

Research Article

Synthesis and Evaluation of Biological Activity of New Arylphosphoramidates

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The synthesis of new substituted arylphosphoramidates is performed in two steps through phosphorylation of the corresponding alcohols followed by aminolysis. The formation of the desired phosphoramidates depends on the subsequent addition of the two alcohols with the amine being added at the last step. The products were obtained in 58–95% yields. They were characterized mainly by multinuclear (¹H, ¹³C, ³¹P, and ¹⁹F) NMR and IR spectroscopy. In addition, the antimicrobial and antiacetylcholinesterase activities were evaluated. The results showed acetylcholinesterase activity by some compounds, whilst no significant inhibitory effect against the tested bacterial strains has been recorded.

1. Introduction

Organophosphorus compounds are widely used as pesticides and chemical weapon agents because of their inhibitory effect on acetylcholinesterase [1]. The development in the field of medicinal chemistry of these compounds is currently characterized by a more marked orientation towards the synthesis of their derivatives as prodrugs for pharmaceutical purposes [2, 3]. Recent studies have shown that phosphoramidates and phosphates can be used as anticancer agents [4, 5], anti-HIV [6], and against Alzheimer's disease [7]. It was shown [8] that some phosphoramidates are active against strains of *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus mutans*. It was also shown [9] that they are bacterial enzyme inhibitors, aspartate semialdehyde dehydrogenase (ASA-DH), which is involved in the biosynthesis of the aspartate family of amino acids. The biological activity of these compounds was also shown to depend significantly on the phosphorus atom substituents [10]. Thus, p-nitrophenylphosphoramidate derivatives were proven to be considerably stronger [11]

than the methamidophos which is known for its acetylcholinesterase (AChE) inhibition and insecticidal property [12]. Furthermore, it was shown that phosphoramidates could be very useful for studying the mechanism of prophylaxis against poisoning by organophosphates and also reported that the p-nitrophenylphosphoramidates protect the guinea pigs against poisoning by Soman neurotoxic gas [13].

Inspired by these results and in continuation of our research on the complexing properties of phosphorylated compounds [14–17], we have already studied in a previous paper the complexes SnCl₄·2L by multinuclear NMR at variable temperature of a series of new arylphosphoramidates with the formula (ArO)P(O)(NR₂)(OR') [18]. We have found that tuning substituents nondirectly bounded at phosphorus atom as R, R', and Ar groups have affected the donor character of the phosphoryl group towards tin atom. In this paper we describe the synthesis of these arylphosphoramidates and their biological activity tests against bacterial strains and acetylcholinesterase enzyme.

2. Materials and Methods

2.1. Chemistry

2.1.1. Synthesis of Phosphoramidates. All reactions were performed under nitrogen using anhydrous solvents. The Et₃N products, POCl₃, Me₂NH, Et₂NH, piperidine, morpholine, p-cresol, nitrophenol, and 2,2,2-trifluoroethanol, are commercial. The liquids are distilled before use and the solvents were dried by conventional methods. The synthesis of CF₃CH₂OP(O)Cl₂ was performed according to the literature [19] and the synthesis of 4-nitrophenyldichlorophosphate was also performed according to literature [20]. The proton NMR spectra (TMS) at 300 MHz, ³¹P (H₃PO₄ 85%) at 121 MHz, and ¹⁹F (CFCl₃) at 282 MHz were recorded on a Bruker AVANCE III-300. HRMS were recorded on Q-Tof 6500 Series.

2.1.2. Synthesis of 4-methylphenyl-2,2,2-trifluoroethylchlorophosphate. In an Erlenmeyer flask surmounted by a funnel under nitrogen, a solution of 2,2,2-trifluoroethyl dichlorophosphate (16 mmol) was introduced into 120 mL of anhydrous ether. The mixture of p-cresol (16 mmol) with triethylamine (16 mmol) in 50 mL of anhydrous ether was added dropwise at room temperature. After 12 hours of stirring, the precipitate was removed by filtration and the filtrate concentrated distilled. A pale yellow liquid was obtained, with yield = 93%, E_{b0,3mmHg} = 80°C. RMN δ: ³¹P: 0.52; ¹H: 2.35 (s, CH₃-Ph); 4.5 (m, OCH₂); 7.5 (m, -Ph-, 4H).

2.1.3. Synthesis of 4-methylphenyl-2,2,2-trifluoroethyl phosphoramidates. A solution of 2,2,2-trifluoroethyl dichlorophosphate (15 mmol) was placed in 100 mL of anhydrous ether in a flask equipped with a funnel under a flow of nitrogen, then HNR₂ (30 mmol) in 20 mL of anhydrous ether is added dropwise. Viscous liquid is obtained without no further purification. The ³¹P NMR spectra show that crude compounds are pure.

