Synthesis, characterization and antimicrobial activity of two α-aminophsphonates esters derived from benzylamine

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Abstract

Synthesis of α-aminophosphonate esters with antimicrobial properties is of great interest in the development of new pharmaceutical products. In this article we present the synthesis of two α-aminophosphonate esters, namely, [(Benzylamino-phenyl-methyl)-phosphonic acid diethyl ester (AM1) and (Benzylamino-p-tolyl-methyl)-phosphonic acid diethyl ester (AM2) under green condition using microwave irradiation. These molecules where then, characterized, and evaluated for their antimicrobial responses. The synthesized compounds were obtained utilizing methylamine, aromatic aldehydes and diethylphosphite *via Kabachnik-Fields reaction*, as well as their structures were established through spectroscopic methods. Additionally, the results of the preliminary screening indicate that the examined molecule showed potent antimicrobial activity, compared to standard drugs. The results showed reasonable microbial activity against the two gram positive bacteria (*Staphylococcus aureus* and *Streptococcus pyogens*) and the two gram negative (*Pseudomonas aureginosa* and *Escherichia coli*) as well as two the fungi strains (*Aspergillus niger* and *Candida albicans*).

Keywords: α-aminophosphonate esters, Kabachnik-Fields reaction, antimicrobial, microwave irradiation.

1. Introduction

Aminophsphonate esters are among the organophsphorus compounds that are widely found in the component of disease treatments [1-3]. They have become increasingly involved in the discovery of new classes of medicines with new mechanisms of action. Aminophsphonic acids occupy an important place among the classes of compounds known for their therapeutic and pharmacological activities [4-6]. These pharmacophores are in fact the basic structure of many natural and synthetic molecules with anticancer, anti-inflammatory, antimicrobial, analgesic, and corrosion inhibitor activities. They are also ubiquitous in several pesticides such as herbicides, fungicides, and insecticides. Indeed, these entities constitute excellent pharmacophores for the development of many drug candidates [3].

In continuation of our previous efforts in the development of new Aminophsphonic acids as antimicrobial candidates [7-11], we report in the present work the synthesis, structural identification, and biological activity assessment of [(Methyl-phosphonomethyl-amino)-methyl]-phosphonic acid. The synthesis of this molecule is carried out from methylamine, formaldehyde and phosphorous acid *via Kabachnik-Fields reaction.* The Kabachnik–Fields reaction involves the condensation of a primary or secondary amine, an oxo compound such as an aldehyde or ketone, and a >P(O)H-containing reagent, which is in most cases a dialkyl phosphate to result in the formation of α-aminophosphonates,. The classical version of the "phospha-Mannich" reaction was discovered independently by Kabachnik and Fields more than sixty years ago [12,13]. The structural identification of the studied compound is established by spectroscopic techniques. In addition, the antimicrobial activity of the synthesized compound is evaluated against four microbial species and compared to that of standard drug.

