Antibacterial Organophosphorus Compounds: Phosphoranilidohydrazones of 5-Nitro-2-furaldehyde

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Abstract □ A series of phosphoranilidohydrazones of 5-nitro-2-furaldehyde was synthesized and evaluated for antibacterial activity. The series was prepared to examine the applicability of phosphoramidic hydrazones as carriers for the antibacterial nitrofuran moiety. Designed as analogues of nitrofurantoin, members of the series were chosen according to the Topliss approach to analogue design. The title compounds were devoid of gram-negative activity but possessed moderate antistaphylococcal activity. The most potent members of the series were equipotent with nitrofurantoin against *Staphylococcus aureus*. The relationship between structure and antistaphylococcal activity is discussed.

The antimicrobial properties of nitrofurans, recognized for nearly 50 years, have been well documented.[1,2] The intrinsic antibacterial activity of the nitrofuran moiety in sensitive organisms is apparently due to its activation to a partially reduced intermediate. Asnis[3] showed that cell-free extracts of a furacin (nitrofurazone)-resistant strain of *Escherichia coli* were devoid of a flavoprotein-mediated reductase found in furacin-sensitive cells. McCalla et al.[4] corroborated these results and proposed that it was perhaps the hydroxylamino analogue that was the active reduced form in sensitive organisms. In a related study, Beckett and Robinson[5] provided evidence that, after contact with *Aerobacter aerogenes*, nitrofurazone was reduced to the corresponding aminofuran derivative. Only limited growth of the organism occurred during the reduction of the drug.

A nitrofuran derivative that is frequently used to treat urinary tract infections when the infective organism is sensitive to it is nitrofurantoin. One hypothesis for the mechanism of antibacterial action of nitrofurantoin is an interaction with DNA secondary to formation of a nitro anion radical via one-electron reduction of the redox-active nitrofuran moiety, as outlined by Holtzman et al.[6] A similar mechanism has been proposed to account for the antimalarial activity of nitrofurans.[7]

The hydantoin portion of nitrofurantoin, though not essential for antibacterial activity, is compatible with structure–activity relationships in this class of compounds.[8] Its role at the molecular level is probably as a carrier to facilitate membrane transport and/or to enhance interaction with essential enzymes or other functional targets. Moreover, the hydantoin carrier undoubtedly influences the pharmacokinetic properties of the compound.[9] The proper choice of carrier for the nitrofuran moiety could result in a compound with superior antibacterial activity, with activity against previously resistant organisms, or with attractive pharmacokinetic properties. In an attempt to gain understanding of the role of the carrier moiety, a series of phosphoranilidohydrazones (2) has been synthesized and evaluated for antibacterial activity.

Experimental Section

Melting points were determined in open glass capillaries with a Thomas-Hoover melting point apparatus and are uncorrected. IR spectra were recorded on a PE 599 IR spectrophotometer. ^{1}H NMR spectra were obtained on a Varian T60 spectrometer. Elemental analyses (C,H,N) were performed by Atlantic Microlab, Inc. (Norcross, GA) or by Schwarzkopf Microanalytical Laboratory (Woodside, NY) and were within ±0.4% of theoretical values. General experimental procedures are provided and experimental data for the compounds synthesized are reported in Table I.

Synthesis of Ethyl *N*-(Substituted phenyl)phosphoramidic Hydrazides (1)—A solution of the appropriate aniline (0.025 mol) in 25 mL of dichloromethane containing triethylamine (0.026 mol) was added in a dropwise manner to a solution of ethyl dichlorophosphate (3 mL, 0.025 mol) in 25 mL of dichloromethane and stirred at room temperature. After 3 h, the solvent was removed under reduced pressure, and the residue was triturated with 25 mL of tetrahydrofuran and filtered to remove triethylamine hydrochloride. The filtrate was added in a dropwise manner to a suspension of anhydrous hydrazine (4 mL) in 25 mL of tetrahydrofuran and stirred at 5 °C. After 18 h, the lower liquid phase of the reaction mixture was removed and discarded, and then the remaining solvent was removed

Table I—Properties of Ethyl *N*-(Substituted phenyl)phosphoramidic Hydrazides (1) and Hydrazones (2)

