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Environmentally benign synthesis and antimicrobial study of novel chalcogenophosphates

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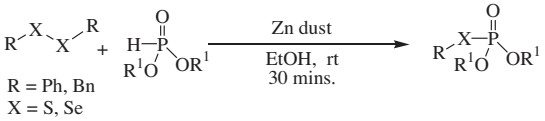
ABSTRACT

We report in this work an environmentally benign zinc mediated synthesis of aryl and benzyl phosphorochalcogenoates in ethanol within a short reaction time. In vitro antimicrobial study along with statistical analysis and seed germination assay were performed. These chalcogenophosphates possess strong antimicrobial activity against the reference strains. The antibacterial activity was determined against four standard strains (*Bacilus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*). The antifungal activity was evaluated against one fungal strain *Candida albicans*.

During the last few years, organochalcogen (Se, S) compounds have been the subject of constant scientific interest due to its broad spectrum of applications in the field of synthesis as well as medicinal biology. The organoselenium compounds found its eligibility as anticancer,[1,2] anti-oxidant,[3–7] anti-inflammatory, anti-allergic agents.[8–11] Organosulfur compounds proved its efficiency in the field of medicinal chemistry as many health benefits have been ascribed to them. They possess several biological activities including antimicrobial,[12,13] antiatherosclerotic,[14,15] lipid and cholesterol lowering effects,[16–18] inhibition of carcinogenesis,[19–24] enhancing the immune system. On the other hand, organophosphorus compounds have important and multifaceted functions in biochemistry of living system. These are associated with antiviral,[25] anticancer,[26] antimicrobial[27–30] activities. They have been found to play an important role in various areas of functional material sciences[31] and particularly, in catalysis as ligands.[32] As both chalcogen and phosphorous scaffolds show strong biological activities, we thought that chalcogenophosphate moieties having both the chalcogen and phosphorus molecules might also show interesting pharmaceutical activities. Therefore, we made an attempt to synthesize various chalcogenophosphate moieties and seek for its biological activities. However, only a few approaches are in literature to synthesize these compounds.[33–36] The major limitations of these reported methods are use of reagents sensitive to air and moisture or long and harsh reaction conditions. Herein, we report an environmentally benign method for the synthesis of some novel *S*-aryl and Se-aryl phosphorochalcogenoates using zinc dust in ethanol solvent at room temperature within a very short time period (Scheme 1).

We made a further antimicrobial study of these scaffolds in order to establish our assumption, against some bacteria as well as fungi. Furthermore, a thorough statistical analysis and seed germination assay was also performed to make our study effective.

Initially we started the reaction with diphenyl diselenide (0.5 mmol) and diethyl phosphite (1 mmol) in presence of catalytic amount of zinc dust (0.2 mmol) under neat condition at room temperature. The desired phenylselenophosphate was obtained in low yield (16%) after 30 min (entry 1, Table 1). The yield of the reaction did not improve after longer time stirring also (24 h). But the formation of the desired product inspired us to proceed further. Increasing the amount of zinc dust from 0.2 to 0.5 mmol, the product yield increased from 16% to 41% (entry 2, Table 1). No significant increment of yield was observed on increasing the



Scheme 1. Synthesis of arylphosphorochalcogenoates.

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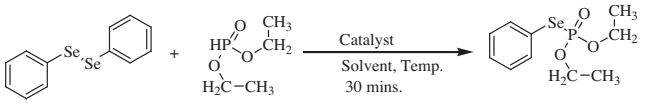
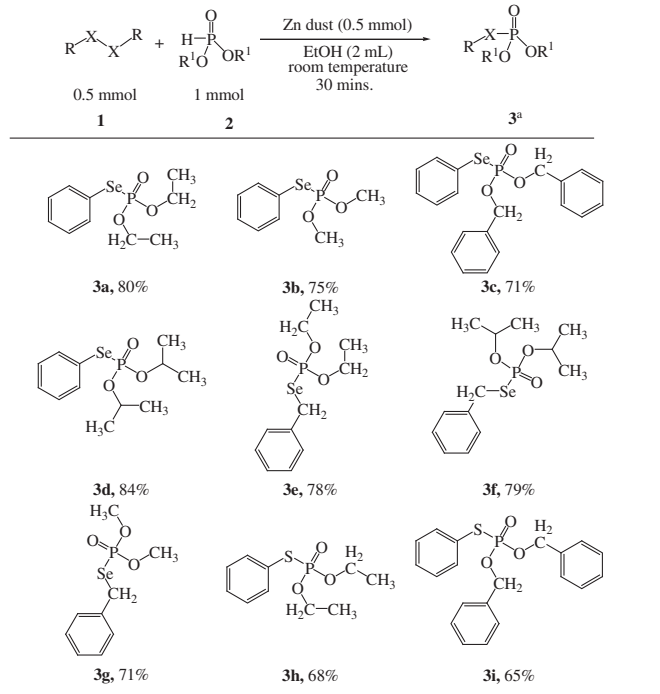
Table 1
Optimization of the reaction conditions[a]