4-Methylphenyl-2,2,2-trifluoroethyl dimethylamidophosphate 3a is as follows: It is a colorless viscous liquid, yielding 92%; ¹H NMR δ: 2.32 (CH₃-Ph); 2.76 (d, CH₃N, 6H, ³J_{H-P} = 12 Hz); 4.32 (m, OCH₂); 7.1 (m, -Ph-, 4H). ¹⁹F NMR δ: -75.27 (t, ³J_{H-F} = 8.5 Hz). ¹³C NMR δ: 20.6 (CH₃Ph); 36.5 (CH₃N); 62.7 (q, CH₂CF₃; J = 37 Hz); 122 (q, CF₃, J = 276 Hz); (117; 130; 134; 148; C_{arom}). ³¹P NMR δ: 6.15 (9 peaks, ³J_{P-H} = 9.7 Hz).

4-Methylphenyl-2,2,2-trifluoroethyl diethylamidophosphate 3b is as follows: It is a colorless liquid, yielding 78%, E_{b0,01mmHg} = 100°C. ¹H NMR δ: 1.0 (t, 3H, CH₃); 2.3 (s, 3H, CH₃-Ph); 3.2 (m, 4H, CH₂N); 4.32 (m, 2H, OCH₂-); 7.1 (m, -Ph-, 4H). ³¹P NMR δ: 5.53 (11 peaks, ³J_{P-H} = 7.3 Hz). ¹⁹F NMR δ: -75.4 (t, ³J_{H-F} = 8.5 Hz). ¹³C NMR δ: 13.8 (CH₃CH₂); 20.7 (CH₃Ph); 39.9 (2NCH₂CH₃); 62.7 (q, CH₂CF₃; ³J = 33 Hz); 122 (q, CF₃, ¹J = 267 Hz); (117; 130; 134; 148; C_{arom}). ESI MS m/z 348 [M+Na]⁺; 673 [2M + Na]⁺; C₁₃H₁₉F₃NO₃P: calc. 325.1055; found 325.1056

4-Methylphenyl-2,2,2-trifluoroethylpiperidin-1-yl-phosphonate 3c is as follows: It is a colorless liquid, yielding 95%, NMR δ: ³¹P: 3.95 (7 raies, ³J_{P-H} = 7.9 Hz); ¹⁹F: -75.4 (t,

³J_{H-F} = 8.5 Hz); ¹³C: 19.6 (CH₃Ph); 23.2 (CH₂); 24.7 (2CH₂); 44.5 (2CH₂N); 62.7 (q, CH₂CF₃; ²J = 37 Hz); 122 (q, CF₃, ¹J = 276 Hz) (117; 130; 134; 148; C_{arom}); ¹H: 2.3 (s, 3H, CH₃-Ph); 7.1 (m, -Ph-, 4H).

4-Methylphenyl-2,2,2-trifluoroethylmorpholin-4-yl-phosphonate 3d is as follows: It is a colorless viscous liquid, with yield 95%, NMR δ: ³¹P: 2.92 (7 raies, ³J_{P-H} = 7.3 Hz); ¹⁹F: -75.4 (t, ³J_{H-F} = 7.3 Hz); ¹³C: 20.5 (CH₃Ph); 44.5 (2CH₂N); 62.9 (q, CH₂CF₃; ²J = 33 Hz); 66.6 (2OCH₂); 122.0 (q, CF₃, ¹J = 267 Hz) (119; 130; 134; 148; H_{arom}); ¹H: 2.3 (s, 3H, CH₃-Ph); 3.2 (m, 4H, CH₂N); 3.6 (m, 4H, OCH₂); 4.32 (m, 2H, OCH₂CF₃); 7.1 (m, -Ph-, 4H).

2.1.4. Synthesis of 2,2,2-trifluoroethyl(4-nitrophenyl)phosphonochloridate. A solution of trifluoroethanol (18 mmol) and triethylamine (18 mmol) in 20 mL was added at 0°C to a solution of p-O₂NPhP(O)Cl₂ (15 mmol) in 120 mL of anhydrous ether under a flow of nitrogen. After 48 h of stirring at room temperature, the precipitate is filtered and the filtrate is concentrated and then distilled. It is a yellow viscous liquid, with yield = 61%, E_{b0,5mmHg} = 138°C, NMR δ: ³¹P: -0.43 (t, J = 9.7 Hz); ¹⁹F: -75.0 (t, J = 8.5 Hz); ¹³C: 65.0 (q, OCH₂CF₃, J = 32 Hz); 122 (q, CF₃, J = 274 Hz); (154, 146, 127, 121 C_{arom}); ¹H: 8.3 et 7.4 (2m, 4H_{arom}); 4.6 (m, 2H, OCH₂CF₃).

2.1.5. Synthesis of 4-nitrophenyl-2,2,2-trifluoroethylphosphoramidates. 6 mmol of 2,2,2-trifluoroethyl (4-nitrophenyl) phosphonochloridate in 50 mL of anhydrous ether was added to 13.2 mmol of triethylamine in 10 mL of anhydrous ether under nitrogen. Stirring is continued for 5 hours. The precipitate is filtered and the filtrate was concentrated to give an oil, which unless otherwise is not further purified.

