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PERSPECTIVE ARTICLE

MULTIDISCIPLINARY APPROACHES FOR THE DESIGN OF HETEROCYCLES AS POTENTIAL ANTIBACTERIAL COMPOUNDS AGAINST MULTIDRUG RESISTANT BACTERIA

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Decades of use and misuse of antibacterial agents have resulted in the development of bacterial resistance to all antibiotics in clinical use independently from the biological target of the drug. Despite this threat to public health, most pharmaceutical companies are leaving the area of antimicrobial development due to insufficient returns with respect to investments in other areas. In this context basic research is needed with the scope of optimizing, by means of chemoinformatic tools, synthesizing and evaluating the structure of new heterocyclic systems in order to overcome the problem of drug-resistance. In general the objective of this type of studies is that of designing and synthesizing new compounds and determining "in vitro" their antibacterial activity against recently isolated antibiotic susceptible and resistant bacteria. Generally, the following aspects are targeted: a) inhibition of the activity of the 23S ribosomal region essential for bacterial protein synthesis and b) inhibition of bacterial genome replication by blocking DNA-gyrase and topoisomerase IV activities. These approaches allow the development of two different groups of compounds: i) structural analogues of linezolid, developed in order to inhibit bacterial protein synthesis at the level of the 23S ribosomal subunit and ii) structure analogues of fluoroquinolones. Both this compounds are biologically active heterocycles. Heterocycles are an important class of organic compounds whose properties are strongly dependent on ring dimension, number and kind of heteroatoms, aromaticity, and ring substituents. Besides constituting part of biological systems (enzymes, vitamins, energy transporters, nucleic acids, etc.), heterocycles can also interact with these systems, affecting their normal function. For this reason, there are uncountable examples of heterocyclic compounds used as drugs. Therefore, the development of new synthetic strategies based on drug design as well as the study of the interaction between the molecule and its biological target continue to be a "hot" research field [1]. As for the antibacterial activity, it is worth to mention that linezolid [2] and fluoroquinolones [3] are amongst the most used heterocyclic compounds. From a structural point of view, the first one, containing an oxazolidinone ring, can be classified as a five-membered heterocyclic system; fluoroquinolones, instead, belong to benzocondensated series of azine systems (or six-membered nitrogen containing heteroaromatics); both compounds possess a fluorine-substituted aromatic moiety. From

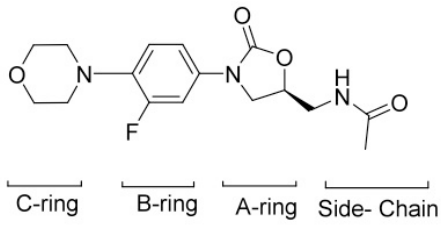
the biological activity point of view, Linezolid is the parent compound of a new generation of antibacterial agents active against Gram-positive resistant pathogens [2].

Fluoroquinolones, instead have been employed against both Gram-positive and Gram-negative pathogens [4]. However, due to the rapid emergence of resistance to every antibiotic [5], irrespective of their target, there is a need for the discovery of new classes of antibacterial agents [6,7] . In spite of the knowledge of the genome sequence of various pathogens [8], the discovery of new antibacterial agents remains challenging [9]. This growing problem has rekindled the interest in the search for new structural classes of antibiotics effective against multi-drug resistant bacteria and possibly towards a better understanding of their mechanism of action at molecular level. As for oxazolidinones, in 2008 the x-ray structure of the linezolid bound to the 50S ribosomal subunit became available [10,11] suggesting possible ligand-receptor interactions related to the mechanisms of action of the above antibacterial at a molecular level. Fluoroquinolones instead irreversibly bind the complexes formed between DNA and topoisomerase II, DNA-gyrase and topoisomerase IV, during bacterial replication, thus altering the chromosomic topology which is crucial in the replication, translation, recombination and repairing of DNA [12]. Although linezolid has been introduced in clinical practice since 2000 and is active against resistant microorganisms such as MRSA [13], VRE and PNSSP, resistance to this antibiotic has already been recently reported [14,15].

