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The Effect of Remote Chirality on the Antibacterial Activity of Indolinyl, Tetrahydroquinolyl and Dihydrobenzoxazinyl Oxazolidinones

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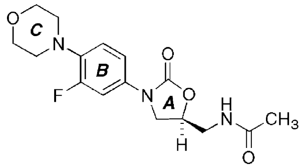
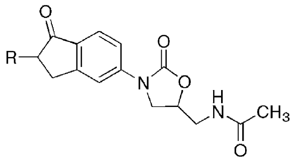
Abstract—The oxazolidinones are promising agents for the treatment of infections caused by gram-positive bacteria, including multidrug-resistant strains. In ongoing studies we have discovered that a strategically placed chiral center of appropriate absolute configuration improves the antibacterial activity of indolinyl oxazolidinone analogues (gram-positive MIC's <0.5 μg/mL for the most potent congeners). The design, synthesis, antibacterial activity and pharmacokinetic profile of a selected series of α-methylated indoline derivatives and a related set of tetrahydroquinolyl and dihydrobenzoxazinyl analogues are discussed.

The oxazolidinones, exemplified by linezolid (**1**), are a promising group of antibacterials that have recently emerged as important therapeutic agents for the treatment of infections caused by gram-positive bacteria.[1] Importantly, linezolid's spectrum of activity includes multidrug-resistant strains of the staphylococci, streptococci and enterococci, including recently described glycopeptide-intermediate *Staphylococcus aureus* (GISA) isolates with reduced susceptibility to vancomycin.[2] Because of their significance, extensive synthetic investigations into the structure–activity relationships (SARs) of the oxazolidinones have been conducted at Pharmacia and elsewhere. Early SAR studies revealed that the incorporation of a strategically placed methyl group in indanone derivatives significantly improved antibacterial activity. For example the MICs of the unsubstituted indanone **2** range from 8 to 16 μg/mL for *S. aureus* whereas the 2-methyl indanone congener **3** (mixture of diastereomers) has MICs = 2–4 μg/mL. Thus, alkyl substitution adjacent to the carbonyl moiety confers enhanced antibacterial activity to the pharmacophore.[3–5]

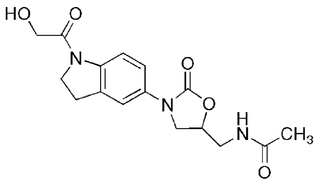
Contemporaneous synthetic studies led to the preparation of closely related indolinyl oxazolidinones such as (±)-**4**.[6] Compound (±)-**4** exhibits interesting levels of gram-positive antibacterial activity (*S. aureus* MIC = 4–8 μg/mL). Moreover, it was found to be very well tolerated in extended rat safety studies, showing no significant adverse events after oral doses of 100 mg/kg bid for 30 days.[7] Based on these results, we decided to



1 (linezolid, PNU-100766)



2: R = H
3: R = CH$_3$



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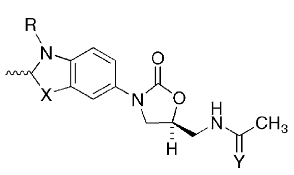

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R = H, CHO, COCH$_2$OH
X = CH$_2$, CH$_2$CH$_2$, CH$_2$O
Y = O, S

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investigate the effects of methylation at the 2-position of indolinyl oxazolidinones and at the analogous position of the related tetrahydroquinoline and dihydrobenzoxazines (see generic structure **5**). Herein we describe the synthesis, antibacterial activity and pharmacokinetic profile of selected examples in this series. Like the indanone series, these compounds exhibit increased levels of antibacterial activity against gram-positive bacteria when compared to their unsubstituted counterparts. In addition, the configuration of the newly introduced stereocenter plays an important role in the antibacterial activity. Furthermore, some analogues have notable activity against the fastidious gram-negative organisms *Haemophilus influenzae* and *Moraxella catarrhalis*.[8]

