Design, Synthesis and Molecular Docking of some Oxazolidinone Compounds

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A series of oxazolidinone compounds have been obtained and characterized by physico-chemical methods and antimicrobial activity against Staphylococcus Aureus ATCC 6538. For the synthesized compounds have been performed calculations of characteristics and molecular properties, using Spartan 14 Software from Wavefunction, Inc. Irvine, CA. and molecular docking studies using CLC Drug Discovery Workbench 2.4 software, to identify and visualize the most likely interaction ligand (oxazolidinone derivatives) with the receptor protein.

Keywords: linezolid, oxazolidinone, antimicrobial activity, molecular docking

Infectious diseases are the second leading cause of death worldwide [1]. In this context, newer drugs have been developed. Linezolid (Figure 1) is a synthetic drug belonging to oxazolidinone class that was approved by the FDA in 1999. This new drug shows a great activity against gram-positive microorganisms, especially methicillin resistant St. Aureus (MRSA), methicillin resistant St. Epidermis (MRSE) and Vancominyc Resistant Enterococci (VRE) [2-7].

Experimental part

Melting points were determined in opened capillary on Melting point apparatus OptiMelt and are uncorrected.

Progress of the reaction was followed by TLG on Merck silica gel 60F254 plates eluted with the solvent system: tetrahydrofuran:dioxan: ammoniac (60:20:30) (v:v:v).

The residue was dissolved in 50 mL methanol, was treated with 0.075 mol (7.15 g) 3,4-difluoronitrobenzene in 50 mL saturated sodium chloride solution followed by stirring 5 h at 50°C. The solution was cooled to room temperature and concentrated in vacuum to afford 85% yield. 1H-NMR(CDCl3, δ ppm, J Hz): 7.94(dd, 1H, H-6, J(F-H6)=8.8); 7.85(dd, 1H, H-2, J(H-2-H6)=2.2, J(F-H2)=8.8); 6.90(t, 1H, H-5, J(H-5-H6)=8.8); 3.88(m, 4H, H-7, H-11); 2.87(m, 2H, H-7, H-11); 1.77(m, 2H, H-8, H-10); 1.60(m, 1H, H-9); 1.39(m, 2H, H-8, H-10); 1.00(m, 3H, H-12, 6.6); 13C-NMR(CDCl3, δ ppm): 152.75(d, C-3, J(F-C3)=247.8 Hz); 146.26(d, C-1, J(F-C1)=7.4 Hz); 139.56(d, C-4, J(F-C4)=8.0 Hz); 121.06(d, C-5, J(F-C5)=2.9 Hz); 116.38(d, C-6, J(F-C6)=4.3 Hz); 113.2(d, C-7, C-11, J(F-C7)=5.2 Hz); 25.81(C-8, C-10); 24.11(C-9).

Synthesis of 1-(2-fluoro-4-nitrophenyl)-3-methyl-piperidine

A solution of 0.03 Mol (5 g) 3,4-difluoronitrobenzene in 50 mL methanol, was treated with 0.075 mol (7.15 g) 3-methyl-piperidine, followed by stirring 5 h at 50°C. The solution was cooled to room temperature and concentrated in vacuum. The residue was dissolved in 50 mL toluene-ethyl acetate : 1(v:v) to yield 6.43 g 1-(2-fluoro-4-nitrophenyl)-3-methyl-piperidine (oil, yield. 90%).

1H-NMR(CDCl3, δ ppm, J Hz): 7.94(dd, 1H, H-6, J(F-H6)=8.8); 7.85(dd, 1H, H-2, J(H-2-H6)=2.2, J(F-H2)=8.8); 6.90(t, 1H, H-5, J(H-5-H6)=8.8); 3.27(m, 4H, 2H-7, 2H-11, syst. A 2B2);1.60. 1.80(m, 6H, 2H-8, 2H-9, 2H-10).

