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Green synthesis and antimicrobial activities of diphenyl substituted aryl phosphoramidates

Santhisudha Sarva[1], Mohan Gundluru[1], Vasudha Kolathur[2] and Suresh Reddy Cirandur[1*]

[1] *Department of Chemistry, Sri Venkateswara University, Tirupati-517 502, A.P., India*

[2]*Department of Chemistry, S.P.W. Degree and PG College, Tirupati-517 502, A.P., India*

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Abstract: A green, facile and an efficient protocol has been used for the synthesis of new series of diphenyl substituted aryl phosphoramidates by the reaction of diphenyl phosphoryl chloride and various primary/secondary amines using THF as solvent. 1,4-dimethylpiperazine (DMP) was entrenched as a suitable base for catalysing the formation of a P−N linkage. ^{1}H-NMR, ^{13}C-NMR, ^{31}P-NMR and mass spectral studies were used to characterize all the title compounds. The newly synthesized phosphoramidates were screened for their antimicrobial activity. Most of the compounds depicted good to moderate antimicrobial activity when compared to the standard.

Keywords: Phosphoramidates; P−N linkage; 1,4-dimethylpiperazine; antimicrobial activity; synthesis. ©2022 ACG Publications. All right reserved.

1. Introduction

Phosphonate chemistry[1-12] is ubiquitous in numerous notable chemical ideas in current and future inducements as it delineated curiosity for future research and considerations. Organophosphorus compounds (OPCs) are drawing the attention of researchers because of their wide variety of applications in medical, biochemical, agricultural and industrial areas as well as in organic synthesis[13-16]. In particular, the P−N bond is a vital chemical link due to its prevalence in many useful compounds such as pharmaceuticals and agrochemicals[17]. Among them one such important class having P-N bond linkage compounds are phosphoramidates. There is significant interest in phosphoramidates as they are important structural scaffolds found in many naturally occurring biologically active compounds such as agrocin 84 and phosmidosine[18]. In addition, phosphoramidates are employed as ligands[19] for catalysts used in asymmetric synthesis and as labelling groups in spectrometric applications[20]. They are also used as labeling groups to improve sensitivity in mass

* Corresponding author: E-Mail: csrsvu@gmail.com

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spectroscopy[21] and as flame retardants[22]. Phosphoramidates are used in agriculture as insecticides, fungicides, and herbicides[23], and in medicine as antifungal, anti-HIV, and antitumor agents[24]. Further, they are precursors to many organic compounds such as imines[25], amines[26], azetidines[27] and aziridines[28].

In 2013, Hayes et al.[29] developed the synthesis of *N*-acylphosphoramidates by copper-catalyzed aerobic oxidative coupling between amines and phosphonates with excellent results. Dhineshkumar et al.[30] developed an elegant synthetic route to phosphoramidates, phosphorus triesters and sulfoximine-derived phosphoramidate by using iodine as a catalyst in a stoichiometric amount and H_2O_2 as an oxidant to obtain the desired products in good yields.

Meazza et al.[31] reported simple P–N bond-forming cross dehydrogenative coupling reactions between amines and phosphites for the synthesis of phosphoramidates in a one-pot manner by utilizing light-emitting diodes (LEDs), an organic dye as the photocatalyst and air as the oxidant. Gupta et al.[32] developed one-pot synthesis of *O,O*-dialkyl *N,N'*-dialkyl phosphoramidates from dialkyl phosphites and dialkyl amines in the presence of trichloroisocyanuric acid. Recently, in the 2018, Reddy et al.[33] prepared 2-(benzo[d]thiazol-2-yl) phenyl-4-nitrophenyl alkyl/aryl substituted phosphoramidates *via* two-step *in situ* methods.

However, these methods have many disadvantages such as long reaction times, utilize moisture-sensitive toxic catalysts, require stoichiometric amounts of catalysts, offer poor product yields and generate large amounts of waste. 1,4-dimethylpiperazine[34-35] has found to be a versatile catalyst indulging in the synthesis of various new OPCs. Taking into account the aforementioned contingent, we have focused our studies on diphenyl phosphoramidates and have developed a new, simple and convenient method for their synthesis using catalytic amounts of 1,4-dimethylpiperazine.

Further, the synthesized compounds were studied for their antimicrobial activity to check whether the active principles (phosphoramidate derivatives) inhibit the growth of microbes, prevent the formation of microbial colonies and destroy microorganisms.

