RSC Advances

REVIEW

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Cite this: *RSC Adv.*, 2016, **6**, 7101

Selected organophosphorus compounds with biological activity. Applications in medicine

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Received 30th November 2015
Accepted 7th January 2016

DOI: 10.1039/c5ra25446a

www.rsc.org/advances

The purpose of this article is to provide an overview of the latest applications of organophosphorus compounds (OPs) that exhibit biological activity. A large family of OPs have become popular in recent years. The practical application of OPs in modern medicine has been attributed to their unique properties. In this article, the methods used to select these compounds will be emphasized. This paper will first outline the findings of a literature review on OPs, including anticancer and antiviral agents, bisphosphonates, phosphorus analogues of amino acids and peptides, and organophosphorus metal complexes, and secondly, it will classify the compounds according to their biological activity and applications in the treatment of diseases.

1. Introduction

Organophosphorus compounds (OPs), which are a wide class of chemical compounds containing organic moieties usually bonded directly to phosphorus or bonded through a heteroatom, such as sulfur, oxygen or nitrogen, are some of the most common chemicals in the human environment. Because of their unique properties and high biological activity, they have largely been used worldwide in agricultural (pesticides),[1] industrial (production of lubricants, hydraulic fluids, and plastics materials),[2] medicinal (drugs against osteoporosis, anticancer and antiviral compounds)[3,4] or veterinary (anthelmintics) applications.[5] The first potent synthetic organophosphorus poison, tetraethyl pyrophosphate (TEPP), was synthesized by Clermont in 1854. At the beginning of the twentieth century, some very toxic compounds were used in many armed conflicts as chemical weapons, known as chemical warfare agents (CWA). Following the German laboratories discovery of soman, sarin and tabun, the United States and England developed VX production technologies. The book "Chemical Warfare Agents"[6] discusses the physicochemical properties of chemical warfare agents, their dispersion and fate in the environment, their toxicology and management of their effects on humans, decontamination, and protective equipment. After the Second World War, OPs have been used mainly as pesticides for plants and animals. Furthermore, OPs have practically contributed to the substantial benefits for efficient food production and the fight against many serious diseases, such as malaria, yellow fever, typhus,[7] or smallpox.[4]

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2. Bisphosphonates in the treatment of osteoporosis

Osteoporosis is one of the most serious health problems in the world, and prevention and treatment are of great interest in the European Union, which issued "Report on Osteoporosis in the European Community – Action for Prevention".[8] The scale of the problem is alarming. One in three women and one in eight men over the age of 50 years will experience at least one fracture due to osteoporosis in their lifetime. The main criterion for selecting an osteoporosis therapy is its impact on the risk of osteoporotic fracture (femoral neck, spine, wrist). The following pharmacological methods are used:

- hormone replacement therapy;
- specific estrogen receptor modulators (SERMs);
- calcitonin;
- vitamin D3 with active metabolites and calcium;
- fluorine.

Bisphosphonates are currently the most important and effective class of drugs developed for the treatment of metabolic bone disorders associated with increased osteoclast-mediated bone resorption, such as osteoporosis[9,10] and Paget's disease.[11,12] They are effective inhibitors of tumor-induced bone destruction and significantly reduce the incidence of skeletal complications in patients with bone metastases from several forms of cancer, including breast and prostate cancer.[13] Bisphosphonates have a high affinity for calcium and therefore specifically target bone mineral, where they are internalized by bone-destroying osteoclasts and inhibit their function.[14] Importantly, potential of bisphosphonates has also been identified in areas ranging from parasite-growth inhibition to immunological and cancer therapeutics.[3] These compounds primarily affect the function of osteoclasts, but recent preclinical evidence indicates that other neighboring cell types, such as

macrophages, monocytes and cancer cells, could also be targets for these drugs.[15] Bisphosphonates have been shown to induce tumor cell apoptosis, to modulate cells in the immune system and to inhibit tumor angiogenesis.

Bisphosphonates used in clinical practice are characterized by a P–C–P structure.[16] This structure allows for a wide variety of variations. The first bisphosphonates were synthesized by German chemists and were first used only for a few industrial applications, such as antiscaling agents. It was only in 1968–1969 that H. Fleisch *et al.* demonstrated that these compounds had biological effects, specifically on calcified tissues.[17–20] Replacing the O atom with a CH_2 group in the pyrophosphoric acid structure created new bioactive phosphorus agents and resulted in a major breakthrough in the treatment of bone disease. It is hypothesized that bisphosphonic acid **1** are isosteric with pyrophosphoric acid **2** but are hydrolytically stable, and if attracted to bone, may block resorption since inorganic pyrophosphate inhibits the formation and dissolution of hydroxyapatite in bone (Fig. 1).

E. Breuer's research group from the Hebrew University of Jerusalem between 1992 and 2005 had a significant impact on the synthesis and biological evaluation of bisphosphonates. E. Breuer's research group authored numerous publications and patents concerning the use of bisphosphonates for the treatment of osteoporosis. These researchers were focused on synthesizing novel bifunctional compounds, such as bisacylphosphonates **3**,[21] tetrakisphosphonates **4**,[22] bisphosphonic acid betaine derivatives **5** (ref. 23) and hydroxyiminophosphonates **6**,[24] and examining their effects compared to other clinically used drugs (Fig. 2).

The drugs clodronate **7**, tiludronate **8** and etidronate **9** were among the first to be used in the clinic.

Attention has recently been drawn to the derivatives with aminoalkyl side chains for the next generation of bisphosphonates, as found in pamidronic acid **10**, alendronic acid **11**, olpadronic acid **12**, neridronic acid **13**, ibandronic acid **14** and risedronic acid **15**.

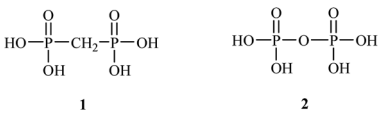


Fig. 1 Chemical structures of bisphosphonic acid **1** and pyrophosphoric acid **2**.

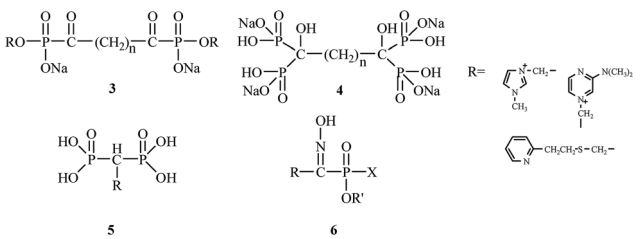


Fig. 2 Chemical structures of bisacylphosphonates **3**, tetrakisphosphonates **4**, bisphosphonic acid betaine derivatives **5** and hydroxyiminophosphonates **6**.

