Bioorganic & Medicinal Chemistry Letters 21 (2011) 5266–5269

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and in vitro activity of novel 1,2,4-triazolo[4,3-*a*]pyrimidine oxazolidinone antibacterial agents. Part II

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ARTICLE INFO

Article history:
Received 8 May 2011
Revised 6 July 2011
Accepted 8 July 2011
Available online 19 July 2011

Keywords:
Oxazolidinones
Antibacterial

ABSTRACT

The synthesis and antibacterial activity of 1,2,4-triazolo[4,3-*a*]pyrimidine oxazolidinones is reported. Compound **3e** with a 2,4-disubstituted thiophene ring was found to be a potent inhibitor of Gram-positive pathogens and was 4–16-fold more potent than Linezolid.

Infections due to Gram-positive bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis* and vancomycin-resistant enterococci (VRE) are the leading causes of death from bacterial infections and, as such, are becoming increasingly problematic in clinical settings.[1–4] Oxazolidinones are the only class of orally active, synthetic antibacterial agents to be introduced since the discovery of Trimethoprim in 1968, which act with a novel mechanism of action at the protein synthesis level.[5,6] Linezolid **1** (Fig. 1), developed by Pharmacia and Upjohn, was the first oxazolidinone derivative to be approved in April, 2000 for use in humans.[7] The commercial success of this drug arises in part from its excellent bioavailability and favorable ADME properties which allow its administration by both oral and intravenous routes.[8] Linezolid, however, has been the subject of some safety concerns,[9] and extended usage (twice daily for more than two weeks) may lead to bone marrow toxicity. In view of the safety concerns of the Linezolid, attempts have been made by the pharmaceutical industry and academia to develop second generation oxazolidinone antibacterial agents which have an improved potency and safety profile.[10–13]

The oxazolidinone class of antibacterial agents requires the presence of a C-5 acylaminomethyl substituent on the oxazolidinone ring for optimal activity.[13] Additionally, it has been reported by many research groups that replacement of the morpholine ring of Linezolid with larger substituents results in compounds with improved potency.[14–18] Triazolopyrimidine derivatives are known to have diverse biological significance.[19–24] In our preceding publication,[25] we reported the synthesis and antibacterial activity of oxazolidinones containing a 1,2,4-triazolo[4,3-*a*]pyrimidine in place of the morpholine. In the present investigation, we disclose a series of related oxazolidinones in which the impact of an additional aromatic ring (A in Fig. 2) resulted in improved antibacterial activity.

A representative compound (**3a**) from the new series was docked[26,27] in the crystal structure of the 50S ribosomal unit of *Escherichia coli* in order to identify its binding mode (Fig. 3). The binding mode of this compound to the ribosome was found to be similar to that of Linezolid and Ranbezolid, as predicted by Raj et al.[28] The oxazolidinone ring forms a stacking interaction with

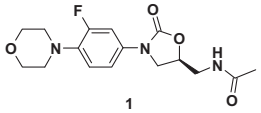

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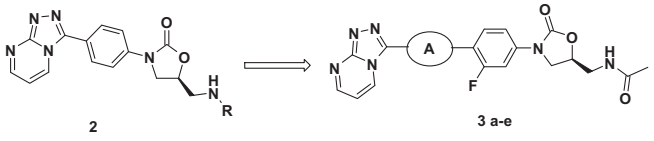
Figure 1. Linezolid.



2 3 a-e

A: aryls (a-e) mentioned in Table 1

Figure 2. Modifications in the series.

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doi:10.1016/j.bmcl.2011.07.034

