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Synthesis and antibacterial activity of potent heterocyclic oxazolidinones and the identification of RBx 8700

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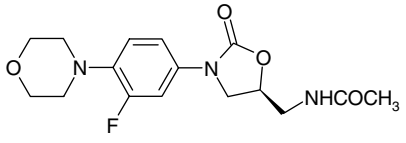
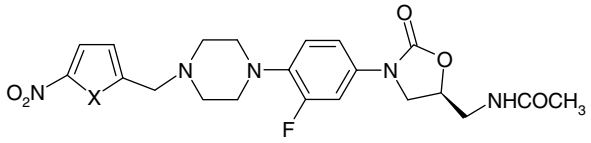
Abstract—Several potent oxazolidinone antibacterial agents were obtained by systematic modification of the linker between the five-membered heterocycle and the piperazinyl ring of RBx 7644 (Ranbezolid, **1**) and its thienyl analogue **2**, leading to the identification of an expanded spectrum compound RBx 8700 (**6b**).

Oxazolidinones have emerged as a new class of antibacterial agents active against Gram-positive nosocomial infections. Linezolid[1] (Zyvox™, Pfizer) is the only drug from the oxazolidinone class of compounds to be approved for use in humans. Linezolid is active against Gram-positive pathogens such as strains of methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant *Staphylococcus epidermidis* (MRSE), vancomycin-resistant *Enterococcus faecium* (VRE), *Streptococcus pyogenes* (*S. pyogenes*) and *Streptococcus pneumoniae* (*S. pneumoniae*).[2] Linezolid is inactive against Gram-negative bacilli and has modest activity against fastidious Gram-negative bacteria such as *Haemophilus influenzae* (*H. influenzae*). Linezolid exhibits its antibacterial activity by inhibiting bacterial protein synthesis via binding to the 50S ribosomal subunit and interfering with the *f*Met-*t*RNA binding to the P-site of the ribosomal peptidyltransferase centre.[3]



Linezolid (Zyvox™/Pfizer)



1: X = O (HCl salt), **(RBx 7644, Ranbezolid)**
2: X = S

We have been interested in furyl and thienyl heterocyclic derivatives of oxazolidinones,[4–8] and have previously reported the discovery of Ranbezolid[9] (RBx 7644, **1**) as a lead molecule active against Gram-positive pathogens. RBx 7644 has a 5-nitrofuryl ring linked with a methylene linker to the piperazin-1-yl-3-phenyloxazolidin-2-one core. Herein, we describe the synthesis and antibacterial activity of a series of compounds **3–6** (Table 1) obtained by varying the linker between the piperazine and five-membered heterocycle of the furyl and thienyl compounds **1** and **2**, respectively. Compound

Keywords: Antibacterial; Oxazolidinone; RBx 8700; RBx 7644; Ranbezolid; Nitrothiophene; Nitrofuran.
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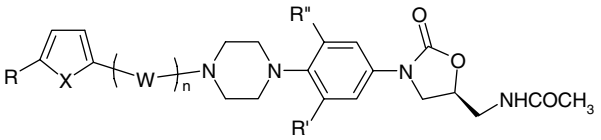
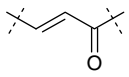
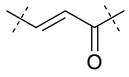
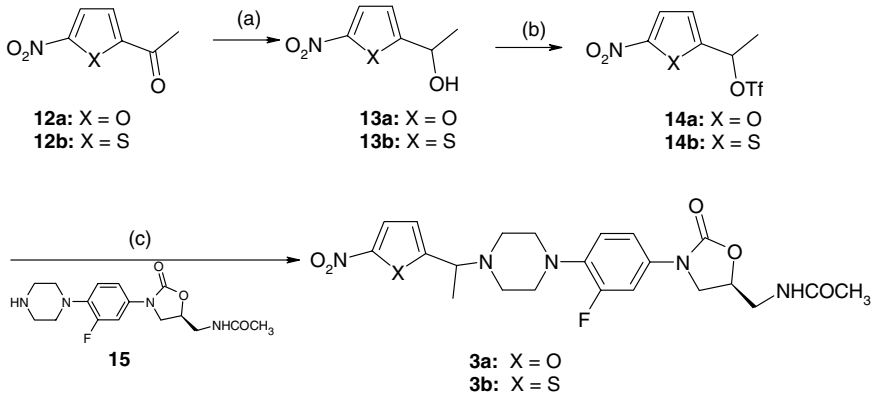
Table 1. Structures of compounds (**3–11**)



