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Short communication

Carbamate prodrugs of *N*-alkylfuramidines

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Abstract

The synthesis and evaluation of 2,5-bis[4-(*N*-ethoxycarbonyl-*N*′-isopropyl)amidinophenyl]furan, 2,5-bis[4-(*N*-2,2,2-trichloroethoxycarbonyl-*N*′-isopropyl)amidinophenyl]furan and 2,5-bis[4-(*N*-cyclopentyl-*N*′-2,2,2-trichloroethoxycarbonyl)amidinophenyl]furan as prodrugs of bis-*N*-alkylamidines are reported. The results show that the bis-2,2,2-trichloroethyl carbamates function effectively in a rat model for *Pneumocystis* pneumonia.

Keywords: Prodrugs; Carbamates; *N*-Alkylfuramidines; *Pneumocystis carinii*

1. Introduction

Amidines are quite basic functional groups and the pKs of arylamidines range from 10 to 12 [1]. Consequently, drugs containing amidine units are protonated at physiological pH and typically exhibit low bioavailability. An extensive number of monoamidines have been evaluated for their utility in blocking various stages of the thrombin cascade and numerous highly potent molecules have been reported [2]. In efforts to markedly improve the oral bioavailability of this class of molecules, various prodrug approaches including conversion to amidoximes, *O*-alkylamidoximes and carbamates have been investigated [3]. A number of these prodrugs have been found to significantly improve oral bioavailability relative to the parent compounds and the amidoxime prodrugs sibrafiban and ximelagatran have undergone clinical trials in an effort to provide orally effective thrombin inhibitors [4]. Since the discovery of the potent antiprotozoan activity of pentamidine aryl diamidines, these compounds have been extensively studied as antimicrobial agents [5]. In an effort to provide orally effective diamidine antimicrobial agents we have explored various prodrug strategies including making bis-amidoximes, bis-*O*-alkylamidoximes and bis-carbamates of potent diamidine systems [6,7]. Currently, pafuramidine, a methoxamidine of furamidine, is in two phase III clinical trials, one against early stage human African trypanosomiasis and the other against human *Pneumocystis jiroveci* (formerly *Pneumocystis carinii*) pneumonia [5b,8]. We have reported that several *N*-alkylfuramidine compounds showed significantly improved activity compared to furamidine against *P. carinii* when administered intravenously in an immunosuppressed rat model [9]. Despite the fact that there does not appear to be a carbamate specific mammalian enzyme [10], carbamates have been successfully used as prodrugs for a number of different functional groups [11]. Presumably one or more of the plethora of mammalian esterases is responsible for carbamate cleavage [12]. Despite a considerable number of investigations focused on development of prodrugs for unsubstituted amidines we are aware of only one such study on bis-*N*-alkylamidines [13]. Thus, it is important to explore approaches to provide oral bioavailability of the highly active bis-*N*-alkylamidines. In view of our success by using carbamates of furamidine as prodrugs [7] we decided

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to examine their effectiveness with *N*-alkylfuramidine analogues. We report here an initial study of carbamates of two active *N*-alkylfuramidines.

2. Chemistry

Given the in vivo effectiveness of bis-2,2,2-trichloroethyl furamidine carbamate we decided to explore the use of this system with the highly active *N*-*i*-propyl and *N*-*c*-pentyl furamidine analogues. Earlier we reported the synthesis of a number of aryl–alkyl carbonates and their synthetic utility in the preparation of carbamate prodrugs of 2,5-bis(4-amidinophenyl)furan [7]. This method was applied to the synthesis of the *N*-alkyl substituted amidines reported here (Scheme 1). The reaction of the *N*-isopropyl amidine **1a** with ethyl 4-nitrophenylcarbonate in DMF at room temperature gave **2a** in 85% yield and when a THF/CH_3CN mixture was used the requisite carbamate was obtained in a 90% yield. When **2b** was prepared by the reaction of 2,2,2-trichloroethyl chloroformate with **1a** only a 56% yield was obtained and the reaction of **1b** with the same chloroformate failed to provide any isolatable carbamate (experimental not presented). However, when **1a** was allowed to react with 4-nitrophenyl 2,2,2-trichloroethylcarbonate in THF/CH_3CN the carbamate **2b** was obtained in an 85% yield. The *N*-cyclopentyl-*N*′-2,2,2-trichloroethyl carbamate **2c** was synthesized in a 76% yield in a similar manner using 4-nitrophenyl 2,2,2-trichloroethylcarbonate in THF/CH_3CN.

