International Journal of Pharmaceutical Science and Research
ISSN: 2455-4685, Impact Factor: RJIF 5.28
www.pharmacyjournal.net
Volume 2; Issue 1; January 2017; Page No. 27-31

$LiClO_4$ catalysed one-pot synthesis of Novel α-aminophosphonates and study of their effects on (MCF7) breast cancer cell line and (HCT) colon carcinoma cell line

[1] Mohamed A Hamed, [2] Ahmed A El Gokha, [3] Eslam M. EL-Deab, [4] Abdel Aleem El Gokha, [5] Ibrahim El-Tantawy El Sayed

[1] Chemistry Department, Faculty of Science, Tanta University, Tanta, Egypt
[2-5] Chemistry Department, Faculty of Science, El-Menoufeia University, Shebin El-Kom, Egypt

Abstract
The syntheses of a novel of N-protected α-aminophosphonates were achieved with high yields through lithium per chlorate catalyzed one-pot three component reaction process. It involves the reaction of benzyl carbamate, aldhydes and triphenyl phosphite using lithium perchlorate as Lewis acid catalyst in aceto nitrile at room temperature. The structures of all new compounds were established by IR, ^{1}HNMR and mass spectral data. On the other hand, Cleavage of the resulted α-aminophosphonates lead to the formation of a mine. Condensation reaction of the resulted amine with mono and disaccharides in presence of ethanol and gacial acetic acid. All the synthesized compounds were screened for in vitro antibacterial activity and most of them showed potency against both gram positive and gram negative bacteria. The chemical structures of all new compounds were established by IR, ^{1}HNMR, and mass spectra data.

Keywords: aldehydes, triphenylphosphite, lewis acid, α-aminophosphonates

1. Introduction
Organophosphorus compounds have found a wide range of applications in the areas of industrial, agricultural, and medicinal chemistry owing to their biological and physical properties as well as their utility as synthetic intermediates [1]. A-Functionalizedphosphonic acids are valuable intermediates for the preparation of medicinal compounds and synthetic intermediates [2, 4]. Among α-functional phosphonic acids, α-aminophosphonic acids are an important class of compounds that exhibit a variety of interesting and useful properties. α-Aminophosphonic acids I, as structural mimics of α-amino acids II (Fig.1), exhibit a broad spectrum of biological activities [5, 12].

These compounds have already been found to act as antibacterial agents, neuroactive compounds, anticancer drugs, and pesticides, with some of them already commercialized [13-15]. We focused on aminophosphinates and its derivatives because it is an important class of compounds and attracted widespread attention due to their pharmacological properties, being reported to have a large spectrum of biological effects, especially antimalarial, anti-bacterial and anticancer properties. In this paper we would like to present the synthesis of novel sa ccharide modified α-aminophosphonates conjugates.

2. Experimental

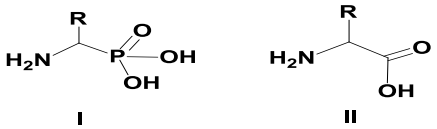

Fig 1

Materials
Determinations of melting points were performed in open glass apillaries using electro thermal BUCHI (B-540) hot storage melting-point apparatus and are uncorrected. Infra-red (IR) spectra were recorded on a Shimadzu 435 Spectrometer, using KBr discs and values were represented in cm^{-1} at the Micro analytical Center, Cairo University. (MS) Mass spectrawere run on Shimadzu QP-2010 spectrometer and Mass spectra were run on Hewlett Packard 5988 spectrometer at the Micro analytical Center, Cairo University, Egypt.[1] HNMR spectra was recorded on Bruker (300MHz) FT-NMR spectro meter using DMSO and the chemical shifts are given in δ (ppm) using tetramethylsilane (TMS) as an internal standard. Splitting patterns were designated as follows: s: singlet; d: doublet and m: multiplet. The anti-cancer analysis was carried out at, Division of National Cancer Institute, Cairo, Egypt. the compounds was made by thin layer chromatography (TLC) on silica gel-precoated aluminum sheets and the spots were detected by the aid of iodine vapour and by exposure to UV lamp at λ254 nm for few seconds. Starting materials, MeOH, DMF, CH_2Cl_2, hexane and diethyl ether were either commercially available as reported in literature.

