Bioorganic & Medicinal Chemistry Letters 21 (2011) 2887–2889

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

Manoj K. Khera [a,*], Ian A. Cliffe [a], Tarun Mathur [b], Om Prakash [c]

[a] Department of Medicinal Chemistry, New Drug Discovery Research, Ranbaxy Laboratories Limited, R&D III, Plot No. 20, Sector-18, Udyog Vihar Industrial Area, Gurgaon 122 001, India
[b] Department of Microbiology, New Drug Discovery Research, Ranbaxy Laboratories Limited, R&D III, Plot No. 20, Sector-18, Udyog Vihar Industrial Area, Gurgaon 122 001, India
[c] Department of Chemistry, Kurukshetra University, Kurukshetra 136 119, India

ARTICLE INFO

*Article history:*
Received 21 January 2011
Revised 21 March 2011
Accepted 22 March 2011
Available online 29 March 2011

*Keywords:*
Oxazolidinones
Antibacterial

ABSTRACT

The synthesis and antibacterial activity of 3-(4-([1,2,4]triazolo[4,3-*a*]pyrimidin-3-yl)phenyl)oxazolidin-2-ones is reported. Thiocarbonyl derivatives were found to be potent inhibitors of Gram-positive pathogens and compound **4l** was two to fourfold more potent than Linezolid.



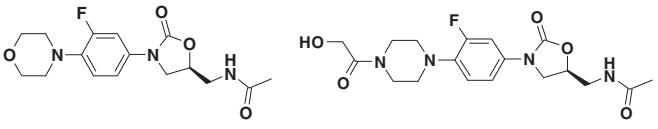
The emergence of bacterial resistance to antibiotics has posed an increasingly serious concern for medical professionals over the last two decades[1] and multi-drug-resistant Gram-positive bacteria,[2] including methicillin-resistant *Staphylococcus aureus* (MRSA),[3] *Staphylococcus epidermidis* and vancomycin-resistant Enterococci (VRE), have now become a major problem.[4] Oxazolidinones are a new class of totally synthetic antibacterial agents which are active against Gram-positive bacteria and anaerobic bacteria.[5,6] Linezolid **1** and Eperezolid **2** (Fig. 1) were the initial lead compounds from the oxazolidinone class and Linezolid **1**, developed by Pharmacia and Upjohn, was the first compound to be approved in 2000.[7] Oxazolidinones inhibit protein synthesis prior to the chain initiation step by binding to the 23S rRNA of the 50S ribosomal subunit, and thereby interfere with the initiator fMet-tRNA binding to the P-site of the ribosomal peptidyltransferase centre.[8,9]

This novel mechanism of action combined with potent antibacterial activity against resistant Gram-positive target pathogens stimulated the pharmaceutical industry to further explore the chemistry of the oxazolidinone class of compounds.

1,2,4-Triazolo[4,3-*a*]pyrimidines have been reported by our group as potent antibacterial agents,[10,11] and compound **3** was also found to be active against Gram-negative strains (*E. coli*). This encouraged us to evaluate the impact on antibacterial activity of this heterocycle when coupled to the oxazolidinone heterocycle of Linezolid (Fig. 2).

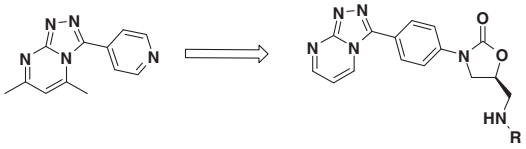
In the present investigation, we disclose a series of 5-aminomethyl-3-(4-([1,2,4]triazolo[4,3-*a*]pyrimidin-3-yl)phenyl)oxazolidin-2-ones **4a–v** as antibacterial agents in which the 5-aminomethyl substituent is attached to a range of functional groups.

