chemistry proceedings

MDPI

Proceeding Paper

Microwave synthesis and antimicrobial evaluation of selected aminophosphonates †

Felkaoui Nourelhouda [1,*], Abdelkader Hellal [2], Khadidja Fadli [3] and Yousra Miloudi [4]

[1] Department of Exact Sciences, University of Constantine 1, Laboratory for Obtaining Therapeutic Substances (L.O.S.T.), Campus ChaabetErsas, 25000 Constantine, Algeria
[2] Department of Process Engineering, Laboratory of Electrochemistry of Molecular Materials and Complex (LEMMC), Faculty of Technology, University of Ferhat ABBAS Setif-1, El-Mabouda Campus, 19000 Sétif, Algeria; abdelkader.hellal@univ-setif.dz
[3] Laboratoire de Chimie, Ingénierie Moléculaire et Nanostructures, Université Ferhat Abbas Sétif 1, Sétif, Algeria; khadidjafadli19@gmail.com
[4] Département de Chimie, Faculté des Sciences, Laboratoire de Recherche sur la Physico-Chimie des Surfaces et Interfaces-LRPCSI, Université 20 Aout 1955, Skikda 21000, Algerie; youssrayoussi97@gmail.com
* Correspondence: felkaouinourelhouda96@gmail.com
† Presented at The 28th International Electronic Conference on Synthetic Organic Chemistry (ECSOC 2024), 15-30 November 2024; Available online: https://sciforum.net/event/ecsoc-28.

Abstract: In the search for new bioactive molecules, a series of new molecules from the phosphonate family were synthesized via the Kabachnik-Fields reaction (phosphonate ester) and the Irani-Moedritzer reaction (phosphonic acids). Their structures were characterized by various spectroscopic methods, including IR and UV-vis. The synthesized compounds were screened for in vitro antimicrobial activity against Gram-positive (Bacillus subtilis and Staphylococcus aureus) and Gram-negative (Escherichia coli and Pseudomonas aeruginosa) bacteria using the well method. The results also showed that all the products synthesized exhibited good activity with a zone of inhibition; D>8, except one product against *S. aureus* bacteria. The three products were tested for their antifungal effects against three pathogenic fungal strains, namely Candida albicans, Aspergillus niger and Penicillium notatum. The results show that the zones of maximum inhibition were observed against *P. notatum* (35.5mm). So the biological tests showed that all the compounds studied exhibited high antibacterial and antifungal activities. The aim of the present work is therefore to synthesize aminophosphonate derivatives using microwaves. Microwaves open up new opportunities for synthetic chemists in the form of new reactions that are difficult to use with conventional heating. Interest in microwave-assisted organic synthesis (SOAM) has been growing in recent years. The short reaction times provided by microwave synthesis make it ideal for rapid reaction screening and optimization of reaction conditions.

Citation: Nourelhouda, F.; Hellal, A.; Fadli, K.; Miloudi, Y. The 28th International Electronic Conference on Synthetic Organic Chemistry . *Chem. Proc.* **2024**, *6*, x. https://doi.org/10.3390/xxxxx

Academic Editor(s): Name

Published: 15 November 2024

1. Introduction

Aminophosphonates are an important class of phosphonate compounds due to their versatile biological activities, which have attracted researchers' attention as they are considered structural analogs of corresponding α-amino acids. They have gained particular interest in the preparation of isosteric or bioisosteric analogs of many natural products.

Various methods for synthesizing α-aminophosphonate esters and α-aminophosphonic acids have been reported. These compounds can be synthesized through various pathways involving amine compounds, aldehydes, and phosphites. However, the one-pot synthesis remains favored due to reduced steps and high-yield reactions.

This study aims to synthesize aminophosphonate derivatives using microwave-assisted methods. Microwaves provide synthetic chemists with new opportunities for reactions that are difficult to perform with conventional heating. Interest in microwave-assisted organic synthesis (MAOS) has grown in recent years. The short reaction times provided by microwave synthesis make it ideal for rapid reaction screening and reaction condition optimization.

