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The Synthesis and Antibacterial Activity of 1,3,4-Thiadiazole Phenyl Oxazolidinone Analogues

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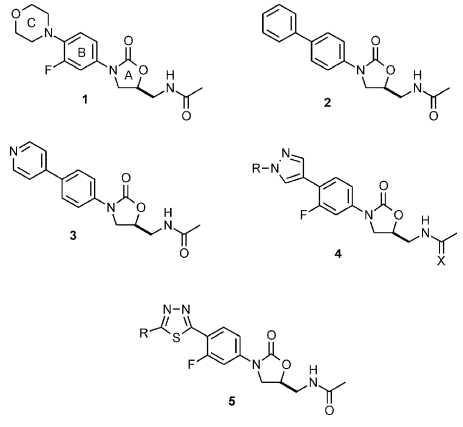
Abstract—Replacement of the morpholine C-ring of linezolid **1** with a 1,3,4-thiadiazolyl ring leads to oxazolidinone analogues **5** having potent antibacterial activity against both gram-positive and gram-negative organisms. Conversion of the C5 acetamide group to a thioacetamide further increases the potency of these compounds.

Decades of antibiotic use have resulted in the development of widespread resistance to commonly prescribed antibacterial agents. Nosocomial infections by methicillin-resistant *Staphylococcus aureus* (MRSA)[1] and vancomycin-resistant *Enterococcus* (VRE)[2] are particularly serious problems. Recently, a glycopeptide-intermediate *S. aureus* (GISA) isolate with reduced susceptibility to vancomycin[3] was reported. The spread of resistance of MRSA to vancomycin would be especially devastating. Within the community, penicillin- and cephalosporin-resistant *Staphylococcus pneumoniae* (PRSP)[4] has become an increasing problem. In view of the increased resistance of gram-positive bacteria to currently available antibacterial agents, there is an urgent need for new antibacterial agents.

The oxazolidinones are a novel class of totally synthetic antibacterial agents. Linezolid (**1**) is the first oxazolidinone approved for the treatment of gram-positive bacterial infections in humans.[5] Linezolid is active against multidrug-resistant strains of staphylococci, streptococci and enterococci, but is only weakly active (or inactive) against gram-negative organisms. Early SAR studies, conducted by workers at DuPont, revealed that oxazolidinone analogues having a phenyl or 4-pyridyl C-ring resulted in oxazolidinone analogues (**2** and **3**) with improved antibacterial potency.[6,7] More recently, Genin and co-workers have reported that replacement of the morpholine C-ring of linezolid with a carbon–carbon linked pyrazole ring system afforded oxazolidinone analogues (**4**) having improved antibacterial potency against fastidious gram-negative organisms.[8] With this in mind, we were interested in preparing oxazolidinone analogues (**5**) having other carbon-linked heteroaromatic C-rings. In this letter, we describe the synthesis and antibacterial activity of oxazolidinone analogues having 1,3,4-thiadiazole C-rings.



In order to facilitate the rapid preparation of multiple 1,3,4-thiadiazolyl phenyl oxazolidinone analogues, an

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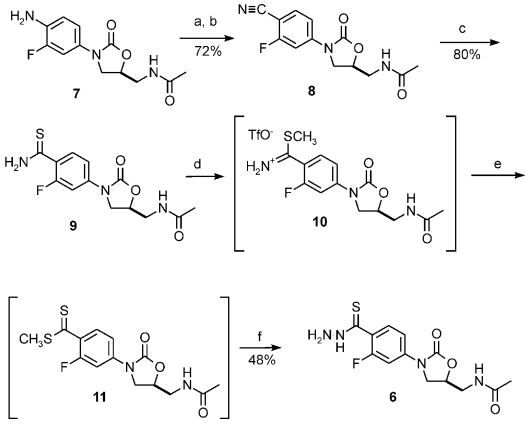
advanced intermediate was sought that could readily be converted to the target compounds. The thiobenzhydrazide **6** was selected as the key intermediate, since the 1,3,4-thiadiazoles would be readily available from **6** by reaction with carboxylic acid chlorides. Synthesis of **6** was carried out as shown in Scheme 1. Thus, diazotization of the known[9] amine **7** followed by reaction with potassium cyanide gave the nitrile **8**. Addition of hydrogen sulfide to **8** in hot DMF afforded the thioamide **9**. Alkylation of **9** with methyl triflate and treatment of the intermediate isothioamide **10** with H_2S led to the dithioester **11**, which was immediately treated with hydrazine to afford the desired thiobenzhydrazide **6**.[10,11]

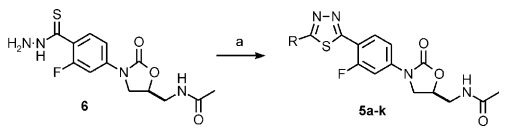
Once in hand, reaction of the thiobenzhydrazide **6** with readily available acid chlorides in refluxing THF afforded a variety of 1,3,4-thiadiazolyl phenyl oxazolidinone analogues in good yields (Scheme 2).