3. Biology

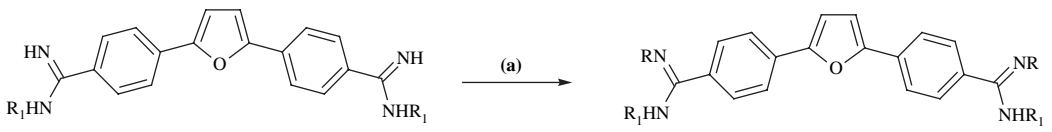
Table 1 contains the results of evaluation of the carbamate prodrugs of *N*-alkylfuramidines in an immunosuppressed rat *Pneumocystis* model. The table also contains results for the control compounds pentamidine, furamidine, pafuramidine and the bis-2,2,2-trichloroethyl carbamate of furamidine. As is well known the parent diamidine, pentamidine and furamidine show limited efficacy on oral administration and similar results were observed with the *N*-*i*-propyl and *N*-*c*-pentyl furamidine analogues **1a** and **1b** (Table 1). The ethyl carbamate of *N*-*i*-propyl furamidine **2a** was not effective in the immunosuppressed rat model (Table 1); similar results were observed for the ethyl carbamate of furamidine [7]. In marked contrast, the 2,2,2-trichloroethyl carbamate of *N*-*i*-propyl furamidine **2b** shows excellent activity on iv dosing and quite good activity on oral administration. These results and those reported earlier [7], suggest that the trichloroethyl carbamate is either better absorbed or metabolized (or both) than its ethyl carbamate analogue in these bis-amidine systems. Only modest activity was found on iv dosing of the 2,2,2-trichloroethyl carbamate of *N*-*c*-pentyl furamidine **2c**, however, on oral administration good activity, approaching to that of **2b**, was observed. Based on these observations we conclude that carbamates of *N*-alkylamidines are suitable for prodrug approaches for these potent and potentially quite useful molecules. No detectable overt toxicity has been noted for numerous other prodrugs of diamidines.

This initial study indicates that carbamates of *N*-alkylamidines can function effectively as prodrugs. Further studies are required to optimize the carbamate structure for maximum delivery of the active compounds.

4. Experimental section

Melting points were recorded using a Thomas-Hoover (Uni-Melt) capillary melting point apparatus and are uncorrected. TLC analysis was carried out on silica gel 60 F_{254} pre-coated aluminium sheets (0.20 mm layer thickness) (E. Merck) and detected under UV light. IR spectra were recorded using Perkin-Elmer Model 337 spectrometer. ^1H and ^{13}C NMR spectra were recorded employing a Varian GX400 or a Varian Unityplus 300 spectrometer and chemical shifts (δ) are in ppm relative to TMS as internal standard. Mass spectra were recorded on a VG Analytical 70-SE spectrometer (Georgia Institute of Technology, Atlanta, GA). IR spectra were recorded using a Perkin-Elmer 2000 instrument. Elemental analyses were obtained from Atlantic Microlab Inc. (Norcross, GA) and are within 0.4% of the theoretical values unless otherwise mentioned. All chloroformates were purchased from Aldrich Chemical Co. Other chemicals and solvents were purchased from either Acros, Aldrich or Fischer Scientific.

Anti-*P. carinii* pneumonia screening was carried out according to published methods [7,14]. Compounds were routinely tested orally at 33 μmol/kg/day and intravenously at 22 μmol/kg/day. Saline and pentamidine treated groups of rats were included as negative and positive controls, respectively.



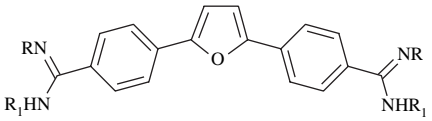
1a R_1 = *i*-Propyl
1b R_1 = *c*-Pentyl

2a R_1 = *i*-Propyl R = $COOCH_2CH_3$
2b R_1 = *i*-Propyl R = $COOCH_2CCl_3$
2c R_1 = *c*-Pentyl R = $COOCH_2CCl_3$

Reagents and conditions: (a) 4-nitrophenyl ethyl carbonate or 4-nitrophenyl 2,2,2-trichloroethyl carbonate, DMF or THF, MeCN, rt.

Scheme 1.

Table 1
In vivo evaluation of prodrugs and comparison with parent molecules versus *P. carinii* in a rat model (DB numbers will be deleted before submission)



