European Journal of Medicinal Chemistry 90 (2015) 68–81

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

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

Ivana Stolić [a, 1], Hana Čipčić Paljetak [b, 1], Mihaela Perić [b, *], Mario Matijašić [b], Višnja Stepanić [c], Donatella Verbanac [b], Miroslav Bajić [a, *]

[a] University of Zagreb, Faculty of Veterinary Medicine, Department of Chemistry and Biochemistry, Heinzelova 55, 10000 Zagreb, Croatia
[b] University of Zagreb School of Medicine, Center for Translational and Clinical Research, Department for Intercellular Communication, Šalata 2, 10000 Zagreb, Croatia
[c] Rudjer Bošković Institute, Division of Molecular Medicine, Laboratory for Epigenomics, Bijenička 54, 10000 Zagreb, Croatia

ARTICLE INFO

Article history:
Received 24 February 2014
Received in revised form
28 October 2014
Accepted 1 November 2014
Available online 4 November 2014

Keywords:
Thiophene
Amidine
Minor groove binders
Minimum inhibitory concentration
Antibacterial activity
DNA binding

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 *Staphyloccocus aureus*, *Streptococcus pneumoniae, Streptococcus pyogenes, Moraxella catarrhalis*, respectively and 4–32 μg/ml against clinical isolates of sensitive and resistant *S. aureus, Staphylococcus epidermidis and Enterococcus faecium*) and also demonstrated the strongest DNA binding affinity (ΔT_m of 15.4 °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.

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 methicillin-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

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

* Corresponding authors.
E-mail addresses: mihaela.peric@mef.hr (M. Perić), mbajic@vef.hr (M. Bajić).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.ejmech.2014.11.003
0223-5234/

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

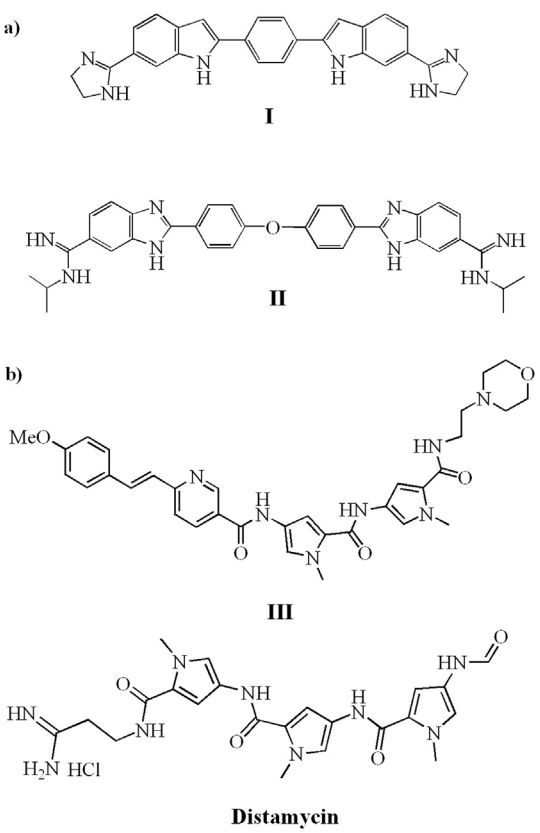

Fig. 1. Structures of a) head-to-head and b) head-to-tail linked MGBs with demonstrated antibacterial activity. MICs against sensitive *S. aureus* are ≤0.5 μg/ml for compounds **I** and **II** and 6.25 and 50 μg/ml for compound **III** and distamycin, respectively.[12,21,23,25].

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* ≤ 0.5 μg/ml for compounds I and II and 6.25 and 50 μg/ml for compound III and distamycin, respectively [21,23,24].

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 *S. aureus* – MRSA and methicillin resistant *Staphylococcus 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