respectively). The MICs of 64 μg/ml against *S. pneumoniae* and *H. influenzae* indicated much lower potency against these species. Two additional compounds from bis-phenyl series, compounds **21** and **23,** showed similar trends in antibacterial activity (Table 2). Compounds from bis-phenyl class also showed good antibacterial activity against clinical strains of staphylococci and enterococci, especially compound **21** with MIC of 16 μg/ml against VRE.

In bis-benzimidazole analogues **18**–**20**, replacement of both phenyl moieties with benzimidazole groups did not improve antibacterial activity. Although all three analogues retained high DNA binding affinities, only compound **19** exhibited significant antibacterial potency. Compound **19** showed substantial activity against Gram-positive bacterial species, including VRE (with the lowest MIC in this set of compounds, 8 μg/ml), and promising activity against MRSA and MRSE strains (MICs 16–32 μg/ml). It was the only compound with noteworthy activity against *S. pneumoniae*, impeding its growth at 2 μg/ml and at the same time inhibiting *S. pyogenes* strain at 0.5 μg/ml. In addition, compound **19** showed the strongest DNA binding affinity of all tested compounds (ΔT_m 15.4 °C).

Replacement of one amidino-benzimidazole group in symmetrical compounds **18**–**20** with substituted phenyl unit yielded asymmetrical compounds **9**–**17** and generally resulted in the decrease of antibacterial activity. Among the three groups: cyano- (**9**–**11**), nitro- (**12**–**14**) and amino-amidine derivatives (**15**–**17**), only two compounds from the nitrile class, **10** and **11,** demonstrated somewhat wider antibacterial spectrum with prominent activity against *M. catarrhalis* (MICs of 0.5 μg/ml), *S. pyogenes* and sensitive strain of *S. aureus* (MICs of 4 μg/ml for both species). This finding is in accordance with the wide use of nitrile groups in many commercial drugs since nitriles often play a key role as hydrogen bond acceptors and polarize the aromatic π-system to optimize π–π interactions between compounds and DNA [52]. Compounds **15**–**17** from the amino-amidine subclass, even though bearing amine groups

as hydrogen bond donors, were found to be less potent. Substitution of amine group present in **15**–**17** with electron-acceptor dipolar nitro group yielded compounds **12**–**14** that were biologically less active.

Expansion of the core by introduction of carboxamide bond between the phenyl group and the central 3,4-ethylenedioxythiophene unit yielded compounds **27**–**34** and resulted in the substantial decrease of the antibacterial activity as well as in the affinity towards DNA. Compounds **31** and **27** (obtained by substitution of 1,4,5,6-tetrahydropyrimidine substituents of compound **31** with amidine groups) showed excellent activities against methicillin-resistant *S. epidermidis* strain with MICs of 0.5 and 1 μg/ml, respectively. Shifting the amidino moiety on the phenyl ring from *para* (compounds **21** and **25**) to *meta* position (compounds **32** and **33**) significantly impaired antibacterial activity against all the tested strains. Compounds **34** and **35**, showing no changes in ΔT_m (Table 2), were antibacterially inactive.

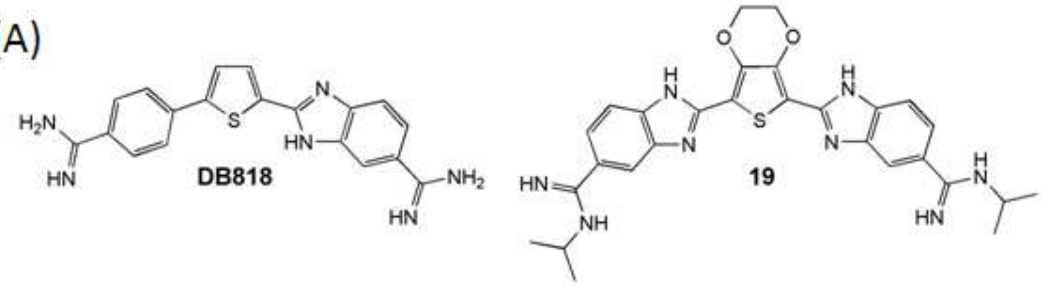
In this study it was observed that in all derivative groups, except carboxamid-amidines, amidine derivatives with alkyl end groups appeared to be more active than their counterparts containing unsubstituted amidine moiety or cyclic amidines as end groups. This might be due to the fact that hydrophobic groups, which are often present in DNA binding agents, maximize contacts with deoxyribose in DNA and thus increase the agent-DNA binding strength [11]. Therefore, structural modifications of the end groups have a decisive effect on the biological activity.

To additionally verify suggested interactions, the binding of here investigated compounds into the DNA minor groove was analysed *in silico* by utilizing the most active compound **19** as a representative. This compound has both the best antibacterial profile and the highest relative binding affinity for DNA. The binding of the molecule **19** to DNA has been suggested by using simple molecular docking approach and the crystal structure of the **DB818-DNA** complex available in the RCSB Protein and Nucleic Acid Data Banks with accession number **1VZK** [53]. **DB818** binds to AT rich minor groove region of the duplex B-DNA [30,39] DNA model used in **1VZK** structure has been $d(CGCGAATTCGCG)_2$, often utilised B-DNA duplex model structure with AATT minor groove site. We supposed that due to structural similarity with **DB818,** compound **19** is likely to share similar binding mode (Figures 2 (A) and (B)).

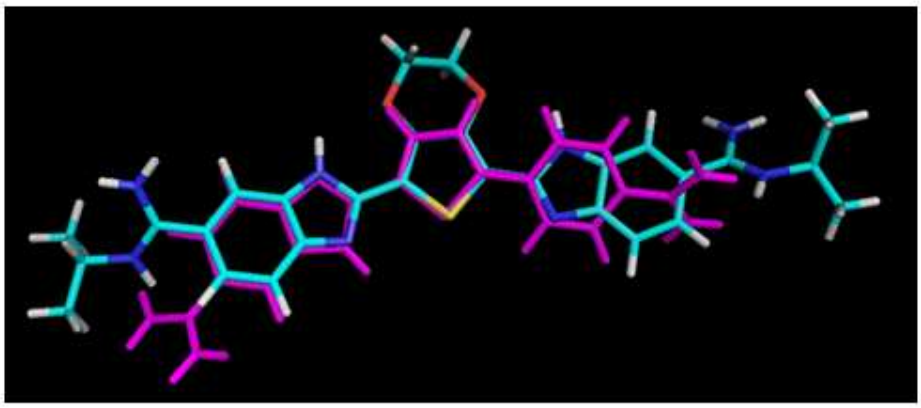
The thiophene compounds **DB818** and **19** have very similar concave inner radius (Figure 2 (B)). Their central phenyl/benzimidazole-thiophene-benzimidazole building blocks

are nearly planar allowing their optimal fit against the five base-pair edges at the floor of the groove. Both compounds form hydrogen bond contacts primarily with the groove floor, specifically AT bases of the DNA model molecule [30]. Their terminal amidine groups are conformationally flexible. In the case of **DB818** these groups make hydrogen bonds with AT bases of DNA [30], while in the case of the larger compound **19** they face sugar-phosphate backbone for hydrogen bonding and electrostatic interactions (Figure 2 (C)). Although compounds **9**-**17** are structurally more similar to **DB818** than compound **19,** the presence of structural groups less prone to form hydrogen bonds such as cyano, amino and nitro, limits their hydrogen bonding capacity and consequently results in a lower DNA binding affinity (i.e. lower ΔT_m values).