Simple functional group manipulations provided additional analogues. For example, hydrolysis of the acetate **5g** ($MeOH/K_2CO_3$) yielded **5l**. Reaction of the ester **5h** with methanolic ammonia gave **5m**. Compound **5n** was prepared by acidic hydrolysis (H_2SO_4/H_2O) of **5j**. Reaction of ketone **5i** with hydroxylamine afforded the oxime **5o**. Oxidation of **5k** with $NaIO_4$ gave the sulfoxide **5p**, while reaction of **5k** with OXONE® yielded the sulfone **5q**.

Reaction of **6** with Fmoc-glycyl chloride or Fmoc-sarcosine acid chloride (prepared from Fmoc-sarcosine and thionyl chloride using a catalytic amount of DMF) in refluxing THF afforded the Fmoc-protected 2-aminomethyl-1,3,4-thiadiazole analogues **5r** and **5s**, respectively. Removal of the Fmoc-protecting group with piperidine afforded **5t** and **5u** (Scheme 3).



Scheme 1. (a) $NaNO_2$, 2 N HCl; (b) CuCN, KCN, EtOAc; (c) H_2S, Et_3N, DMF, 100 °C; (d) $CF_3SO_3CH_3$, THF, CH_2Cl_2; (e) H_2S, pyridine, THF, CH_2Cl_2; (f) $NH_2NH_2{\cdot}H_2O$, THF, CH_2Cl_2.

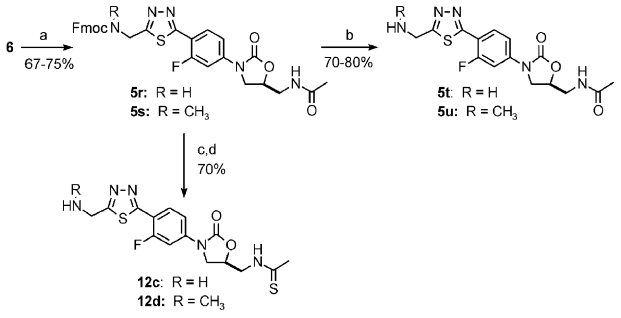


Scheme 2. (a) RCOCl, THF, reflux, 60–95%.

Oxazolidinone analogues bearing a C-5 thioacetamide group are known to have dramatically increased potency against both gram-positive and gram-negative organisms.[12,13] In order to evaluate this modification in 1,3,4-thiadiazolyl phenyl oxazolidinones, several examples were converted to a C-5 thioacetamide. Reaction of **5a** and **5q** with Lawesson's Reagent in refluxing dioxane afforded the thioamides **12a** and **12b**. Thioamides **12c** and **12d** were prepared by reaction of the Fmoc-protected acetamide oxazolidinones **5r** and **5s** with Lawesson's Reagent followed by deprotection with piperidine (Scheme 3).

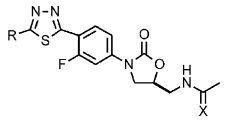
All of the analogues were tested in vitro against a panel of gram-positive and fastidious gram-negative bacteria.[14] Selected compounds were also evaluated for in vivo efficacy against *S. aureus* in a mouse bacteremia model.[15] The in vitro and in vivo activity of the 1,3,4-thiadiazole analogues **5a–u** and **12a–d** are shown in Table 1. All of these analogues exhibited good to excellent antibacterial activity, including good activity against fastidious gram-negative organisms. In many cases, the compounds had superior activity to linezolid. As seen in Table 1, the in vitro activity of the 1,3,4-thiadiazolyl phenyl oxazolidinones is relatively insensitive to the nature of the substituent at the 2-position. As expected, the thioacetamide analogues (**12a–d**) are extremely potent against both gram-positive and fastidious gram-negative organisms. Several of the 1,3,4-thiadiazole analogues had in vivo activity comparable to linezolid, but the thioamides lacked oral activity presumably due to metabolism of the thioamide. For example, in rat pharmacokinetic studies, **12c** was found to have a clearance of 34.3 mL/min/kg and its bioavailable fraction was only 14%.

