Synthesis, Antibacterial and Antifungal Screening of Three new of Alpha-aminophosphonic acids

Hellal Abdelkader*, Chafaa Salah, Chafai Nadjib

Abstract—Due to the medicinal importance of α-aminophosphonic acid derivatives, the biological property of three synthesized α-aminophosphonic acids derivatives were screened in vitro against some Gram-positive and Gram-negative pathogenic bacteria and some pathogenic fungi. The results obtained are compared with those of the starting compounds. In this study, three compounds ([(4-Hydroxyphenyl) phosphonomethyl-amino]-methyl)-phosphonic acid (S1), ([(3-Hydroxyphenyl) phosphonomethyl-amino]-methyl)-phosphonic acid (S2) And ([(2-Hydroxyphenyl) phosphonomethyl-amino]-methyl)-phosphonic acid (S3) have been synthesized from 2-aminophenol (2AP), 3-aminophenol (3AP) and 4-aminophenol (4AP). The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were assessed by serial dilution technique. The synthesized compounds showed good antimicrobial and antifungal activity against all the tested organisms and the results are comparable to standard antibiotic chloramphenicol. The low MIC and MBC values and high sensitivity of pathogenic microorganisms to the compounds led to conclude that the α-aminophosphonic acids derivatives have potential antimicrobial and antifungal properties

Index Terms— α-aminophosphonic acid, Antibacterial Activity, Antifungal Activity, MIC, MBC.

1 INTRODUCTION

ONE of the main causes of morbidity and mortality in immunocompromised patients in the developing countries is mainly due to the frequent life threatening infective diseases originated from various pathogenic microorganisms. A large number of drugs have been discovered so far to combat such situation. But none of these drugs could completely destroy such microorganisms in some cases. It is mainly because these organisms are developing resistance towards such drugs. As a result, the drugs already in use are gradually losing their effectiveness. The discovery of novel antibiotics which are much more effective against such microorganisms is essential. α-aminophosphonates are an important class of compounds since they are considered as structural analogues of the corresponding α-amino acids, and their utilities as enzyme inhibitors, antibiotics and pharmacological agents [1]. Aminophosphonates are widely used as imaging agents and as antitumor, antihypertensive and antibacterial agents [2].

α-aminophosphonates applications are significant in agriculture as plant regulators, herbicides [3], pesticides and in medicine as anticancer agents [4], enzyme inhibitors [5], peptide mimics [6], antibiotics and pharmacological agents [7]. These compounds have already been found to act as antibacterial agents, neuroactive compounds, with some of them already commercialized [8]. Synthesis of α-aminophosphonic acids has been a focus of considerable attention in synthetic organic chemistry as well as in medicinal chemistry [9-13]. The aminophosphonic acids are generally obtained by hydrolysis of esters via Kabachnik-Fields reaction [14]. This reaction can be activated by microwave irradiation [15], nevertheless there are often secondary reactions during the hydrolysis [14]. The synthesis of Irani-Moedritzer [16] is less general than the Kabachnik-Fields reaction, but it has the advantage of obtaining the aminophosphonic acid without a step of hydrolysis. In this context, these biological data prompted to investigate the biological activity of synthesized ([(2-Hydroxyphenyl) phosphonomethyl-amino]-methyl)-phosphonic acid (S1), ([(3-Hydroxyphenyl) phosphonomethyl-amino]-methyl)-phosphonic acid (S2) And ([(4-Hydroxyphenyl) phosphonomethyl-amino]-methyl)-phosphonic acid (S3). The results obtained are compared with those of the starting products (scheme 1).