The obtained products were characterized using IR and UV-visible spectroscopic methods. We then evaluated their biological activity, including antibacterial and antifungal activities.

2. Methodology

Phosphonates, due to their biological importance, have encouraged organic chemists to explore various synthetic routes to obtain new derivatives of α-aminophosphonate esters and α-aminophosphonic acids. In this work, three products from the phosphonate family were synthesized: one α-aminophosphonate ester derivative and two α-aminophosphonic acid derivatives, using microwave-assisted chemical synthesis. These products were characterized by common physicochemical methods, including melting point measurement, UV-Vis spectrophotometry, and IR spectroscopy.

Finally, an in vitro determination of biological activities was conducted, including antioxidant, antibacterial, and antifungal activities.

A. Synthesis

Two synthesis procedures were followed to obtain an α aminophosphonate ester derivative via the Kabachnik-Fields reaction, and two α-aminophosphonic acid derivatives via the Moedritzer-Irani reaction.

The following compounds were used:

- Two amines as nucleophilic base molecules:4-chlorophenylhydrazine and thiourea,
- Three aldehydes with electrophilic character:benzaldehyde,4hydroxybenzaldehyde, and cinnamaldehyde,
- Phosphorous acid and triethyl phosphite.

B. Characterization of Synthesized Molecules

Different physicochemical analysis techniques were used to identify the synthesized compounds:

- Melting Point
- Thin Layer Chromatography (TLC)

After several trials, the proper elution system was found to be n-hexane/dichloromethane (1/1, v/v) for Nb and No, and n-hexane/ethyl acetate (3/2, v/v) for TC. TLC was used to monitor the progress of the reactions and to verify the purity by determining the retention factor (Rf).

UV-Visible Spectrophotometry Analysis

The UV-Vis spectrophotometer used was the SHIMADZU UV-1800. The synthesized molecules were dissolved in ethanol.

Infrared Spectroscopy (IR) Analysis

The IR spectra of the synthesized compounds were recorded using the FT-IR4200 JASCO spectrophotometer in the range of 4000-500 cm^{-1} at room temperature. The measurement chamber was flushed with nitrogen gas to limit the effects of atmospheric absorption.

C. Antibacterial and Antifungal Activity

The method used for antibacterial testing was the agar well diffusion method, as described by Berghe and Vlietinck (1991). Mueller Hinton agar medium was poured into Petri dishes at a thickness of 4 mm. After inoculation with a swab of a microorganism dilution prepared according to a McFarland scale (MC 0.5), wells with a diameter of 6 mm were created concentrically on the medium, and 80 µL of each concentration was placed in the center of each well. After a pre-diffusion of 45 minutes at room temperature, the strains were incubated at 37 °C for 24 h, after which the diameters of the inhibition zones were measured.

The same procedure was followed for antifungal activity testing, except the culture medium was PDA (potato dextrose agar). The incubation period for the antifungal test

was 48 hours. The compound is considered active when an inhibition zone greater than 6 mm in diameter is observed around the well.

Tested Strains

Antibacterial and antifungal activity was evaluated on various microorganisms: 4 bacterial strains (2 Gram-positive and 2 Gram-negative) and 3 fungal strains (2 fungi and 1 yeast). The 4 bacterial strains used were Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Staphylococcus aureus ATCC 25923, and Bacillus cereus ATCC 14579, which are responsible for various infections (urinary, intestinal, respiratory, etc.). These bacterial strains were provided by the CHU of Setif and maintained by subculturing on a nutrient agar medium for 24 h in the dark at 37 °C.

3. Results

The objective of this study was to obtain one α-aminophosphonate ester via the Kabachnik reaction and two α-aminophosphonic acids via the Irani-Moedritzer reaction. These are three-component, one-pot reactions, in which two reagents have nucleophilic character and the third is an electrophilic carbonyl compound.