2,2,2-Trifluoroethyl-4-nitrophenyl-N,N-dimethylphosphoramidate 6a is as follows: It is colorless viscous liquid, with yield (94%), NMR δ: ³¹P: 4.85; ¹³C: 36.6 (CH₃N); 63.5 (q, CH₂CF₃, J = 33 Hz); 122 (q, CF₃, J = 275 Hz); (164, 157, 156, 145, 126, 121, C_{arom}); ¹H: 2.8 (d, 2CH₃); 4.4 (m, CH₂); 7.4 et 8.2 (2m, 4H_{arom}).

2,2,2-Trifluoroethyl-4-nitrophenyl-N,N-diethylphosphoramidate 6b is as follows: It is a yellow viscous liquid, with yield (58%), E_{b0,01mmHg} = 152°C, NMR δ: ³¹P: 5.0 (hept. J = 7.4 Hz); ¹³C: 36.6 (CH₃N); 63.5 (q, CH₂CF₃, J = 33 Hz); 122 (q, CF₃, J = 275 Hz); (154, 146, 126, 121, C_{arom}); ¹⁹F: -73.6 (t, J = 8.5 Hz), ¹H: 1.0 (t, CH₃); 3.2 (m, CH₂CH₃); 4.4 (m, CH₂); 7.4 et 8.2 (2m, 4H_{arom}).

2,2,2-Trifluoroethylmorpholin-4-yl(4-nitrophenyl)phosphoramidate 6c is as follows: It is a yellow solid, with yield (95%), RMN δ: ³¹P: 2.4 (hept. J = 7.9 Hz); ¹³C: 45.0 (2CH₂N); 62.7 (q, CH₂CF₃; ²J = 35 Hz); 67.0 (2OCH₂); 122 (q, CF₃, J = 275 Hz); (163, 156, 144, 126, 121, C_{arom}); ¹⁹F: -75.6 (t, J = 8.5 Hz); ¹H: 3.1 (m, 4H, CH₂N); 3.6 (m, 4H, OCH₂); 4.32 (m, 2H, OCH₂CF₃); 7.4 et 8.2 (2m, 4H_{arom}).

2.2. Biological Activity

2.2.1. Antimicrobial Activity. Different bacterial strains are maintained by subculture on BHI agar (Brain Heart Infusion,

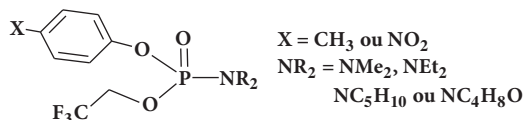


FIGURE 1: Designed phosphoramidates.

agar and brain-heart infusion) favorable to their growth for 24 hours *C. B. cereus* at 37°C with the exception of *L. monocytogenes* and incubated at a temperature of 30° grown on nutrient agar at 30°C. The agar diffusion method (method of disc). Filter paper disc was impregnated by different tested compounds and deposited on the surface of agar petri dishes. Minimal inhibitory concentrations were determined by the dilution method in solid medium.

2.2.2. Anticholinesterase Activity. Chemicals: Acetylcholinesterase (AChE) type VI-S, from electric eel 137 U/mg solid, 217 U/mg protein, 5,5'-dithiobis[2-nitrobenzoic acid] (DTNB), acetylthiocholine iodide (AChI), tris[hydroxymethyl] aminomethane (tris buffer), and dimethylsulfoxide (DMSO) were supplied from Sigma-Aldrich. Acetylcholinesterase enzymatic activity was measured by the Ellman test [21]: 98 μL (50mM/L) tris-HCl buffer (pH 8), 30 μL of the sample, and 7.5 μL of the acetylcholinesterase solution containing 0.26 U/mL were well mixed in 96-well microplates and incubated for 15 min. Subsequently, 22 μL of (3mmol/L) DTNB was added. The absorbance at 405 nm was read when the reaction reached the equilibrium. A control reaction using DMSO instead the sample and a blank with Tris-HCl buffer instead of enzyme solution were used. Tests were carried out in duplicate.

Inhibition, in %, was calculated in the following way: $I (\%) = 100 - (A \text{ sample}/A \text{ control}) * 100$,

where A sample is the absorbance of the sample containing reaction and A control the absorbance of the reaction control.

3. Results and Discussion

3.1. Synthesis. The design of arylphosphoramidates in this work (Figure 1) is based on phosphoramidate structures already used as prodrugs. (NR_2) is the masking group which hydrolyzes first. (Ar-X) is the leaving group and (OR) is the active group that should be supplied to the cell to be treated, avoiding hydrolysis thereof to the surface of the cell by the NR_2 group in Figure 1.