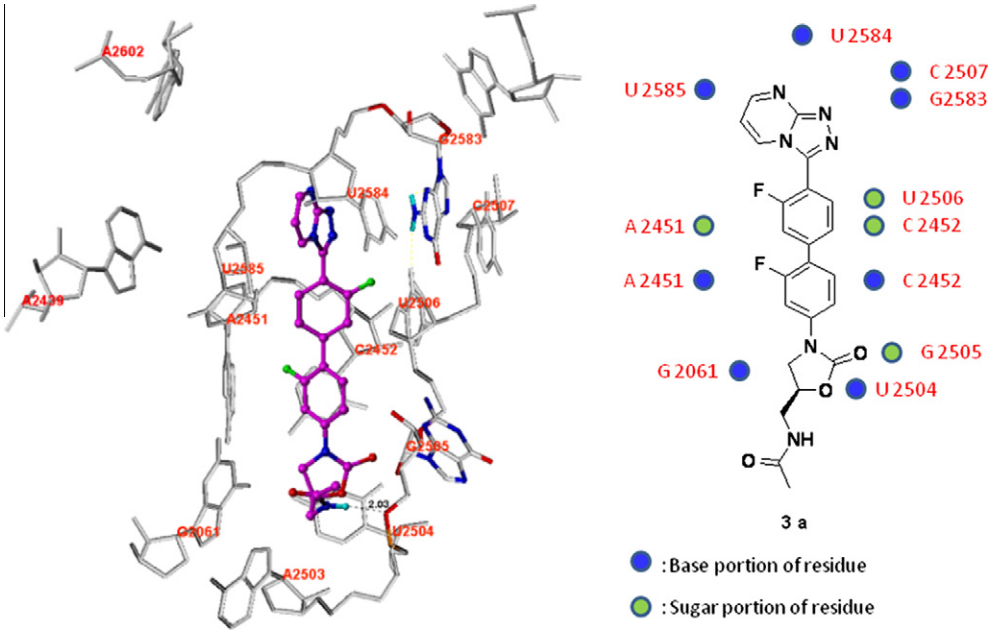
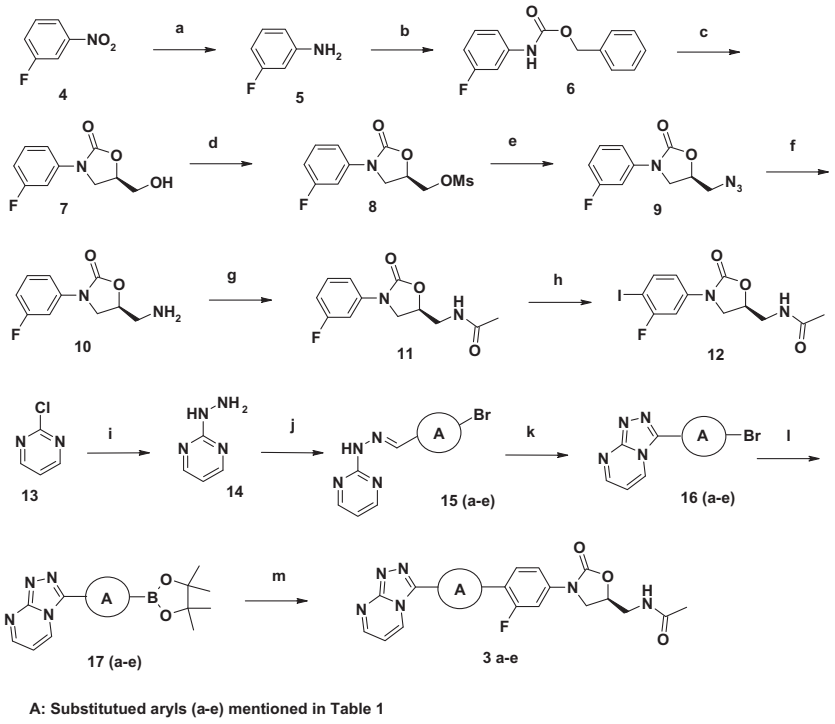


Figure 3. Molecular docking studies of compound **3a**.

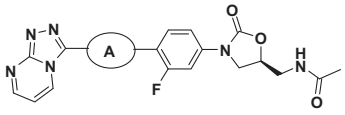


Scheme 1. Reagents and conditions: (a) H_2, Pd-C, THF; (b) benzyl chloroformate, aqueous $NaHCO_3$, acetone, 0 °C; (c) *n*-BuLi, (*R*)-glycidyl butyrate, THF, −78 °C–rt; (d) MsCl, Et_3N, DCM; (e) NaN_3, DMF, 70 °C; (f) Ph_3P, THF–H_2O (3:1), rt–50 °C; (g) $(CH_3CO)_2O$, Et_3N, DCM, 0 °C–rt; (h) CF_3COOAg, I_2, $CHCl_3$–CH_3CN (3:1); (i) $N_2H_4{\cdot}H_2O$, pyridine–DCM (1:1), 0 °C–rt; (j) bromoaryl carboxaldehyde, EtOH, HOAc (cat.), reflux; (k) $PhI(OAc)_2$, DCM, rt; (l) bis-(pinacolato)diboron, KOAc, $Pd(dppf)Cl_2$, dioxane, 80 °C; (m) $Pd(dppf)Cl_2$, K_2CO_3, **12**, MeCN, 80 °C.

the face of the uracil base of U2504. Other residues interacting with the oxazolidinone ring are the sugar of G2505 and the base of G2061. The amide side-chain of compound **3a** is folded over the oxazolidinone ring and the amide N–H makes a hydrogen bond interaction with the 5′-oxygen atom of G2505**.** The aromatic ring proximal to the oxazolidinone ring is stabilized by the bases A2451 and C2452, while the aromatic ring distal to the oxazolidinone ring is stabilized by Van der Waals interactions with the sugar residues A2451, C2452 and U2506**.** Additionally, the triazolopyrimidine ring is stabilized by residues C2507, G2583, U2584 and U2585.