The syntheses were carried out according to the following reaction sequences:

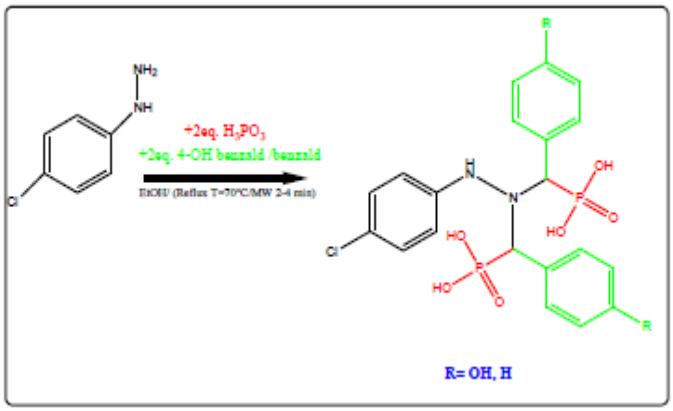

Figure 1. Reaction for synthesizing α-aminophosphonic acids via the Irani-Moedritzer reaction.

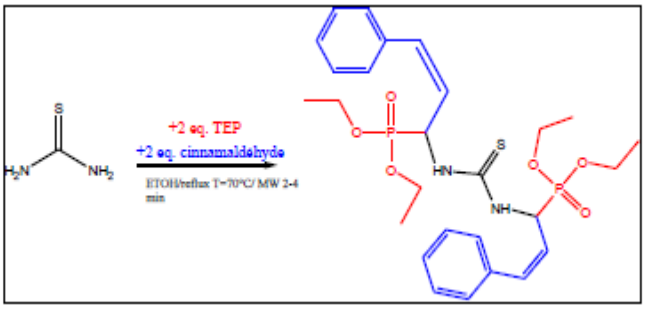

Figure 2. Reaction for synthesizing the α-aminophosphonate ester via the Irani-Moedritzer reaction.

It should be noted that the use of microwaves accelerated the reaction by more than 30 times compared to conventional synthesis under reflux and prevented waste of the starting materials.

For ease of reference, abbreviations were assigned to the synthesized products as follows:

- Nb: obtained from 4-chlorophenylhydrazine and benzaldehyde.
- No: obtained from 4-chlorophenylhydrazine and 4-hydroxybenzaldehyde.
- Tc: obtained from thiourea and cinnamaldehyde.

A. Melting Points

The melting points of the synthesized compounds were determined using a Köfler bench. The results are summarized in Table 1

Table 1. Melting points of synthesized compounds and corresponding starting materials.

Products	Tf (°C)
Thioureé	180–182
TC	Gel
4 cl Ph-hydrazine	210–212
No	46–48
Nb	50–52

B. Thin Layer Chromatography (TLC) Analysis

To characterize our products, we calculated the retention factor (Rf), which is characteristic of the compound, the plate material, and the elution system. The appearance of a single spot for each synthesized product, different from the starting material, indicated purity and confirmed the end of the reaction and the formation of new products. The retention factor values of the synthesized products are shown in the following table.

Table 2. Retention factors of synthesized products.

Product	Retention Factor
Nb	**0.87**
No	**0.16**
TC	**0.72**

C. UV-Visible Spectrophotometry Characterization

The UV-Vis spectra were used to observe significant differences between the starting materials and the synthesized products by comparing the obtained spectra. The UV-Vis spectra of the synthesized products and starting materials, recorded in the range of 200–800 nm in absolute ethanol, are presented below.

- Case of TC:

The starting material (T) shows a single absorption band (λmax = 243 nm) corresponding to the π-π* transition.

The synthesized product (TC) also shows a single absorption band at λmax=273.56 nm, corresponding to the π-π* transition but shifted to longer wavelengths (a bathochromic effect). This may be due to a structural modification of the starting material (substitution of the aminophosphonate fragment).

