Journal of Medicinal Chemistry

PERSPECTIVE

pubs.acs.org/jmc

Remarkable Potential of the α-Aminophosphonate/Phosphinate Structural Motif in Medicinal Chemistry

Artur Mucha, Paweł Kafarski, and Łukasz Berlicki*

Department of Bioorganic Chemistry, Faculty of Chemistry, Wrocław University of Technology, Wybrzeże Wyspiańskiego 27, 50-370 Wrocław, Poland

INTRODUCTION

α-Aminophosphonic acids are broadly defined as analogues of amino acids in which the carboxylic group is replaced by a phosphonic acid or related group (usually phosphonous or phosphinic acids). This results in the presence of the characteristic N−C−P scaffold (Scheme 1). The biological activity and natural occurrence of these compounds (often called α-aminophosphonates) were discovered half a century ago.[1,2] Since then, the chemistry and biology of this class of compounds have been developed into a distinct branch of phosphorus chemistry.[3] It is generally acknowledged that α-aminophosphonates possess a broad capability of influencing physiologic and pathologic processes, with applications ranging from agrochemistry to medicine. In some cases, these compounds have been commercialized. A number of excellent reviews on various aspects of their activity in natural systems have been published.[4−12]

The mode of action of aminophosphonates primarily involves the inhibition of enzymes of different class and origin. Despite its long history, this area of research remains intensively explored and frequently delivers new promising lead compounds in medicinal chemistry. The N−C−P molecular fragment and its chemistry offer many possibilities for structural modifications, which have resulted in broad biological relevance (Scheme 1).

Often, α-aminophosphonic and phosphinic acids are considered simple analogues of their natural counterparts, carboxylic acids. Although carboxylic and phosphonic acid groups differ in shape (tetrahedral at phosphorus versus planar at carbon), acidity (with phosphonic acid being significantly more acidic), and steric bulk (the phosphorus atom has a much larger atomic radius than carbon), they frequently exhibit similar properties, with the phosphonic acid being recognized by enzymes or receptors as false substrates or inhibitors.

However, the tetrahedral geometry of substituents around the phosphorus moiety causes it to resemble the high-energy transition state (TS) of ester and amide bond hydrolyses. The tetrahedral transition state is believed to be specifically stabilized in enzyme active sites, which has inspired numerous studies on their applications in regulating the activity of proteases. This approach has been most successful in the case of metalloproteases, which have an organophosphorus moiety in their active sites that facilitates the chelation of metal ions. This approach has resulted in the development of many potent inhibitors of various enzymes, such as the antihypertensive drug fosinopril, an angiotensin I converting enzyme (ACE) inhibitor. Recently, the N−C−P scaffold has been used to construct extended transition state analogues of amide bond synthesis or hydrolysis to find potent inhibitors of enzymes such as glutamine synthetase or urease.

Reactive peptidyl phosphonate diaryl esters have been successfully used to covalently modify members of the serine hydrolase superfamily. This approach exploits their ability to phosphonylate the hydroxyl residue of the active-site serine of these enzymes. They act as competitive, irreversible inhibitors, which, after the formation of an initial enzyme−substrate complex, bind to the active site via a transesterification reaction and thus block its catalytic function. The activity and selectivity of the interactions of inhibitors with target enzymes can be adjusted by structural optimization of the S1 residues and/or by the development of an extended peptide chain.

Finally, aminomethylenebisphosphonic acids form a separate class of medicinally important compounds bearing the N−C−P skeleton. They are hydrolytically stable analogues of pyrophosphate characterized by a common P−C−P fragment in which a carbon−phosphorus bond replaces an oxygen−phosphorus bond. Their primary medical application is in combating osteoporosis. They exhibit very high affinity to bone tissue, being rapidly adsorbed at the bone surface, and they regulate the bone remodeling process. Because the action of bisphosphonates is limited to osseous tissue, they have also been used to deliver conjugated chemotherapeutic agents to bone. Likely because of their strong chelating properties, bisphosphonates also exhibit inhibitory properties toward a wide variety of metalloenzymes.

In this Perspective, we present the key features of the N−C−P molecular fragment that govern the activity of the molecules that incorporate it. A general overview of known modes of action and target enzyme classes is briefly presented. Recent representative medicinal chemistry projects are described and discussed, including the achievements of our research group on leucine aminopeptidase and urease. Particular attention is given to the molecular aspects of the N−C−P mechanism of action and to the rational design of new compounds based on three-dimensional structures. The potential future applications of this class of compounds are also discussed.

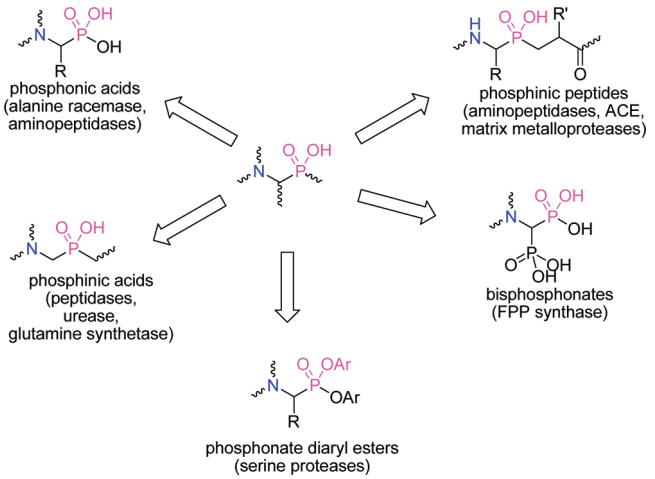
AMINOCARBOXYLIC ACID MIMETICS

Aminophosphonates represent one of the best suited compound classes for contructing false substrates or inhibitors of enzymes involved in amino acid metabolism. Although the significant differences between carboxylic and phosphonic acid moieties defy the typical understanding of structural analogy, this replacement frequently results in mimetics of amino acids that display potent activity toward the chosen receptors.[13] This approach has been most widely used for the preparation of

Received: May 11, 2011
Published: July 22, 2011

dx.doi.org/10.1021/jm200587f | J. Med. Chem. 2011, 54, 5955–5980

Scheme 1. Representative Classes of Compounds Containing the N−C−P Scaffold and Selected Molecular Targets of Medicinal Relevance



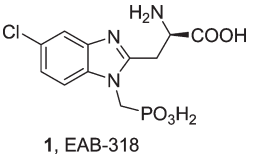
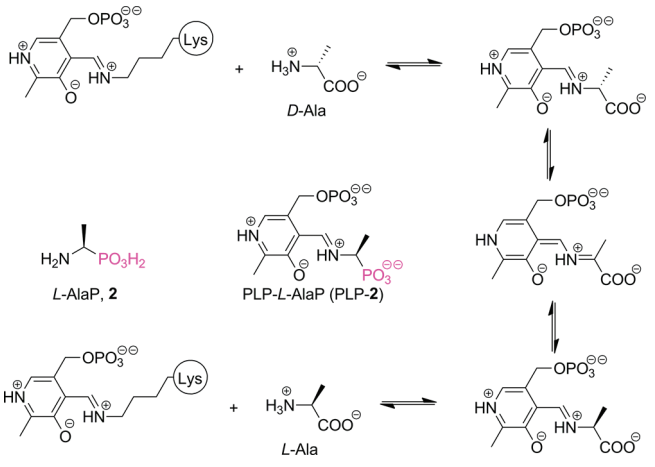


Figure 1. Noncompetitive antagonist of NMDA receptor.

Scheme 2. Mechanism of Alanine Racemization Showing that the Inhibitory Complex of PLP and AlaP Binds Analogously to the Reaction Intermediate



neuroactive analogues of the central nervous system neurotransmitters glutamic and γ-aminobutyric acids. Notably, compounds built with the N−C−P scaffold rarely exhibit this activity. One example of this approach is *R*-α-amino-5-chloro-1-(phosphonomethyl)-1*H*-benzimidazole-2-propanoic acid (**1**, EAB-318, Figure 1), a potent noncompetitive antagonist of synaptic NMDA receptors.[14] It is expected to be developed as a drug to prevent strokes.

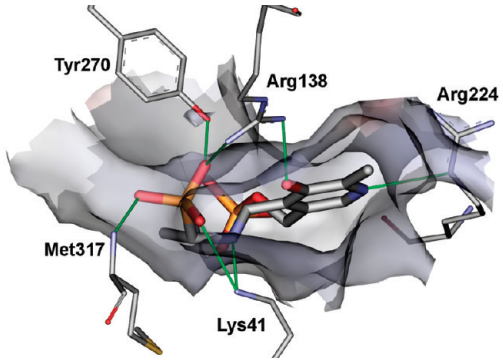


Figure 2. Phosphonic analogue of L-alanine (**2**) bound to *Bacillus anthracis* alanine racemase (PDB entry 2VD9). The phosphonate group of L-AlaP is fixed by interactions between the phosphonate O atoms and the amide N atom of Met317, the released N^{ζ} atom of Lys41, the guanidinium N atoms of Arg138, and the O^{η} atom of Tyr270 (all hydrogen atoms are omitted). Hydrogen bonds are marked by solid green lines. The surface is colored according to the interpolated charge. Only the residues interacting with the ligand are shown (this remark will hold true for all subsequent figures as well).

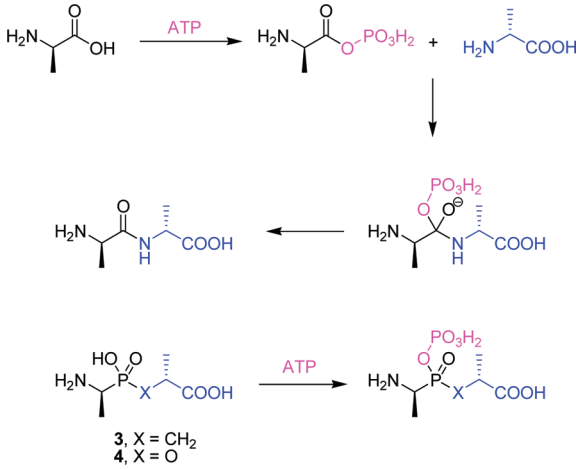
The phosphonic acid moiety is believed to bind to the affected protein more strongly than the corresponding carboxylic acid because of its dianionic character. However, this assumption has only been fully confirmed in the case of inhibition of some kinds of L-alanine racemase by their phosphonic acid counterpart, L-aminoethanephosphonic acid (**2**, L-AlaP, Scheme 2), a component of the antibacterial drug alamecin (alafosfalin, L-alanyl-L-aminoethanephosphonic acid). This phosphonate behaves like a substrate and forms an external aldimine with the bound pyridoxal 5′-phosphate (PLP) cofactor, but it is not efficiently hydrolyzed. Instead, it binds strongly to the enzyme with significant participation of the phosphonic acid group (Figure 2).[15−18] The inhibitor-bound structure of *Bacillus anthracis* alanine racemase was obtained by soaking apoenzyme crystals with L-AlaP. The largest conformational change occurs at Lys41, which no longer forms an aldimine linkage with the pyridoxal phosphate (PLP), and the PLP itself rotates by about 15° in the plane of the pyridoxal ring to form a new aldimine link to the inhibitor. The electron density for the inhibitor-bound structure, while generally good, is not continuous around the C1 atom of L-AlaP. Detailed inspection of this phenomenon revealed that most likely the PLP-L-AlaP moiety is itself a tightly binding substrate of alanine racemase and has been partially converted to PLP-D-AlaP.[18]

Although the details vary (depending on the source of the enzyme), the generally accepted mechanism of the L-alanine racemase reaction proceeds as depicted in Scheme 2.[19] The active-site pyridoxal phosphate condenses with Ala to form an external Schiff base. The subsequent α-hydrogen abstraction by an active-site base (presumably lysine) results in the formation of a resonance-stabilized deprotonated intermediate. When reprotonation occurs on the opposite face of the planar intermediate, D-alanine is formed. The inhibitory action of L-AlaP results from a combination of its substrate-like behavior and from the electrostatic interaction of the phosphonate moiety with the enzyme.

D-Alanyl-D-alanine ligase is one of the key enzymes in peptidoglycan biosynthesis and is an important target for antibacterial drugs. The enzyme catalyzes the condensation of two D-Ala molecules using ATP to produce D-Ala-D-Ala (Scheme 3), which

dx.doi.org/10.1021/jm200587f |*J. Med. Chem.* 2011, 54, 5955–5980

Scheme 3. Mechanism of Condensation of Two D-Alanine Molecules Catalyzed by D-Alanyl-D-Alanine Ligase[a]



[a] A similar reaction of its suicide substrates results in formation of a real inhibitor-mimicking phosphorylated transition state analogue of the enzymatic reaction.

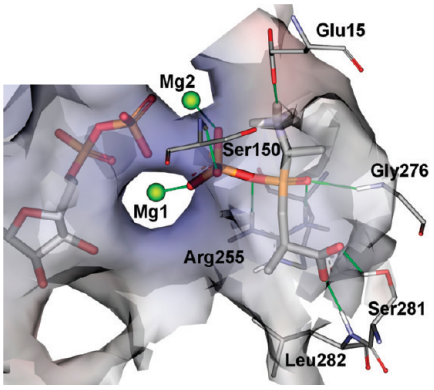


Figure 3. Crystal structure of the phosphorylated transition state analogue (**3**) bound to the *Escherichia coli* D-Alanyl-D-alanine ligase (PDB entry 2DLN).

is the terminal peptide of a peptidoglycan monomer.[20] Phosphonic analogues of the substrate of the enzyme act as very weak inhibitors,[21] whereas D-alanyl-D-alanine analogues in which the peptide bond was replaced by a phosphinate or phosphonate moiety (usually considered transition state analogues; compounds **3** and **4**, respectively) appear to be promising and potent mimetics.[21,22] Their mode of action, however, is different and quite complex because they were shown to act as suicide substrates on the enzyme. As determined by kinetic studies,[22] X-ray,[23,24] and molecular modeling,[25] the inhibitor reacts with ATP to produce ADP and a tight-binding phosphorylated transition state analogue, which exerts inhibitory action against the enzyme (Scheme 3 and Figure 3).

