Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 4261–4267
Bioorganic & Medicinal Chemistry Letters

Synthesis and SAR of novel oxazolidinones: Discovery of ranbezolid☆

Biswajit Das,[a,*] Sonali Rudra,[a,*] Ajay Yadav,[a] Abhijit Ray,[a] A. V. S. Raja Rao,[a] A. S. S. V. Srinivas,[a] Ajay Soni,[a] Suman Saini,[a] Shalini Shukla,[a] Manisha Pandya,[b] Pragya Bhateja,[b] Sunita Malhotra,[b] Tarun Mathur,[b] S. K. Arora,[a] Ashok Rattan[b] and Anita Mehta[a]

[a]*Department of Medicinal Chemistry, Ranbaxy Research Laboratory, Plot-20, Sector-18, Udyog Vihar, Gurgaon 122001, India*
[b]*Department of Microbiology, Ranbaxy Research Laboratory, Plot-20, Sector-18, Udyog Vihar, Gurgaon 122001, India*

Received 2 June 2005; revised 20 June 2005; accepted 22 June 2005
Available online 27 July 2005

Abstract—Novel oxazolidinones were synthesized containing a number of substituted five-membered heterocycles attached to the 'piperazinyl–phenyl–oxazolidinone' core of eperezolid. Further, the piperazine ring of the core was replaced by other diamino-heterocycles. These modifications led to several compounds with potent activity against a spectrum of resistant and susceptible Gram-positive organisms, along with the identification of ranbezolid (**RBx 7644**) as a clinical candidate.

1. Introduction

Infections due to Gram-positive bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecium* (VRE), and penicillin-resistant *Streptococcus pneumoniae* (PRSP) are the leading cause of morbidity and mortality in hospital settings and community today. Oxazolidinones are a new class of totally synthetic antibacterial agents active against Gram-positive infections.[1,2] Linezolid[3] (Zyvox™, Pharmacia/Pfizer, Fig. 1) is the only drug in this class, approved in the United States and Europe for treatment of Gram-positive nosocomial and community-acquired pneumoniae and skin infections. Oxazolidinones inhibit the bacterial protein synthesis prior to the chain initiation step, by binding to the 23S rRNA of 50S ribosomal subunit, and interfering with the initiator fMet–tRNA binding to the P-site of the ribosomal peptidyltransferase centre.[4,5]

Eperezolid (Fig. 1) was another oxazolidinone developed by Pharmacia concurrently with linezolid, up to Phase II clinical trial. Both linezolid and eperezolid are inactive against Gram-negative bacteria, require multi-dosing regimen, and have serious side effects.[6] Thus, to broaden the antibacterial spectrum, improve the PK parameters, and obtain better efficacy and safety, a research programme was initiated in oxazolidinones. As eperezolid structure allowed scope for structural refinement, the 'piperazinyl–phenyl–oxazolidinone' core structure of eperezolid was attached to various five-membered heterocycles with a methylene linker, to obtain compounds **1**–**18** (for a general representation see Fig. 2). Further, the piperazine ring was replaced with other diamino-heterocycles to obtain compounds **19**–**27**.

2. Chemistry

Eight different core compounds **35a**–**h** were prepared as intermediates by the synthetic route outlined in Scheme 1, wherein W represents (throughout this publication) eight different diamino-heterocycles as given in Figure 3. Following the procedures similar to that described by Pharmacia[7] (Scheme 1), the nitro group of Boc-protected-nitrofluorobenzene **28a**–**h**, was reduced

Keywords: Antibacterial; Oxazolidinone; Ranbezolid; **RBx 7644**; Nitro-furan derivative.
☆ This work was presented in 227th ACS National Meeting, 2004, Anaheim, CA, USA.
* Corresponding authors. Tel.: +91 124 2342001 10 10, x4926/4716; fax: +91 124 2343545 (S.R.); e-mail addresses: biswajit.das@ranbaxy.com; sonali.rudra@ranbaxy.com

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.06.063

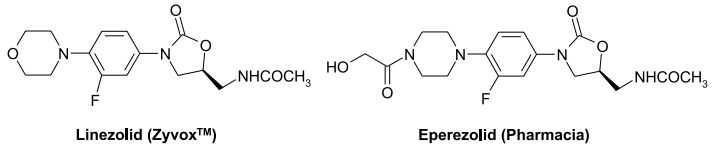


Figure 1.

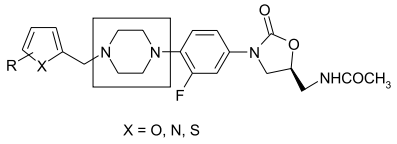


Figure 2.