(A)



(B)



(C)

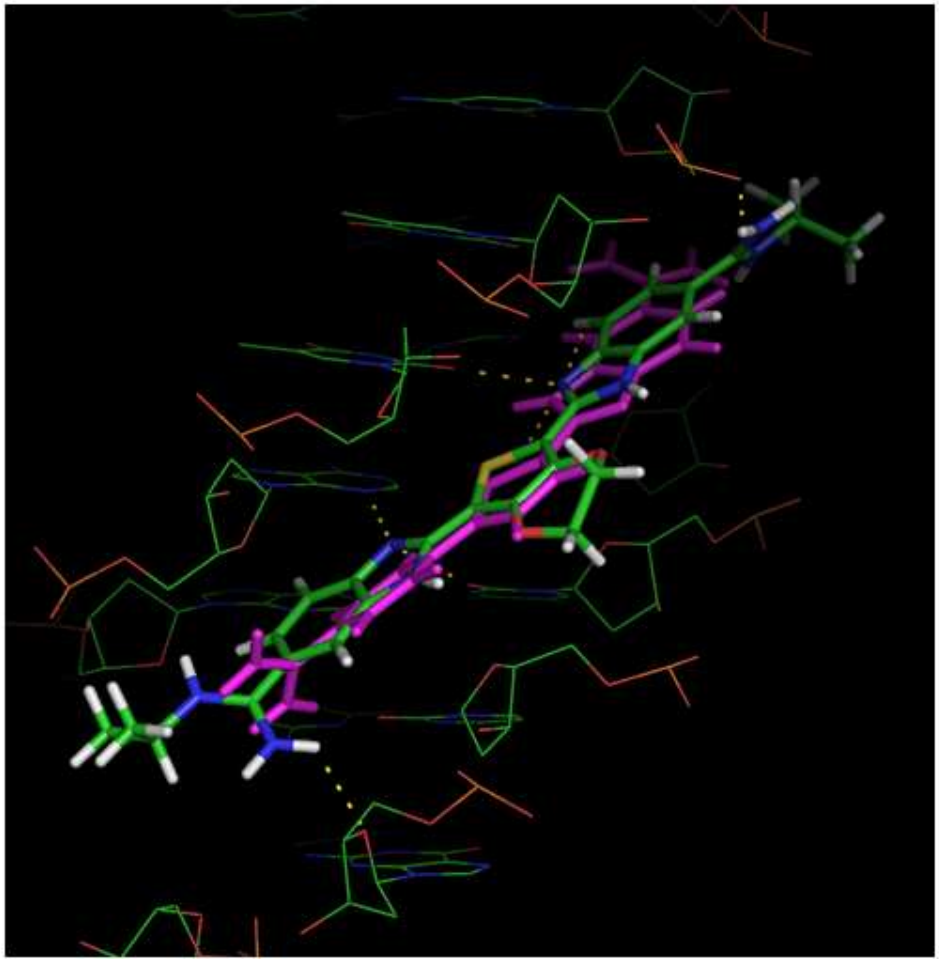


Figure 2. (A) 2D structures of compounds **DB818** and **19**. (B) Alignment of the crystal structure of **DB818** (magenta) and the optimized, B3LYP/6-31+G** structure of **19**. The more stable conformer of **19** with two intramolecular hydrogen bonds between imidazoles and 3,4-ethylenedioxy fragment, is shown. (C) Overlay of diprotonated structures of **DB818** and **19** (shown in (B)) within the AATT binding site of $d(CGCGAATTCGCG)_2$ [30]. Possible interactions between compound **19** and DNA are shown in yellow dots.

From the results of this study, it could be generally suggested that DNA binding represents the mechanism of antibacterial action of here reported compounds. Nevertheless, no direct correlation between ΔT_m values and antibacterial activity for all the compounds could be observed. A number of possible explanations for this could be proposed. Additional biological and cellular aspects including different ability of the compounds to passively cross bacterial cell wall and/or engagement of different influx/efflux transport mechanisms might lead to poor accumulation within the cell and consequently impede DNA-compound interactions. The potential for the distinct substrate specificity for closely related bis-indoles was already suggested for Multidrug and Toxic Compound Extrusion (MATE) family of efflux pumps [24] indicating that altered compound cell accumulation could influence the discrepancies observed between target and whole-cell activities. Previous studies of MGBs have made similar observations and conclusions, mainly emphasizing that target activity is just one of the factors influencing biological activity and that physicochemical properties, membrane interactions and transport play significant part in the overall drug potency [11,12].

2.5. In vitro cytotoxic activity

Nine new amidine derivatives **9**–**17** were assessed on their cytotoxic effect against two human cell lines: HepG2 (hepatocellular carcinoma) and THP-1 (acute monocytic leukemia) to determine whether the new compounds have inhibitory influence on host cells. By using MTS assay, metabolic activity of the cells was evaluated and none of the nine compounds inhibited cell proliferation i.e. IC_{50} values for all compounds and for both cell lines were >95 μM. New derivatives **9**-**17**, in general, affected eukaryotic cells to a lesser extent than previously synthesized compounds bis-benzimidazoles **18**–**20** [26], bis-phenyls **21**–**26** [27] and carboxamid-amidines **27**–**34** [28] that showed cytotoxic activity against several different human cell lines. Out of the subsets of previously synthesized molecules, compounds **25** and **27** were more cytotoxic than their counterparts displaying cytotoxic effect in the low

micromolar concentrations across all the tested cell lines, while compound **19**, although being the most effective in its subset, was not as potent and consistent against all the cell lines. Overall, SAR between different biological effects, primarily with regard to cytotoxicity and antibacterial potency, could not be explicitly claimed.

3. CONCLUSIONS

In this study 9 new amidine derivatives of 3,4-ethylenedioxythiophene (**9**–**17**) were synthesized. Their DNA binding properties and cytotoxicity were measured. Here presented new derivatives, together with our previously reported compound classes, were for the first time evaluated for their antibacterial potency against sensitive and resistant bacterial strains.

Most of the compounds have shown moderate activity against selected bacterial species with antibacterial spectrum extended against resistant gram positive strains (MRSA, MRSE, VRE). The bis-phenyl derivatives (**21**–**27**) were the most active subset of compounds with promising activity against both sensitive and resistant strains and compounds **21**–**24** being the most potent. Compound **19** from bis-benzimidazole class exhibited the widest antibacterial spectrum of activity (with MIC of 4, 2, 0.5 and ≤0.25 μg/ml against laboratory strains *S. aureus, S. pneumoniae, S. pyogenes, M. catarrhalis*, respectively and 4-32 μg/ml against clinical isolates of sensitive and resistant *S. aureus, S. epidermidis and E. faecium*) and demonstrated the strongest DNA binding affinity (ΔT_m of 15.4 °C). Asymmetrically designed compounds and carboxamid-amidines were, in general, less active although nitriles **10** and **11** demonstrated wider antibacterial spectrum of activity. Also, in this class of asymmetric compounds, alkyl derivatives have shown higher potency. Simple molecular docking indicated that the shape of here presented compounds and their ability to form multiple electrostatic and hydrogen bonds correspond to the DNA binding mode of other MGBs. For compounds, although no direct correlation between DNA binding and antibacterial action was established, it can be suggested that DNA binding plays an important role in the mechanism of action.

Herein reported results encourage further derivatization and investigation of this compound class as novel antibacterial MGBs.

4. EXPERIMENTAL SECTION

4.1. Chemistry

The compounds **18**–**35** have been described previously [26-28]. The synthesis and physical properties of novel compounds are given as follows.

Solvents were distilled from appropriate drying agents shortly before use. TLC was carried out on DC-plastikfolien Kieselgel 60 F254, Merck. Melting points were determined on a Büchi 510 melting point apparatus and were uncorrected. IR spectra [ν_{max}/cm^{-1}] were obtained on a Bruker Vertex 70 spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 300 MHz spectrometer. Chemical shifts (δ/ppm) were referred to TMS. Mass spectra were recorded on a Waters Micromass Q-ToF micro. Elemental analyses were performed by the Applied Laboratory Research Department at Ina, d.d., Research and Development Sector, Central Analytical Laboratory.

4.1.1. 5-(4-cyanophenyl)-2,3-dihydrothieno[3,4-*b*][1,4]dioxine (**2**)

To a stirred solution of 3,4-ethylenedioxythiophene (**1**) (0.27 g, 1.90 mmol) in freshly distilled THF (20 mL), 2.5 M n-BuLi in hexane (1 mL, 2.50 mmol) was slowly added under the nitrogen atmosphere at –78 °C. The resulting solution was stirred until it reached temperature of –40 °C and Bu_3SnCl (0.7 mL, 0.84 g, 2.59 mmol) was added dropwise. The vigorously stirred mixture was left overnight at room temperature. The solvent was removed under reduced pressure, the residue suspended in anhydrous hexane and the suspension was filtered off. The filtrate was concentrated to dryness under reduced pressure and the obtained yellow liquid (0.81 g, 2.79 mmol) was dissolved in freshly distilled THF (20 mL) under a nitrogen atmosphere. 4-Bromobenzonitrile (0.34 g, 2.86 mmol) and [$Pd(PPh_3)_4$] (0.11 g, 0.1 mmol) were added to the solution. The vigorously stirred mixture was refluxed for 7 days under a nitrogen atmosphere. The solvent was partially evaporated, and obtained product was purified by column chromatography (CH_2Cl_2:petrolether 1:1) to yield 0.39 g (85.7 %) of white powder, mp 100–101 °C; IR (KBr) (ν_{max}/cm^{-1}): 2927, 2856, 2224, 1606, 1511, 1485, 1179, 1066, 910, 845, 819, 711, 681, 554; ^{1}H NMR (DMSO-d_6) δ/ppm: 7.75 (d, 2H, J = 8.60 Hz, ArH), 7. 77 (d, 2H, J = 8.60 Hz, ArH), 6.36 (s, 1H, ArH), 4.38 + 4.27 (m + m, 4H, OCH_2CH_2O). MS *m/z* 244.00 $(M+H)^+$.