Oxazolidinone analogues that contain a 1,3,4-thiadiazole C-ring represent a new class of oxazolidinone antibacterial agents having excellent activity against both gram-positive and fastidious gram-negative organisms. These compounds could be prepared in a straightforward manner from the key intermediate thiobenzhydrazide **6**. Reaction of **6** with a variety of acid chlorides leads to 1,3,4-thiadiazoles in good yield. 1,3,4-Thiadiazolyl phenyl oxazolidinones that incorporate a C-5 thioacetamide moiety are extraordinarily potent antibacterial agents. The 1,3,4-thiadiazolyl phenyl oxazolidinones are useful extensions of the SAR of the oxazolidinone class of antibacterial agents.



Scheme 3. (a) FmocNH(R)CH_2COCl, THF, reflux; (b) piperidine, rt, 30 min; (c) Lawesson's, dioxane, reflux; (d) piperidine, rt, 30 min.

Table 1. In vitro and in vivo activity of 1,3,4-thiadiazole oxazolidinone analogues against selected organisms



Compd	R	X	MIC (μg/mL)[a]							ED_{50} (mg/kg)
			SA[b]	SA[c]	SE[d]	SP[e]	EF[f]	HI[g]	MC[h]	SA[i]
5a[k]	H	O	1	1	0.25	0.25	1	4	4	2.2 (1.8)
5b[l]	OH	O	1	1	0.5	0.125	1	4	4	13 (3.8)
5c	CH_3	O	1	0.5	<0.125	<0.125	0.5	2	2	6.2 (3.5)
5d	CH_3CH_2	O	1	0.5	0.25	0.25	0.5	4	2	9.2 (3.8)
5e	FCH_2	O	1	1	0.25	0.25	1	4	2	8.6 (4.0)
5f	CH_3OCH_2	O	2	1	4	0.25	1	8	4	5.8 (2.2)
5g	$AcOCH_2$	O	1	1	0.25	<0.125	1	4	2	18.5 (3.7)
5h	EtO_2C	O	8	8	2	<0.125	8	>16	16	NT[j]
5i	$CH_3CO(CH_2)_2$	O	1	1	0.25	<0.125	0.5	4	4	7.6 (2.2)
5j	$NCCH_2$	O	2	1	0.25	0.25	1	4	4	>20 (2.9)
5k	CH_3SCH_2	O	1	1	0.25	0.25	1	>16	4	NT[j]
5l	$HOCH_2$	O	1	1	0.25	<0.125	1	4	2	8.7 (3.1)
5m	H_2NCO	O	0.5	0.5	0.25	0.25	1	16	4	12.4 (2.0)
5n	H_2NCOCH_2	O	4	4	0.5	<0.125	2	4	8	>20 (3.5)
5o	$HON=C(CH_3)(CH_2)_2$	O	2	2	0.5	<0.125	1	4	4	>20 (5)
5p	$CH_3S(O)CH_2$	O	8	8	0.5	0.25	4	8	8	NT[j]
5q	$CH_3SO_2CH_2$	O	4	4	1	<0.5	2	4	4	NT[j]
5t	H_2NCH_2	O	8	4	1	<0.125	1	2	2	21.3 (11.5)
5u	$MeHNCH_2$	O	4	4	1	<0.5	2	4	4	NT[j]
12a	H	S	0.25	<0.125	<0.125	<0.125	0.125	1	0.25	>20 (5.6)
12b	$CH_3SO_2CH_2$	S	0.25	0.5	0.25	0.06	0.25	2	0.5	>20 (5.0)
12c	H_2NCH_2	S	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	>20 (8)
12d	CH_3NHCH_2	S	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	>20 (4.3)
1	—		2–4	2–4	1–2	0.5–1	1–2	16	8–16	