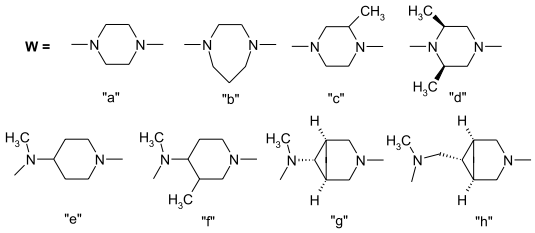
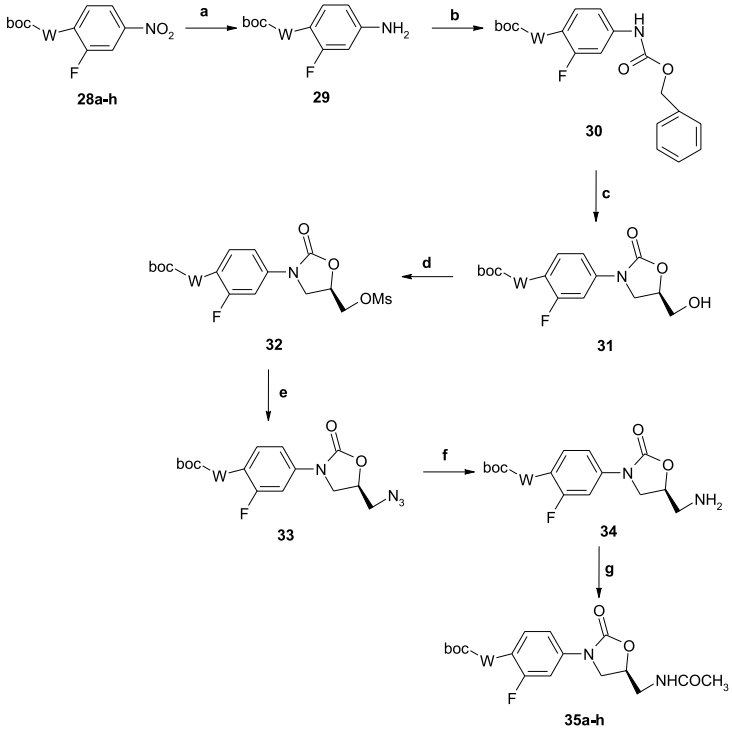


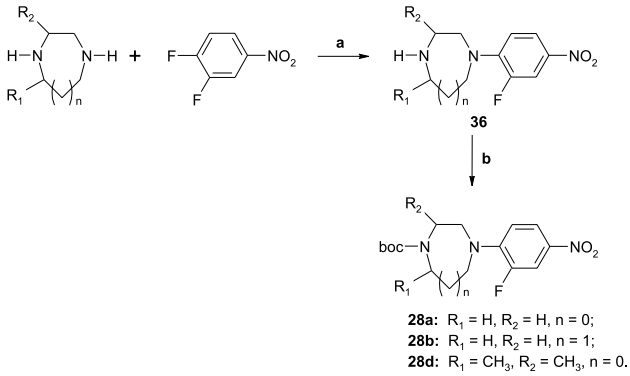
Figure 3. Eight different diamino-heterocycles.

to the corresponding amino derivative **29**, by hydrogenation in Parr apparatus with catalytic palladium on carbon. The amino group was derivatized to the benzyloxycarbamate derivative **30**, on treatment with benzylchloroformate and sodium bicarbonate in THF. Next, the oxazolidinone ring was formed by reacting **30** with *n*-butyl lithium in THF at −78 °C followed by addition of (*R*)-glycidyl butyrate, to obtain **31**. The alcohol **31** was reacted with methanesulfonyl chloride to yield **32,** followed by treatment with sodium azide to obtain the azide **33**. The azide was reduced to amine **34** by hydrogenation in Parr apparatus with catalytic palladium on carbon. Finally, the derivative **34** was transformed to acetamide **35** on treatment with acetic anhydride.