The synthesis of compounds (Scheme 1) **3a**–**e** was initiated by the reduction of 1-fluoro-3-nitrobenzene under hydrogenation conditions and the resulting aniline **5** was converted to the benzyl

Table 1
In vitro MIC (μg/ml) of compounds with variation in the aryl ring



Compound	A	*E. faecalis* ATCC 29212	*S. aureus* MRSA 43300	*S. aureus* ATCC 25923	*S. epidermedis* ATCC 12228
3a		0.5	0.125	0.5	0.5
3b		0.5	0.125	0.25	0.5
3c		4	1	4	4
3d		16	8	16	>16
3e		0.06	0.125	0.125	0.25
11	—	>16	16	>16	16
	Linezolid	2	2	2	1

carbamate **6**. Deprotonation of the benzyl carbamate **6** followed by reaction with (*R*)-glycidyl butyrate yielded the oxazolidinone alcohol **7**. The alcohol derivative **7** was converted to the corresponding azide **9** by a series of nucleophilic reactions. The azide derivative **9** was converted to the amino analog **10** with triphenylphosphine in aqueous THF with heating.[29] Amine **10** was treated with acetic anhydride under basic conditions to afford the acetamide derivative **11**. Selective iodination[30] of intermediate **11** led to the iodo derivative **12**. Intermediates **16a–e** were prepared in a three-step sequence starting from 2-chloropyrimidine. Nucleophilic attack of hydrazine on 2-chloropyrimidine **13** afforded 2-hydrazinopyrimidine **14**,[31] which was treated with various bromoaryl carboxaldehydes to obtain the Schiff bases **15a–e**. The Schiff bases were cyclized using iodobenzene diacetate[32] to produce the triazolopyrimidine derivatives **16a–e**. Miyaura borylation[33] of bromo compounds **16a–e** led to the corresponding borate esters **17a–e**, which were successfully coupled with the iodo compound **12** under Suzuki conditions to give the target compounds **3a–e**. It should be noted that the reverse Suzuki coupling reactions of the borate ester of iodo compound **12** with the bromo derivatives **16a–e** were unsuccessful.

Compounds **3a–e** were screened[34] against Gram-positive target pathogens including *E. faecalis* ATCC29212, *S. aureus* MRSA 43300, *S. aureus* ATCC 25923 and *S. epidermidis* ATCC 12228 (Table 1). In accordance with the docking studies, compound **3a** displayed excellent in vitro activity against all the target pathogens tested. It was observed that the potency was retained by replacing the 2-fluorophenyl ring (distal to the oxazolidinone) by a pyridyl group (compound **3b**). Such a successful change may be useful in manipulating the physico-chemical and ADME properties of the series. Interestingly, changing the disubstitution pattern of the phenyl ring from 1,4- (compound **3a**) to 1,3- (compound **3c**) led to diminished activity. Compound **3c** is a nonlinear molecule in which the N-(3-fluorophenyl)oxazolidinone and the 1,2,4-triazolo [4,3-*a*]pyrimidine ring systems are attached in a 1,3-disposition to the aromatic ring A. The orientation angle between the two substituents is therefore 120°. Such a bent constitution may hinder the binding of compound **3c** to the 50S ribosomal unit and result in relatively weak antibacterial activity: this effect is not seen in compound **3a** in which the orientation angle is 180°.

Further loss in activity was observed with the introduction of an additional methoxy substituent (compound **3d**) at the *ortho*-position with respect to the triazolopyrimidine ring: this may be due to loss in the planarity of the aromatic ring systems. It was gratifying to see that the 2,4-disubstituted thiophene system was well tolerated and the resulting compound **3e** was very potent. Whilst compound **3e** like compound **3c** is a nonlinear molecule, the orientation of the two substituents attached to a thiophene ring[35] in a 2,4 pattern is 140.5°. Thus, compound **3e** is less bent than compound **3c** and is able, therefore, to adopt an adequate binding pose with the ribosome: of course, electronic effects may also play a part in the potent activity of the compound. In order to further substantiate this argument, we performed an overlay[36] of the energy minimized structures of compounds **3a**, **3c** and **3e** (Fig. 4). As expected, compound **3a** overlapped much better with compound **3e** than with compound **3c**. Compound **11** was also tested for antibacterial activity and found to have an MIC of ≥16 μg/ml against all the strains, which indicates that the 1,2,4-triazolo[4,3-*a*]pyrimidinyl-aryl portion of compounds **3a–e** has an important part to play in the antibiotic effect. In order to establish the spectrum of activity, the most potent compound **3e** was tested against other Gram-positive strains (Fig. 5) and found to be 4–16-fold more potent than Linezolid.