2 Materials and methods

2.1 Chemistry

2.1.1 Chemicals and Materials

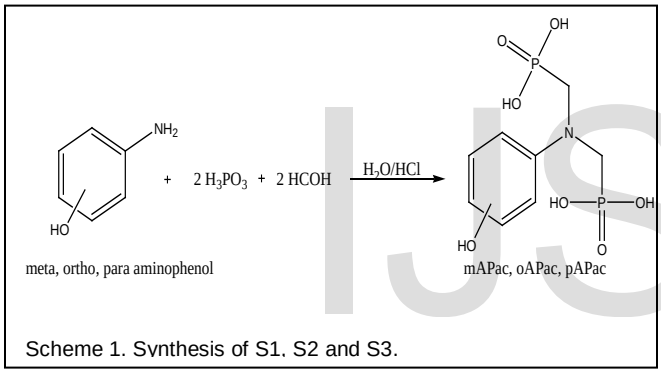
All chemicals used throughout the research were purchased from Aldrich, and used without further purification. Solvents were distilled from the appropriate drying agents and stored under nitrogen atmosphere. Melting points were determined on a digital apparatus Koefler Banc. All reactions were monitored using thin–layer chromatography (TLC) carried out on 0.25–mm E Merck silica gel plates (60F–254) and the spots were visualized by UV light. Infrared spectra were recorded on FT/IR JASCO 300 E (4000-400 cm-1). NMR spectra were recorded on a Bruker Avance 300 apparatus operating at 300 MHz with TMS as the internal standard and D_2O as solvent. Chemical shifts are given in parts per million (ppm). Multiplicities are shown as the abbreviations: s (singlet), brs (broad singlet),

- *Hellal Abdelkader is currently pursuing Magister degree program in Faculty of Technology, Departement of Ingeneering Process in Farhat Abbas Sétif1, Algeria, PH-0774835272. E-mail: haekphbarm@yahoo.fr*
- *Chafaa Salah is currently pursuing Professor degree program in Faculty of Technology, Departement of Ingeneering Process in Farhat Abbas Sétif1, Algeria, PH-0659280909.. E-mail: S,chafaa@yahoo.fr*
(*This information is optional; change it according to your need.*)

d (doublet), t (triplet), m (multiplet). ^{31}P NMR chemical shifts were referenced to external H_3PO_4 (85% w/w). The percentages of carbon, hydrogen and nitrogen were determined by Elemental analyses using Perkin Elmer 2400 CHN Elemental Analyzer.

2.1.2 General procedure for the synthesis of α-aminophosphonic acids (S1, S2, S3):

The α-aminophosphonic acids were synthesized according to the method explained by Irani- Moedritzer [16]. In a three-neck flask, a mixture of 0.1 mol of H_3PO_3 50% and 0.2 mol of Ortho-, Para- or Meta-aminophenol was dissolved into 100 ml water and 50 mL of hydrochloric acid. The mixture was allowed to be refluxed for 3h at 110°C while 0.4 mol of paraformaldehyde 37% solution was added drop wise to the reaction mixture. After adding, the mixture was kept at this temperature for an additional two hours. Completion of the reaction was monitored by TLC analysis. After completion of the reaction, solvent was removed in a rotaevaporator. The crude product was obtained, and the further pure product was received by recrystallization in ethanol (Scheme 1).



Scheme 1. Synthesis of S1. S2 and S3.

2.2 Biological activity

2.2.1 Microorganisms

Antimicrobial activity, Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Antifungal activity were determined against four gram-positive bacteria (*Bacillus subtilis (*ATCC 6633), *Bacillus megaterium* (ATCC 14581), *Stapphylococcus aureus (*ATCC 25923),and *Enterobacter aerogenes (*ATCC13048), four gram-negative bacteria (*Escherichia coli (*ATCC 25922), *Pseudomonas aeruginosa (*ATCC 27853), *Salmonella typhimurium* (ATCC 23564) and *Klebsiella pneumoniae* (ATCC 700603) and against three fungi (*Candida albicans* (ATCC 90028), *Aspergillus fumigatus* (ATCC 204305) And *Aspergillus flavus* (ATCC 204304)). All the organisms were collected from the Microbiology Laboratory of Ain Defla Hospital and Khemis Miliana University.

2.2.1 Preparation of inoculums

Suspension of organism was prepared as per McFarland nephelometer standard. A 24 hour old culture was used for the preparation of bacterial suspension. Suspension of organism was made in a sterile isotonic solution of sodium chloride (0.9% w/v) and the turbidity was adjusted so that it contained approximately 1.5×10^8 cells/ml. It was obtained by adjusting the optical density of the bacterial suspension to that of a solution of 0.05 ml of 1.175% of barium chloride and 9.95 ml of 1% sulphuric acid.