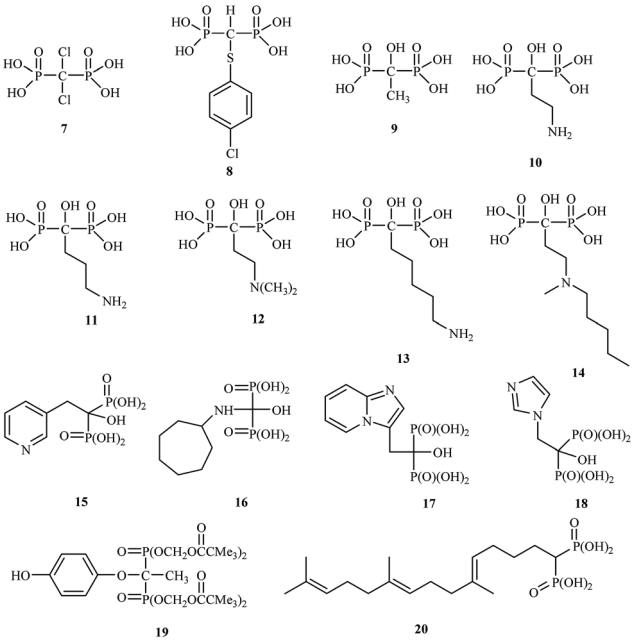


Fig. 3 Chemical structures of bisphosphonates **7**–**20**.

The research field is currently very active, and many new discoveries in bisphosphonate bone-disease therapy have been published.[25] Bisphosphonates are currently in the third generation, which includes incadronic acid **16**, minodronic acid **17** and zoledronic acid **18**. In addition, several bisphosphonates act as antidepressant **19** and antihypercholesterolemic agents **20** (Fig. 3).[26,27]

3. Organophosphorus compounds as anticancer drugs

Recent research findings suggest that a number of OPs are used as anticancer drugs or have potential anticancer properties. They are usually used in oncology as alkylating chemotherapeutic agents. These compounds react with DNA, RNA and some enzymes. *N*,*N*′,*N*″-Triethylenethiophosphoramide **21**, sold under the trade name thioTEPA, is a compound used as an anticancer chemotherapeutic drug that binds to DNA, cross-links the two strands and prevents cell duplication. *N*,*N*′,*N*″-Triethylenethiophosphoramide, developed in the 1950s, is a trifunctional alkylating agent with a broad spectrum of antitumor activity.[28] This drug is used to treat many diseases, including breast cancer, ovarian cancer, lymphosarcoma, superficial papillary carcinoma of the urinary bladder, and Hodgkin's disease (Fig. 4).[29]

Several derivatives of *N*,*N*′,*N*″-triethylenethiophosphoramide have been synthesized, and their antitumor activity was evaluated. McCracken and co-workers carried out the synthesis of *N*-[bis(1-aziridinyl)phosphoro]-carbamate **22**.[30] *In vivo* studies demonstrated that the synthesized drugs had a higher toxicity against cancer cells, and they did not invade other tissues. In 1963, Chernov *et al.*[31] published the synthesis and biological evaluation of phosphazine **23**. Chernov's research

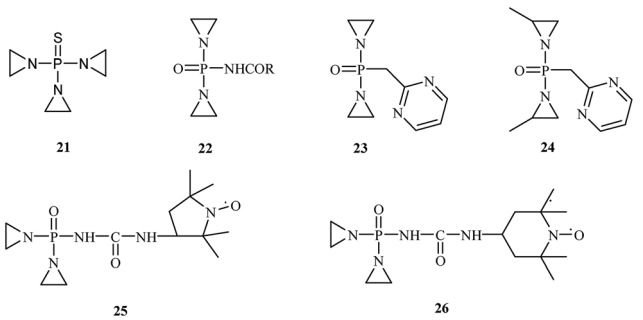


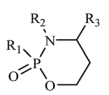
Fig. 4 Chemical structures of organophosphorus alkylating agents 21–26.

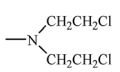
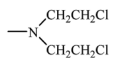
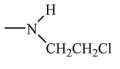
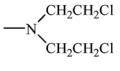
demonstrated phosphazine's high activity **23** against transplanted carcinoma in mice, rats, and rabbits. According to these investigations, phosphazine is toxic; however, its toxicity is much lower than thio-phosphamide (thio-TEPA) or dipin (*N*,*N*′-bis(diaziridinylphosphinylidyne)piperazine). In 1968, Noell *et al.*[32] synthesized methylphosphazine (*P*,*P*-bis(2-methyl-1-aziridinyl)-*N*-2-pyrimidinylphosphophosphinic amide) **24**, which demonstrated excellent activity against both leukemia L1210 and Walker 256 and was more active and less toxic than several clinical drugs, such as thioTEPA and phosphazine. In the 1980s, further structural modifications were tested. Sosnovsky synthesized the nitroxyl-labeled analogues of tiamide (TEPA).[33] These studies showed that compounds **25** and **26** possess therapeutic indexes 8- to 12-fold higher than those of thio-TEPA and TEPA.

Several of the most important OPs that exhibit anticancer properties are oxazaphosphorines. Even today, 50 years after its introduction, this class of compounds is one of the most widely used cytostatics. The more than 35 000 scientific publications regarding this compound demonstrate the wide interest it has received.

These compounds (Table 1), classified as alkylating agents, are therapeutically inactive prodrugs that must be activated to induce their cytotoxicity.[35,36] Currently, cyclophosphamide **27** and ifosfamide **29** are used commonly for the treatment of non-Hodgkin lymphomas and a variety of bone and soft tissue sarcomas.[37] Compared to many other anticancer drugs, cyclophosphamide exhibits relatively little non-hematopoietic toxicity.

Table 1 Structures of oxazaphosphorines[34]



Substance	R_1	R_2	R_3
Cyclophosphamide **27**		H–	H–
Mafosfamide **28**		H–	$^{-}O_3S–H_2C–CH_2–S–$
Ifosfamide **29**		Cl–H_2C–CH_2–	H–
Trofosfamide **30**		Cl–H_2C–CH_2–	H–

In 2000, a series of naphthoquinone **31** and benzimidazolequinone **32** phosphorodiamidates were synthesized and studied as potential cytotoxic prodrugs activated by DT-diaphorase,[38] and an activation process was proposed (Fig. 5). To activate the synthesized prodrugs, the quinone moiety is reduced to form phosphoramide mustard. All compounds were excellent substrates for human DT-diaphorase. The naphthoquinones **31** showed high toxicity towards both HT-29 and BE human colon cancer cell lines and were 1- to 2-fold more active than the benzimidazolequinone derivatives **32**.