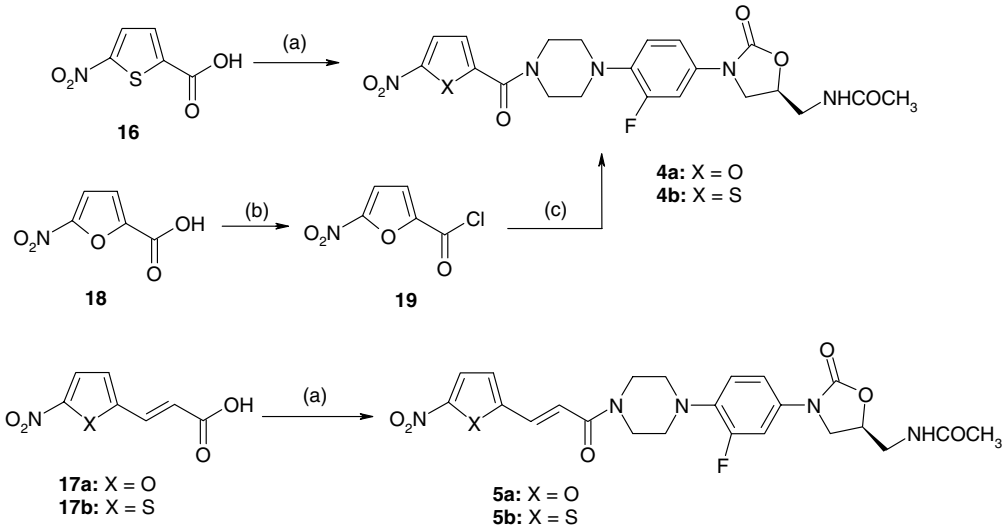
Compound	X	R	W	*n*	R′	R″
3a	O	NO_2		1	F	H
3b	S	NO_2		1	F	H
4a	O	NO_2		1	F	H
4b	S	NO_2		1	F	H
5a	O	NO_2		1	F	H
5b	S	NO_2		1	F	H
6a	O	NO_2	Direct bond	0	F	H
6b	S	NO_2	Direct bond	0	F	H
7	S	$COCH_3$	Direct bond	0	F	H
8	S	CN	Direct bond	0	F	H
9	S	CHO	Direct bond	0	F	H
10	S	NO_2	Direct bond	0	F	F
11	S	NO_2	Direct bond	0	H	H

6b (RBx 8700, Table 1) has a five-membered 5-nitrothiophene attached directly to the piperazine ring, and modification of this lead compound led to compounds **7–11**.

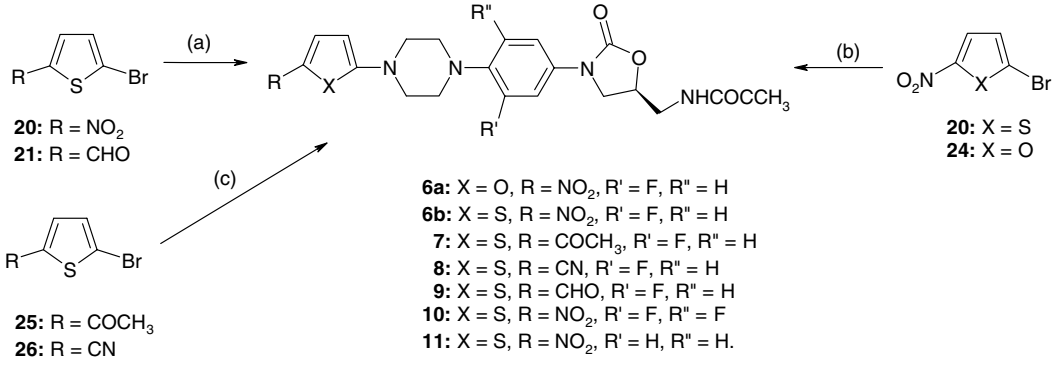
The synthesis of compounds **3–11**[10] is depicted in Schemes 1–3. As shown in Scheme 1, 2-acetyl-5-nitrofuran (**12a**) and 2-acetyl-5-nitrothiophene (**12b**) were reduced to their corresponding alcohols (**13a–b**) with sodium borohydride. These secondary alcohols were converted to triflate derivatives **14a–b** using triflic anhydride. The triflate derivatives were used to alkylate the piperazin-1-yl-3-phenyloxazolidin-2-one compound **15**[11] to give compounds **3a** and **3b**. Using achiral reverse phase HPLC (C18 Kromasil™ column, UV detector), compounds **3a** and **3b** were found to have purities of 96.1% (mobile phase: 20 mM sodium acetate, pH 5.5,



Scheme 1. Reagents and conditions: (a) $NaBH_4$, THF-H_2O, 0 °C, 3 h; (b) triflic anhydride, triethylamine, dichloromethane, 0 °C to rt, 6 h (for **14a**), 3 h (for **14b**); (c) **15**, triethylamine, THF, 0 °C to rt, 24 h, 31% (for **3a**), 30% (for **3b**).