General procedure for the synthesis of (3, 12, 13, 14, 15and 16).
Carbonyl compound 1 (1 mmol), benzylcarbamate 2 (1 mmol) and triphenylphosphite 3 were dissolved in acetonitrile (5 ml) and stirred at r.t about 15 minutes. Then the lewis acid,$LiClO_4$(10 mmol%) was added in one portion.The reaction mixture was stirred at r.t until the starting materials were consumed as monitored by TLC (5 days) using hexae,CH_2Cl_2 as eluent.After the completion of the reaction the precipitated product 4 was filtered off.
Recrystallization by using ethanol gave light pink precipitate.

Synthesis of Benzyl ((diphenoxyphosphoryl) (phe nyl) methyl) carbamate (3).
Show the following data; MP. =135 °C Yield= 80.2%, Infra-red

spectra of compound (3) show: 3430.74 (NH), 1250.61 (P=O),1075.12 (P-O), 766.566 (P-C) $cm^{-1,}$ ^{1}HNMR (DMSO-300 MHz): δ ppm = 7.61-7.63(m,2H,C-H Phenyl),7.1(d,2H,N-H), 5.5 (d,2H,CHP),5.16 (s, 1H, CH_2OC), The mass spectra show the molecular ion peak at m/e = 473.6 $[M]^+$,33.9 %).

Synthesis of Diphenyl (amino (phenyl) methyl) phosphonate (4)

Cleavage of Benzyl ((diphenoxyphosphoryl) (phenyl) methyl) carbamate (1mmol) by using 3:4 drops of HBr\Acetic acid in presence of triethylamine (3 ml) to give the appropriate (4), stirring at room temperature for (3h). The white precipitate was filtered off and recrystallized by using ethanol.

Show the following data; MP. =157 °C Yield= 82%, Infra-red spectra of compound (4) show: 3746.05 (NH2), 1251.58 (P=O),1026.91 (P-O), 767.53 (P-C) cm-1, 1HNMR (DMSO-300 MHz): δ ppm = 7.61-7.63(m,2H,C-H Phenyl), 5.15(d,2H,N-H2), 5.63 (t,3H,CHP), The mass spectra show the molecular ion peak at m/e = 339 $[M]^+$,4.98 %).

General procedure for the synthesis of (5, 6, 7, 8, 9, 10and 11).

Condensation of Diphenyl (amino (phenyl) methyl) phosphonate (1mmol) with suger (1mmol) in ethanol (5 ml) in presence of drops of glacial acetic acid. The mixture was refluxed for (2h) at 70C The mixture, until TLC analysis showed the complete consumption of amine in hexan, The product was precipitated from this solution by the evaporation of ethanol. To make sure we have the product, Tlc paper have to be burnt by dissolving in a solution of ethanol 80: 20 H_2SO_4 then burnt on the heater, the points on the tlc paper will burn and turn into brown colour.

Synthesis of Diphenyl (phenyl ((E)-((2S, 3R)-2, 3, 4, 5-tetrahydroxypentylidene) amino) methyl) phosphonate (5)

Show the following data; MP. =160 °C Yield= 79.3%, Infra-red spectra of compound (5) show: 1672.95 (C=N), 1251.58 (P=O),1205.29 (P-O), 760.78 (P-C) cm^{-1}, ^{1}HNMR (DMSO-300 MHz): δ ppm = 7.34-7.62(m,2H,C-H Phenyl), 6.54(s,1H,HC =N-), 5.76 (s,1H,CHP), 4.85 (s, 1H, OH). The mass spectra show the molecular base ion peak at m/e = 471 $[M]^+$, 0.32 %).

Synthesis of Diphenyl (((E)-((2S, 3S, 4s)-2, 3, 4, 5, 6-pentahydroxylidene) amino) (phenyl) methyl) phosphonate (6).