2. Experimental

2.1. General Experimental Methods

Reagents were purchased from commercial sources. All solvents were purified and dried by standard procedures. All the reactions were monitored by thin-layer chromatography (TLC) on silica gel GF254 plates from Qingdao Haiyang Chemical Co. Ltd (China) and visualized in an iodine chamber with UV lamp (254 nm). Column chromatography was performed using merck silica gel (100–200 mesh). The melting points of the products were determined on a Guna Digital melting point apparatus (China) and are uncorrected. The IR spectra were recorded on a Bruker Alpha ECO-ATR FTIR (attenuated total reflection-Fourier transform infrared) interferometer with a single reflection sampling module equipped with a Zn-Se crystal. Elemental analysis was performed on an Elementar Vario-III CHN analyzer. NMR spectra were recorded on a JEOL (model no. JNM-ECS400) (400 MHz for ^{1}H, 100 MHz for ^{13}C, 125 MHz for ^{31}P) using $CDCl_3$ or DMSO-d_6 as solvent. TMS ($\delta = 0$) served as an internal standard for ^{1}H NMR and ^{13}C NMR and H_3PO_4 ($\delta = 0$) was used as an external standard for ^{31}P NMR. HRMS spectra were recorded on a Micromass Q-TOF spectrometer using electrospray ionization**.**

*2.2. General Procedure for the Synthesis of Phosphoramidates **3a-l***

To a 50mL round-bottomed flask containing diphenyl phosphoryl chloride (**1**) and the aromatic/substituted piperazine amine, 20mL THF was added. To this mixture, 1,4-dimethylpiperazine (DMP) was added and the mixture was stirred vigorously at 60°C for 1-3 h. The progress of the reaction was monitored by TLC. After completion of the reaction, DMP.HCl was removed by filtration, the filtrate was concentrated under vacuum and the resulting solid was purified by passing

through a column of silica gel using hexane/ethyl acetate (2:1) as the eluent to afford the title compound.

2.3. Spectral Data of Representative Compounds

Diphenyl (4-(benzo[d]isothiazol-3-yl)piperazin-1-yl)phosphonate (***3a***): Dark brown solid, mp: 210°C. IR: (KBr, cm^{-1}) ν 1248.20, 1193.52, 761.35; ^{1}H NMR: ($CDCl_3$, 400 MHz), δ: 7.84-7.17 (m, 14H), 3.52 (s, 4H), 3.42 (s, 4H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 165.0, 152.8, 150.8, 129.8, 127.8, 125.1, 124.1, 120.7, 120.2, 120.1, 50.2, 44.6; ^{31}P NMR ($CDCl_3$, 125 MHz) δ: 0.68; MS (ES): Calculated for $C_{23}H_{22}N_3O_3PS$ *m/z*: 451.11, found 451.01.

*Diphenyl quinolin-8-yl-phosphoramidate (**3b**):* Dark brown solid, mp: 240°C. IR: (KBr, cm^{-1}) ν 3460.89, 1220.78, 1209.60, 768.56; ^{1}H NMR: ($CDCl_3$, 400 MHz), δ: 8.86-7.21 (m, 16H), 6.65 (s, 1H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 148.8, 143.4, 136.5, 134.6, 132.6, 130.5, 127.3, 121.5, 121.2, 120.8, 119.2, 117.3, 113.4; ^{31}P NMR ($CDCl_3$, 125 MHz) δ: 0.84; MS (ES): Calculated for $C_{21}H_{17}N_2O_3P$ *m/z*: 376.10, found 376.02.

Diphenyl (4-(pyrimidin-2-yl)piperazin-1-yl)phosphonate (***3c***): Dark brown solid, mp: 207 °C. IR: (KBr, cm^{-1}) ν 1248.90, 1193.98, 761.45; ^{1}H NMR: ($CDCl_3$, 400 MHz), δ: 8.27-6.46 (m, 13H), 3.35 (s, 4H), 2.82 (s, 4H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 161.5, 157.8, 150.8, 129.8, 125.1, 120.2, 110.4, 44.6, 43.8; ^{31}P NMR ($CDCl_3$, 125 MHz) δ: 0.92; MS (ES): Calculated for $C_{20}H_{21}N_4O_3P$ *m/z*: 396.14, found 396.03.

Diphenyl (2-fluoro-4-((2-(methylcarbamoyl)pyridin-4-yl)oxy)phenyl)phosphoramidate (***3d***): Dark brown solid, mp: 160 °C. IR: (KBr, cm^{-1}) ν 3420.89, 1230.35, 1209.79, 758.37; ^{1}H NMR: ($CDCl_3$, 400 MHz), δ: 8.48 (s, 1H), 7.57-6.73 (m, 16H), 6.16 (s, 1H), 3.06 (s, 3H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 173.2, 157.2, 157.1, 150.2, 148.6, 133.1, 130.1, 128.5, 121.3, 120.5, 120.3, 117.6, 111.8, 25.8; ^{31}P NMR ($CDCl_3$, 125 MHz) δ: -11.2; MS (ES): Calculated for $C_{25}H_{21}FN_3O_5P$ *m/z*: 493.11, found 492.99.