Scheme 2. Reagents and conditions: (a) **15**, EDAC, NMM, HOBt, DMF, 0 °C to rt, 18 h, 93% (for **4b**), 35% (for **5a**), 13% (for **5b**); (b) SO_2Cl_2, Et_3N, dichloromethane, 0 °C to rt, 4 h; (c) **15**, K_2CO_3, DMF, rt, 18 h, 21%.



Scheme 3. Reagents and conditions: (a) **15** (trifluoroacetate salt) or **22**, *N*-ethyldiisopropylamine, acetonitrile, 60 °C, 4 h, 46% (for **6b**); 80 °C, 30 h, <10% (for **9**); 60 °C, 17 h, 55% (for **10**); (b) **23**, K_2CO_3, DMSO, 18 h, 16% (for **11**), rt; **15** (hydrochloride salt), NaOH, DMF, 18 h, 59% (for **6a**), rt; (c) **15** (trifluoroacetate salt), *rac*BINAP, Cs_2CO_3, $Pd_2(dba)_3$, DMF, 95 °C, 10 h, 12% (for **7**), <10% (for **8**).

and methanol, 60–40–60% gradient) and 88.8% (mobile phase: 20 mM sodium acetate, pH 5.5, and methanol, 80–20–80% gradient), respectively, with no other major peak >5% being detected. The diastereomeric purities of compounds **3a** and **3b** were not determined.

An amide bond was formed between the *N*-piperazinyl group of compound **15** and 5-nitrothiophene-2-carboxylic acid (**16**), 3-(5-nitro-2-furyl)acrylic acid (**17a**) and 3-(5-nitro-2-thienyl)acrylic acid (**17b**), respectively, using *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide

hydrochloride (EDAC), *N*-methylmorpholine (NMM) and 1-hydroxy-benzotriazole (HOBt) to obtain compounds **4b**, **5a** and **5b** (Scheme 2). 5-Nitrofuran-2-carboxylic acid (**18**) was treated with thionyl chloride to generate the acid chloride **19**, which was then used to acylate the compound **15** to produce compound **4a**.

The synthesis of compounds **6–11** is shown in Scheme 3. Nucleophilic substitution of the bromine of 2-bromo-5-nitrothiophene (**20**) and 5-bromothiophene-2-carboxaldehyde (**21**) by compound **15** (trifluoroacetate salt) using *N*-ethyldiisopropyl amine in acetonitrile at elevated temperatures led to the synthesis of compounds **6b** and **9**, respectively. Similarly, nucleophilic substitution of **20** by the difluorophenyl analogue **22** ((*S*)-*N*-[[3-[3,5-difluoro-4-(piperazin-1-yl)phenyl]-2-oxo-5-oxazolidinyl]methyl]-acetamide trifluoro-acetate) led to the compound **10**. For nucleophilic substitution of **20** by the phenyl without fluorine analogue **23**((*S*)-*N*-[[3-[4-(piperazin-1-yl)phenyl]-2-oxo-5-oxazolidinyl]methyl] -acetamide trifluoroacetate), and 2-bromo-5-nitrofuran (**24**) by **15** (hydrochloride salt), the reaction was carried out with inorganic bases such as potassium carbonate or sodium hydroxide at room temperature to produce compounds **11** and **6a**, respectively. Buchwald's method of C–N bond formation[12,13] was employed for the reaction of 2-acetyl-5-bromothiophene (**25**) or 2-bromo-5-cyanothiophene (**26**) with compound **15** (trifluoroacetate salt) to give compounds **7** and **8**, respectively.

The in vitro[14] activity (against a panel of resistant and susceptible Gram-positive pathogens) and in vivo[15] activity (against MRSA 562) of the compounds are presented in Table 2. Replacing the methylene linker of RBx 7644 (**1**) or the thienyl analogue **2** with a 1-alkylmethylene linker gave the highly active in vitro compounds **3a** and **3b**. Compound **3a** was also active in vivo. Changing the methylene linker to an oxo linker gave amides **4a** and **4b** which were very active in vitro and in vivo, with the thienyl analogue **4b** being several fold more active than its methylene analogue **2**. Extension of the oxo linker to an acryloyl linker produced highly active in vitro compounds (**5a** and **5b**), but with loss of in vivo activity. The absence of a linker, wherein a 5-nitrofuryl or 5-nitrothienyl ring is attached directly to the piperazine compound **15**, led in the case of the thiophene analogue to a compound (**6b**, RBx 8700) having high activity against staphylococci, enterococci and streptococci strains, and in the case of the furan analogue to a compound (**6a)** having low activity.

