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PYRROLNITRIN AND RELATED PYRROLES ENDOWED WITH ANTIBACTERIAL ACTIVITIES AGAINST *MYCOBACTERIUM TUBERCULOSIS*

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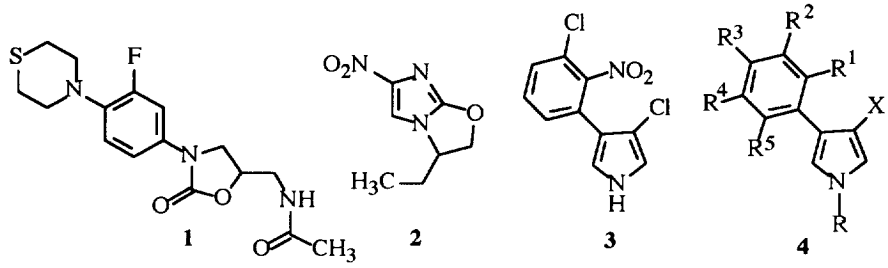
Abstract. During development of nitroheterocycles with potential antimycobacterial activities we have tested against *Mycobacterium tuberculosis* a number of pyrroles strictly related to pyrrolnitrin, an antifungal antibiotic isolated from *Streptomyces pyrrocinia*. Some of the tested arylpyrrole derivatives and pyrrolnitrin have shown appreciable inhibiting activity against *M. tuberculosis* and *M. avium*. SAR studies well correlate antimycobacterial potency with the presence of halogens in the phenyl ring and a nitro group at position 3 of pyrrole.

The embitterment of *Mycobacterium tuberculosis* in recent years all over the world, after several decades in which the tuberculosis was deemed to be wiped out, has led to an urgent need for novel improved therapeutics to hinder this insidious disease. In fact, the World Health Organization (WHO) has recently estimated that within ten years about 30 million people world wide will die from tuberculosis.[1,2]



R = H, Me; R^1, R^2, R^3, R^4, R^5= H, Cl, F, OMe

X = NO_2, COOEt, CH_2OH, COOH, $CONH_2$, $CONHNH_2$

Also alarming has been the rise of multi-drug resistant (MDR) tuberculosis. In fact, mycobacteria are known to easily acquire resistance to streptomycin (SM), isoniazid (INH), ethambutol (EB) and rifampicin (RFP), the most effective agents currently used in tuberculosis chemotherapy.[3,4] Moreover, these conventional antitubercular agents are also known to exhibit only restricted activity against atypical mycobacteria. For example, clinical management of human acquired immune deficiency syndrome (AIDS) is greatly hindered by the development of opportunistic infections, particularly those involving the *Mycobacterium avium*

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complex and *Mycobacterium tuberculosis*. In particular, the *M. avium* complex is very difficult to treat because of its diversified drug resistance patterns to the majority of the antimycobacterial agents employed in the clinics.[5]

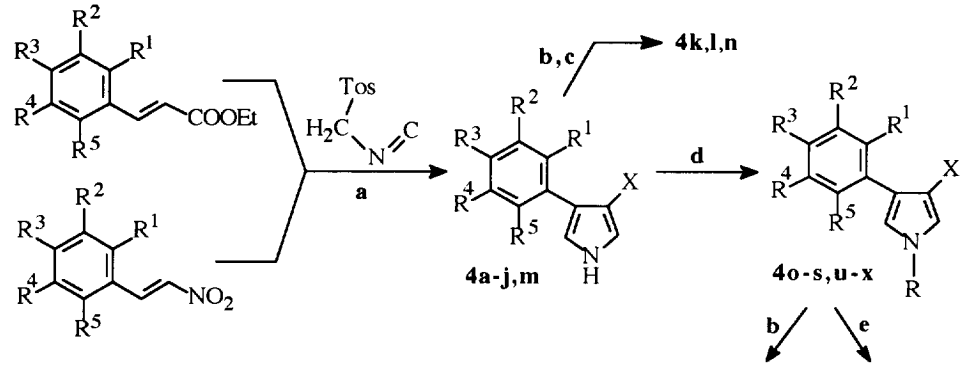
Despite the urgent need for new chemotherapeutic agents created by the recent outbreak of drug-resistant strains of tuberculosis, only a few number of substances with good antimycobacterial activities have been discovered in the recent years. These include the Upjohn oxazolidinone **1** (U-100480)[6] and the bicyclic nitroimidazole **2** (CGT 17341),[7] which have been described as novel promising tuberculostatic agents.