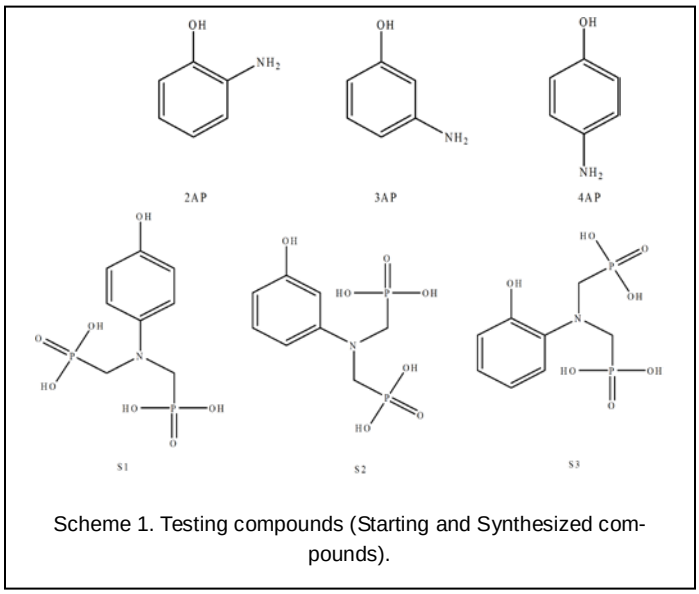
2.2.2 Antimicrobial screening

Newly synthesized compounds (S1-S3) and starting compounds (2AP, 3AP and 4AP) were screened *in vitro* for their antimicrobial activity against four gram-negative and four gram-positive bacterial strains using disc diffusion method [17, 18]. Briefly, three calculated amount of the compounds S1, S2 and S3 were dissolved in DMSO in three different vials for getting solutions having concentrations of 50 µg/ml, 75 µg/ml and 100 µg/ml respectively. They were then applied on filter paper disc. Standard chloramphenicol (100 µg/ml) was used as positive control and DMSO as negative control. Both experimental and control discs were placed in petridishes seeded with organism in nutrient agar medium. The petridishes were kept in a refrigerator at 4°C for 24 hours to ensure diffusion of the test materials. Finally, they were incubated at 37±1°C for 24 hours and all experiments were done as triplicates. The antibacterial activity was determined by measuring the diameter of zone of inhibition in mm.

2.2.3 Determination of MIC and MBC

Each bacteria has a level of antibiotic which will inhibit growth but not kill the organisms. This is called the minimum inhibitory concentration (MIC). Related to this, a higher antibiotic concentration will kill the organisms. This is called the minimum bactericidal concentration (MBC). By understanding the concepts in determining antibiotic concentrations compared to the MIC and MBC, we can make rational decisions in determining how successful antibiotic treatment is likely to be [19]. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of S1, S2, S3, 2AP, 3AP and 4AP were determined by serial dilution technique against the above mentioned pathogenic bacteria [19, 20].

S1, S2, S3, 2AP, 3AP and 4AP (Scheme 2) were used from a concentration of 0,25 µg/ml to 256 µg/ml. A control test-tube containing only medium (nutrient broth medium) was used to confirm the sterility of the medium. Bacterial suspension (10 µl) containing 10^7cells/ml was inoculated into all tubes. All of the test tubes were incubated at 37±1°C and observed for bacterial growth for 24 hours for MIC and 96 hours (4 days) for MBC determinations after inoculation for 24 hours, the test tube with no visible growth of the microorganism was taken to represent the MIC value of the sample in µg/ml. MBC, in which no viable organism occurred was determined by keeping the test tubes which was used for MIC determination for four days. After four days, bacterial growth was observed and MBC was determined at lowest concentrations where no bacterial growth was observed.



Scheme 1. Testing compounds (Starting and Synthesized compounds).

2.2.4 Evaluation of bactericidal and bacteriostatic capacity

The action of an antibacterial on the bacterial strains can be characterized with two parameters such as Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC). According to the ratio MBC/MIC, we appreciated antibacterial activity. If the ratio MBC/MIC≤4, the effect was considered as bactericidal but if the ratio MBC/MIC >4, the effect was defined as bacteriostatic [21, 22].

