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Oxazolidine-2-thiones: a molecular modeling study☆

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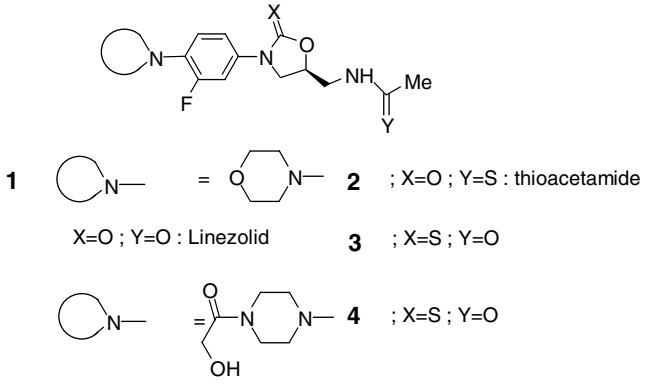
Abstract—Two oxazolidine-2-thiones, thio-analogs of linezolid, were synthesized and their antibacterial properties evaluated. Unlike oxazolidinones, the thio-analogs did not inhibit the growth of Gram positive bacteria. A molecular modeling study has been carried out to aid understanding of this unexpected finding.

1. Introduction

Linezolid **1** has been in clinical use since 2000 for treating infection caused by terminal Gram +ve organisms. However, toxicity as well as emergence of resistance in some patients receiving prolonged treatment has been reported.[1] A number of attempts have been made by various research groups to obtain potent and safer analogs without much success.[2] Replacement of oxygen by sulfur in several pharmacophores has led to dramatic improvements in efficacy, pharmacokinetics, and to a lower toxicity in the drug candidate. In the case of linezolid itself, replacement of the acetamide group in the side-chain by thioacetamide **2** led to a significant improvement of in vitro MIC values.[3] This encouraged us to explore the effect of replacing the carbonyl group of the oxazolidinone ring (X = O) by a thiocarbonyl group (X = S).



Seneci et al. have reported replacement of the carbonyl group of oxazolidinone by a thiocarbonyl group in *N*-[3-(4-acetyl-phenyl)-2-oxo-oxazolidin-5-ylmethyl]-acetamide (DuP 721).[4] We decided to synthesize oxazolidine-2-thiones **3** and **4**, as analogs of linezolid, to evaluate their antibacterial potential. Compounds **3** and **4** were synthesized according to the method outlined in Scheme 1. The yield reported in Scheme 1 is for compound **3**.[5]

Evaluation of thio-analogs **3** and **4** in an in vitro MIC assay against a panel of Gram +ve bacteria (*Enterococcus faecium*, *E. faecalis*, *Bacillus cereus*, *B. subtilis*, *Staphylococcus epidermidis*, *S. aureus*, and *B. pumilus*) led to surprising results. The compounds were found to be completely ineffective against all the organisms including the most sensitive, *B. subtilis* (MIC value >64 μg/mL using the standard assay as described in NCCLS guidelines). In contrast, replacement of 'O' by 'S' in the acetamide side-chain of linezolid **2** led to superior antibacterial activity against all the organisms we studied.[3] In order to understand this contrasting behavior, we decided to carry out modeling studies of linezolid **1**, thioacetamide **2** and thio-analog **3** in the potential binding pocket.

Keywords: Oxazolidine-2-thiones; Linezolid; Molecular modeling; 23S rRNA; Antibacterial; Piecewise linear potential (PLP); Thioacetamide.
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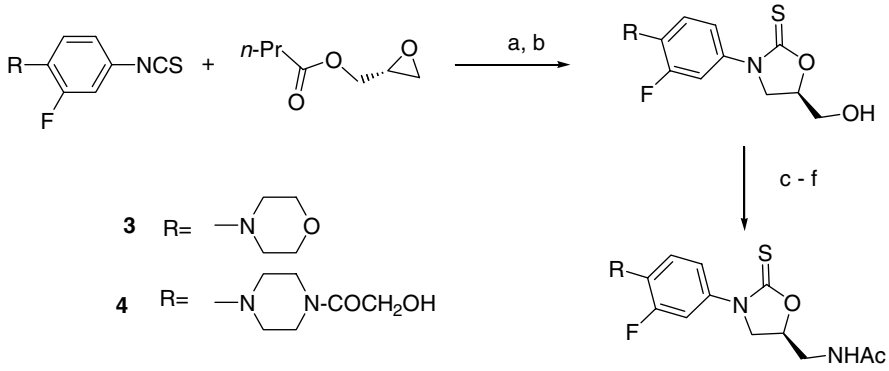

Scheme 1. Reagents and conditions: (a) LiBr, Ph_3PO, xylene, 110–115 °C, 1 h; (b) NaOMe, MeOH, 26–28 °C, 2 h, overall yield for steps a and b is 100%; (c) $MeSO_2Cl$, TEA, DCM, 0–5 °C, 30 min, 70%; (d) NaN_3, DMF, 60–70 °C, 1 h, 81%; (e) Ph_3P, 1,4-dioxane, MeOH, aq NH_3, 26–28 °C, 1 h; (f) Ac_2O, pyridine, 0–5 °C, overall yield steps e and f is 21%.