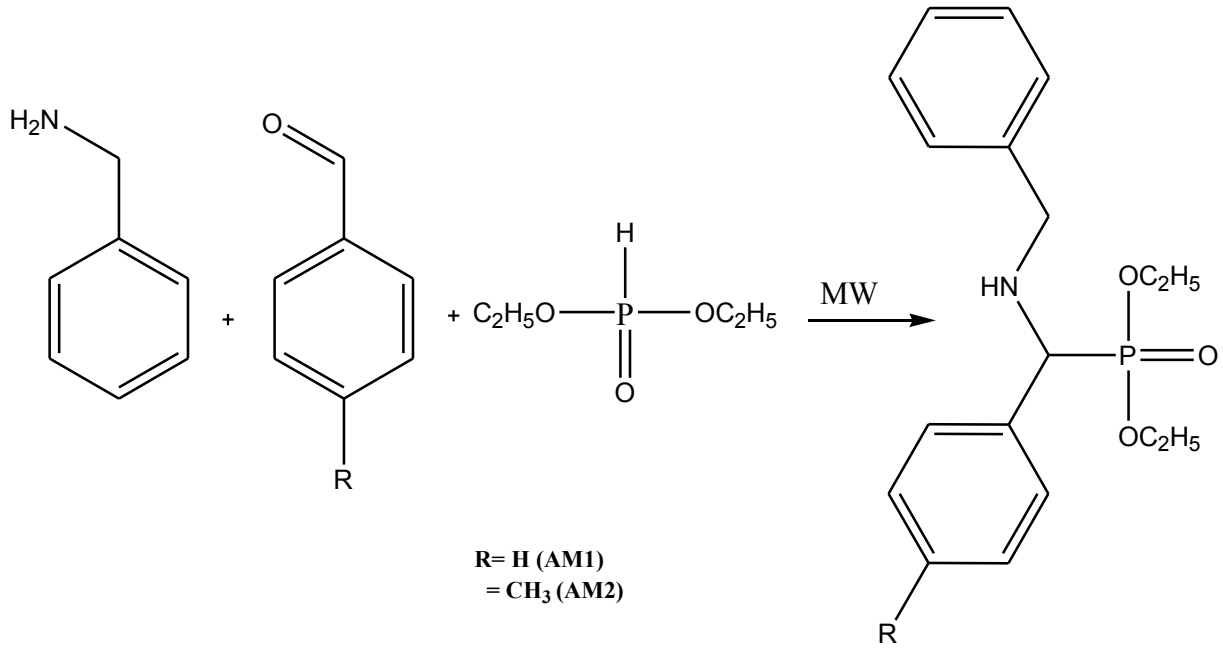
2. Materials and Methods

2.1. Experimental

All of the chemical substances used in this research were acquired from Sigma-Aldrich and used without additional purification. UV–vis spectra were obtained using a JASCO V-650 UV–Vis spectrophotometer with quartz cells of 1 cm path length in ethanol between 200 and 800 nm. FT-IR spectra were obtained using a JASCO 4200 spectrometer between 4000 and 400 cm^{-1}. Finally, the ^{31}P-NMR spectra were obtained using a Bruker AV III 300 MHz in DMSO-d_6 with tetramethylsilane (TMS) as an internal reference.

2.2. Chemical Synthesis of α-Aminophosphonates (AM1 and AM2)

A mixture of 1.0 mmol benzylamine, 1.0 mmol substituted aromatic aldehyde (benzaldehyde; p-methylbenzaldehyde) and 1.2 mmol diethyl phosphite was irradiated in a sealed tube in a Microwave reactor at the frequencies and for the times shown in Table 1. The volatile components were removed under reduced pressure. The residue so obtained was purified by flash column chromatography using silica gel and 3% MeOH in CH_2Cl_2 as the eluent to afford α-aminophosphonates (AM1 and AM2) as powder (Scheme 1).



Scheme 1. Synthetic route of AM1 and AM2 *via* the Kabachnik-Fields reaction.

2.3 Antimicrobial activity

The antibacterial activity of the AM1 and AM2 were examined against two gram positive bacteria (*Staphylococcus aureus* and *Streptococcus pyogens*) and two gram negative (*Pseudomonas aureginosa* and *Escherichia coli*) by agar disc diffusion method at concentration of 50-100 µg/mL. The bacterial cultures were

inoculated in nutrient broth (inoculation medium) and incubated overnight at 37°C. Inoculated medium containing 24h grown culture was added aseptically to the nutrient medium and mixed thoroughly to get a uniform distribution. This solution was poured (25mL in each disc) and then allowed to attain room temperature. Discs (6mm in diameter) were punched carefully using a sterile cork borer and were filled with test solution 200µL. The plate was allowed to stand for an hour in order to facilitate the diffusion of the drug solution, and incubated at 37°C for 24h and the diameter of the zone of inhibition was measured [14]. The results were compared with that of standard drug Gentamicin under identical conditions. Their antifungal activity was evaluated against *Candida albicans* and *Asperigillus niger* at concentration of 200 µg/ml. Griseofulvin was used as the reference compound. Fungal cultures were grown on potato dextrose broth at 25 °C and finally spore suspension was adjusted to 10^5 spores mL^{-1} [15].