The synthesis of compounds **4a–v** (Scheme 1) was initiated by the nucleophilic displacement of 2-chloropyrimidine **5** with hydrazine.[12] The 2-hydrazinopyrimidine **6** was treated with 4-nitrobenzaldehyde to obtain the Schiff base **7**, which was cyclized using iodobenzene diacetate to obtain the triazolopyrimidine derivative **8**.[13] At this stage, the nitro containing compound **8** was converted to the corresponding aniline **9** using stannous chloride and further converted to the carbamate derivative **10**.



**1** **2**

**Figure 1.** Structures of Linezolid **1** and Eperezolid 2.

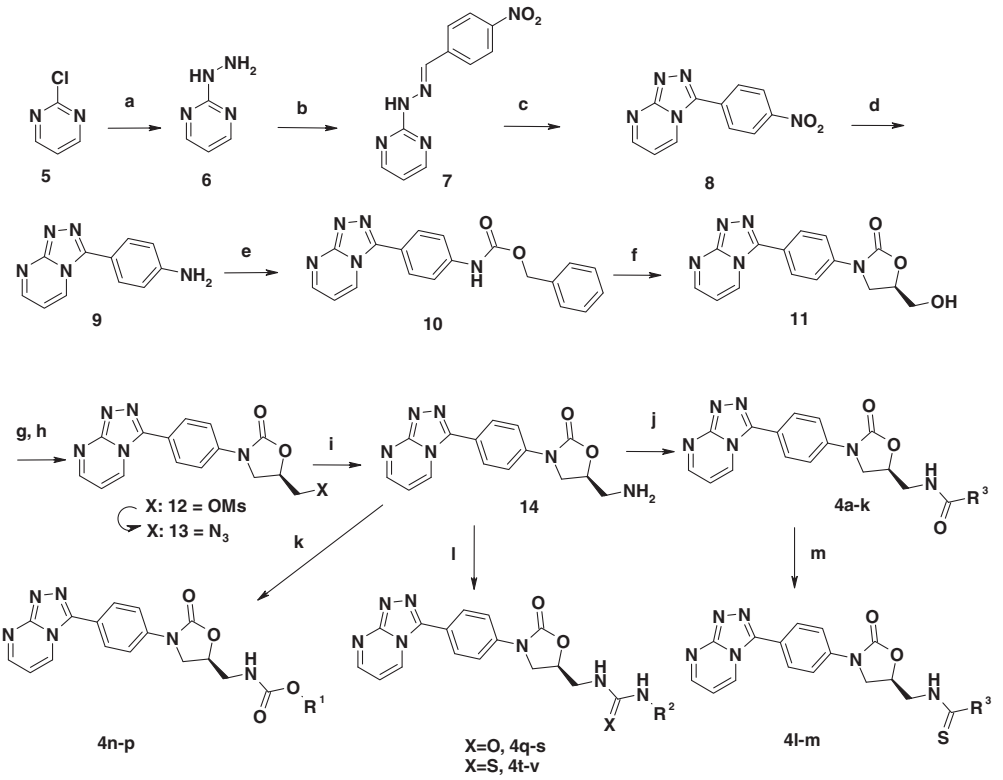


**3** **4a-v**

**Figure 2.** Modifications in the series.

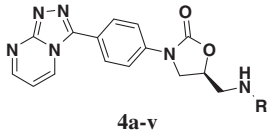
* Corresponding author. Tel.: +91 124 2848718; fax: +91 124 2397546.
*E-mail address:* manoj.khera.c4@dsin.co.in (M.K. Khera).

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.03.075




**Scheme 1.** Reagents and conditions: (a) $N_2H_4{\cdot}H_2O$, pyridine/DCM (1:1), 0 °C–rt; (b) 4-nitrobenzaldehyde, ethanol, acetic acid (catalytic), reflux; (c) $PhI(OAc)_2$, DCM, rt; (d) $SnCl_2{\cdot}H_2O$, EtOAc, reflux; (e) benzyl chloroformate, aqueous $NaHCO_3$, acetone, 0 °C; (f) *n*-BuLi, (*R*)-glycidyl butyrate, THF, −78 °C–rt; (g) MsCl, $Et_3N$, DCM/DMF (1:1), 0 °C–rt; (h) $NaN_3$, DMF, 70 °C; (i) $Ph_3P$, THF, $H_2O$, rt–50 °C; (j) $R^3COCl$, $Et_3N$, DCM; (k) $R^1OCOCl$, $Et_3N$, DCM; (l) $R^2NCO$ or $R^2NCS$, $Et_3N$, THF; (m) Lawesson's reagent, dioxane, reflux.

