Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 24, pp. 3017-3020, 1996

0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00557-4

ANTI-PNEUMOCYSTIS ACTIVITY OF BIS-AMIDOXIMES AND BIS-O-ALKYLAMIDOXIMES PRODRUGS

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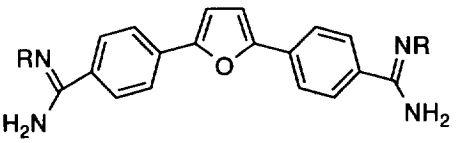
Abstract. We report the synthesis of 2,5-bis-[4-amidinophenyl]furan bis-amidoxime(**2**), the corresponding bis-O-methylamidoxime(**3**) and the corresponding bis-O-ethylamidoxime(**4**) and their evaluation as prodrugs against *Pneumocystis carinii* pneumonia. Copyright © 1996 Elsevier Science Ltd

A number of aromatic diamidines have been shown to exhibit excellent activity against *Pneumocystis carinii* pneumonia in the immunosuppressed rat model.[1-4] Limited oral bioavailability and both acute and chronic toxicity have slowed the development of this class of compounds. Although certain amidines, such as pentamidine, berenil, and stilbamidine, have been used for decades as therapeutic agents[5-7] and continue to serve as the basis for new compound synthesis,[8-10] it was only recently that prodrug strategies have been reported for the amidine functional group. Work from the laboratories of Clement[11] and of Hall and Tidwell[12] have demonstrated that the bis-amidoxime of pentamidine is metabolized in vivo to pentamidine, thereby demonstrating that amidoximes can function as prodrugs. Recently, Eldred and coworkers[13] reported the oral bioavailability of an amidoxime of a monoamidine fibrinogen receptor antagonist. Weller and coworkers[14] demonstrated that amidoximes and carbamate derivatives of amidines as prodrugs provide significantly improved oral bioavailability for fibrinogen antagonists. Interestingly, in a recent patent application Clement has suggested that amidoximes can function as prodrugs for any pharmacologically active amidine.[15] In a study of diamidoximes of pentamidine, pentamidine analogs, and bis-benzimidazoles, Hall, Tidwell, and coworkers have demonstrated, for the first time, that all amidoximes do not function as prodrugs.[16] For example, the bis-amidoximes of compounds in a very promising class of bis-benzimidazoles lacked both intravenous and oral activity in the immunosuppressed rat model.[16] These investigations prompted this report of results from our studies focused on improving the bioavailability of the aryl diamidines that show promising antimicrobial activity.

Recently, we reported that 2,5-bis-[4-amidinophenyl]furan **1** was more active and less toxic than pentamidine against *Pneumocystis carinii* in the immunosuppressed rat model on intravenous dosage.[1] However, the activity of **1** in the rat model was significantly less on oral administration.[1] In view of the promising activity of **1** we have investigated approaches to improve the bioavailability of this compound. The

results of studies with 2,5-bis-[4-amidinophenyl]furan bis-amidoxime(**2**), the bis-O-methylamidoxime(**3**) and the bis-O-ethylamidoxime(**4**) are reported here.



1 R = H
2 R = OH
3 R = OCH_3
4 R = OCH_2CH_3

There are two commonly used approaches for preparation of amidoximes. One method involves direct conversion of a nitrile into the amidoxime by the reaction with hydroxylamine in the presence of base.[17] The second approach involves a two step process using Pinner methodology to convert a nitrile into an imidate ester followed by reaction of the imidate ester with hydroxylamine.[18] In our hands, the reaction of 2,5-bis[4-cyanophenyl]furan **5** with hydroxylamine employing the first method yielded mixtures of products, which were difficult to separate. In contrast, the second process resulted in good yields of **2-4**.[19]

Table 1 contains the results of evaluation of the three potential prodrugs **2-4** against *Pneumocystis carinii* pneumonia in the immunosuppressed rat model on both oral and intravenous administration. Previous data for **1** are also included in Table 1 for comparison purposes.[1] The results shown in Table 1 clearly demonstrate that compounds **2** and **3** are effective when given orally by gavage when compared to saline control. The data also show that amidoximes can function as prodrugs in the furan series. The values for the cyst counts suggest that drug uptake on oral dosage for **2** and **3** is superior to that of the parent dicationic compound **1**. Unexpectedly, the bis-O-methylamidoxime **3** is more effective than the bis-amidoxime **2.** On intravenous administration of both **2** and **3**, the acute toxicity[16] generally noted for diamidines((hypotension, hypoactivity, dyspnea, etc.) was absent; however, for compound **2** considerable inflammation and necrosis of the tail vein at the injection site is observed even at the relatively low dosage of 5.5 μmol/kg/day. In contrast, the bis-O-methylamidoxime **3** is well tolerated; inflammation and necrosis at the injection site is absent even on intravenous administration of 22.0 μmol/kg/day.