Chemistry

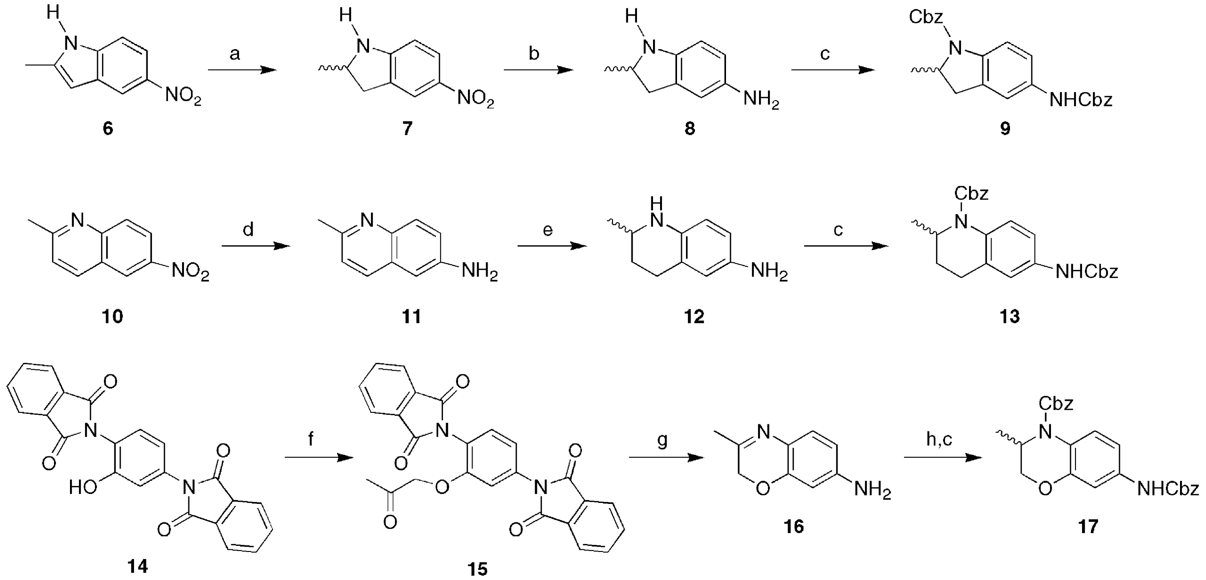
The synthesis of the desired analogues is outlined in Schemes 1–3. Requisite bis-Cbz protected intermediates were prepared as shown in Scheme 1. The known indole **6**[9] was reduced to indoline **7** with sodium cyanoborohydride. Reduction of the nitro group via catalytic hydrogenation afforded **8**, which was bis-protected with Cbz–Cl to give **9**. The tetrahydroquinoline **13** was prepared from the commercially available quinoline **10**. Reduction of the nitro group with $SnCl_2 \cdot 2H_2O$ gave **11**, which was converted to the tetrahydro intermediate **12** via hydrogenation using PtO_2/MeOH. This material was bis-protected as above to give **13**. The benzoxazine bis-carbamate **17** was constructed from the known precursor **14**.[10] Alkylation of the phenol with chloroacetone gave **15**. Phthalimide deprotection using excess hydrazine is accompanied by ring-formation to give the intermediate imine **16**. The imine was then reduced with $NaBH_4$ and protected to afford **17**.

Compounds **9**, **13** and **17** were converted to the corresponding (5*R*)-(hydroxymethyl)oxazolidinones **18**–**20** via chemistry previously described (Scheme 2).[11] Preparative scale separation of the diastereomeric mixtures on a Chiralcel® OJ column provided the pure diastereomers (*R,R*)-**18**–**20a** and (*S,R*)-**18**–**20b** ($X = CH_2$, CH_2CH_2, CH_2O). The **a** and **b** descriptors refer to the absolute configuration of the stereocenter in the indoline, tetrahydroquinoline or dihydrobenzoxazine ring, with **b** corresponding to the configuration depicted in Figure 1 (vide infra). Several X-ray structure determinations enabled an unequivocal assignment of the absolute configurations of the indoline and tetrahydroquinoline C-2 chiral centers and the dihydrobenzoxazine C-3 chiral center. A representative example, diastereomer **18b**, is shown (Fig. 1).

The individual indoline diastereomers **18a** and **b** were converted to the acetamide derivatives (*R,S*)-**21a** and (*S,S*)-**21b**, respectively (Scheme 3). Cbz-deprotection gave **22a** and **22b** which were converted to the formyl and hydroxymethylacetyl analogues **23a**,**b** and **24a**,**b** as shown. The preparation of analogues bearing C-5 thioamide side chains was also examined and in this regard, two approaches gave desired analogues. Initial reaction of **21a** and **21b** with Lawesson's Reagent followed by Cbz-deprotection with HBr/HOAc and formylation gave thioamides **25a** and **25b**. Alternatively, **22a** was treated with Lawesson's Reagent followed by acylation and deprotection to afford the hydroxymethylacetyl analogue **26a**. The desired analogues in the tetrahydroquinoline and benzoxazine series were prepared in a similar fashion yielding compounds **27**–**30a** and **27**–**29b**.