Entry	Catalyst (mmol)	Solvent	Temp (°C)	Yield[b] (%)
1	Zn (0.2)	—	rt	16
2	Zn (0.5)	—	rt	41
3	Zn (0.5)	—	80	43
4	Zn (1.0)	—	rt	44
5	Zn (0.5)	DCM	rt	35
6	Zn (0.5)	DMSO	rt	50
7	Zn (0.5)	Toluene	rt	30
8	Zn (0.5)	CH_3CN	rt	25
9	Zn (0.5)	PEG-400	rt	42
10	Zn (0.5)	H_2O	rt	60
11	Zn (0.5)	Dioxane	rt	20
12	Zn (0.5)	EtOH	rt	80 (81)[c]
13	$ZnCl_2$ (0.5)	EtOH	rt	<5
14	$Zn(OTf)_2$ (0.5)	EtOH	rt	<5
15	$FeCl_3$ (0.5)	EtOH	rt	—
16	CuI (0.5)	EtOH	rt	<5
17	Cu (0.5)	EtOH	rt	18
18	Fe (0.5)	EtOH	rt	27
19	—	EtOH	rt	—

[a] Diphenyl diselenide (0.5 mmol), diethyl phosphite (1 mmol), solvent 2 mL.
[b] Isolated yield.
[c] Reflux condition.

Table 2
Substrate scope



[a] Isolated yield.

temperature from room temperature to 80 °C (entry 3, Table 1). Further we increased the amount of zinc to 1 mmol and similar yield (44%) was obtained (entry 4, Table 1). Next we varied the reaction conditions with various protic and aprotic solvents (entries 5–12, Table 1) using 0.5 mmol zinc dust. A remarkable variation in yield was observed on going from aprotic to protic solvents. Maximum yield was obtained in ethanol (80%). After choosing ethanol as suitable solvent for this reaction, other catalysts were surveyed (entries 13–16, Table 1). However, no improvement of yield was observed on varying these Zn, Fe and Cu catalysts. Lower yields were obtained in case of Cu and Fe dust also (entries 17 and 18, Table 1). The reaction did not proceed at all in absence of Zn dust even after 24 h (entry 19, Table 1). Finally the optimization of the reaction condition was done using 0.5 mmol zinc dust in ethanol solvent under room temperature for 30 min (entry 12, Table 1).

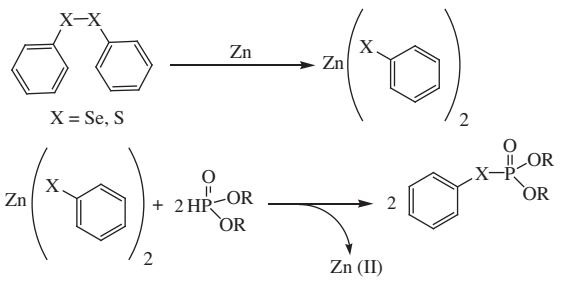
Under the optimized reaction conditions, various chalcogenophosphates were synthesized and the results are summarized in Table 2. Both diphenyl diselenide and dibenzyl diselenide reacted well under the present reaction conditions. A wide range of phosphites such as dimethyl phosphite, diethyl phosphite, diisopropyl phosphite and dibenzyl phosphite were tested and all the phosphites produced moderate to high yields. Phenylthiophosphates were also obtained in moderate yields (**3h** and **3i**, Table 2).

A plausible reaction pathway is outlined in Scheme 2. Initially Zn reacts with diphenyl dichalcogenide to form $Zn(XPh)_2$.[37,38] Probably $Zn(XPh)_2$ reacts rapidly with dialkyl phosphite to form the desired product at room temperature.

Antimicrobial activity of the synthesized compounds was assessed in vitro against two gram positive bacteria *Bacilus subtilis* (MTCC121) and *Staphylococcus aureus* (MTCC1430), two gram negative bacteria *Escherichia coli* (MTCC1610) and *Pseudomonas aeruginosa* (MTCC424) and one fungus *Candida albicans* (MTCC227).