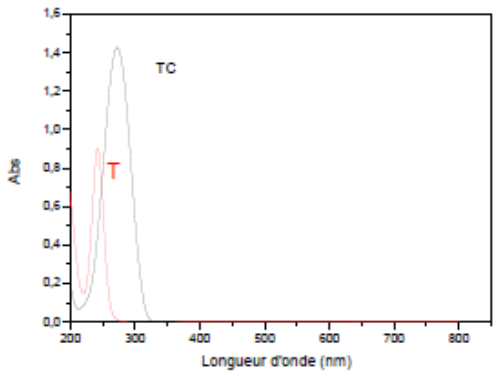

Figure 3. UV-vis spectra of Thiourea (T) and the product obtained (TC).

- Case of No and Nb:

The starting material, 4-chlorophenylhydrazine, presents three absorption bands: the first band at λmax = 236.29 nm (π-π* transition), the second at λmax = 277.71 nm (π-π* transition), and the third band with a weak intensity at λmax = 351.50 nm (n-π* transition).

The synthesized product (Nb) presents two characteristic bands: one absorption band at λmax = 270.48 nm (π-π* transition) and another weak intensity band at λmax = 355.12 nm (n-π* transition).

The spectrum of (No) presents a single absorption band at λmax = 272.28 nm.

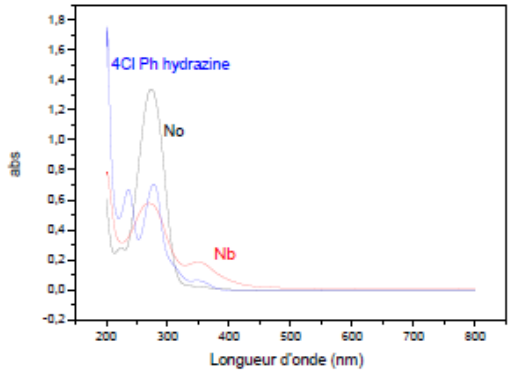

Figure 4. UV-vis spectra of 4Clph Hydrazine, No and Nb.

D. Infrared Spectroscopy (IR) Characterization:

The figures below show peaks corresponding to different groups and indicate the stretching or bending vibrations of these functions.

- Case of the α-aminophosphonate ester (TC):

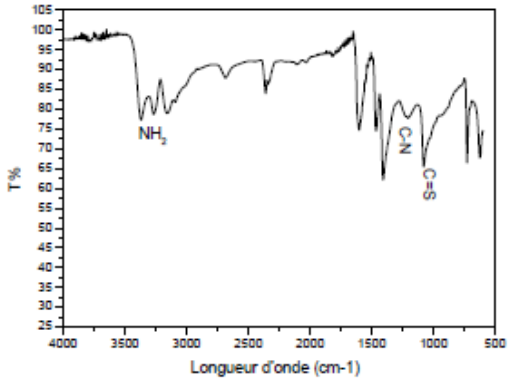

Figure 5. Infrared spectrum of the starting product(Thiourea: T) .

The IR spectrum of the starting material (thiourea) shows bands corresponding to the following vibrations:

- (NH_2): 3264 and 3364 cm^{-1} (stretching), 1598 cm^{-1} (bending)
- C=S: 1076 cm^{-1} (stretching)
- C-N: 1207 cm^{-1} (stretching)

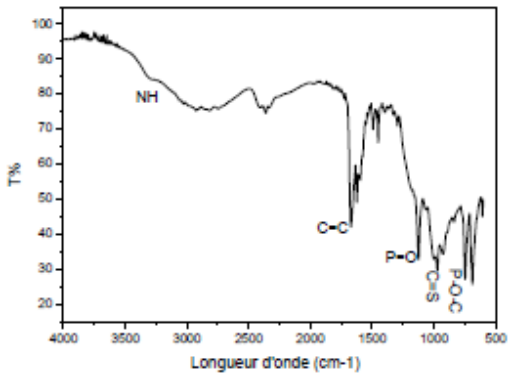

Figure 6. Infrared spectrum of the synthesized product TC.