Closely related enzymes found in vancomycin-resistant Gram-positive bacteria using alternative substrates, such as D-serine or D-lactate, are also inhibited by the same phosphinophosphate analogue of the reaction intermediate, which is formed upon reaction with ATP.[21,26]

Analogues of peptides containing C-terminal α-aminophosphonates have also been found in nature. The first was phosphonic

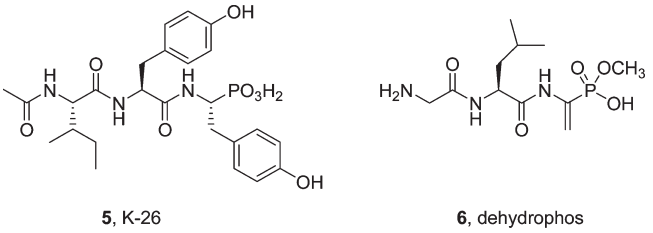


Figure 4. Naturally occurring α-aminophosphonates: **5** and dehydrophos.

tripeptide analogue **5** (K-26, Figure 4), which was discovered unexpectedly during angiotensin-converting enzyme bioactivity-guided fractionation of the extracts of a soil dwelling prokaryote, actinomycete strain.[27] It has been reported to possess an ACE inhibitory activity comparable to the widely prescribed antihypertensive drug captopril. Analogues of **5** were also found to strongly inhibit ACE activity.[28] Esterification of the phosphonic moiety was found to be a critical determinant of activity, with the resulting diethyl phosphonate ester exhibiting a 1500-fold increase in ACE inhibition.[29]

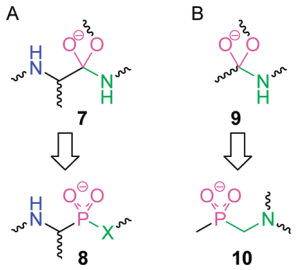
Dehydrophos (**6**, Figure 4), produced by *Streptomyces luridus* and discovered in 1984, exhibits a broad spectrum of activity against Gram-positive and Gram-negative bacteria.[30] Its structure, unknown until 2007, was determined by comparison with synthetic samples, which revealed that the C-terminal component of the peptide is a monomethyl ester of the phosphonic analogue of dehydroalanine.[31] Approaches undertaken to modify its structure to obtain better antibacterial agents have so far been unsuccessful.[32]

As far as pharmacokinetic properties of α-aminophosphinic acids are concerned, the most comprehensive studies have been probably performed for alafosfalin. The released active metabolite L-AlaP interferes with cell wall biosynthesis as described above. Alafosfalin is rapidly absorbed after parenteral administration. Peak plasma concentration is achieved within 20 min of subcutaneous dosing to rats, and the area under the plasma concentration−time curve is 25 μg·h/mL for a single 20 mg/kg dose.[33] Plasma half-life ranges from 20 min in rats to 1 h in humans. The drug is also rapidly absorbed by oral administration, but this route is approximately 8 times less effective, as the dipeptide is extensively hydrolyzed. An intramuscular dose of 200 mg and an oral dose of 500 mg produce adequate inhibitory concentration in human plasma for many pathogens. The compound is readily cleared via the renal route, in both forms, as the dipeptide and the amino acid L-AlaP (predominantly), with the P−C bond intact. Alafosfalin is well tolerated and of a low toxicity to mammals.

TRANSITION STATE ANALOGUES

One of the most fundamental abilities of enzymes is decreasing the energy of activation of the catalyzed processes, regardless of their exact mechanisms. To achieve this, the strongest possible ligand−protein interactions must occur in the transition states (TSs) of the reactions. The concept of evolutional arrangement of the residues in the active sites to stabilize these systems has been suggested as a potential approach to construct effective inhibitors that share structural and electronic resemblance to the TSs.[34,35] This classical concept relies on the exchange of amino acid residues in the ligand structure (**7**) with their

Scheme 4. Similarities between the Transition State of Amide Bond Hydrolysis (Structures 7 and 9) and an Aminophosphonate (Structures 8 and 10) in the Case of Classical (A) and Extended (B) Approaches



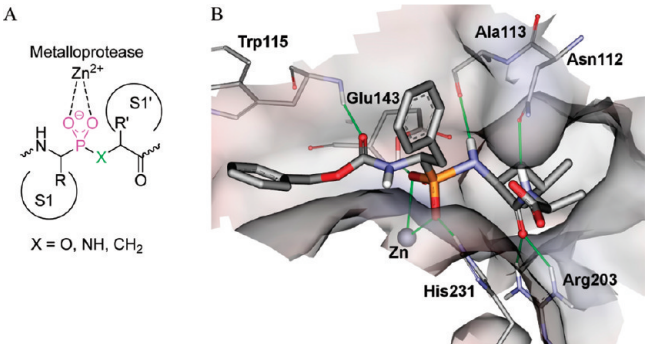


Figure 5. (A) General structure of phosphonodepsipeptides (X = O) and phosphonamidate (X = NH) and phosphinic (X = CH_2) pseudopeptides based on the α-amino phosphorus motif, and their schematically represented interactions with the active site of zinc proteases. (B) The crystal structure of thermolysin complexed with an extremely tight-binding transition state phosphonamidate inhibitor Cbz-Phe[P(O)-(OH)NH]Leu-Ala, K_i = 68 pM (PDB entry 4TMN).

phosphonic/phosphinic analogues (**8**) (Scheme 4A). Proteolytic enzymes (proteases, peptidases) appeared to be obvious targets for the application of this idea. Another approach, called extended transition state analogy, is based on the replacement of the amide moiety (**9**) with an aminophosphinate/phosphonate (**10**), thus introducing an additional methylene group inside the structure (Scheme 4B).

Peptidases cleave the peptide bond via different mechanisms. Serine (or threonine) and cysteine proteases use the active nucleophilic residue (hydroxyl or thiol, respectively) to attack the amide carbonyl and produce a hemi(thio)acetal intermediate that is subsequently hydrolyzed by a water molecule. Both the formation and decomposition of the covalent acyl enzyme proceed via a tetrahedral TS stabilized in the oxyanion hole. Aspartic (or glutamic) and metalloproteases use the activated water/hydroxide ion to perform hydrolysis in a one-step process. Numerous peptide analogues with a phosphorus-containing moiety replacing the scissile amide bond have been found to regulate the activity of proteases from different families (this topic was comprehensively reviewed in 2000).[5,36] The cysteine peptidases remain the only exception. During these studies, it became evident that simple tetrahedral resemblance was not the only factor necessary to ensure the activity of N−C−P peptidomimetics. For example, for serine-dependent enzymes, the presence of a diaryl phosphonate moiety ensured covalent bonding to the catalytic serine in the active site, whereas the peptide fragment occupied the Sn subsite. Structurally extended phosphinic peptides were successfully employed to develop effectors of aspartic proteases such as renin, pepsin, and HIV-1 protease. In the case of HIV-1 protease, the C_2-symmetrical structure of the inhibitors was inspired by the architecture of the target active site.[37] However, regulation of metalloproteases (predominantly zinc-dependent proteases) by organophosphorus compounds has been the most extensively and successively studied area. In particular, three types of transition state peptide mimetics, namely, phosphonodepsipeptides (−P(O)-(OH)O−), phosphonamidates (−P(O)(OH)NH−), and phosphinates (−P(O)(OH)CH_2−) (Figure 5A), have been described. For these compounds, the TS analogy has been associated with the formation of bidentate complexes between the pseudopeptide phosphoryl moiety and a metal (zinc) ion(s). In addition to chelation at the central warhead portion, the structures of the Pn and Pn′ residues of the backbone can be optimized to dock favorably in the binding pockets (Figure 5B).[38] Numerous papers have been published to illustrate these applications, with thermolysin and carboxypeptidase A being the most prototypically studied peptidases.[39] Recent approaches to the design and optimization of selected inhibitors of metalloproteases (aminopeptidases, matrix metallopeptidases, and angiotensin-converting enzyme) are presented below.

The M17 cytosolic leucine aminopeptidase (EC 3.4.11.1, LAP) and the M1 microsomal alanyl aminopeptidase (EC 3.4.11.2, APN/CD13) are among the most recognized representatives of metal-containing exopeptidases of biomedical significance.[40−44] These multifunctional, broad-specificity enzymes are detected in many mammalian tissues and organs and in lower organisms such as plants and bacteria. Although they belong to different clans, LAP and APN are frequently compared to each other because of their related substrate specificities. Both aminopeptidases efficiently catalyze the release of hydrophobic amino acids from the N-termini of peptides or proteins; the preferences of APN also include basic residues. Accordingly, in mammals, these hydrolases are involved in the metabolism of regulatory and bioactive (mostly neuroactive and hormonal) peptides of diverse cell types, antigen presentation, angiogenesis control (APN), and other processes. Functions related to tumorigenesis and invasion make them molecular targets for the development of potential anticancer drugs. The recognized role of APN in the pathogenesis of hypertension provides an opportunity for regulating arterial blood pressure by affecting its activity. In single-cell organisms, aminopeptidases are largely responsible for digestive proteolysis. For example, the *Plasmodium falciparum* species uses M1 and M17 during the final stages of hemoglobin degradation in the host erythrocytes.[45,46] Hemoglobin degradation is directly connected with the clinical symptoms of malaria, the most prevalent parasitic disease in humans.

The design and development of pseudopeptidic inhibitors of aminopeptidases are greatly facilitated by two factors. First, the results of extensive structure−activity relationship studies, available for a wide collection of α-aminophosphonic acids, have defined the requirements of the S1 binding pockets of LAP and APN.[47−49] Both enzymes apparently prefer bulky aliphatic (e.g., isobutyl) or, even more favorably, noncoded arylalkyl (e.g., phenylethyl) P1 substituents. Somewhat surprisingly, the data obtained for the most efficiently cleaved substrates (methionine or norleucine amides for LAP) cannot be simply translated into

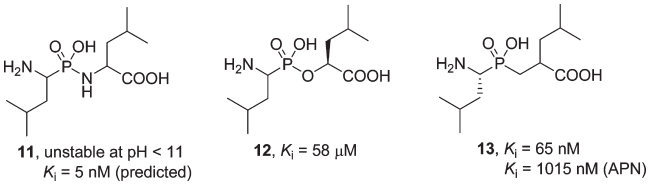
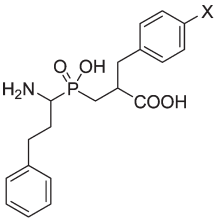


Figure 6. Structures and activities of phosphorus containing Leu-Leu dipeptide analogues studied as inhibitors of leucine aminopeptidase (LAP).



Compound	K_i [nM]			
	M1		M17	
	Porcine kidney (APN)	rec. *P. falciparum*	Porcine kidney (LAP)	rec. *P. falciparum*
14 (X = H)	276	78.4	66	13.2
R,S-**14**			45	
15 (X = OH)	36	232.2	67	10.4

Figure 7. Structures of phosphinic analogues of hPhe-Phe (**14**) and hPhe-Tyr (**15**) and their inhibitory activities toward the alanyl and leucine aminopeptidases of mammalian (porcine kidney) and protozoan (*Plasmodium falciparum*) origins.

an inhibitor structure.[50] A recent study of the correlation between the kinetic parameters for the alanyl aminopeptidase explained this inconsistency. The strength of substrate binding (K_m), and not the turnover velocity (k_{cat}/K_m), allowed for more accurate predictions of the structures of potent inhibitors.[51]

The second factor is that computer-aided analysis of numerous crystal structures available for LAP, including the complex with the phosphonic analogue of leucine,[52] has pointed to this enzyme as a primary molecular target for extending and optimizing interactions within the S1′ pocket. According to these computed predictions (using the LUDI program), the phosphonamidate analogue of Leu-Leu (**11**) appears to be the most promising compound (theoretically calculated K_i = 5 nM).[53] This dramatic, 4 orders of magnitude difference in activity compared to the activity of a previously studied phosphonate derivative (**12**, Figure 6)[47] was explained by an energy gain originating from differences in binding details. Unfavorable entropic effects and electrostatic repulsions with the oxygen atom of Ala113 weakened the docking of phosphonate **12** in the active site, whereas the P−N compound formed a privileged hydrogen bond (NH···O═C) to Leu360 that is very similar to that of a substrate in the transition state. The corresponding relationship between the pseudopeptide structures and their activity have been previously validated for thermolysin and Cbz-Gly[P(O)(OH)X]Leu-Leu derivatives.[54] Disappointingly, in the case of LAP, the data predicted for the phosphonamidate were not confirmed experimentally. The deprotected P−N analogue **11** appeared to be hydrolytically unstable at pH levels below 11. The adjacent amino group, although crucial for the effective binding, significantly increased the susceptibility of the phosphonamidate to hydrolysis.[55]

Phosphinic pseudodipeptide **13** appeared to be a promising alternative. It was stable in water and exhibited an inhibition constant in the nanomolar range. The diastereomerically enriched sample gave K_i = 65 nM, which ranks it among the most effective ligands of the enzyme reported to date.[53] The affinity to the referenced APN was measured at 1 μM.

Further optimization of the P1 and P1′ residues led to phosphinate hPhe-Phe and hPhe-Tyr mimetics (**14** and **15**, Figure 7) with nearly the same K_i values (although measured for the mixture of four diastereomers). The potency of the individual stereoisomers of **14**, separated by a chiral chromatography, was also determined. The data, including K_i = 45 nM for the most active *R,S* (L,L) configuration, indicate that all four isomers are good to excellent ligands.[56] Importantly, this observation implies that there is no need to prepare these derivatives in a stereoselective manner. Although phenylalanine **14** and tyrosine analogue **15** showed equipotent activity toward LAP, the latter compound was found to be a remarkably effective inhibitor of APN (K_i = 36 nM). The strong preference for the *p*-hydroxybenzyl P1′ side chain was rationalized using a model of the APN active site homologous to the leukotriene A_4 hydrolase. The formation of a specific hydrogen bond between a phenolic OH group and the carboxylate of Glu413 was suggested as the rationale for this observation.[53]

Importantly, N−C−P−C pseudodipeptides appear to be excellent inhibitors when applied to *Plasmodium falciparum* M1 and M17 aminopeptidases, the protozoan counterparts of APN and LAP. Compounds **14** and **15** both suppress the activity of recombinant enzymes, with particularly tight-binding to *Pf*M17 (K_i of 13.2 and 10.4 nM for **14** and **15**, respectively, Figure 7).[57] The potency of **14** measured for *Pf*M1 was also improved (K_i = 78.4 nM) when compared to mammalian APN (K_i = 276 nM), but the exceptional results of **15** were not reproduced for the protozoan enzyme. Nevertheless, both phosphinates efficiently controlled the growth of *P. falciparum* in cultures, including those of malaria cell lines resistant to chloroquine. The IC_{50} varied in the range of 13−75 μM, which is similar to the well-known natural inhibitor of aminopeptidases, bestatin (IC_{50} = 8−21 μM). The phosphinic dipeptides were much more active in vivo. Upon treatment of mice with 2 mg (via interperitoneal injection) twice daily for 7 consecutive days, compound **14** reduced malaria infections (*P. chabaudi* murine model) by 92% compared with the control (the same doses of bestatin caused a reduction of only 34%).[57] Although neither the half-life nor the tissue and cellular penetrations of the phosphinate are known, they are expected to be significantly greater than those of bestatin (>2 h in mice and 0.3%/min in vitro, respectively) because of its increased hydrophobicity. These results positively validate *P. falciparum* M1 and M17 aminopeptidases as potential targets for a novel treatment of malaria and identify new leads with antiparasite potential.[46] The rational design and optimization process was further facilitated by subsequent resolution of the X-ray structures of both enzymes cocrystallized with compound **14** (Figure 8).[58,59]

Pseudodipeptidic derivatives do not cover different possibilities of structural modifications of phosphinic inhibitors. Two variations described for the alanyl aminopeptidase are worth mentioning. Peptide chain elongation represents the most obvious variation. Thus, a series of tripeptide analogues, extended to the S2′ residue, were found to be nanomolar effectors of APN (for example, compound *R,S,S*-Ala[P(O)(OH)CH_2]-Phe-Phe displayed K_i of 2.2 and 1.5 nM for mammalian and bacterial enzymes, respectively).[60,61] Phosphinic tripeptides exhibited promising antinociceptive activity because of their dual action against APN and neprilysin.