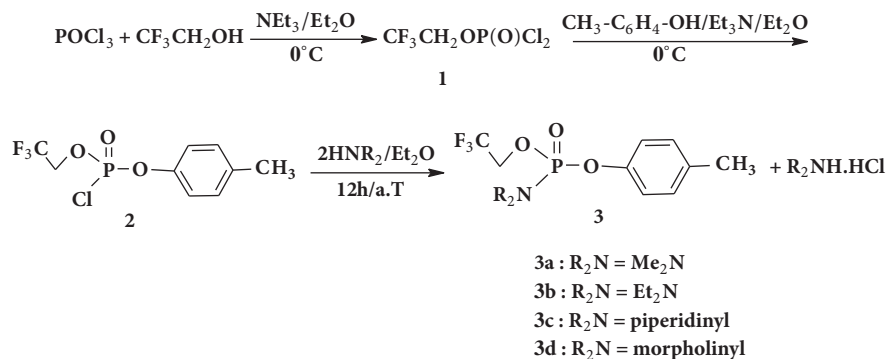
For the synthesis of the designed arylphosphoramidates, several attempts have been carried out. On the basis of the reported literature by [2], we have attempted the synthesis of the phosphoramidates (R_2N)P(O)(OCH_2CF_3)(OphCH_3) in a one pot by mixing phosphorus oxychloride, alcohols (HOphCH_3 , HOCH_2CF_3), and the amine (R_2NH) as shown in Scheme 1. However, in addition to the expected phosphoramidates, the ^{31}P NMR spectrum showed signals relating to the formation of several byproducts such as $\text{CF}_3\text{CH}_2\text{OP(O)Cl}$, $(\text{CF}_3\text{CH}_2\text{O})_2\text{P(O)}(\text{OphCH}_3)$, and

$\text{P(O)}(\text{OphCH}_3)_3$. These byproducts could not be separated by distillation. The reaction was then undertaken in multisteps with several assays: first, the p-cresol and triethylamine are added to phosphorus oxychloride in anhydrous ether at -10°C and kept at room temperature for 12 hours. The corresponding ^{31}P NMR spectrum showed the corresponding dichlorophosphate in addition to other unknown phosphorus compounds. Then, the addition of alkylamine on phosphorus oxychloride followed by the addition of HOCH_2CF_3 gave the desired dialkylphosphoramidic dichloride. However the addition of $\text{CF}_3\text{CH}_2\text{OH}$ in the presence of DMAP as catalyst afforded the expected phosphoramidate in low proportion with the appearance of a new compound due to the substitution of the -NR_2 group by -OR group located at -6 ppm. Finally, we have reacted POCl_3 with $\text{CF}_3\text{CH}_2\text{OH}$ in presence of triethylamine in anhydrous ether for 12 hours at room temperature and subsequent addition of p-cresol and amine afforded the desired compounds **3** with good yields and satisfactory purity. In these optimized conditions, the other amines were used and gave the corresponding phosphoramidates (Scheme 1).

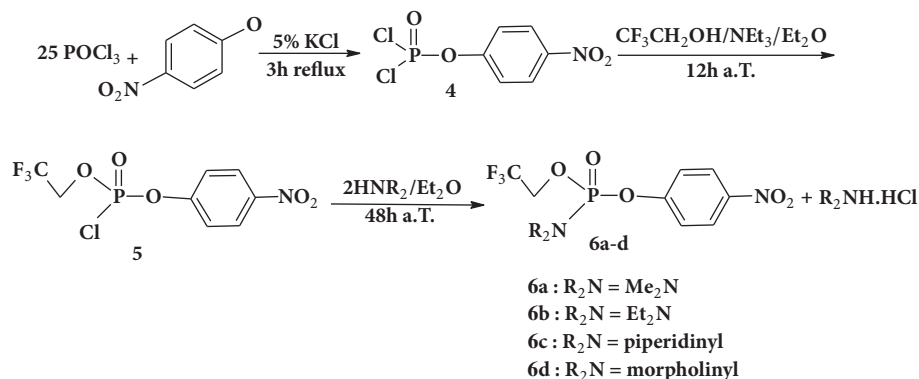
The ^{31}P NMR coupled to ^1H spectrum of the compound **2** showed a triplet at 0.5 ppm with a coupling constant value $^3J_{\text{H-P}} = 8 \text{ Hz}$ with the two protons of the methylene group. The reaction of two equivalents of amine in anhydrous ether with compound **2** for 12 hours at room temperature gave the pure arylphosphoramidates **3**. The ^1H NMR spectrum of compound **3a** shows a doublet at 2.8 ppm resulting from the coupling with the phosphorus atom. The methylene group shows a multiplet at 4.4 ppm due to the coupling with both fluorine and phosphorus atoms. The corresponding ^{31}P NMR spectrum (Figure 1) shows a multiplet of 9 peaks resulting from the coupling between the phosphorus atom and 8 protons (CH_2O and 2CH_3). ^{19}F NMR spectrum shows a triplet due to coupling of the fluorine atom with the methylene group (Figure 2).

