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Synthesis and biological evaluations of a series of novel azolyl, azinyl and azepinyl phosphonates

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Abstract

A facile synthetic methodology of novel azolyl, azinyl and azepinyl phosphonates as cyclic α-aminophosphonates was described. The methodology depends on reaction of 6-methyl-3-formylchromone, and nitrogen nucleophiles in the presence of diethyl phosphite in one-pot three component under solvent-free conditions.The products were evaluated for their antimicrobial activities and antioxidant properties.

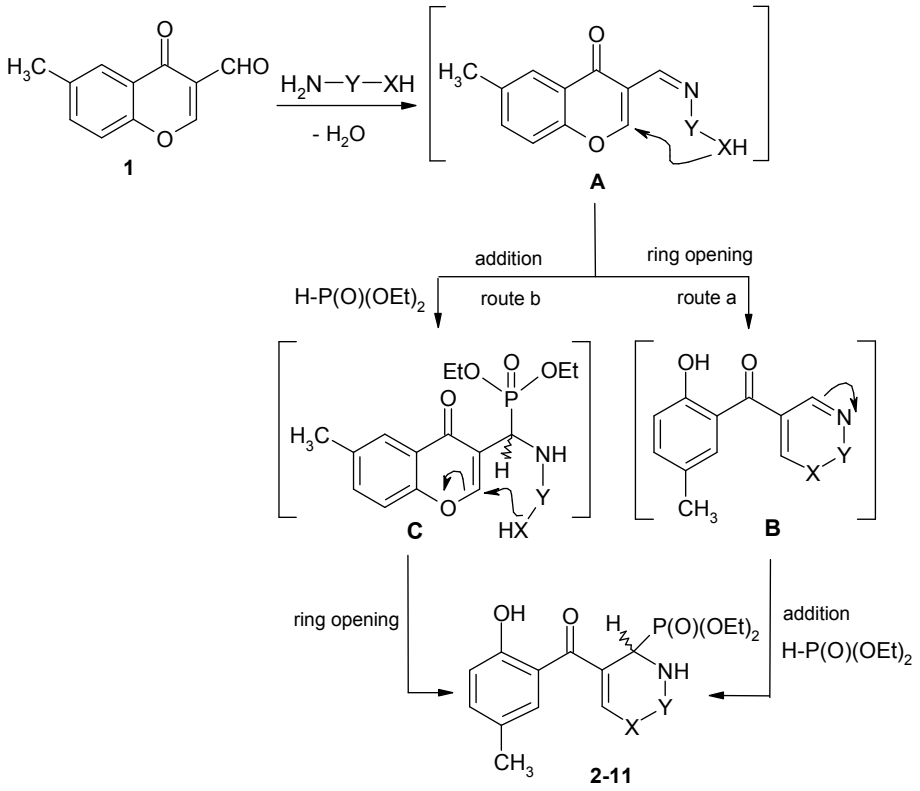
Keywords: α-Aminophosphonate, Phosphonate, Chromone, Ring opening and Ring closure, Antimicrobial, Antioxidant.

Introduction

α-Aminophosphonic acid diesters, as phosphorus analogues of α-aminocarboxylic acids, are of great interest due to their reported biological activities.[1] Some representatives of α-amino-phosphonates have demonstrated promising enzyme inhibitory activities, as for example, HIV protease antagonists[2] and collagenase inhibitor.[3] Also, they have an important anticancer,[4] antibacterial[5] and antiviral activities.[6] These biological properties are mostly associated with the tetrahedral structure of the phosphonyl group acting as "a transition-state analogue".[7] In the last decades, intensive synthetic articles were performed in the preparation of α-aminophosphonic acids and their esters.[8–13] The Kabachnik-Fields method is the most noteworthy and remarkable, generally using amines, dialkyl phosphites and carbonyl compounds.[14,15] Although, a number of different methods have been reported for the preparation of acyclic α-aminophosphonates,[16–21] there is still a need to search for new methods for the preparation of cyclic α-aminophosphonates which have found promising biological applications.[22,23] As a part of our continuing interest in the preparation of acyclic and cyclic α-aminophosphonates,[24–27] we describe a facile methodology to prepare some novel cyclic α-aminophosphonates. The method is based on the reaction of 6-methyl-3-formylchromone with nitrogen *bi*-nucleophiles in the presence of diethyl phosphite in one step under solvent-free conditions. The antimicrobial activities and antioxidant properties of the synthesized compounds were also evaluated.