The second type of modification involves the introduction of a heteroatom, which could then be involved in metal complexation, adjacent to the phosphinate moiety. This change could increase the coordination number of the substrate and provide an

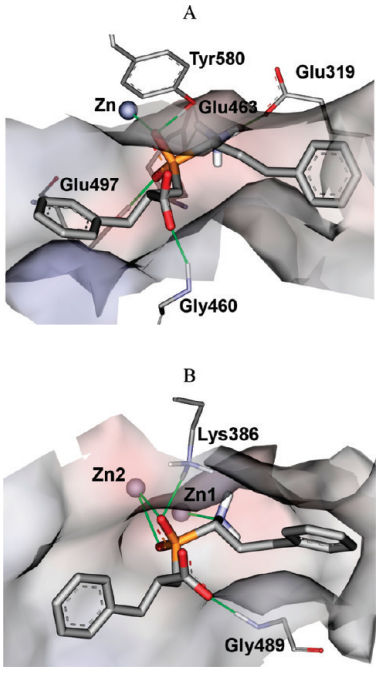


Figure 8. Architecture of the active sites of *P. falciparum* M1 (A) and M17 aminopeptidases (B) based on the crystal structures of their complexes with phosphinate inhibitor hPhe[P(O)(OH)CH_2]Phe (**14**) (PDB entries 3EBI and 3KR5, respectively).

additive effect to the strength of the inhibitor binding. Four such changes were envisioned, synthesized, and tested for the alanyl aminopeptidase: hydroxyalkylated (e.g., **16**, Figure 9), aminoalkylated, carbamoylated, and thiocarbamoylated α-aminoalkanephosphinic acids (e.g., **17**).[62−64] A combination of hydrophobic residues provided low micromolar or submicromolar IC_{50}. Interestingly, the lack of a C-terminal carboxyl group did not significantly detract from the activity of the compounds when compared to classical phosphinic pseudodipeptides.

Phosphinic pseudopeptides have also clearly revealed their potential for the regulation of matrix metalloproteinases (MMPs, matrixins), endopeptidases implicated in the breakdown of the extracellular matrix.[65] Cleavage of the matrix component (collagen, lamanin, elastin, gelatin, etc.) is physiologically essential for tissue remodeling processes such as morphogenesis, embryogenesis, and reproduction. Imbalanced MMP-mediated proteolysis leads to pathological states such as osteoarthritis, rheumatoid arthritis, and inflammation, but it is most associated with tumorigenesis (tumor growth, tumor invasion, and metastasis). The alteration of matrixin activity represents one the most promising approaches to cancer therapy.[66−69] However, despite enormous efforts in research and development in this field, the outcome in the drug market has so far been unsatisfactory. The spectacular failure of the clinical trials of first generation MMPs inhibitors, mainly due to a lack of selectivity that caused side effects, required careful reinvestigation of the precise roles of each particular MMP member in tumorigenesis and associated processes such as angiogenesis, cell growth, and signaling.[66,69] Advanced generations of inhibitors should instead be directed at individual targets.[70,71]

Phosphinic peptide inhibitors of matrix metalloproteinases have been known in the literature for over 2 decades,[72,73] but the development of solid-phase synthetic methods has greatly

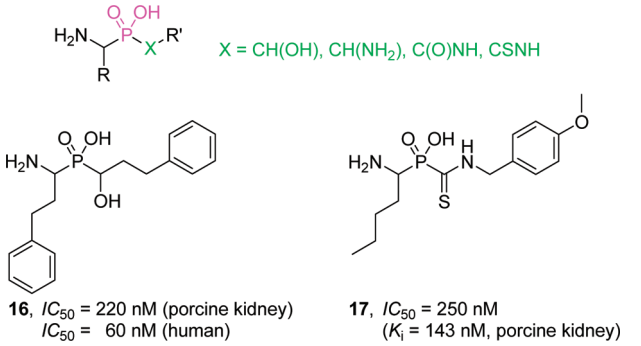


Figure 9. General structure of phosphinic acids functionalized with an additional heteroatom to enhance the chelation power toward a catalytic zinc ion in the enzyme active site, and the activities of selected representatives (**16** and **17**) toward alanyl aminopeptidases.

accelerated the availability of compounds obtained in parallel and combinatorial fashion.[74,75] Among the extensive series of pseudotripeptides prepared via solid-phase synthesis and primarily targeted toward MMP-11 (stromelysin-3), several very potent analogues have been identified, including compound **18** (RXP03, Figure 10).[76,77] This canonical compound inhibits different matrixins with K_i in the nanomolar range. MMP-1 (colagenase 1) and MMP-7 (matrylisin) remain two exceptions. SAR studies have demonstrated the importance of an unnatural side chain, such as phenylpropyl, in the P1′ position for achieving tight binding. The more extended heteroatom *p*-methoxybenzylthiomethyl (*p*-CH_3O-$C_6H_4CH_2SCH_2$−) residue gave slightly improved results. Bulky P1 and P2′ side chains were also favorable but not as selective as at P1′. This difference was rationalized by the architecture of the S1′ specificity loop. In the majority of MMPs, the cavity is shaped as a long open channel and is able to accommodate extended residues.[78] In the cases of MMP-1 and MMP-7, steric hindrance from arginine and tyrosine (the position typically occupied by leucine) limits the size of the loop.

The quest for a selective ligand of stromelysin 3 has only proven partially successful. The selectivity for MMP-11 versus other matrixins was improved by 2 or more orders of magnitude using the arylthiomethyl P1′ substituent in compound **19** (Figure 10).[79] To develop this compound, a unique Gln residue (characteristic only in stromelysin 3), located inside the S1′ cavity and in proximity with this particular residue, was targeted. However, this modification led to a significant drop in overall potency when compared to the lead compound. Another example of an extremely long P1′ side chain containing inhibitor, compound **20**, that gives unusually high selectivity (approximately 180-fold) for MMP-13 over MMP-14 is also presented in Figure 10.[80]

It has become apparent that the differentiation of matrix metalloproteinases using active and specific inhibitors is a nontrivial task. Additionally, the application of broad-spectrum agents gives inconsistent results, as evidenced by compound **18** when tested for treating tumors in mice.[81] The pharmacokinetic parameters are advantageous, and the major fraction of the compound remains intact in plasma and tumor tissue or undergoes biliary excretion. Treatment with a dose of 100 μg/day maintains the plasma concentration after 24 h over the K_i values determined for MMP-11, MMP-8, and MMP-13. However, the influence of **18** on primary tumors (induced C26 colon carcinoma) depends on the doses and treatment schedules and can even lead to stimulation under certain conditions.

dx.doi.org/10.1021/jm200587f |*J. Med. Chem.* 2011, 54, 5955–5980

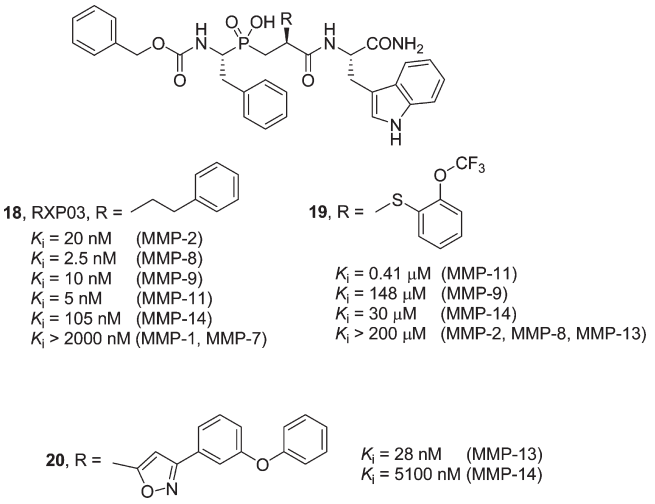


Figure 10. Structures of **18**, a canonical phosphinic tripeptide inhibitor of matrix metalloproteinases, and its modifications (**19** and **20**) to explore interactions with the S1′ specificity loop. Activities are provided for selected members of the family.

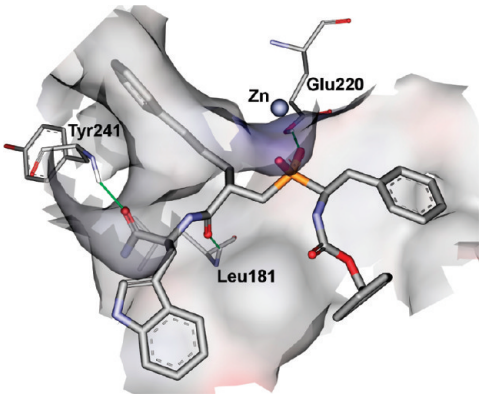


Figure 11. Crystal structure of MMP-11 complexed with phosphinic inhibitor **18** that contains an extended P1′ substituent, which explores the S1′ specificity loop of matrix metalloproteinases (PDB entry 1HV5).

The chemistry of phosphinic peptides has offered new insights toward developing highly specific inhibitors of matrix metalloproteinases. Such ligands are indispensable tools for studying the structure (Figure 11)[82] and the role of these enzymes at different stages of complex tumorigenesis processes. Without this precise knowledge, anticancer strategies based on MMP inhibition are unlikely to provide important therapeutic benefits.

Angiotensin-converting enzyme (ACE, EC 3.4.15.1), a zinc dipeptidyl carboxypeptidase, is a key enzyme of the renin−angiotensin system. ACE has two primary functions: the conversion of angiotensin I to hypertensive angiotensin II and the degradation of bradykinin and many other bioactive peptides. Because of its activity profile, ACE is one of the major therapeutic targets for controlling hypertension and related cardiovascular diseases. The somatic, membrane-bound angiotensin-converting enzyme contains two extracellular domains (N and C) of high homology but distinct ligand specificity.[83] Recent achievements, including the development of highly selective phosphinic pseudopeptide inhibitors, have provided insight into the functional and structural features of the domains, which had previously been considered identical.

A phosphinic tetrapeptide analogue **21** (RXP407, Figure 12), was the first reported inhibitor of angiotensin-converting enzyme that was able to discriminate between the two active sites.[84] The compound was very potent toward the N-terminal domain, with a K_i in the low nanomolar range, whereas its affinity to the C-terminal domain was 3 orders of magnitude weaker. The application of **21** in vivo blocked the degradation of the regulatory peptide Ac-Ser-Asp-Lys-Pro[85] and revealed differences in the mechanisms of angiotensin I and bradykinin cleavage.[86] The Tyr369 and Arg381 residues of the S2 binding pocket were suggested to be responsible for the N-domain selectivity of **21**.[87] In contrast, phosphonic tripeptide Cbz-Phe-[P(O)(OH)CH_2]Pro-Trp (RXP380, **22**, Figure 12), exhibited the opposite selectivity profile.[88] Its remarkable affinity for the C-domain was explained by favorable interactions between the hydrophobic residues defining the S2′ pocket and the indole ring of tryptophan.[88−90]

Further exploration of the structure of this ligand led to identification of a dual inhibitor of angiotensin-converting enzyme (C-domain) and endothelin-converting enzyme 1 (ECE) (**23**, Figure 12).[91] Interestingly, the specific stereochemistry of the P1′ residue is crucial for its remarkable selectivity versus neprilysin. When dosed intravenously to hypertensive rats (10 mg/kg), the phosphinate **23** reduced arterial blood pressure by 24 mmHg.

These results provide an important base of knowledge on the precise roles of the individual domains in ACE activity and have identified the C-domain as the major site of angiotensin I cleavage. None of the currently available drugs are selective in this context, targeting both homologous domains. The discovery that these sites differ in architecture and function in vivo is of great significance for developing new specific inhibitors and for understanding their clinical effects.[83,92]

As peptide analogues, phosphinates are not ideal drug candidates. They are resistant to hydrolysis in plasma, even when possessing two and more amide bonds. However, they are also characterized by poor bioavailability, particularly those with hydrophilic sequences. Nevertheless, this disadvantage can be addressed to improve structural optimization. Such was the case with fosinopril, an inhibitor of ACE now marketed under the trade name Monopril. Its lead structure (**24**, Figure 13) also incorporates an aminophosphinic fragment, although in the β-position rather than the α-position. To optimize the pharmacokinetic properties, the benzamide fragment was removed, and the proline fragment was substituted with a cyclohexyl residue.[93] Finally, to improve the bioavailability, the phosphinic acid was esterified (**26**). The ester is cleaved in vivo to generate the active compound (**25**, fosinoprilat).

After oral administration fosinopril undergoes rapid hydrolysis to its active diacid form. A single, daily recommended, 10 mg dose is not entirely absorbed (approximately 30% to healthy volunteers) but completely inhibits plasma ACE activity within 1 h. Peak plasma concentration ranges from 100 to 180 ng/mL. Time to the peak concentration is approximately 3 h, and the area under the plasma concentration−time curve ranges from 1.0 to 1.5 μg·h/mL.[94,95] In hypertensive patients, single and multiple oral doses (10−40 mg/day) reduces arterial, systolic, and diastolic blood pressure (typically 10−15%). Fosinoprilat is cleared slowly from the body (total body clearance ranges from 1.55 to 2.35 L/h).[94] It has a dual mode of excretion and is eliminated via a hepatic and renal pathway. The terminal elimination half-life has been estimated at 12 h. Generally,

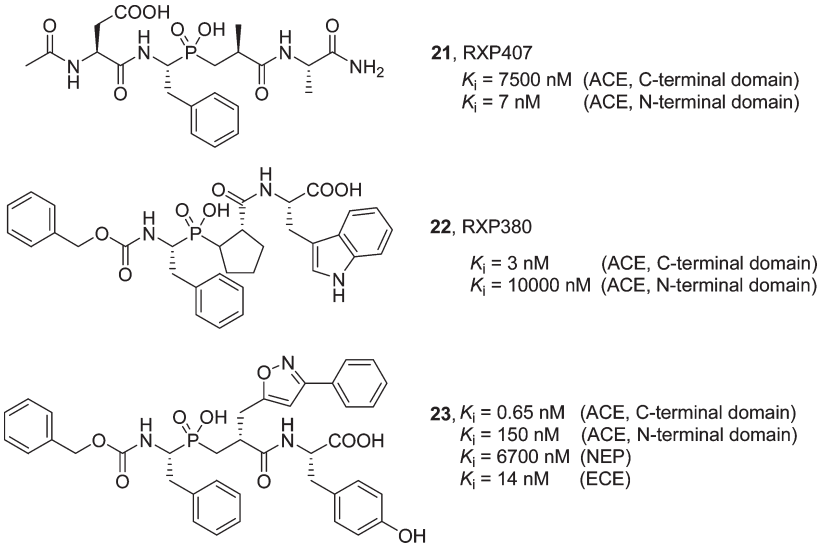


Figure 12. Structures of highly selective phosphinic peptide inhibitors of angiotensin-converting enzyme, which are able to distinguish between the N- and C-domains of ACE (**21** and **22**), and a dual inhibitor of angiotensin- and endothelin-converting enzymes (**23**).

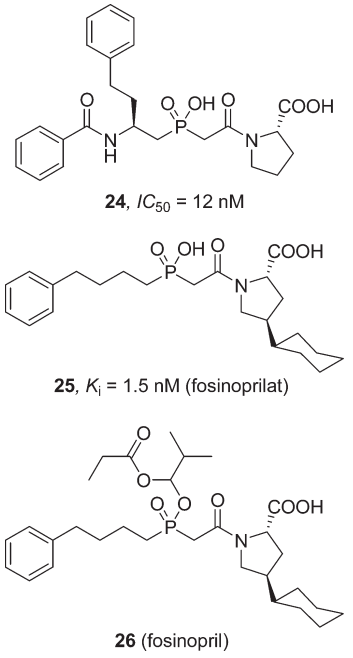


Figure 13. Structure and activity of the lead inhibitor of angiotensin-converting enzyme (**24**) that was modified to commercially available fosinopril (**26**), the esterified prodrug of antihypertensive fosinoprilat (**25**).

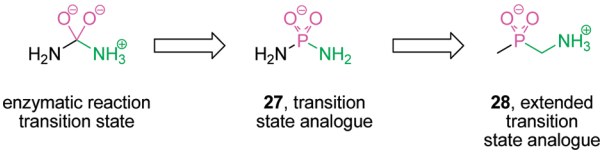
fosinopril is well tolerated in placebo controlled studies (data for over 1500 treated individuals). Mild and rare side effects are dizziness, headache, fatigue, and gastrointestinal disorders.