In 2008, Jian-Xin Duan *et al.* synthesized new phosphoorganic compounds with anticancer activity based on 2-nitroimidazole derivatives.[39] These compounds are hypoxia-activated achiral phosphoramidate mustards synthesized based on the DNA cross-linking toxin from the prodrug ifosfamide. Hypoxia-activated phosphoramidates were introduced by Borch and coworkers.[40] The most successful were the 5-nitrothiophene- and 5-nitrofuran-triggered prodrugs of phosphoramidate toxins. The synthetic methods (Fig. 6) were very straightforward and high yielding. The most promising antitumor agent was TH-302 **33**, which demonstrated excellent *in vivo* efficacy and is currently in clinical trials.

In 2003, Jain *et al.* proposed a new class of 1,2-benzisoxazole phosphorodiamidate compounds.[41] As expected, *in vivo* studies showed that the compounds **34**, **35**, and **36** were 3- to 5-fold more active than analogues **37** and **38** (Fig. 7).

Organophosphorus compounds are also used as prospective treatments for hormone-dependent breast cancer. They are included in the list of steroid sulfatase (STS) inhibitors, an enzyme involved in the biosynthesis of estrogen in the mammary glands. The WHO lists estrogen as an important factor in the development of breast cancer. Breast cancer is the

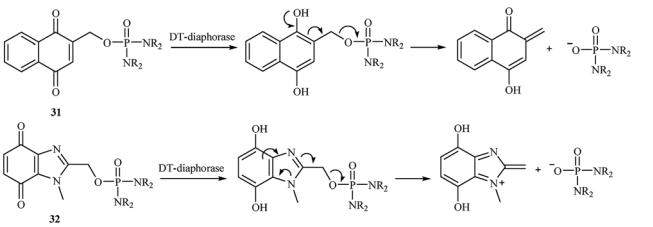


Fig. 5 Activation process of naphthoquinone **31** and benzimidazolequinone **32** phosphorodiamidates.

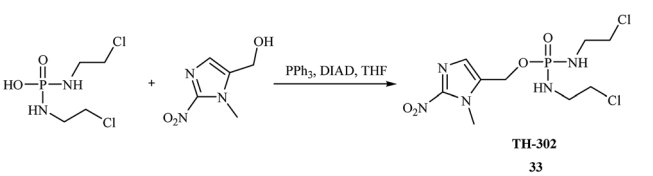


Fig. 6 Synthesis of TH-302 **33**.

most frequently diagnosed cancer in the female population of industrialized countries. Estimates for 2014 show more than 230 000 diagnosed cases of breast cancer and more than 40 000 deaths (according to National Cancer Institute data). Inhibitors based on OPs show potential as new breast cancer therapies because they are structurally similar to the natural steroid sulfatase substrate and have excellent binding affinities to the enzyme active site.[42]

The initial strategy employed for generating a lead STS inhibitor involved replacement of the sulfate group (OSO_3) on the natural enzyme substrate with surrogates or mimics such as phosphates or thiophosphates. Recently, Demkowicz *et al.* synthesized new phosphate and thiophosphate esters of tricyclic coumarin **39**,[43,44] *N*-alkanoyl tyramine **40**,[45] biphenyl **41** (ref. 46) and flavone **42** (ref. 47) derivatives as potent STS inhibitors. The most active compound, 4-(2-dodecanoylaminoethyl)-phenyl dimethyl phosphate, demonstrated the greatest inhibitory effect, with IC_{50} values of 390 nM in enzymatic assay with STS isolated from the human placenta. Although the mechanism of activity is unknown, docking studies conducted to explore the potential interactions between synthesized compounds and the active site of STS suggest a phosphate group transfer to one of the key amino acid residue involved in the enzymatic reaction (FGly75) or methylation of this residue during the inactivation process.

In 2015, the same research group synthesized a series of bicoumarin **43** (ref. 48) and biflavone **44** (ref. 47) thiophosphate derivatives as STS inhibitors. The most active compound, bis-(6-oxo-7,8,9,10-tetrahydro-6*H*-benzo[*c*]chromen-3-yl) hydrogenthiophosphate, inhibit STS activity with IC_{50} values of 860 nM in enzymatic assay with purified STS. Although the mechanism of inhibition also remains unclear, molecular modeling suggests a completely different manner of binding to the active site of STS in comparison to previous organophosphorus inhibitors of STS. Indeed, they can adopt conformations that are able to fill the whole cavity and prevent the substrate's access to the catalytic amino acid residues.

In 2001, David *et al.* demonstrated the utility of a new anticancer drug called apomine **45**, a *per os*-active, apoptosis-inducing agent that recently entered clinical trials in cancer patients.[49] The clinical trial findings revealed that the drug can selectively inhibit cell proliferation and induce tumor cell apoptosis through the farnesoid X receptor. *In vitro* assay results showed that 63 and 91% of ovarian cancers were sensitive to apomine at concentrations of 10 and 20 μM, respectively. This compound also inhibits the mevalonate/isoprenoid pathway of cholesterol synthesis and may also prove effective as a skin cancer chemoprevention therapy.[50]

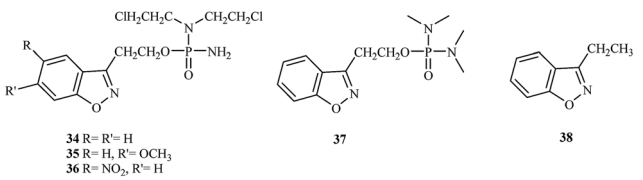


Fig. 7 Structures of benzisoxazole phosphorodiamidates **34–37** and reference compound **38**.

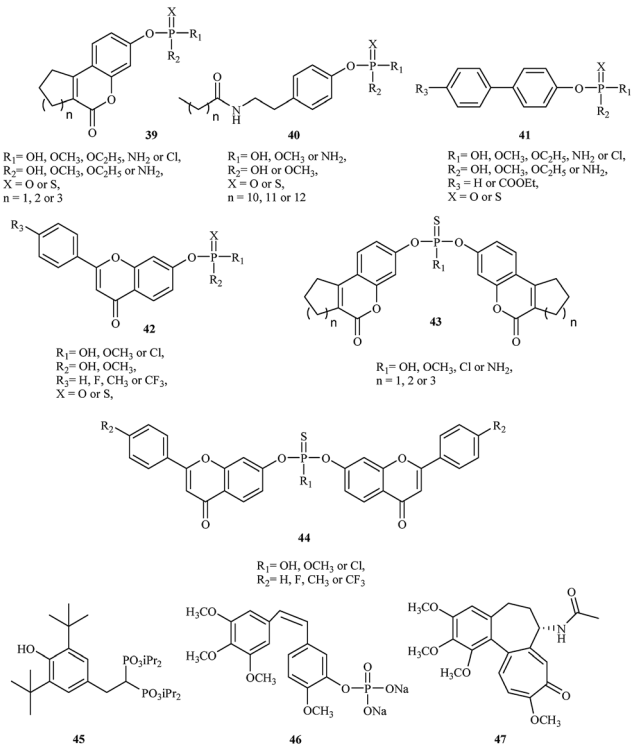


Fig. 8 Chemical structures of organophosphorus anticancer agents **39–47**.