The starting Boc-protected-nitrofluorobenzene **28a**–**h** derivatives were prepared by methods given in Schemes 2–4. The synthesis of compounds **28a**, **b** and **d** were carried out as outlined in Scheme 2. The appropriate diamino-heterocycles, that is, piperazine, homopiperazine and 2,6-dimethylpiperazine, were condensed with 1,2-difluoro-4-nitrobenzene in the presence of a base *N*-ethyldiisopropylamine to yield **36**. Compound **36** was treated with Boc-anhydride to obtain **28a**, **b** and **d**. For the synthesis of compounds **28e**–**h**, 4-(*tert*-butoxycarbonyl)amino-piperidine, 3-methyl-4-(*tert*-butoxycarbonyl)aminopiperidine,[8] (1α,5α,6α)-6-*tert*-butoxycarbonyl-amino-3-azabicyclo[3.1.0]hexane[9] and (1α,5α,6α)-6-(*tert*-butoxycarbonyl)amino-methyl-3-azabicyclo[3.1.0]-hexane[10] were condensed with 1,2-difluoro-4-nitrobenzene to obtain **37** (Scheme 3). Further, compound **37**



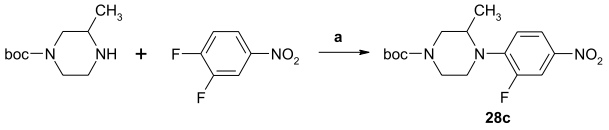
Scheme 1. Reagents and conditions: (a) cat. Pd/C, H_2 55 psi, MeOH, rt; (b) $NaHCO_3$, benzylchloroformate, THF; (c) 15% *n*-BuLi, THF, −78 °C, 1.5 h, (*R*)-glycidyl butyrate, overnight; (d) CH_3SO_2Cl, triethylamine, CH_2Cl_2, 0 °C → rt; (e) sodium azide, DMF, 80 °C; (f) cat. Pd/C, H_2 55 psi, MeOH, rt; (g) acetic anhydride, triethylamine, CH_2Cl_2, 0 °C → rt.



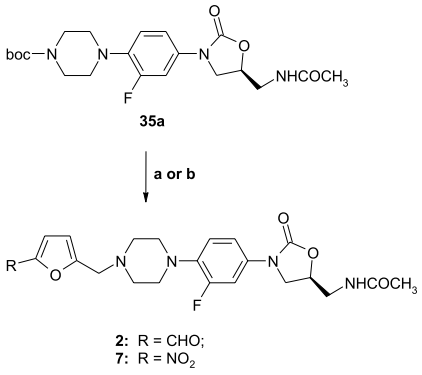
Scheme 2. Reagents and conditions: (a) *N*-ethyldiisopropylamine, acetonitrile, 80 °C; (b) Boc-anhydride, triethylamine, CH_2Cl_2, 0 °C → rt.

was methylated with methyl iodide and *n*-tetrabutylammonium iodide in the presence of sodium hydride to yield **28e–h**. As shown in Scheme 4, 1-*tert*-butoxycarbonyl-3-methylpiperazine was condensed with 1,2-difluoro-4-nitrobenzene and potassium carbonate in DMF at 80 °C to obtain **28c**.

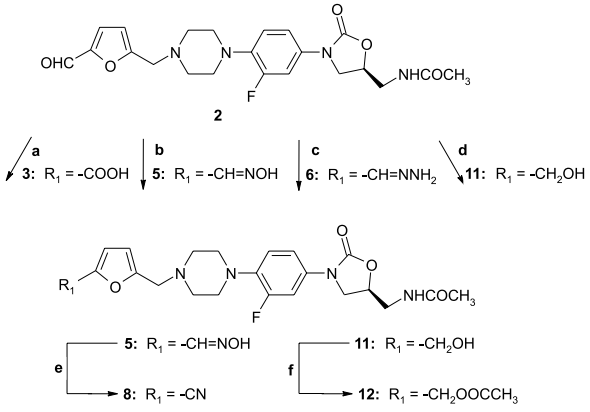
For the synthesis of targets **1–27**, the Boc-protected cores **35a–h** were key intermediates and used as starting materials for linking the heterocycles (representative examples are given in Scheme 5). Suitably substituted five-membered heterocycles were linked by a methylene linker to the Boc-deprotected cores, either by Method A: alkylations with the corresponding chloromethyl-heterocycles (compounds **1**, **2**,[11] **4**, **9** and **19** were prepared by this method); or by Method B: reductive amination with the corresponding heterocyclic carboxaldehydes (compounds **7**,[12] **10**, **13–18**, **20–22**, **24–27** were prepared by this method). The aldehyde group of compound **2** was further derivatized to the corresponding carboxylic acid (**3**), oxime (**5**), hydrazone (**6**) and methyl alcohol (**11**) by conditions given in Scheme 6. The oxime **5** was converted to nitrile derivative (**8**) on treatment with triflic anhydride and the alcohol **11** was acylated to obtain **12**. Compound **38**, was synthesized by the procedures described by Yamada et al.,[13] and further derivatized (Scheme 7).