4.1.2. 5-(4-nitrophenyl)-2,3-dihydrothieno[3,4-*b*][1,4]dioxine (**3**)

To a stirred solution of 3,4-ethylenedioxythiophene (**1**) (3 g, 21.1 mmol) in freshly distilled THF (230 mL), 2.7 M n-BuLi in hexane (8 mL, 21.6 mol) was slowly added under the nitrogen atmosphere at –78 °C. The resulting solution was stirred till –40 °C when Bu_3SnCl (6 mL, 7.2 g, 22.2 mol) was added dropwise. The vigorously stirred mixture was left over night at room temperature. The solvent was removed under reduced pressure. The residue was suspended in anhydrous hexane and suspension was filtered off. The filtrate was concentrated to dryness under reduced pressure and the was dissolved in freshly distilled THF (200 mL) under a nitrogen atmosphere and then 4-bromo-1-nitrobenzen (4.26 g, 21.1 mmol) and [$Pd(PPh_3)_4$] (1.22 g, 1.06 mmol) were added. The vigorously stirred mixture was refluxed for 3 days under a nitrogen atmosphere. The solvent was evaporated, and the precipitate was dissolved in mixture of CH_2Cl_2:petrolether 1:1, and purified by column chromatography. Yield 2.74 g (49.4 %) of yellow powder, mp 189–190 °C; IR (ν_{max}/cm^{-1}): 1587, 1498, 1474, 1336, 1175, 1060, 851, 753, 705, 664, 526, 461; ^{1}H NMR ($CDCl_3$) δ/ppm: 8.25 (d, 2H, J = 9.01 Hz, ArH), 7.89 (d, 2H, J = 9.05 Hz, ArH), 6.88 (s, 1H, thiophene), 4.41 + 4.29 (m + m, 4H, OCH_2CH_2O); ^{13}C NMR (DMSO-d_6) δ/ppm: 145.4, 142.9, 141.6, 139.9, 125.9, 124.8, 114.1, 102.3, 65.6, 64.5.

4.1.3. 7-(4-cyanophenyl)-2,3-dihydrothieno[3,4-*b*][1,4]dioxine-5-carbaldehyde (**4**)

The solution of phosphorous oxychloride (16 mL, 26.40 g, 0.17 mol) and dimethylformamide (13 ml, 12.2 g, 0.17 mol) was stirred at 0 °C for 1.5h. Then solution of compound **2** (8.14 g, 0.03 mmol) in dry DMF (150 mL) was added and the reaction mixture was stirred under reflux for 5 h. After cooling, the solution was poured into ice and Na_2CO_3 is added until pH 8. Resulting solid is filtered off and washed with water till pH 7. Recrystallization from ethanol yield 6.02 g (66.9 %) of product, mp 262–263 °C; IR (KBr) (ν_{max}/cm^{-1}): 2925, 2855, 2229, 1658, 1606, 1516, 1488, 1452, 1371, 1277, 1082, 834, 672, 538; ^{1}H NMR (DMSO-d_6) δ/ppm: 9.97 (s, 1H, CHO), 7.88 (d, 2H, J = 8.37 Hz, ArH), 7.67 (d, 2H, J = 8.33 Hz, ArH), 4.43 (s, 4H, OCH_2CH_2O).; ^{13}C NMR (DMSO-d_6) δ/ppm: 179.6, 149.2, 140.0, 135.7, 132.9, 126.9, 123.5, 118.4, 116.4, 110.7, 65.3, 65.0; MS *m/z* 272.1 $(M+H)^+$.

4.1.4. 7-(4-nitrophenyl)-2,3-dihydrothieno[3,4-*b*][1,4]dioxine-5-carbaldehyde (**5**)

The solution of phosphorous oxychloride (0.4 mL, 0.66 g, 4.31 mmol) and dimethylformamide (0.33 ml, 0.31 g, 4.31 mmol) was stirred at 0 °C for 1.5 h. Then solution of compound **3** (0.19 g, 0.72 mmol) in dry DMF (8 mL) was added and the reaction mixture

was stirred under reflux for 5 h. After cooling, the solution was poured into ice and Na_2CO_3 is added until pH 8. Resulting solid is filtered off and wash with water till pH 7. Recrystallization from ethanol yield 0.14 g (69 %) of product, mp 253–254 °C; IR (KBr) (ν_{max}/cm^{-1}): 1652, 1595, 1498, 1449, 1336, 1077, 842, 681, 461; ^{1}H NMR (DMSO-d_6) δ/ppm: 9.94 (s, 1H, ArH), 8.31 (d, 2H, J = 9.02 Hz, ArH), 8.04 (d, 2H, J = 9.03 Hz, ArH), 4.5 (s, 4H, OCH_2CH_2O); ^{13}C NMR (DMSO-d_6) δ/ppm: 180.4, 149.7, 147.0, 141.0, 138.1, 127.7, 124.9, 123.4, 117.1, 65.8, 65.5;

4.1.5. 2-(7-(4-cyanophenyl)-2,3-dihydrothieno[3,4-*b*][1,4]dioxine-5-yl)-1*H*-benzo[*d*]imidazole-6-carboximidamide hydrochloride (**9**)

A solution of compound **4** (0.07 g, 0.26 mmol), 3,4-diaminobenzimidamide hydrochloride (**6**) (0.05 g, 0.27 mmol), and 1,4-benzoquinone (0.03 g, 0.28 mmol) in ethanol (30 mL) was heated at reflux for 4h (under nitrogen). The reaction mixture was cooled to room temperature. Dark solid collected by filtration was washed with anhydrous ether. Crude solid was dissolved in water and by addition of acetone precipitate is formed. It was dissolved in HCl-saturated ethanol and stirring overnight. Green solid was collected by filtration, washed with anhydrous ether and dried under vacuum to yield 0.04 g (11.1 %) of brown powder, mp > 290 °C; IR (ν_{max}/cm^{-1}): in KBr: 3099, 2219, 1596, 1481, 1315, 1083, 815, 538; ^{1}H NMR (DMSO-d_6) δ /ppm: 12.63 (s, 1H, NH), 9.27 (s, 2H, NH), 8.93 (s, 2H, NH), 8.09 (s, 1H, ArH), 7.93 (d, 4H, J = 1.26 Hz, ArH), 7.75 (d, 1H, J = 7.89 Hz, ArH), 7.65 (d, 1H, J = 8.09 Hz, ArH), 4.58 + 4.54 (s + s, 4H, OCH_2CH_2O); ^{13}C NMR (DMSO-d_6) δ /ppm: 166.1, 142.1, 140.2, 136.3, 132.9, 126.0, 122.1, 121.4, 118.8, 116.6, 109.2, 106.5, 65.1, 65.0. HRMS: calcd. for $C_{21}H_{16}N_5O_2S$ $(M+H)^+$: 402.1025; found: 402.1000. Anal. calcd. for $C_{21}H_{15}N_5O_2S \times HCl \times 2.5H_2O$ (M_r = 482.95): C 52.23, H 4.38, N 14.50; found: C 52.51, H 4.29, N 14.11%.