Another drug used in cancer therapy is combretastatin A-4 phosphate (CA4P) **46**.[51] Combretastatin A-4 phosphate is a novel microtubule destabilizing drug, a type of vascular-targeting agent designed to damage the vasculature (blood vessels) of cancer tumors and cause central necrosis. It is the first of a series of combretastatin analogs to enter the clinic (Fig. 8).

The structure of CA4P is similar to colchicine **47**, and it binds the colchicine-binding site on tubulin and inhibits tubulin polymerization.[52] Clinical studies showed that CA4P's toxicity profile is consistent with a drug that is vascularly active and devoid of traditional cytotoxic side effects.[49]

The latest achievement in the treatment of cancer is the use of antisense drugs.[53–55] Antisense therapy is based on oligonucleotides expected to stop or reduce the expression of selected genes using different approaches based on sequence specific targeting of nucleic acids.[56] This new anticancer strategy has been widely used to treat cancers such as colorectal carcinoma, lung cancer, pancreatic carcinoma, malignant glioma or malignant melanoma. Most therapies have not yet produced significant clinical results, and application of this method is the subject of intensive investigations.

4. Organophosphorus metal complexes

Research about the applications of metal complexes in medicine is one of the most integrated areas of science, combining

data regarding structure, properties of metal complexes, and control of the body's vital processes. The design of new drugs based on metal complexes is an important contribution to the development of more efficient chemotherapy methods. These metal complexes are used in the treatment of many diseases. However, it was not until the early 1960's that the biological effect of cisplatin was discovered.[57] Primary research by Rosenberg indicated that metal ions were capable of binding to nucleic acids, thereby altering their conformation and biological function.[58] Metal complexes play an important role in many biological processes[59] including cell division and gene expression, as well as processes such as carcinogenicity or toxicity.[60] Among the synthesized compounds, many very important complexes are based on OPs. The literature indicates that most studies consider OPs to have potential anti-cancer aspects. Their activity against many types of cancer is currently the subject of intensive research. Organophosphorus metal complexes include platinum, ruthenium, palladium, gold and copper metal centers.

High *in vitro* activities of ruthenium complexes against different cancer cell lines suggest that they are the most promising in anticancer therapy and may play a dominant role as antitumor drugs compared to others metal complexes.[61] Among, a number of ruthenium complexes, NAMI-A and KP1019 are currently entered into a clinical trials.[62,63] Although, the mechanism of action for these compounds is not fully understood,[64] recent reports indicate that antiproliferative activity of Ru complexes is strengthened with the interactions with DNA and different cellular proteins.[65] Among the many synthesized ruthenium compounds that exhibit antitumor activity, the arene PTA ruthenium(II) complexes (RAPTA) **48–56** are of great interest (Fig. 9).[66] The RAPTA complexes **48–56** were found to exhibit pH-dependent DNA damage. Although the cytotoxicity of the new complexes proved to be lower compared to cisplatin, progress has been made in designing new and selective drugs without the harsh side effects of cisplatin.

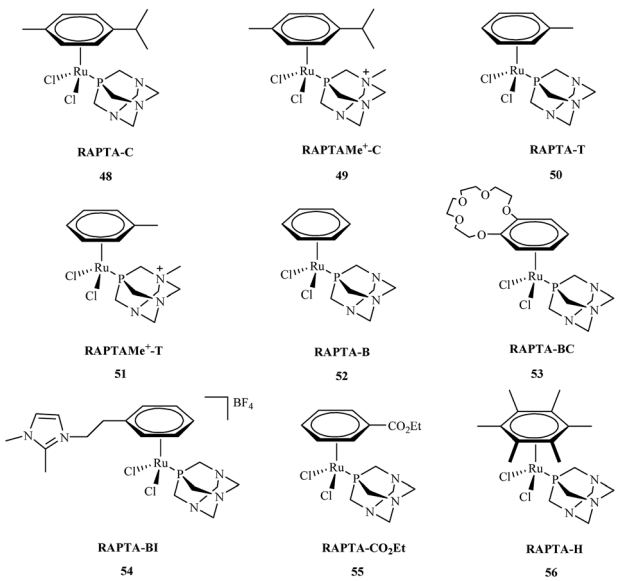


Fig. 9 Chemical structures of RAPTA complexes **48–56**.

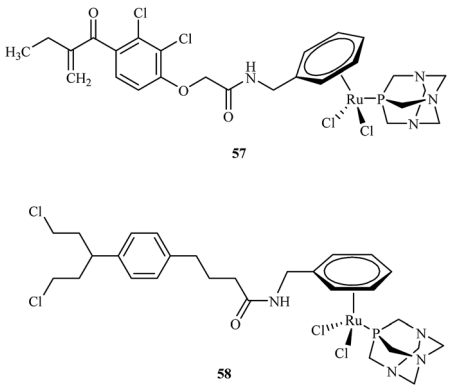


Fig. 10 Chemical structures of ethaRAPTA **57** and **58** complexes.

Efforts to synthesise of ruthenium complexes that exhibit potent anticancer properties led to the discovery of other RAPTA derivatives. [Ru(η^6-*p*-phenylethacrynate)Cl_2(pta)] – ethaRAPTA **57**, a derivative of RAPTA-C **48** is one of the most promising (Fig. 10). Chakree *et al.* characterized the ethaRAPTA **57** interactions with DNA and found out that ethaRAPTA exposed a higher efficiency in comparison to RAPTA-C in inhibiting the breast cancer suppressor gene 1.[67]

Recent advances in RAPTA complexes developments led to synthesis of compounds that were the modification of RAPTA scaffold with chlorambucil.[68] Chlorambucil is well known DNA alkylating agent whereas RAPTA complexes coordinate to amino acid residues of proteins. Biological activities of tested RAPTA derivatives were in the low μM range against A2780, A2780R and MCF-7 cell lines. Among a series of newly obtained RAPTA complexes, the most potent was **58** with IC_{50} values for A2780, A2780R and MCF-7 cell lines of 8.3, 10.0 and 12.0 μM, respectively.