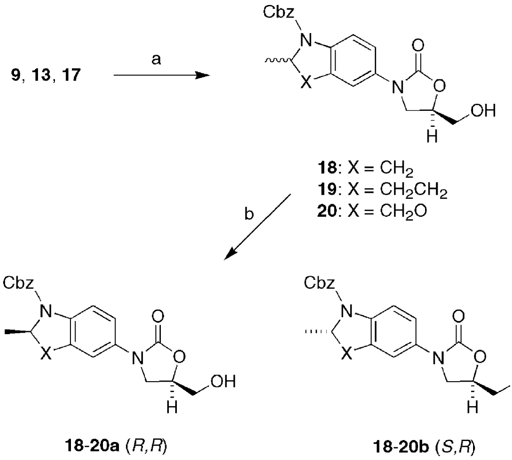
Biology

The analogues prepared above were tested in vitro versus a panel of gram-positive and fastidious gram-negative bacteria (Table 1). Minimum inhibitory concentration (MIC) values were determined using standard agar dilution methods.[12] Also shown are comparator compounds



Scheme 1. (a) $NaBH_3CN$; (b) H_2, Pd/C, MeOH; (c) CbzCl, $NaHCO_3$, acetone; (d) $SnCl_2 \cdot H_2O$/EtOH, reflux; (e) H_2, PtO_2, MeOH/H_2SO_4; (f) KI, K_2CO_3, $ClCH_2COCH_3$, reflux; (g) $NH_2NH_2 \cdot H_2O$, THF, reflux; (h) $NaBH_4$, MeOH.

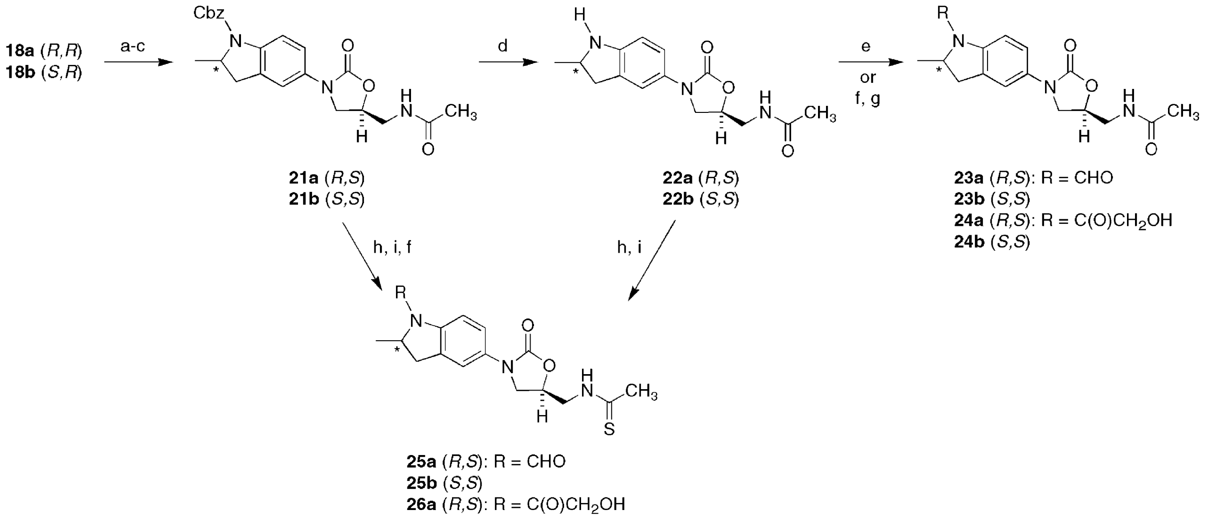
linezolid and **4**. Considering the indoline series first, it can be seen that methylation at the 2-position imparts a 2- to 4-fold increase in activity (**24a** vs **4**). This is in agreement with what we predicted based on historical observations for the indanones **2** and **3** (vide supra). Moreover, the stereochemistry of the new chiral center is critical. Only the appropriate absolute configuration at the 2-position of the indolinyl ring leads to an enhancement in MICs. Thus, in all instances the (*R*)-isomer is approximately 4- to 8-fold more active than the corresponding (*S*)-isomer (e.g., **23a** vs **23b**). Exploring substitution on the indoline nitrogen it was found that the formyl group was preferred over the hydroxymethyl acetyl moiety, and analogue **23a** exhibits potent antibacterial activity with gram-positive MICs in the range of <0.5–1 μg/mL. Furthermore, the thioamide side-chain analogue of this congener, **25a**, is even more effective against gram-positive organisms and also has very interesting activity against the fastidious gram-negative organisms. The MICs of **25a** against *H. influenzae* and *M. catarrhalis* are 2 and 1 μg/mL, respectively.