4.1.6. 2-(7-(4-cyanophenyl)-2,3-dihydrothieno[3,4-*b*][1,4]dioxine-5-yl)-*N*-isopropyl-1*H*-benzo[*d*]imidazole-6-carboximidamide hydrochloride (**10**)

A solution of compound **4** (0.1385 g, 0.51 mmol), 3,4-diamino-*N*-isopropylbenzimidamide hydrochloride (**7**) (0.117 g, 0.51 mmol), and 1,4-benzoquinone (0.05 g, 0.51 mmol) in ethanol (50 mL) was heated at reflux for 9 h (under nitrogen). The reaction mixture was cooled to room temperature. Dark precipitate collected by filtration was washed with anhydrous ether. Crude solid was dissolved in water and by addition of acetone precipitate was formed. It was dissolved in HCl-saturated ethanol and stirred overnight. Green

solid was collected by filtration, washed with anhydrous ether and dried under vacuum to yield 0.12 g (54.6 %) of green powder, mp 271–272 °C; IR (ν_{max}/cm^{-1}): 3093, 227, 2595, 1482, 1441, 1352, 1077, 535; ^{1}H NMR (DMSO-d_6) δ /ppm: 9.50 (s, 1H, NH), 9.37 (s, 1H, NH), 8.96 (s, 1H, NH2), 8.00 (s, 1H, ArH), 7.93 (s, 4H, ArH), 7.74 (d, 1H, J = 7.94 Hz, ArH), 7.54 (d, 1H, J = 7.64 Hz, ArH), 4.59 + 4.57 (br.s + br s, 4H, OCH_2CH_2O) 4.07 (m, 1H, CH), 1.30 (d, 6H, J = 6.35 Hz, CH_3); ^{13}C NMR (DMSO-d_6) δ /ppm: 162.9, 147.5, 142.6, 140.7, 136.8, 133.4, 126.5, 123.3, 122.8, 119.3, 117.0, 109.7, 107.1, 65.6, 65.5, 45.5, 21.8. HRMS: calcd. for $C_{24}H_{22}N_5O_2S$ $(M+H)^+$: 444.1494; found: 444.1489. Anal. calcd. for $C_{24}H_{21}N_5O_2S \times HCl \times 3H_2O$ (M_r = 534.04): C 53.98, H 5.28, N 13.11; found: C 53.73, H 5.14, N 13.12 %.

4.1.7. 2-(7-(4-cyanophenyl)-2,3-dihydrothieno[3,4-*b*][1,4]dioxine-5-yl)-6-(4,5-dihydro-1*H*-imidazol-2-yl)-1*H*-benzo[*d*]imidazole hydrochloride (**11**)

A solution of compound **4** (0.05 g, 0.18 mmol), 4-(4,5-dihydro-1*H*-imidazol-2-yl)benzene-1,2-diamine hydrochloride (**8**) (0.04 g, 0.18 mmol), and 1,4-benzoquinone (0.02 g, 0.18 mmol) in ethanol (40 mL) was heated at reflux for 13 h (under nitrogen). The reaction mixture was cooled to room temperature and the dark solid collected by filtration was washed with anhydrous ether. It was dissolved in water and by addition of acetone precipitate was formed. The precipitate was dissolved in HCl-saturated ethanol and stirred overnight. Green solid was collected by filtration, washed with anhydrous ether and dried under vacuum to yield 0.06 g (81.8 %) of green powder, mp 274–275 °C; IR (ν_{max}/cm^{-1}): in KBr: 3091, 2221, 1587, 1355, 1271, 1078, 1006, 849, 702, 538; ^{1}H NMR (DMSO-d_6) δ/ppm: 10.42 (s, 2H, NH), 7.93 (s, 1H, NH), 7.93 (s, 4H, ArH), 7.77 (s, 3H, ArH), 4.60 + 4.56 (br s + br s, 4H, OCH_2CH_2O), 4.01 (s, 4H, NCH_2CH_2N); ^{13}C NMR (DMSO-d_6) δ/ppm: 166.1, 147.0, 143.0, 142.8, 140.7, 140.5, 136.8, 133.4, 126.6, 123.1, 119.2, 115.9, 109.8, 106.9, 65.6, 65.5, 44.7, 40.5. HRMS: calcd. for $C_{23}H_{18}N_5O_2S$ $(M+H)^+$: 428.1181; found: 428.1197. Anal. calcd. for $C_{23}H_{17}N_5O_2S \times HCl \times 2.5H_2O$ (M_r = 508.99): C 54.28, H 4.55, N 13.76; found: C 54.46, H 4.20, N 13.87.

4.1.8. 2-(7-(4-nitrophenyl)-2,3-dihydrothieno[3,4-*b*][1,4]dioxine-5-yl)-1*H*-benzo[*d*]imidazole-6-carboximidamide hydrochloride (**12**)

A solution of compound **5** (0.2186 g, 0.75 mmol), 3,4-diaminobenzimidamide hydrochloride (**6**) (0.1686 g, 0.75 mmol), and 1,4-benzoquinone (0.09 g, 0.75 mmol) in absolute ethanol (200 mL) was heated at reflux for 2 days (under nitrogen). The reaction mixture was cooled to room temperature and the dark solid was collected by filtration and

washed with anhydrous ether. The solid was suspended in HCl-saturated ethanol and stirred overnight. Green solid was collected by filtration, washed with anhydrous ether, and dried under vacuum to yield 0.22 g (64.4 %) of green powder, mp > 290 °C; IR (ν_{max}/cm^{-1}): in KBr: 3077, 1685, 1588, 1482, 1442, 1337, 1086, 851; ^{1}H NMR (DMSO-d_6) δ /ppm: 9.35 (s, 2H, NH), 9.11 (s, 2H, NH), 8.31 (d, 2H, J = 8.80 Hz, ArH), 8.11 (s, 1H, ArH), 8.00 (d, 2H, J = 8.79 Hz, ArH), 7.76 (d, 1H, J = 7.52 Hz, ArH), 7.67 (dd, 1H, J_1 = 8.38 Hz, J_2 = 0.84 Hz ArH), 4.61 + 4.58 (br s+ br s, 4H, OCH_2CH_2O); ^{13}C NMR (DMSO-d_6) δ /ppm: 166.2, 147.0, 145.6, 142.4, 140.7, 138.3, 126.2, 124.4, 122.3, 121.6, 116.4, 106.9, 65.1; HRMS: calcd. for $C_{20}H_{16}N_5O_4S$ $(M+H)^+$: 422.0918; found: 422.0917. Anal. calcd. for $C_{20}H_{15}N_5O_4S \times HCl \times 2H_2O$ (M_r = 493.93): C 48.63, H 4.08, N 14.18; found: C 48.87, H 4.10, N 14.22.

4.1.9. 2-(7-(4-nitrophenyl)-2,3-dihydrothieno[3,4-*b*][1,4]dioxine-5-yl)-*N*-isopropyl-1*H*-benzo[*d*]imidazole-6-carboximidamide hydrochloride (**13**)

A solution of compound **5** (0.098 g, 0.34 mmol), 3,4-diamino-*N*-isopropylbenzimidamide hydrochloride (**7**) (0.077 g, 0.34 mmol), and 1,4-benzoquinone (0.04 g, 0.34 mmol) in ethanol (110 mL) was heated at reflux for 3 days (under nitrogen). The reaction mixture was cooled to room temperature and the dark solid was collected by filtration and washed with anhydrous ether. The solid was dissolved in HCl-saturated ethanol and stirred overnight. Green solid was collected by filtration, washed with anhydrous ether, and dried under vacuum to yield 0.12 g (70.6 %) of green powder, mp > 290 °C; IR (ν_{max}/cm^{-1}): in KBr: 3085, 2357, 1580, 1482, 1337, 1087, 851, 673; ^{1}H NMR (DMSO-d_6) δ /ppm: 9.49 (d, 1H, J = 7.61 Hz, NH), 9.35 (s, 1H, NH), 8.94 (s, 1H, NH), 8.31 (d, 2H, J = 8.94 Hz, ArH), 8.02 (d, 2H, J = 8.95 Hz, ArH), 7.98 (s, 1H, ArH), 7.76 (d, 1H, J = 8.34 Hz, ArH), 7.54 (dd, 1H, J_1 = 8.37 Hz, J_2 = 1.40 Hz, ArH), 4.61 + 4.59 (br s+ br s, 4H, OCH_2CH_2O), 4.16 (m, 1H, CH), 1.31 (d, J = 6.39 Hz, 6H); ^{13}C NMR (DMSO-d_6) δ /ppm: 163.2, 147.4, 146.4, 143.3, 141.5, 139.1, 127.1, 125.2, 123.9, 123.4, 117.3, 107.5, 65.9, 45.8, 22.1. HRMS: calcd. for $C_{23}H_{22}N_5O_4S$ $(M+H)^+$: 464.1387; found: 464.1341. Anal. calcd. for $C_{23}H_{21}N_5O_4S \times HCl \times 2.5H_2O$ (M_r = 545.02): C 50.69, H 4.99, N 12.85; found: C 50.35, H 4.92, N 12.83.