The extended transition state analogue approach has also been used with the N−C−P scaffold for construction of urease inhibitors. Urease is an enzyme that catalyzes the hydrolysis of urea to ammonia and carbamate, and the latter product spontaneously decomposes to give a second molecule of ammonia and carbon dioxide.[96,97] This enzymatic activity is closely related to two severe human infections: *Helicobacter pylori* colonization of the gastric tract, and *Proteus* and related species infections of the urinary tract.[98−100] In the first case, the release of ammonia causes a local increase of the pH of gastric juice and allows for survival of pathogenic bacteria. In the case of urinary tract infections, the alkalinization of urine by the enzymatic decomposition of urea, which is caused by infectious bacteria, leads to serious medical consequences ranging from the rapid formation of urinary stones (struvite and carbonate apatite) via pyelonephritis to hepatic encephalopathy. The most active inhibitors of urease are based on phosphordiamidate structure (**27**), which is a classical transition state analogue (Scheme 5).[101] Unfortunately, phosphoramidates are relatively unstable in aqueous solutions, particularly at low pH.[102] The aminophosphinate scaffold has been used for the development of extended transition state analogues that show high hydrolytic stability.[103]

Aminomethane-*P*-methylphosphinic acid (**28**), which has versatile possibilities for modification, is a lead compound of high interest, despite showing only weak inhibitory activity (K_i = 340 μM against *Bacillus pasteurii* enzyme). Simple N-methylation of the parent structure **28** to compound **29** gives a 20-fold increase in the inhibitory activity (K_i = 18 μM, Figure 14). Interestingly, bis-N-methylation leads to further significant enhancement of the potency (K_i = 0.62 μM for structure **30**).[104] The differences in the free energies of binding ($\Delta\Delta G$) between compounds **28** and **29** and between **29** and **30**, as estimated from inhibitory constants, are similar (1.7 and 2.0 kcal/mol, respectively) and correspond to the energy of a single hydrogen bond. The total balance of the hydrogen bonds formed and lost by inhibitor between its solution and bound form is the most favorable for compound **30**, reflected by the highest K_i. Molecular modeling results that illustrate the mode of binding of **30** to bacterial urease (Figure 15) indicate that the phosphinic group is bound by two nickel ions and forms hydrogen bonds with His222 and Asp363, whereas the amine group forms a hydrogen bond with Ala366.

Later, dipeptides containing **28** were investigated, and the *N*-glycyl derivative **31** was found to be the most potent of the studied compounds (Figure 14).[103] A substantial improvement in the activity was achieved by modification of the phosphinic moiety with a sulfur atom (inhibitor **32**), which likely interacts strongly with the nickel ion present in the active site.

Scheme 5. Rationale the for Construction of Organophosphorus Inhibitors of Urease



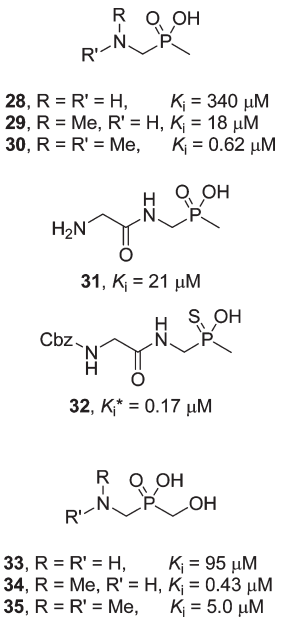


Figure 14. Selected structures of aminomethanephosphinic inhibitors of *B. pasteurii* urease.

Computer-aided, structure-based inhibitor design provides the possibility of further improving the characteristics of active compounds. The introduction of a hydroxyl group at the *P*-methyl position resulted in additional interactions with the enzyme.[105] Interestingly, the activity profiles of *P*-hydroxymethyl compounds **32**−**35** were different from those observed for *P*-methyl compounds **28**−**30** (Figure 14). In the former set of structures, *N*-methyl derivative **34** was the most active (K_i = 0.43 μM). This result was explained on the basis of molecular modeling that showed an intramolecular hydrogen bond between the amine and hydroxyl groups.

The phosphinates described above are also effective inhibitors of urease in intact cells of the human pathogen *Proteus mirabilis*.[104] Compound **29** gives IC_{50} = 36 μM, and its efficiency is similar to that of acetohydroxamic acid (IC_{50} = 64.4 μM). The latter inhibitor is the only urease inhibitor on the market but is unfortunately characterized by severe side effects such as teratogenicity.[106]

IRREVERSIBLE INHIBITORS

The irreversible inhibition of enzyme activity by organophosphorus compounds is not frequently found. The phosphorylation of acetylcholinesterase, the principal molecular mechanism of nerve agents, is the most infamous example.[107] The α-aminophosphonate scaffold has also been developed into a successful,

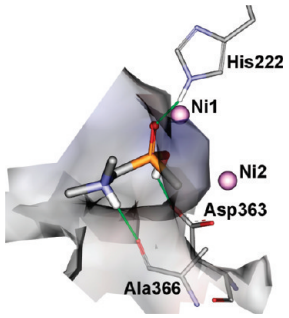


Figure 15. Modeled mode of binding of inhibitor **28** to *B. pasteurii* urease.

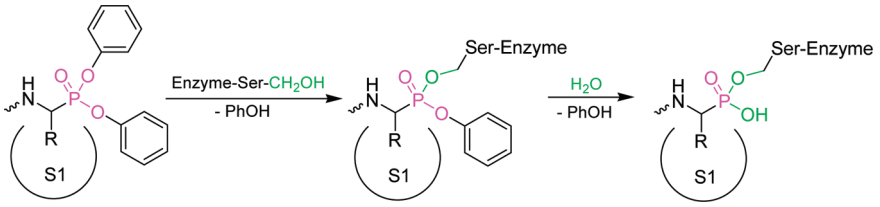
important application in this field. P-Terminal amino acids and peptide diaryl phosphonates are potent inhibitors of serine proteases. The biomedical potential of these peptidomimetics, together with the current state of the art, has been thoroughly summarized by the principal inventors in a series of updated reviews.[108−110] Therefore, only selected issues will be discussed here.

Diaryl phosphonates can be classified as competitive transition state analogue inhibitors. On a molecular level, they act as transesterification/phosphonylation agents directed specifically toward the catalytic serine residue (Ser195 in trypsine-like enzyme numbering, clan PA, previously SA). Their covalent binding blocks the catalytic triad Asp102-His57-Ser195 responsible for the formation of enzyme−substrate acyl intermediate and its further hydrolysis. Both steps of substrate conversion proceed through tetrahedral transition states. The structural and mechanistic aspects of serine protease inhibition by diphenyl phosphonates were the most thoroughly visible in X-ray crystallography studies of trypsin complexed with diphenyl *N*-benzyloxycarbonylamino(4-amidinophenyl)methanephosphonate.[111] According to the commonly accepted mechanism, this process involves substitution on the phosphorus atom (addition of the Ser195 hydroxyl accompanied by the release of one phenoxy group) (Scheme 6). In transition state models, both reactive oxygen atoms are favorably located in apical positions. The covalent reaction, which follows the initial reversible binding, can be easily detected by means of ^{31}P NMR.[112] The mixed ester then undergoes nucleophilic attack by a water molecule to eliminate the second phenoxy leaving group. The aging process is completed within a time scale of days to weeks. The final Ser-O-phosphonate monoester demonstrates the presence of the covalent enzyme−inhibitor complex (Figure 16A).[111] The P1 fragment forms specific interactions with the S1 binding pocket. The phosphonate oxygen atoms are arranged in a tetrahedral geometry, and one is favorably located in the oxyanion cavity formed by the amides of Gly193, Asp194, and Ser195. Interestingly, only the natural L enantiomer binds to the enzyme.

The mode of action of phosphonates toward serine proteases is not yet fully elucidated and proceeds with minor variations depending on the target and conditions. Both substitution steps must involve the formation of pentacoordinate transition states. However, their precise geometry is not clear because certain distortions from the bipyramidal arrangement must occur in the crowded environment of the active site. Pseudorotation is also allowed, as evidenced by application of structurally extended compounds (to interact with the S′ subsite), including diastereomeric monophenyl phosphonate[113] and phosphonamidate[114]

dx.doi.org/10.1021/jm200587f |J. Med. Chem. 2011, 54, 5955–5980

Scheme 6. Schematic Illustration of the Mechanism of Action of the Diphenyl α-Aminophosphonate Inhibitors of Serine Proteases



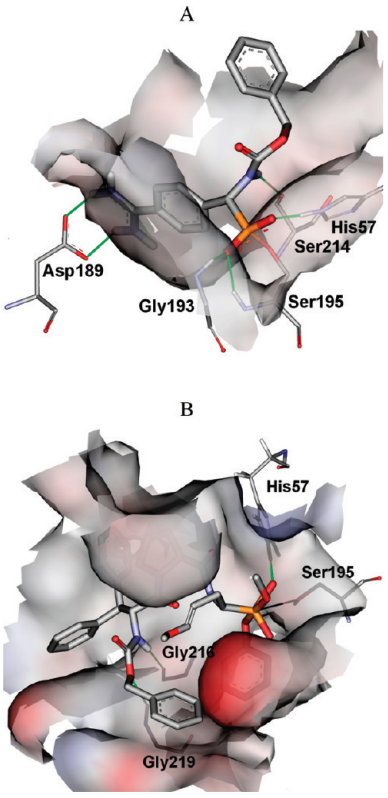


Figure 16. Structural features of serine proteases inhibition by diphenyl phosphonates visualized by X-ray crystallography: (A) trypsin-Ser-*O*-phosphonate monoester that represents a mature covalent modification of the enzyme using diphenyl *N*-benzyloxycarbonylamino(4-amidinophenyl)methanephosphonate (PDB entry 1MAX);[111] (B) a "trapped" bipyramidal pentacoordinate transition state resulting from the postulated nucleophilic attack of a water molecule on the phosphorus atom of the tripeptidic (*N*-Cbz-D-(β,β-diphenylalanyl)prolyl-(4-methoxypropyl)glycine)diphenyl phosphonate analogue and formation of the monoester prior to covalent bonding to human α-thrombin (PDB entry 1H8D), with one phenyl group disordered.[116]

pseudopeptides. The defined configuration of the phosphorus atom did not greatly affect the reactivity of these inhibitors. Additionally, the ligand−enzyme complexes of the mixed ester Boc-Ala-Ala-Pro-Val[P(O)(OPh)O]Ala-Ala-OMe diastereoisomers with α-lytic protease exhibit exactly the same final stereochemical arrangements (with overall retention or inversion of the configuration).[115]

Interestingly, nucleophilic attack of a water molecule (located in a deacylating H_2O position) and formation of the monoester prior to covalent bonding to the enzyme have also been postulated.[116] This type of distorted bipyramidal intermediate (Figure 16B) has been observed in the structure of human α-thrombin complexed with a tripeptide diphenyl phosphonate obtained from "immature" crystals (grown in less than 1 week at a decreased temperature). This assumption seems to correlate well with the results of the inhibition of β-lactamases by monophenyl phosphonates.[117,118] Although much less electrophilic than diesters, monoaryl phosphonates are capable of enzyme O-phosphonylation, likely via a dianionic transition state with the release of a phenol molecule. Apparently, it collapses to exactly the same definitive phosphonate−enzyme complex as in the general mechanism described above.

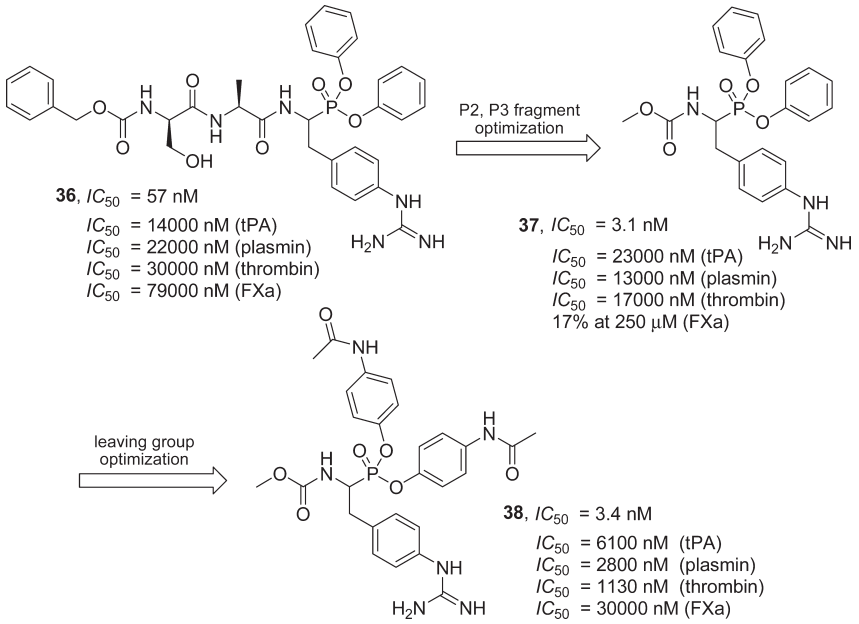
In parallel to fundamental studies on the application of diphenyl phosphonates to the inhibition of prototypical serine proteases such as trypsin, chymotrypsin, and thrombin, they have also been tested as potential molecular targets for therapeutic interventions. When considering the diverse biomedical potential of diaryl α-aminophosphonates, treatments of various pathologies have emerged as attractive opportunities: cancer and metastasis (e.g., by targeting urokinase, human neutrophil elastase, and seprase), inflammatory diseases (human neutrophil elastase, mast cell chymase and tryptase, granzymes, and cathepsin G), emphysema (granzymes and mast cell tryptase), hypertension (mast cell chymase), type 2 diabetes, immunological and fibrogenic disorders (proline-specific dipeptidyl peptidases), and infections (a range of viral and bacterial proteases). The lead compounds were generally inspired by the substrate preferences of the enzymes, but they were frequently optimized to provide more effective compounds. Below, the relevance of diaryl α-aminophosphonates in this context is illustrated on inhibition studies of two selected serine-dependent catalytic proteins: urokinase-type plasminogen activator and dipeptidyl peptidase IV.