In a previous work[8] we stated how it would be worthwhile to design and develop novel chemotherapeutic agents that would be active against both mycobacteria and fungi and we reported the synthesis of *N*-substituted 1-aryl-2-(1*H*-imidazol-1-yl)-1-ethanamines related to miconazole which were endowed with broad spectrum *in vitro* antimycobacterial and antifungal activities. Pursuing such a goal, we decided to explore whether pyrrolnitrin (**3**), a natural antibiotic used in topical antifungal diseases,[9] and some related arylpyrroles containing a nitro group (**4**) would exhibit antimycobacterial activity against a range of mycobacteria, including pathogenic *M. tuberculosis* and *M. avium*. To our knowledge nitropyrroles, such as compounds **4**, have not particularly been studied up to now as antimycobacterial agents.

Chemistry

Pyrrolnitrin was prepared according to the synthetic procedure described by Nakano.[9] Preparation of 4-aryl-3-nitropyrroles **4a-i** (X = NO_2) (Scheme 1) was reported in a previous work.[10] Methylation of **4a,c,d,f** pyrroles was performed by reaction with methyl iodide in alkaline medium. 4-Aryl-3-carbethoxypyrroles (X = COOEt) **4j**[11] and **4m**[12] were synthesized by (3,2)cycloaddition of tosylmethylisocyanide (TosMIC)[13] to the appropriate ethyl cinnamates or nitrovinyl compounds and then were subjected to N-alkylation to afford compounds **4s,u-x**.

SCHEME 1

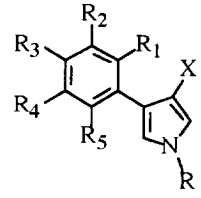


X = NO_2, COOEt, CH_2OH, $CONH_2$, $CONHNH_2$
R = H, Me, $PhCH_2$, 4-F-$PhCH_2$, 2-Cl-$PhCH_2$, 4-Cl-$PhCH_2$
R^1, R^2, R^3, R^4, R^5 = H, Cl, F, OMe

Legend: a: NaH; **b**: NaOH; **c**: $(imidazolyl)_2CO$, NH_3 or NH_2NH_2; **d**: R-Hal, K_2CO_3; **e**: $LiAlH_4$.

Hydrolysis of **4s** gave **4t**. Esters **4j** and **4m** were hydrolyzed to the corresponding acids, which were reacted with 1,1'-carbonylimidazole and then treated with ammonium hydroxide or hydrazine hydrate to give pyrroles **4k**, **4n** and **4l**, respectively. Carbinol derivative **4y** was obtained by lithium aluminum hydride reduction of the corresponding ester.[11] Chemical and physical data of the newly synthesized derivatives are reported in Table 1. ^{1}H-NMR spectra ($CDCl_3$, TMS as internal standard, Brucker AC 200 spectrometer) of compounds **4k,l,n,y** were in agreement with the proposed structures.

Table 1. Structures of Derivatives **4a-j,m,o-x,z** and Chemical and Physical Data of Compounds **4k,4l,4n,4y**