**Table 1**
In vitro MIC values (μg/ml) of compounds with variation in the acetamide portion



**4a-v**

| Compound | R | *E. faecalis* ATCC 29212 | *S. aureus* MRSA 43300 | *S. aureus* ATCC 25923 | *S. epidermedis* ATCC 12228 | *E. coli* ATCC 25922 |
|---|---|---|---|---|---|---|
| **4a** | $-COCH_3$ | 16 | 16 | 16 | 8 | >16 |
| **4b** | $-COCH_2CH_3$ | 16 | 16 | 16 | 16 | >16 |
| **4c** | $-CO(CH_2)_2CH_3$ | >16 | 16 | >16 | 16 | >16 |
| **4d** | $-CO(CH_2)_3CH_3$ | >16 | 16 | >16 | 16 | >16 |
| **4e** | $-COCH_2CH(CH_3)_2$ | >16 | 16 | >16 | 16 | >16 |
| **4f** | $-COCH(CH_3)_2$ | >16 | 16 | >16 | >16 | >16 |
| **4g** | $-COCH_2Cl$ | 16 | 4 | 4 | 4 | >16 |
| **4h** | $-COCHCl_2$ | 8 | 8 | 4 | 4 | >16 |
| **4i** |  | 16 | 8 | 16 | 8 | >16 |
| **4j** |  | >16 | 16 | >16 | 16 | >16 |
| **4k** | –COPh | >16 | >16 | >16 | >16 | >16 |
| **4l** | $-CSCH_3$ | 1 | 0.5 | 1 | 0.5 | >16 |
| **4m** | $-CSCH_2CH_3$ | 4 | 2 | 4 | 2 | >16 |
| **4n** | $-COOCH_3$ | >16 | 16 | 16 | 16 | >16 |
| **4o** | $-COOCH_2CH_3$ | >16 | 16 | 16 | >16 | >16 |
| **4p** | –COOPh | >16 | >16 | >16 | >16 | >16 |
| **4q** | $-CONHCH(CH_3)_2$ | >16 | >16 | >16 | >16 | >16 |
| **4r** | $-CONHC(CH_3)_3$ | >16 | >16 | >16 | >16 | >16 |
| **4s** | $-CONH(CH_2)_3CH_3$ | >16 | >16 | >16 | >16 | >16 |
| **4t** | $-CSNHCH(CH_3)_2$ | >16 | 16 | 16 | 16 | >16 |
| **4u** | $-CSNH(CH_2)_3CH_3$ | 16 | 8 | 16 | 8 | >16 |
| **4v** | $-CSNH(CH_2)_2OCH_3$ | >16 | 16 | >16 | 8 | >16 |
| **1** | Linezolid | 2 | 2 | 2 | 1 | >16 |