In contrast with **2** and **3**, the bis-O-ethylamidoxime **4** is only marginally effective by both routes of administration. Hall and Tidwell suggest for diamidoxime prodrugs to be effective against extracellular parasites they must enter host cells, be reduced to the active amidine form, be extracellularly released, and then be taken up by the infecting organism.[16] Certain diamidoximes, which are not effective as prodrugs, have been shown by Hall and Tidwell to exhibit significantly reduced cellar uptake.[16] Since the O-alkylamidoximes **3** and **4** differ only by two methylene groups it seems unlikely that uptake differences between the two can be attributed to differences in passive uptake. Consequently, it appears that transport enzymes or the reductases,

presumably responsible for reduction of oxime to the parent amidine, exhibit considerable specificity. Detailed metabolism studies are underway exploring uptake and other aspects of the pharmacology of these active furan compounds.

Table 1. In vivo Activity of Prodrugs of Bis-2,5-[4-Amidinophenyl]furan vs. *Pneumocystis carinii*.

	IV Dosing			Oral Dosing		
Compd	Dosage [a] (μmol/kg/day)	Toxicity [b]	%Sal. Ctrl. ± S.E.[c]	Dosage [d] (μmol/kg/day)	Toxicity [b]	%Sal. Ctrl. ± S.E.c
Saline Control		0	100.0 ± 9.51	Not done		
Pentamidine	22.0	+2	3.94 ± 1.49	Not done		
1[e]	26.6	+1	NA[f]	66.3	0	25.2 ± 9.84
	13.3	0	0.79 ± 0.34	39.8	0	42.3 ± 12.6
	2.7	0	7.26 ± 3.56			
	0.3	0	6.92 ± 3.36			
	0.03	0	26.3 ± 5.0			
2[g]	22.0	+1	0.67 ± 0.46	33.0	0	3.1 ± 1.31
	11.0	+1	1.37 ± 0.61	11.0	0	197.6 ± 64.4
	5.5	+1	42.3 ± 34.4	5.5	0	127.0 ± 48.4
	2.7	0	54.4 ± 27.4			
	0.3	0	203.6 ± 56.4			
3[g]				33.0	0	1.82 ± 0.53
	22.0	0	14.3 ± 5.14	22.0	0	12.0 ± 11.5
				11.0	0	34.7 ± 21.2
				5.5	0	264.2 ± 72.9
4[g]	22.0	+1	104.7 ± 49.9	33.0	0	121.5 ± 48.4

(a) Dosage by tail vein injection, see ref 2. (b) See ref 2 for detailed explanation of toxicity scale, generally the larger the value the more severe the toxicity, values greater than +2 indicates death of some animals. (c) Cysts counted in lung tissue employing a blinded protocol; reported as percentage of saline-treated controls, see ref 2. (d) Oral dosage by gavage. (e) See ref 1. (f) Not available; experiment was suspended on day eight due to severe necrosis of the tail at the injection site. (g) Compounds were administered as the dimaleate salts.

We have found that the bis-amidoxime **2** and the bis-O-methylamidoxime **3** in the 2,5-bis-phenylfuran series are effective antipneumocystis agents on both oral and intravenous administration. The bis-O-methylamidoxime **3** shows significantly less toxicity on intravenous dosage than the bis-amidoxime **2**. We report here, for the first time, that O-methylamidoximes can function as prodrugs for amidines.

Acknowledgment

This work was supported by NIH Grant AI-33363 and by Pharm-Eco Laboratories, Inc. The Instrumental Program of NSF (CHE 8409599) provided partial support for acquisition of the Varian VXR400 spectrometer.

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19. New compounds reported here gave analytical data (C, H, N) in agreement with calculated values and their spectroscopic data(^{1}H and ^{13}C NMR; IR; MS) were in accord with the assigned structures.

(*Received in USA* 8 *October* 1996; *accepted* 18 *November* 1996)