We have also used nitrophenol instead of p-cresol following the same sequence described for the synthesis of compounds **3**. However the purification of the reaction products was tedious and gave a mixture of products together with the desired phosphoramidates. We have therefore attempted to do the synthesis using a different sequence where the addition of the amine with phosphorus oxychloride was followed by nitrophenol and then by trifluoroethanol allowing the desired phosphoramidate but the reaction took 5 days. Finally using the starting compound **4** described in the literature [20], the reaction with trifluoroethanol gave the corresponding chlorophosphoramidate in a good yield. The reaction of the compound **5** with two equivalents of amine in anhydrous ether at room temperature for 48 hours led to the desired phosphoramidates with yields ranging from 58 to 95% (Scheme 2).

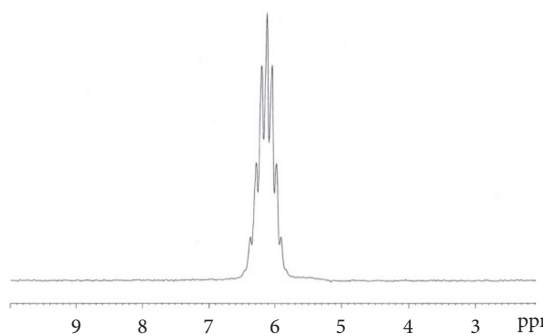
The ^1H NMR spectrum of the compound **6a** shows the coupling with methyl protons with the phosphorus atom at 2.8 ppm (Figure 3). On the other hand, the ^{31}P NMR spectrum of **6d** shows a multiplet of seven peaks reflecting the coupling between phosphorus atom and methylene protons at 2.4 ppm, at lower field compared to



SCHEME 1: Synthesis of p-tolylphosphoramidates 3.



SCHEME 2: Synthesis of p-nitrophenol phosphoramidates 6.

FIGURE 2: ³¹P NMR of **3a** in CDCl₃ at 298 K.

p-tolylphosphoramidate. The spectroscopic data of these arylphosphoramidates are shown in Table 1.

The above results in Schemes 1 and 2 show that the formation of phosphoramidates is sensitive to the nature of the alcohols used. Thus preparing phosphoramidates **3** is in the order CF₃CH₂OH followed by p-cresol and the amine, whilst for phosphoramidates **6**, the addition of the aromatic alcohol, nitrophenol, should be the first step then the addition of the second alcohol CF₃CH₂OH and amine as the final step.

3.2. Biological Activity

3.2.1. Antimicrobial Activity. The phosphoramidates **3a**, **3c**, and **3d** have been tested towards different Gram negative

and Gram positive bacteria. Chloramphenicol was taken as reference to study the effect of different substituents on biological activity. The compound 2,2,2-trifluoroethyl N,N,N',N'-tetramethylphosphorodiamidate (TMP) [22] has also been tested in order to evaluate the effect of electrodonating effect on the phosphorus atom. The reactivity of each compound was evaluated towards the different bacterial strains by the agar diffusion method. The inhibition diameters of bacterial growth area are summarized in Figure 4.

The results show that all the tested compounds in a pure state have diameters of inhibition zone of bacterial growth ranging between 6 and 10 mm for all strains of Gram negative and Gram positive bacteria. The compounds **3a**, **3c**, **3d**, and TMP do not exhibit a particular antimicrobial activity. To better assess the sensitivity of the strains towards the activity of these compounds, their minimum inhibitory concentration (MIC) was determined by the dilution method on solid medium. The results show that these values (i.e., 1000 to 2000 μg/mL) are high compared to those of usual therapeutic agents. The lack of antimicrobial activity may be due to the low solubility in water [23, 24], as well as instability in alkaline hydrolysis [25].

3.2.2. Antiacetylcholinesterase Activity. The determination of the inhibitor activity of acetylcholinesterase (AChE) of compounds **3a**, **c**, **d**, and TMP with galantamine, taken as a reference, was performed according to the method of Ellman

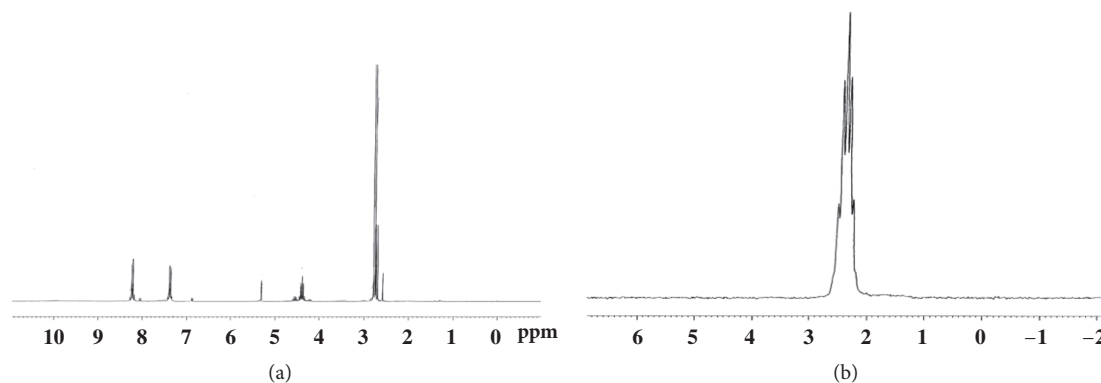


FIGURE 3: (a) ^1H NMR of **6a** in CDCl_3 and (b) ^{31}P NMR coupled to ^1H of **6d** in CDCl_3 .