The broad spectrum activity of compound **3e** supports the case for using such a compound in the treatment of skin and soft tissue and upper respiratory tract bacterial infections. Compound **3e** was also profiled for its cytochrome P450 (CYP) inhibition liability (Table 2) and it was found to have no CYP-related liabilities up to a concentration of 10 μM.[37]

In summary, we have described the initial results from a study of 1,2,4-triazolo[4,3-*a*]pyrimidine oxazolidinones having a biaryl system between the triazolopyrimidine and the oxazolidine

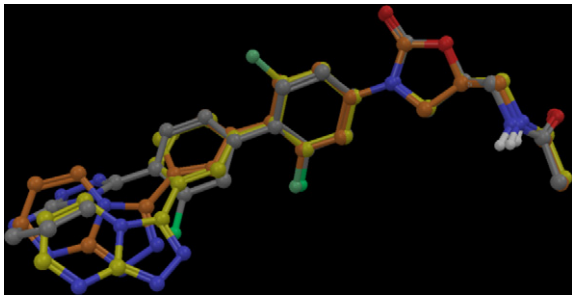


Figure 4. Overlay of compounds **3a** (grey), **3c** (yellow) and **3e** (orange).

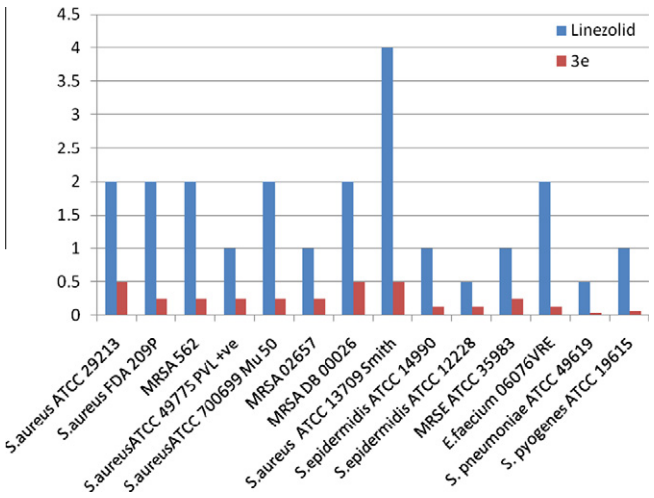


Figure 5. In vitro MIC (μg/ml) of compound **3e** and Linezolid against a set of Gram-positive pathogens.

Table 2
Human CYP inhibition profile of compound **3e**

Compound	% CYP inhibition (at 10 μM)				
	1A2	2C9	2C19	2D6	3A4
3e	4.3	40.9	36.2	5.6	37.7

heterocycles. The acetamide derivatives **3a**, **3b** and **3e** were found to display an excellent antibacterial activity profile against all the Gram-positive pathogens tested. Compound **3e** was found to be 4–16-fold more potent than Linezolid and also was devoid of any CYP liability up to a concentration of 10 μM. Further modifications in this series of compounds including the impact on antibacterial activity of additional fluorine atoms on the aromatic rings and substitutions on the triazolopyrimidine ring will be the subject of further communications.

Acknowledgments

We thank the analytical chemistry, microbiology and drug metabolism and pharmacokinetics departments of Ranbaxy Research Laboratories for their support. We also thank Ms Rajni Miglani for performing the molecular docking studies.

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37. Cytochrome P450s (CYPs) are the hemoproteins that play a critical role in the metabolism of a wide variety of xenobiotic substances; compromising the normal activity of these enzymes results in pharmacokinetic, toxicokinetic and drug–drug interaction related issues. It is imperative that the adverse effects of drug compounds be detected at an early stage to improve the efficiency and cost-effectiveness of the drug discovery process. CYP inhibition was evaluated in vitro using a standard commercial kit (BD-Gentest) comprising recombinant human CYPs, fluorogenic substrates, standard inhibitors, buffers and stop reagents. The recombinant CYP metabolizes the (nonfluorescent) fluorogenic substrate into a fluorescent product, whose fluorescence is measured by a fluorescence plate reader. The concentration-dependent ability of compounds to reduce this fluorescence for each individual CYP is measured and reported as CYP inhibition.