4.1.10. 6-(4,5-dihydro-1*H*-imidazol-2-yl)-2-(7-(4-nitrophenyl)-2,3-dihydrothieno[3,4-*b*][1,4]dioxine-5-yl)-1*H*-benzo[*d*]imidazole hydrochloride (**14**)

A solution of compound 5 (0.087 g, 0.29 mmol), 4-(4,5-dihydro-1*H*-imidazol-2-yl)benzene-1,2-diamine hydrochloride (**8** (0.062 g, 0.29 mmol), and 1,4-benzoquinone (0.03 g, 0.29 mmol) in ethanol (110 mL) was heated at reflux for 3 days (under nitrogen). The

reaction mixture was cooled to room temperature and dark precipiate was collected by filtration and washed with anhydrous ether. The solid was dissolved in HCl-saturated ethanol and stirred overnight. Green solid was collected by filtration, washed with anhydrous ether and dried under vacuum to yield 0.12 g (80.8 %) of green powder, mp > 290 °C; IR (ν_{max}/cm^{-1}): 3093, 1588, 1478, 1337, 1087, 844, 681; ^{1}H NMR (DMSO-d_6) δ /ppm: 10.66 (s, 2H, NH), 8.30 (s, 1H, ArH), 8.28 (d, 2H, J = 8.72 Hz, ArH), 7.97 (d, 2H, J = 8.87 Hz, ArH), 7.84 (dd, 1H, J_1 = 8.59 Hz, J_2 = 0.70 Hz, ArH), 7.76 (d, 2H, J = 8.44 Hz, ArH), 4.61 + 4.58 (br s + br s, 4H, OCH_2CH_2O), 4.01 (s, 4H, NCH_2CH_2N). ^{13}C NMR (DMSO-d_6) δ /ppm: 166.2, 148.2, 146.4, 143.1, 141.4, 139.1, 136.0, 127.0, 125.2, 123.4, 117.2, 116.3, 107.8, 65.9, 45.0. HRMS: calcd. for $C_{22}H_{18}N_5O_4S$ $(M+H)^+$: 448.1074; found: 448.1043. Anal. calcd. for $C_{22}H_{17}N_5O_4S \times HCl \times 2.5H_2O$ (M_r = 528.97): C 49.95, H 4.38, N 13.24; found: C 49.94, H 3.97, N 13.01.

4.1.11. 2-(7-(4-aminophenyl)-2,3-dihydrothieno[3,4-*b*][1,4]dioxine-5-yl)-1*H*-benzo[*d*]imidazole-6-carboximidamide dihydrochloride (**15**)

A suspension of compound **12** (0.1526 g, 0.33 mmol) and 10 % Pd-C (0.028g) in methanol (80 mL) was shaked in the hydrogen atmosphere overnight. The solvent was removed under reduced pressure, and the residue was suspended in methanol. Addition of ether resulted in precipitation of brown product. Solid was collected by filtration, washed with anhydrous ether, and dried under vacuum to yield 0.07 g (49.2 %) of brown powder, mp >290 °C; IR (ν_{max}/cm^{-1}): 3060, 2817, 2575, 1595, 1498, 1458, 1085, 494; ^{1}H NMR (DMSO-d_6) δ/ppm: 9.36 (s, 2H, NH), 9.11 (s, 2H, NH), 8.10 (s, 1H, ArH), 7.78 (d, 2H, J = 8.55 Hz, ArH), 7.75 (d, 1H, J = Hz, ArH), 7.69 (d, 1H, J = 8.51 Hz, ArH), 7.30 (d, 2H, J = 7.90 Hz, ArH), 4.58 + 4.51 (br s + br s, 4H, OCH_2CH_2O), 3.82 (br. s, 3H, NH_3^+ + D_2O); ^{13}C NMR (DMSO-d_6) δ/ppm: 166.3, 147.7, 142.7, 137.1, 127.0, 122.0, 121.2, 120.1, 118.3, 102.2, 65.2, 64.6. HRMS: calcd. for $C_{20}H_{18}N_5O_2S$ $(M+H)^+$: 392.1181; found: 392.1152. Anal. calcd. for $C_{20}H_{17}N_5O_2S \times 2HCl \times 2.5H_2O$ (M_r = 509.41): C 47.16, H 4.75, N 13.75; found: C 46.95, H 4.53, N 13.78.

4.1.12. 2-(7-(4-aminophenyl)-2,3-dihydrothieno[3,4-*b*][1,4]dioxine-5-yl)-*N*-isopropyl-1*H*-benzo[*d*]imidazole-6-carboximidamide trihydrochloride (**16**)

A suspension of compound **13** (0.1567 g, 0.31 mmol) and 10 % Pd-C (0.024g) in methanol (80 mL) was shaked in the hydrogen atmosphere overnight. The solvent was removed under reduced pressure, and the residue was suspended in methanol. Addition of

ether resulted in precipitation of brown product. Solid was collected by filtration, washed with anhydrous ether and dried under vacuum to yield 0.15 g (99.2 %) of brown powder, mp > 290 °C; IR (ν_{max}/cm^{-1}): 2835, 2583, 1588, 1499, 1442, 1369, 1337, 1086, 818, 713; ^{1}H NMR (DMSO-d_6) δ/ppm: 9.49 (d, 1H, J = 8.10 Hz, NH), 9.35 (s, 1H, NH), 8.96 (s, 1H, NH), 8.01 (d, 1H, J = 1.24 Hz, ArH), 7.82 (d, 2H, J = 8.67 Hz, ArH), 7.78 (d, 1H, J = 8.60 Hz, ArH), 7.59 (dd, 2H, J_1 = 8.50 Hz, J_2 = 1.58 Hz, ArH), 7.37 (d, 2H, J = 8.57 Hz, ArH), 4.60 + 4.52 (br s + br s, 4H, OCH_2CH_2O), 4.09 (m, 1H, CH), 3.62 (br s, 3H, NH_3^+ + D_2O), 1.30 (s, 6H, CH_3); ^{13}C NMR (DMSO-d_6) δ/ppm: 162.8, 147.1, 143.8, 138.7, 127.6, 123.8, 123.3, 122.8,116.1, 114.8, 65.8, 65.3, 45.5, 21.7. HRMS: calcd. for $C_{23}H_{24}N_5O_2S$ $(M+H)^+$: 434.1651; found: 434.1652. Anal. calcd. for $C_{23}H_{23}N_5O_2S \times 3HCl \times 3H_2O$ (M_r = 596.96): C 46.28, H 5.40, N 11.73; found: C 46.60, H 5.28, N 11.83.

4.1.13. 2-(7-(4-aminophenyl)-2,3-dihydrothieno[3,4-*b*][1,4]dioxine-5-yl)-6-(4,5-dihydro-1*H*-imidazol-2-yl)-1*H*-benzo[*d*]imidazole trihydrochloride (**17**)

A suspension of compound **14** (0.1461 g, 0.30 mmol) and 10 % Pd-C (0.024g) in methanol (80 mL) was shaken in the hydrogen atmosphere overnight. The solvent was removed under reduced pressure and the residue was suspended in methanol. Addition of ether results in precipitation of brown product. Solid was collected by filtration, washed with anhydrous ether and dried under vacuum to yield 0.13 g (98.2 %) of brown powder, mp > 290 °C; IR (ν_{max}/cm^{-1}): 3109, 2874, 1588, 1490, 1361, 1272, 1078, 818; ^{1}H NMR (DMSO-d_6) δ/ppm: 10.73 (s, 2H, NH), 8.31 (d, 1H, J = 1.36 Hz, ArH), 7.89 (dd, 1H, J_1 = 8.55 Hz, J_2 = 1.68 Hz, ArH), 7.77 (d, 2H, J = 8.64 Hz, ArH), 7.75 (d, 1H, J = 8.40 Hz, ArH), 7.31 (d, 2H, J = 8.66 Hz, ArH), 4.59 + 4.52 (br s + br s, 4H, OCH_2CH_2O), 4.5–3.5 (br s, NH_3^+ + D_2O), 4.06 (s, 4H, NCH_2CH_2N); ^{13}C NMR (DMSO-d_6) δ/ppm: 165.8, 148.1, 143.5, 138.5, 127.5, 123.3, 122.1, 117.0, 116.1, 115.0, 65.7, 65.2, 44.7. HRMS: calcd. for $C_{22}H_{20}N_5O_2S$ $(M+H)^+$: 418.1338; found: 418.1286. Anal. calcd. for $C_{22}H_{19}N_5O_2S \times 3HCl \times 3H_2O$ (M_r = 580.92): C 45.49, H 4.86, N 12.06; found: C 45.18, H 4.55, N 11.83.