Moreover, recent papers focused also on the mechanisms of resistance to the fluoroquinolones [4] and the alteration of molecular targets.

The most common mechanism consists of mutational alterations in the Quinolones Resistance Determining Regions (QRDRs) at the level of pharmacological targets of molecules (*gyr*A, *gyr*B, *par*C, *par*E) [12]. Another mechanism involves the reduction of accumulated intracellular fluoroquinolone amounts through efflux systems coded at the chromosomic level and mediated by plasmids [16] .Until the first decade of the new millennium, structural modifications of linezolid and fluoroquinolones have been based on classical strategies of "lead compound variations" leading to several derivatives without operating any preliminary virtual screening assay. Accordingly to modern strategies for drug design, in fact, the design of new antibiotics able to overcome the problem of antibacterial resistance is based on a better understanding of the complex relationships between structure and drug activity. However, in spite of significant advances in the field, the overall structural basis for multidrug bacterial resistance has not been fully clarified. In general, chemoinformatic strategies possess great potentialities in modelling the interactions between biomolecules and ligands. Molecular recognition plays in fact a fundamental role in drug-receptor interactions. In the lack of pharmacological targets, in previous studies [17] we adopted a Virtual Receptor Site (VRS) approach, where ligands interact with a complex receptor of unknown structure, aimed at identifying pieces of the structure which could be valuable for improving the antibacterial activity. The Molecular Interaction Field (MIF) approach which uses Grid Independent Descriptors (GRIND) [18,19] calculated using the program ALMOND [20], is based on the assumption that the process of ligand-receptor interaction can be represented with the help of the MIF. However, it is worth mentioning that an increased drug-receptor interaction does not necessarily imply an increase in biological activity. It is also very important to design new structures which exhibit ADME (Adsorption, Distribution, Metabolism, Elimination) properties assuring an acceptable bioavailability. A high-throughput chemoinformatic approach should therefore include modelling of ADME properties in the design of new structures. For this purpose, a new method, called VOLSURF [21], able to correlate 3D molecular structures with physico-chemical properties, and highly efficient in predicting the biological activities, appears to be appropriate. According to the above mentioned advances achieved in the past two years due to the availability of the x-ray structures for a few drug targets, molecular modelling by docking of new ligands to the receptor active sites appears nowadays an appropriate strategy in the design of new antibacterials against multidrug resistant strains. In this context, a recently developed algorithm called Fingerprints for Ligands and Proteins (FLAP) could be used to describe proteins and ligands based on a common reference framework

[22]. FLAP is able to explore the 3D-pharmacophoric space of ligands and proteins and to provide quantitative information for the complementarity of their interactions to allow ligand-ligand, ligand-protein, or protein-protein comparison. The program relies on the GRID force-field to calculate the Molecular Interaction Fields, from which possible pharmacophoric points can be obtained. The synthetically accessible functional variables, preliminarly designed by virtual screening, will be different depending on the kind of molecules considered as parent or lead compound. The appropriate functional groups, to derivatize the main molecular skeleton, can be therefore chosen on the basis of the combination of computational and preliminary experimental in vivo activity assays. By using this approach, the synthesis of a series of new heterocyclic compounds which reflect the stereoelectronic, chemical and physical-chemical features of Linezolid has been exploited leading to improved efficiency against drug-resistant Gram-positive bacteria [24,25,26]. In particular, by referring to the structure and nomenclature of Linezolid below (see figure 1)



N-(((*S*)-3-(3-fluoro-4-morpholinophenyl)-2-oxooxazolidin-5-yl)methyl)acetamide

Fig.1

the following modifications have been achieved:
a) Ring A modifications by replacing the oxazolidinonic ring with other five-membered heterocycles containing atoms of nitrogen and oxygen, in various positions[23].
b) Ring C modifications [24] and c) Modifications in the side-chain [25]. The data showed that the active moiety of the oxazolidinone scaffold can benefit from secondary supramolecular interactions involving the C ring.
These results open the way to further studies and validated the virtual screening approach. New research to apply this multidisciplinary methodology for the design of antibiotics against drug resistant Gram negative bacteria is currently under investigation.

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