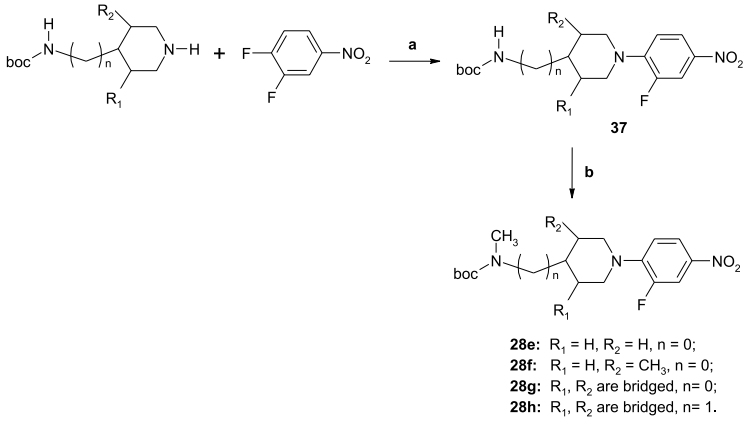
Scheme 4. Reagents and conditions: (a) potassium carbonate, DMF, 80 °C.



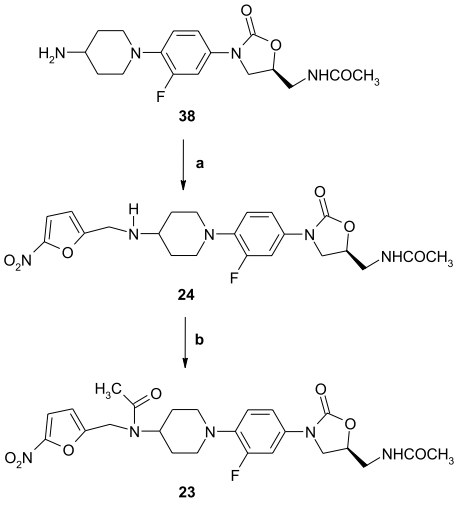
Scheme 5. Reagents and conditions: (a) Method A: TFA, CH_2Cl_2, 0 °C → rt; 5-chloromethyl-2-furaldehyde, potassium carbonate, DMF, rt; or (b) Method B: TFA, CH_2Cl_2, 0 °C → rt; 5-nitrofuran-2-carboxaldehyde, sodiumtriacetoxyborohydride, THF, molecular sieves 3 Å, rt.



Scheme 6. Reagents and conditions: (a) $AgNO_3$, aq NaOH, EtOH, rt, 17 h; (b) hydroxylamine hydrochloride, pyridine, rt, 4 h; (c) hydrazine hydrate, cat. sulfuric acid, EtOH, rt, 48 h; (d) sodium borohydride, EtOH, 6 h; (e) triflic anhydride, triethylamine, dichloromethane, −78 °C → rt, 3 h; (f) acetic anhydride, triethylamine, rt, 24 h.



Scheme 3. Reagents and conditions: (a) *N*-ethyldiisopropyl amine, acetonitrile, 80 °C; (b) sodium hydride 60% w/w, tetrabutylammoniumiodide, methyl iodide, THF, 0 °C.



Scheme 7. Reagents and conditions: (a) 5-nitro-2-furaldehyde, sodiumtriacetoxyborohydride, THF, molecular sieves 3 Å; (b) acetic anhydride, pyridine, rt.

3. Biology

3.1. In vitro

Minimum inhibitory concentrations (MIC) were determined by agar dilution method using doubling dilutions in cation adjusted Mueller Hinton agar over a concentration range of 16–0.015 μg/mL and incubation in air at 35 °C for 24 h.

3.2. In vivo

Swiss albino mice weighing 20 ± 2 g were used in the study. There were six mice in each group. Water and rodent feed was provided ad libitum throughout the study. Lethal systemic infection was caused in mice by injecting intraperitoneally MLD inoculum of methicillin-resistant *Staphylococcus aureus* (MRSA) 562 mixed 1:1 with 10% mucin. Compounds were administered orally at 30 min and 4 h post-infection. The ED_{50} was calculated by the Spearman Karber method on day 7-post-infection.

4. Results and discussion

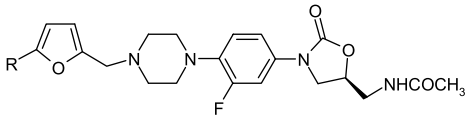
Compounds (**2**, **5**, **6** and **7**; Table 1) bearing polar groups have good antibacterial activity, with compound **7** (**RBx 7644**) being most potent.[8,14,15] Replacement of the nitro group of **7**, with other electron-withdrawing groups (compounds **3**, **8**, **9** and **10**) or electron-donating groups (compounds **11**, **12** and **13**) led to decreased activity. Nitro group was the optimum substitution on furan.