4.2. Spectroscopic experiments

Electronic absorption spectra were recorded on a Varian Cary 100 Bio and PerkinElmer Lambda 25 spectrometer using quartz cuvettes (1 cm). Measurements were performed in an aqueous buffer solution (pH 7; sodium cacodylate buffer, I = 0.05 mol dm^{-3}). Polynucleotides were purchased from Sigma and Aldrich and were dissolved in sodium cacodylate buffer (I = 0.05 mol dm^{-3}, pH 7). Calf thymus (ct-DNA) was additionally

sonicated and filtered through a 0.45 µm filter. Their concentration was determined via spectroscopic measurements as the concentration of total phosphates [54]. DNA-melting experiments were carried out by monitoring the absorbance of ct-DNA at 260 nm at varying temperature in the absence and presence of amidines **9**–**35**, at r = 0.1 compound to polynucleotide ratio with a ramp rate of 0.5 °C min^{-1} using a Peltier system attached to the UV-Vis spectrophotometer. Absorbance of the ligands was subtracted from every curve, and the absorbance scale was normalized. T_m values are the midpoints of transition curves, determined from the maximum of the first derivative and checked graphically by the tangent method [54]. ΔT_m values were calculated subtracting T_m of the free nucleic acid from T_m of the complex. Every ΔT_m value here reported was the average of at least two measurements, the error in ΔT_m was ± 0.5 °C.

4.3. Biological experiments

4.3.1. Antibacterial testing

Determination of minimal inhibitory concentrations (MIC) was performed as described previously [55]. Testing was performed by the standard broth microdilution method with azithromycin, oxacillin, ampicillin and vancomycin (all obtained from USP) as comparator antibiotics. Bacterial strains used as the primary screening panel were all obtained from ATCC and included three fully sensitive strains of gram-negative species, *Escherichia coli* (ATCC 25922), *Moraxella catarrhalis* (ATCC 23246), *Haemophilus influenzae* (ATCC 49247) and three fully sensitive strains of gram-positive species *Staphylococcus aureus* (ATCC 29213), *Streptococcus pneumoniae* (ATCC 49619), *Streptococcus pyogenes* (ATCC 700294). The panel used for additional screening included six of gram-positive clinical isolates: two methicillin resistant *Stapylococcus aureus* (B0008 and B0967), methicillin sensitive (B0423) and methicillin resistant (B0674) *Staphylococcus epidermidis* and vancomycin sensitive (B0557) and vancomycin resistant (B0085) *Enterococcus faecium* which are part of our internal strain collection. Bacteria were grown on appropriate agar plates (Becton Dickinson, USA): Columbia agar with 5% sheep blood for streptococci and enterococci, Mueller Hinton chocolate agar for *H. influenzae* and Mueller Hinton agar for staphylococci. Minimum inhibitory concentrations (MICs) were measured according to guidelines of the Clinical Laboratory Standards Institute [56], with minor modifications of medium for testing streptococci, where lysed horse blood was substituted with 5 % horse

serum. Compounds were tested as double dilutions (128–0.5 μg/mL concentration range) in 96-well microtitre plates. Bacterial inocula were prepared by direct colony suspension method and plates inoculated with 5×10^4 cfu/well. Results were determined by visual read-out after overnight incubation at 37 °C in ambient air.

4.3.2. Cytotoxic activity

A HepG2 human hepatocellular carcinoma cell line (ATCC, HB-8065) and THP-1 human acute monocytic leukemia cell line (ATCC, TIB-202) were purchased from ATCC and maintained in RPMI 1640 medium (Sigma, R7388) supplemented with 10 % Fetal Bovine Serum (Sigma, R7524) at 37 °C in 5 % CO_2 atmosphere. Cytotoxicity assay was performed using MTS CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega, G3580). Double dilutions of tested compounds in 96-well microtiter plates were prepared in 100–0.8 μM concentration range and 5×10^4 cells were added per each well followed by overnight incubation at 37 °C in 5 % CO_2 atmosphere. Subsequently, MTS reagent was added and plates were incubated at 37 °C in 5 % CO_2 atmosphere for and additional period of 1h and 6h for HepG2 and THP-1, respectively. Absorbance was recorded at 490 nm using a 96-well Wallac Victor2 plate reader and results were analyzed in GraphPad Prism software.

4.4. In silico experiments

The equilibrium geometries of the minor groove binder **DB818** [30], and compound **19** were fully optimized in the gas phase by applying density functional theory (DFT) model B3LYP/6-31+G(d,p). Both compounds had both amidine groups protonated. The minima on the potential energy surfaces were confirmed by vibrational frequency calculations. All calculations were done with the Gaussian 09 program suite [57].

The alignments of the optimized conformations of compound **19** with the reference **DB818** as well as analyses of feasible interactions with the **1VZK** DNA model were done by using the program PyMOL (The PyMOL Molecular Graphics System, Version 1.0r1, Schrödinger, LLC).

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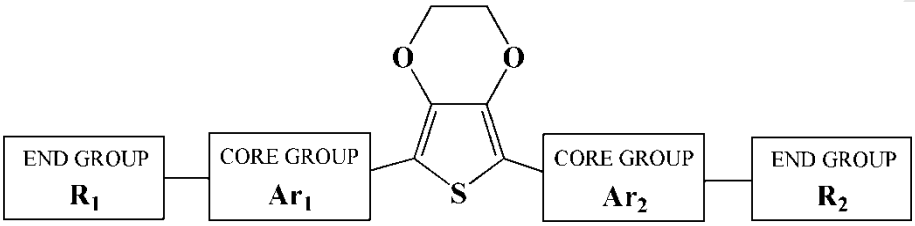
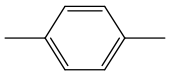
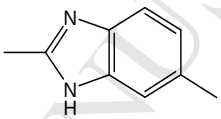
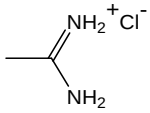
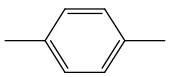
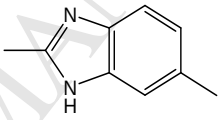
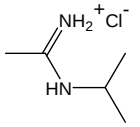
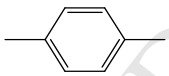
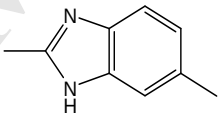
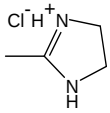
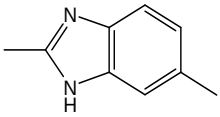
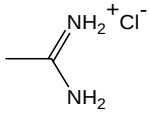
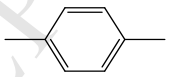
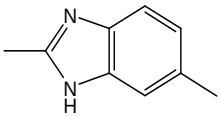
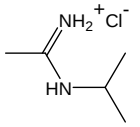
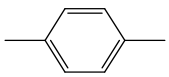
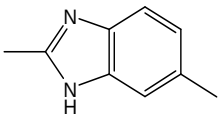
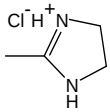
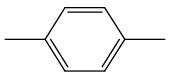
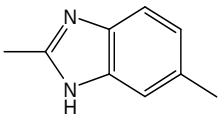
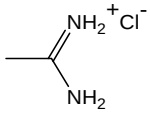
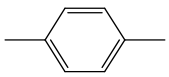
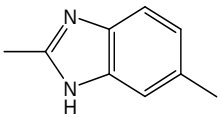
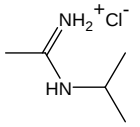
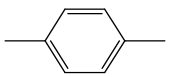
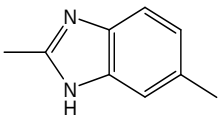
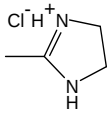
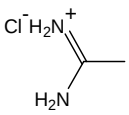
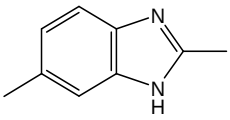
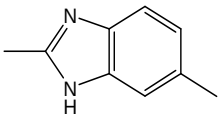
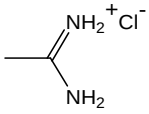
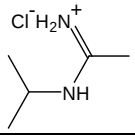
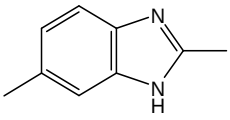
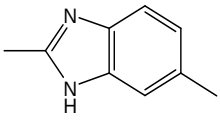
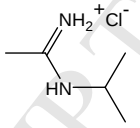
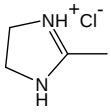
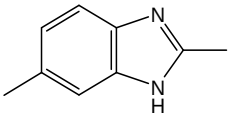
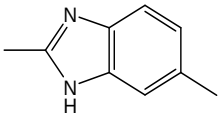
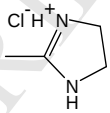
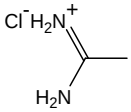
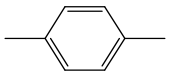
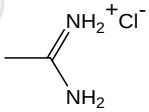
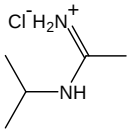
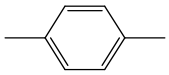
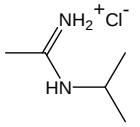
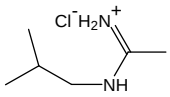
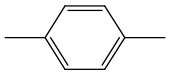
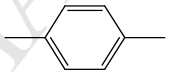
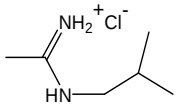
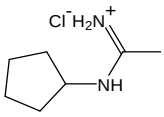
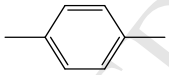
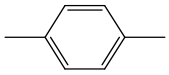
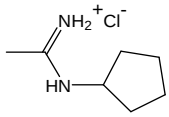
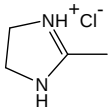
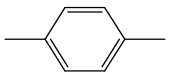
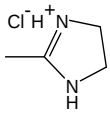
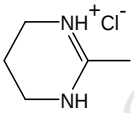
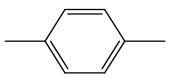
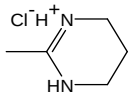
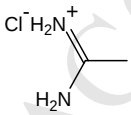
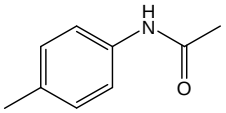
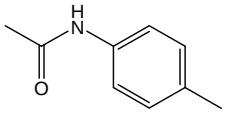
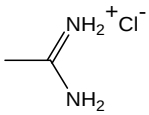
Table 1. Structures of tested compounds illustrated with their major building blocks: 3,4-ethylenedioxythiophene central unit, core groups (Ar_1 and Ar_2) and end groups (R_1 and R_2). **A**. Structures of newly synthesized compounds. **B**. Structures of previously synthesized compounds [26-28].