3. Results and Discussion

3.1. Synthesis and Spectral Analysis

Microwave irradiation has become a very useful tool in organic synthesis and has been explored extensively since the last decade. Microwave irradiation often leads to a remarkable decrease in reaction time, increased yields and easier workup matching with green chemistry protocols. The synthetic pathway employed to prepare the target molecules is outlined in (Scheme 1), in a *one pot* process. Scheme 1 illustrates the synthesis route for the preparation of α-aminophosphonates (AM1 and AM2). This simple *one-pot* reaction occurs between amine compound (Benzyamine), both aromatic aldehyde (Benzaldehyde or p-methybenzaldehyde) and diethylylphosphite. The treatment and purification of the reaction mixture allowed us to isolate the corresponding compounds, in good yields. The reactions of benzylamine, benzaldehyde (or p-benzaldehyde) and diethyl phosphite were carried out under microwave (MW) irradiation is ethanol as solvent. α-Aminophosphonate AM1 and AM2 were obtained from benzaldehyde and diethyl phosphite at 200 W after a 15 min irradiation and they were isolated in a yields of 90% and 85% for AM1 and AM2 , respectively (Table 1-Entry 1 and 2). It is obvious that electron-donating substituent ($-CH_3$) in para position of the benzaldehyde slow down the condensation and the formation of AM2.

Entry	n	Ald.	Freq.(W)	t (min)	Yield (%)	Product
1	1	H	200	15	90	AM1
2	1	p-Me	200	15	55	AM2

Table 1. Times/Yields data of the obtained α-aminophosphonates.

The identification of the structure of the obtained products is established by usual spectroscopic methods, such as infrared (IR), UV-vis ^{31}P-NMR, and microanalysis. FTIR spectroscopy was used for characterizing the functional groups on the aminophosphonates. The reaction of amine, aldehyde with diethylphosphite leads to the formation of α-aminophosphonate, which is confirmed by the appearance of specific peaks on FTIR spectra, especially assigned to P-C, P=O, P-O-C groups. In the IR spectra of the products AM1 and AM2, we observe an absorption band around 3344 cm^{-1} characteristics of the vibration of the NH bond of the secondary amine; which highlights the substitution of the primary amine of the benzylamine. The bands had been observed at 3020-3070cm^{-1} and 2899-2905 cm^{-1} in the spectra of both aminophosphonates (AM1 and AM2) aliphatic sp^3 C-H (CH_3) and sp^1 C-H (N-CH-P) vibrations, respectively. The peaks at 1280–1291 cm^{-1} and 920–930 cm^{-1}, which are assigned to υ(–P—O) and υ(P-O-C) vibrations. The symmetric stretching vibrations of the P=O group for AM1 and AM2 appeared at 1,244–1,242 cm^{-1}. The ^{31}P -NMR spectra of AM1 and AM2 display a singlet signal at 23.16 and 23.44 for AM1 and AM2, respectively, belongs to the two phosphorus atoms of the phosphonate groups. The appearance of a singlet for AM1 and AM2, confirms the formation of α-aminophosphonate moiety. This resulting chemical shift value agrees with the chemical shift in literature. Table 2 summarizes the ^{31}P-NMR data required for the identification of α aminophosphonates AM1 and AM2 [16, 17].

Compounds	^{31}P (ppm)	$^{31}P_{lit}$ (ppm)	Reference
AM1	23.16	23.7	[16]
AM2	23.44	23.6	[17]

Table 2. ^{31}P-NMR Identification of the synthesized aminophosphonates.

C, H, N and O were estimated by using elemental analyzer Microanalysis is an important tool to get information about how the product is formed and which can be used to evaluate the purity of the synthesized products. In the present study, Microanalyses were performed using a LECO-elemental analyzer. The structure of AM1 and AM2 were further confirmed by elemental analysis (Table 3). *For* such data, *agreement of* calculated and *found* values within 0.4%. *Elemental analysis* values *of* the resulting aminophosphonates are in *good agreement with* the calculated values *for* the proposed structures

Compounds	M(g/mol)	Anal. calc. (%)	Found. (%)
AM1 ($C_{18}H_{24}NO_3P$)	333.15	C, 64.85; H, 7.26; N, 4.20; O, 14.40;	C, 64.77; H, 7.31; N, 4.27; O, 14.56;
AM2 ($C_{19}H_{26}NO_3P$)	347.17	C, 65.69; H, 7.54; N, 4.03; O, 13.82	C, 65.07; H, 7.59; N, 4.11; O, 13.42

Table 3. Elemental analysis identification of the synthesized aminophosphonates.