2. Molecular modeling studies with oxazolidinones and oxazolidine-2-thiones

It has been reported that eperezolid binding to the 50S ribosomal subunit is competitively inhibited by chloramphenicol.[6] This suggests that oxazolidinones share proximate binding sites on the 50S ribosome with chloramphenicol but the mode of action of oxazolidinones is distinctly different from chloramphenicol. Although oxazolidinones inhibit bacterial protein synthesis, they have no effect on peptidyl transferase unlike chloramphenicol or lincomycin.[6] Colca et al.[7] have shown that oxazolidinone is cross-linked specifically to 23S rRNA, tRNA, and two polypeptides and a specific oxazolidinone binding site is formed in the translating ribosome in the immediate vicinity of the peptidyl transferase centre. Since the exact site and mechanism of action of oxazolidinones is highly debatable, we modeled the oxazolidinone ring in a potential binding pocket on the 23S rRNA using the X-ray co-ordinates from 1K01[8] and Discovery Studio SBD 1.2 (www.accelrys.com) using the Ligandfit module[9] on Windows platform. Before docking, the co-ordinates at a distance of 20 Å from the ligand chloramphenicol were extracted as shown in Figure 1. The docking protocols were validated by docking chloramphenicol in the binding pocket, which generated hydrogen bonds with various nucleotides of the peptidase transferase cavity.[6] The structures **1**, **2** and **3** were docked using CFF and random Monte–Carlo simulations in the binding pocket. Docking analyses were observed on the basis of PLP[10] (piecewise linear potential) scores and hydrogen bonds (at a distance of 2.5 Å) of the inhibitors with the 23S ribosomal subunit.

3. Results and discussions

Oxazolidinones act by inhibiting protein synthesis. Shinabarger[11] and Hutchinson[2a] have comprehensively compiled the studies reported to elucidate the mechanism of action of oxazolidinones. Ganoza and co-workers[12] have suggested that linezolid interferes with the binding of fMet-tRNA to the ribosomal P site while

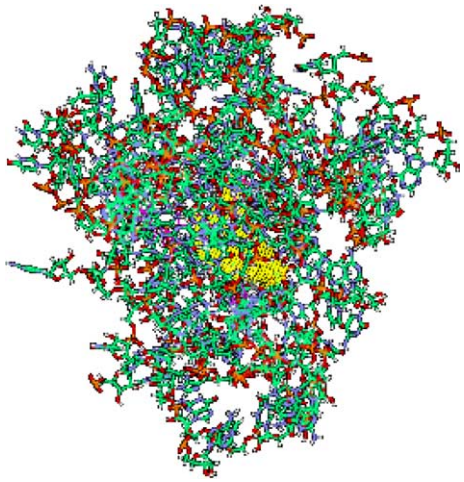

Figure 1. Chloramphenicol binding pocket (shown in yellow) in the 23S ribosomal structure. Crystal co-ordinates were extracted along a radius of 20 Å from the binding pocket.[4]

Colca et al.[7] have provided evidence that a binding site for the oxazoldinone is formed near the peptidyl transferase centre in actively translating bacterial ribosomes. Initiation of protein synthesis takes place when the 30S ribosomal subunit, mRNA, fMet-tRNA and 50S combine whilst oxazolidinones prevent initiation of protein synthesis through binding to 23S rRNA of the 50S subunit and inhibit fMet-tRNA from attaching to the ribosome.[13] Various modifications in the ring system of oxazolidinones and its structure–activity relationships have been reported. We report here an unusual binding pattern shown by the modified oxazolidinone ring through molecular modeling. The docking results show the 23S rRNA-ligand binding affinities (Table 1). The

Table 1. Results of modeling of the 23S ribosome with linezolid **1**, thioacetamide **2** and thio-analog **3**