Show the following data MP. = 150 °C Yield = 57%, Infra-red spectra of compound **(6)** show: 1635.34 (C=N), 1251.58 (P=O),767.53(P-C),1070.3 (P-O),3416.28 (C-OH) cm^{-1}, ^{1}HNMR (DMSO-300 MHz): δ ppm = 7.34-7.62(m,2H,C-H Phenyl), 6.24(s,1H, HC=N-), 5.76 (s,1H,CHP), 4.62 (s, 1H, OH)., The mass spectra show the molecular ion peak at m/e = 501 $[M]^+$,0.22 %).

Synthesis of Diphenyl (phenyl ((E)-((2S,3S)-2, 3, 4, 5-tetrahydroxypentylidene) amino) methyl) phosphonate (7).

Show the following data MP. = 165 °C, Yield = 60.9%, Infra-red spectra of compound **(7**) show: 1635.34 (C=N), 1251.58 (P=O),1035.59 (P-O), 761.744 (P-C) 3423.03 (C-OH) cm^{-1}, ^{1}HNMR (DMSO-300 MHz): δ ppm = 7.32-7.41(m,2H,C-H Phenyl), 6.34(s,1H,HC =N-), 5.76 (s,1H,CHP), 4.72 (s, 1H, OH).The mass spectra show the molecular ion peak at m/e= 471 $[M]^+$, 1.27%).

Synthesis of Diphenyl (((E)-((2S, 3S, 4R)-2, 3, 4, 5, 6-pentahydroxypentylidene) amino (phenyl) phosphonate (8).

Show the following data MP. = 175°C, Yield = 61.6 %, Infra-red spectra of compound **(8)** show: 1630.52 (C=N), 1249.65 (P=O),1072.23 (P-O), 764.637 (P-C) 3396.99 (C-OH) cm^{-1}, ^{1}HNMR (DMSO-300 MHz): δ ppm = 7.32-7.65(m,2H,C-H Phenyl), 6.11(s,1H,HC =N-), 5.76 (s,1H,CHP), 4.92 (s, 1H, OH).The mass spectra show the molecular ion peak at m/e = 501 $[M]^+$,1 %).

Synthesis of Diphenyl ((((2R, 3R, 4R, 5S, 6R)-3, 4-dihydroxy-6-(hydroxymethyl)-5-(((2R, 3R, 4S, 5S, 6R)-3, 4, 5-trihydroxy-6-(hydroxymethyl) tetra hydro-2H-pyran-2-yl) amino) (phenyl) methyl) phosphonate (9).

Show the following data MP = 185 °C, Yield = 70%, Infra-red Spectra of compound **(9)** show3414.35 (NH), 1251.58 (P=O), 1076.08 (P-O), 766.566 (P-C) cm^{-1}, ^{1}HNMR (DMSO-300 MHz): δ ppm = 7.31-7.41(m,2H,C-H Phenyl), 6.67(d,2H,NH), 5.76 (s,1H, CHP). The mass spectra show the molecular ion peak at m/e=681$[M]^+$,0.24 %).

Synthesis of Diphenyl ((((2R, 3R, 4R, 5S, 6R)-3,4-dihydroxy-6-(hydroxymethyl)-5-(((2S, 3R, 4S, 5R, 6R)-3, 4, 5-trihydroxy-6-(hydroxymethyl) tetrahy dro-2H-pyran-2-yl) oxy) tetrahydro-2Hyl) amino) (phenyl) methyl) phosphonate (10).

Show the following data MP. = 200°C, Yield = 80 %, Infra-red spectra of compound (10) 3452.92 (NH), 1253.5 (P=O), 1072.23 (P-O), 766.566 (P-C) cm^{-1}, ^{1}HNMR (DMSO-300 MHz): δ ppm = 7.31-7.38(m,2H,C-H Phenyl), 6.66(d,2H,NH), 5.76 (s,1H, CHP). The mass spectra show the molecular ion peak at m/e = 681 $[M]^+$, 0.27 %).