Many metal complexes with triphenylphosphine and other tertiary phosphines have been reported to be catalysts for various processes, such as polymerization of alkenes and acetylenes, Wilkinson catalyst,[69] oxo hydroformylation of alkenes with hydrogen and CO,[70] asymmetric Pauson–Khand[71] and Morita–Baylis–Hillman[72] reactions, synthesis of enantiomerically enriched cyclohexadiene by reaction of terminal dieneyne using a chiral iridium complex[73] and asymmetric allylation and propargylation of ketones.[74] Many phosphines and diphosphines are optically active due to an asymmetric phosphorus or a carbon atom and have been used to for asymmetric hydrogenations.[75]

5. Phosphorus analogs of amino acids and peptides

Aminophosphinic and aminophosphonic acids are a very important group of chemical compounds, and their synthesis has attracted considerable attention in medicinal chemistry. Although the biological importance of these compounds was discovered in the 1950's, they still represent a promising class of potential drugs. They are classified as antimetabolites, which

compete with their aminocarboxylic acid analogues in the active sites of enzymes and other cell receptors.[76–79]

5.1. Aminophosphinic acid derivatives

V. Dive synthesized and evaluated a series of cyclic peptides containing a phosphinic bond as potential zinc bacterial collagenase inhibitors.[80] Studying collagenases or other proteases helps to better understand the pathology and treatment of human diseases such as rheumatoid arthritis, tissue repair, metastasis, angiogenesis or cirrhosis. Among the synthesized pseudopeptides with different sized cyclic rings, they found two compounds, (cyclo[Gly-Pro-Phe-ψ(PO_2CH_2)-Gly-Pro-Ahx]) and (cyclo[βAla-Pro-Pheψ(PO_2CH_2) Gly-Pro-Ahx]), exhibited very potent inhibition activity with K_i values of 120 and 90 nM, respectively. The authors found that the stereochemistry and conformation of the pseudophenylalanine residue determined the potency of these cyclic peptides.

Bartlett and coworkers reported the synthesis and application of phosphinic acid peptide analogues, potent slow-binding inhibitors of aspartic peptidases.[81] They showed that incorporation of a phosphorus-containing analogue of the amino acid statine into the oligopeptide sequences significantly increased inhibition of the prototypical aspartic peptidase pepsin. During the course of the investigations, they discovered that the more effective inhibitor for each pair of the diastereomers was the L-configuration (Table 2).

Several aminophosphonic acids were found to be very selective and potent inhibitors toward other endopeptidases. B. Vincent's research group published the biological studies of a series of selective and potent phosphinic peptide inhibitors of endopeptidase 3.4.24.16.[82] This research showed that the most selective peptide analog, Pro-Phe-ψ(PO_2CH_2)-Leu-Pro-NH_2, displayed a K_i value of 12 nM. In comparison to the related endopeptidase 3.4.24.15 tested, the inhibitor was 5540-fold less effective; furthermore, compared to other enzymes (endopeptidase 3.4.24.11, aminopeptidases B and M, dipeptidylaminopeptidase IV or proline endopeptidase), no inhibition was observed.

Phosphinic acid derivatives have also been utilized as inhibitors of metalloproteinases. Matrix metalloproteinases (MMPs) are zinc-dependent proteolytic enzymes. They contain a zinc cation (Zn^{2+}) that plays both a catalytic and structural role.[83] There are over 30 types of metalloproteinases involved in many physiological and pathological remodeling and degradation processes of extracellular matrix components.[84] In many cases, a change in MMP function causes a pathological state; they are also implicated in many diseases including cancer,[85] osteoporosis,[86] arthritis,[87] arteriosclerosis,[88] multiple sclerosis,[89] and liver cirrhosis.[90] Fosinopril **61** was one of the first phosphorus-based MMP inhibitors clinically used for the treatment of hypertension and some types of chronic heart failure by inhibition of ACE (angiotensin converting enzyme).[91] Fosinopril is administered as a prodrug and converted *in vivo* to the active form fosinoprilat **62**. Direct administration of the drug in its active form does not guarantee effective treatment because fosinoprilat is ionic under physiological conditions, and its oral bioavailability is thus very low.

In 1999, Vassiliou and coworkers reported the synthesis and biological studies of a series of phosphinic pseudo-tripeptide inhibitors against MMP-1, MMP-2, MMP-7, MMP-8, MMP-11, and MMP-14.[92] They demonstrated the structure–activity relationships regarding the influence of different substituents at the P1′, P2 and P2′ position. The inhibitors were highly potent towards MMPs, displaying nanomolar K_i values. For MMP-8, replacement of the benzyl group at the P1′ position by phenylpropyl (RXP03) **63** caused a 30-fold increase in potency (Fig. 11).

Yiotakis and coworkers published the synthesis of a phosphinic pseudopeptide series containing a variety of P1-side chains.[93] The new compounds were tested as potential selective inhibitors against MMP-2, MMP-7, MMP-8, MMP-9, MMP-11, MMP-13, and MMP-14. Several phosphinic inhibitors displayed high selectivity toward MMP-11; the greatest potency was exhibited by compound **64** with a K_i value of 0.23 μM.

In 2003, scientists from France and Greece reported the solution-phase synthesis of new phosphinopeptide inhibitors.[94] Promising results were observed for RXP03, which was modified at the P1′ position by introducing an isoxazole group to obtain **65**.

Table 2 Binding of tripeptide and tetrapeptide analogues **59–60** to pepsin

Inhibitor	K_i
Iva-D-Sta^P-Ala-Iaa **59A**	25 μM
Iva-L-Sta^P-Ala-Iaa **59B**	0.9 μM
Iva-Val-D-Sta^P-Ala-Iaa **60A**	200 nM
Iva-Val-L-Sta^P-Ala-Iaa **60B**	<0.07 nM

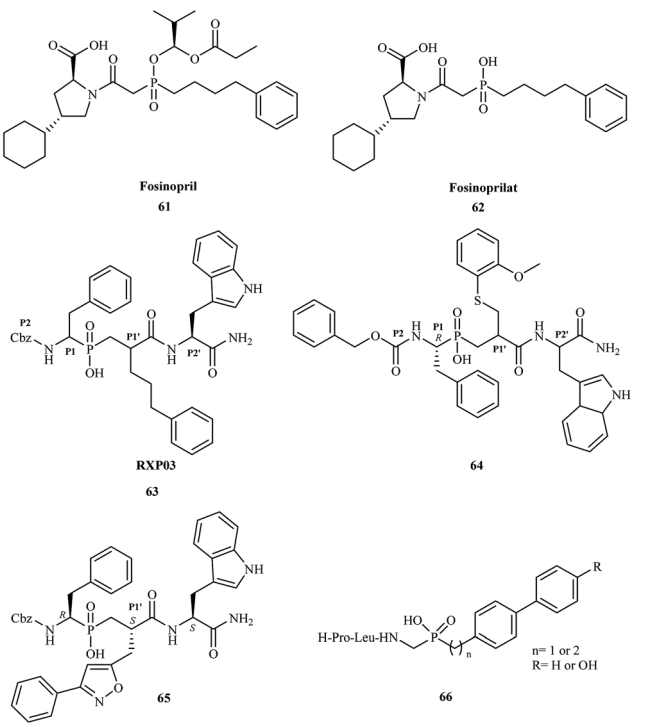


Fig. 11 Chemical structures of aminophosphinic acid derivatives **61–66**.

