Synthesis and in vitro Antimycobacterial Activity of Novel 3-(1H-Pyrrol-1-yl)-2-oxazolidinone Analogues of PNU-100480

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Synthesis and in vitro antimycobacterial activity of novel 3-(1*H*-pyrrol-1-yl)-2-oxazolidinone analogues of PNU-100480

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Abstract—Pursuing our search program for new antitubercular drugs we decided to explore the potentiality of oxazolidinone moiety by synthesizing novel 3-(1*H*-pyrrol-1-yl)-2-oxazolidinone analogues of PNU-100480. The new derivatives were tested against atypical mycobacteria as well as against drug resistant *Mycobacterium tuberculosis* and some of them exhibited a fairly good activity against *Mycobacterium avium* complex (MAC).

Tuberculosis (TB) is the leading infectious cause of death in the world today, with approximately three million patients deceasing every year. Nearly one third of the world's population is infected with *Mycobacterium tuberculosis* and the World Health Organization (WHO) estimates that about 30 million people will be infected within next 20 years.[1] Moreover, the resurgence of TB in industrialized countries and the worldwide increase in the prevalence of *Mycobacterium avium* complex (MAC) infections in immunocompromised hosts (often accompanied by other bacterial infections) as well as the appearance of multidrug-resistant (MDR) strains of *M. tuberculosis* have prompted the quest for new drugs acting both as antibacterial and antimycobacterial, without cross-resistance with known antituberculous agents.[2]

The oxazolidinones[3] are a new class of totally synthetic antibacterial agents, active against a variety of clinically important susceptible[4,5] and resistant Gram-positive organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecium* (VRE), and penicillin-resistant *Streptococcus pneumoniae* (PRSP).[6] These compounds have been shown to inhibit translation at the initiation phase of protein synthesis[7] in bacteria by selectively and uniquely binding to the central loop of domain of 23S rRNA of 50S ribosomal subunit, interfering with initiator fMet-tRNA binding to the P-site of the ribosomal peptidyltransferase center.[8] The oxazolidinones were originally discovered by researchers at DuPont in the late 1980s but development of **DuP-721** (Fig. 1), the drug candidate emerged from early studies, was discontinued following Phase I clinical trials.[3] Subsequently, researchers at Pharmacia Corporation identified two clinical candidates, **eperezolid** and **linezolid** (Fig. 1), the latter currently being marketed for the treatment of multidrug resistant Gram-positive infections. The thiomorpholine analogue of linezolid, **PNU-100480** showed an interesting antimycobacterial activity.[3,9]

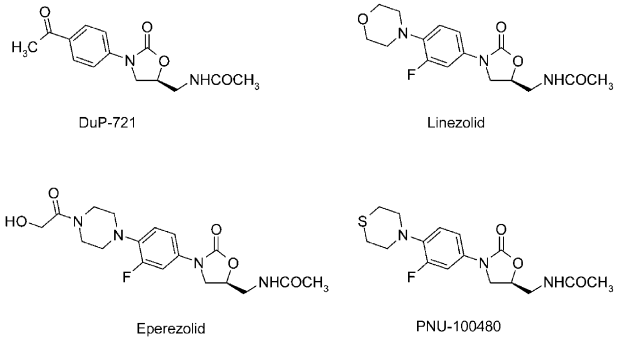

Figure 1.

Keywords: Oxazolidinones; Tuberculosis; MAC; Antitubercular drugs.
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During our antituberculosis studies we previously investigated the activity of different classes of derivatives against *M. tuberculosis* and atypical mycobacteria.[10] Pursuing this research program and taking account the fairly good antimycobacterial activity of PNU-100480, we decided to further explore the antitubercular efficacy of oxazolidinone moiety.

Starting from 1995, many groups have reported the synthesis and the biological activity of novel oxazolidinones,[3,11] but chemical modifications concerned the substituent(s) placed on the C5 methylene group of the 2-oxazolidinone ring (A ring)[12] or in the various positions of the phenyl ring (B ring). In particular the introduction of azole moieties resulted in compounds endowed with high antibacterial (both Gram-positive and Gram-negative) potency as well as favourable pharmacokinetic profiles.[13] Recently[14] researchers at Bayer A.G. turned their attention to bioisosteric replacement of the phenyl ring, but no article describing a substitution with a pyrrole ring has been reported thus far. As pyrrole moiety is a pharmacophore contained in many antimicrobial drugs (i.e., pyrrolnitrin) often able to improve the bioavailability of the compounds, we herein describe the synthesis and the antimycobacterial activity of 3-(1*H*-pyrrol-3-yl)-2-oxazolidinones **1a–g** (Fig. 2).