Synthesis of Diphenyl (((E)-((2S, 3R, 4R)-2, 3, 4, 5, 6-pentahydroxyhexylidene) amino) (phenyl) methyl) phosphonate (11)

Show the following data MP. = 170°C. Yield = 81.1 %, Infra-red spectra of compound (11) 1637.27 (C=N), 1251.58 (P=O), 1029.8 (P-O), 768.494 (P-C) 3400.85 (C-OH) cm^{-1}, ^{1}HNMR (DMSO-300 MHz): δ ppm = 7.34-7.36(m,2H,C-H Phenyl), 6.57(s,1H, HC=N-), 5.76 (s,1H,CHP), 4.27 (s, 1H, OH).The mass spectra show the molecular ion peak at m/e = 501 $[M]^+$,0.19 %).

Synthesis of (1-Benzyloxycarbonylamino-1, 4, 5, 6, 7-pentahydroxy-heptyl)-phosphonic acid diphenyl ester (12)

Show the following data MP. = 138°C. Yield = 73.3 %, Infra-red spectra of compound **(12**) 3414.35 (NH), 1686 (C=O), 1187.94(P=O), 760 (P-C) cm^{-1}, ^{1}HNMR (DMSO-300 MHz): δ ppm = 7.35-7.69(m,2H,C-H Phenyl), 5.76(d,2H,NH), 5.63 (s,1H, CHP), 5.16 (s, 1H, CH_2O). The mass spectra show the molecular ion peak at m/e = 517 $[M]^+$,1 %).

Synthesis of (1-Benzyloxycarbonylamino-1, 4, 5, 6, 7, 8-hexahydroxy-octyl)-phosphonic acid diphenyl ester (13).

Show the following data MP. = 140°C. Yield = 83.3 %, Infra-red spectra of compound **(13**) 3393.14 (NH), 1244.83 (P=O),1067 (P-O), 768.49 (P-C) cm^{-1}, ^{1}HNMR (DMSO-300 MHz): δ ppm = 7.35-7.37(m,2H,C-H Phenyl), 6.48(d,2H,NH), 5.76 (s,1H, CHP), 4.91 (s, 1H, CH_2O).The mass spectra show the molecular ion peak at m/e = 547 $[M]^+$, 9 %).

Synthesis of (1-Benzyloxycarbonylamino-1, 4, 5, 6, 7, 8-hexahydroxy-octyl)-phosphonic acid diphenyl ester (14)
Show the following data MP. = 143°C. Yield = 97.2 %, Infra-red spectra of compound **(14**) 3406.64 (NH), 1240.97 (P=O),1071.26 (P-O), 774.279 (P-C) cm^{-1}, ^{1}HNMR (DMSO-300 MHz): δ ppm = 7.35-7.39(m,2H,C-H Phenyl), 6.22(d,2H,NH), 5.76 (s,1H, CHP), 4.88 (s, 1H, CH_2O). The mass spectra show the molecular ion peak at m/e = 547 $[M]^+$,0.91 %).

Synthesis of (1-Benzyloxycarbonylamino-1, 4, 5, 6, 7-pentahydroxy-heptyl)-phosphonic acid diphenyl ester (15)
Show the following data MP. = 139°C. Yield = 84.4 %, Infra-red spectra of compound (15) 3425.92 (NH), 1291.11 (P=O), 772.351 (P-C) cm^{-1}, ^{1}HNMR (DMSO-300 MHz): δ ppm = 7.80-7.82 (m,2H, C-H Phenyl), 6.96(d,2H,NH), 5.83 (s,1H, CHP), 4.46 (s, 1H, CH_2O). The mass spectra show the molecular ion peak at m/e = 517 $[M]^+$, 0.54 %).