For antimicrobial study, agar diffusion as say cup method was used. The agar plates were prepared by pouring 20 mL of molten nutrient agar medium into sterile petri plates. The plates were allowed to solidify and 0.1% cell suspension (10^6 CFU/mL) of test organisms were spread uniformly and kept for 15 min. After solidification, the wells (6 mm diameter) were prepared. The samples (100 μg/mL) were dissolved in DMSO and poured into the wells prepared in the respective plates. After 24 h of incubation at 30–35 °C, the zone of inhibition was calculated and all experiments were conducted in parallel sets of triplicate. The values calculated as mean ± standard deviation. Data were analyzed by the analysis of variance (ANOVA) using the Origin pro 8 statistical software and the mean differences were separated using Turkey's studentized range test at the 1% level of probability.

The investigation of the antimicrobial screening data along with the statistical analysis shown in Table 3 revealed that the synthesized compounds showed promising results against the microorganisms. For gram positive bacteria *B. subtilis*, compound **3d** acts as the most effective one and is mostly comparable to the effectiveness of the marketed antibacterial drug chlorampenicol. The phenylthiophosphates (**3h** and **3i**) show the antibacterial activity with insignificant variation upto 1% level. Statistically insignificant variation is shown by compound **3d**, **3g** and the marketed drug chlorampenicol, though compound **3d** shows greater activity than



Scheme 2. Plausible mechanism.

Table 3
Inhibitory activity of the compounds **3a–i** expressed as zone of inhibition (mm)[a]

Compound	Gram positive		Gram negative		Fungus
	Bacilus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*P. aeruginosa*	*C. albicans*
3a	31.6 ± 0.2a	30.2 ± 0.3a	24.3 ± 0.5a	32.1 ± 0.4a	34.0 ± 0.3a
3b	22.1 ± 0.3b	28.4 ± 0.3b	22.5 ± 0.3b	25.6 ± 0.6b	28.3 ± 0.7b
3c	23.4 ± 0.4c	20.3 ± 0.1c	27.3 ± 0.2c	24.3 ± 0.3dc	25.9 ± 0.1c
3d	33.1 ± 0.3d	30.5 ± 0.4a	35.1 ± 0.1d	33.4 ± 0.2d	38.6 ± 0.6d
3e	29.9 ± 0.3e	25.4 ± 0.4dg	30.2 ± 0.6e	27.5 ± 0.1e	30.2 ± 0.2e
3f	21.8 ± 0.3b	25.9 ± 0.3d	25.7 ± 0.3f	26.9 ± 0.4f	27.4 ± 0.5bc
3g	32.8 ± 0.1d	31.8 ± 0.5e	22.6 ± 0.4b	25.4 ± 0.2bc	30.5 ± 0.3e
3h	20.6 ± 0.3f	22.5 ± 0.2f	21.0 ± 0.3g	23.6 ± 0.2c	26.4 ± 0.4c
3i	19.8 ± 0.6f	25.1 ± 0.1g	22.9 ± 0.1b	25.4 ± 0.3bc	28.1 ± 0.1b
Chlorampenicol	33.4 ± 0.3d	30.9 ± 0.4a	35.0 ± 0.1d	33.6 ± 0.2d	—
Nystatin	—	—	—	—	39.2 ± 0.6d

Each value represents a mean ± standard deviation (SD) of three replications. Values followed by the same letter(s) in each column are not statistically different according to Turkey's test ($P < 0.01$).

[a] The zone diameters have been calculated in mm by digital vernier caliper.

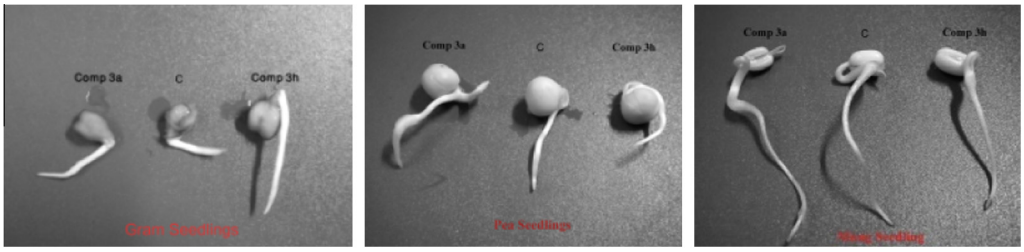


Figure 1. Seed germination assay of compound **3a** and **3h** on gram, pea and mung seeds.