2.2.5 Antifungal activity

For antifungal screening, each sample was tested at concentrations of 100, 200, 400 µg/ml. The experimental protocol was almost same as antibacterial screening except the plates were incubated at 37±1°C for 48h and Nystatin disc was used as positive control. All experiments were done in triplicates.

2.2.6 Statistical analysis

The experimental results have been expressed as the mean ± SEM (Standard Error of Mean). Statistical analysis was performing with Origin 6.0 software;

3. Results and Discussion

3.1. Chemistry

The α-aminophosphonic acids (**S1-S3)** used in this study were synthesized via Irani-Moedritzer reaction by treatment of the formaldehyde with the primary amine (Ortho-, Meta- and Para-aminophol) and phosphorous acid in strong acidic medium (concentrated hydrochloric acid) under reflux. After filtration and evaporation, the α-aminophosphonic acids were isolated in almost quantitative yields. The results showed that the α-Aminophosphonic acids (**S1-S3**) are synthesized in good yields (73-88%). Thin layer chromatography (TLC) was employed to monitor reaction progress and to determine the purity of the products. All the title compounds (S1-S3) readily dissolve in polar solvents and melted in the range of 134-161°C. The structures of the title compounds **S1-S3** were established by their spectroscopical data. The structures of the obtained compounds were identified and characterized by elemental analysis, FT-IR, and by ^{1}H NMR, ^{13}C NMR and ^{31}P NMR spectroscopy. The percentages of carbon, hydrogen and nitrogen were determined by using CHN analyzer. The found and calculated data is in good agreement with the proposed molecular formulae. The presence of α-aminophosphonic acids was confirmed by FTIR measurement: All the compounds (S1-S3) showed absorption bands of the saturation carbon (sp^3) at 2930-2290 cm^{-1}. The α-aminophosphonic acids display characteristic bonds for phosphonic group (P–O–H) at 3300-3400 cm^{-1}. The peak at 1645 is due to (P(O)–OH) stretching and the absorption bands at 1256 cm^{-1} and 957 cm^{-1} are assigned to the stretching modes of (P=O) and (P–O-H) respectively. The strong peak at 1378 cm^{-1} is ascribed to the (P-CH_2) bending that could be taken advantage for confirming the formation of a bond between phosphorus acid and methanal. The peaks at 1510 and 1604 cm^{-1} are assigned to the vibrations of aromatic ring. The broad absorption band from 3500~3600 cm^{-1} arises from stretching (–OH) of phenol group. NMR spectral characteristics of α-aminophosphonic acids are an important tool for determination of structure and identification of new compounds. The structure of these compounds was confirmed on the basis of their spectroscopic characteristics. In the ^{1}H NMR spectrum of (S1), the methylene protons resonate as singlet at 1.96ppm. The other consecutive signals correspond to the phosphonic group resonate as singlet at 4.56ppm. The aromatic protons resonate as multiplet at 5.7-6.2ppm. The phenol proton resonates as singlet at 6.68ppm. On the other hand the methylene carbon appeared as singlet at 46.42 ppm in the ^{13}C NMR, the aromatics carbon showed resonance at 116 and 156.6ppm. The ^{1}H NMR spectrum of (S2) shows a multiplet in the region 5.60-6.07 ppm for the aromatic protons. The protons of the methylene group (N-CH_2) appeared as a singlet at 0.987 ppm while the proton of the phosphonic group (P-OH) appears as a singlet at 4.83 ppm. The phenolic protons appeared at the region 6.7 ppm. The ^{13}C NMR shows signals at 116-140ppm for aromatics cabrons. The methylene carbon appeared as singlet at 30.10 ppm which confirms the structure of the product. The ^{1}H NMR spectrum of compound (S3) demonstrated a singlet at 4.25ppm due to phosphonic protons, the singlet at 1.75ppm was assigned to the methylene protons. The aromatic protons appeared as a multiplet at 5.7-6.64ppm. The phenolic proton appeared as singlet at 6.8ppm. The ^{13}C NMR spectrum exhibited the following signals: methylene carbon at 46.52ppm and the aromatic carbons at 116.4–156ppm. The ^{31}P-NMR spectroscopy is the most precise method for determining the structure of the phosphorus-containing compounds. Chemical shifts for ^{31}P of the three α-aminophosphonic acids (S1-S3) synthesized depend on imbalance of σ-bonds caused by the difference in electronegativity of the atoms and by the effect of the free electron pairs, degree of occupation of phosphorus *d*-orbitals, and deviation from geometric symmetry [23]. The phosphorus atom of the three α-aminophosphonic acids (S1-S3) in the ^{31}P NMR spectrum appears at 1.017–1.081ppm. From these results, we confirm the successful synthesis of α-aminophphosphonic acids (S1-S3) with high purity.