Diphenyl (4-(6-fluorobenzo[d]isoxazol-3-yl)piperidin-1-yl)phosphonate (***3e***): Dark brown solid, mp: 242 °C. IR: (KBr, cm^{-1}) ν 1262.24, 1207.56, 756.98; ^{1}H NMR: ($CDCl_3$, 400 MHz), δ: 7.47-6.93 (m, 13H), 3.32 (s, 4H), 2.13 (s, 1H), 1.78 (s, 4H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 166.3, 164.3, 150.8, 147.3, 131.3, 123.4, 121.3, 120.3, 45.5, 31.5, 27.4;^{31}P NMR ($CDCl_3$, 125 MHz) δ:-8.8; MS (ES): Calculated for $C_{25}H_{21}FN_3O_5P$ *m/z*: 452.13, found 452.01.

Diphenyl (4-(2-fluorophenyl)piperazin-1-yl)phosphonate (***3f***): Dark brown solid, mp: 190°C. IR: (KBr, cm^{-1}) ν 1230.67, 1209.89, 811.02; ^{1}H NMR: ($CDCl_3$, 400 MHz), δ: 7.47-6.51 (m, 14H), 3.42 (s, 4H), 2.77 (s, 4H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 150.8, 150.2, 137.4, 130.1, 125.2, 122.3, 121.3, 120.3, 52.6, 46.5; ^{31}P NMR ($CDCl_3$, 125 MHz) δ: -8.6; MS (ES): Calculated for $C_{22}H_{22}FN_2O_3P$ *m/z*: 412.14, found 412.00.

Diphenyl (3-fluoro-4-morpholinophenyl)phosphoramidate (***3g***): Dark brown solid, mp:170 °C. IR: (KBr, cm^{-1}) ν 3216.15, 1259.80, 1212.57, 814.05; ^{1}H NMR: ($CDCl_3$, 400 MHz), δ: 7.40-6.13 (m, 13H), 6.13 (s, 1H), 3.72 (s, 4H), 3.18 (s, 4H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 156.3, 150.2, 130.8, 130.1, 127.3, 121.3, 120.3, 116.7, 112.8, 105.7, 66.3, 53.3; ^{31}P NMR ($CDCl_3$, 125 MHz) δ:9.4; MS (ES): Calculated for $C_{22}H_{22}FN_2O_4P$ *m/z*: 428.41 found 428.12.

Diphenyl (4-methylbenzo[d]thiazol-2-yl)phosphoramidate (***3h***): Dark brown solid, mp:187 °C. IR: (KBr, cm^{-1}) ν 3420.98, 1240.95, 1210.25, 760.24; ^{1}H NMR: ($CDCl_3$, 400 MHz), δ: 7.92-7.21 (m, 13H), 5.45 (s, 1H), 1.92 (s, 3H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 174.5, 150.2, 147.2, 131.4, 130.5, 126.6, 125.9, 124.4, 123.6, 120.3, 118.5, 16.1; ^{31}P NMR ($CDCl_3$, 125 MHz) δ: -12.2; MS (ES): Calculated for $C_{20}H_{17}N_2O_3PS$ *m/z*: 396.12 found 396.02.

*Diphenyl (3-(trifluoromethyl)phenyl)phosphoramidate (**3i**)*: Dark brown solid, mp:215°C. IR: (KBr, cm^{-1}) ν 3450.67, 1230.46, 1209.49, 758.08; ^{1}H NMR: ($CDCl_3$, 400 MHz), δ: 7.30-6.83 (m, 14H), 6.76(s, 1H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 150.2, 140.2, 132.5, 131.8, 130.1, 129.5, 124.3, 124.1, 122.5, 115.1, 112.5; ^{31}P NMR ($CDCl_3$, 125 MHz) δ: -11.2; MS (ES): Calculated for $C_{19}H_{15}F_3NO_3P$ *m/z*: 393.07 found 393.01.

Diphenyl (1H-imidazo[1,5-c]imidazol-2(3H)-yl)phosphonate (***3j***): Dark brown solid, mp:182 °C. IR: (KBr, cm^{-1}) ν 3465.69, 1230.45, 1209.79, 758.58; ^{1}H NMR: ($CDCl_3$, 400 MHz), δ: 7.47-7.23 (m, 12H), 3.46 (s, 2H), 3.16 (s, 2H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 152.1, 137.5, 130.1, 126.2, 121.9, 121.3, 120.3, 64.1, 43.4; ^{31}P NMR ($CDCl_3$, 125 MHz) δ: -8.2; MS (ES): Calculated for $C_{17}H_{16}N_3O_3P$ *m/z*: 341.30 found 341.19.