Synthesis of 1-(2-fluoro-4-nitrophenyl)-4-methyl-piperidine

A solution of 0.03 Mol (5 g) 3,4-difluoronitrobenzene in 50 mL methanol, was treated with 0.075 mol (7.15 g) 3-methyl-piperidine, followed by stirring 5 h at 50°C. The solution was cooled to room temperature and concentrated in vacuum. The residue was dissolved in 50 mL toluene-ethyl acetate : 1(v:v) to yield 6.43 g 1-(2-fluoro-4-nitrophenyl)-4-methyl-piperidine (oil, yield. 90%).

1H-NMR(CDCl3, δ ppm, J Hz): 7.99(dd, 1H, H-6, J(F-H6)=8.8); 7.91(dd, 1H, H-2, J(H-2-H6)=2.2, J(F-H2)=8.8).

Synthesis of 1-(2-fluoro-4-nitrophenyl)-morpholine

A solution of 0.03 Mol (5 g) 3,4-difluoronitrobenzene in 50 mL methanol, was treated with 0.075 mol (7.15 g) 3-methyl-piperidine, followed by stirring 5 h at 50°C. The solution was cooled to room temperature and concentrated in vacuum. The residue was dissolved in 50 mL toluene-ethyl acetate : 1(v:v) to yield 6.43 g 1-(2-fluoro-4-nitrophenyl)-4-methyl-piperidine (oil, yield. 90%).

1H-NMR(CDCl3, δ ppm, J Hz): 7.99(dd, 1H, H-6, J(F-H6)=8.8); 7.91(dd, 1H, H-2, J(H-2-H6)=2.2, J(F-H2)=8.8).
3-fluoro-4-morpholiny-aniline: (m.p. 120.2-122.2°C, yield 88%). 1H-NMR (CDCl₃, δ ppm): 156.72 (d, C-3, J(F-C3) = 244.7 Hz); 143.20 (d, C-1, J(F-C1) = 10.5 Hz); 132.99 (d, C-4, J(F-C4) = 10.2 Hz); 131.32 (d, C-6, J(F-C6) = 2.8 Hz); 107.59 (d, C-2, J(F-C2) = 23.5 Hz); 67.06 (C-13); 51.69 (C-7, C-11); 34.44 (C-8, C-10); 30.58 (C-9); 21.94 (C-20).

A solution of 0.03 Mol (7.12 g) 1-(2-fluoro-4-nitrophenyl)-1H-benzo[d]imidazole in 100 mL of acetic anhydride was treated with 90 mL of pivaldehyde and the mixture was refluxed for 20 min. The acetic anhydride was then removed by distillation under reduced pressure, the residue was filtered and dried to give 0.033 mol (5.63 g) benzyl N:8.22%. Found C: 70.22% H: 6.91% N: 8.53%.

Synthesis of N-Benzoyloxycarbonyl-3-fluoro-4-(3-methyl-piperidinyl)-aniline

A solution of 0.02 mol (1.52 g) 3-fluoro-4-methyl-1H-benzo[d]imidazole in 150 mL of acetic anhydride was treated with 0.02 mol (1.52 g) benzyl N-carbonyl chloride. The mixture was refluxed for 20 min. The acetic anhydride was then removed by distillation under reduced pressure, the residue was filtered and dried to give 0.03 mol (1.52 g) benzyl N-carbonyl chloride.