Keeping the nitro group constant and changing the heteroatom on the five-membered heterocycle led to inferior activity, in vitro rank order being O > NH, N–CH_3 > S (Table 2). The activity decreased with increase in aromaticity.

Varying the position of the linker led to decrease in activity (Table 3), suggesting 5-nitro-2-furan derivative (compound **7**) to be optimally substituted with the basic nitrogen of the piperazine ring acting inductively through the methylene linker to the electron sink (nitro-furan ring).

Replacing the piperazine ring with one carbon homologue compound **19** (Table 4), retained in vitro activity,

Table 1. Optimization of 5-substituted furans

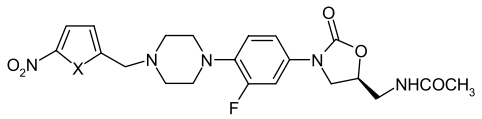


Compound	R	% Yield[a]	MIC (μg/mL) (organisms)[b]					
			S.a	MRSA	*E.fa*	VRE	*S.py*	*S.pn*
1	H	96	8	8	8	8	4	8
2	–CHO	25	1	1	8	8	8	4
3	–COOH	85	>16	>16	>16	>16	>16	>16
4	$CH_3CH_2OC(=O)$–	87	>16	>16	>16	>16	>16	>16
5	–CH=NOH	97	2	2	8	16	4	8
6	$–CH=NNH_2$	69	2	1	8	8	8	8
7 (RBx 7644)	$–NO_2$.HCl	55	1	1	2	2	0.125	0.125
8	–CN	33	16	16	16	16	16	16
9	–Br	53	>16	>16	>16	>16	>16	>16
10	–Cl	87	>16	>16	>16	>16	>16	>16
11	$–CH_2OH$	70	>16	>16	>16	>16	>16	>16
12	$–CH_2OC(=O)CH_3$	64	>16	>16	>16	>16	>16	>16
13	$–CH_3$	20	>16	>16	>16	>16	>16	>16
	Linezolid		2	2	2	2	2	2
	Vancomycin		1	0.5	0.5	>16	0.5	0.5

[a] % Yield: Chemical yield in the final step of the synthetic sequence.

[b] Organisms: *S.a*, *Staphylococcus aureus* ATCC 25923; MRSA, methicillin-resistant *Staphylococcus aureus* 562; VRE,vancomycin-resistant *Enterococcus faecium* 6A; *E.fa*, *Enterococcus faecalis* ATCC 29212; *S.py*, *Streptococcus pyogenes* ATCC 19615; *S.pn*, *Streptococcus pneumoniae* ATCC 6303.

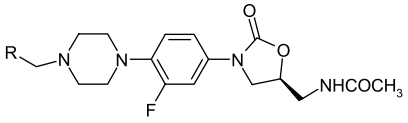
Table 2. Optimization of nitro-heterocycles

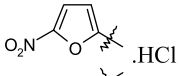
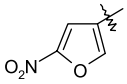
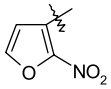


Compound	X	% Yield[a]	MIC (μg/mL) (organisms)[b]					
			S.a	MRSA	*E.fa*	VRE	*S.py*	*S.pn*
7 (RBx 7644)	O	—	1	1	2	2	0.125	0.125
	.HCl	—	1	1	2	2	0.125	0.125
14	S	27	8	8	8	8	1	8
15	NH	61	4	4	4	4	1	4
16	N–CH_3	24	4	4	4	4	2	8

[a] % Yield: Chemical yield in the final step of the synthetic sequence.
[b] Organisms: *S.a*, *Staphylococcus aureus* ATCC 25923; MRSA, methicillin-resistant *Staphylococcus aureus* 562; VRE, vancomycin-resistant *Enterococcus faecium* 6A; *E.fa*, *Enterococcus faecalis* ATCC 29212; *S.py*, *Streptococcus pyogenes* ATCC 19615; *S.pn*, *Streptococcus pneumoniae* ATCC 6303.