Synthesis of (1-Benzyloxycarbonylamino-1, 4, 5, 6, 7, 8-hexahydroxy-octyl)-phosphonic acid diphenyl ester (16)
Show the following data MP. = 145°C. Yield = 91.7 %, Infra-red spectra of compound (16) 3410.49 (NH), 1225.54 (P=O), 1051.01 (P-O), 774.279 (P-C) cm^{-1}, ^{1}HNMR (DMSO-300 MHz): δ ppm = 7.34-7.57(m,2H,C-H Phenyl), 6.20(d,2H,NH), 5.57 (s,1H, CHP), 4.90 (s, 1H, CH_2O). The mass spectra show the molecular ion peak at m/e = 547 $[M]^+$, 3.92 %).

3. Results and Discussion

Substituted Substituted α- aminophosphonate (3) was prepared by stirring benzylcarbamate, benzaldhyde and triphenyl phosphite in presence of lewis acid such as lithium perchlorate for 5 days. Cleavage of α- aminophosphonate (3) using triethyl amine and HBr\Aceticacid afforded Diphenyl (amino (phenyl) methyl) phosphonate (4) in good yields and high purity. The structures were confirmed on the basis of IR, HNMR and mass spectral data accordind to scheme (1).

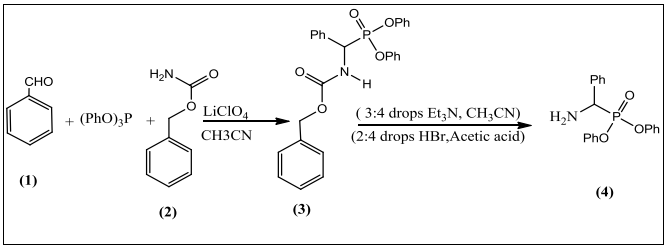

Fig 2

Diphenyl(amino(phenyl)methyl)phosphonate (4) was condensed with various mono and disaccharides by heating in ethanol at 70°C in presence of glacial acetic acid for 2h. The structures were confirmed on the basis of IR,HNMR and mass spectral data according to scheme (2).

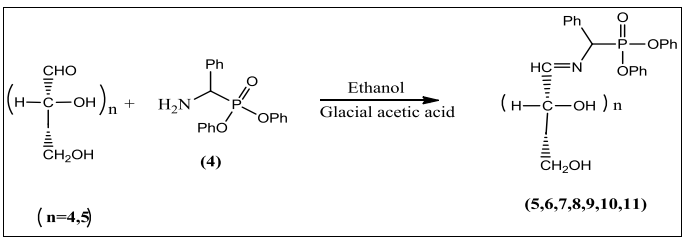

Fig 3

Having diverse types of mono saccharides affording the opportunity to obtain avarious structures diversity of α-aminophosphonates by afast and convenient one-pot three component reaction. The structures were confirmed on the basis of IR, HNMR and mass spectral data according to scheme (3).

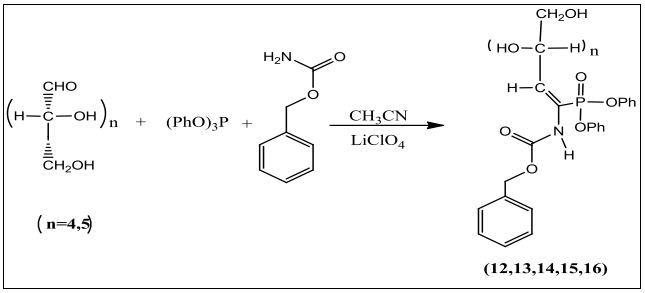

Fig 4

A possible mechanism for involve the reaction of carbonyl group with the amine through schiff base reaction then nucleophile attack occur via nucleophile phosphate which lead to the formation of phosphonium intermediate. Reaction of phosphonium intermediate with water affords the target compound in scheme (4). Reaction with benzyl corbamated proteation or cleavage of the phenyl oxycarbonyl group by acidic hydrolysis using HBr/acidic cleanly affords the free α-aminophosphonates in high yields at scheme (5).