compd	R	R_1	R_2	R_3	R_4	R_5	X	m.p. (°C)	recrytst.[a] solvent	yield (%)	ref.
4a	H	Cl	H	H	H	H	NO_2	-	-	-	10
4b	H	H	Cl	H	H	H	NO_2	-	-	-	10
4c	H	H	H	Cl	H	H	NO_2	-	-	-	10
4d	H	Cl	H	OCH_3	H	H	NO_2	-	-	-	10
4e	H	Cl	Cl	H	H	H	NO_2	-	-	-	10
4f	H	Cl	H	Cl	H	H	NO_2	-	-	-	10
4g	H	Cl	H	H	H	Cl	NO_2	-	-	-	10
4h	H	H	Cl	Cl	H	H	NO_2	-	-	-	10
4i	H	H	Cl	H	Cl	H	NO_2	-	-	-	10
4j	H	H	H	Cl	H	H	$COOC_2H_5$	-	-	-	11
4k	H	H	H	Cl	H	H	$CONH_2$	194-196	a	100	-
4l	H	H	H	Cl	H	H	$CONHNH_2$	240-242	b	73	-
4m	H	Cl	H	Cl	H	H	$COOC_2H_5$	-	-	-	12
4n	H	Cl	H	Cl	H	H	$CONH_2$	232-234	c	65	-
4o	CH_3	Cl	H	H	H	H	NO_2	-	-	-	10
4p	CH_3	H	H	Cl	H	H	NO_2	-	-	-	10
4q	CH_3	Cl	H	OCH_3	H	H	NO_2	-	-	-	10
4r	CH_3	Cl	H	Cl	H	H	NO_2	-	-	-	10
4s	CH_3	H	H	Cl	H	H	$COOC_2H_5$	-	-	-	11
4t	CH_3	H	H	Cl	H	H	COOH	-	-	-	11
4u	CH_3	H	H	OCH_3	H	H	$COOC_2H_5$	-	-	-	11
4v	$PhCH_2$	H	H	OCH_3	H	H	$COOC_2H_5$	-	-	-	11
4w	4-F-$PhCH_2$	H	H	OCH_3	H	H	$COOC_2H_5$	-	-	-	11
4x	4-F-$PhCH_2$	H	H	H	H	H	$COOC_2H_5$	-	-	-	11
4y	2-Cl-$PhCH_2$	H	H	Cl	H	H	CH_2OH	122-123	d	61	-
4z	4-Cl-$PhCH_2$	H	H	Cl	H	H	COOH	-	-	-	11

[a]Recrystallization solvents. a: ethanol/toluene. b: ethyl acetate. c: ethanol. d: benzene.

Antimycobacterial Activity and SAR Studies

Pyrrole derivatives **4** were submitted for the evaluation of their *in vitro* activities against a variety of *Mycobacterium* strains using previously reported procedures.[14,15] The mycobacteria used were *M. tuberculosis*

CIP 103471, *M. avium* CIP 103317, *M. smegmatis* CIP 103599, *M. gordonae* CIP 6427 and *M. marinum* CIP 6423. The cytotoxicity of the test derivatives was also tested on VERO cells after dissolution in DMSO at the initial concentration of 10 mg/mL. Data on the cytotoxicity and antimycobacterial activities of derivatives **4** are reported in Tables 2 and 3. As a result, the majority of compounds were cytotoxic for VERO cells at concentrations near to the active doses. In the *in vitro* assays streptomycin and isoniazid were used as reference drugs.

Pyrrolnitrin showed inhibiting activity against *M. tuberculosis* and *M. avium*, although at MIC doses higher than those of streptomycin and isoniazid. Only three derivatives (**4f, 4h** and **4i**) of nitropyrrole series showed appreciable activity against *M. tuberculosis*. In particular, compound **4f** was two and four times less potent than SM and INH, respectively. This compound was also active against *M. avium* with MIC near to those of controls. Methylation of **4f** at position 1 of pyrrole ring abated antimycobacteral activity. Similarly, removal of one of chlorine atoms from benzene ring of **4f** led to monochloro derivatives **4a** and **4c** deprived of activity. From data of Table 2 it is evident that none of test 4-(monochlorophenyl)-3-nitropyrroles and their 1-methyl derivatives are active against *Mycobacterium* species used in the *in vitro* assays. Among the 4-(dichloro phenyl) analogues the activity is retained by 2,3-, 2,4-, 3,4- and 3,5-dichloro derivatives with the highest potency for 2,4-disubstitution, whereas the 2,6-analog is totally inactive.