Scheme 2. (a) *n*-BuLi, (*R*)-glycidyl butyrate, THF; (b) chiral HPLC.

Methylation of the corresponding tetrahydroquinolinyl (**27**–**28**) and dihydrobenzoxazinyl (**29**–**30**) oxazolidinones also confers enhanced antibacterial activity to these pharmacophores and in all cases the (*R*)-isomer is more active. As was seen with the indolines, formyl and hydroxyacetyl appendages at the ring nitrogen increase potency, with formyl substitution being favored. Formyl substituted tetrahydroquinolines (e.g., **28a**) were comparable to the indolines while the benzoxazine analogues were slightly less active. Consistent with previously studied oxazolidinones, thioacetamide derivatives are significantly more active than acetamides.[13]

Selected compounds were also evaluated for in vivo efficacy against *S. aureus* in a lethal systemic infection model in the mouse (Table 2).[14] With ED$_{50}$s of 1.8 and 7.5 mg/kg, respectively, the formyl and hydroxymethyl acetyl indoline analogues, **24a** and **25a**, are comparable in activity to linezolid. These compounds were also subjected to a single-dose pharmacokinetic performance assessment in the male Sprague–Dawley rat (Table 2). All of the selected analogues have reasonable oral bioavailabilty ($F = 30.8$–56.0%). However, the maximum serum concentrations (C_{max}) are generally



Scheme 3. (a) NosCl, Et$_3$N, CH$_2$Cl$_2$; (b) NH$_4$OH, CH$_3$CN/IPA; (c) Ac$_2$O; (d) H$_2$, Pd/C; (e) HCO$_2$H, (i-Pr)$_2$NEt, (EtO)$_2$P(O)CN; (f) (1) ClCO-CH$_2$OAc; (2) K$_2$CO$_3$/MeOH; (g) chiral HPLC; (h) Lawesson's reagent; (i) (1) HBr/HOAc; (2) HCO$_2$H, (i-Pr)$_2$NEt, Ac$_2$O.

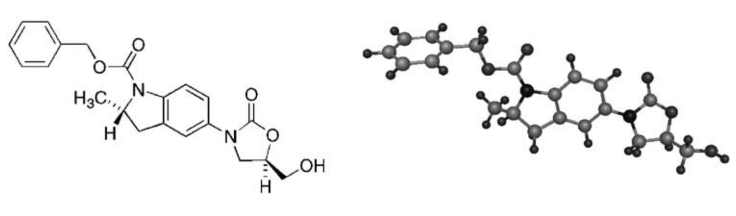
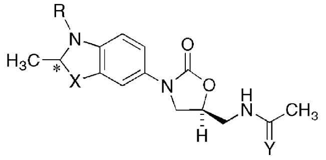


Figure 1. X-ray structure of intermediate **18b**.

Table 1. In vitro activity of indolinyl, tetrahydroquinolinyl and dihydrobenzoxazinyl oxazolidinones