Compound	X	% Yield	mp, °C	Recrystallization Solvent[a]	Formula[b]
1a	3,4-Cl_2	20	137–138	U	$C_8H_{12}Cl_2N_3O_2P$
1b	4-Cl	37	135–136	V	$C_8H_{13}ClN_3O_2P$
1c	H	31	119–120	U	$C_8H_{14}N_3O_2P$
1d	4-CH_3	14	104–105	U	$C_9H_{16}N_3O_2P$
1e	4-OCH_3	57	125–126	U	$C_9H_{16}N_3O_3P$
2a	3,4-Cl_2	71	96–99	W/V	$C_{13}H_{13}Cl_2N_4O_5P \cdot 1/2\ H_2O$
2b	4-Cl	66	112–115	X/V	$C_{13}H_{14}ClN_4O_5P \cdot 1/2\ H_2O$
2c	H	91	117–120	Y/V	$C_{13}H_{15}N_4O_5P \cdot 1/2\ H_2O$
2d	4-CH_3	77	95–98	Z/V	$C_{14}H_{17}N_4O_5P \cdot 1/2\ H_2O$
2e	4-OCH_3	50	88–91	Z	$C_{14}H_{17}N_4O_6P \cdot H_2O$

[a] U = toluene, V = water, W = dioxane, X = acetonitrile, Y = acetone, Z = methanol. [b] Satisfactory analytical data (± 0.4% for C,H,N) were reported for all compounds listed in the table.

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under reduced pressure. Trituration of the residue with ether and chilling gave the crystalline hydrazide, which was recrystallized from an appropriate solvent.

Synthesis of Ethyl *N*-(Substituted phenyl)phosphoramidic Hydrazones of 5-Nitro-2-furaldehyde (2)—A mixture of hydrazide 1 (2–3 mmol) and equimolar 5-nitro-2-furaldehyde in 5 mL of methanol per mmol of 1 was stirred at reflux for 3 h. The reaction mixture was concentrated under reduced pressure, and the residue was crystallized from the appropriate solvent(s).

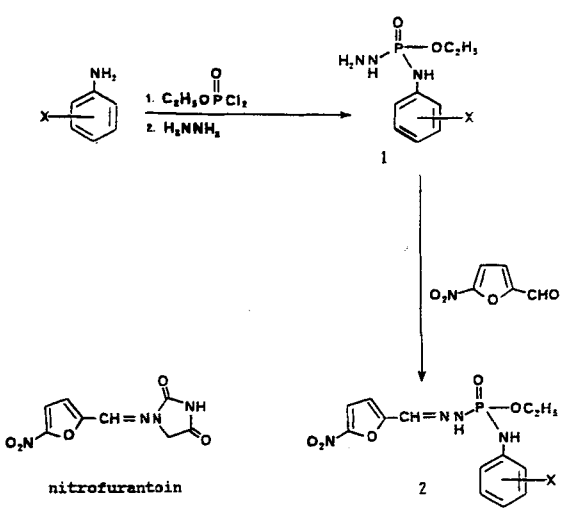
Microbiology—The antimicrobial activity of the phosphoranilidohydrazones was compared with that of nitrofurantoin by the agar disk diffusion method.[10,11] Disks were prepared by applying 20 μL of a solution of hydrazone in methanol (15 mg/mL) to a blank disk. The disk was dried, and antimicrobial activity was assayed by a standard disk diffusion method with Mueller–Hinton agar.[12]

Quantitative comparison of the activity of the compounds with nitrofurantoin was examined by a broth dilution technique against *S. aureus* ATCC 25923. The compounds were dissolved in dimethyl sulfoxide (DMSO) and then added to Mueller–Hinton broth to yield a solution of 32–64 μg/mL (1.25% DMSO). Serial twofold dilutions were performed, and tubes were inoculated with log phase cultures of *S. aureus* to yield a final inoculum of 10^5–10^6 colony-forming units. The tubes were incubated overnight at 37 °C, and the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by the method of Pearson et al.[13]

Results and Discussion

The title compounds were synthesized according to Scheme I. The appropriately substituted anilines in dichloromethane containing triethylamine were phosphorylated with ethyl dichlorophosphate to give the corresponding phosphoramidic monochlorides that were not isolated. After removal of triethylamine hydrochloride, the crude monochloride reaction mixture in tetrahydrofuran was used directly in the next step, which involved treatment with excess hydrazine to give the desired hydrazides (1) in yields of 14–57% based on ethyl dichlorophosphate. Condensation of 1 with 5-nitro-2-furaldehyde afforded the target hydrazones (2) in moderate yields (50–91%; Table I).