Code	R	R_1	Dosage[a] (μmol/kg/day)	Cysts/g of lung[b] (% of control)	Toxicity[c]
Saline	NA	NA	iv	100.0 ± 12.72	0
			po	100.00 ± 35.16	0
Pentamidine[d]	NA	NA	iv at 22.0	2.75 ± 1.27	++
			oral at 33.0	67.78 ± 20.49	0
Furamidine[e]	H	H	iv at 13.3	0.86 ± 0.52	0
			oral at 39.8	15.67 ± 6.07	0
Pafuramidine[f]	OMe	H	iv at 22.0	13.99 ± 5.03	0
			oral at 33.0	1.21 ± 0.35	0
Furamidine trichloroethyl carbamate[g]	$COOCH_2CCl_3$	H	iv at 22.0	1.72 ± 1.66	0
			oral at 33.0	5.45 ± 5.02	0
1a	H	*i*-Propyl	iv at 10.8	0.26 ± 0.23	0
			oral at 33.0	78.52 ± 24.52	0
2a	$COOCH_2CH_3$	*i*-Propyl	iv at 11.0	82.21 ± 43.74	0
			oral at 33.0	51.92 ± 19.53	0
2b	$COOCH_2CCl_3$	*i*-Propyl	iv at 22.0	0.02 ± 0.01	++
			oral at 33.0	2.01 ± 0.92	0
1b	H	*c*-Pentyl	iv at 9.4	0.04 ± 0.02	+
			oral at 33.0	125.30 ± 39.98	0
2c	$COOCH_2CCl_3$	*c*-Pentyl	iv at 22.0	39.84 ± 2.81	0
			oral at 33.0	3.70 ± 2.22	0

[a] Evaluation of iv dosage (by tail vein injection) of the compounds against *P. carinii* in rats as described in Refs. [7,13]; oral dosage by gavage.
[b] Lower numbers of cysts/g reflect higher activity versus *P. carinii*. Values below 1% of control are within experimental error and can be considered a complete cure of the infection in the immunosuppressed rats. Mean cyst count for pooled controls: saline ($n = 94$) 50.63×10^6 cysts/g of lung tissue, pentamidine ($n = 89$) 1.39×10^6 cysts/g of lung tissue for iv dosing and saline ($n = 11$) 74.19×10^6 cysts/g of lung tissue, pentamidine ($n = 6$) 50.28×10^6 cysts/g of lung tissue for po dosing.
[c] Toxicity of the compounds tested is evaluated according to a six level scale which ranges from 0 to +4. The toxicity noted should not be considered definitive and is essentially anecdotal. However, these values do allow for comparison of relative toxicities among tested compounds and the control drug pentamidine.
[d] Ref. [13].
[e] Ref. [9].
[f] Ref. [6a].
[g] Ref. [7].

*4.1. 2,5-Bis[4-(N-ethoxycarbonyl-N′-isopropyl)-amidinophenyl]furan (**2a**)*

To a suspension of the *N*-isopropyl amidine (**1a**) [9] (0.4 g, 0.001 mol) in DMF (15 mL) at room temperature was added ethyl 4-nitrophenylcarbonate (0.47 g, 0.0023 mol) and the mixture was stirred over night. The solution was then cooled in an ice bath, water (20 mL) was added and the resulting solid was filtered, washed with water (3 × 40 mL), ether (2 × 30 mL) and dried. The crude product was crystallized from CH_2Cl_2/ether mixture to afford pure bis-carbamate **2a** (0.46 g, 85% yield) as a pale yellow solid: mp 186–188 °C; IR (KBr) 3440–3140 (br, NH str), 3114 (m, CH-Ar str), 3080 (m, CH-Ar str), 3050 (m, CH-Ar str), 2984 (s, CH-Alip str), 2935 (s, CH-Alip str), 1675 (s, C=O str) cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.96 (s, 2H, NH), 7.88 (d, $J = 8.20$ Hz, 4H, Ar-CH), 7.42 (d, 4H, $J = 8.1$ Hz, Ar-CH), 7.22 (s, 2H, CH-furan), 4.05 (m, 2H, C*H*Me_2), 3.85 (q, 4H, OCH_2Me, $J = 6.97$ Hz), 1.20 (d, 6H, $J = 6.7$ Hz, CH_3 of isopropyl), 0.99 (t, 6H, $J = 6.9$ Hz, OCH_2CH_3); ^{13}C NMR (DMSO-d_6) δ 180.8, 178.3, 177.2, 156.4, 155.0, 152.1, 148.1, 134.5, 91.1, 88.4, 39.0, 24.9; MS *m/z* (FAB, thioglycerol) 533 (M + 1), 492, 428, 402, 356, 330, 315, 288, 271. Anal. calcd. for $C_{30}H_{36}N_4O_5$: theory: C, 74.35; H, 7.49; N, 11.56. Found: C, 74.21; H, 7.60; N, 11.30.