3.2 Biological assay

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3.2.1 Antibacterial activity

All the compounds were screened against Gram-positive bacteria and Gram-negative bacteria at three concentrations, 50, 75 and 100µg/ml by the disc diffusion method and the results were compared with the standard drug. Standard antibiotics (Chloramphenicol) were used as positive control and DMSO used as negative control. The results for the antibacterial activity of S1, S2, S3; 2AP, 3AP and 4AP as well as the standard and DMSO have been presented in Table 1. DMSO had no effect on the bacteria in the concentrations studied. All these compounds showed higher zone of inhibition when tested with higher doses (Table 1). A poor activity was shown by 2AP, 3AP and 4AP against Gram positive bacteria and Gram negative bacteria compared to the reference compound and the synthesized compounds (S1, S2 and S3). The synthesized compound presenting the strongest antimicrobial activity than the starting products and broadest range of action were, in descending order, S3, S2, S1, 2AP, 3AP and 4AP. For the S1, S2, S3 products, The highest activity (diameter of zone of inhibition >27mm) was demonstrated by all compounds against all tested bacteria at high concentration (100µg/ml), while the lowest activity (diameter of zone of inhibition <16mm) was demonstrated by the products at low concentration (50µg/ml) against *gram-negative bacteria.* The good activity of the newly synthesized compounds is attributed to the presence of pharmacologically active aminophosphonic groups attached to phenyl group at 2, 3 and 4th position. When the substitution of these groups is replaced by hydrogen, a sharp decrease in activity against most of strains was observed. Compounds **S1**, **S2**, **S3** exhibited better activity compared to that of standard Chloramphenicol against all the bacterial strain. Further the result showed that Gram- positive exhibited better activity than Gram-negative organism.

TABLE 1
ANTIBACTERIAL ACTIVITY OF 4AP, 3AP, 2AP, S1, S2, S3 AND CHLORAMPHENICOL.

		S1 4AP			S2 3AP			S3 2AP			Standard (+ Ctrl)
	Testing organisms	50 µg/ml	75 µg/ml	100 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml	100 µg/ml
Gram-Positive	*B. subtilis*	16.6±0.4	19.9±0,4	25.6±0,4	14.6±0.4	19.8±0,8	32.6±0,4	16.9±0.4	22.9±0,4	35.0±0.0	32.8±0.0
		R	11.0±0.8	16.3±0.0	10.4±0.0	12.6±0.0	20.4±0.5	R	12.9±0.2	17.4±0.0	
	B.megaterium	15.3±0.7	20.6±0,5	25.6±0.3	16.0±0.9	18.6±0,7	29.6±0.3	15.1±0.7	21.6±0,0	34.0±0.5	34.5±0.9
		R	13.4±0.7	14.1±0.0	06.2±0.8	11.4±0.6	19.4±0.6	R	13.4±0.6	19.4±0.1	
	S.aureus	15.0±0.0	16.3±0,4	24.3±0.4	17.0±0.1	22.3±0,1	33.3±0.4	17.0±0.0	23.0±0,4	32.6±0.2	30.1±0.0
		R	12.7±0.0	16.4±0.7	R	11.9±0.8	19.4±0.0	R	11.5±0.6	18.4±0.4	
	E. aerogenes	15.0±0.0	18.0±0,4	20.0±0.0	15.5±0.0	17.6±0,3	25.0±0.0	14.0±0.9	18.0±0,4	30.0±0.0	29.7±0.8
		08.4±0	09.0±0.1	14.0±0.8	R	13.4±0.9	16.4±0.1	R	14.8±0.5	20.4±0.8	
	E. coli	14.0±0.0	19.9±0,4	25.0±0.0	14.0±0.8	17.9±0,5	31.0±0.7	14.0±0.9	19.9±0,4	34.0±0.0	30.8±0.0
		R	09.4±0.6	14.7±0.0	09.0±0.1	11.0±0.7	18.4±0.8	R	10.4±0.8	21.4±0.6	
Gram-Negative	*P. aeruginosa*	12.3±0.7	17.0±0,2	23.0±0.5	12.3±0.0	19.6±0,9	25.0±0.5	13.3±1.2	19.6±0,5	30.3±0.8	29.5±0.9
		R	12.8±0.5	15.9±0.8	R	10.4±0.8	17.4±0.0	R	11.2±0.0	22.4±0.1	
	S. typhi	11.0±0.0	16.3±0,3	24.0±0.6	13.0±0.6	19.3±0,7	29.0±0.0	11.0±0.4	19.3±0,4	32.7±0.6	28.9±0.0
		R	11.0±0.9	13.2±0.7	08.9±0.6	10.1±0.3	15.4±0.9	R	10.4±0.3	19.0±0.8	
	K. pneumoniae	10.3±0.7	15.0±0,0	22.0±0.0	12.3±0.7	22.0±0,0	27.0±0.9	11.3±0.7	23.0±0,4	33.6±0.9	29.7±0.0
		R	10.3±0.5	15.4±0.7	R	11.2±0.1	15.4±0.6	R	11.9±0.7	22.7±0.0	
	DMSO (- Ctrl)	R	R	R	R	R	R	R	R	R	R