The multi-step synthesis of derivatives **1a–g**[15] is outlined in Scheme 1.

In particular, 3-(1-phenyl-1*H*-pyrrol-3-yl)-2-oxazolidinones **1a–f** were obtained (Scheme 1) starting from properly substituted anilino derivatives (commercially available or synthesized by us following the procedure illustrated in reference 9) which were condensed with 2,5-dimethoxytetrahydrofuran-3-carboxyaldehyde in the presence of acetic acid to the corresponding 1-phenyl-1*H*-pyrrole-3-carboxyaldehydes **2a–f**. These derivatives were then oxidized with silver oxide to obtain the carboxylic acids **3a–f** which, by reaction with diphenylphosphoryl azide in the presence of triethylamine, were converted to acyl azide intermediates, further undergoing a modified Curtius reaction (in the presence of benzyl alcohol) to give benzylcarbamates **4a–f** directly. The oxazolidinone ring was formed by a stereospecific reaction of Cbz derivatives **4a–f** with (*R*)-glycidyl butyrate in the presence of *n*-butyllithium to afford the alcohols **5a–f**.

Functional group manipulation of alcohols **5a–f** yielded the desired acetamide derivatives **1a–f** in several steps in good overall yield.

3-[1-(4-Morpholino)-1*H*-pyrrol-3-yl)-2-oxazolidinone **1g** was obtained starting from commercially available 4-aminomorpholine following an identical procedure.

The pyrryloxazolidinones **1a–g** were evaluated in vitro against atypical mycobacterial strains (*Mycobacterium fortuitum, Mycobacterium smegmatis* and *M. avium* complex (MAC)), *M. tuberculosis* strains (ATCC 27294 and clinical isolate 1104) and also against a panel of drug-resistant strains of *M. tuberculosis* (ATCC 35820, ATCC 35828, and ATCC 35837). PNU-100480 and isoniazid were used as reference drugs.

Results of the in vitro evaluation of antimycobacterial activity of the tested compounds are reported in Tables 1 and 2.

When tested against mycobacteria all derivatives **1a–g** resulted active but less potent than reference compounds. The introduction of a fluorine atom on the phenyl ring (compounds **1b–d**) enhanced antimycobacterial activity in the order *para* > *ortho* > *meta*, whereas the introduction of a morpholine or a thiomorpholine group resulted in derivatives **1e** and **f** (respectively) which exhibited an activity comparable to unsubstituted phenyl derivative **1a**. On the contrary, when the morpholine group was directly connected with the pyrrole ring the resulting derivative **1g** was completely inactive. It was noteworthy that *o*-fluoro and, above all, *p*-fluorophenyl derivatives **1b** and **d** exhibited an interesting activity against MAC, comparable to that of PNU-100480 ($MIC_{50} = 2.0$ μM and $MIC_{50} = 1.4$ μM,