In the in vitro experiments, the rank order of potencies of the thienyl analogues was **6b** (the linker is –), **4b** (–CO–) > **5b** (–CH_2=CH_2CO–), ∼**3b** (–CH(CH_3)–) > **2** (–CH_2–). For the furyl analogues, the rank order was **5a** (–CH_2=CH_2CO–) > **1** (–CH_2–), ∼**3a** (–CH(CH_3)–) > **4** (–CO–) > **6a** (–). It is speculated that the observed decrease in activity in the thiophene series may be associated with a decrease in the planarity of the heterocycle–linker–piperazinyl system. The decrease in activity in the furan series appears to be less associated with the presence of mesomeric interactions between the heterocycle, linker and piperazinyl groups, and more related to either a decrease in the steric size of the linker group (and a concomitant decrease in the size of the heterocycle–linker–piperazinyl system) or a decrease in the flexibility of the linker.

A comparison of the furan-containing compounds with their thiophene analogues reveals some interesting differences. In the case of compounds **6a** and **6b**, in which there exists a direct attachment of the piperazine ring nitrogen atom to the 2-position of the 5-membered heteroaromatic ring, the thienyl compound **6b** has higher potency. It may be that the activity seen correlates with charge delocalization from the 5-membered heteroaro-

Table 2. In vitro and in vivo activity

Compound	MIC (μg/ml) (organisms)[a]							ED_{50} mg/kg (po) MRSA 562
	S.a 25923	MRSA 562	MRSA 33	VRE 6A	E.fa 29212	S.py 19615	S.pn 6303	
1	1	1	2	2	2	0.125	0.125	4.33
2	8	8	8	8	8	1	8	>25
3a	1	1	1	0.25	0.125	0.06	1	9.1
3b	0.5	0.5	0.5	0.5	0.5	2	2	>25
4a	1	n.d.	0.5	1	1	2	2	8.24
4b	0.125	0.06	0.06	0.5	0.5	0.125	0.5	9.77
5a	0.5	0.25	0.25	<0.125	0.5	<0.125	0.5	>25
5b	0.5	1	0.5	0.5	1	0.5	1	>25
6a	16	16	8	8	8	4	4	n.d.
6b	0.25	0.25	0.25	0.25	0.25	0.125	0.125	11.15
7	>16	>16	>16	>16	>16	>16	>16	n.d.
8	4	2	2	n.d.	2	0.25	0.5	n.d.
9	1	0.5	0.5	1	<0.25	0.5	0.5	>25
10	0.25	0.25	0.25	0.25	0.25	0.5	0.5	>25
11	2	1	1	1	1	0.5	0.5	n.d.
Linezolid	2	2	2	2	2	2	2	5.6
Vancomycin	1	0.5	0.5	>16	4	0.5	0.5	8.8 (s.c.)

[a] Organisms: S.a 25923, *Staphylococcus aureus* ATCC 25923; MRSA 562, methicillin-resistant *Staphylococcus aureus* 562; MRSA 33, methicillin-resistant *Staphylococcus aureus* 33; VRE 6A, vancomycin-resistant *Enterococcus faecium* 6A; E.fa 29212, *Enterococcus faecalis* ATCC 29212; S.py 19615, *Streptococcus pyogenes* ATCC 19615; S.pn 6303, *Streptococcus pneumoniae* ATCC 6303.

matic ring into the 5-nitro group. Such an effect is more favoured in a 5-nitrothiophene than a 5-nitrofuran because the development of positive charge (in the ground state) on the heteroatom is more favourable with thiophene than with furan. The charge delocalization is expected to be assisted by the presence of the 2-amino group. In compounds **4a** and **4b**, the thienyl compound **4b** also has higher potency. Once again, delocalization of charge from the heteroaromatic ring into the 5-nitro group is anticipated, but in this case such delocalization can also occur into the 2-carboxamido group. As explained above, the amount of delocalization will be greater for the thiophene compound, but here the explanation is confused by the possible existence of rotational isomers arising from the presence of syn and anti amide groups, leading to the possibility that the two compounds may adopt different ground-state conformations.