A

Cpd	R_1	Ar_1	Ar_2	R_2
9	NC–			
10	NC–			
11	NC–			
12	O_2N–			
13	O_2N–			
14	O_2N–			
15	$Cl^-H_3^+N$–			
16	$Cl^-H_3^+N$–			
17	$Cl^-H_3^+N$–			

B

Cpd	R_1	Ar_1	Ar_2	R_2
18[a]				
19[a]				
20[a]				
21[b]				
22[b]				
23[b]				
24[b]				
25[b]				
26[b]				
27[c]				
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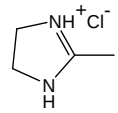
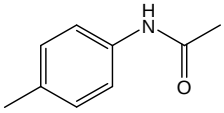
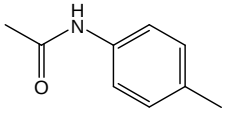
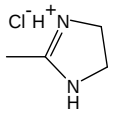
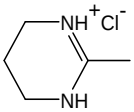
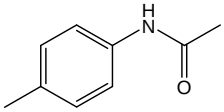
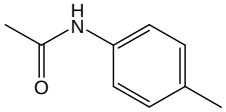
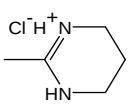
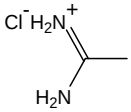
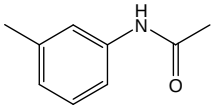
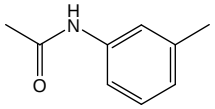
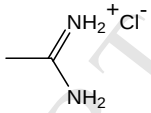
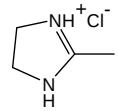
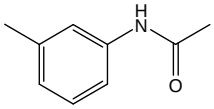
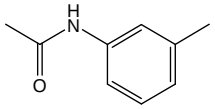
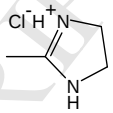
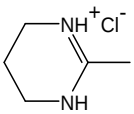
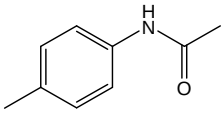
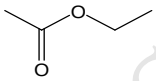
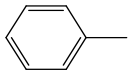
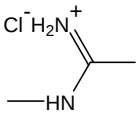
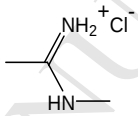
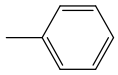
29[c]				

30[c]				
31[c]				
32[c]				
33[c]				
34[c]				-
35[b]				

Synthesis presented in: [a] Ref. [26], [b] Ref. [27], [c] Ref. [28].

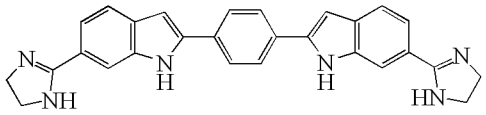
Table 2. Biological activity of compounds **9**–**35**: the influence of the compounds on the calf thymus DNA stability expressed as the change in the melting temperature (ΔT_m) and inhibition of the *in vitro* bacterial growth expressed as the MIC (minimal inhibitory concentration) values. ΔT_mvalues for compounds **18**–**35** are from [26-28].

		MIC (μg/ml)					
Cpd	**ΔT_m (° C)**	***S. aureus ATCC 13709***	***S. pneumoniae ATCC 49619***	***S. pyogenes ATCC 700294***	***M. catarrhalis ATCC 23246***	***E. coli ATCC25922***	***H. influenzae ATCC 49247***
9	3.8	> 128	> 128	> 128	32	> 128	> 128
10	2.2	4	64	4	0.5	> 128	> 128
11	1.1	4	16	4	0.5	128	> 128
12	2.2	> 128	> 128	> 128	32	> 128	> 128
13	3.1	32	> 128	> 128	1	> 128	> 128
14	2.3	> 128	> 128	> 128	32	> 128	> 128
15	2.0	64	> 128	> 128	2	> 128	> 128
16	1.8	32	> 128	16	2	> 128	> 128
17	1.1	> 128	> 128	4	1	> 128	> 128
18	14.4	128	> 128	4	2	> 128	> 128
19	15.4	4	2	0.5	≤ 0.25	> 128	> 128
20	11.9	> 128	> 128	16	64	> 128	> 128
21	12.2	4	> 128	0.5	≤ 0.25	32	128
22	8.4	8	128	2	0.5	32	64
23	9	4	64	2	≤ 0.25	16	32
24	12.3	2	64	0.5	≤ 0.25	8	64
25	10.8	16	64	2	2	128	> 128
26	11.8	16	> 128	4	1	32	32
27	1.3	8	> 128	4	8	64	> 128
28	1.6	> 128	> 128	> 128	> 128	> 128	> 128
29	2.1	> 128	> 128	> 128	> 128	> 128	> 128
30	1.2	64	> 128	4	16	>128	> 128
31	1.4	64	> 128	32	1	>128	> 128
32	0.8	128	> 128	32	2	> 128	> 128
33	1.8	64	128	16	32	> 128	> 128
34	0	> 128	> 128	> 128	64	> 128	> 128
35	0	> 128	> 128	> 128	> 128	> 128	> 128
azithromycin		1	0.25	0.125	0.06	8	2

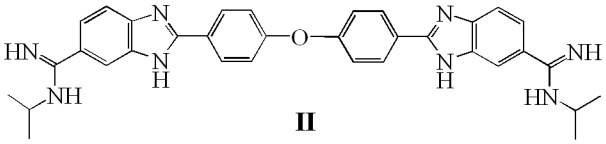
Table 3. Antibacterial activity of selected active compounds against additional clinical strains of Gram-positive organisms.

	MIC (μg/ml)					
Compound	***S. aureus B0008 MRSA***	***S. aureus B0967 MRSA***	***S. epidermidis B0423 MSSE***	***S. epidermidis B0674 MRSE***	***E. faecium B0557 VSE***	***E. faecium B0085 VRE***
10	8	>128	32	64	128	32
11	4	128	16	16	32	16
12	>128	>128	>128	128	128	64
13	16	>128	128	128	128	64
15	128	>128	128	128	128	128
16	64	>128	64	128	128	128
17	>128	>128	>128	>128	>128	128
18	128	>128	128	64	32	32
19	16	32	16	16	4	8
20	128	128	128	128	32	32
21	8	64	8	4	2	16
22	16	128	4	64	32	>128
23	8	32	16	16	32	64
24	8	32	8	16	2	32
25	32	32	16	32	32	32
26	64	64	32	32	32	128
27	8	32	4	1	128	32
30	64	16	32	16	64	32
31	32	128	64	0.5	128	64
32	128	128	16	32	128	64
33	64	>128	32	32	128	64
oxacillin	>128	>128	≤0.25	>128	32	>128
ampicillin	128	128	≤0.25	64	0.5	64
vancomycin	1	2	2	1	0.25	>16
azithromycin	>128	>128	1	1	≤0.25	32

a)