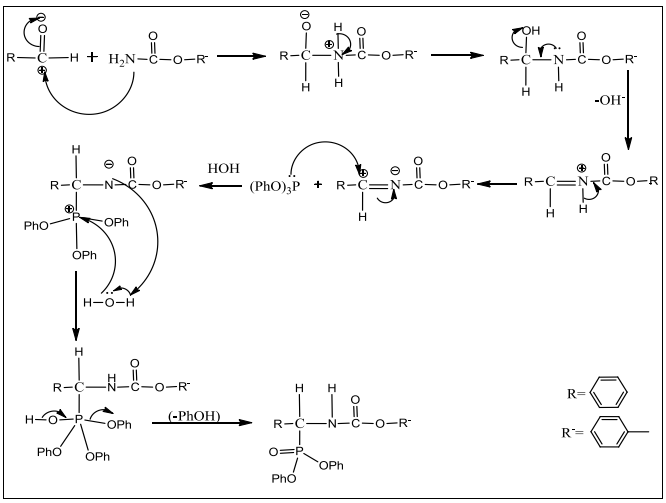

Fig 5

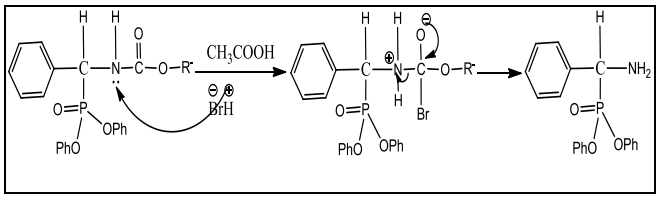

Fig 6

4. Anticancer Screening

Potential cytotoxicity of the compounds were tested using the method of SKehan et al. (1990**)** [16, 17].
*****Cells were plated in96-multiwell plate (10000 cells\well) for 24hrs before treatment with the compounds to allow attachment of cell to the wall of the plate.

*Different concentration of the compound under test (0, 5, 12.5, 25, 50ug\ml) were added to the cell monolayer triplicate wells were prepared for each.

Individual dose. *Monolayer cells were incubated with the compounds for 48hrs at 37C and inatomo-sphere of 5% CO_2.

- After 48 hrs, cells were fixed,washed and stained with Sulfo-Rhodamine-B stain.
- Excess stain was washed with with acetic acid and attached stain was recovered with Tris EDTA buffer.
- Color intensity was measured in an ELISA reader.
- The relation between surviving fraction and drug conc.is plotted to get the survival curve of each tumor cell line after the specified compound.

Table 1: Drug Cytotoxicity

conc.ug/ml	MCF7-6-
0.000	1.000
5.000	0.990
12.500	0.712
25.000	0.471
50.000	0.251

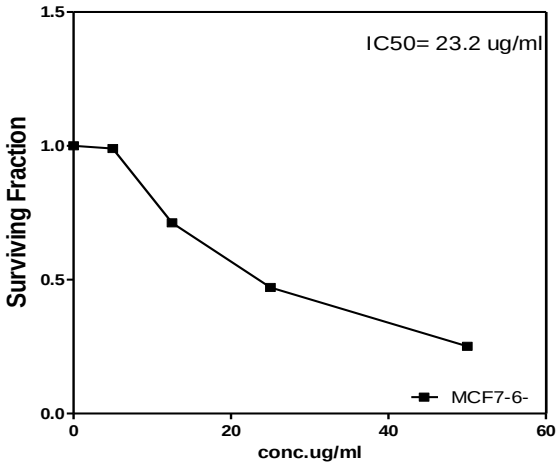

Fig 7

Table 2: Drug Cytotoxicity

conc.ug/ml	MCF7-7-
0.000	1.000
5.000	0.885
12.500	0.630
25.000	0.305
50.000	0.356