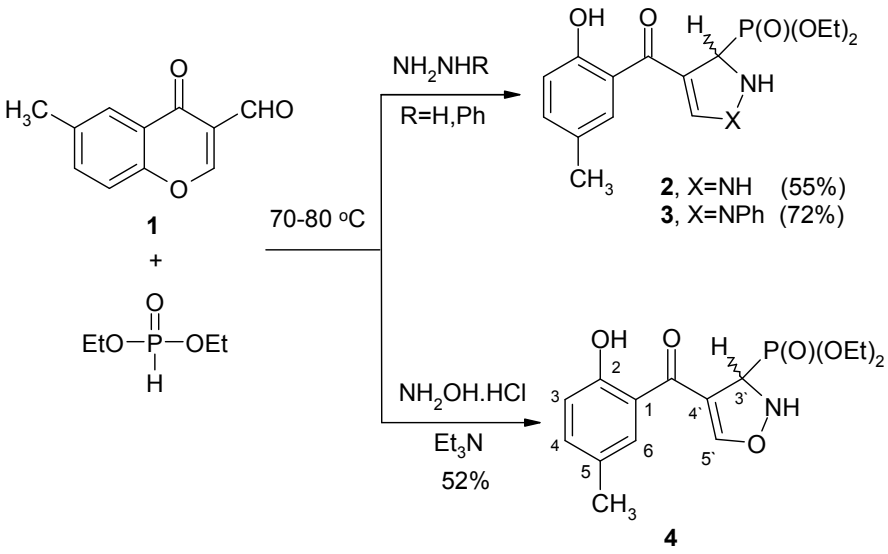
Results and discussion

3-Formylchromones have attracted attention long ago as highly reactive compounds, which can serve as starting substances in the synthesis of a whole series of heterocycles. 3-Formylchromones have useful chemical properties due to presence of three strong electrophilic centers at C−2, $C=O_{pyrone}$ and formyl group.[28,29] In the present article, the synthetic utility of 6-methyl-3-formylchromone (**1**) is derived from its reaction with nitrogen *bi*-nucleophiles that starts predominately from the attack on the formyl group to give the nonisolable condensation product **A** then pyrone ring opening at the unsubstituted C−2 forming the intermediate **B**. The latter intermediate **B** undergoes addition of diethyl phosphite at the cyclic azomethine bond to form the target phosphonates (route a, Scheme 1). Also, these phosphonates may be formed *via* addition of diethyl phosphite at the acyclic azomethine bond of the intermediate **A** leading to the formation of the nonisolable intermediate **C**, which undergoes pyrone ring opening (route b, Scheme 1).[30] The resulted compounds gave characteristic red, green and blue colors with an alcoholic ferric chloride solution which support pyrone ring opening.