Table 2. Cytotoxicity and Antimycobacterial Activities of Pyrrolnitrin and Nitroarylpyrroles **4a-i,o-r**

Compd	Cytotoxicity[a] MTD_{50} (μg/mL)	Antimycobacterial Activities, MIC (μg/mL)[a]				
		Mycobacterium				
	VERO Cells	*tuberculosis* CIP 103471	*avium* CIP 103317	*smegmatis* CIP 103599	*gordonae* CIP 6427	*marinum* CIP 6423
3	8	4	8	16	>16	>16
4a	nd[b]	>16	>16	>16	>16	>16
4b	nd	>16	>16	>16	>16	>16
4c	nd	>16	>16	>16	>16	>16
4d	2	>16	16	>16	>16	>16
4e	1	16	>16	>16	>16	>16
4f	1	1	16	>16	>16	>16
4g	nd	>16	>16	>16	>16	>16
4h	2	8	16	>16	>16	>16
4i	1	8	16	>16	>16	>16
4o	nd	>16	>16	>16	>16	>16
4p	nd	>16	>16	>16	>16	>16
4q	2	>16	16	>16	>16	>16
4r	nd	>16	>16	>16	>16	>16
Streptomycin	128	0.50	8	8	16	32
Isoniazid	32	0.25	32	64	32	16

a: The assays were done in triplicate. The degree of variation was found always within 50%.
b: nd = not determined.

Adverse results were generally obtained when the nitro group bound at the position 3 of pyrrole ring was replaced by a carbethoxy group. Differently from **4c**, however, the 3-carbethoxy analog **4j** showed appreciable inhibiting activity against either *M. tuberculosis* or *M. avium*. In the last case the activity was two and eight times lower than that of SM and INH, respectively. Unfortunately, these good MIC values were negatively counterbalanced by the higher cytotoxicity showed by **4j** in comparison with the controls.

Transformation of carbethoxy group into carbinol gave **4y** endowed with some activity (16 times less potent than SM) against *M. tuberculosis*. Methylation at N-1 (compound **4s**) and hydrolysis of ester group to the related carboxylic acid (compound **4t**) gave inactive products. Also derivatization of COOEt to the related amide **4k** or hydrazide **4l** produced compounds deprived of activity.

Differently from dichlorophenylnitropyrroles none of the tested dichlorophenylpyrrole derivatives (**4m** and **4n**) displayed any activity against mycobacteria. Replacement of 4-chloro substituent with a methoxy and introduction of a methyl or an arylmethyl moiety led to derivatives with some activity against *M. tuberculosis* and *M. avium* (compounds **4u**, **4v**, **4w**, **4x** and **4z**).

Table 3. Cytotoxicity and Antimycobacterial Activities of Carbethoxyarylpyrroles **4j,m,s,u-x**, Related Derivatives **4k,l,n,t,z** and Carbinol **4y**

Compd	Cytotoxicity[a] MTD_{50} (μg/mL)	Antimycobacterial Activities, MIC (μg/mL)[a]				
		Mycobacterium				
	VERO Cells	*tuberculosis* CIP 103471	*avium* CIP 103317	*smegmatis* CIP 103599	*gordonae* CIP 6427	*marinum* CIP 6423
4j	4	16	4	>16	>16	>16
4k	16	>16	>16	>16	>16	>16
4l	16	>16	>16	>16	>16	>16
4m	2	>16	>16	>16	>16	>16
4n	0.125	>16	16	>16	>16	>16
4s	nd[b]	>16	>16	>16	>16	>16
4t	nd	>16	16	>16	>16	>16
4u	4	>16	>16	>16	>16	>16
4v	8	8	>16	>16	>16	>16
4w	2	16	>16	>16	>16	>16
4x	4	>16	16	>16	>16	>16
4y	15	8	>16	>16	>16	>16
4z	8	16	16	>1	>16	>16
Streptomycin	128	0.50	8	8	16	32
Isoniazid	32	0.25	32	64	32	16

a: The assays were done in triplicate. The degree of variation was found always within 50%.
b: nd = not determined.

Conclusion

Although nitroheterocycles have been largely studied during search on chemotherapeutic agents with antifungal, antiprotozoal and antibacterial activities, only few nitropyrroles have been reported to have antimicrobial activities. These include pyrrolnitrin and pyrrolomycins A and B. However, to our knowledge no previous mention has been made up to now of nitropyrroles with antimycobacterial activities. We now for the first time describe the *in vitro* antimycobacterial activities of nitropyrroles, including pyrrolnitrin and some related pyrrole derivatives. Although they are not very potent against *M. tuberculosis*, some of the here reported pyrroles were also moderately active against *M. avium*, an opportunistic mycobacterium resistant to currently used antitubercular drugs. This interesting result warrants further investigations of nitropyrroles, a novel promising class of simple antimycobacterial agents, and other related pyrrole derivatives.