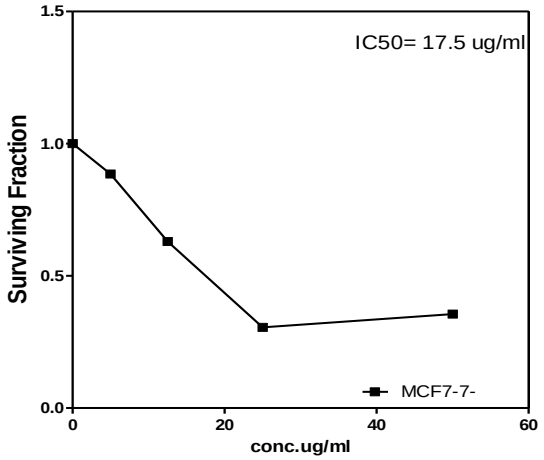

Fig 8

Table 3: Drug Cytotoxicity

conc.ug/ml	MCF7-8-
0.000	1.000
5.000	0.963
12.500	0.772
25.000	0.479
50.000	0.424

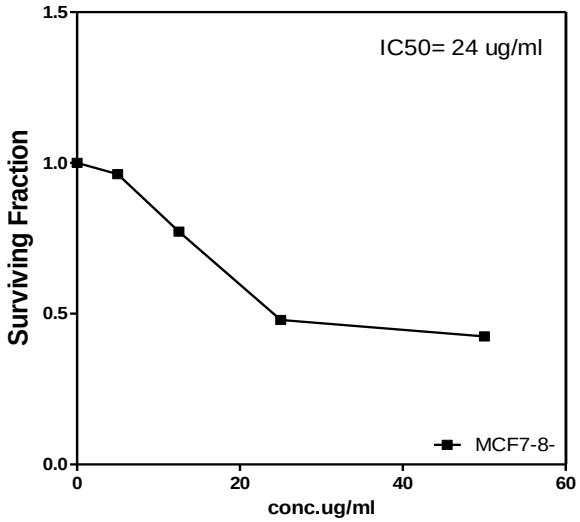

Fig 9

Table 4: Drug cytotoxicity

Conc.ug/ml	HCT-ISLAM 9
0.000	1.000
5.000	0.844
12.500	0.659
25.000	0.329
50.000	0.347

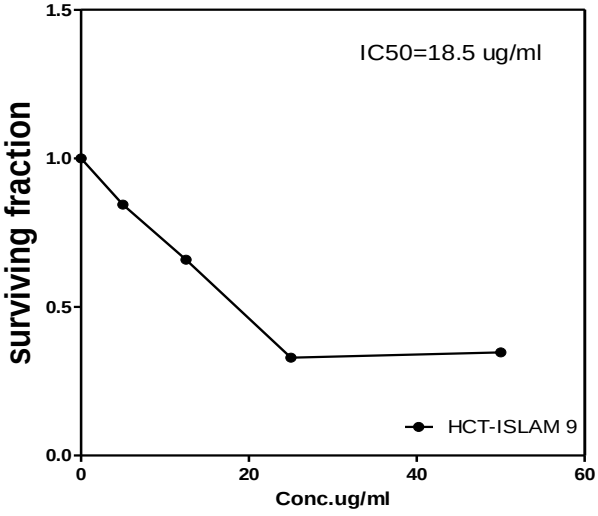

Fig 10

Table 5: Drug Cytotoxicity

Conc.ug/ml	HCT-ISLAM12
0.000	1.000
5.000	0.964
12.500	0.838
25.000	0.323
50.000	0.240

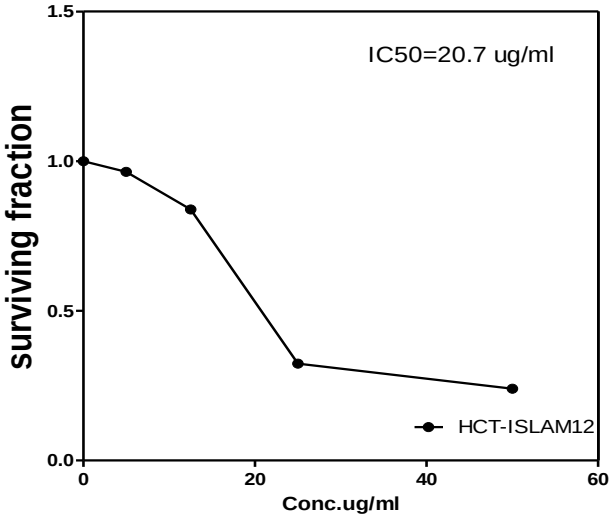

Fig 11

5. Conclusion

We have reported one-pot three-component synthesis of α-aminophosphonate derivatives as a valuable bioactive compounds to be investigated starting from aldhydes, amines and triphenyl phosphite using $LiClO_4$ as a catalyst. The biological assays show that the tested compounds showed promising anticancer activity.