Diphenyl (4-6-difluorobenzo[d]thiazol-2-yl)phosphoramidate (***3k***): Dark brown solid, mp:190°C. IR: (KBr, cm^{-1}) ν 3400.45, 1230.86, 1180.39, 758.68; ^{1}H NMR: ($CDCl_3$, 400 MHz), δ: 7.57-6.73 (m, 12H), 5.46 (s, 1H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 174.5, 163.2, 157.2, 150.1, 130.5, 130.1, 128.5, 121.3, 120.5, 103.6, 99.1; ^{31}P NMR ($CDCl_3$, 125 MHz) δ: -18.2; MS (ES): Calculated for $C_{19}H_{13}F_2N_2O_3PS$ *m/z*: 418.14 found 418.02.

Diphenyl (4-(4-chlorophenyl)piperazin-1-yl)phosphonate (***3l***): Dark brown solid, mp: 222 °C. IR: (KBr, cm^{-1}) ν 3465.82, 1230.48, 1209.95, 758.57. ^{1}H NMR: ($CDCl_3$, 400 MHz), δ: 7.40-6.71 (m, 14H), 3.46 (s, 4H), 2.77 (s, 4H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 150.8, 147.2, 130.1, 129.2, 127.3, 121.3, 120.3, 115.7, 52.6, 46.5; ^{31}P NMR ($CDCl_3$, 125 MHz) δ: -8.9; MS (ES): Calculated for $C_{22}H_{22}ClN_2O_3P$ *m/z*: 428.11 found 428.01.

2.4. *In vitro Antimicrobial Activity*

2.4.1. Antibacterial Activity

The antibacterial activity of compounds **3a-l** was studied using the agar diffusion method[22]. The test samples of compounds **3a-l** and the standard streptomycin (positive control) were prepared by taking 1 mg of each compound in dimethyl sulfoxide (DMSO) (negative control) and diluting further to the required concentration of 50 g/mL. A reference standard was taken (2.5 mg dissolved at 250 µL of distilled water) and Nutrient Agar (Hi-media) (7.0 g) was dissolved in distilled water (250 mL). The medium was sterilized under 15 lb of pressure for 15 minutes in an autoclave. Next, 30 mL of this sterilized semi-solid nutrient agar medium was added to pre-sterilized 90 mm glass petri plates under aseptic conditions in a laminar airflow chamber. The plates were allowed to cool at room temperature to solidify the medium. After 5 min, a sterile filter paper disc (6 mm) containing 5 µL of the compound was placed on the surface of a plate. The plate was incubated at 37 ºC for 24 hours. The antibacterial activity of the test compounds were expressed by measuring the diameter of the inhibition zone (DIZ).

2.4.2. Antifungal Activity

The antifungal potency of the products **3a-l** was investigated against *Aspergillus niger* and *Rhizopus oryzae* using the agar diffusion technique[23]. Test samples of the products and the standard fluconazole were prepared by dissolving 2.5 mg of the test compound in dimethylsulfoxide (250 mg) and diluting them to the required concentration of 50 g/mL. Test samples were mixed with sterilized potato dextrose agar medium (PDA) in separate flasks and transferred to the Petri plates.

Potato dextrose agar (Hi-media) (9.75 g) was dissolved in 250 mL of distilled water. The medium was sterilized under 1.03 barof pressure for 15 minutes in an autoclave. Next, 30mL of this sterilized semi-solid nutrient agar medium was poured in pre-sterilized 90 mm glass Petri plates under aseptic conditions in a laminar air flow chamber. The plates were cooled at room temperature to solidify the medium. By using the agar diffusion technique, the components of an extract or a pure compound diffuse through an agar plate towards a growing colony of fungus. If there are active compounds

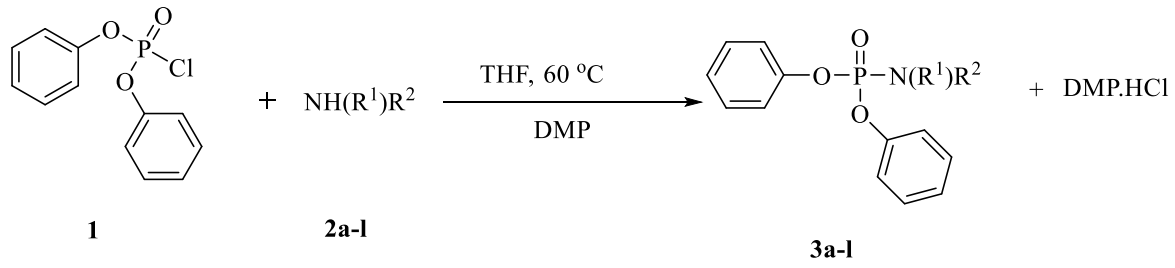
present, the growth of the fungus is either slowed or stopped, resulting in the deformation of the colony. Two principal assay techniques commonly employed are the disc- diffusion technique and the agar-well technique.

Both assays require agar plates to be prepared, which should contain a fixed amount of thoroughly homogenous medium. Between 15 and 20 mL is enough to fill special syringes available for dispensing fixed volumes of agar. It is common practice to store the agar plates for 24 h after pouring to allow any excess moisture to escape prior to the assay 24 h and the zone of inhibition is then measured.