Urokinase-type plasminogen activator (uPA, urokinase, clan PA, family S1, EC 3.4.21.73) is a key serine protease of the uPA system that is involved in the degradation of the basement membrane and the extracellular matrix. Upon binding to its cognate receptor, urokinase activates its primary physiological substrate, proenzyme plasminogen. The activation of plasmin triggers an endogenous proteolytic cascade that, under neoplastic conditions, facilitates tumor cell invasion and contributes to metastasis.[119,120] The uPa system markers indicate a strong negative diagnosis in a variety of tumor types. Currently, diverse pharmaceutical strategies are being investigated to inhibit the expression and/or activity of uPA.[121,122]

A series of tripeptidic diphenyl phosphonates based on the substrate-like peptide Z-D-Ser-Ala-Arg scaffold have given interesting results in SAR studies.[123] In particular, alkyl, cycloalkyl, and aryl side chains with terminal guanidyl groups have been explored as P1 substituents. Only structures of precisely defined

dx.doi.org/10.1021/jm200587f |*J. Med. Chem.* 2011, 54, 5955–5980

Scheme 7. Optimization of the Structure of Diaryl Phosphonate Inhibitors of Plasmin, Based on the Highly Selective *p*-Guanidinebenzyl P1 Substituent[123,124]



length are well accommodated in the S1 pocket. This structural restriction particularly concerns the arginine analogue, which inhibits uPA at IC_{50} = 61 nM. The removal of only a single methylene group from this inhibitor decreases its activity by nearly 2 orders of magnitude, whereas the homoarginine analogue gives a 4-fold loss of potency. The *p*-guanidinobenzyl group appears to be the most promising (IC_{50} = 57 nM, k_{app} = 6700 M^{-1} s^{-1}, **36**, Scheme 7), and the only positive, modification of the original arginine structure. This compound exhibits a selectivity factor greater than 240 toward tissue plasminogen activator, thrombin, plasmin, and factor Xa protease.[123] In continuation of these studies, the extensive P3−P2 fragment was optimized by installation of the relatively small *N*-methoxycarbonyl group.[124] This structural reduction increased the potency of **37** by 1 order in magnitude compared to **36** to achieve low nanomolar range activity (IC_{50} = 3.1 nM, k_{app} = 62 000 M^{-1} s^{-1}, Scheme 7). As the kinetic parameters obtained for referencing proteases varied within the same range as before, the selectivity index was further improved to achieve impressive values greater than 4300. Finally, replacement of the cytotoxic phenol leaving group by paracetamol retains the activity of compound **38** toward uPa (IC_{50} = 3.4 nM, Scheme 7) but diminishes the selectivity. When tested against a rat mammary carcinoma model (BN472 breast tumors), compounds **37** and **38** showed significant antimetastatic effects. Daily administration at 0.1 and 1 mg/kg resulted in a non-dose-dependent inhibition of tumor growth (10−18%) and a dose-dependent decrease in the number of lung foci (29−70%) and the weight of axillary lymph nodes (46−73%).[124] These preclinical data positively validate the potential utility of selective, irreversible diaryl phosphonate inhibitors of uPA in anticancer therapy.

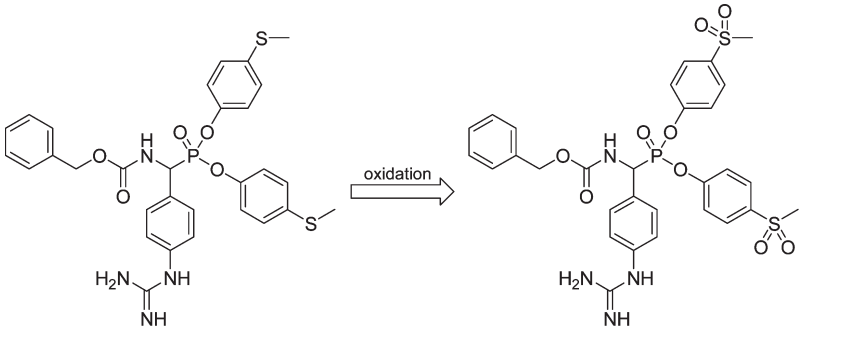
The influence of the structure of the released aryl group on the affinity of Cbz-protected *p*-guanidinophenylglycine phosphonates to urokinase and trypsin has been comprehensively studied by Sienczyk and Oleksyszyn.[125] The most significant inhibitory effect was achieved with *p*-*S*-methyl derivative **39** (Scheme 8). Its IC_{50} was calculated as 26 nM for plasmin and 8.7 nM for trypsin. Although this dual activity could be promising in the context of anticancer applications, selectivity toward plasmin versus trypsin could also be easily achieved. Simple sulfide oxidation to sulfone **40** (Scheme 8) retains the anti-plasmin properties (2-fold decrease) and nearly eliminates the anti-trypsin affinity (the activity decreased by a factor of 3000). The reactivity of the corresponding dinaphthyl phosphonate toward trypsin has been reported to be entirely suppressed.[125]

Dipeptidyl peptidase IV (DPP IV, EC 3.4.14.5, CD26) is a proline-specific member of clan SC of serine proteases, which contain catalytic residues in the order Ser, Asp, His. DPP IV exhibits numerous physiological functions in eukaryotes. The regulation of glucose homeostasis by the degradation of the incretin insulinotropic hormones glucagon-like peptide 1 and gastric inhibitory polypeptide is among its most important activities. DPP IV inhibition stimulates insulin secretion, which in turn reduces the blood glucose level.[126,127] Thus, inhibitors of dipeptidyl peptidase IV have emerged as an important novel class of therapeutic agents to treat type 2 diabetes.[128,129] Their pharmaceutical relevance is also correlated with only a very low risk of hypoglycemia development and no risk of weight gain. The membrane-bound peptidase CD26 plays a crucial role in the immune system by activating T-cells.[130,131]

Not surprisingly, diaryl phosphonate analogues of Xaa-proline dipeptides have been predominantly studied as potential inhibitors of dipeptidyl peptidase IV (for a selection of their structures and activities see compounds **41**−**48** in Figure 17).[132,133] According to the post proline dipeptidylpeptidase specificity, the corresponding P1 substituent must be rigorously conserved. Nevertheless, the six-member ring of homoproline appeared to be a privileged alternative to proline in this position (compare compounds **43** and **44** to **41** and **42**). The gain in activity was 1 order in magnitude in favor of a more extended cycle. The affinity of the compounds could then be further modified by the choice of an appropriate P2 residue and by adding appropriate groups to

dx.doi.org/10.1021/jm200587f |*J. Med. Chem.* 2011, 54, 5955–5980

Scheme 8. Influence of Oxidation of the Sulfide Group on Affinity of Diaryl *N*-Benzyloxycarbonylamino(*p*-guanidinebenzyl)-phosphonates toward Plasmin and Trypsin[125]



39, IC_{50} = 26 nM
IC_{50} = 8.7 nM (trypsin)

40, IC_{50} = 53 nM
IC_{50} = 23000 nM (trypsin)

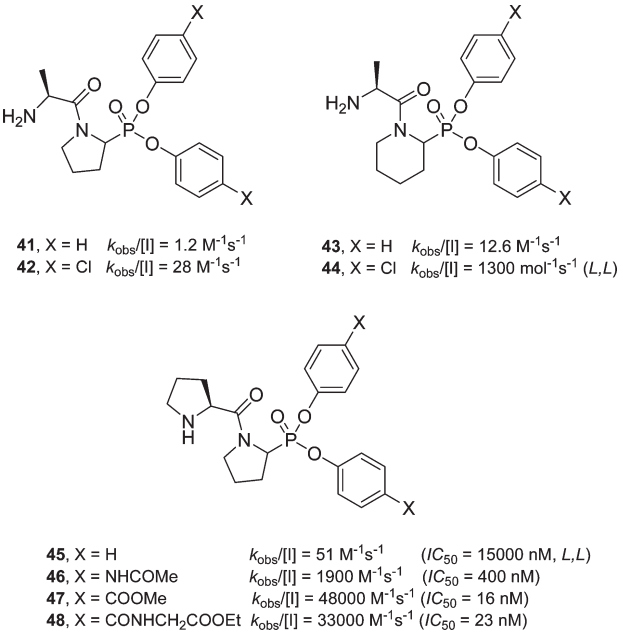
the phenyl ring in order to increase electrophilicity of the phosphorus atom. *p*-Chlorophenyl esters **42** and **44** are up to 2 orders of magnitude more potent than unsubstituted phenyl esters **41** and **43** (and demonstrate a noteworthy selectivity versus trypsin, elastases, acetylcholinesterase, papain, and cathepsin G).[132] This trend is even more pronounced for various Pro-Pro diaryl phosphonate analogues. Compounds **46**−**48**, bearing amide and carboxylate substituents in the para position of the leaving group, inhibit DPP IV with an IC_{50} in the nanomolar range, whereas unsubstituted diphenyl ester **45** is much less potent.[133]

A characteristic feature of diaryl phosphonate peptidomimetics is persistent irreversible inhibition, even with compounds of relatively low or moderate k_{on}. For example, after the application of compound **45** to dipeptidyl peptidase IV in vivo, only a 10% recovery of activity was observed after 4 weeks. In in vivo experiments, a single 1−10 mg dose of **45**, intravenously injected into rabbits, reduced 80% of the enzymatic activity in plasma. Three weeks were then required to recuperate the starting level of DPP IV digestion.

Selected structural elements can also influence the susceptibility of diaryl phosphonates to hydrolysis. Dipeptidic homoproline *p*-chlorophenyl esters **42** and **44** are characterized by remarkable stability, with half-lives exceeding 2 days in a buffer of pH 7.8. In contrast, electron-withdrawing groups, while increasing the affinity of compounds **46**, **47**, and **48**, greatly reduce their stability ($t_{1/2}$ of 320, 19, and 35 min, respectively). These electronic effects have been thoroughly discussed in review articles and the references cited therein.[110,134,135]

Despite its limited stability, significant in vivo effects were observed after DPP IV/CD26 targeted administration of phosphonate **46** after lung transplantation rats. The inhibitor (initial dose of 100 mg/kg, followed by 60 (mg/kg)/day until day 4 after surgery) eliminated acute pulmonary rejection by the suppression of DPP IV serum activity (>95% after initial injection and >90% until day 5).[136] Regulation of the enzymatic activity also preserved early graft functions and attenuated ischemia/reperfusion injury.[137] No systemic or local toxicity was observed.

Inhibition of other Xaa-Pro specific serine dipeptidylpeptidases related to DPP IV, such as DPP II, DPP8, DPP9, and fibroblast activation protease-α (FAPα)/seprase, has recently been studied by means of peptidic diaryl phosphonates.[135,138,139] Initial drug discovery programs view these enzymes as promising



41, X = H $k_{obs}/[I]$ = 1.2 $M^{-1}s^{-1}$
42, X = Cl $k_{obs}/[I]$ = 28 $M^{-1}s^{-1}$

43, X = H $k_{obs}/[I]$ = 12.6 $M^{-1}s^{-1}$
44, X = Cl $k_{obs}/[I]$ = 1300 $mol^{-1}s^{-1}$ (*L*,*L*)

45, X = H $k_{obs}/[I]$ = 51 $M^{-1}s^{-1}$ (IC_{50} = 15000 nM, *L*,*L*)
46, X = NHCOMe $k_{obs}/[I]$ = 1900 $M^{-1}s^{-1}$ (IC_{50} = 400 nM)
47, X = COOMe $k_{obs}/[I]$ = 48000 $M^{-1}s^{-1}$ (IC_{50} = 16 nM)
48, X = $CONHCH_2COOEt$ $k_{obs}/[I]$ = 33000 $M^{-1}s^{-1}$ (IC_{50} = 23 nM)

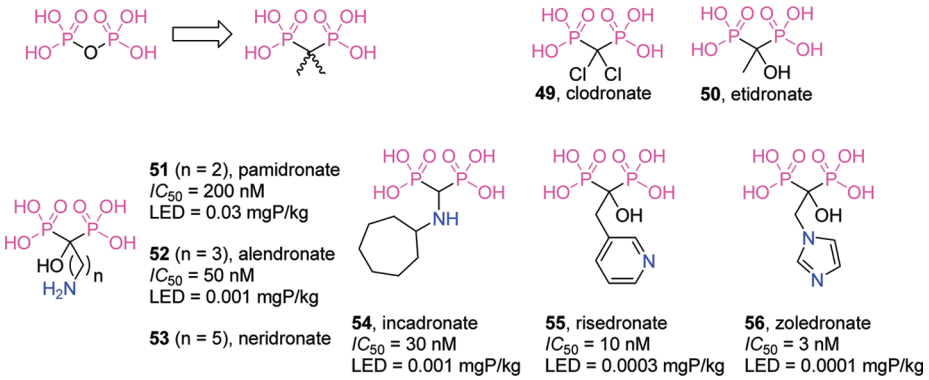
Figure 17. Structure and reactivity of diaryl phosphonate analogues of Xaa-Pro and Xaa-hPro dipeptides as inhibitors of dipeptidyl peptidase IV.[132,133]

targets for the treatment of cancer, fibrogenic diseases, inflammatory states, and hematopoietic disorders.[135,140]

In summary, the statement that diaryl phosphonates, which covalently bind to their receptors,[141] are excellent drug candidates seems to be justified. They possess moderate, well-balanced, and highly specific reactivity. They target serine protease exclusively, whereas other oxygen and sulfur nucleophiles, including cysteine proteases, remain intact. These properties can be relatively easily tuned by appropriate structure selection and optimization, considering both the peptide residues (particularly the critical P1 side chain) and the leaving groups. Additionally, they are easily synthesized. The starting amino acid analogues of the diaryl phosphonates are prepared in a convenient three-component condensation of an aldehyde, a benzyl carbamate, and a triaryl phosphite. After N-deprotection, the P2−P*n* fragment can be developed by standard peptide chemistry.

dx.doi.org/10.1021/jm200587f |*J. Med. Chem.* 2011, 54, 5955–5980

Scheme 9. Analogy between Pyrophosphate and Bisphosphonates, and Examples of Various Bisphosphonate Structures[a]



[a] IC_{50} values are given for studies with recombinant human FPPS, and lowest effective dose (LED) is for inhibition of bone resorption in rats in vivo.[155]

Finally, these phosphonate ester inhibitors exhibit promising pharmacokinetic properties. Although frequently developed based on a peptide structure, they demonstrate very good stability in buffer and plasma. Possessing an irreversible mode of action (except for the initial noncovalent binding step), they demand neither large nor frequent doses despite their modest inactivation rates. The diaryl phosphonate groups in these compounds act as prodrug-type functions, which involves protection of the molecule against unwanted proteolysis, shielding the hydrophilic character of the phosphonate (which can block passage through cell membranes) and finally revealing its specific reactivity in precisely defined target locations. The resulting low concentrations of the released phenol appear to be cytotoxically insignificant. However, the leaving group can also be modified into a nontoxic structure. Several diaryl phosphonate inhibitors of dipeptidyl peptidase IV are under investigation in clinical trials for the treatment of type 2 diabetes,[134] but more detailed pharmacokinetic data are not available yet.

BONE TARGETING AGENTS

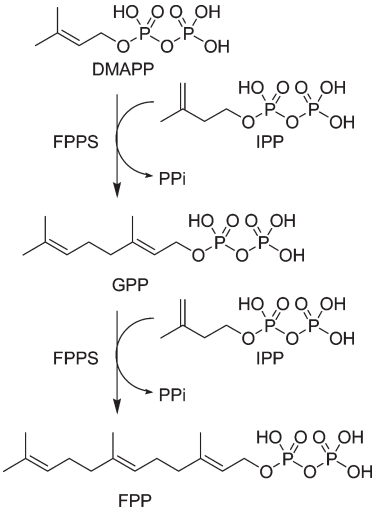
One of the most important features of the aminophosphonate moiety is its ability to bind metal ions.[142] Polyphosphonates containing α-aminomethylene fragments are now industrially used, in massive quantities, as antiscaling and anticorrosive agents. The chelation of metal cations is also crucial for the diverse biological activity of aminophosphonates, aminomethylenebisphosphonates, and related structures. Among these compounds, bisphosphonic acids are considered to be hydrolytically stable analogues of pyrophosphate in which a carbon−phosphorus bond replaces an oxygen−phosphorus bond (Scheme 9). It was only in the 1960s that their potential for the treatment of various bone diseases was realized after Fleisch and co-workers discovered that bisphosphonates hinder the formation and dissolution of calcium phosphate crystals in vitro.[143] Today, they have emerged as the primary standard of care for patients with osteoporosis or bone metastases from advanced solid tumors or from bone lesions caused by multiple myelomas.[144−146] Mainly, they act by inhibiting osteoclastic action on bone resorption after being absorbed by bone tissue and bound to hydroxyapatite crystals.[147] Moreover, aside from the direct action on bone function and bone cells, they can directly inhibit the proliferation of, and induce the death of, cancer cells themselves.[148,149] Thus, there is strong preclinical rationale and clinical evidence to support the hypothesis that modifying the bone microenvironment using bisphosphonates reduces the risk of developing bone metastases.