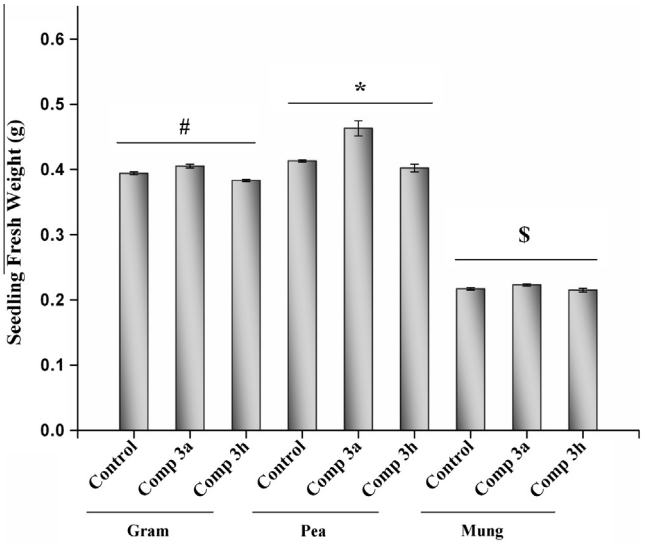


Figure 2. Seed germination assessment: seedling fresh weight.

3g. Compounds **3a** and **3d** varies insignificantly with chlorampenicol though compound **3g** shows the highest activity against *S. aureus*. Compound **3d** shows the highest zone of inhibition and is statistically insignificant with the clinical drug against the reference strain of *E. coli*. Similar conclusion can be drawn that compound **3d** shows the best result against another gram negative bacterial strain *P. aeruginosa*. For the study of antifungal activity, *C. albicans* was chosen and compound **3d** shows almost comparable activity with the clinical drug nystatin. From analysis of variance it can be concluded that compound **3d** (selenophosphoric acid *O*,*O*′-diisopropyl ester Se-phenyl ester) and the marketed drugs chlorampenicol as well as nystatin are not statistically different by its activities upto 1% level of significance, which we found mostly supportive by our assumption on the synthesized products.

After screening for the antimicrobial properties, further seed germination experiments were carried out to investigate the adverse effects of the Se and S containing reference compounds (**3a** and **3h**) on plant system. To perform the toxicity test, compounds **3a** and **3h** were taken for study on gram, pea and mung seeds along with a control experiment. The seeds were kept in the solution of the test compounds and the control seed batch was kept in water. The seedling fresh weight and seedling length was calculated after

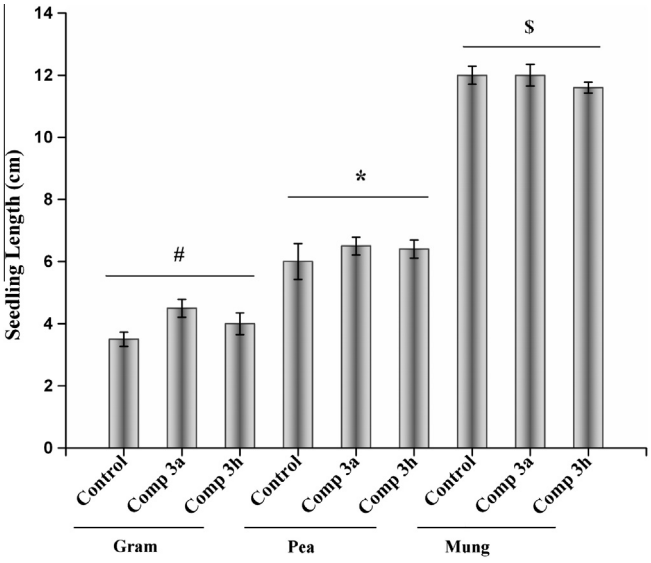


Figure 3. Seed germination assay: seedling length.

48 hours of incubation. Figure 1 gives a pictorial representation of the outcome of the experiment. The results are shown in Figures 2 and 3.

Figures 2 and 3 display the results of the seed germination assay. The bar showed the mean ± standard deviation (SD) (n=3). The #, $*$ and $ indicates the insignificant difference at $P \leqslant 0.05$ level in gram, pea and mung, respectively, between the treatments and the control. Both the graphs indicate that no adverse effects of these compounds on seed germination were observed upto 5% level of significance in compare to the control. Similarly no adverse effect was observed in case of another potent compound **3d**.

In conclusion, we have developed an environmentally benign and convenient method for the synthesis of chalcogenoposphonates. These derivatives possess strong antimicrobial activity. To the best of our knowledge bioactivity study of these compounds has not been reported yet. We believe chalcogenophosphates will be useful in pharmaceutical industries. More bioactivity studies are currently ongoing in our laboratory.

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Supplementary data

Supplementary data (characterization data and NMR spectra) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.03.008.

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