Compd	*	R	X	Y	MIC (μg/mL)						
					SA1	SA2	SE	SP	EF	HI	MC
22a	(*R*)–	H	–CH_2–	O	8	8	4	4	16	> 64	64
22b	(*S*)–	H	–CH_2–	O	32	32	16	—	64	> 64	> 64
23a	(*R*)–	CHO	–CH_2–	O	1	1	< 0.5	< 0.5	1	8	4
23b	(*S*)–	CHO	–CH_2–	O	8	8	4	4	16	> 64	64
24a	(*R*)–	$C(O)CH_2OH$	–CH_2–	O	4	2	0.5	0.5	2	8	8
24b	(*S*)–	$C(O)CH_2OH$	–CH_2–	O	8	8	2	1	8	> 16	16
25a	(*R*)–	CHO	–CH_2–	S	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	2	1
25b	(*S*)–	CHO	–CH_2–	S	2	2	1	< 0.5	2	32	16
26a	(*R*)–	$C(O)CH_2OH$	–CH_2–	S	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	8	2
27a	(*R*)–	CHO	–$(CH_2)_2$–	O	2	1	< 0.5	< 0.5	1	32	4
27b	(*S*)–	CHO	–$(CH_2)_2$–	O	8	4	2	2	8	64	16
28a	(*R*)–	CHO	–$(CH_2)_2$–	S	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	4	1
28b	(*S*)–	CHO	–$(CH_2)_2$–	S	1	1	< 0.5	< 0.5	2	16	2
29a	(*R*)–	CHO	–CH_2O–	O	4	2	1	1	4	64	8
29b	(*S*)–	CHO	–CH_2O–	O	8	8	4	4	32	64	32
30a	(*R*)–	CHO	–CH_2O–	S	< 0.5	< 0.5	< 0.5	0.5	1	32	2
4	—	$C(O)CH_2OH$	–CH_2–	O	8	4	2	1	4	16	8
Linezolid (**1**)	—	—	—	—	2–4	2–4	1–2	0.5–1	1–2	16	8–16

MIC, minimum inhibitory concentration; SA1, *Staphylococcus aureus* UC®9213 (MSSA); SA2, *S. aureus* UC®12673 (MRSA, CIPRO^R, RIF^R, IMIPEN^R); SE, *Staphylococcus epidermidis* UC®12084 (MRSE); SP, *Streptococcus pneumoniae* UC®9912 (PSSP); EF, *Enterococcus faecalis* UC®9217 (VSEF); HI, *Haemophilus influenzae* 30063 (AMP^R, β-lactamase +); MC, *Moraxella catarrhalis* 30607 (β-lactamase +).

Table 2. Efficacy in a murine model and single-dose pharmacokinetics in rats

Compd	ED_{50} (mg/kg)[a]	Route	Dose[d] (mg/kg)	C_{max}[e] (μg/mL)	t_{max}[f] (h)	$t_{1/2}\beta$[g] (h)	V_{ss}[h] (L/kg)	CL[i] (mL/min/kg)	F[j] (%)
24a	1.8 (2.5)[b]	iv	9.92	15.1	na[k]	1.8	1.28	24	na
		po	20.73	3.8	0.5	2.8	na	nc[l]	55.4
23a	Not Tested	iv	9.84	16.1	na	1.7	1.27	18.4	na
		po	19.76	7.2	0.5	0.8	na	nc	56
25a	7.5 (4.0)[b]	iv	9.83	13.6	na	0.1	0.64	28.9	na
		po	19.2	1.13	0.5	0.61	na	nc	30.8
Linezolid	5.6 (3.9)[c]	iv	10	nc	na	0.95	0.72	10.5	na
		po	25	15.8	0.3	1.05	na	nc	109

[a] *S. aureus* UC®9213 systemic infection mouse model, po administration.

[b] Orally administered linezolid control in parentheses.

[c] Subcutaneously administered vancomycin control in parentheses.

[d] $N = 3$.

[e] Maximum plasma concentration.

[f] Time at which C_{max} achieved.

[g] Harmonic mean apparent terminal disposition half-life.

[h] Steady state volume of distribution.

[i] Clearance.

[j] Absolute oral bioavailability assuming linear pharmacokinetics.

[k] na, not applicable.

[l] nc, not calculated.

lower and the clearance (CL) typically higher than the comparator.

In summary, we have prepared a series of α-methylated indolinyl, tetrahydroquinolyl and dihydrobenzoxazinyl oxazolidinone derivatives which demonstrate a unique stereochemical relationship with antibacterial activity. The (*R*)-configurational isomers are always more active and represent an interesting structure–activity trend. It might be inferred that the methyl of the (*R*)-diastereomer is either occupying a hydrophobic pocket and/or promoting a more energetically stable conformation for adjacent nitrogen substituents that is preferred by the binding site. In addition, the indoline derivatives (**23**–**25a**) have reasonable oral bioavailability as well as in vivo efficacy comparable to that of linezolid. With these results in hand, further evaluation of related oxazolidinones appears to be warranted.

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