Table 3. Nitro-furan linked at different position



Compound	R	% Yield[a]	MIC (μg/mL) (organisms)[b]					
			S.a	MRSA	*E.fa*	VRE	*S.py*	*S.pn*
7		—	1	1	2	2	0.125	0.125
17		17	8	4	8	4	4	8
18		48	8	4	4	4	1	1

[a] % Yield: Chemical yield in the final step of the synthetic sequence.
[b] Organisms: *S.a*, *Staphylococcus aureus* ATCC 25923; MRSA, methicillin-resistant *Staphylococcus aureus* 562; VRE, vancomycin-resistant *Enterococcus faecium* 6A; *E.fa*, *Enterococcus faecalis* ATCC 29212; *S.py*, *Streptococcus pyogenes* ATCC 19615; *S.pn*, *Streptococcus pneumoniae* ATCC 6303.

but in vivo activity was lost (Table 5). Methyl groups on the piperazine ring as in compounds **20** and **21**, are not well tolerated. Amino-piperidine derivatives, compounds **22** and **24**, were a good bioisosteric replacement for the piperazine ring. Amino-piperidine **24** and methyl-aminopiperidine **22** were onefold superior in in vitro to **RBx 7644** (**7**). The in vivo activity of compound **22** was comparable to that of **RBx 7644** (**7**). An electron-withdrawing group such as acetyl (compound **23**) on the amino group of the amino-piperidine ring reduces basicity of the nitrogen atom and was found to be inactive. Thus, basicity of the nitrogen is important for activity.

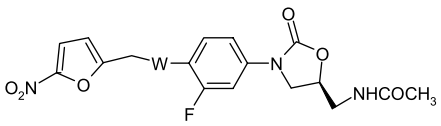
5. Conclusion

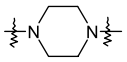
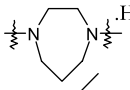
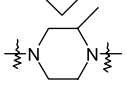
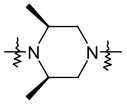
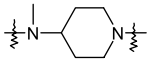
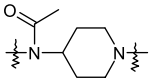
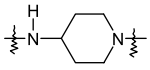
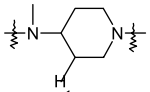
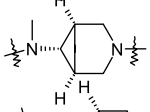
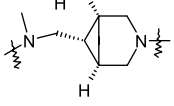
2-Substituted-5-nitro-furyl derivative, **RBx 7644** (**7**) was the optimal substitution and was the most in vivo active compound in this series, with in vitro activity similar or slightly superior to linezolid. **RBx 7644** also has activity against all anaerobes (Gram-positive or Gram-negative).[16] Methyl-amino-piperidine derivative (**22**) was an equipotent bioisosteric replacement for the piperazine derivative and warrants further investigation.

Objective of this work was to make oxazolidinones, which are active against Gram-positive bacteria and SAR conclusions are based upon it. Unlike other nitro-furan compounds (furazolidinone and nitrofurantoin), **RBx 7644** showed no evidence of DNA damaging activity in Ames test, micronucleus test, chromosomal aberration test and macromolecular synthesis in anaerobes.[17] We think that **RBx 7644** is not effluxed out in anaerobes, resulting in its anaerobic activity.[18]

In preclinical studies ranbezolid (**7**, **RBx 7644**) exhibited favorable pharmacokinetic and safety profile[16] and thus was selected as a clinical candidate.

Table 4. Replacement of piperazine ring with mimetics



Compound	W	% Yield[a]	MIC μg/mL (organisms)[b]					
			S.a	MRSA	*E.fa*	VRE	*S.py*	*S.pn*
7	 .HCl	—	1	1	2	2	0.125	0.125
19	 .HCl	3	2	1	2	2	1	1
20		26	4	2	4	4	4	8
21		46	16	8	8	8	16	16
22		52	1	0.5	0.5	0.25	0.125	0.5
23		74	>16	>16	>16	>16	>16	>16
24		67	1	1	0.5	0.5	<0.25	2
25		27	>16	>16	>16	>16	>16	>16
26		20	2	—	2	1	0.06	2
27		29	2	1	2	4	0.125	—

[a] % Yield: Chemical yield in the final step of the synthetic sequence.

[b] Organisms: *S.a*, Staphylococcus aureus ATCC 25923; MRSA, methicillin-resistant *Staphylococcus aureus* 562; VRE, vancomycin-resistant *Enterococcus faecium* 6A; *E.fa*, *Enterococcus faecalis* ATCC 29212; *S.py*, *Streptococcus pyogenes* ATCC 19615; *S.pn*, *Streptococcus pneumoniae* ATCC 6303.

Table 5. In vivo activity

Compound	ED_{50} (MRSA 562) mg/kg BW p.o.
1	>25
2	>25
7 (RBx 7644)	4.33
Linezolid	5.6
19	>25
22	7.43
27	>25
Vancomycin	— (8.8 sc)

Acknowledgments

We are thankful to Prof. Upendra K. Pandit, University of Netherland and Dr. Dharam Vir, Ranbaxy Research Laboratories for their helpful suggestions. We would also like to thank the Analytical Department, Ranbaxy Research Laboratories for the spectral data.