TABLE 1: Spectroscopic data of arylphosphoramidates $\delta^{31}\text{P}$ (ppm), ^3J (Hz), and $\nu_{\text{P=O}}$ (cm^{-1}).

Phosphoramidates	yield%	$\delta^{31}\text{P}$ (ppm)	^3J (Hz)	$\nu_{\text{P=O}}$ (cm^{-1})
3a	91	6.15	9,7	1167
3b	80	5.53	7,3	1165
3c	95	3.95	7,9	1165
3d	90	2.92	7,3	1168
6a	60	4.85	9,7	1180
6b	58	5.0	7,4	1181
6c	90	3.1	7,5	1180
6d	95	2.4	7,9	1178

[21]. The results of optical density measurements of all the tested compounds are shown in Figure 5.

As can be seen from Figure 3, the negative values of the phosphoramidates **3a** and **3d** indicate that the compounds have no inhibitory activity against AChE. The compounds **3c** and **TMP** exhibit some AChE activity. The compound **3c** is more active than the compound **3d** probably due to the hydrophobicity and the more electrodonating character of **3d**. However, the difference in activity found between phosphoramidates **3a** and **3c** both bearing electrodonating groups could be mainly due to steric hindrance which would enhance AChE activity in **3c**. On the other hand, the direct substitution of phosphorus atom by amine group in **TMP** can enhance sensitively of the AChE inhibitory effect. This electrodonating group enhances nucleophilic character which facilitates the nucleophilic attack on the phosphorous atom and the elimination of the leaving group. This is consistent with the literature [26, 27] which showed that the AChE inhibition increases when the polarity of the amine group increases related to the electrostatic attraction between this group and the enzyme which becomes stronger.

Therefore the inhibitor-enzyme interaction would be influenced mainly by the reactivity of the phosphorus atom, which determines the rate of the phosphorylation reaction and the ease of bonding between the inhibitor and the enzyme to form a complex before the phosphorylation step and the electronic and steric effects of hydrophobic moieties directly bounded to the phosphorus atom. The binding affinity is determined by the structural features in

particular the instability of the $\text{P}=\text{O}$ bond as reported in the literature [28, 29] which may also influence the cholinesterase activity.

4. Conclusions

In this paper, we have synthesized new phosphoramidates $\text{R}_2\text{N}(\text{pX-ArO})\text{P}(\text{O})\text{OR}'$ using convenient steps. All synthesized phosphoramidates were characterized by ^{31}P NMR, ^1H , and ^{13}C NMR, IR spectroscopy. The biological study of some of arylphosphoramidates did not show particular antibacterial activity even when the phosphorus atom was directly substituted by an electrodonating group ($-\text{N}(\text{Me})_2$). However the AChE activity has shown that the directly substituted electrodonating group on the phosphorus atom has some AChE inhibitory effect. Therefore the substituents nondirectly bounded to the phosphorus atom did not affect sensitively the reactive sites of the arylphosphoramidates towards AChE enzyme.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

There are no conflicts of interest in this manuscript.

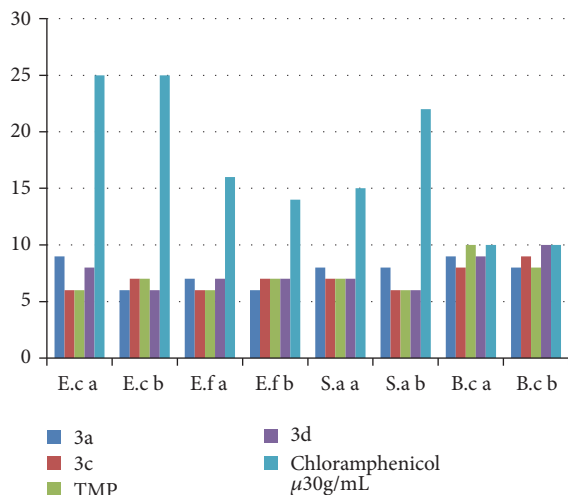


FIGURE 4: Inhibition diameters zones of bacterial growth (mm) of phosphoramidates **3a**, **c**, **d**, and **TMP**. *E.c a*: *Escherichia coli* ATCC 8739; *E.c b*: *Escherichia coli* DH5 α ; *E.f a*: *Enterococcus faecalis* ATCC 29212; *E.f b*: *Enterococcus faecium* ATCC19436; *S.a a*: *Staphylococcus aureus* PIC 4.83; *S.a a*: *Staphylococcus aureus* ATCC 25923; *B.c a*: *Bacillus cereus* 49; *B.c b*: *Bacillus circulans* (ATCC: American Type Culture Collection; PIC: Pasteur Institute Collection).