The synthesized product (TC) shows the following main bands:

- C=S: 998 cm^{-1}
- C=C: 1640 cm^{-1}
- P-O-C: 743 cm^{-1}
- P=O: 1120 cm^{-1}
- NH: 3309 cm^{-1}
- Case of α-aminophosphonic acids:

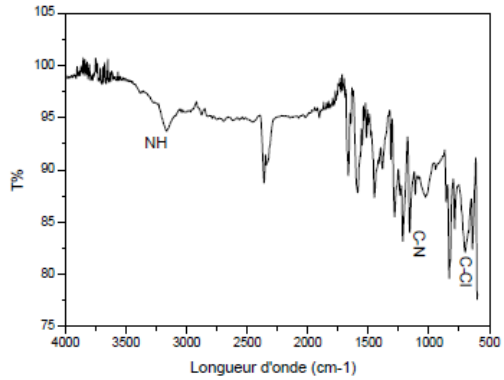

Figure 7. Infrared spectrum of the starting product (4Cl Ph hydrazine).

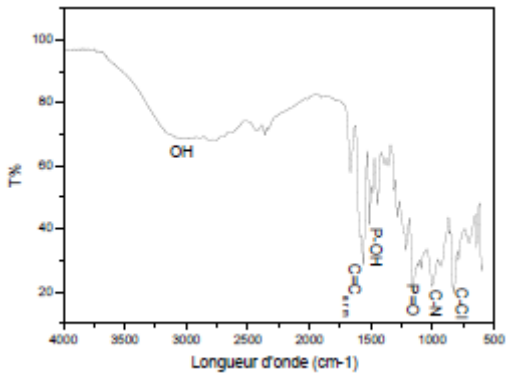

Figure 8. Infrared spectrum of the synthesized product (No).

Table 3. IR band assignments for the studied products.

Products	Function	Frequencies (cm^{-1})	Vibration
4Cl Ph Hydrazine	NH	3163	stretching
	C-N	1151	

	C-Cl	700	
No	OH	≈2871–3176	stretching
	C=Carm	1558	
	P=O	1151	
	P-OH	1507	
	C-N	1008	
	C-Cl	815	
Nb	Carm	2963	stretching
	P-OH	1487	
	P=O	1090	
	C-N	927	
	C-Cl	815	

E. Antibacterial and Antifungal Activity Evaluation

Our synthesized products were tested in vitro to evaluate their antibacterial and antifungal activities.

Antibacterial Activity

The antimicrobial activity against the microorganisms studied in this research was evaluated qualitatively based on the presence or absence of inhibition zones, the diameter of the zone (DD), compared to Chloramphenicol (Table 5). In this study, the well diffusion method was used to study the antibacterial activity of our products at different concentrations. This method provides a preliminary idea of a product's ability to inhibit microbial growth. The compounds were tested against two types of Gram-positive bacteria (Bacillus subtilis and Staphylococcus aureus) and two types of Gram-negative bacteria (Escherichia coli and Pseudomonas aeruginosa).

The estimation scale of antimicrobial activity is given by Moreira et al. (2005), who classified the diameter of microbial growth inhibition zones as follows:

- D < 8 mm: bacteria are non-sensitive,
- 9 < D < 14 mm: bacteria are sensitive,
- 15 < D < 19 mm: bacteria are highly sensitive,
- D > 20 mm: bacteria are extremely sensitive.

Our antibacterial test results are presented in Table 4:

Table 4. Inhibition zone diameters (in mm) of the tested products.

Products	TC			Nb			No			Standard (Chloramphenicol)
Conc. µg/mL	200	400	800	200	400	800	200	400	**800**	**800**
E. coli(G–)	11	11	13	9	9	9	12	12	12	0
P.aeruginosa (G–)	10	10	11	9	9	10	-	-	-	20
S. aureus(G+)	13	17	17	7	7	7	9	11	13	25
B. cereus(G+)	12	13.5	14.5	10.5	10.5	12	8	10	12	25

Note: The negative control (Ctrl-) was DMSO, which showed no activity against bacteria.