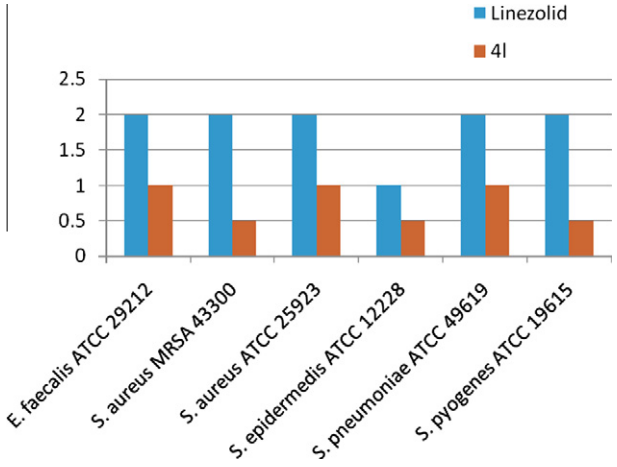



**Figure 3.** MIC values of **4l** and Linezolid against Gram-positive strains.

Deprotonation followed by reaction with (*R*)-glycidyl butyrate yielded the oxazolidinone alcohol **11**. This was converted to the corresponding azide **13** by a series of nucleophilic reactions. The azide derivative was converted to the corresponding amine **14** with triphenyl phosphine in aqueous THF with heating.[14] It should be noted that hydride-containing reagents (e.g. LAH) or hydrogenation methods were not suitable for this transformation owing to the reactivity of the triazolopyrimidine ring. The amine derivative **14** was converted to the corresponding amides **4a–k**, carbamates **4n**–**p**, ureas **4q–s** and thioureas **4t–v** using standard procedures. The amides **4a–b** were also converted to the corresponding thioamides **4l–m** using Lawesson's reagent.[15]

Final compounds **4a–v** were screened[16] against target pathogens including both Gram-positive (*E. faecalis* ATCC 29212, *S. aureus* MRSA 43300, *S. aureus* ATCC 25923 *S. epidermidis* ATCC 12228) and Gram-negative (*E. Coli* ATCC 25922) strains (Table 1). Compounds showed varying degrees of activity against Gram-positive pathogens; however, all the compounds were found to be inactive against *E. coli*. A weak enhancement of activity was observed among the alkyl amide derivatives **4a–k** when the alkyl group was relatively small in size (compare acetamide **4a** and cyclopropyl amide **4i** with the larger alkylamides **4b–f**, **4j–k**). The surprisingly moderate to good activity of relatively large chloroacetamide derivatives **4g–h** may arise from electronic effects such as enhancement of double bond character within the carbonyl group of the amide. Such enhancement of electron density in this region would be expected to occur when the carbonyl oxygen atom is replaced by sulphur and it was gratifying to see that the thioamide derivatives **4l–m** displayed excellent potency against all Gram-positive target pathogens tested. Indeed, thioacetamide **4l** was found to be two- to four- fold more potent than Linezolid. As expected, the sterically demanding and less electron rich carbamate, urea and thiourea systems **4n–v** had moderate to low activity against Gram-positive pathogens. In order to establish the spectrum of activity, the most potent compound **4l** was tested against other Gram-positive strains including *Streptococcus pneumoniae* and *Streptococcus pyogenes* (Fig. 3). Compound **4l**, being active against these strains, indicates that it could be useful in treating upper respiratory tract bacterial infections. This compound was also profiled for its CYP liability[17] and metabolic stability[18] and found to be stable in human liver microsome and to have no CYP-related liabilities up to a concentration of 10 μM (Table 2). Interestingly, the predicted permeability[19] of amide derivatives (**4a** and **4b**) was low; however, the corresponding thioamide derivatives (**4l** and **4m**) were found to have values closer to that of Linezolid.

**Table 2**
CYP profile and metabolic stability of **4l**

| Compound | % CYP inhibition (at 10 μM) | | | | | Metabolic stability ml/min/g liver HLM |
|---|---|---|---|---|---|---|
| | 1A2 | 2C9 | 2C19 | 2D6 | 3A4 | |
| 4l | 0 | 15 | 30 | 13 | 28 | 0.3 |

In summary, we have described initial results from a study of 3-(4-([1,2,4]triazolo[4,3-*a*]pyrimidin-3-yl)phenyl)oxazolidin-2-ones. The thioamide derivatives **4l** and **4m** were found to display a good antibacterial activity profile against all the Gram-positive pathogens tested. Compound **4l** was also found to be metabolically stable and devoid of any CYP liability up to a concentration of 10 μM. Further modifications in this series of compounds including the impact of additional fluorine atom substitution in the phenyl ring and substitutions on the triazolopyrimidine ring will be the subject of further communications.

## Acknowledgements

We thank the analytical chemistry department of New Drug Discovery Research, Ranbaxy Research Laboratories for support. We also thank the metabolism and pharmacokinetics department for ADME related support.