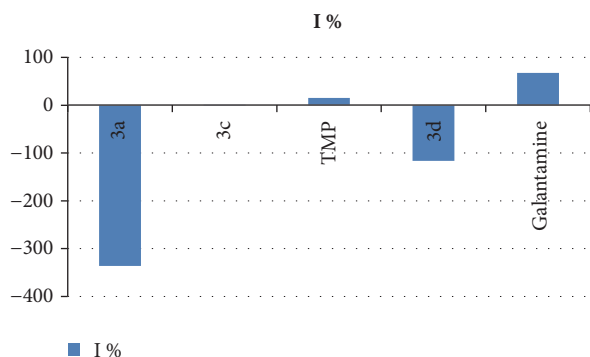


FIGURE 5: Measurements of optical density DO406.

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References

- [1] E. C. Meek, H. W. Chambers, A. Coban et al., "Synthesis and in vitro and in vivo inhibition potencies of highly relevant nerve agent surrogates," *Toxicological Sciences*, vol. 126, no. 2, pp. 525–533, 2012.
- [2] H. Garrido-Hernandez, K. D. Moon, R. L. Geahlen, and R. F. Borch, "Design and synthesis of phosphotyrosine

peptidomimetic prodrugs," *Journal of Medicinal Chemistry*, vol. 49, no. 11, pp. 3368–3376, 2006.

- [3] M. J. Berridge and R. F. Irvine, "Inositol trisphosphate, a novel second messenger in cellular signal transduction," *Nature*, vol. 312, no. 5992, pp. 315–321, 1984.
- [4] H. Chapman, M. Kernan, E. Prisbe et al., "Practical synthesis, separation, and stereochemical assignment of the PMPA prodrug GS-7340," *Nucleosides, Nucleotides and Nucleic Acids*, vol. 20, no. 4–7, pp. 621–628, 2001.
- [5] H. Chapman, M. Kernan, J. Rohloff, M. Sparacino, and T. Terhorst, "Purification of PMPA amidate prodrugs by SMB chromatography and x-ray crystallography of the diastereomerically pure GS-7340," *Nucleosides, Nucleotides and Nucleic Acids*, vol. 20, no. 4–7, pp. 1085–1090, 2001.
- [6] C. McGuigan, R. N. Pathirana, J. Balzarini, and E. De Clercq, "Intracellular Delivery of Bioactive AZT Nucleotides by Aryl Phosphate Derivatives of AZT," *Journal of Medicinal Chemistry*, vol. 36, no. 8, pp. 1048–1052, 1993.
- [7] J. Patocka, "Acetylcholinesterase inhibitors - From nervous gas to Alzheimer's disease therapeutics," *Chemické listy*, vol. 92, pp. 1016–1019, 1998.
- [8] K. Gholivand and N. Dorosti, "Synthesis, spectroscopic characterization, crystal structures, theoretical studies, and antibacterial evaluation of two novel N-phosphinyl ureas," *Monatshefte für Chemie - Chemical Monthly*, vol. 142, no. 2, pp. 183–192, 2011.
- [9] L. A. Adams, R. J. Cox, J. S. Gibson, M. B. Mayo-Martin, M. Walter, and W. Whittingham, "A new synthesis of phosphoramidates: Inhibitors of the key bacterial enzyme aspartate semi-aldehyde dehydrogenase," *Chemical Communications*, no. 18, pp. 2004–2005, 2002.
- [10] C. A. Roman, J. Balzarini, and C. Meier, "Diastereoselective synthesis of aryloxy phosphoramidate prodrugs of 3'-deoxy-2',3'-didehydrothymidine monophosphate," *Journal of Medicinal Chemistry*, vol. 53, no. 21, pp. 7675–7681, 2010.
- [11] J. P. Langenberg, L. P. A. De Jong, M. F. Otto, and H. P. Benschop, "Spontaneous and oxime-induced reactivation of acetylcholinesterase inhibited by phosphoramidates," *Archives of Toxicology*, vol. 62, no. 4, pp. 305–310, 1988.
- [12] L. P. A. de Jong, G. Z. Wolring, and H. P. Benschop, "Reactivation of acetylcholinesterase inhibited by methamidophos and analogous (di)methylphosphoramidates," *Archives of Toxicology*, vol. 49, no. 2, pp. 175–183, 1982.
- [13] J. P. Langenberg, L. P. A. De Jong, and H. P. Benschop, "Protection of guinea pigs against soman poisoning by pretreatment with p-nitrophenyl phosphoramidates," *Toxicology and Applied Pharmacology*, vol. 140, no. 2, pp. 444–450, 1996.
- [14] J. L. Petersen and L. F. Dahl, "Synthesis and structural characterization by x-ray diffraction and EPR single-crystal techniques of (dichloro)bis(η^5 -methylcyclopentadienyl)vanadium and (dichloro)bis(η^5 -methylcyclopentadienyl)titanium. Spatial distribution of the unpaired electron in a V(η^5 -C₅H₅)₂L₂-type complex," *Journal of the American Chemical Society*, vol. 97, no. 22, pp. 6422–6433, 1975.
- [15] M. T. Ben Dhia, M. A. M. K. Sanhoury, L. C. Owono Owono, and M. R. Khaddar, "Phosphine oxide adducts of tin(IV) chloride: Experimental NMR and DFT computational study," *Journal of Molecular Structure*, vol. 892, no. 1–3, pp. 103–109, 2008.
- [16] M. A. Sanhoury, M. T. Ben Dhia, and M. R. Khaddar, "Synthesis, characterization and solution behaviour of phosphoryl complexes of tin tetrafluoride," *Journal of Fluorine Chemistry*, vol. 132, no. 11, pp. 865–869, 2011.