The results showed that the standard antibiotic produced larger inhibition zones than the tested products. Additionally, the results indicated that all the synthesized products exhibited good activity with D > 8, except Nb against ***S. aureus***. The highest levels of

activity were recorded by product TC against Gram-positive bacteria, particularly **S. aureus** (inhibition zone diameter of 17.5 mm), followed by slightly less activity against Gram-negative bacteria. According to the scale given by Moreira et al. (2005), **S. aureus** is highly sensitive to this product, while the other products (acids) showed good activity against all tested strains.

Antifungal Activity

The antifungal activity of the compounds was also determined at five different doses (50, 100, 200, 400, 800 μg/mL) against three pathogenic fungal strains:Candida albicans, Aspergillus niger, and Penicillium notatum. The results regarding antifungal activity (inhibition zone diameters) of the compounds on the fungal strains are shown in Table 5.

Table 5. Inhibition zone diameters (in mm) for TC, Nb, No against fungi (in mm) of the tested products.

Products	**TC**			**Nb**			**No**			**Standard (Chloramphenicol)**
Conc.	100	200	400	800	100	200	400	800	100	200
A. niger	R	R	R	R	8	8	10	13.5	9.5	11
P. notatum	10	11	11	12.5	8	8	15	**20**	9	10
C. albicans	12	12	12	14	8	8	15	**21**	13	15
Conc.	100	200	400	800	100	200	400	800	100	200

Regarding antifungal activity, the maximum inhibition zone was observed for No against *P. notatum* (35.5 mm), followed by Nb against *P. notatum* and *C. albicans* (20 mm and 21 mm). The results also showed that TC was inactive against A. niger at all concentrations and moderately active against the other fungi.

4. Conclusions

In this study, we synthesized three new molecules from the phosphonate family: one α-aminophosphonate ester (TC) via the Kabachnik-Fields reaction, and two α-aminophosphonic acids (Nb and No) via the Irani-Moedritzer reaction. These molecules were synthesized using microwave-assisted techniques. The products were obtained in a short time and with good yields using microwave irradiation. The purity of the compounds was confirmed through TLC, with each product having a unique retention factor (RfNb = 0.87, RfNo = 0.16, RfTc = 0.72).

The synthesized products were characterized by determining some physicochemical properties using standard characterization techniques such as melting point determination, IR spectroscopy, and UV-Vis spectrophotometry. The melting points of the synthesized products (TfNb = 50 °C, TfNo = 46 °C, TfTc (gel)) were entirely different from those of the starting materials (Tfthiourea = 180 °C, Tf4-ClPh hydrazine = 210 °C).

The confirmation of our products was achieved through IR spectroscopy and UV-Vis spectrophotometry. Infrared spectroscopy was used to observe the disappearance of bands characteristic of primary amines (4-chlorophenylhydrazine, thiourea) and the appearance of new functions in the synthesized molecules, identifying key functional groups such as (P-C, P=O, N-C, phosphonates, etc.). The UV-Vis analysis provided qualitative data through comparison of spectra between the starting materials and the synthesized products. The results indicated the formation of different spectra compared to the starting materials, with new bands in the synthesized products Nb and No and a bathochromic effect for TC.

The antibacterial activity was evaluated against Gram-positive bacteria (Bacillus subtilis and Staphylococcus aureus) and Gram-negative bacteria (Escherichia coli and

Pseudomonas aeruginosa) using the well diffusion method. The results showed that all synthesized products exhibited good activity with an inhibition zone (D > 8), except for Nb against *S. aureus*.

The antifungal activity of the three products was tested against three pathogenic fungal strains (Candida albicans, Aspergillus niger, and Penicillium notatum). The results showed that the maximum inhibition zones were observed for No against *P. notatum* (35.5 mm) and Nb against *P. notatum* and *C. albicans* (20 mm and 21 mm). The results also showed that TC was inactive against A. niger at all concentrations and moderately active against the other fung.

Author Contributions:

Funding:

Institutional Review Board Statement:

Informed Consent Statement:

Data Availability Statement:

Conflicts of Interest: .