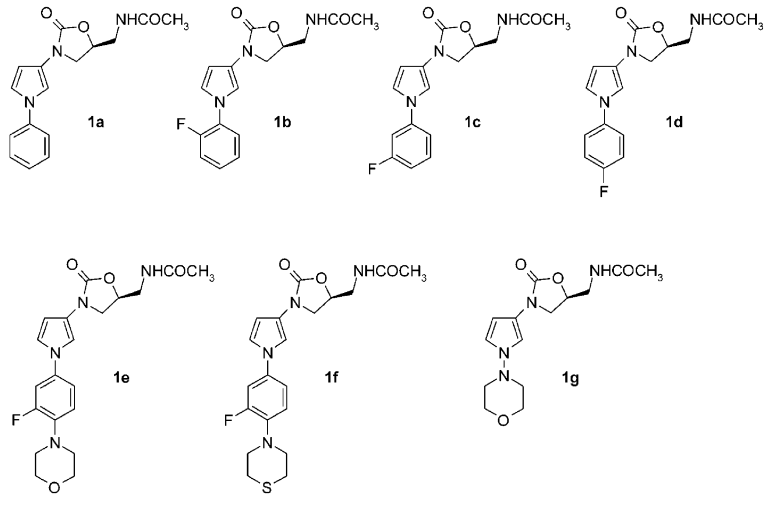
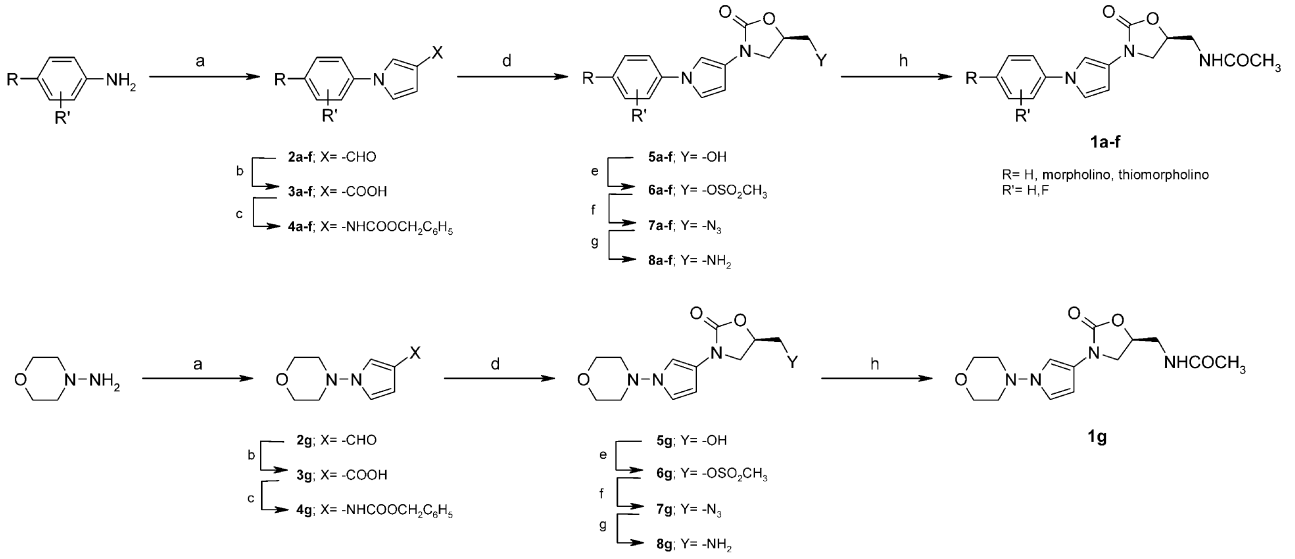


Figure 2.



Scheme 1. (a) 1.2 equiv 2,5-dimethoxytetrahydrofuran-3-carboxyaldehyde, AcOH, reflux, 15 min; (b) 1.6 equiv $AgNO_3$, 6 N $NaOH_{aq}$, MeOH, reflux, 5 h; (c) 1.1 equiv diphenylphosphoryl azide, 1.1 equiv triethylamine, 1.2 equiv benzyl alcohol, benzene, reflux, 7 h; (d) 1.1 equiv 2.5 M *n*-BuLi, THF, −78 °C, 30 min; 1.1 equiv (*R*)-glycidyl butyrate, overnight; (e) 1.2 equiv CH_3SO_2Cl, 1.5 equiv triethylamine, CH_2Cl_2, 0 °C, 20 min; (f) 5 equiv NaN_3, DMF, 65 °C, 16 h; (g) cat. Pd/C, H_2 55 psi, MeOH, rt, 2 h; (h) 1.2 equiv CH_3COCl, 1.5 equiv triethylamine, CH_2Cl_2, 0 °C, 10 min.

Table 1. In vitro antimycobacterial activity of compounds **1**

Compd	CC_{50}[a]	MIC_{50}[b]/MIC_{90}[c]				
	MT-4	*M. tuberc.* ATCC 27294	*M. tuberc.* C.I. 1104	MAC	*M. smegmatis*	*M. fortuitum*
1a	>100	19/74	10.5/77.8	4.0/32.8	9.8/41.3	>100
1b	33.3	3.6/24.7	ND	2.0/8.9	4.2/15.7	88.6/>100
1c	>100	12.9/91.4	9.4/76.9	4.6/39.8	14.5/56.3	>100
1d	100	1.9/19.7	ND	1.4/5.8	10.3/45.8	>100
1e	>100	12.9/≈100	49/>100	16.5/≈100	42.6/>100	>100
1f	20	9.8/25	12.2/31	5.8/11.8	34/>100	>100
1g	>100	>100	>100	>100	>100	>100
PNU-100480	>100	0.1/0.9	0.5/2.5	0.7/5.0	0.5/1.6	>100
Isoniazid	>100	0.09/9.8	0.06/0.2	1.4/4.7	1.8/6.7	>100