[a]MIC, minimum inhibitory concentration.
[b]SA, *Staphylococcus aureus* UC®9213 (methicillin-susceptible *S. aureus*).
[c]SA, *S. aureus* UC®12673 (methicillin-resistant *S. aureus*, Ciprofloxicin® resistant, Rifampin® resistant, Imipenem® resistant).
[d]SE, *Staphylococcus epidermidis* UC®12084 (methicillin-resistant *S. epidermidis*).
[e]SP, *Streptococcus pneumoniae* UC®9912 (penicillin-sensitive *S. pneumoniae*).
[f]EF, *Enterococcus faecalis* UC®9217 (vancomycin-resistant *E. faecalis*).
[g]HI, *Haemophilus influenzae* 30063 (Ampicillin® resistant, β-lactamase +).
[h]MC, *Moraxella catarrhalis* 30607 (β-lactamase +).
[i]Against *S. aureus* UC®9213 in mice. Oral administration. Value in parentheses is the ED_{50} for linezolid, which is used as a control.
[j]Not tested.
[k]Prepared using formic acid as the solvent.
[l]Prepared using triphosgene in refluxing THF.

Preparation of (*S*)-4-[5-[(acetylamino)methyl]-2-oxo-3-oxazolidinyl]-2-fluorobenzenecarbothioic acid hydrazide (6)[16]. To a stirred solution of the aniline **7** (10.0g, 37.4 mmol) in 2 N HCl (44 mL), cooled to 0 °C, was added a solution of $NaNO_2$ (3.9 g, 56.1 mmol) in H_2O (24 mL). The reaction mixture was stirred at 0 °C for 90 min, then neutralized with solid $NaHCO_3$ (pH 7). In a separate flask, a mixture of copper cyanide (4.4 g, 48.6 mmol) and potassium cyanide (3.6 g, 56.1 mmol) was suspended in H_2O (36 mL) and ethyl acetate (73 mL). The resulting mixture was cooled to 0 °C and the neutralized diazonium salt was added via cannula over 20 min. The dark reaction mixture was stirred at 0 °C for 45 min and then filtered through a pad of Celite. The filter cake was washed with ethyl acetate (5×100 mL). The filtrate phases were separated and the aqueous phase was extracted with ethyl acetate (300 mL). The combined organic phases were dried ($MgSO_4$), filtered and concentrated to a small volume (75 mL). The orange solution was purified by filtration through a plug of silica gel using 5% CH_3OH in ethyl acetate as the eluent to afford 7.42 g (26.8 mmol, 72%) of the nitrile **8** as a yellow solid. Mp 164–167 °C. ^1H NMR (DMSO-d_6) δ 8.23 (m, 1H), 7.92 (t, $J=9$ Hz, 1H), 7.74 (dd, $J=14$, 2 Hz, 1H), 7.52 (dd, $J=9$, 2 Hz, 1H), 4.76 (m, 1H), 4.14 (t, $J=9$ Hz, 1H), 3.75 (dd, $J=9$, 6 Hz, 1H), 3.40 (t, $J=5$ Hz, 2H), 1.80 (2, 3H); ^{13}C NMR (DMSO-d_6) δ 170.4, 161.0 (d, $J=251$ Hz), 154.2, 145.0 (d, $J=11$ Hz), 134.8, 114.5 (d, $J=14$ Hz), 105.3 (d, $J=25$ Hz), 94.0 (d, $J=15$ Hz), 72.5, 47.6, 41.7, 22.8; IR (mull) 3361, 2417, 2363, 2232, 2180, 2114, 1757, 1745, 1664, 1625, 1513, 1442, 1410, 1219, 1207 cm^{-1}. $[\alpha]_D^{25}=-35.5°$ (*c* 1.06, DMSO) Anal. calcd for $C_{13}H_{12}FN_3O_3$: C, 56.32; H, 4.36; N, 15.16. Found: C, 56.16; H, 4.34; N, 14.83.

To a stirred solution of the nitrile **8** (8.84, 31.9 mmol) in DMF (35 mL) was added triethylamine (11.2 mL, 79.8 mmol). The reaction was heated to 100 °C and H_2S was bubbled into the flask for 1 h. The excess H_2S was swept out of the reaction mixture with a stream of N_2 over 30 min, while the mixture cooled to 60 °C. The reaction mixture was then poured onto ice (50 mL) and stirred