- [17] L. Bahri, T. Barhoumi-Slimi, R. Mallek, M. A. K. Sanhoury, B. Crousse, and M. T. Ben Dhia, "One-pot synthesis of new highly substituted allylic phosphorodiamidates," *Journal of Fluorine Chemistry*, vol. 189, pp. 96–101, 2016.
- [18] T. Barhoumi-Slimi, M. A. Sanhoury, M. T. Ben Dhia, and M. R. Khaddar, "Tin(IV) Chloride Complexes of β -Chlorovinyl Aldehydes: A Multinuclear Nmr Characterization in Solution," *Phosphorus, Sulfur, and Silicon and the Related Elements*, vol. 188, no. 9, pp. 1220–1227, 2013.
- [19] C. M. Timperley and M. Waters, "Fluorinated phosphorus compounds: Part II. The reactions of some fluorinated amines with dialkyl and bis(fluoroalkyl) phosphorochloridates," *Journal of Fluorine Chemistry*, vol. 126, no. 8, pp. 1144–1149, 2005.
- [20] T. Hata, Y. Mushika, and T. Mukaiyama, "New phosphorylating reagent. I. Preparation of alkyl dihydrogen phosphates by means of 2-chloromethyl-4-nitrophenyl phosphorodichloridate," *Journal of the American Chemical Society*, vol. 91, no. 16, pp. 4532–4535, 1969.
- [21] A. Ferreira, C. Proença, M. L. M. Serralheiro, and M. E. M. Araújo, "The *in vitro* screening for acetylcholinesterase inhibition and antioxidant activity of medicinal plants from Portugal," *Journal of Ethnopharmacology*, vol. 108, no. 1, pp. 31–37, 2006.
- [22] C. Timperley, *Best Synthetic Methods: Organophosphorus(V) Chemistry*, Elsevier, 2015.
- [23] N. A. Minton and V. S. G. Murray, "A Review of Organophosphate Poisoning," *Medical Toxicology and Adverse Drug Experience*, vol. 3, no. 5, pp. 350–375, 1988.
- [24] C. Bismuth, "Description and risks of chemical weapons," *Reanimation Urgences*, vol. 2, no. 6, pp. 625–633, 1993.
- [25] K. D. Grimes, Y.-J. Lu, Y.-M. Zhang et al., "Novel acyl phosphate mimics that target PlsY, an essential acyltransferase in gram-positive bacteria," *ChemMedChem*, vol. 3, no. 12, pp. 1936–1945, 2008.
- [26] P. William, J. G. Purcell, P. Ronald, A. Quintana, and J. Singer, *Biochem*, vol. 43, 2008.
- [27] T. Mohamed, X. Zhao, L. K. Habib, J. Yang, and P. P. Rao, "Design, synthesis and structure–activity relationship (SAR) studies of 2,4-disubstituted pyrimidine derivatives: Dual activity as cholinesterase and A β -aggregation inhibitors," *Bioorganic & Medicinal Chemistry*, vol. 19, no. 7, pp. 2269–2281, 2011.
- [28] S. Di Giovanni, A. Borloz, A. Urbain et al., "In vitro screening assays to identify natural or synthetic acetylcholinesterase inhibitors: thin layer chromatography versus microplate methods," *European Journal of Pharmaceutical Sciences*, vol. 33, no. 2, pp. 109–119, 2008.
- [29] K. Gholivand, Z. Hosseini, S. Farshadian, and H. Naderi-Manesh, "Synthesis, characterization, oxidative degradation, antibacterial activity and acetylcholinesterase/butyrylcholinesterase inhibitory effects of some new phosphorus(V) hydrazides," *European Journal of Medicinal Chemistry*, vol. 45, no. 11, pp. 5130–5139, 2010.