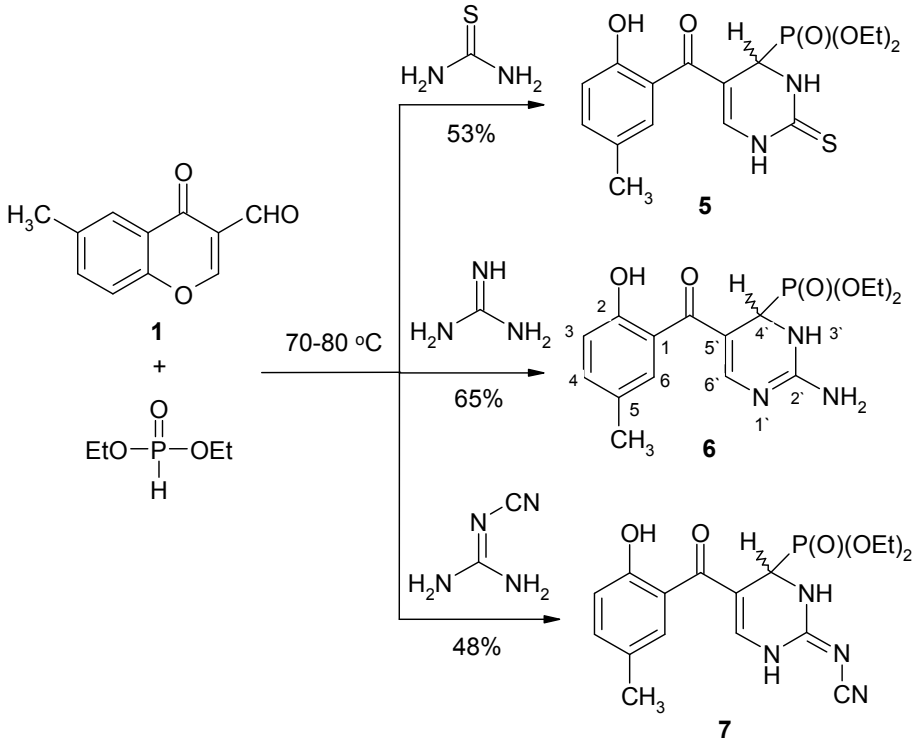
Scheme 1

When 6-methyl-3-formylchromone (**1**) was treated with some nitrogen 1,2-*bi*-nucleophiles such as hydrazine hydrate, phenyl hydrazine and hydroxylamine hydrochloride in the presence of diethyl phosphite at 70−80 ^{o}C for two hours afforded the corresponding novel azolyl phosphonates **2**−**4**, respectively, as cyclic α-aminophosphonates (Scheme 2). Structures of the products **2**−**4** were elucidated from their spectroscopic data and elemental analysis.



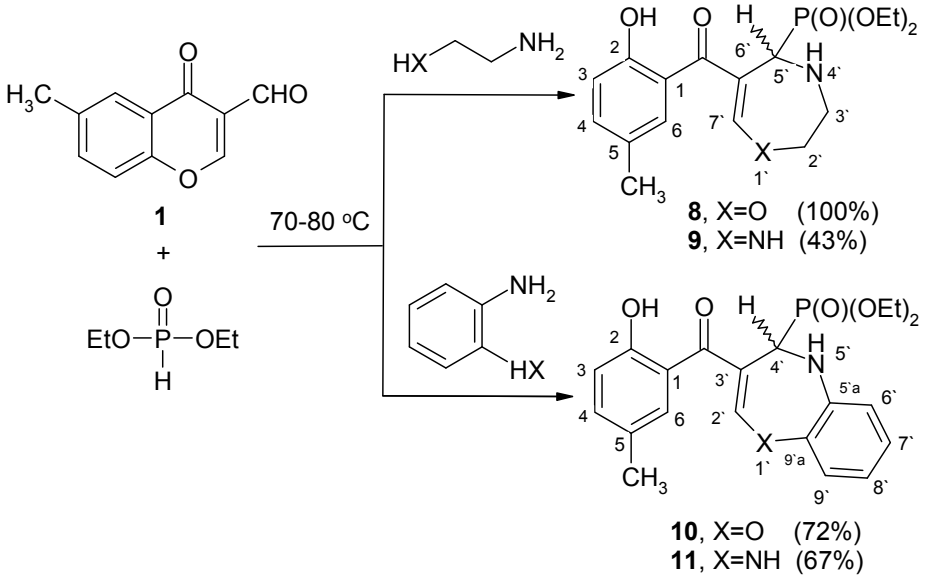
Scheme 2

In the present investigation, several subtypes of 1,3-*bi*-nucleophiles were used for recyclization of compound **1**, in the presence of diethyl phosphite leading to the formation of some novel pyrimidinyl phosphonates as cyclic α-aminophosphonates. Thus, fusion of the aldehyde **1** with thiourea, guanidinium carbonate and cyanoguanidine in the presence of diethyl phosphite at 70−80 °C yielded a first type of pyrimidinyl phosphonates **5**−**7**, respectively (Scheme 3).



Scheme 3

Seven-membered heterocycles with two heteroatoms in 1,4-distance are known to possess main fold biological activity.[31–33] The present study was extended to investigate the behavior of the aldehyde **1** with classical nitrogen 1,4-*bi*-nucleophiles in the presence of diethyl phosphite with a view to synthesizing phosphonates beard on seven-membered heterocyclic systems. Thus, treatment of aldehyde **1** with each one of nitrogen 1,4-*bi*-nucleophiles such as ethanolamine, ethylenediamine, 2-aminophenol and 1,2-phenylene-diamine in the presence of diethyl phosphite at 70−80 °C for 4 hours furnished the corresponding phosphonate derivatives of 1,4-oxazepine **8**, 1,4-diazepine **9**, 1,5-benz-oxazepine **10** and 1,5-benzodiazepine **11**, respectively (Scheme 4).