Many bisphosphonates have been marketed as antiosteoporotic drugs, such as clodronate (Bonefos, Loron, and Clodron, **49**), etidronate (Didronel, **50**), pamidronate (Aredia, **51**), alendronate (Fosamax, **52**), incadronate (Bisphonal, **54**), risedronate (Actonel, **55**), and zoledronate (Zometa, Zomera, Aclasta, and Reclast, **56**, Scheme 9). Importantly, structures containing a nitrogen atom (**51**−**56**) are much more potent. Among these compounds, incadronate (**54**) is the only structure containing the N−C−P scaffold, and it was introduced as a drug against malignant hypercalcaemia in Japan in 1997.[150,151] Incadronate is also active against cancer development in vitro.[152] Although bisphosphonates have been used extensively for the successful treatment of various bone conditions, they also show non-negligible side effects that could prevent their clinical use.[149,153] The most serious reported side effect is osteonecrosis of the jaw, which was observed in up to 10% of patients undergoing cancer IV therapy.[154] These drawbacks could be overcome by searching for new drugs lacking these side effects or by application of safe and efficient galenic formulations for these compounds.

Significant effort to elucidate the molecular mode of bisphosphonate action has led to their classification into two groups.[144,156] Non-nitrogen containing bisphosphonates (e.g., **49** and **50**, Scheme 9) are likely incorporated into nonhydrolyzable analogues of ATP, and the accumulation of its metabolites causes inhibition of osteoclast functions and induces their apoptosis.[157,158] The higher potency of nitrogen-containing bisphosphonates (N-BP, e.g., structures **51**−**56**, Scheme 9) is the result of farnesyl diphosphate synthase (FPPS, EC 2.5.1.10) inhibition.[159−161] This enzyme catalyzes the reaction of dimethylallyl diphosphate (DMAPP) and isopentenyl diphosphate (IPP) to form geranyl diphosphate (GPP, Scheme 10). The addition of a second molecule of IPP gives farnesyl diphosphate (FPP). These compounds (GPP and FPP) are then used for protein prenylation, which is one of the crucial methods for controlling cell survival and signaling pathways. The nanomolar level of FPPS inhibition evidenced by the N-BPs is well correlated with the in vivo inhibition of bone resorption (Scheme 9).[155,162]

Further studies have determined a structural basis for the inhibitory action of bisphosphonates.[163−166] As expected, the bisphosphonate group of zoledronate **56** is bound in a manner analogous to the diphosphate of natural substrates by interactions

Scheme 10. Reactions catalyzed by farnesyl diphosphate synthase (FPPS)



with three magnesium ions and positively charged residues Arg112, Lys257, and Lys200 (Figure 18). The positive charge of the bisphosphonate side chain, formed by protonation of the nitrogen atom, has been described as being analogous to the carbocation in the reaction transition state. Additionally, bisphosphonate is locked in the enzyme active site by flap closure and is IPP-bound at the active site entrance (Figure 18).[165] This atypical mode of binding causes exceptionally high efficacy in vivo, in contrast to the classical competitive inhibitors in which the accumulated level of the enzyme substrate reduces the inhibitor efficiency.

The inhibitory activity of aminomethylenebisphosphonates against FPPS has also led to their development as antiparasitic agents.[167−171] The growth of *Trypanosoma brucei*, *Trypanosoma cruzi*, *Leishmania donovani*, *Toxoplasma gondii*, *Entamoeba histolytica*, and *Plasmodium falciparum* was inhibited at low micromolar levels (e.g., compound **57**, Figure 19, exhibited IC_{50} = 0.7 μM against growth of *T. brucei*). Subsequently, large libraries of bisphosphonates were tested against parasitic FPPS, with many compounds giving inhibitory constants in the nanomolar range (e.g., **57** showed K_i = 11 nM against FPPS from *L. major*), and 3D QSAR models were constructed.[172]

Further studies have demonstrated that bisphosphonates are able to inhibit other parasitic enzymes: pyrophosphatase,[173] hexokinase,[174,175] 1-deoxyxylulose-5-phosphate reductoisomerase,[176] and geranylgeranyl diphosphate synthase (GGPPS).[177]

Anticancer treatment is another highly important application of the bisphosphonates.[178,179] Several clinical studies have been performed on the combination of known bisphosphonates (e.g., pamidronate **51** and zoledronate **56**, Scheme 9) with existing anticancer drugs (e.g., interleukin 2 and aromatase inhibitors) to find more effective therapeutic strategies.[180−182] These detailed studies indicate that the inhibition of FPPS and GGPPS is a crucial factor in their anticancer activities because the accumulation of IPP and DMAPP (substrates of the aforementioned enzymes) triggers activation of $\gamma\delta$-T-cells.[183] An extensive 3D-QSAR study on various bisphosphonates has also shown that the positive charge on the aromatic ring correlates well with improved inhibitory activity against FPPS.[184] Additionally, an increase in hydrophobicity should enhance cell and tissue penetration. Indeed, compound **58** (BPH-715, Figure 19), which

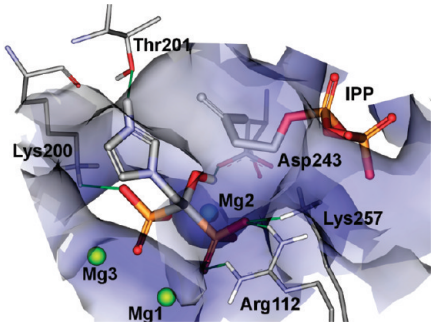


Figure 18. Crystal structure of the ternary complex of FPPS, zoledronate (**56**), and IPP (PDB entry 2F8Z).[165]

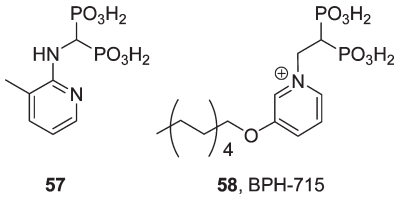


Figure 19. Bisphosphonic acid inhibitors of FPPS with potent antiparasitic (**57**) and anticancer (**58**) activities.

combines both of the required features, kills tumor cell lines (MDA-MB-231 invasion in a Matrigel system) with IC_{50} = 100 nM. This activity represents at least a 2 orders of magnitude greater potency than that found for one of the most effective bisphosphonates, zolendronate.[183,185]

In general, bisphosphonates are fairly potent therapeutic agents, but their shortcomings include their exceptionally low bioavailability and their intrinsic, dose-dependent toxicity. Pharmacokinetics of nitrogen containing bisphosphonates were extensively analyzed, but unfortunately, mostly short time studies (up to 1 month) were undertaken.[186,187] The bioavailability of N-BP is poor and variable, typically around 1%. It can be additionally reduced (approximately by a 10-fold) because of interaction with food; thus, for oral dosing it is necessary to consume pills on empty stomach at least 1 h before food intake. Bisphosphonate compounds are rapidly removed from circulation (within hours), and their skeleton uptake is dependent on the rate of bone turnover and renal function. In the case of patients with osteoporosis, approximately half of absorbed dose reaches the skeleton, while the rest is excreted unchanged with urine.

These limitations can be dealt with by the design and application of special forms of administration. Liposomes are good candidates for this purpose, as demonstrated by the inhibition of human cancer cell line (MDA-MB-231) migration and invasion using liposome-encapsulated neridronate (**53**, Scheme 9).[188] Another possibility is binding bisphosphonate to a biodegradable polymer, which has been reported in the case of poly(lactic-*co*-glycolide) and alendronate **52**.[189]

Bisphosphonates are specifically targeted to osseous tissue and exhibit significant binding capability to hydroxyapatite. This property has been used to target chemotherapeutic agents to bone after their conjugation to bisphosphonates through hydrolyzable linkages. This concept is called the osteoporotic drug delivery system. Recent successful examples of this strategy include targeting estrogenic estradiol and raloxifene (structures

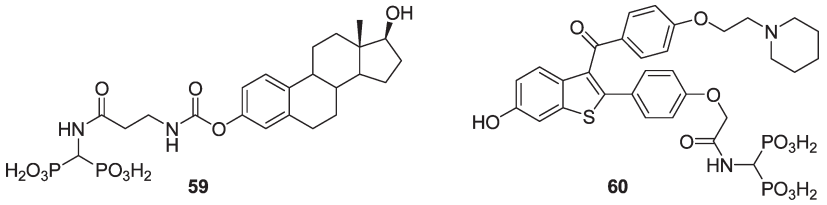


Figure 20. Osteoporotic delivery systems for estradiol (**59**) and raloxifene (**60**).

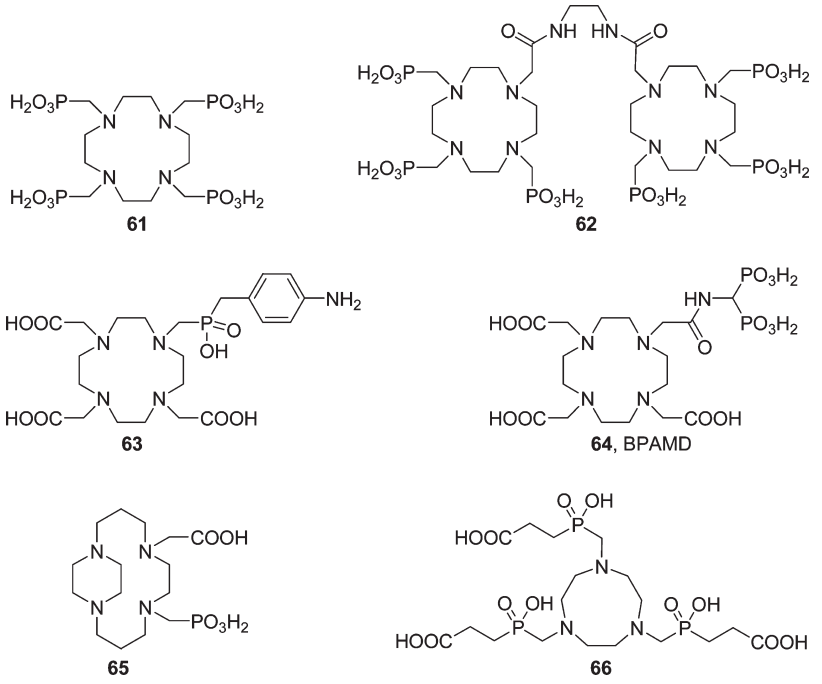


Figure 21. Structural variety of polyaminopolyphosphonate chelators of radionuclei.

59 and **60**, respectively, Figure 20),[190,191] the glycopeptide antibiotics vancomycin and oritavacin,[192] the tumor necrosis factor osteoprotegerin,[193] and the calcification-regulating protein fetuin.[194]

The increased prevalence of bone metastases arising from primary tumors, such as those of breast, lung, and prostate cancers, has made skeletal imaging and palliation a major priority in nuclear medicine, as bone metastases are the most common cause of potentially debilitating pain in patients with advanced cancers.[195] The mechanism by which most lanthanide complexes actively relieve this pain is not yet understood.[196]

Macrocyclic polyaminophosphonate ligands can firmly encapsulate trivalent lanthanide ions to form monomeric and water-soluble complexes. They are thermodynamically stable and kinetically inert and thus have been explored for medical applications as contrast agents in magnetic resonance imaging or as bifunctional chelating agents for molecular imaging and/or targeted radiotherapy. Chelators containing phosphonate side chains are particularly adept bone-seeking agents to be used as MRI contrast agents or for bone pain palliation and therapy of bone cancer. Thus, a variety of cyclic polyaminopolyphosphonates are currently being evaluated as potential radiopharmaceuticals in animal models. The most recent and successful examples of these ligands (**61**−**66**) are shown in Figure 21.[197−201] Nuclei such as ^{68}Ga, ^{90}Y, ^{99}Tc, ^{105}Rh, ^{111}In, ^{140}Ln, ^{153}Sm, ^{161}Tb, ^{166}Dy/^{166}Ho, ^{177}Lu, ^{188}Re, ^{212}Pb/^{212}Bi, ^{225}Ac, and ^{227}Th have been tested. Additionally, the first human trial has been performed using [^{68}Ga]BPAMD (ligand **64** shown in Figure 21). This facile, new bone-imaging positron emission tomography tracer has the advantages of very high target to soft tissue ratios and fast clearance.[202]

■ SUMMARY AND OUTLOOK

The α-aminophosphonate/phosphinate constitutes a unique motif for the construction of various classes of low molecular weight enzyme inhibitors. The fundamental structural and electronic properties of the N−P−C fragment are as follows: (a) acidic and basic functions arranged analogously to the natural amino acids and thus maintaining the capability of forming a specific network of hydrogen bonds, (b) negatively charged oxygen atoms (two or three) and/or other heteroatoms distributed tetrahedrally around the central phosphorus atom, and (c) good metal cation complexation properties. Various combinations of these features, together with additional active site directed substituents, provide the possibility of creating highly effective, selective, structurally diverse inhibitors targeted toward catalytic proteins of all systemic classes.

The characteristics of the targets affected by compounds containing the N−C−P scaffold (Table 1) clearly indicate that

Table 1. Medicinally Relevant Enzymes Systematically Classified and Listed with the Catalyzed Reaction and the Type of Inhibition by N−C−P Compounds[a]

enzyme/reaction	inhibitor type	reference
Oxidoreductases		
1-deoxyxylulose 5-phosphate reductoisomerase [1.1.1.267]	S/P	176
2-methyl-D-erythritol-4-phosphate + NADP → 1-deoxy-D-xylulose 5-phosphate + NADPH		
tyrosinase [1.14.18.1]	S/P	208
L-Tyr + O_2 → Dopa + O_2 → dopaquinone		
Transferases		
aspartate transcarbamoylase [2.1.3.2]	S/P	209
carbamoyl phosphate + L-Asp → carbamoyl-L-Asp + Pi		
ornithine transcarbamoylase [2.1.3.3]	ETS	210
carbamoyl phosphate + L-Orn → L-cytruline + Pi		
betaine−homocysteine *S*-methyltransferase [2.1.1.5]	ND	211, 212
betaine + L-hCys → dimethyl-Gly + L-Met		
farnesyl diphosphate synthase [2.5.1.10]	S/P	172
IPP + DMAPP → GPP + PPi		
GPP + IPP → FPP + PPi		
geranylgeranyl diphosphate synthase [2.5.1.29]	S/P	177
FPP + IPP → GGPP + PPi		
hexokinase [2.7.1.1]	S/P	174
D-hexose + ATP → D-hexose 6-phosphate + ADP		
Hydrolases		
esterases	ITS	213
RCOOR′ + H_2O → RCOOH + R′OH		
carboxylesterase [3.1.1.1]		
neuropathy target esterase [3.1.1.5]		
acetylcholineesterase [3.1.1.7]		
butyrylcholinesterase [3.1.1.8]		
***S*-adenosyl-L-homocysteine hydrolase [3.3.1.1]**	S/P	214, 215
S-adenosyl-L-hCys + H_2O → adenosine + L-hCys		
proteases		
R-CONH-R′ + H_2O → RCOOH + H_2NR′		
metalloproteases	S/P, TS	reviews: 5, 36, 39, 40, 42, 46, 72, 73, 75, 77
leucine aminopeptidase [3.4.11.1]		
aminopeptidase N [3.4.11.2]		
aminopeptidase A [3.4.11.7]		
renal dipeptidase [3.4.13.11]		
angiotensin I converting enzyme [3.4.15.1]		
carboxypeptidase A [3.4.17.1]		
glutamate carboxypeptidase II (NAALADase) [3.4.17.21]		
bacterial collagenase [3.4.24.3]		
enkephalinase, neprylisin [3.4.24.11]		
thimet oligopeptidase [3.4.24.15]		
endopeptidase 24.16 [3.4.24.16]		
astacin [3.4.24.21]		
thermolysin [3.4.24.27]		
endothelin converting enzyme 1 [3.4.24.71]		
interstitial collagenase (MMP-1) [3.4.24.7]		
gelatinase A (MMP-2) [3.4.24.24]		
stromelysin 1 (MMP-3) [3.4.24.17]		
matrilysin (MMP-7) [3.4.24.23]		
neutrophil collagenase (MMP-8) [3.4.24.34]		