R= Resistance.

(- Ctrl) Negative control.

(+ Ctrl) Positive control.

MIC and MBC values of these compounds against pathogenic bacteria are shown in Table 2. For the synthesized compounds, MIC values ranged between 8 and 32µg/ml against gram-positive and gram-negative, and MBC values, between 32 and 256 µg/ml. Almost as a rule, MBC values were several folds higher than those of MIC, suggesting a better inhibitory than bactericidal activity. The exception was the case of the starting compounds where the MICs

against all tested strains were lowest values. 2AP, 3AP and 4AP presented higher MIC and MBC values than the other evaluated. These compounds showed weak to moderate activity against both Gram-positive and Gram-negative bacteria (MIC between 32 and 256µg/ml and MBC between 64 and 256µg/ml). Antibacterial potency of S1, S2 and S3 against these bacteria expressed in MIC indicated the synthesized products are more effective against gram-positive at lower concentration than that against gram-negative bacteria. The strong antibacterial activity of S3 was confirmed by the lowest MIC and MBC values (8-16µg/ml and 16-128 µg/ml respectively) observed against all tested microorganisms.

TABLE 2
MINIMUM INHIBITORY CONCENTRATION MIC AND MBC OF S1, S2, S3, 4AP, 2AP AND 3AP.

	S1			*S2*			*S3*		
	4AP			*3AP*			*2AP*		
Testing organisms	MIC	MBC	**MBC/MIC (Effect)**	MIC	MBC	**MBC/MIC (Effect)**	MIC	MBC	**MBC/MIC (Effect)**
Bacillus subtilis	8	32	4 (+)	16	32	2(+)	8	16	2(+)
	16	128	8(-)	32	256	8(-)	32	256	4(+)
Bacillus megaterium	16	64	4(+)	16	128	4(+)	8	16	2(+)
	32	256	8(+)	32	128	4(+)	16	128	4(+)
Stapphylococcus aureus	32	128	4(+)	16	128	4(+)	8	32	8(-)
	64	256	4(+)	16	256	16(-)	32	64	2(+)
Enterobacter aerogenes	16	32	2(+)	32	128	4(-)	16	128	8(-)
	32	256	8(-)	64	256	4(+)	64	128	4(+)
Escherichia coli	16	32	2(+)	32	128	4(+)	8	32	4(+)
	64	128	2(+)	128	256	2(+)	64	256	4(+)
Pseudomonas aeruginosa	32	128	4(+)	16	128	4(+)	16	32	2(+)
	64	128	4(+)	32	128	8(-)	32	128	4(+)
Salmonella typhimurium	32	64	2(+)	32	128	4(+)	16	128	4(+)
	64	256	4(+)	64	256	4(+)	32	64	2(+)
Klebsiella pneumoniae	16	128	4(+)	32	128	4(+)	16	128	4(+)
	32	128	4(+)	64	128	4(+)	32	256	8(-)