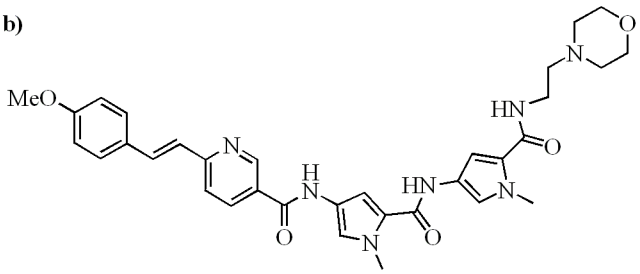


I

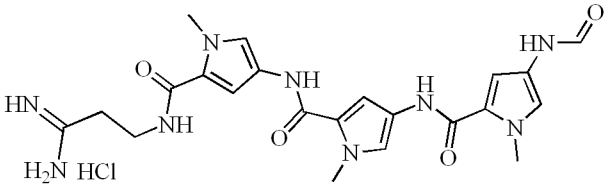


II

b)

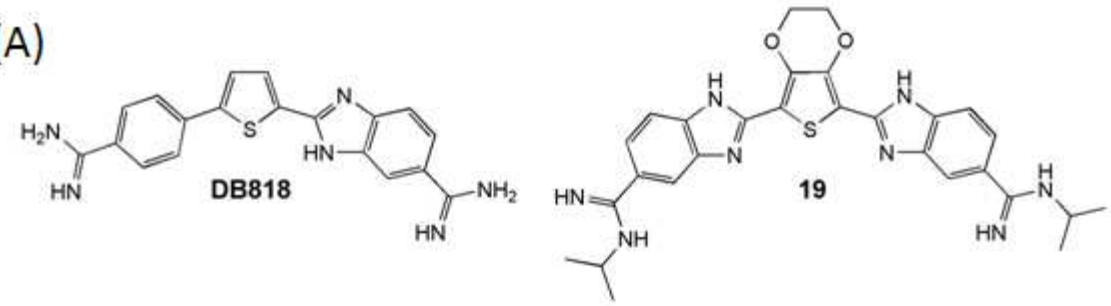


III

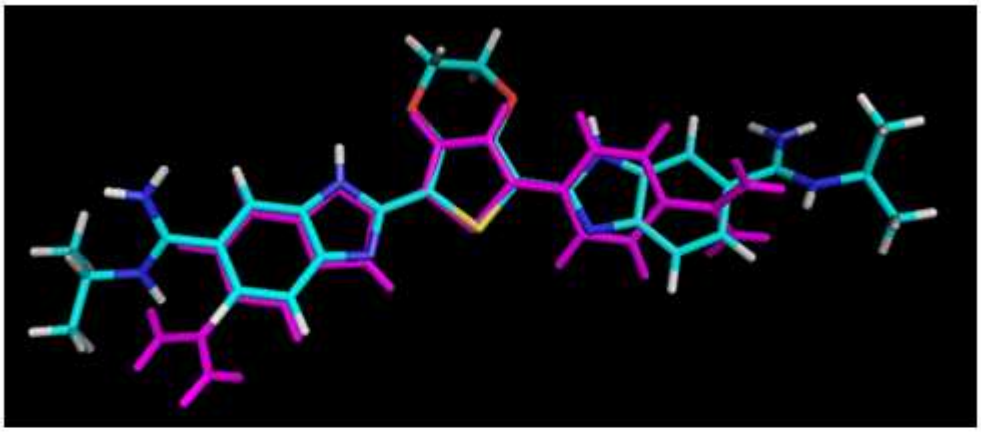


Distamycin

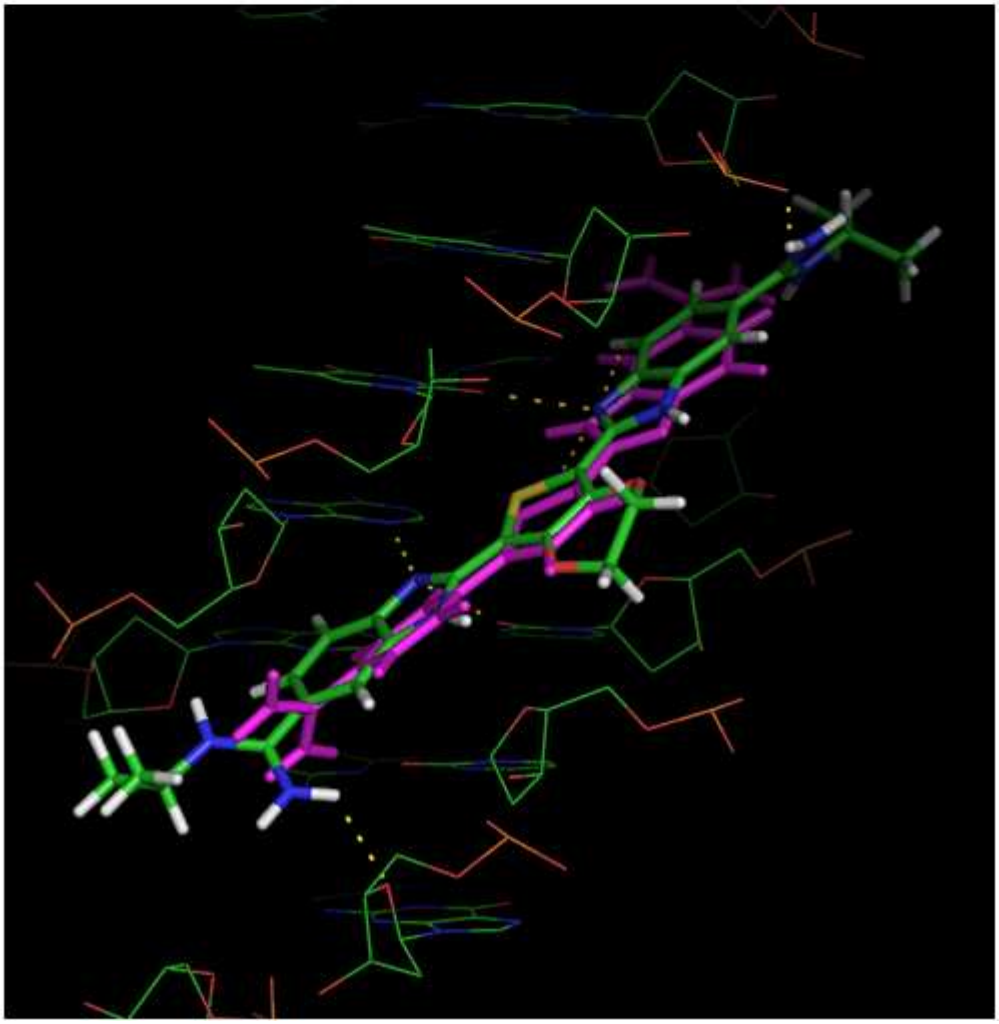
(A)

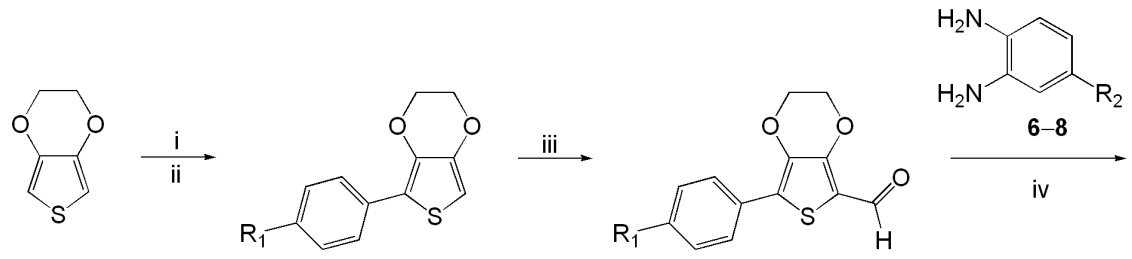


(B)



(C)

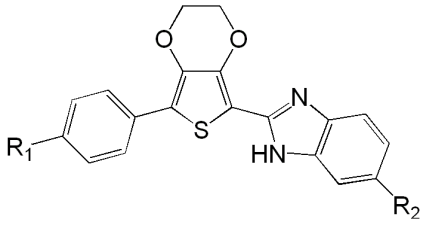




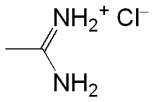
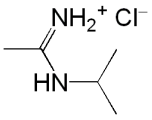
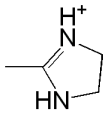
1

2 R_1 = CN
3 R_1 = NO_2

4 R_1 = CN
5 R_1 = NO_2



9–11 R_1 = CN
12–14 R_1 = NO_2
15–17 R_1 = NH_2 — v

6, 9, 12, 15 R_2 =  ; **7, 10, 13, 16** R_2 =  ; **8, 11, 14, 17** R_2 =  Cl^-