Based on the results of zone of inhibition, the minimum inhibitory concentration (MIC) of compounds **3a-l** against all bacterial and fungal strains were determined by liquid dilution method. Stock solutions of tested compounds with 200, 100, 50, 25, 12.5 and 6.25 μg/cm^3 concentrations were prepared with DMSO solvent. The solutions of standard drugs, streptomycin and flucanozole were prepared in the same concentrations. Inoculums of the fungal culture were also prepared. A series of tubes containing 1 mm^3 of title compound solution at various concentrations and 0.2 mm3 of inoculums were added. Further 3.8 mm^3 of the sterile water was added to each of the test tubes. These test tubes were incubated for 24 h at 37°C and observed for the presence of turbidity. This method was repeated by changing title compounds with standard drugs streptomycin and flucanozole for comparison. The minimum inhibitory concentration at standard drug flucanozole for comparison. The minimum inhibitory concentration at which no growth was observed was taken as the MIC value.

3. Results and Discussion

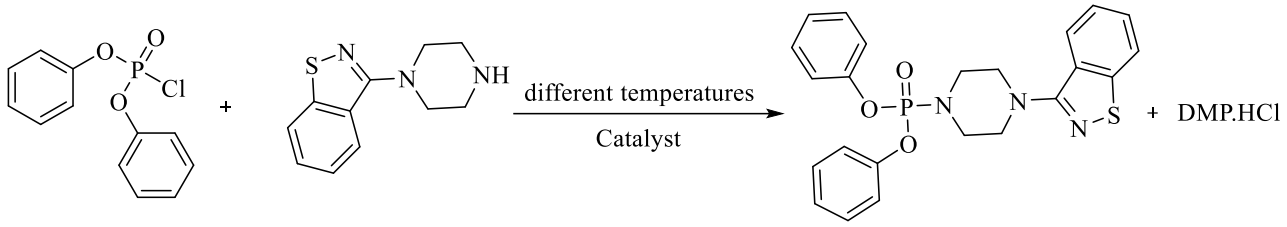
The title compounds 3a-l were synthesized by the reaction of equimolar quantities of diphenyl phosphoryl chloride 1 with various aromatic and substituted piperazine amines **2a-l** in THF at 60°C in the presence of 1,4-dimethylpiperazine (DMP) as a base for 1-3 h. The corresponding diphenyl phosphoramidates **3a-l** were obtained in yields of 82-95%. All the synthesized compounds were purified by column chromatography using hexane/ethyl acetate (1:1) as the eluent.



Scheme 1. Synthesis of diphenyl phosphoramidates **3a-l**

In order to optimize the experimental conditions, a model reaction was carried out for the synthesis of diphenyl (4-(benzo[*d*]isothiazol-3-yl)piperazin-1-yl)phosphonate (**3a**) by the reaction of diphenyl phosphorochloridate (**1**) and 3-(piperazin-1-yl)benzo[*d*]isothiazole (**2a**). At the outset, the reaction under catalyst-free conditions with conventional heating resulted in very low yields, even after 24 h (Entry 1, Table 1). Next, we investigated a wide range of catalysts, including tetramethylguanidine (TMG), Et_3N, DBU, cyclodextrin, diethylamine, pyrrolidine, thiomorpholine, morpholine and dimethylpiperzine (DMP) (Entries 2-11, Table 1) for the reaction. Among all the catalysts DMP (20 mol%) worked effectively and provided an excellent yield of the desired product **3a**. Decreased amounts of the catalyst from 10 mol% gave lower yields, even after prolonged reaction times while increased mol% quantities did not lead to higher product yields or shorter reaction times. The reusability of the DMP catalyst was also examined for this reaction.

Table 1. Optimization of the reaction conditions for the synthesis of compound **3a**.[a]



1 **2a** **3a**

Entry	Catalyst (mol%)	Temperature (°C)	Time (h)	Yield (%)[b]
1	None	60	24	trace
2	TMG (10)	70	12	70
3	Et_3N (10)	65	12	70
4	DBU (10)	60	10	65
5	cyclodextrin (10)	65	10	60
6	diethylamine (10)	60	8	70
7	pyrrolidine (10)	65	8	65
8	thiomorpholine (10)	60	6	75
9	morpholine (10)	60	8	75
10	dimethylpiperzine (DMP) (10)	60	2	85
11	dimethylpiperzine (DMP) (20)	60	1	90

[a] Reaction of diphenyl phosphorochloridate (**1**), 3-(piperazin-1-yl)benzo[*d*]isothiazole (**2a**) using different catalysts and temperatures. [b] Isolated yields.