(+): bactericidal effect, (-) bacteriostatic effect

The MIC values of S1 were found to be between 8-32 µg/ml and the MBC values for S1 were 32-128 µg/ml. On the other hand the MIC and MBC values of S2 and S3 were also found to be between 8-32 and 64-128 µg/ml in Table 2. The bactericidal and bacteriostatic effect of the tested compounds was determined using the ratio MBC/MIC. Antimicrobial substances are considered as bacteriostatic agents when the ration MBC/MIC>4 and bactericidal agents when the ration MBC/MIC≤4. The summary of the microcide and the microbiostatic effects were given in Table 2. In the present study, S1, S2, S3 and standard showed the ratio MBC/MIC≤4, suggesting that these compounds act as bactericidal agents on these strains. Most Starting products showed MBC/MIC ratio of more than 4, which may be classified as bacteriostatic agents.

3.2.2 Antifungal activity

Antifungal activity of the compounds were also determined at three different doses (100, 200, 400 µg/ml) against three pathogenic fungi such as *Candida albicans Aspergillus flavus* and *Aspergillus fumigatus*. At lower doses all these organisms were almost insensitive to the starting compounds but at higher doses the compounds showed mild to moderate antifungal activities which are given in Table 3. For the synthesized products, Compound S3 showed highest activity against *Aspergillus fumigatus* (zone of inhibition 30.0 ± 0.5 mm at 400 µg/ml) whereas S1 and *S*2 were effective to same extent against *Aspergillus flavus* (zone of inhibition 29 ±0.3 mm at 400 µg/ml) and *Aspergillus fumigatus* (zone of inhibition 28±3 mm at 400 µg/ml) respectively.

TABLE 3
ANTIFUNGAL ACTIVITY OF S1, S2, S3, 4AP, 2AP, 3AP AND NYSTATIN.

	S1			S2			S3			Standard
	4AP			3AP			2AP			
Testing organisms	100 µg/ml	200 µg/ml	400 µg/ml	100 µg/ml	200 µg/ml	400 µg/ml	100 µg/ml	200 µg/ml	400 µg/ml	400 µg/ml
Candida albicans	12.9±0,9	17.9±0,4	26.6±0,4	14.6±0.4	19.8±0,8	25.6±0,4	10.9±0.4	20.0±0,4	27.0±0.0	27.8±0.0
	R	10.0±0.8	12.3±0.0	R	12.6±0.0	15.4±0.5	R	12.9±0.2	16.4±0.0	
Aspergillus fumigatus	11.9±0,0	16.6±0,5	25.6±0.3	16.0±0.9	18.6±0,7	25.6±0.3	15.1±0.7	19.6±0,0	30.0±0.5	26.5±0.9
	R	09.4±0.7	10.1±0.0	R	10.4±0.6	13.4±0.6	R	11.4±0.6	17.4±0.1	
Aspergillus flavus	11.8±0,9	14.3±0,4	29.3±0.4	17.0±0.1	22.3±0,1	28.3±0.4	17.0±0.0	20.0±0,4	22.6±0.2	24 .9±0.0
	R	08.7±0.0	11.4±0.7	R	10.9±0.8	13.4±0.0	R	11.5±0.6	16.4±0.4	

R= Resistance

4 CONCLUSION

In this paper three novel aminophosphonic acids derivatives containing aminophenol side groups were synthesized and evaluated their antimicrobial and antifungal activities. The structure of the obtained compounds was confirmed by different spectral methods and elemental analysis. Testing *in vitro* of these new compounds for their biological activity against several type strains, it was noticed that {[(4-Hydroxyphenyl) phosphonomethyl-amino]-methyl}-phosphonic acid *(S3)* showed the best antibacterial an antifungal actions on both gram negative and gram positive bacterial species. It is clear that the synthesized compounds have broad spectrum of antimicrobial activity than the starting compounds. The results obtained indicate that the synthezised compounds (S1, S2 and S3) could be potential chemotherapeutics and also further pharmacological investigation is needed in this area. Further experiments are required to investigate the actual mechanism of bioactivities and their probable effects on animal model.

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