[a] Compound concentration (μM) required to reduce the viability of mock-infected MT-4 cells by 50%, as determined by the MTT method.
[b] Minimum inhibitory concentration (μM) required to reduce the number of viable Mycobacteria by 50%, as determined by the MTT method.
[c] Minimum inhibitory concentration (μM) required to reduce the number of viable Mycobacteria by 90%, as determined by the MTT method.

respectively). The need for drugs effective against MDR strains prompted us to test derivatives **1a–g** against *M. tuberculosis* strains resistant to streptomycin (SM^R, ATTC 35820), pirazynamide (PZA^R, ATCC 35828) and ethambutol (EB^R, ATCC 35837). In general, while all derivatives resulted less effective than PNU-100480, fluoro-substituted derivatives **1b–d** retained their antimycobacterial efficacy against resistant strains (*ortho* ≥*meta* >*para*), thus confirming the importance of this substituent for biological activity. Again, the introduction of morpholine or thiomorpholine group on phenyl ring (derivatives **1e** and **f**, respectively) or even directly on the pyrrole ring (derivative **1g**) did not improve antimycobacterial efficacy of compounds.

In conclusion, a series of 3-(1*H*-pyrrol-1-yl)-2-oxazolidinone analogues of PNU-100480 were synthesized and tested against both wild-type and drug-resistant strains. SAR studies confirmed the importance of the fluorine pharmacophore on the phenyl ring, having fluoro-substituted derivatives good antimycobacterial efficacy against

Table 2. In vitro antimycobacterial activity of compounds **1** against drug-resistant strains of *M. tuberculosis*

Compd	CC_{50}[a]	MIC_{50}[b]/MIC_{90}[c]		
	MT-4	*M. tuberc.* ATCC 35820 (SM^R)	*M. tuberc.* ATCC 35828 (PZA^R)	*M. tuberc.* ATCC 35837 (EB^R)
1a	>100	5.6/≥100	50.3/>100	23.8/100
1b	33.3	1.1/45	10/58.7	7.0/23.9
1c	>100	0.9/>100	18.4/63.9	7.0/24.9
1d	100	0.8/39.9	21.5/73.7	13.5/56.6
1e	>100	4.9/>100	>100	63.5/>100
1f	20	ND	ND	ND
1g	>100	>100	>100	>100
PNU-100480	>100	5.6×10^{-3}/0.4	0.1/0.7	0.06/0.3
Isoniazid	>100	1.3/>100	0.1/>100	0.2/13

[a] Compound concentration (μM) required to reduce the viability of mock-infected MT-4 cells by 50%, as determined by the MTT method.
[b] Minimum inhibitory concentration (μM) required to reduce the number of viable Mycobacteria by 50%, as determined by the MTT method.
[c] Minimum inhibitory concentration (μM) required to reduce the number of viable Mycobacteria by 90%, as determined by the MTT method.

both wild-type and MDR mutant strains as well as against MAC. Surprisingly *para*-substitution proved more effective than *meta*-substitution. Moreover the introduction of the pyrrole moiety as a spacer between oxazolidinone pharmacophore and phenyl ring or as a replacement of the latter group resulted in a diminished antimycobacterial activity. Both results suggest that the alteration of the geometry of PNU100480 lead to less favorable interactions of synthesized compounds with the mycobacterial target.