Table 1. Continued

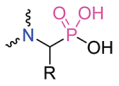
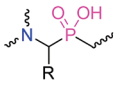
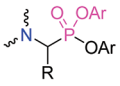
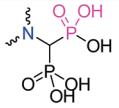
enzyme/reaction	inhibitor type	reference
gelatinase B (MMP-9) [3.4.24.35]		
stromelysin 3 (MMP-11) [3.4.24.B3]		
macrophage elastase (MMP-12) [3.4.24.65]		
collagenase 3 (MMP-13) [3.4.24.B4]		
membrane-type MMP-1 (MMP-14) [3.4.24.80]		
aspartic proteases	TS	reviews: 5, 37, 75
pepsin [3.4.23.1]		
renin [3.4.23.15]		
HIV-1 protease [3.4.23.16]		
penicillopepsin [3.4.23.20]		
β-secretase (BACE1) [3.4.23.46]		206, 207
serine proteases	ITS	
DPP II [3.4.14.2]		reviews: 134, 135
DPP IV [3.4.14.5]		
chymotrypsin [3.4.21.1]		reviews: 109, 110
trypsin [3.4.21.4]		
thrombin [3.4.21.5]		
cathepsin G [3.4.21.20]		
human neutrophil elastase [3.4.21.37]		
mast cell chymase [3.4.21.39]		
mast cell tryptase [3.4.21.59]		
urokinase-type plasminogen activator [3.4.21.73]		
granzyme A and B [3.4.21.78 and 3.4.21.79]		
seprase [3.4.21.B28]		
hepacivirin (HCV NS3 protease) [3.4.21.98]		216, 217
alkaline phosphatase [3.1.3.1]	ND	218
phosphate monoester + H_2O → alcohol + Pi		
urease [3.5.1.5]	ETS	103, 105
urea + H_2O → carbamate + NH_4^+		
***β*-lactamase [3.5.2.6]**	ITS	117, 118, 219
β-lactam + H_2O → β-amino acid		
pyrophosphatase [3.6.1.1]	S/P	173
PPi + H_2O → 2 Pi		
Lyases		
dialkylglycine decarboxylase [4.1.1.64]	S/P	220
dialkyl-Gly → dialkylketone + CO_2		
pyruvate → L-Ala		
KDOP synthase [4.1.2.16]	TS	221
D-arabinose 5-phosphate + phosphoenolpyruvate → 3-deoxy-D-*manno*-2-octulosonate 8-phosphate (KDOP)		
Isomerases		
alanine racemase [5.1.1.1]	S/P	15, 16
L-Ala → D-Ala		
Ligases		
alanine t-RNA ligase [6.1.1.7]	TS	222
ATP + L-Ala + t-RNAAla → ADP + L-Ala-t-RNAAla + Pi		
glutamine synthetase [6.3.1.2]	ETS	223
L-Glu + NH_4^+ + ATP → L-Gln + ADP + Pi		
glutathione synthetase [6.3.2.3]	TS	224
γ-L-Glu-L-Cys + Gly + ATP → γ-L-Glu-L-Cys-Gly + ADP + Pi		
D-Alanyl-D-alanine ligase [6.3.2.4]	TS	21, 22
ATP + 2 D-Ala → D-Ala-D-Ala + ADP + Pi		
glutathionylspermidine synthetase [6.3.1.8]	TS	225

Table 1. Continued

enzyme/reaction	inhibitor type	reference
GSH + SPD + ATP → GSP + ADP + Pi		
UDP-*N*-acetylmuramoyl-L-alanine-D-glutamate ligase, MurD [6.3.2.9]	TS	226, 227
UDPMurNAc-L-Ala + D-Glu + ATP → UDPMurNAc-L-Ala-γ-D-Glu + ADP + Pi		
D-Ala-D-Ala adding enzyme [6.3.2.15]	TS	228
D-Ala-D-Ala + UDPMurNAc-L-Ala-γ-D-Glu-*m*-DAP + ATP →		
UDPMurNAc-L-Ala-γ-D-Glu-*m*-DAP-D-Ala-D-Ala + ADP + Pi		

[a] Inhibitor types: S/P, substrate/product analogue; TS, transition state analogue; ETS, extended transition state analogue; ITS, irreversible transition state inhibitor; ND, not defined.

Table 2. General and Pharmacological Characteristics and the Representative Drug Examples of Phosphonate/Phosphinate Chemotypes

feature	chemotype			
				
synthetic availability	very good	complex	good	very good
activity and selectivity	moderate	high	high	high
reversibility	yes	yes	no	yes
chemical and metabolic stability	very good	very good	very good	very good
bioavailability	low	structure-dependent	good	very low
clearance	fast	structure-dependent	slow	very fast
therapeutic effect duration	short	short	long	long
side effects, toxicity	no	no	no	yes
representative example	alafosfalin (a prodrug of antibiotic *L*-AlaP)	fosinopril (antihypertensive drug)	prodipine (diabetes type 2, clinical trials)[134]	incadronate (antiosteoporotic drug)

this approach has been particularly successful for enzymes that catalyze hydrolysis (proteases, EC 3.4) or the formation of amide bonds (ligases, EC 6.3). Reversible transition state analogues of metallo-dependent proteases exhibit particularly strong potential due to the comprehensive employment of the many discussed features of the N−C−P structure, combined with systematic (rational computer-aided design and parallel or combinatorial synthesis) side chain optimization. The studies described in this review have yielded many compounds, mostly phosphinic pseudopeptides, for medicinal applications that target such important human diseases as cancer (development and metastasis), hypertension, and malaria. The other specific mechanism of efficient proteolysis suppression involves irreversible phosphonylation of serine proteases by diaryl aminophosphonates. These amino acids, or short peptide phosphonate esters, covalently bind to the serine residue in the active site and thus represent potential drug candidates for the treatment of cancer, type 2 diabetes (currently in advanced phases of clinical trials), hematopoetic disorders, and some viral infections. Several other specific enzymatic activities among hydrolases and ligases, responsible for various pathological states, have been recently targeted by regular or extended transition state analogue inhibitors. Potential applications of these methods could provide new therapies for tuberculosis (glutamine synthetase), bacterial and protozoan infections (urease, ligases), and HIV (HIV-1 protease).

Diphosphate- or triphosphate-dependent proteins represent another important group of enzymes inhibited by the title compounds. The key structural motif of such inhibitors is supplemented by an additional phosphorus-containing group to form a bisphosphonic moiety (the P−C−P analogue of pyrophosphate). This tetraanionic fragment is responsible for strong binding to bone hydroxyapatite, and the presence of the side chain nitrogen atom(s) is essential for the high affinity to appropriate osteoclastic transferases that down-regulate the resorption action. Consequently, nitrogen-containing bisphosphonates are clinically used for the treatment of osteoporosis and related bone diseases. Recently, new applications of N-BP to cancer and parasitic infections have also been described.

Given the current level of knowledge in this field, it is relatively easily to predict that several aminophosphonate inhibitors of novel, important targets remain to be developed. It seems increasingly apparent that any metallo-dependent or serine protease can be specifically affected by compounds designed on the basis of the N−C−P scaffold. For example, the members of ADAMs and ADAMTSs (disintegrins and metalloproteinases with thrombospondin motifs) families of proteases, which control several cell functions such as adhesion, fusion, migration and proliferation, have emerged as promising, novel targets for treating cancer and neurodegenerative diseases.[203−205] The latter pathologies can be also treated by the inhibition of β-secretase (BACE 1), an aspartyl protease involved in amyloid plaque formation, the regulation of which has also been recently investigated using N−P−C phosphinic psedopeptides.[206,207] Finally, aminomethylenebisphosphonates possess vast potential for structural explorations, not only in the context of antiosteoporotic applications but in the development of anti-ATP-dependent

enzyme inhibitors. However, their rapid introduction to the market seems to be limited by the existence of many bisphosphonate drugs already on the market.

The results of fundamental studies performed on α-aminophosphonates in vitro cannot generally be translated into a final drug structure, with perhaps only a single exception, the diaryl phosphonate esters. These compounds irreversibly inhibit serine proteases and typically show superior activity in vivo and pharmacokinetic properties (Table 2). Somewhat surprisingly in this context, no representatives of diaryl phosphonates have been introduced to the market. The other variants of the N−C−P compounds (containing an unprotected phosphonate/phosphinate) are charged, highly polar, and poorly bioavailable species. For example, only a small fraction of a bisphosphonate drugs reach the target bone tissue. This limitation is compensated by exceptionally tight binding to the target enzyme. However, more general methods can be used to mask the unwanted properties of such lead compounds. One option relies on structural refinements that retain a reasonable potency while increasing the hydrophobicity and/or protecting the functional group(s) to form a prodrug. An alternative method involves coupling the active molecules to a drug delivery system. Such opportunities further enhance the present attractiveness of α-aminophosphonates and open novel perspectives for the construction of bioactive compounds.

AUTHOR INFORMATION

Corresponding Author

*Phone: +48-71-320-4080. Fax: +48-71-320-2427. E-mail: lukasz.berlicki@pwr.wroc.pl.

BIOGRAPHIES

Artur Mucha is Associate Professor in Department of Bioorganic Chemistry, Wrocław University of Technology, Poland. He obtained his Ph.D. in Chemistry in 1994 with Prof. Roman Tyka on synthesis and transformations of α-aminophosphinic acids. During a 2-year postdoctoral fellowship in Département d'Ingénierie et d'Etudes des Protéines, CEA Saclay, France, with Dr Vincent Dive, he worked on phosphinic peptides as inhibitors of matrix metalloproteinases. His current scientific interests are focused on synthesis of organophosphorus compounds, mainly peptidomimetics, and their application in drug discovery and medicinal chemistry. The main field of his activity relates to regulation of the action of aminopeptidases as a potential anticancer and antimalarial targeted strategy.

Paweł Kafarski received his Ph.D. (1977, with Prof. Przemysław Mastalerz) from Wrocław University of Technology, Poland, and he rose through the ranks to Professor of Chemistry. Simultaneously, in 1983 he was employed at University of Opole, Poland. He currently holds the positions of Professor at both universities. His research focuses on chemistry and biology of aminophosphonic acids and their short peptides, namely, the rational design (based on reaction mechanisms and crystal structure of enzymes) and synthesis of biologically active substances coiled as potential drugs and herbicides and search for new, including stereospecific, methods of their synthesis. As a scientific hobby he also studies natural substances present in various branches of honeys and in hallucinogenic mushrooms.

Łukasz Berlicki received his Ph.D. in Chemistry from Wrocław University of Technology (Poland) in 2004 (on design and synthesis of glutamine synthetase inhibitors, with Prof. P. Kafarski). During a Marie Curie Fellowship at University of Regensburg (Germany), he worked on structural and biological aspects of peptides constrained with cyclopentane-containing amino acid residues. Now he works as Assistant Professor at Department of Bioorganic Chemistry, Wrocław University of Technology, Poland, on development of organophosphorus inhibitors of medicinally relevant enzymatic targets. In particular, he investigates aminophosphinic inhibitors of urease as possible agents against ureolytic bacteria.

ACKNOWLEDGMENT

Financial support by Ministry of Science and Higher Education (Grant No. 681/N-COST/2010/0) is gratefully acknowledged. The use of software resources (including the Accelrys programs) at the Supercomputing and Networking Center in Wrocław, Poland, is kindly acknowledged.

ABBREVIATIONS USED

ACE, angiotensin I converting enzyme; ADAMs and ADAMTSs, disintegrins and metalloproteinases with thrombospondin motifs; APN/CD13, M1 microsomal alanyl aminopeptidase; BACE 1, β-secretase; DMAPP, dimethylallyl diphosphate; DPP IV, dipeptidyl peptidase IV; ECE, endothelin-converting enzyme 1; FPP, farnesyl diphosphate; FPPS, farnesyl diphosphate synthase; GPP, geranyl diphosphate; IPP, isopentenyl diphosphate; LAP, M17 cytosolic leucine aminopeptidase; LED, lowest effective dose; MMPs, matrix metalloproteinases, matrixins; N-BP, nitrogen-containing bisphosphonates; PLP, pyridoxal 5′-phosphate; TS, transition state; uPA, urokinase-type plasminogen activator, urokinase

REFERENCES

(1) Hiroguchi, M.; Kandatsu, M. Isolation of 2-Aminoethanephosphonic Acid from Rumen Protozoa. *Nature* **1959**, *184*, 901–902.

(2) Mastalerz, P. Inhibition of Glutamine Synthetase by Phosphonic Analogues of Glutamic Acid. *Arch. Immunol. Ter. Dosw.* **1959**, *7*, 201–210.

(3) Kukhar, V. P.; Hudson, H. R. *Aminophosphonic and Aminophosphinic Acids: Chemistry and Biological Activity*; John Wiley & Sons: Chichester, U.K., 2000.

(4) Kafarski, P.; Lejczak, B. Biological Activity of Aminophosphonic Acids. *Phosphorus, Sulfur Silicon Relat. Elem.* **1991**, *63*, 193–215.

(5) Collinsová, M.; Jiráček, J. Phosphinic Acid Compounds in Biochemistry, Biology and Medicine. *Curr. Med. Chem.* **2000**, *7*, 629–647.

(6) Kafarski, P.; Lejczak, B. Aminophosphonic Acids of Potential Medical Importance. *Curr. Med. Chem.: Anti-Cancer Agents* **2001**, *1*, 301–312.

(7) Berlicki, Ł.; Kafarski, P. Computer-Aided Analysis and Design of Phosphonic and Phosphinic Enzyme Inhibitors as Potential Drugs and Agrochemicals. *Curr. Org. Chem.* **2005**, *9*, 1829–1850.

(8) Wardle, N. J.; Bligh, S. W. A.; Hudson, H. R. ω-Phosphinyl-α-amino Acids: Synthesis, Development towards Use as Therapeutic Agents. *Curr. Org. Chem.* **2007**, *11*, 1635–1651.

(9) Ecker, S. J.; Erion, M. D. Prodrugs of Phosphates and Phosphonates. *J. Med. Chem.* **2008**, *51*, 2328–2345.

(10) Lejczak, B.; Kafarski, P. Biological Activity of Aminophosphonic Acids and Their Short Peptides. *Top. Heterocycl. Chem.* **2009**, *20*, 31–63.

(11) Orsini, F.; Sello, G.; Sisti, M. Aminophosphonic Acids and Derivatives. Synthesis and Biological Applications. *Curr. Med. Chem.* **2010**, *17*, 264–289.

(12) Azema, L.; Baron, R.; Ladame, S. Targeting Enzymes with Phosphonate-Based Inhibitors: Mimics of Tetrahedral Transition States and Stable Isosteric Analogues of Phosphates. *Curr. Enzyme Inhib.* **2006**, *2*, 61–72.