Scheme 4

Biological evaluations

1. Antimicrobial activity

All the newly synthesized compounds were evaluated in *vitro* for their antibacterial activities against *Staphylococcus aureus* (ATCC 25923) and *Bacillus subtilis* (ATCC 6635), as representatives of Gram–positive bacteria and *Escherichia coli* (ATCC 25922) and *Salmonella typhimurium* (ATCC 14028) as examples of Gram–negative bacteria. They were also examined against *Candida albicans* (ATCC 10231) as yeast and *Aspergillus fumigatus* as fungus. Agar diffusion technique was used for the determination of the preliminary antibacterial and antifungal activities.[34,35] Cephalothin, Chloramphenicol and Cycloheximide were used as reference drugs (30 µg/mL) for Gram positive bacteria, Gram negative bacteria and fungi, respectively. The minimum inhibitory concentration (MIC, µg/mL) for some selected compounds against some species of microbes was also determined. The tube dilution technique[36] was applied for the determination of MIC of the tested compounds against microbes. Dilution series were set up with 250, 125, 62.5……3.25 µg/mL of nutrient broth medium to each tube, 100 mL of standardized suspension of the test microbes (107 cell/ mL) were added and incubated at 37 °C for 24 hours.

The obtained results on the antimicrobial activities of the compounds and control drugs are given in Table 1. In general, the tested compounds recorded variable antimicrobial activities towards the used microorganism. The most compounds recorded low to moderate inhibitory effects towards all the microorganisms. The antimicrobial spectrum of the synthesized compounds against Gram-negative bacteria demonstrated very low inhibitions. Similarly, compounds **4** and **7** recorded moderate inhibitions against *Bacillus subtilis* with high MIC value >250 μg/ml. All the tested compounds exhibited relatively low to high inhibitory activities against *Candida albicans*. Furthermore, compounds **2–5**, and **7** exhibited relatively moderate inhibitory activities against *Candida albicans* especially compounds **4** and **5** which recorded MIC values at 250 μg/ml.

2. Antioxidant activity

The nitrogen centered stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) has often been used to characterize antioxidants. It is reversibly reduced and the odd electron in the DPPH free radical gives a strong absorption maximum at λ 517 nm, which is purple in color. This property makes it suitable for spectrophotometric studies. A radical scavenging antioxidant reacts with DPPH stable free radical and converts into 1,1-diphenyl-2-picrylhydrazine. The resulting decolorization is stoichiometric with respect to the number of electrons captured. The change in the absorbance produced in this reaction has been used to measure antioxidant properties. The solutions of tested compounds (150, 300 and 450 μmol L^{-1}) were added to DPPH (100 μmol L^{-1}) in DMSO/ethanol. The tubes were kept at an ambient temperature for 20 minutes and the absorbance was measured at λ 517 nm. The difference between the test and the control experiments was taken and expressed as the percent scavenging of the DPPH radical using the following formula % inhibition = (*AB*−*AA*/*AB*) x 100 where *AB* = absorption of blank and *AA* = Absorption of tested compound. The radical scavenging activity of ascorbic acid was also measured and compared with that of the different synthesized compounds.[37,38] The observed data on the antioxidant activities of the compounds and control are shown in Table 2 and illustrated in Figure 1. The results of scavenging the stable DPPH radical recorded variable antioxidant activities towards the synthesized compounds at the different concentrations 150, 300 and 450 μmol L^{-1}. Compounds **3**, **7**, **8** and **11** showed moderate activities. On the other hand, compounds **5** and **9** proved to exhibit potent antioxidative activities. The structure activity relationships (SARs) of the tested compounds demonstrated that all the synthesized compounds recorded remarkable inhibition activities in range 37.81−78.35% at the different concentrations due to the presence of 4-methylphenol group in all the compounds. The cyclic α-amino-phosphonates **2**–**11** recorded noticeable antioxidative properties. This may due to the presence of free phenolic OH groups in compounds **2**–**11** which can scavenge the DPPH radical. The appearance of isoxazole unit in compound **4** exhibited greater activity than those having pyrazole units in compounds **2** and **3**. Similarly, the thioxopyrimidinyl derivative **5** was more active than the other amino/cyanoiminopyrmidinyl derivatives **6** and **7**. Amongst compounds having seven-membered rings **8**–**11**, the diazepinyl derivative **9** exhibited the highest inhibition activity. In this study, the systems **5** and **9** displayed the higher scavenging

activities. However, the result exemplified that compound **9** having the diazepinyl unit in combination with phosphonic diester moiety is the most powerful antioxidant agent.

Table 1: In *vitro* antimicrobial activities of the synthesized compounds **2-11** at 500 and 1000 μg/mL and the MIC values for some selected compounds.