Compound	PLP1[10a]	PLP2[10b]
1	−59.94	−77.03
2	−39.76	−25.55
3	−17.19	−15.3

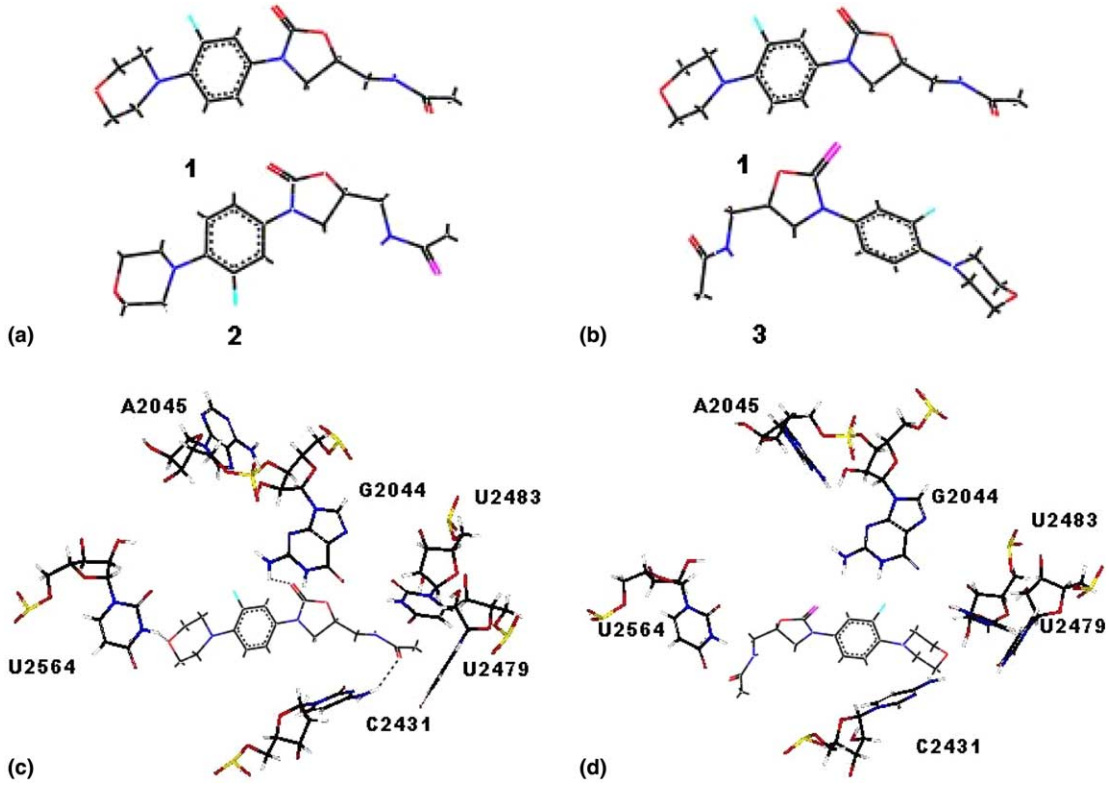

Figure 2. The hydrogen bonds are represented by black dotted lines and the binding nucleotides in stick representation. Nucleotide numbering is according to the *D. radiodurans* sequence. Nucleotides A2430, U2485 and G2484 are not shown for clarity. (a) Energy minimized conformation of linezolid **1** and thioacetamide analog **2**. (b) Energy minimized conformation of linezolid **1** and thio-analog **3**. (c) Binding pocket interaction of linezolid **1** in 23S rRNA. (d) Binding pocket interaction of thio-analog **3** in 23S rRNA.

PLP scores are in arbitrary units of energy. Lower PLP scores indicate better rRNA-ligand binding (higher pK_i values).

In general, the acetamide group in linezolid **1** is optimal for antibacterial activity. However, the thioacetamide derivative **2** is equally as good as linezolid in its antibacterial activities. From modeling studies of linezolid **1** and thioacetamide **2**, it is clear that both orientate similarly (Fig. 2a) when docked in the binding pocket (Fig. 2c), the oxygen atom of the morpholine ring hydrogen bonds with U2564Dr in both cases (docking of linezolid **1** only is shown). The oxygen atom of the carbonyl of the oxazolidinone ring in **1** and **2** is hydrogen bonded to the nitrogen of G2044Dr (G2061Ec) of 23S rRNA.[14] Similarly, the acetamide of **1** and thioacetamide of **2** form hydrogen bonds with the C2431Dr (C2452Ec) nucleotide. These interactions result in the formation of a tertiary complex, which inhibits protein synthesis (Fig. 2c). Nucleotides A2430Dr (A2451Ec), U2485Dr (U2506Ec) and G2484Dr (G2502Ec) are not shown for clarity. Nucleotide numbering is according to the *D. radiodurans* sequence with *E. coli* nucleotides labeled in parentheses. Interestingly, Rib-X Pharmaceuticals[15] have reported the crystal structure of linezolid bound to the 23S ribosome of various organisms including humans. The crystal bound linezolid appears to adopt a similar configuration to that, which we obtained in the energy minimization studies.

In contrast, and to our surprise, when oxazolidine-2-thione **3** was docked using the same model, it adopted a completely reversed orientation (Fig. 2b) in which the side-chain of the acetamide of **3** lies over the morpholine ring of **1** and the oxazolidine-2-thione moiety of **3** overlaps the aromatic ring of **1**. Thus, **1** and **3** have a 180° opposite orientation (Fig. 2b). Docking **3** in the 23S rRNA binding pocket did not show any of the interactions that were observed with oxazolidinone **1** (Fig. 2d). Thio-analog **3** did not bind to the 23S rRNA in the expected way. Thus, compound **3** may not be inhibiting protein synthesis, because it cannot form the desired tertiary structure needed for inhibition of the enzyme. Similar modeling was carried out with thio-analog of eperezolid, **4** with no improvement in the results. Thus, these modeling studies support our experimental observations on the antibacterial activity of **3** and **4** and also suggest that thio-analogs simply do not fit into the binding pocket of 23S ribosomes.

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