References and notes

1. Brickner, S. J. *Curr. Pharm. Des.* **1996**, *2*, 175.
2. Phillips, O. A. *Curr. Opin. Invest. Drugs* **2003**, *4*, 117.
3. Brickner, S. J.; Hutchinson, D. K.; Barbachyn, M. R.; Manninen, P. R.; Ulanowicz, D. A.; Garmon, S. A.; Grega, K. C.; Hendges, S. K.; Toops, D. S.; Ford, C. W.; Zurenko, G. E. *J. Med. Chem.* **1996**, *39*, 673.
4. Swaney, S. M.; Aoki, H.; Ganoza, M. C.; Shinabarger, D. L. *Antimicrob. Agents Chemother.* **1998**, *42*, 3251, and references cited therein.
5. 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, and references cited therein.
6. Ford, C. W.; Zurenko, G. E.; Barbachyn, M. R. *Curr. Drug Targets: Infect. Disord.* **2001**, *1*, 181.
7. Hutchinson, D. K.; Brickner, S. J.; Gammill, R. V.; Patel, M. V. US 5700799
8. Mehta, A.; Rudra, S.; Rao, A. V. S.; Das, B.; Ray, A.; Srinivas, A. S. S. V.; Arora, S. K.; Rattan, A. WO 03/008389.