Compd.	Conc. (μg/ml)	Zone of inhibition in mm* and (MIC values in μg/ml)					
		Bacteria Gram (+) ve		Bacteria Gram (−) ve		Yeast	Fungi
		S. aureus	*B. subtilis*	*S. typhimurium*	*E. coli*	*C. albicans*	*A. fumigatus*
2	500	-	8	-	8	13 (>250)	-
	1000	-	9	-	9	17	-
3	500	-	-	-	-	10 (>250)	-
	1000	-	-	-	-	13	-
4	500	-	9 (>250)	-	-	17 (250)	-
	1000	-	14	-	-	20	-
5	500	-	-	-	-	14 (250)	-
	1000	-	-	-	-	20	-
6	500	7	-	7	8	8	-
	1000	10	-	8	10	9	-
7	500	-	10 (>250)	-	-	13 (>250)	-
	1000	-	12	-	-	18	-
8	500	7	9	9	7	7	7
	1000	8	10	11	10	8	11
9	500	7	8	-	-	8	-
	1000	8	9	-	-	11	-
10	500	-	8	-	-	11	-
	1000	-	9	-	-	15	-
11	500	-	8	-	-	7	-
	1000	-	9	-	-	14	-
Standard drug	500	26	25	28	27	28	26
	1000	35	35	36	38	35	37

* Low active: 6–12 mm; moderately active: 13–19 mm; highly active: 20–30 mm; –: No inhibition or inhibition less than 5 mm.

Table 2: DPPH radical scavenging activities of the synthesized compounds **2-11** at 150, 300 and 450 μmol L^{-1}.

Compd. No.	DPPH % inhibition antioxidant ± SD		
	150 μmol L^{-1}	**300 μmol L^{-1}**	**450 μmol L^{-1}**
2	51.79 ± 0.25	53.46 ± 0.12	58.58 ± 0.06
3	48.84 ± 0.12	52.89 ± 0.06	56.16 ± 0.06
4	55.35 ± 0.06	58.02 ± 0.18	62.46 ± 0.06
5	61.53 ± 0.38	64.70 ± 0.25	67.96 ± 0.06
6	56.16 ± 0.12	59.13 ± 0.06	62.69 ± 0.24
7	48.56 ± 0.06	51.79 ± 0.06	53.69 ± 0.06
8	49.97 ± 0.18	52.92 ± 0.06	57.01 ± 0.18
9	71.47 ± 0.12	73.56 ± 0.06	78.35 ± 0.06
10	52.28 ± 0.24	55.79 ± 0.24	58.45 ± 0.25
11	44.68 ± 0.18	49.37 ± 0.06	52.16 ± 0.06
ascorbic acid	43.00	50.70	55.20

Conclusion

In conclusion, we have explored one-pot three component reaction, which furnished novel classes of functionalized heterocyclic analogues of cyclic α-aminophosphonates from readily available 6-methyl-3-formylchromone, nitrogen *bi*-nucleophiles and diethyl phosphite. The procedure is efficient and general. The reactions have been shown to display relatively good functional group tolerance and good yields. We hope that this approach may be value to others seeking novel synthetic fragments with unique properties for medicinal chemistry.

Experimental

The melting point was determined in an open capillary tube on a digital Stuart SMP-3 apparatus. Infrared spectra were measured on FT-IR (Nicolet IS10) spectrophotometer using KBr disks. ^{1}H-NMR spectra were measured on Gemini-300BB spectrometer (300 MHz), using DMSO-d_6 as a solvent and TMS (δ) as the internal standard. ^{13}C-NMR spectra were measured on Mercury-300BB (75 MHz using DMSO-d_6 as a solvent) and Bruker-600 (150 MHz using $CDCl_3$ as a solvent) spectrometer and TMS (δ) as the internal standard. ^{31}P-NMR spectra were registered on a Bruker-600 (242 MHz) spectrometer at room temperature using DMSO-d_6 as a solvent and TMS as internal standard and 85% H_3PO_4 as external reference. Mass spectra recorded on a Gas Chromatographic GCMSqp 1000 ex Shimadzu instrument at 70 ev. Elemental microanalyses were performed Perkin-Elmer 2400II at the Chemical War department, Ministry of Defense. The purity of the synthesized compounds was checked by thin layer chromatography (TLC). 6-Methyl-3-formylchromone (**1**) was prepared according to the reported method.[39]

General procedure for the preparation of target compounds 2-11

A mixture of 6-methyl-3-formylchromone (**1**) (5 mmol, 0.94 g), nucleophile (5 mmol) and diethyl phosphite (10 mmol, 1.38 ml) was heated under reflux at 70−80 ^{o}C for 2−8 hours. The reaction mixture was cooled then poured into ice and left for complete precipitation. The precipitate formed was filtered off, dried and crystallized from the proper solvent.

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