A possible mechanism is demonstrated *via* the reaction of benzaldehyde, benzylamine, and diethylphosphite. The first step consists of the formation of an imine-intermediate, followed by the attack of this intermediate by nucleophilic phosphite (leading to the formation of phosphonium ion). In the last step of the process, the reaction of phosphonium intermediates with water promotes the elimination of phenol and the formation of the relevant α-aminophosphonates. This interpretation of resin synthesis is supported by a series of analyses (see below).

3.2 Bioactivities evaluation

The increase in the mortality rate associated with infectious diseases is directly related to bacteria that exhibit multiple resistance to antibiotics. The lack of effective treatments is the main cause of this problem [18,19]. The development of new antibacterial agents with novel and more efficient mechanisms of action is definitely an urgent medical need [20]. Aminophsphonates have been pointed to as promising antibacterial agents. In the present study, the in vitro antimicrobial activity of the α- aminophsphonates was tested against two gram positive bacteria (*Staphylococcus aureus* and *Streptococcus pyogens*) and two gram negative (*Pseudomonas aureginosa* and *Escherichia coli*) as well as two fungi strains (*Aspergillus niger* and *Candida albicans*). The antibacterial effect of the four synthesized compounds against the four strains was investigated by using the diffusion method, and the results are reported in Table 4. The obtained data showed clearly that the AM2 compound possesses significant activity against the whole strains compared to the AM1. We also observed that the AM2 was more actives against *Pseudomonas aureginosa* and *Escherichia coli*, while the AM1 compound showed a substantial activity only against *Staphylococcus aureus*. On the other side, no inhibition zone was detected with vehicle control (DMSO).

Compounds	Zone of inhibition (mm)							
	Staphylococcus aureus		*Streptococcus pyogens*		*Escherichia coli*		*Pseudomonas aureginosa*	
	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml
AM1	8	12	-	-	11	15	13	16
AM2	10	15	11	15	13	17	14	18
Gentamicin (100µg/ml)	14	15	16	21	18	24	14	20
DMSO	-	-	-	-	-	-	-	-

Table 4. Antibacterial activity of α-aminophosphonates (AM1 and AM2).

The same AM1 and AM2 were screened for their antifungal activity (Table 5) against *Aspergillus niger* and *Candida albicans* species along with the standard fungicide Griseofulvin at three different concentrations (200µg/ml). It is gratifying to observe that the two compounds exhibited moderate antifungal activity when compared with the Griseofulvin reference.

Compounds	Zone of inhibition (mm)	
	Aspergillus niger	*Candida albicans*
	200 µg/ml	200 µg/ml
AM1	8	11
AM2	14	17
Griseofulvin (300 µg/ml)	24	26
DMSO	-	-

Table 5. Antifungal activity of α-aminophosphonates (AM1 and AM2).

4. Conclusions

The present study highlighted the successful synthesis of two α-aminophosphonate, under green condition using microwave irradiation in good yields. These molecules where then, characterized, and evaluated for their antimicrobial responses. The synthesized compounds were obtained utilizing methylamine, aromatic aldehydes and diethylphosphite *via Kabachnik-Fields reaction*. The molecular structures of newly synthesized compounds were characterized by analytical and spectral techniques. Additionally, the results of the preliminary screening indicate that the examined molecule showed potent antimicrobial activity, compared to standard drugs. The results showed reasonable microbial activity against the two gram positive bacteria (*Staphylococcus aureus* and *Streptococcus pyogens*) and the two gram negative (*Pseudomonas aureginosa* and *Escherichia coli*) as well as two the fungi strains (*Aspergillus niger* and *Candida albicans*). Further antimicrobial studies should be extended to other bacterial and fungal strains. As a result of, our study indicate that test compounds have similarly affected against test microorganisms as compared to antibiotics. The results of our study may help to obtain new antibiotics in advanced pharmacological research.

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