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Synthesis and Biological Activity of Some N-Substituted Organic Phosphorotriamidates

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In present communication, we describe the synthesis of N,N′,N″-tri-*o*-methoxy phenyl phosphorotriamidate, N,N′,N″-tri-*o*-ethoxy phenyl phosphorotriamidate and 3,4-dichloro-N,N′,N″-triphenyl phosphoro triamidate by phosphorylation method. These phosphorotriamidates were used for screening effect on some selected species of fungi (*Mucor mucedo, Pythium aphadermatam* and *Rhizoctonia solani*) and bacteria (*E. coli, Shigella boydii* and *Citrobacter frundi*) in minimum concentration 0.0005 M in dioxane-water mixture (20:80 v/v).

INTRODUCTION

Microorganiams, the viruses, bacteria, algae, fungi and protozoa are very important in the various processes. The larger organisms namely fungi are filamentous organisms that live on the dead bodies of plants and animals. Another group of microorganisms *i.e.* bacteria which are somewhat smaller than the fungi are found in all natural waters. Mostly they live on soluble organic matter such as food or any particular matter. The growth of fungi and bacteria was screened by organic phosphates and phosphorotriamidates by various workers.[1–3] Some snythesised organophosphorotriamidates were used to evaluate their toxicity against some selective species of fungi and bacteria. Observed results show that phosphorotriamidates were effective in lower concentration to completely check the growth of fungi and bacteria. The solvent used was dioxane-water mixture in these cases. Triamidates on hydrolysis produce the original compound and inorganic phosphates; therefore, they do not not have a prolonged effect on the environment. It is one of the important criteria for safeguard against environmental degradation.

EXPERIMENTAL

The synthesis of N,N′,N″-tri-*o*-methoxy phenyl phosphoro triamidate, N,N′,N″-tri-*o*-ethoxy phenyl phosphorotriamidate and 3,4- dichloro-N,N′,N″-triphenyl phosphoro triamidate has been performed by phosphorylation method[4].

For the determination of antifungal activity Streak Agar technique[5] was used against *Mucor mucedo, Pythium aphadermatum* and *Rhizoctonia solani* fungi. All triamidates were prepared at 5.0×10^{-4} M concentration in aqueous dioxane mixture 20:80% (v/v). Six different concentrations of prepared solution were

TABLE 1
ANTIFUNGAL ACTIVITY OF PHOSPHORO TRIAMIDATES

Name of phosphoro triamidates	Name of Fungus species								
	Mucor mucedo			*Pythium aphadermatum*			*Rhizoctonia solani*		
	Sensitivity	Conc.	Growth in %	Sensitivity	Conc.	Growth in %	Sensitivity	Conc.	Growth in %
N,N′,N″-tri-*o*-methoxy phenyl phosphoro triamidate	+ +	1.0 mL	90	+ +	1.0 mL	65	+ +	1.0 mL	70
	+ +	1.5 mL	50	+ +	1.5 mL	50	+ +	1.5 mL	60
	+ +	2.0 mL	25	– –	2.0 mL	45	+ +	2.0 mL	40
	– –	2.5 mL	0	– –	2.5 mL	30	– –	2.5 mL	10
	– –	3.0 mL	0	– –	3.0 mL	0	– –	3.0 mL	0
N,N′,N″-tri-*o*-ethoxy phenyl phosphoro triamidate	+ +	1.0 mL	70	+ +	1.0 mL	60	+ +	1.0 mL	75
	+ +	1.5 mL	60	+ +	1.5 mL	50	+ +	1.5 mL	50
	+ +	2.0 mL	50	– –	2.0 mL	30	+ +	2.0 mL	25
	– –	2.5 mL	0	– –	2.5 mL	0	– –	2.5 mL	0
	– –	3.0 mL	0	– –	3.0 mL	0	– –	3.0 mL	0
3,4-dichloro-N,N′,N″-tri-phenyl phosphoro triamidate	+ +	1.0 mL	75	+ +	1.0 mL	90	+ +	1.0 mL	90
	+ +	1.5 mL	50	+ +	1.5 mL	80	+ +	1.5 mL	75
	– –	2.0 mL	25	+ +	2.0 mL	65	+ +	2.0 mL	60
	– –	2.5 mL	0	+ +	2.5 mL	25	+ +	2.5 mL	30
	– –	3.0 mL	0	– –	3.0 mL	0	– –	3.0 mL	0