The target compounds were designed as structural analogues of nitrofurantoin. Whereas nitrofurantoin is weakly acidic (pK_a = 7.5; K_a is the association constant), 2 would be expected to be essentially neutral. Moreover, whereas the hydantoin ring in nitrofurantoin bonds strongly to hydrogen and conveys water-solubilizing properties, the carrier moiety in 2 is expected to be highly lipophilic. The overall effect of the aforementioned changes in regard to antibacterial activity was evaluated by testing the series against various nitrofurantoin-sensitive organisms.



Scheme I

The disk susceptibility data indicate that members of the series are effective only against gram-positive organisms (Table II). Qualitatively, the spectrum differences between 2 and nitrofurantoin probably reflect the requirement for a hydrophilic/weakly acidic carrier moiety for transport into gram-negative organisms, perhaps via porins.[14]

To provide a quantitative measure of antibacterial activity, MIC and MBC data were measured against *Staphylococcus aureus* ATCC 25923 (Table II). Compound 2a was the most active, with an MIC and MBC of 8.0 μg/mL.

The choice of phenyl substituents in this series was made according to the manual method for applying the Hansch approach to drug design as developed by Topliss.[15] We had hoped that an antistaphylococcal potency order would emerge for this initial group of compounds, which would allow comparison with tabulated potency orders calculated for various hydrophobic, electronic, and steric parameters. Because of the possibility of cumulative errors inherent in serial dilution tests, no significance is usually attached to less than fourfold differences in activity levels. Therefore, it is not possible to derive an unequivocal potency order for our compounds.

The MIC data demonstrate that the test compounds are at least as potent as nitrofurantoin and, perhaps, more potent than nitrofurantoin on a molar basis. There is a significant difference between the MIC for dichloro analogue 2a and the corresponding methylether 2e. Considering these data and applying the Topliss method, it appears that for inhibition of growth the most probable operative parameter is either σ or a combination of σ and π. If this is true, optimization of activity should be realized with the synthesis of analogues bearing suitable phenyl substituents (e.g., 4-CF_3 and 4-cyclohexyl). However, because the present compounds display poor water solubility, it is unlikely that quantitative data can be obtained on more lipophilic analogues without considerable difficulty. The MBC data show that the dichloro analogue is essentially bactericidal and, in this regard, at least four times more potent than the other phosphorohydrazones and nitrofurantoin.

There has been a resurgence of interest in the investigation of nitrofuran derivatives as antiinfective agents as evidenced by a number of recent reports.[16–18] The results of this study suggest that phosphorohydrazones can be effective and versatile carriers of the antibacterial nitrofuran moiety. The focus of future studies should be the introduction of gram-negative activity through judicious selection of carrier moieties.

Table II—In Vitro Activities of Phosphorohydrazones (2) and Nitrofurantoin (NF) against Gram-Positive Bacteria

Compound	Zone of Inhibition, mm[a,b]		MIC/MBC, g/mL
	S. aureus	*E. faecalis*	*S. aureus* (ATCC 25923)
2a	20	18	8/8
2b	20	19	8/64
2c	—	—	16/64
2d	21	18	16/32
2e	21	20	32/>32
NF	19	22	16/32

[a] Values reported are zones of inhibition (diameter in mm including 6-mm disk); a value >17 indicates a nitrofurantoin-susceptible organism. [b] None of the organophosphorus compounds were active against *Escherichia coli*, *Pseudomonas aeruginosa*, *Pseudomonas mirabilis*, *Serratia marcescens*; nitrofurantoin (NF) was active against *E. coli*, but inactive against *Proteus aeruginosa*, *Proteus mirabilis*, *S. marcescens*.

References and Notes

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