Subsequently, we investigated the effect of different non-polar solvents (Entries 1-5, Table 2) polar protic solvents (Entries 6-11, Table 2) and polar aprotic solvents (Entries 12-17, Table 2) for this reaction in the presence of DMP (20 mol%) as the catalyst. The non-polar solvents afforded very low product yields. Polar protic solvents furnished the desired product **3a** in moderate yields. Improved results were obtained with polar aprotic solvents and DMP as the catalyst. The best result was obtained with THF as the solvent and DMP (20 mol%) as the catalyst. Under these conditions, the yield was improved to 95% in Table 2 and the time was reduced to 1 h.

Table 2. Solvent effect on the synthesis of **3a** in the presence of DMP

Entry	Solvent	Time (h)	Temp (°C)	Yield (%)[b]
1	diethyl ether	12	40	30
2	benzene	12	60	40
3	$CHCl_3$	12	65	55
4	1,4-dioxane	12	80	50
5	toluene	12	80	55
6	acetic acid	8	90	60
7	*n*-butanol	8	65	65
8	ethanol	8	70	60
9	methanol	8	60	60
10	*n*-propanol	8	65	70
11	isopropanol	8	65	65
12	dimethylformamide	8	80	65
13	dichloromethane	8	60	70
14	ethyl acetate	5	65	72
15	acetone	5	65	75
16	acetonitrile	5	70	80
17	tetrahydrofuran	1	60	95

[a] Reaction of diphenyl phosphorochloridate (**1**) and 3-(piperazin-1-yl)benzo[d]isothiazole (**2a**) using different solvents, temperatures and times. [b]Isolated yields.

Synthesis of diphenyl substituted aryl phosphoramidates

The optimization of the required quantity of the catalyst DMP using THF as the solvent was carried out at 60ºC (Table 3, entries 1–9) over 1-2 h. We obtained a 95% with 20 mol% of the catalyst in 1h. On increasing the quantity of catalyst to 20mol%, the rate of the reaction and yield of the product did not improve.

Table 3. Optimization of the catalyst loading for the synthesis of **3a**.

Entry	Cat (mol%)	Time (h)	Yield (%) [b]
1	1	2	70
2	3	2	75
3	5	2	80
4	7	2	85
5	10	2	86
6	15	1	88
7	20	1	95
8	25	1	95
9	30	1	95

[a] Reaction of diphenyl phosphorochloridate (**1**) and 3-(piperazin-1-yl) benzo[*d*]isothiazole (**2a**) using different catalysts; [b] Isolated yields.

The different substrates (**2a-l**) and the synthesized diphenyl phosphoramidates structures (**3a-l**) were given in the Table 4.

Table 4. Synthesis of phosphoramidates in the presence of DMP using THF as the solvent.

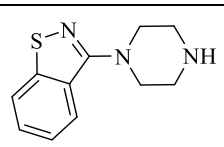
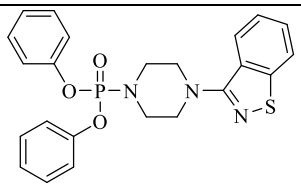
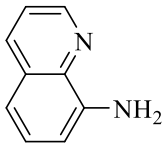
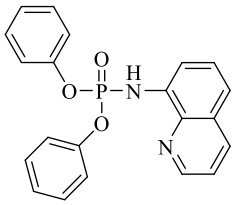
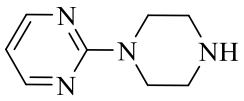
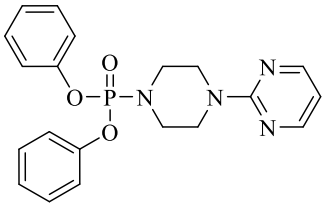
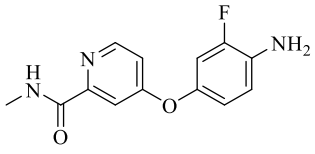
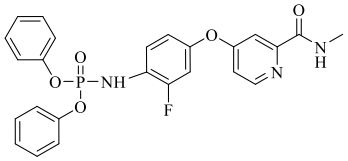
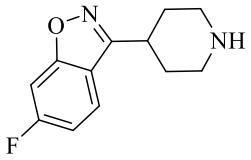
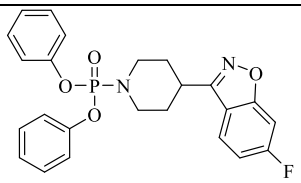
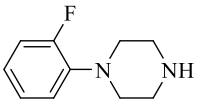
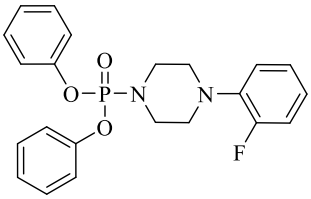
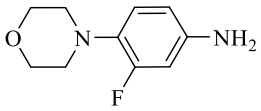
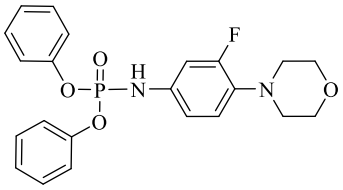
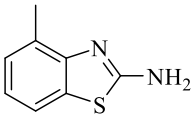
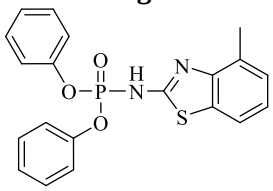
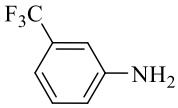
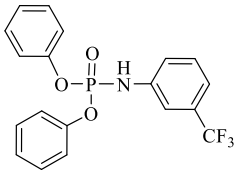
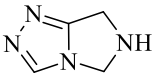
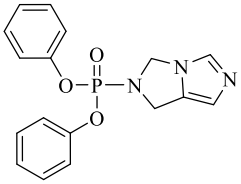
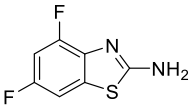
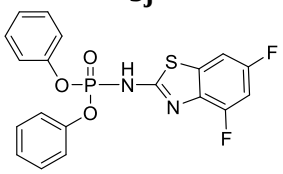
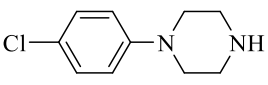
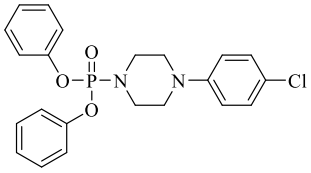
Entry	Substrate	Product	Time (h)[a]	Yield (%)[b]
1	 **2a**	 **3a**	1	95
2	 **2b**	 **3b**	1	85
3	 **2c**	 **3c**	1.5	84
4	 **2d**	 **3d**	3	88