Synthesis of 3-fluoro-4-(4-methyl-piperidinyl)-aniline

A solution of 0.02 mol (1.52 g) 3-fluoro-4-(4-methyl-piperidinyl)-aniline in 150 mL of acetic anhydride was treated with 0.02 mol (1.52 g) benzyl N-carbonyl chloride. The mixture was refluxed for 20 min. The acetic anhydride was then removed by distillation under reduced pressure, the residue was filtered and dried to give 0.03 mol (1.52 g) benzyl N-carbonyl chloride.
Synthesis of (R)-[N-3-(3-fluoro-4-(3-methyl-piperidinyl-phenyl)-2-oxo-5-oxazolidinyl)methanol (Alcohol 4)

To a mixture of 0.0207 mol (7.08 g) of N-Benzoylcarbonyl-3-fluoro-4-(3-methyl-piperidinyl)-aniline in 100 mL tetrahydrofuran anhyd. at -78°C, under argon was added 0.207 mol (8.4 mL) 2.5M nBuLi - hexane dropwise over 30-30 min. After 30 min. 0.021 mol (3.05 g) of (R)-glycidyl butyrate was added and the mixture allowed to stir at -78°C for 40 min. and then at room temperature for 24 h. Saturated aqueous ammonium chloride (30 mL) was added followed by 60 mL ethyl acetate and 20 mL water. The layers were separated, and the aqueous layer was extracted with ethyl acetate (3 x 30 mL). The combined layers were separated, and the aqueous layer was extracted with CH2Cl2 (3 x 30 mL). The combined extracts were washed with brine and then dried over Na2SO4. The solvent was removed, and the residue was chromatographed on silica gel (30% ethyl acetate in hexane) and 5% ethyl acetate in hexane, to give 0.0204 mol (7.02 g) of product as a colorless oil. The analysis gave the following: δ ppm (J, Hz): 7.38 (dd, 1H, H-7, J(F-H6)=1.9, J(H5-H6)=9.1); 6.93(t, 1H, H-5, J(F-H5)=9.1); 4.70(m, 1H, H-13); 4.00(d, 1H, H-15A, 8.7); 3.97(dd, 1H, H-15B, 8.7, 6.8); 3.96(d, 1H, H-14A, 12.6); 3.74(dd, 1H, H-14B, 12.6, 4.2); 2.70(bs, 1H, OH); 1.70(qv, 4H, H-8, H-10, 5.6); 1.58(m, 2H, H-9eq or H-8ax, H-10ax); 0.98(d, 3H, H-9', 5.8).

Synthesis of (R)-[N-3-(3-fluoro-4-(morpholinyl-phenyl)-2-oxo-5-oxazolidinyl)methanol (Alcohol 1)

Synthesis of (R)-[N-3-(3-fluoro-4-(morpholinyl-phenyl)-2-oxo-5-oxazolidinyl)methanol: (m.p. 116.4-118.5°C, yield 75%). 1H-NMR(CDCl3, δ ppm, J, Hz): 7.38(dd, 1H, H-7, J(F-H6)=1.9, J(H5-H6)=9.1); 6.93(t, 1H, H-5, J(F-H5)=9.1); 4.70(m, 1H, H-13); 4.00(d, 1H, H-15A, 8.7); 3.97(dd, 1H, H-15B, 8.7, 6.8); 3.96(d, 1H, H-14A, 12.6); 3.74(dd, 1H, H-14B, 12.6, 4.2); 2.70(bs, 1H, OH); 1.70(qv, 4H, H-8, H-10, 5.6); 1.58(m, 2H, H-9eq or H-8ax, H-10ax); 0.98(d, 3H, H-9', 5.8).

Results and discussions

It were obtained some oxazolidinone derivatives evaluated for in vitro activity by determining minimum inhibitory concentration against S. aureus ATCC6538 by agar dilution method [8].

Biological Assays: The oxazolidinone derivatives were evaluated for in vitro activity by determining minimum inhibitory concentration against S. aureus ATCC6538 by agar dilution method [8].

Molecular mechanics calculations: Molecular, topological, conformational characteristics on 3D oxazolidinone derivatives optimized structure were calculated using Spartan 14 Software [10].

Docking studies: Molecular docking approach, using CLC Drug Discovery Workbench Software was conducted in order to achieve accurate predictions on optimized conformation for both, the oxazolidinone compounds (as ligand) and their target receptor protein (Staphylococcus Aureus ribosomal subunit, PDB ID: 4WFA) to form a stable complex.
All compounds exhibit moderate activity from Staphylococcus aureus, MIC > 64 µg/mL.