poured in presterilized petridishes. Control plates containing culture media (without test substance) were also prepared for comparison. Both the test and control plates were incubated at 28°C for 36 h and after incubation period results were noted (Table 1).

The antibacterial activity was determined by paper disc method.[6] For this purpose filter paper (Whatman No. 41) discs (5 mm diameter) saturated with the solution of tested triamidates were placed on nutrient agar plates[7] (1.56% agar, 0.5% NaCl, 0.5% glucose and 2.5% peptone). After drying up the solvent each plate was put in an incubator for bacterial growth and the plates were incubated at the optimum growth temperature (37°C) for 24 h and the zones of inhibition around the discs were measured after 24 h (Table 2).

TABLE 2
ANTIBACTERIAL ACTIVITY OF PHOSPHORO TRIAMIDATES

S. NO.	Name of triamidates	Conc. mL/disc	*E.coli*	*Shigella boydii*	*Citrobacter frundi*
1.	N,N',N''-tri-*o*-methoxy phenyl phosphoro triamidate	A	++	+	++
		B	++	+	++
		C	+	–	–
2.	N,N',N''-tri-*o*-methoxy phenyl phosphoro triamidate	A	+	++	–
		B	+	++	+
		C	–	+	–
3.	3,4-dichloro-N,N',N''-tri-phenyl phosphoro triamidate	A	++	++	++
		B	++	++	+
		C	+	+	+

Note: A = each disc contains 0.5 mL of test triamidate
B = each disc contains 0.2 mL of test triamidate
C = each disc contains 0.1 mL of test triamidate

RESULTS AND DISCUSSION

All of the phosphoro triamidates were tested against six microorganisms *i.e.* three bacteria and three fungi. The bacterial growth occurred after an incubation period of six days while all the three fungi showed growth within three days. All phosphoro triamidates were tested *in vitro* against growth of fungi *i.e. Mucor mucedo, Phythium aphadermatum* and *Rhizoctonia solani* and growth of bacteria *i.e. E. coli, Shigella boydii* and *Citrobacter frundi.*

As recorded in Table 1, N,N',N''-tri-*o*-methoxy phenyl phosphoro triamidate were less toxic to fungi *Mucor mucedo* and *Rhizoctoma solani* while more toxic to *Pythium aphadermatum.* N,N',N''-tri-*o*-ethoxy phenyl phosphoro triamidate was more toxic to *Pythium aphadermatum* and less toxic to *Mucor mucedo* and *Rhizoctonia solani.* 3,4-Dichloro-N,N',N''-triphenyl phosphoro triamidate showed more activity to *Mucor mucedo* and less activity to *Pythium aphadermatum* and *Rhizoctonia solani* [Fig. 1].

As inferred from Table 2, N,N',N''-tri-*o*-methoxy phenyl phosphoro triamidate was more toxic to bacteria *E. coli* and *Citrobacter frundi* and less active to