Research showed that compound **65** was 36-fold more active than RXP03 **63** towards MMP-14. The investigation suggested that the P1′ substituent was responsible for the high potency and selectivity of these MMP inhibitors. Compound **65** was highly stereospecific; biological studies showed that the (*R*, *S*, *S*) diastereoisomer was 100-fold more active than the (*R*, *R*, *S*) isomer.

In 2005, Italian scientists reported the synthesis of three novel peptidomimetic phosphinate inhibitors and evaluated their biological activity towards metalloproteinases MMP-2 and MMP-8.[95] All of the compounds were highly potent showing IC_{50} values in the micromolar range. The greatest activity was observed for inhibitors **66** against MMP-2.

The latest research reported the application of aminophosphinic acid derivatives for the treatment of Alzheimer's disease. They are classified as the inhibitors of β-secretase, an aspartic acid protease important in the formation of myelin sheaths in peripheral nerve cells.[96] Until 2007, several pharmaceutical companies were in the early stages of testing new drugs for the treatment of Alzheimer's disease. In 2012, Merck reported the results of a phase I trial for MK-8931 **67** and started a new clinical trial to evaluate the safety and effectiveness of this compound in patients with mild-to-moderate Alzheimer's disease. Phosphinic acid derivatives **68** as potential β-secretase inhibitors were first described in 2006.[97] The authors of the patent reported that the synthesized inhibitors had high activity toward the enzyme with IC_{50} values in the range of 0.01–0.2 μM (Fig. 12).

5.2. α-Aminophosphonic acid derivatives

α-Aminophosphonic acids derivatives are a significant type of compounds that contain a P–C bond. These class of molecules are known as effective chelating agents. The presence of a $-NH_2C-P(O)(OH)_2$ group increases their metal binding abilities. Many synthetic methods for the preparation of aminophosphonic acid derivatives have been described.[98] Although aminophosphonic acids were mainly used as insecticides, herbicides, and plant-growth regulators, they have also been applied as effective chemotherapeutic agents.[99]

A number of reports have been published on phosphonic acid derivatives used as MMP inhibitors. Hunter and coworkers reported the synthesis of a series of peptidomimetic α-aminophosphonic acid derivatives with high inhibitory properties against human fibroblast collagenase[100] and carried out *in vitro* tests for these compounds. Introduction of a bromonaphthalimidoethyl group into the structure led to a very potent inhibitor **69** with an IC_{50} value of 0.02 μM. The (*R*, *S*, *S*) isomer of **69** was much more potent (80-fold) than the (*S*, *S*, *S*) isomer towards MMP-1.

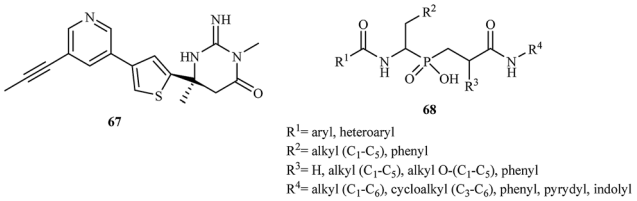


Fig. 12 Structures of β-secretase inhibitors MK-8931 **67** and **68**.

In 1999, D'Alessio and coworkers synthesized and evaluated peptidomimetic *N*-(furan-2-yl)carbonyl-Leu-Trp-OH analogues.[101] The compounds were tested for their activity against MMP-2, MMP-3, MMP-8, and MMP-9. The study showed that most peptidomimetic derivatives exhibited low potency towards MMPs. Nevertheless, compound **70** exhibited high activity with a satisfactory IC_{50} value of 60 μM.

Gallina *et al.* prepared a series of new phosphonic acid derivatives **71** by modifying the previously reported phosphotryptophan derivative L-Pro-L-Leu-L-(P)Trp$(OH)_2$.[102] By replacing the aminoterminal L-Pro with amino acid residues bearing small side chains, the affinity to MMP-2 and MMP-8 was increased, and the derivatives showed different selectivity profiles (Fig. 13).

In 2009, Tortorella and coworkers obtained new α-sulfonylaminophosphonate analogues.[103] Preliminary activity screening of these compounds was investigated against MMP-2, MMP-8, MMP-13, and MMP-14. Most of the synthesized analogues were very effective MMP inhibitors, exhibiting IC_{50} values in the nanomolar range. Derivative **72** proved to be the most potent inhibitor towards MMP-2 with an $IC_{50} = 60$ nM.

The Mazza research group prepared new α-arylsulfonylamino phosphonates and tested them as stereoselective inhibitors of MMP-8.[104] The mechanism of binding in the active site for both the *R*- and *S*-enantiomers (**73** and **74**, respectively) was explained by analyzing the crystal structures of the complexes with MMP-8. The study showed that enantiomer *R* **73** was much more potent than the *S* enantiomer **74** with an IC_{50} value in the nanomolar range.

Another class of phosphonic acid-based MMP inhibitors are the carbamoylphosphonic acid derivatives. A collaboration of scientists from Israel and Germany reported the synthesis, characterization and biological evaluation of the alkyl and cycloalkylcarbamoylphosphonic acid analogs.[105] Their inhibition potency was tested in *in vitro* models with MMP-1, MMP-2, MMP-3, MMP-8, and MMP-9. The cycloalkylcarbamoylphosphonic acid derivatives exhibited higher

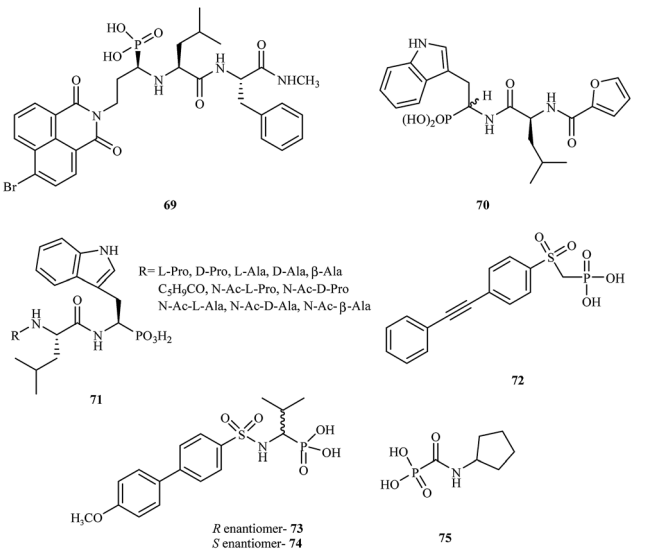


Fig. 13 Chemical structures of aminophoshonic acid derivatives **69–75**.

potency compared to the open-chain alkyl analogs. Most of the analogs showed good selectivity against MMP-2. The optimal activity against MMP-2 was observed for compound **75** with an IC_{50} value of 0.08 μM. In 2005, the same research group reported[106] that cycloalkylcarbamoylphosphonic acid-based inhibitors that block MMP-2 activity have a significant impact on the inhibition of tumor cell growth and reduce lung metastasis. This investigation proved that new carbamoyl phosphonate matrix metalloproteinase inhibitors might be effective drug candidates for cancer treatment.