Table 4 continued..

5	 **2e**	 **3e**	2	86
6	 **2f**	 **3f**	2	88
7	 **2g**	 **3g**	1	86
8	 **2h**	 **3h**	3	82
9	 **2i**	 **3i**	3	84
10	 **2j**	 **3j**	3	86
11	 **2k**	 **3k**	2	88
12	 **2l**	 **3l**	2	86

[a] Reactions of diphenyl phosphorochloridate (1.5 mmol) **1** and (1 mmol) N-Substituted amines and aromatic and non-aromatic piperazines **2a-l** in the presence of 20 mol% of DMP as the catalyst in THF. [b] Isolated yields.

The chemical structures of all the products **3a-l** were determined by IR, ^{1}H NMR, ^{13}C NMR and ^{31}P NMR Spectroscopy. The IR spectra of the products showed absorption bands for NH stretching vibrations at 3465-3216 cm^{-1}; -P=O and -P-O stretching vibrations at 1262-1220 and 1210-1180 cm^{-1} and -P-N stretching vibrations at 814-756 cm^{-1}. A singlet is observed in the region from 6.76-5.45 ppm for the NH Proton. For the aromatic protons, chemical shifts were observed in the region from 8.86-6.13 ppm. The peaks were observed in the region from 3.57-2.77 ppm for the $(N\text{-}CH_2)_2$ protons. The chemical shifts

of the aromatic and aliphatic carbons appeared between 174 and 16 ppm in the ^{13}C NMR spectra. The ^{31}P NMR chemical shifts of the products occurred as singlets between -12.2 and 0.68 ppm.

The antibacterial activity[36-38] of compounds **3a-l** was studied using the agar diffusion method.[39] The antibacterial activities of the newly synthesized products **3a-l** were screened against two Gram-positive bacteria, *Bacillus subtilis* (MTCC-441) and *Staphylococcus aureus* (MTCC-737) and two Gram-negative bacteria, *Escherichia coli* (MTCC- 443) and *Pseudomonas aeruginosa* (MTCC-741), using the paper disk diffusion method (Table 5). Minimum inhibitory concentration (MIC) of all compounds was determined, which is defined as the lowest concentration of inhibitor at which bacterial growth was not visually apparent.

Compounds **3a-l** exhibited moderate to good activity against both the Gram +ve and Gram –ve bacteria in comparison with the standard streptomycin. Compounds **3k** and **3a** showed high and significant activity against Gram +ve and Gram –ve bacteria when compared to the standard streptomycin. The SAR of antibacterial activity of the synthesized compounds (3a-l) discloses that the compounds **3k** and **3a** having thiazole group and heteroatom fluorine showed good antibacterial activities when compared to others. Compounds **3d** and **3e** showed better activity when compared to the standard against Gram +ve and Gram (-) bacteria. This might be due to the presence of different heteroatoms having nitrogen, oxygen and fluorine. The remaining compounds showed low activity compared to the standard.