Ligand preparation: The ligands have been prepared using SPARTAN’14 software package [10]. In this study, the DFT/B3LYP/6-31 G’ level of basis set has been used for the computation of molecular structure, vibrational frequencies and energies of optimized structures (fig. 2-5). In order to perform structure-activity relationship (SAR) studies, some electronic properties (table 1), such as HOMO (Highest Occupied Molecular Orbital) and LUMO (Lowest Unoccupied Molecular Orbital) energy values, HOMO and LUMO orbital coefficients distribution,

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Dipole moment</th>
<th>EHOE [eV]</th>
<th>LLUMO [eV]</th>
<th>EHOE-LUMO</th>
<th>Polarity</th>
<th>Log P</th>
<th>EBA count</th>
<th>HBA count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol 1</td>
<td>3.82</td>
<td>-3.44</td>
<td>-0.18</td>
<td>5.72</td>
<td>62.87</td>
<td>30.368</td>
<td>1.44</td>
<td>1.03</td>
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<tr>
<td>Alcohol 3</td>
<td>3.21</td>
<td>-3.29</td>
<td>-0.16</td>
<td>5.45</td>
<td>65.81</td>
<td>42.451</td>
<td>1.45</td>
<td>2.16</td>
</tr>
<tr>
<td>Alcohol 4</td>
<td>3.15</td>
<td>-3.27</td>
<td>-0.34</td>
<td>5.41</td>
<td>65.08</td>
<td>42.482</td>
<td>1.48</td>
<td>2.36</td>
</tr>
<tr>
<td>Alcohol 5</td>
<td>3.18</td>
<td>-3.29</td>
<td>-0.16</td>
<td>5.45</td>
<td>65.08</td>
<td>42.472</td>
<td>1.48</td>
<td>2.49</td>
</tr>
</tbody>
</table>

Fig. 2. The optimized geometry (2a), electrostatic potential pattern of the surface of Alcohol 1 (red- negative, high electron density, blue-positive area, low electron density) (2b) and local ionization potential map (2c)

Fig. 3. The optimized geometry (3a), electrostatic potential pattern of the surface of Alcohol 3 (red- negative, high electron density, blue-positive area, low electron density) (3b) and local ionization potential map (3c)

Fig. 4. The optimized geometry (4a), electrostatic potential pattern of the surface of Alcohol 4 (red- negative, high electron density, blue-positive area, low electron density) (4b) and local ionization potential map (4c)

Fig. 5. The optimized geometry (5a), electrostatic potential pattern of the surface of Alcohol 5 (red- negative, high electron density, blue-positive area, low electron density) (5b) and local ionization potential map (5c)
molecular dipole moment, polar surface area (PSA), the ovality, polarizability, the octanol water partition coefficient (logP), the number of hydrogen-bond donors (HBDs) and acceptors (HBAs). The polarizability is useful to predict the interactions between non-polar atoms or groups and other electrically charged species, such as ions and polar molecules having a strong dipole moment.

Compounds molecular properties

**Molecular Docking:** The steps to go through to explore protein-ligand interaction using docking are: setup the binding site in a Molecule Project, dock ligands imported to a Molecule Table, inspect the docking results. The docking studies have been carried out using CLC Drug Discovery Workbench Software. The score and hydrogen bonds formed with the amino acids from group interaction atoms are used to predict the binding modes, the binding affinities and the orientation of the docked compounds (fig. 6) in the active site of the protein-receptor (table 2). It was realized molecular docking studies in order to identify and visualize the most likely interaction, the binding affinities and the orientation of the docked ligands at the active site of *Staphylococcus aureus* ribosomal subunit (PDB ID: 4WFA) [11].