In 2004, Breuer and coworkers prepared novel MMP inhibitors based on Ca(II), Mg(II), Zn(II) and Cu(II) complexes of cyclopentylcarbamoylphosphonic and 2-(*N*,*N*-dimethylamino) ethylcarbamoylphosphonic acids (**76** and **77**, respectively).[107] Both carbamoyl phosphonates showed very high MMP-2 activity with IC_{50} values of 80 nM for compound **76** and 25 nM for analog **77**. During the study, it was found that the dimethylamino group on compound **77** enhanced the binding potency of zinc binding group (ZBG) in aqueous solutions (Fig. 14).

In 2008, the Hoffman research group presented the synthesis of *cis*-2-aminocyclohexylcarbamoylphosphonic acid (*cis*-ACCP) **78** and analyzed its pharmacodynamic and pharmacokinetic properties.[108] *Cis*-ACCP was evaluated in *in vitro* and *in vivo* cancer metastasis models and classified as a medium-potency MMP inhibitor; because of its good pharmacological profile, this molecule entered phase 2 and 3 clinical trials (Fig. 15).

Recently, this laboratory[109] presented the synthesis of seven 4-phenoxybenzenesulfonamidopolymethylene carbamoyl phosphonates **79** containing polymethylene chains. Biological evaluation of these compounds showed the highest potency for analogues with $(CH_2)_{5,6}$ groups, which exhibited antimetastatic activity in a murine melanoma model. Compounds with shorter polymethylene linkers were selective towards MMP-2 in contrast to the analogs bearing chains with 7 or 8 methylene groups, for which no inhibitor activity was observed.

The latest achievements in the development of aminophosphonic acids with biological activity are the synthesis and application of chiral thiourea derivatives. They are an important class of compounds that demonstrate potential antibacterial, fungicidal, antiviral (including HIV) and anticancer activity. Several of them have found practical application as herbicidal compounds or plant growth regulators.[110] Currently, the use of chiral thiourea derivatives to treat disease is the subject of intense research.

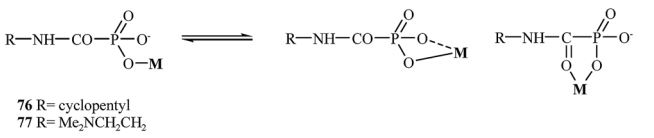


Fig. 14 Structures of MMP inhibitors **76** and **77** based on metal complexes.

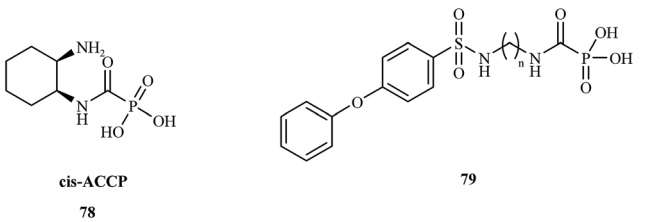


Fig. 15 Structures of *cis*-ACCP **78** and MMP inhibitors **79**.

6. Phosphorus compounds with antiviral activity

Development of new antiviral therapy is needed to treat viral infections that are not amenable to prophylaxis by vaccination or did not fulfill its promises for complete protection, but is also highly desirable for those infections where vaccination has not been implemented. The beginning of the antiviral era is marked by the description in 1959 of the synthesis of 5-iodo-2′-deoxyuridine (IDU).[111] IDU was actually synthesized as a potential antitumor agent, but later became commercialized as the first antiviral drug to be used in the topical treatment of herpetic eye infections. Nowadays, among the drugs used in treatment of some viral infections [*e.g.* human immunodeficiency virus (HIV), hepatitis C virus (HCV), hepatitis B virus (HBV), cytomegalovirus (CMV), smallpox (variola virus)] there are many compounds containing a phosphorus atom.

Nowadays, AIDS, caused by HIV infection is one of the major medical problems. According to the WHO data, 36.9 million people were living with HIV and 1.9 million people newly enrolled on antiretroviral treatment in 2014. In 1985, E. de Clercq *et al.*, described the activity of 9-(*R*)-(2-phosphonomethoxypropyl)adenine (tenofovir) **80** against HIV in cell culture.[112] Tenofovir is a nucleotide (nucleoside monophosphate) analogue with activity against retroviruses, including HIV-1, HIV-2 and hepadnaviruses in a variety of cell types, including resting cells. Tenofovir is administered to patients in the form of a prodrug – tenofovir disoproxil fumarate (tenofovir DF) **81**. Following absorption, tenofovir DF is rapidly converted to tenofovir (Fig. 16), which is metabolised intracellularly to its active anabolite, which is a competitive inhibitor of HIV-1 reverse transcriptase and terminates the growing DNA chain.[113]

Continuing research into more active agents led to the discovery of other derivatives of tenofovir with better distribution into lymphoid tissues. GS-7340 **82** is a prototype molecule representing a novel class of tenofovir monophosphonoamidate prodrugs. Unlike tenofovir, GS-7340 contains phenol and alanine isopropyl ester as the phosphonate masking groups. Relative to parent tenofovir, GS-7340 exhibits 500- to 1000-fold enhanced activity against HIV-1 in

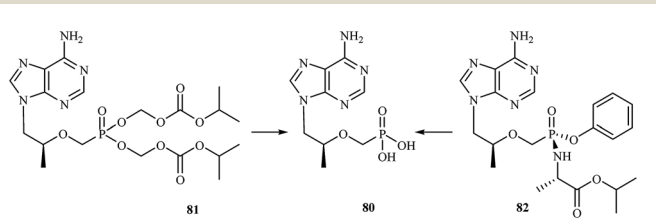


Fig. 16 Conversion of tenofovir DF **81** and GS-7340 **82** to tenofovir **80**.