The furan-containing compound **1** is the more potent of the pair of methylene-linked compounds **1** and **2**. Whilst mesomeric interactions between the piperazine nitrogen atoms and the heteroaromatic groups cannot occur in these cases, the 5-aminomethyl group would be expected to have an electron-withdrawing inductive effect upon the aromatic ring. Of more importance, however, may be the conformational differences arising from rotation around the methylene linker bonds. Branching of the methylene linkers by a methyl group results in equipotent compounds, with the thienyl analogue **3b** being onefold more potent than the furyl analogue **3a** against staphylococci, and **3a** being one- or more fold potent than **3b** against enterococci and streptococci. The significant gain in potency of compounds **3a** and **3b** over their methylene counterparts **1** and **2** cannot be explained by electronic effects, and the introduction of the methyl groups is probably having a profound effect upon the compounds, conformational preferences. In addition, compounds **3a** and **3b** may exist as diastereoisomers, and it may be that the isomers possess differing antibacterial activities.

Compounds **5a** and **5b** were essentially equipotent, and it is speculated that a complex mixture of mesomeric, steric and conformational effects is playing major roles in defining the compounds, activities. It is interesting to note that Lohray et al.[17] have reported on a series of compounds containing a phenyl group linked to a piperazine ring nitrogen atom via an extended conjugated linker and shown that electron-donating substituents such as methoxy and electron-withdrawing substituents such as acetamido have no effect on antibacterial activity.

In the in vivo experiments, compounds **3a**, **4a**, **4b** and **6b** were all active against MRSA 562, but slightly less potent than Ranbezolid. The reasons for the potency differences were not investigated further, but may be due to differing pharmacokinetic and metabolic profiles. Compound **6b** showed moderate activity against fastidious Gram-negative bacteria such as *H. influenzae* (MIC_{50} = 8 μg/ml) and good activity against *M. catarrhalis* (MIC_{50} = 2 μg/ml), thus extending the spectrum of oxazolidinones.[18] Compound **6b** was also highly active against sensitive and multi-drug resistant (MDR) *Mycobacterium tuberculosis* (*M. tuberculosis)* strains[19,20] with MIC_{90} values of 0.25 and 1 μg/ml, respectively.

Compound **6b** exhibited an expanded range of microbiological activity, so the nitro group was replaced with other electron-withdrawing groups (compounds **7–9**). The results were disappointing with the acetyl derivative **7** showing no activity at less than 16 μg/ml, the nitrile derivative **8** showing only modest activity, and the formyl derivative **9** showing high activity but no activity in vivo. Changing the fluorophenyl ring of **6b** to a difluorophenyl ring gave a compound (**10**) with similar in vitro activity but reduced in vivo activity. The simple phenyl analogue **11** was inferior to the monofluorophenyl compound **6b** in all aspects.

In summary, changing the methylene linker of compounds **1** (RBx 7644) and **2** led to many compounds with superior in vitro activities. Compound **6b** (RBx 8700) exhibited an expanded spectrum in vitro activity along with in vivo efficacy.

Acknowledgments

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10. Analytical data of (**6b**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80 (d, 1H, J = 4.8 Hz, thienyl-*H*), 7.5 (dd, 1H, Ar-*H*), 7.11 (dd, 1H, Ar-*H*), 6.97 (t, 1H, J = 9.1 Hz, Ar-*H*), 6.01 (d, 1H, J = 4.8 Hz, thienyl-*H*), 5.94 (t, 1H, N*H*), 4.77 (m,

1H, C_5-*H*), 4.02 (t, 1H, $J = 9$ Hz), 3.85–3.5 (m, 7H), 3.23 (m, 4H, piperazinyl-*H*), 2.03 (s, 3H, –COCH_3); MS *m/z* [rel. int.]: $(M+H)^+ = 464.1$ [30%]; $(M+Na)^+ = 486.1$, [65%]; $(M+K)^+ = 502.2$, [35%]; $(M-NO_2)^+ = 418.3$ [100%]; HPLC purity = 98.44%; mp = 171–174 °C.

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15. In vivo: Swiss albino mice weighing 20 ± 2 g were used in the study. There were six mice in each group and water and rodent feed were provided ad libertum throughout. Lethal systemic infection was caused in mice by injecting intraperitoneally MLD inoculum of methicillin-resistant *Staphylococcus aureus* (MRSA) 562 mixed with 1:1 10% mucin. Compounds were administered orally at 30 min and 4 h post infection. The ED_{50} values were calculated by the Spearman Karber[16] method on day 7 post infection.
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