until the ice melted. The resulting yellow precipitate was filtered and dried in a vacuum oven (30 °C, 12 h) to give 7.9 g (25.4 mmol, 80%) of the thioamide **9**. Mp 116–119 °C. ^{1}H NMR (DMSO-d_6) δ 10.1 (s, 1H), 9.5 (s, 1H), 8.24 (t, J = 6 Hz, 1H), 7.74 (t, J = 9 Hz, 1H), 7.50 (dd, J = 13, 2 Hz, 1H), 7.31 (dd, J = 9, 2 Hz, 1H), 4.76 (m, 1H), 4.14 (t, J = 9 Hz, 1H), 3.76 (dd, J = 9, 7 Hz, 1H), 3.43 (t, J = 5 Hz, 2H), 1.83 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 195.8, 170.5, 157.3 (d, J = 246.8 Hz), 154.4, 141.7 (d, J = 7.9 Hz), 132.3, 124.8 (d, J = 13.6 Hz), 113.4, 105.2 (d, J = 28.4 Hz), 72.4, 47.7; 41.8, 22.9; IR (mull) 3344, 3153, 1755, 1734, 1669, 1644, 1616, 1550, 1506, 1409, 1395, 1342, 1219, 1206, 1197 cm^{-1}. HRMS (EI) calcd for $C_{13}H_{14}FN_3O_3S$ 311.0740, found 311.0744. $[\alpha]_D^{25} = -32$ (c 0.99, DMSO). Anal. calcd for $C_{13}H_{14}FN_3O_3S$: C, 50.15; H, 4.53; N, 13.50; S, 10.30. Found: C, 50.54; H, 4.70; N, 13.04; S, 9.60.

To a stirred suspension of the thioamide **9** (5.0 g, 16.1 mmol) in dry THF (80 mL) and CH_2Cl_2 (80 mL) was added methyl trifluoromethanesulfonate (2.0 mL, 17.7 mmol). The reaction was stirred at rt for 30 min, during which time the reaction mixture became homogeneous. Pyridine (3.9 mL, 48.3 mmol) was added and then H_2S gas was bubbled through the reaction mixture for 1 h during which time the reaction mixture turned red-orange in color. Excess H_2S was swept out of the reaction with a stream of nitrogen over 30 min. Hydrazine monohydrate (2.6 mL, 53.1 mmol) was added and the reaction mixture was stirred at room temperature for an additional h during which time the red-orange color disappeared. The reaction mixture was concentrated. The resulting residue was dissolved in THF/CH_3OH, adsorbed onto silica gel and purified by silica gel chromatography using 2.5–5% CH_3OH in CH_2Cl_2 as the eluent to afford 2.51 g (7.7 mmol, 48%) of **6** as a yellow solid. Mp 207–208 °C. ^{1}H NMR (DMSO-d_6) δ 12.1 (s, 1H), 8.23 (t, J = 6, Hz, 1H), 7.54 (t, J = 9, Hz, 1H), 7.45 (dd, J = 14, 2 Hz, 1H), 7.29 (dd, J = 9, 2, Hz, 1H), 6.2 (s, 1H), 4.73 (m, 1H), 4.11 (t, J = 9, Hz, 1H), 3.77 (dd, J = 9, 6, Hz, 1H), 3.40 (t, J = 5, Hz, 2H), 1.81 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 176.3, 170.5, 157.1 (d, J = 245 Hz), 154.4, 140.0 (d, J = 11 Hz), 131.9 (d, J = 4 Hz), 123.4 (d, J = 14 Hz), 113.5 (d, J = 3 Hz), 105.2 (d, J = 28 Hz), 72.3, 47.6, 41.8, 22.9; IR (mull) 3369, 3353, 3297, 1752, 1666, 1620, 1556, 1507, 1419, 1401, 1218, 1190, 1153, 1109, 865 cm^{-1}; $[\alpha]_D^{25} = -25°$ (c 1.05, DMSO). Anal. calcd for $C_{13}H_{15}FN_4O_3S$: C, 47.85; H, 4.63; N, 17.17; S, 9.82. Found: C, 48.03; H, 4.72; N, 16.86; S, 9.39.

General procedure for preparation of 1,3,4-thiadiazole oxazolidinone analogues from acid chlorides. To a stirred suspension of the thiobenzhydrazide **6** (1.0 mmol) in dry THF (10 mL) was added the acid chloride (1.2–2.0 mmol). The reaction mixture was heated at reflux for 1–6 h or until complete reaction was evident by TLC. The cooled reaction mixture was concentrated. The resulting residue was dissolved in CH_3OH/CH_2Cl_2, adsorbed onto silica gel and purified by silica gel chromatography using 2.5–5% CH_3OH in CH_2Cl_2 as the eluent to afford the desired thiadiazole.

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