T-cells, activated peripheral blood mononuclear lymphocytes and macrophages.[114]

There are currently a large number of agents in development across a variety of classes for the treatment of HCV infections. One class for which promising *in vitro* results have been reported is represented by the nucleoside/nucleotide analogs. These compounds share properties with the intracellular nucleoside substrates of the target HCV enzymes involved in the transcription of the viral genome and, when phosphorylated to the nucleoside-triphosphate, lead to premature termination of the growing HCV RNA chain during viral replication.[115] One of nucleotide analog, sofosbuvir **83** is a phosphoramidate prodrug that is metabolized within the liver into the active antiviral agent 2′-deoxy-2′-α-fluoro-β-*C*-methyluridine-5′-monophosphate **84** which is further phosphorylated to the active 2′-deoxy-2′-α-fluoro-β-*C*-methyluridine-5′-triphosphate **85** (Fig. 17).[116] The triphosphate serves as a defective substrate for the NS5B protein, which is the viral RNA polymerase, thus acts as an inhibitor of viral RNA synthesis.[117] In 2013, the Food and Drug Administration (FDA) approved sofosbuvir in combination with ribavirin for oral dual therapy of HCV genotypes 2 and 3, and for triple therapy with injected pegylated interferon and ribavirin for treatment-naive patients with HCV genotypes 1 and 4. In 2014 a combination of sofosbuvir with the viral NS5A inhibitor ledipasvir was approved.

Hepatitis B is another viral infection that attacks the liver and can cause both acute and chronic disease. According to the WHO data, an estimated 240 million people worldwide are chronically infected with HBV. More than 780 000 people die every year due to complications of hepatitis B, including cirrhosis or liver cancer.[118] Adefovir **86** is a drug used to treat infections with hepatitis B virus. It is a nucleotide analog with reverse transcriptase inhibitory activity. Adefovir is orally administrated as a prodrug – adefovir dipivoxil **87**. After administration adefovir dipivoxil is hydrolysed to adefovir and phosphorylated to its active diphosphorylated form adefovir dipivoxil **88** (Fig. 17). Upon phosphorylation, adefovir DP competes with dATP for incorporation by the HBV reverse transcriptase. The lack of a 3′-hydroxyl group causes chain-termination when adefovir is incorporated into viral transcripts.[119] Dose of 10 mg per day of adefovir dipivoxil significantly improved histological, biochemical and virological outcomes in HBeAg-positive and – negative patients, and serological outcomes in HBeAg-positive patients.[120]

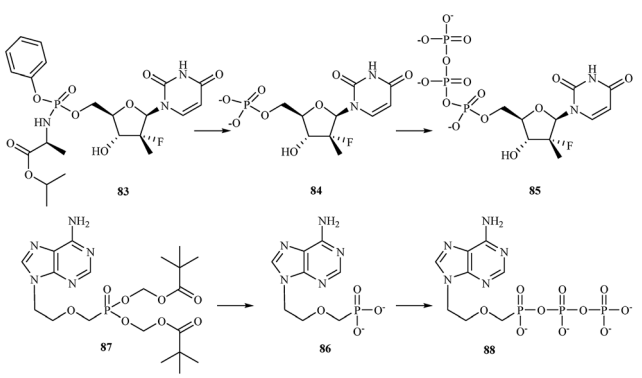


Fig. 17 Activation mechanism of sofosbuvir **83** and adefovir **86**.

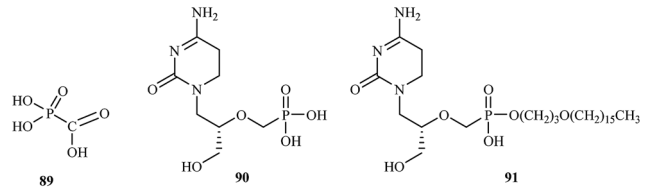


Fig. 18 Structures of foscarnet **89**, cidofovir **90** and CMX-001 **91**.

Treatment of CMV infections in immunosuppressed patients based upon several compounds including foscarnet **89** and cidofovir **90**. Both compounds are administered intravenously [foscarnet at 180 mg kg^{-1} per day for induction therapy and at 120 mg kg^{-1} per day for maintenance therapy; cidofovir at 5 mg kg^{-1} per week during the first 2 weeks (induction therapy), and then 5 mg kg^{-1} every other week (maintenance therapy)]. Foscarnet and cidofovir are targeted at the viral DNA polymerase. Foscarnet interacts directly with the pyrophosphate binding site of the DNA polymerase, whereas cidofovir must be first phosphorylated to its diphosphate derivative, which then interact as competitive inhibitor/alternate substrates. As alternate substrate phosphorylated cidofovir is incorporated into the growing DNA chain and block chain elongation (Fig. 18).[121]

With the declaration by WHO in 1980 that smallpox had been eradicated from the earth, any attempts to develop a potentially active anti-poxvirus drug were abandoned. In 2001, the fear that variola virus might emerge again as the consequence of a terrorist attack. In the US a program was launched to identify antiviral agents that could be used prophylactically or therapeutically against orthopoxviruses, which could be employed as a biological weapon or to give arise to an inadvertent outbreak. One of the foremost candidate to be used in such scenario is CMX-001 **91**. CMX-001 is the hexadecyloxypropyl ester of cidofovir, which had already been reported as an antiviral agent active against vaccinia virus. CMX-001 is a highly promising compounds to treat pathogenic orthopoxvirus infection in humans and should not only be intended for therapeutic use against smallpox, but also for progressive vaccinia as the consequence of the smallpox vaccination (in immunosuppressed patients); monkeypox, where cidofovir has proved more efficacious than vaccination; and even molluscum contagiosum (due to molluscipoxvirus).[4]

7. Conclusions

This literature review has emphasized and described the importance of organophosphorus derivatives, which are a wide class of chemical compounds containing organic moieties usually bonded directly to phosphorus or bonded through a heteroatom, such as sulfur, oxygen or nitrogen. As discussed in this literature review, medical applications of organophosphorus compounds as drugs or drug candidates against many diseases have expanded greatly in recent years. OPs are used in clinical practice as compounds with anticancer or antiviral

properties. Bisphosphonates are currently the most important and effective class of drugs developed for the treatment of metabolic bone disorders associated with increased osteoclast-mediated bone resorption, such as osteoporosis. Aminophosphinic and aminophosphonic acids also are a very important group of chemical compounds, and their synthesis has attracted considerable attention in medicinal chemistry. They are classified as antimetabolites, which compete with their aminocarboxylic acid analogues in the active sites of enzymes and other cell receptors. It is without a doubt because of the unique properties and various applications of organophosphorus compounds that will continue to make them the subject of intense research investigations around the world.

List of abbreviations

ACE	Angiotensin converting enzyme
CA4P	Combretastatin A-4 phosphate
CMV	Cytomegalovirus
CWA	Chemical warfare agents
FDA	Food and drug administration
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
IDU	5-Iodo-2′-deoxyuridine
MMPs	Matrix metalloproteinases
OPs	Organophosphorus compounds
PAN	Pesticide action network
SERMs	Specific estrogen receptor modulators
STS	Steroid sulfatase
TEPP	Tetraethyl pyrophosphate
thioTEPA	*N*,*N*′,*N*″-Triethylenethiophosphoramide
WHO	World health organization
ZBG	Zinc binding group

Acknowledgements

We gratefully acknowledge the National Science Centre (Poland) for financial support (grant no. 2011/03/D/NZ7/03985).

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