*4.2. 2,5-Bis[4-(N-2,2,2-trichloroethoxycarbonyl-N′-isopropyl)amidinophenyl]furan (**2b**)*

To a suspension of *N*-isopropyl amidine (**1a**) [9] (1.0 g, 0.0026 mol) and diisopropylethylamine (1 g, 0.0077 mol) in THF/CH_3CN mixture (20 mL, 1:1 v/v) at room temperature, was added a solution of 4-nitrophenyl 2,2,2-trichloroethylcarbonate (1.76 g, 0.0056 mol) in THF (10 mL) and stirred over night (16 h). The solution was concentrated on a rotary evaporator at 30 °C under reduced pressure and the residue was cooled in an ice bath, triturated with anhydrous diethyl ether (20 mL), filtered, washed with ether (3 × 20 mL), dried and crystallized from CH_2Cl_2/ether mixture to obtain 2,2,2-trichloroethyl carbamate **2b** (1.4 g, 90% yield) as a pale yellow solid: TLC (R_f) 0.68 ($CHCl_3$/MeOH/NH_4OH, 4:1:0.2, v/v/v); mp 156–158 °C; IR

(KBr) 3445–3140 (s, NH str), 3071 (m, CH-Ar str), 3050 (m, CH-Ar str), 2978 (m, CH-Alip str), 2934 (m, CH-Ar str), 1669 (s, C=O str) cm^{-1}. ^1H NMR ($CDCl_3$) δ 10.01 (s, 2H, NH), 7.79 (d, $J = 7.73$ Hz, Ar-CH), 7.74 (d, $J = 7.30$ Hz, Ar-CH), 7.55 (d, $J = 7.61$ Hz), 7.50 (d, $J = 8.41$ Hz, Ar-CH, 4H), 6.84 (s, CH-furan), 5.33 (d, 2H, $J = 7.30$ Hz, NH), 4.81 (s, CH_2CCl_3), 4.68 (s, CH_2CCl_3), 4.37 (m, NHC*H*), 3.85 (m, NHC*H*), 1.31 (d, $J = 6.34$ Hz, CH_3), 1.24 (d, $J = 6.19$ Hz, CH_3); ^{13}C NMR ($CDCl_3$) δ 171.0, 164.8, 162.9, 161.3, 160.8, 153.1, 153.0, 133.5, 133.3, 133.1, 132.5, 128.5, 127.8, 124.2, 124.0, 109.3, 95.7, 95.7, 75.7, 75.4, 48.0, 44.6, 24.1, 22.6; MS *m/z* (FAB, thioglycerol) 740 (M + 1, six isotopic peaks), 739, 738, 589, 563, 487, 415, 389, 356, 330, 314, 288, 271, 245. Anal. calcd. for $C_{30}H_{30}Cl_6N_4O_5 \cdot H_2O$: theory: C, 47.58; H, 4.25; N, 7.40. Found: C, 47.35; H, 4.41; N, 7.25.

*4.3. 2,5-Bis[4-(N-cyclopentyl-N′-2,2,2-trichloroethoxycarbonyl)amidinophenyl]furan (**2c**)*

To a suspension of the cyclopentyl amidine **1b** [9] (0.75 g, 0.0017 mol) and diisopropylethylamine (0.52 g, 0.004 mol) in a THF/CH_3CN mixture (20 mL, 1:1 v/v) at room temperature, was added a solution of 4-nitrophenyl-2,2,2-trichloroethylcarbonate (1.17 g, 0.0048 mol) in THF (10 mL) and stirred for 24 h. Workup as described above for the isopropyl analogue yielded 2,2,2-trichloroethyl carbamate **2c** (1.02 g, 76% yield) as a yellow solid: mp 134–135 °C; IR (KBr) 3628–3168 (s, NH str), 3100 (m, CH-Ar str), 2960 (m, CH-Alip str), 2871 (m, CH-Alip str), 1674 (s, C=O str) cm^{-1}. ^1H NMR ($CDCl_3$) δ (tautomeric or geometric mixture) 10.21 (s, 2H, NH), 7.82 (d, $J = 7.93$ Hz, Ar-CH), 7.75 (d, $J = 7.14$ Hz, Ar-CH), 7.50 (d, $J = 7.93$ Hz, Ar-CH), 6.87 (s, CH-furan), 6.86 (s, CH-furan), 5.45 (d, 2H, $J = 6.82$ Hz, NH), 4.82 (s, CH_2CCl_3), 4.69 (s, CH_2CCl_3), 4.50 (m, NHC*H*), 3.97 (m, NHC*H*), 2.16 (m, *c*-pentyl), 1.96 (m, *c*-pentyl), 1.77 (m, *c*-pentyl), 1.60–1.76 (br m, *c*-pentyl); ^{13}C NMR ($CDCl_3$) δ (tautomeric mixture) 171.1, 165.2, 163.0, 160.8, 153.3, 134.0, 133.33, 133.1, 132.6, 128.9, 127.9, 124.2, 123.9, 109.3, 95.8, 75.8, 75.4, 57.3, 54.2, 34.7, 33.2, 24.0; MS *m/z* (FAB, thioglycerol) 792 (M + 1, six isotopic peaks), 641, 615, 573, 532, 493, 467, 425, 382, 356. Anal. calcd. for $C_{34}H_{34}Cl_6N_4O_5$: theory: C, 51.60; H, 4.33; N, 7.08. Found: C, 51.41; H, 4.42; N, 6.90.

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