6. Acknowledgement

The authors are thankful to the Department of organic chemistry of Menoufia University, Egypt for providing literature survey facility to carry out the work.

7. References

1. El Sayed SM, El Kosy MF, Abdel Magied MA, Hamed A, Abdel A, El Gokha, *et al.* One-pot Synthesis of Novel α-Aminophosphonate Derivatives Containing a Pyrazole Moiety ; Journal of American Science, 7, 604-608 (2011) and references cited therein.
2. El Sayed S, El Kosy M, Hawata MA, El Gokha AA, Tolan A, Abd El-Sattar MM. Journal of American Science. 2011, 7:357.
3. Aydenova ED, Todorov PT, Trove KD, Amino Acids, Lejczak try Department, Faculty of Science, El Menoufeia University, Shebin El Koom, Egypt, 2010; 38:23.
4. Kafarski PB. Topics in Heterocyclic Chemistry. 2009; 20-31.
5. Casteleijn M, Alahuhta M, Groebel K, El-Sayed I, Augustyns K, Lambeir A, *et al.* Functional Role of the Conserved Active Site Pro line of Triosephosphate Isomerase, Biochemistry. 2006; 45(51):15483-15494.
6. Van der Veken P, El Sayed IJ, Joossens CV, Stevens K. Augustyns, Haemers A. The Lewis acid catalyzed synthesis of N-protected diphenyl 1-aminoalkylphosphonates, Synthesis. 2005, 634-638.
7. Pudovik AN, Doklady Akad, Nauk SSSR. 1952, 83:865; Chem. Abstr. 1953; 47:4300.
8. Kabachinik MI, Medved TY, Doklady Akad, Nauk SSSR. 83 (1952) 689; Chem. Abstr. 47(1953) 2724. ((b) Kabachinik M. I., Medved T. Y, Izv. Akad.,Nauk SSSR, Ser. Chim. (1953) 1126. ((c) Fields E., Journal of American Chemical Society, 1952, 74:1528.
9. Cherkasov RA, Galkin VI. Russian Chemistry Review, 67(1998) 857; Chem. Abstr. 1953; 47:2724b.
10. Galkina I, Zvereva E, Galkin V, Sobanov A, Cherkasov R, Zhur. Obshch. Khim. Russian Journal of General Chemistry), 68 (1998) 1453. (b) Galkina, I., Sobanov A., Galkin V, Cherkasov R, Zhur. Obshch. Khim, Russian Journal of General Chemistry. 1998, 68:1465.
11. Corbridg D. Phosphorous. Elsevier, New York, USA, 1990.
12. Hartly FR. The Chemistry of Organophosphorus Compounds. John Wiley and Sons, New York, USA, 1990.
13. Kafarski P, Lejczak B. Aminophosphonic acids of potential medical importance, Current Medicinal Chemistry Anti-Cancer Agents. 1,301, 2001. (b) Phosphonates in Living Systems, CRC, Boca Raton, 1983.
14. Engel R. Synthesis of Carbon-Phosphorus Bond, CRC: Boca Raton, 1988.
15. Kukhar VP, Hudson HR. Ed., Amino phosphonic and Aminophosphinic Acids chemistry and biological activity, Wiley: New York, 2000.
16. Gandavaram SP, Gollapalli NR. Synthesis of biologically active α-Aminophosphonates. J Mod Med Chem. 2013; 1:49.
17. Skehan P, Storeng R, *et al.* Newcolormertic cytotoxicity assay for anti-cancer drug screening. J Natl Cancer Inst. 1990; 82:1107-1112.