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References and Notes

1. Duncan, K. *Exp. Opin. Ther. Patents* **1997**, *7*, 129-137.
2. *WHO Report on the Tuberculosis Epidemic*, 1995, World Health Organization.
3. Daniel, T.M. *J. Lab. Med.* **1990**, *116*, 277-282.
4. Dooley, S.W.; Jarvis, W.R.; Martone, W.J.; Snider, D.E. *Ann. Internal Med.* **1992**, *117*, 257-259.
5. Collins, F.M. *Clin. Microbiol. Rev.* **1989**, *2*, 360-377.
6. (a) Niccolai, D.; Tarsi, L.; Thomas, R.J. *Chem. Commun.* **1997**, 2333-2342. (b) Ashtekar, D.R.; Costa-Pereira, R.; Shrinivasan, T.; Ayyer, R.; Vishvanathan, N.; Rittel, W. *Diagn. Microbiol. Infect. Dis.* **1991**, *14*, 465-471. (c) Barbachyn, M.R.; Hutchinson, D.K.; Brickner, S.J.; Cynamon, M.H.; Kilburn, J.O.; Klemens, S.P.; Glickman, S.E.; Grega, K.C.; Hendges, S.K.; Toops, D.S.; Ford, C.W.; Zurenko, G.E. *J. Med. Chem.* **1996**, *39*, 680-685.
7. (a) Nagarajan, K.; Shankar, R.G.; Rajappa, S.; Shenoy, S.; Costa-Pereira, R. *Eur. J. Med. Chem.* **1989**, *24*, 631-633. (b) Ashtekar, D.R.; Costa-Pereira, R.; Nagarajan, K.; Vishvanathan, N.; Bhatt, A.D.; Rittel, W. *Antimicrob. Agents Chemother.* **1993**, *37*, 183-186.
8. Fioravanti, R.; Biava, M.; Porretta, G.C.; Artico, M.; Lampis, G.; Deidda, D.; Pompei, R. *Med. Chem. Res.* **1997**, *7*, 87-97.
9. (a) Arima, K.; Imanaka, H.; Kousaka, M.; Fukuda, A.; Tamura, G. *J. Antibiotics* **1965**, Ser. A *18*, 201-204. (b) Nakano, H.; Umio, S.; Kariyone, K.; Tanaka, K.; Kishimoto, T.; Noguchi, H.; Ueda, I.; Nakamura, H.; Morimoto, Y. *Tetrahedron Lett.* **1966**, 737-740.
10. Massa, S.; Di Santo, R.; Costi, R.; Mai, A.; Artico, Marino; Retico, A.; Apuzzo, G.; Artico, Marco; Simonetti, G. *Med. Chem. Res.* **1993**, *3*, 192-199.
11. Massa, S.; Di Santo, R.; Mai, A.; Botta, M.; Artico, M.; Panico, S.; Simonetti, G. *Farmaco* **1990**, *45*, 833-846.
12. Di Santo, R.; Costi, R.; Artico, M.; Massa, S.; Musiu, C.; Milia, C.; Putzolu, M.; La Colla, P. *Med. Chem. Res.* **1997**, *7*, 98-108.
13. (a) Di Santo, R.; Massa, S.; Artico, M. *Farmaco* **1993**, *48*, 209-229. (b) van Leusen, A.M. *Lectures in Heterocyclic Chemistry* **1980**, *5*, S-111-S-122.
14. *National Comittee for Clinical Laboratory Standard*, Antimycobacterial susceptibility testing. Proposed standard M24-P National Comittee For Clinical Laboratory Standard, 1995, Villanova, PA, .
15. Clark, B.; Salfinger, M. Antimicrobial Agents and Susceptibility Tests: Mycobacteria. Manual of Clinical Microbiology, V ed., 1996, 1385-1404. Murray P.R.; Baron E.J.; Pfaller, M.A.; Tenover, F.C.; Volken R.H. in American Society for Microbiology, Washington, D.C .