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15. **1a**: MS (EI, 70 ev) *m/z*: 299; IR (cm^{-1}, KBr) 1714 ($CH_3C{=}O$), 1722 (C=O), 3282 (NH); ^1H NMR (200 MHz, $CDCl_3$) δ 1.986 (3H, s); 3.627 (3H, m, overlapped signals); 3.956 (1H, t, $J = 9.0$ Hz); 4.679 (1H, m); 6.098 (1H, m, exchang. with D_2O); 6.302 (1H, dd, $J = 3.3$, 1.8 Hz); 6.950 (1H, dd, $J = 3.3$, 2.6 Hz); 7.218 (2H, m, overlapped signals); 7.346 (4H, m, overlapped signals). Anal. calcd for $C_{16}H_{17}N_3O_3$: C, 64.20, H, 5.72, N, 14.04; Found C, 64.34, H, 5.74, N, 13.91.
1b: MS (EI, 70 ev) *m/z*: 317.12; IR (cm^{-1}, KBr) 1716 (CH_3 C=O), 1724 (C=O), 3279 (NH); ^1H NMR (200 MHz, $CDCl_3$) δ 2.006 (3H, s); 3.661 (3H, m, overlapped signals); 3.968 (1H, t, $J = 9.3$ Hz); 4.786 (1H, m); 6.387 (1H, m); 6.537 (1H, m, exchang. with D_2O); 6.920 (1H, m); 7.250 (5H, m, overlapped signals). Anal. calcd for $C_{16}H_{16}FN_3O_3$: C, 60.56, H, 5.08, F, 5.99, N, 13.24; Found C, 60.64, H, 5.13, F, 5.75, N, 13.04.
1c: MS (EI, 70 ev) *m/z*: 317.12; IR (cm^{-1}, KBr) 1744 ($CH_3C{=}O$, C=O), 3294 (NH); ^1H NMR (200 MHz, $CDCl_3$) δ 1.988 (3H, s); 3.643 (3H, m, overlapped signals); 3.946 (1H, t, $J = 8.9$ Hz); 4.753 (1H, m); 6.343 (1H, m); 7.118 (7H, m, overlapped signals). Anal. calcd for $C_{16}H_{16}FN_3O_3$: C, 60.56, H, 5.08, F, 5.99, N, 13.24; Found C, 60.68, H, 5.13, F, 5.88, N, 13.07.
1d: MS (EI, 70 ev) *m/z*: 317.12; IR (cm^{-1}, KBr) 1696 ($CH_3C{=}O$), 1714 (C=O), 3303 (NH); ^1H NMR (200 MHz, $CDCl_3$) δ 2.009 (3H, s); 3.625 (3H, m, overlapped signals); 3.962 (1H, t, $J = 9.0$ Hz); 4.787 (1H, m); 6.319 (1H, dd, $J = 2.9$, 1.6 Hz); 6.563 (1H, m, exchang. with D_2O); 6.880 (1H, m); 7.075 (3H, m, overlapped signals); 7.271 (2H, m, overlapped signals). Anal. calcd for $C_{16}H_{16}FN_3O_3$: C, 60.56, H, 5.08, F, 5.99, N, 13.24; Found C, 60.38, H, 5.01, F, 6.15, N, 13.45.
1e: MS (EI, 70 ev) *m/z*: 402.17; IR (cm^{-1}, KBr) 1725 ($CH_3C{=}O$, C=O), 3290 (NH); ^1H NMR (200 MHz, $CDCl_3$) δ 1.981 (3H, s); 3.040 (4H, m); 3.629 (3H, m, overlapped signals); 3.857 (5H, m, overlapped signals); 4.748 (1H, m); 6.283 (2H, m); 7.013 (5H, m, overlapped signals). Anal. calcd for $C_{20}H_{23}FN_4O_4$: C, 59.69, H, 5.76, F, 4.72, N, 13.92; Found C, 59.78, H, 5.84, F, 4.50, N, 13.78.
1f: MS (EI, 70 ev) *m/z*: 418.15; IR (cm^{-1}, KBr) 1735 ($CH_3C{=}O$, C=O), 3280 (NH); ^1H NMR (200 MHz,

$CDCl_3$) δ 1.986 (3H, s); 2.774 (4H, m); 3.279 (4H, m); 3.624 (3H, m, overlapped signals); 3.938 (1H, t, $J = 8.7$ Hz); 4.782 (1H, m); 6.288 (2H, m); 6.995 (5H, m, overlapped signals). Anal. calcd for $C_{20}H_{23}FN_4O_3S$: C, 57.40, H, 5.54, F, 4.54, N, 13.39, S, 7.66; Found C, 57.53, H, 5.57, F, 4.43, N, 13.12, S, 7.73.

1g: MS (EI, 70 ev) *m*/*z*: 308.15; IR (cm^{-1}, KBr) 1730 ($CH_3C{=}O$, C=O), 3290 (NH); ^1H NMR (200 MHz, DMSO-d_6) δ 1.779 (3H, s); 2.954 (4H, m); 3.283 (2H, m, overlapped signals); 3.385 (1H, m); 3.666 (4H, m); 3.837 (1H, t, $J = 9.6$ Hz) 4.596 (1H, m); 6.005 (1H, dd, $J = 3.3$, 1.8 Hz); 6.879 (1H, dd, $J = 3.3$, 2.6 Hz); 6.971 (1H, dd, $J = 2.6$, 1.8 Hz); 8.182 (1H, m, exchang. with D_2O). Anal. calcd for $C_{14}H_{20}N_4O_4$: C, 54.54, H, 6.54, N, 18.17; Found C, 54.28, H, 6.48, N, 18.35.