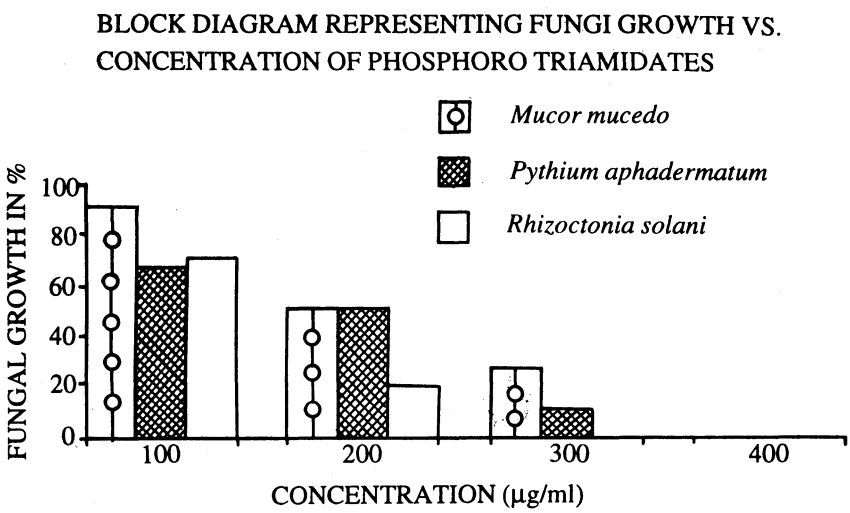

Fig. 1 (a) N,N′,N″-Tri-o-methoxy phenyl phosphoro triamidate

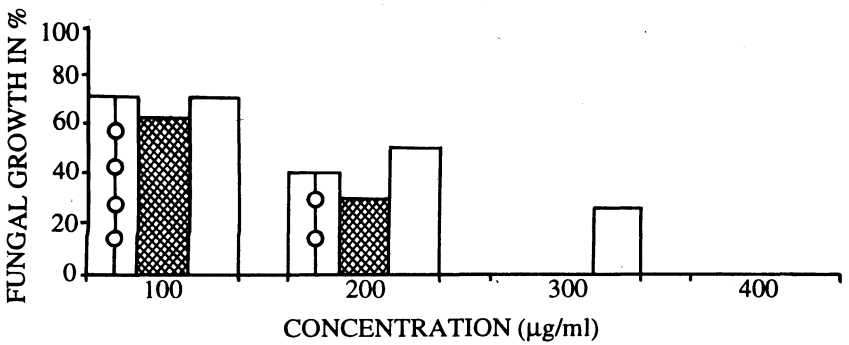

Fig. 1 (b) N,N′,N″-Tri-o-methoxy phenyl phosphoro triamidate

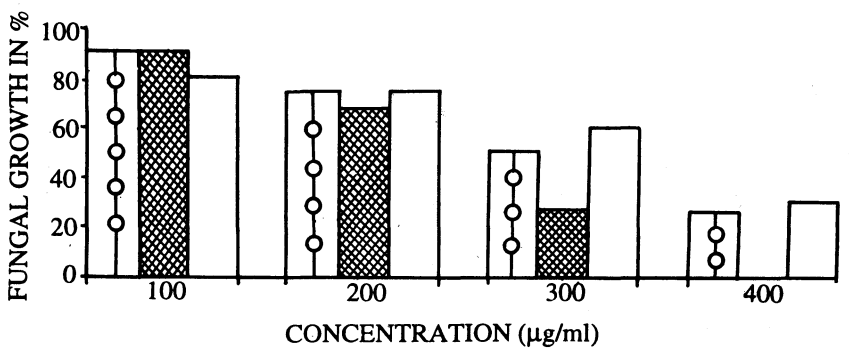

Fig. 1 (c) 3,4-Dichloro-N,N′,N″-Tri-phenyl phosphoro triamidate

Shigella boydii. N,N′,N″-tri-*o*-ethoxy phenyl phosphoro triamidate was more toxic to *Shigella boydii* and less toxic to *Citrobacter frundi* and *E. coli*, while 3,4- dichloro-N,N′,N″-triphenyl phosphoro triamidate was more active to *Shigella*

boydii and *E. coli* and less active to *Citrobacter frundi.* Thus all three phosphorotriamidates can be used as alternative fungistatus and bacteriostatus.

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