## References and notes

1. Service, R. F. *Science* **1995**, *270*, 724.
2. Swartz, M. N. *Proc. Natl. Sci. U.S.A.* **1994**, *91*, 2420.
3. Tomasz, A. N. *Eng. J. Med.* **1994**, *330*, 1247.
4. Selvekumar, N.; Srinivas, D.; Khera, M. K.; Kumar, M. S.; Mamidi, N. V. S. R.; Sarnaik, H.; Chandershekhar, C.; Rao, B. S.; Raheem, M. A.; Das, J.; Iqbal, J.; Rajagopalan, R. *J. Med. Chem.* **2002**, *45*, 3953.
5. Brickner, S. J. *Curr. Pharm. Des.* **1996**, *2*, 175.
6. Phillips, O. A. *Curr. Opin. Invest. Drugs* **2003**, *4*, 117.
7. Brickner, S. J.; Hutchinson, D. K.; Barbachyn, M. R.; Manninen, P. R.; Ulanowicz, D. A.; Garmon, S. A.; Grega, K. C.; Henges, S. K.; Toops, D. S.; Ford, C. W.; Zurenko, G. E. *J. Med. Chem.* **1996**, *39*, 673.
8. Swaney, S. M.; Aoki, H.; Ganoza, M. C.; Shinabarger, D. L. *Antimicrob. Agents Chemother.* **1998**, *42*, 3251.
9. Aoki, H.; Ke, L.; Poppe, S. M.; Poel, T. J.; Weaver, E. A.; Gadwood, R. C.; Thomas, R. C.; Shinabarger, D. L.; Ganoza, M. C. *Antimicrob. Agents Chemother.* **2002**, *46*, 1080.
10. Prakash, O.; Bhardwaj, V.; Kumar, R.; Tyagi, P.; Aneja, K. R. *Eur. J. Med. Chem.* **2004**, *39*, 1073.
11. Kumar, R.; Nair, R. R.; Dhiman, S. S.; Sharma, J.; Prakash, O. *Eur. J. Med. Chem.* **2009**, *44*, 2260.
12. Srinivasan, V.; Jebaratnam, D. J.; Budil, D. E. *J. Org. Chem.* **1999**, *64*, 5644.
13. Sadana, A. K.; Mirza, Y.; Aneja, K. R.; Prakash, O. *Eur. J. Med. Chem.* **2003**, *38*, 533.
14. Vaultier, A.; Knouzi, N.; Carrie, R. *Tetrahedron Lett.* **1983**, *24*, 763.
15. Cava, M. P.; Levinson, M. I. *Tetrahedron* **1985**, *41*, 5061.
16. Minimum inhibitory concentrations (MICs) were determined as per the CLSI guidelines **2009**, *29*(*2*), M07-A8.
17. CYP inhibition was evaluated in vitro using a standard commercial kit (BD-Gentest) comprising recombinant CYPs, fluorogenic substrates, standard inhibitors, buffers and stop reagents. The recombinant CYP metabolizes the (non-fluorescent) fluorogenic substrate into a fluorescent product, whose fluorescence is measured in a fluorescent plate reader. The concentration-dependent ability of compounds to reduce this fluorescence for each individual CYP is measured and reported as CYP inhibition.
18. Compound **4l** (10 μM concentration) was incubated with liver microsomes (1 mg/mL), obtained from BD Gentest, at 37 °C in phosphate buffer (pH 7.4) with UDPGA and an NADPH regenerating system. Samples were withdrawn at specific time points up to 30 min. Reaction was immediately quenched and the parent compound quantified by LCMS. The data was analyzed using GRAPHPAD PRISM software.
19. Apparent Caco-2 cell permeability was calculated using an in-silico ADME prediction tool (Qik Prop from Schrodinger molecular modeling suite). The predicted permeability values for compounds **4a**, **4b**, **4l**, **4m** and Linezolid were found to be 55, 81, 277, 311 and 520 nm/s, respectively.