9. Brighty, K. E.; Castaldi, M. J. *Synlett* **1996**, 1097.
10. Brighty, K. E. US 5164402.
11. Synthesis of compound **2**: To a solution of (*S*)-*N*-[[3-[3-fluoro-4-(*N*-4-*tert*-butoxycarbonyl-piperazin-1-yl)phenyl]-2-oxo-5-oxazolidinyl]methyl]acetamide (**28a**, 0.3 g, 0.69 mmol) in dichloromethane (4 mL) cooled to 5 °C, trifluoroacetic acid (1 mL) was added and stirred for 2 h, while allowing the reaction mixture to warm to rt. The reaction mixture was evaporated in vacuo and the residue dissolved in DMF (5 mL). To the resulting mixture, potassium carbonate (0.4 g, 2.89 mmol) added and stirred at rt for 10 min. Then, 5-chloromethyl-2-furaldehyde[19] (0.3 g, 2.1 mmol) was added and stirred for 1 h. The reaction mixture was filtered over Celite and the filtrate evaporated in vacuo. The residue was purified by column chromatography (1–5% methanol in chloroform) to obtain (*S*)-*N*-[[3-[3-fluoro-4-[*N*-4-(5-formyl-2-furylmethyl)-piperazin-1-yl]phenyl]-2-oxo-5-oxazolidinyl]methyl]acetamide (**2**, 0.1 g, yield 32%) as a sticky solid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.62 (s, 1H, –C*H*O), 7.41 (dd, J = 2.2 Hz, 14.3 Hz, 1H, phenyl-*H*), 7.21 (d, J = 3.4 Hz, 1H, furyl-*H*), 7.06 (app d, J = 8.3 Hz, 1H, phenyl-*H*), 6.92 (t, J = 9.0 Hz, 1H, phenyl-*H*), 6.48 (d, J = 3.3 Hz, 1H, furyl-*H*), 6.08 (t, 1H, –N*H*CO–), 4.75 (m, 1H, oxazolidinone ring C_5–*H*), 4.0 (t, J = 9.0 Hz, 1H), 3.80–3.55 (m, 6 H), 3.10 (m, 4H, piperazine–*H*), 2.72 (m, 4H, piperazine–*H*), 2.01 (s, 3H, –COCH_3). HPLC purity: 90.69%.
12. Synthesis of compound **7**: (*S*)-*N*-[[3-[3-Fluoro-4-(*N*-4-*tert*-butoxycarbonyl-piperazin-1-yl)phenyl]-2-oxo-5-oxa-zolidinyl]-methyl]acetamide (**28a**, 3.65 kg, 8.37 mol) was dissolved in dichloromethane (30.86 L) and cooled to 5 °C. To it trifluoroacetic acid (6.17 L) added dropwise and stirred for 14 h allowing the reaction mixture to warm to rt. The reaction mixture was evaporated in vacuo and the residue dissolved in tetrahydrofuran (58 L) followed by addition of molecular sieves 4 Å (4.2 kg). To the resulting mixture 5-nitro-2-furaldehyde (1.5 kg, 10.77 mol) was added followed by sodium triacetoxyborohydride (5.32 kg, 25.1 mol) and stirred for 14 h. The reaction mixture was filtered over Celite and filtrate evaporated in vacuo. The residue was dissolved in ethylacetate (85.6 L) and washed with satd sodium bicarbonate solution (36 L) and water (36 L). The organic layer was dried over anhyd sodium sulfate (3 kg) and evaporated in vacuo. The crude residue was purified by column chromatography (1–3% methanol in ethylacetate) to obtain (*S*)-*N*-[[3-[3-fluoro-4-[*N*-4-(5-nitro-2-furylmethyl)-piperazin-1-yl]phenyl]-2-oxo-5-oxa-zolidinyl]methyl]acetamide (**39**, 2.6 kg, yield 67%). Mp: 136 °C. ^{1}H NMR ($CDCl_3$): δ 7.42 (dd, 1H, phenyl–*H*), 7.29 (m, 2H, furyl–*H*), 7.07 (d, 1H, phenyl–*H*), 6.92 (t, 1H, phenyl–*H*), 6.51 (d, 1H, furyl–*H*), 6.11 (t, 1H, –N*H*CO–), 4.77 (m, 1H, oxazolidinone ring C_5–H), 4.01 (t, 1H), 3.85–3.45 (m, 5H), 3.09 (m, 4H, piperazine–*H*), 2.72 (m, 4H, piperazine–*H*), 2.02 (s, 3H, –COCH_3). MS *m*/*z* (rel. int.): 462.1 [$(M+H)^+$, 100%], 484 [$(M+Na)^+$, 25%], 500.2 [$(M+K)^+$, 20%]. HPLC purity: 98%. Compound **39** (3.6 kg, 7.81 mol) was dissolved in abs ethanol (53.8 L) by heating to 60 °C. The resulting solution was cooled to 45 °C and ethanolic hydrochloride (1.48 L, 7.9 N) was added dropwise in 10 min. The mixture was then cooled to 10 °C and stirred for 4 h and the precipitate formed was filtered and washed with ethanol and dried to obtain (*S*)-*N*-[[3-[3-fluoro-4-[*N*-4-(5-nitro-2-furylmethyl)-piperazin-1-yl]phenyl]-2-oxo-5-oxazolidinyl]-methyl]acetamide hydrochloride (**7**, 3.2 kg, yield from **39**: 82%, yield from **28a**: 55%). Mp: 207–209 °C. ^{1}H NMR (DMSO, 300 MHz): δ 8.30 (t, 1H, –N*H*CO–), 7.75 (d, J = 3.3 Hz, 1H, furyl–*H*), 7.52 (dd, 1H, phenyl–*H*), 7.3–7.0 (m, 3H, phenyl–*H*, furyl–*H*), 4.70 (m, 1H, oxazolidinone ring C_5–*H*), 4.63 (s, 2H), 4.08 (t, J = 8.8 Hz, 1H, –CH_2–), 3.73 (t, J = 7.5 Hz, 1H), 3.43 (br m, piperazine–*H* merged with H_2O in DMSO), 1.83 (s, 3H, –COCH_3). HPLC purity: 98%. Anal. Calcd for $C_{21}H_{25}ClN_5O_6$·0.5-H_2O: C, 50.76; H, 5.48; N, 14.09. Anal. Found: C, 50.83; H, 5.17; N, 13.83.
13. Yamada, H.; Munesada, K.; Taniguchi, M. WO 95/21706.
14. Mehta, A.; Arora, S. K.; Das, B.; Ray, A.; Rudra, S.; Rattan, A. WO 03/007870.
15. Mehta, A.; Arora, S. K.; Das, B.; Ray, A.; Rudra, S.; Rattan, A. WO 02/06278.
16. Rattan, A. *Drugs Future* **2003**, *28*, 1070.
17. The effect of **RBx 7644** on whole cell macromolecular synthesis in anaerobes was examined recently. In each case a specific inhibitor with a known mechanism of action was included as a positive control (Trovafloxacin and Metronidazole for DNA and linezolid for protein). The results indicate that the compound is inhibitory towards protein synthesis but no effect was observed towards DNA synthesis with 1X and 4XMIC upto 6 h. However, the effect of **RBx 7644** on SOS response and ability to act as a substrate for nitroreductase enzymes from Gram-negative bacteria was not investigated.
18. **RBx 7644** (**7**) had MIC of 2 μg/mL when tested against Acr AB pump deficient and Tol C pump deficient strains, but the MIC was $\geqslant$16 μg/mL when tested against wild type *Escherichia coli* strains. It is possible that the excellent activity of **RBx 7644** against anaerobes is because it is not effluxed out.
19. Chakraborty, T. K.; Tapadar, S.; Kumar, S. K. *Tetrahedron Lett.* **2002**, *43*, 1317.