### Table 2

<table>
<thead>
<tr>
<th>Comp.</th>
<th>Score/ RMSD</th>
<th>Interacting group</th>
<th>Hydrogen bond</th>
<th>Length bond</th>
</tr>
</thead>
<tbody>
<tr>
<td>LZD co-crystalized</td>
<td>-32.73/ 1.88</td>
<td>ARG 33:1, GLY 34:1, HIS 35:1, LYS 36:1, GLY 37:1, GLN 38:1, LYS 39:1, ARG 41:1, ALA 42:1</td>
<td>N sp²(N4)-O sp²(NH2) in GLN 38:1</td>
<td>2.989 A</td>
</tr>
<tr>
<td>Alcohol 1</td>
<td>-35.52/ 0.08</td>
<td>LYS 35:1, ALA 40:1, GLN 38:1, HIS 39:1, LYS 40:1, SER 42:1, ARG 41:1</td>
<td>O sp²(O3)-N sp²(NH2) in ALA 40:1</td>
<td>2.837 A</td>
</tr>
<tr>
<td>Alcohol 3</td>
<td>-32.74/ 0.53</td>
<td>LYS 35:1, ALA 40:1, GLN 38:1, HIS 39:1, LYS 40:1, SER 42:1, ARG 41:1, GLY 42:1</td>
<td>O sp²(O3)-N sp²(NH2) in LYS 39:1</td>
<td>3.085 A</td>
</tr>
<tr>
<td>Alcohol 4</td>
<td>-37.36/ 0.08</td>
<td>LYS 35:1, ALA 40:1, GLN 38:1, HIS 39:1, LYS 40:1, SER 42:1, ARG 41:1, GLY 42:1</td>
<td>O sp²(O3)-N sp²(NH2) in ALA 40:1</td>
<td>3.278 A</td>
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<tr>
<td>Alcohol 5</td>
<td>-38.11/ 0.06</td>
<td>LYS 35:1, ALA 40:1, GLN 38:1, HIS 39:1, LYS 40:1, SER 42:1, ARG 41:1, GLY 42:1</td>
<td>O sp²(O3)-N sp²(NH2) in ALA 40:1</td>
<td>3.948 A</td>
</tr>
</tbody>
</table>

**Docking method validation**

The ensure that the ligand orientations and position obtained from the molecular docking studies are valid and reasonable potential binding modes of ligands, the docking methods and parameters used have been validated by redocking (fig. 7).

**Calculate molecular properties**

Using the Calculate Molecular Properties tool it have been calculated commonly used properties of small molecules, such as Lipinski’s rule of five [12]: number of hydrogen bond donors less than 5 (the total number of nitrogen-hydrogen and oxygen-hydrogen bonds), number of hydrogen bond acceptors less than 10 (the total number of nitrogen and oxygen atoms), the molecular weight less than 500 Daltons; Log P (octanol-water partition coefficient) less than 5. The calculation of the log P is based on the XLOGP3-AA method [13]. The number of violations of the Lipinski rules gives an indication of how drug-like for a molecule is. In general, orally active drugs have fewer than two violations. These properties can be useful for identifying potential drug-like molecules, or for removing non-drug-like molecules from a compound library before starting a large virtual screening experiment (table 3).
Drug-likeness of the oxazolidinone compounds

According to the data presented in table 3, all oxazolidinone compounds have zero violation of all the parameters involved in Lipinski’s rule of five.

Conclusions

We have synthesized some oxazolidinone compounds and we have investigated their antibacterial activity. For the synthesized oxazolidinone derivatives, a study of the characteristics and molecular properties has been realized. The docking studies revealed that all the compounds showed good docking score. The docking score is a measure of the antimicrobial activity of the studied compounds.

Acknowledgements: This paper has been financed through the NUCLEU Program, which is implemented with the support of ANCSI, Project no. PN 16-27 01 01

References

10. SPARTAN14 WAVEFUNCTION, INC. IRVINE, CA.

Manuscript received: 14.12.2017