Table 5. Antibacterial activity of the compounds **3a-l**[a]

Compound	**Gram +ve**		**Gram –ve**	
	Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*
3a	32.4 ± 0.06	28.6 ± 0.02	30.2 ± 0.04	36.8 ± 0.05
3b	14.6 ± 0.04	18.8 ± 0.02	8.4 ± 0.02	13.8 ± 0.04
3c	18.6 ± 0.06	16.4 ± 0.04	12.8 ± 0.04	11.3 ± 0.05
3d	28.6 ± 0.02	25.4± 0.02	28.8 ± 0.04	33.5 ± 0.04
3e	28.4 ± 0.02	24.9 ± 0.06	28.6 ± 0.02	32.8 ± 0.02
3f	12.6 ± 0.04	8.6 ± 0.04	9.2 ± 0.05	13.1 ± 0.03
3g	16.2 ± 0.02	12.6 ± 0.06	11.8 ± 0.07	16.8 ± 0.03
3h	19.8 ± 0.06	16.6 ± 0.02	10.4 ± 0.02	18.3 ± 0.06
3i	16.6 ± 0.04	9.7 ± 0.04	8.0 ± 0.06	9.6 ± 0.03
3j	8.5 ± 0.02	12.6 ± 0.02	14.4 ± 0.02	8.7 ± 0.02
3k	30.6 ± 0.06	27.8± 0.06	18.2 ± 0.04	34.8 ± 0.05
3l	6.5 ± 0.04	14.8 ± 0.02	29.6 ± 0.02	6.8 ± 0.02
streptomycin	26.8 ± 0.05	24.6 ± 0.04	28.4 ± 0.06	32.6 ± 0.05

[a] Zone of inhibition.

The antifungal potencies[36-38] of newly synthesized products **3a-l** were investigated against the fungi *Aspergillus niger* and *Rhizopus oryzae* using the agar diffusion technique.[40] Minimum inhibitory concentration (MIC) of all compounds was determined, which is defined as the lowest concentration of inhibitor at which fungal growth was not visually apparent.

The antifungal activity profiles (Table 6) showed that **3a, 3d and 3e** have significantly higher activity when compared to standard flucanozole. This is due to the presence of thiazole group and presence of different heteroatoms like nitrogen, oxygen and fluorine in the compound respectively.

Table 6. Antifungal activity of compounds **3a-l**

Compound	*Aspergillus niger*	*Rhizopus oryzae*
3a	30.4 ± 0.04	28.8 ± 0.04
3b	8.8 ± 0.04	8.5 ± 0.03
3c	9.6 ± 0.02	13.8 ± 0.02
3d	29.6 ± 0.03	26.6 ± 0.02
3e	28.4 ± 0.02	24.2 ± 0.04
3f	12.3 ± 0.04	9.4 ± 0.03
3g	18.6 ± 0.02	11.6 ± 0.02
3h	16.6 ± 0.03	12.3 ± 0.04
3i	17.4 ± 0.03	14.6 ± 0.02
3j	11.2 ± 0.02	11.4 ± 0.04
3k	8.6 ± 0.04	12.2 ± 0.03
3l	16.4 ± 0.02	18.6 ± 0.02
fluconazole	27.8 ± 0.04	24.8 ± 0.06

Table 7. *In vitro* antimicrobial activity studies of phosphoramidates **(3a-l)**

Minimum inhibitory concentration (MIC) in µg/mL							
S. No.	**Tested Samples**	***Bacterial Strains***				***Fungal Strains***	
		Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Aspergillus niger*	*Rhizopus oryzae*
1	**3a**	6.25	6.25	6.25	6.25	31.25	31.25
2	**3b**	100	100	100	100	125	125
3	**3c**	200	200	200	200	500	500
4	**3d**	6.25	6.25	6.25	6.25	31.25	31.25
5	**3e**	6.25	6.25	6.25	6.25	31.25	31.25
6	**3f**	100	100	100	100	250	250
7	**3g**	200	200	200	200	125	125
8	**3h**	100	100	200	100	250	250
9	**3i**	200	200	100	200	500	64.50
10	**3j**	100	100	100	100	125	500
11	**3k**	6.25	6.25	6.25	6.25	500	64.50
12	**3l**	100	100	100	100	125	500
13	**streptomycin**	6.25	6.25	6.25	6.25	-	-
14	**fluconazole**	-	-	-	-	31.25	31.25

4. Conclusion

1,4-Dimethylpiperazine (DMP) is found to be a highly efficient catalyst for the synthesis of phosphoramidates at ambient temperature. The operational simplicity, high yields and simple work-up make this a green alternative to currently existing protocols. The antibacterial and antifungal properties of the synthesized compounds **3a-l** were evaluated and the compounds **3k**, **3a**, **3d** and **3e** exhibited noticably higher antibacterial activity and the compounds **3a**, **3d** and **3e** manifested significant antifungal activity than the standard.

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Supporting Information

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ORCID ID

Santhisudha Sarva : 0000-0002-5474-9784
Mohan Gundluru : 0000-0003-1456-4774
Vasudhakolathur : 000-0002-5262-6764
Suresh Reddy Cirandur : 0000-0002-9804-9683

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