(13) Macchiarulo, A.; Pellicciari, R. Exploring the Other Side of Biologically Relevant Chemical Space: Insights into Carboxylic, Sulfonic and Phosphonic acid Bioisosteric Relationships. *J. Mol. Graphics Modell.* **2007**, *26*, 728–739.

(14) Sun, L.; Chiu, D.; Kowal, D.; Simon, R.; Smeyne, M.; Zukin, R. S.; Olney, J.; Baudy, R.; Lin, S. Characterization of Two Novel *N*-Methyl-D-aspartate Antagonists: EAA-090 (2-[8,9-Dioxo-2,6-diazabicyclo[5.2.0]non-1(7)-en2-yl]ethylphosphonic Acid) and EAB-318 (*R*-α-Amino-5-chloro-1-(phosphonomethyl)-1*H*-benzimidazole-2-propanoic Acid Hydrochloride). *J. Pharmacol. Exp. Ther.* **2004**, *310*, 563–570.

(15) Badet, B.; Inagaki, K.; Soda, K.; Walsh, C. T. Time-Dependent Inhibition of *Bacillus stearothermophilus* Alanine Racemase by (1-Aminoethyl)phosphonate Isomers by Isomerization to Noncovalent Slowly Dissociating Enzyme-(1-aminoethyl)phosphonate Complexes. *Biochemistry* **1986**, *25*, 3275–3282.

(16) Stamper, C. G. F.; Morollo, A. A.; Ringe, D. Reaction of Alanine Racemase with 1-Aminoethylphosphonic Acid Forms a Stable External Aldimine. *Biochemistry* **1998**, *37*, 10438–10445.

(17) Shaw, J. P.; Petsko, G. A.; Ringe, D. Determination of the Structure of Alanine Racemase from *Bacillus stearothermophilus* at 1.9 Å. *Biochemistry* **1997**, *36*, 1329–1342.

(18) Au, K.; Ren, J.; Walter, T. S.; Harlos, K.; Nettleship, J. E.; Owens, R. J.; Stuart, D. I.; Esnouf, R. M. Structures of an Alanine Racemase from *Bacillus anthracis* (BA0252) in the Presence and Absence of (*R*)-1-Aminoethylphosphonic acid (L-Ala-P). *Acta Crystallogr., Sect. F: Struct. Biol. Cryst. Commun.* **2008**, *64*, 327–333.

(19) Watanabe, A.; Kurosawa, Y.; Yoshimura, T.; Kurihata, T.; Soda, K.; Esaki, N. Role of Lysine 39 of Alanine Racemase from *Bacillus stearothermophilus* That Binds Pyridoxal 5′-Phosphate, Chemical Rescue Studies of Lys39-Ala Mutant. *J. Biol. Chem.* **1999**, *274*, 4189–4194.

(20) Barreteau, H.; Kovač, A.; Boniface, A.; Sova, M.; Gobec, S.; Blanot, D. Cytoplasmic Steps of Peptidoglycan Biosynthesis. *FEMS Microbiol. Rev.* **2008**, *32*, 168–207.

(21) Tytgat, I.; Colacino, E.; Tulkens, P. M.; Poupaert, J. H.; Prévost, M.; Van Bambeke, F. DD-Ligases as a Potential Target for Antibiotics: Past, Present and Future. *Curr. Med. Chem.* **2009**, *16*, 2566–2580.

(22) Ellsworth, B. A.; Tom, N. J.; Bartlett, P. A. Synthesis and Evaluation of Inhibitors of Bacterial D-Alanine:D-alanine Ligases. *Chem. Biol.* **1996**, *3*, 37–44.

(23) Fan, C.; Park, I. S.; Walsh, C. T.; Knox, J. R. D-Alanine:D-alanine Ligase: Phosphonate and Phosphinate Intermediates with Wild Type and the Y216F Mutant. *Biochemistry* **1997**, *36*, 2531–2538.

(24) Wu, D.; Zhang, L.; Kong, Y.; Du, J.; Chen, S.; Chen, J.; Ding, J.; Jiang, H.; Shen, X. Enzymatic Characterization and Crystal Structure Analysis of the D-Alanine-D-alanine Ligase from *Helicobacter pylori*. *Proteins* **2008**, *72*, 1148–1160.

(25) Neuhaus, F. C. Role of Arg301 in Substrate Orientation and Catalysis in Subsite 2 of D-Alanine:D-alanine (D-Lactate) Ligase from *Leuconostoc mesenteroides*: A Molecular Docking Study. *J. Mol. Graphics Modell.* **2010**, *28*, 728–734.

(26) Kuzin, A. P.; Sun, T.; Jorczak-Baillass, J.; Healy, V. L.; Walsh, C. T.; Knox, J. R. Enzymes of Vancomycin Resistance: The Structure of D-Alanine−D-Lactate Ligase of Naturally Resistant *Leuconostoc mesenteroides*. *Structure* **2000**, *8*, 463–470.

(27) Seto, H.; Kuzuyama, T. Bioactive Natural Products with Carbon−Phosphorus Bond and Their Biosynthesis. *Nat. Prod. Rep.* **1999**, *16*, 589–596.

(28) Akif, M.; Ntai, I.; Sturrock, D.; Isaac, R. E.; Bachmann, B. O.; Acharya, K. R. Crystal Structure of a Phosphonotripeptide K-26 in Complex with Angiotensin Converting Enzyme Homologue (AnCE) from *Drosophila melanogaster*. *Biochem. Biophys. Res. Commun.* **2010**, *398*, 532–536.

(29) Ntai, I.; Bachmann, B. Identification of ACE Pharmacophore in the Phosphonopeptide Metabolite K-26. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3068–3071.

(30) Johnson, R. D.; Gordee, R. S.; Kastner, R. M.; Larsen, S. H.; Ose, E. E. Antibiotic A53868 and Process for Production Thereof. U.K. Patent 2,127,413, 1984; Eli Lilly. *Chem. Abstr.* **1984**, *101*, 88837.

(31) Whitteck, J. T.; Ni, W.; Griffin, B. M.; Eliot, A. C.; Thomas, P. M.; Kelleher, N. L.; Metcalf, W. W.; van der Donk, W. A. Reassignment of the Structure of the Antibiotic A53868 Reveals an Unusual Amino Dehydrophosphonic Acid. *Angew. Chem., Int. Ed.* **2007**, *46*, 9089–9909.

(32) Kuemin, M.; van der Donk, W. A. Structure−Activity Relationships of the Phosphonate Antibiotic Dehydrophos. *Chem. Commun.* **2010**, *46*, 7694–7696.

(33) Allen, J. G.; Havas, L.; Leicht, E.; Lenox-Smith, I.; Nisbet, L. J. Phosphonopeptides as Antibacterial Agents: Metabolism and Pharmacokinetics of Alafosfalin in Animals and Humans. *Antimicrob. Agents Chemother.* **1979**, *16*, 306–313.

(34) Pauling, L. Chemical Achievement and Hope for the Future. *Am. Sci.* **1948**, *36*, 51–58.

(35) Wolfenden, R. Transition State Analogues for Enzyme Catalysis. *Nature* **1969**, *223*, 704–705.

(36) Kafarski, P.; Lejczak, B. The Biological Activity of Phosphono- and Phosphinopeptides. In *Aminophosphonic and Aminophosphinic Acids. Chemistry and Biological Activity*; Kukhar, V. P., Hudson, H. R., Eds.; John Wiley & Sons: Chichester, U.K., 2000; pp 407−442.

(37) Peyman, A. Aminophosphonic and Aminophosphinic Acids in the Design and Synthesis of HIV Protease Inhibitors. In *Aminophosphonic and Aminophosphinic Acids. Chemistry and Biological Activity*; Kukhar, V. P., Hudson, H. R., Eds.; John Wiley & Sons: Chichester, U.K., 2000; pp 559−577.

(38) Holden, H. M.; Tronrud, D. E.; Monzingo, A. F.; Weaver, L. H.; Matthews, B. W. Slow- and Fast-Binding Inhibitors of Thermolysin Display Different Modes of Binding: Crystallographic Analysis of Extended Phosphonamidate Transition-State Analogs. *Biochemistry* **1987**, *26*, 8542–8553.

(39) Matthews, B. W. Structural Basis of the Action of Thermolysin and Related Zinc Peptidases. *Acc. Chem. Res.* **1988**, *21*, 333–340.

(40) Mucha, A.; Drąg, M.; Dalton, J. P.; Kafarski, P. Metalloaminopeptidase Inhibitors. *Biochimie* **2010**, *92*, 1509–1529.

(41) Matsui, M.; Fowler, J. H.; Walling, L. L. Leucine Aminopeptidases: Diversity in Structure and Function. *Biol. Chem.* **2006**, *387*, 1535–1544.

(42) Grembecka, J.; Kafarski, P. Leucine Aminopeptidase as a Target for Inhibitor Design. *Mini-Rev. Med. Chem.* **2001**, *1*, 133–144.

(43) Luan, Y.; Xu, W. The Structure and Main Functions of Aminopeptidase N. *Curr. Med. Chem.* **2007**, *14*, 639–647.

(44) Zhang, X.; Xu, W. Aminopeptidase N (APN/CD13) as a Target for Anti-Cancer Agent Design. *Curr. Med. Chem.* **2008**, *15*, 2850–2865.

(45) Stack, C. M.; Lowther, J.; Cunningham, E.; Donnelly, S.; Gardiner, D. L.; Trenholme, K. R.; Skinner-Adams, T. S.; Teuscher, F.; Grembecka, J.; Mucha, A.; Kafarski, P.; Lua, L.; Bell, A.; Dalton, J. P. Characterization of the *Plasmodium falciparum* M17 Leucyl Aminopeptidase. A Protease Involved in Amino Acid Regulation with Potential for Antimalarial Drug Development. *J. Biol. Chem.* **2007**, *282*, 2069–2080.

(46) Skinner-Adams, T. S.; Stack, C. M.; Trenholme, K. R.; Brown, C. L.; Grembecka, J.; Lowther, J.; Mucha, A.; Drag, M.; Kafarski, P.; McGowan, S.; Whisstock, J. C.; Gardiner, D. L.; Dalton, J. P. *Plasmodium falciparum* Neutral Aminopeptidases: New Targets for Anti-Malarials. *Trends Biochem. Sci.* **2010**, *35*, 53–61.

(47) Giannousis, P. P.; Bartlett, P. A. Phosphorus Amino Acid Analogues as Inhibitors of Leucine Aminopeptidase. *J. Med. Chem.* **1987**, *30*, 1603–1609.

(48) Lejczak, B.; Kafarski, P.; Zygmunt, J. Inhibition of Aminopeptidases by Aminophosphonates. *Biochemistry* **1989**, *28*, 3549–3555.

(49) Drąg, M.; Grembecka, J.; Pawełczak, M.; Kafarski, P. alpha-Aminoalkylphosphonates as a Tool in Experimental Optimization of P1

Side Chain Shape of Potential Inhibitors in S1 Pocket of Leucine and Neutral Aminopeptidases. *Eur. J. Med. Chem.* **2005**, *40*, 764–771.

(50) Pícha, J.; Liboska, R.; Buddšínský, M.; Jiráček, J.; Pawełczak, M.; Mucha, A. Unusual Activity Pattern of Leucine Aminopeptidase Inhibitors Based on Phosphorus Containing Derivatives of Methionine and Norleucine. *J. Enzyme Inhib. Med. Chem.* **2011**, *26*, 155–161.

(51) Drąg, M.; Bogyo, M.; Ellman, J. A.; Salvesen, G. S. Aminopeptidase Fingerprints. An Integrated Approach for Identification of Good Substrates and Optimal Inhibitors. *J. Biol. Chem.* **2010**, *285*, 3310–3318.

(52) Strater, N.; Lipscomb, W. N. Transition State Analogue L-Leucinephosphonic Acid Bound to Bovine Lens Leucine Aminopeptidase: X-ray Structure at 1.65 Å Resolution in a New Crystal Form. *Biochemistry* **1995**, *34*, 9200–9210.

(53) Grembecka, J.; Mucha, A.; Cierpicki, T.; Kafarski, P. The Most Potent Organophosphorus Inhibitors of Leucine Aminopeptidase. Structure-based Design, Chemistry, and Activity. *J. Med. Chem.* **2003**, *46*, 2641–2655.

(54) Morgan, B.; Scholtz, J. M.; Ballinger, M. D.; Zipkin, I. D.; Bartlett, P. A. Differential Binding Energy: A Detailed Evaluation of the Influence of Hydrogen-Bonding and Hydrophobic Groups on the Inhibition of Thermolysin by Phosphorus-Containing Inhibitors. *J. Am. Chem. Soc.* **1991**, *113*, 297–307.

(55) Mucha, A.; Grembecka, J.; Cierpicki, T.; Kafarski, P. Hydrolysis of the Phosphonamidate Bond in Phosphono Dipeptide Analogues: The Influence of the Nature of the N-Terminal Functional Group. *Eur. J. Org. Chem.* **2003**, 4797–4803.

(56) Mucha, A.; Laemmerhofer, M.; Lindner, W.; Pawełczak, M.; Kafarski, P. Individual Stereoisomers of Phosphinic Dipeptide Inhibitor of Leucine Aminopeptidase. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1550–1554.

(57) Skinner-Adams, T. S.; Lowther, J.; Teuscher, F.; Stack, C. M.; Grembecka, J.; Mucha, A.; Kafarski, P.; Trenholme, K. R.; Dalton, J. P.; Gardiner, D. L. Identification of Phosphinate Dipeptide Analog Inhibitors Directed against the *Plasmodium falciparum* M17 Leucine Aminopeptidase as Lead Antimalarial Compounds. *J. Med. Chem.* **2007**, *50*, 6024–6031.

(58) McGowan, S.; Porter, C. J.; Lowther., J.; Stack, C. M.; Golding, S. J.; Skinner-Adams, T. S.; Trenholme, K. R.; Teuscher, F.; Donnelly, S. M.; Grembecka, J.; Mucha, A.; Kafarski, P.; Degori, R.; Buckle, A. M.; Gardiner, D. L.; Whisstock, J. C.; Dalton, J. P. Structural Basis for the Inhibition of the Essential *Plasmodium falciparum* M1 Neutral Aminopeptidase. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 2537–2542.

(59) McGowan, S.; Oellig, C. A.; Birru, W. A.; Caradoc-Davies, T. T.; Stack, C. M.; Lowther, J.; Skinner-Adams, T.; Mucha, A.; Kafarski, P.; Grembecka, J.; Trenholme, K. R.; Buckle, A. M.; Gardiner, D. L.; Dalton, J. P.; Whisstock, J. C. Structure of the *Plasmodium falciparum* M17 Aminopeptidase and Significance for the Design of Drugs Targeting the Neutral Exopeptidases. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 2449–2454.

(60) Chen, H.; Noble, F.; Mothé, A.; Meudal, H.; Coric, P.; Danascimento, S.; Roques, B. P.; George, P.; Fournié-Zaluski, M.-C. Phosphinic Derivatives as New Dual Enkephalin-Degrading Enzyme Inhibitors: Synthesis, Biological Properties, and Antinociceptive Activities. *J. Med. Chem.* **2000**, *43*, 1398–1408.

(61) Fournié-Zaluski, M.-C.; Poras, H.; Roques, B. P.; Nakajima, Y.; Ito, K.; Yoshimoto, T. Structure of Aminopeptidase N from *Escherichia coli* Complexed with the Transition-State Analogue Aminophosphinic Inhibitor PL250. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2009**, *65*, 814–822.

(62) Drąg, M.; Grzywa, R.; Oleksyszyn, J. Novel Hydroxamic Acid Related Phosphinates: Inhibitor of Neutral Aminopeptidase N (APN). *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1516–1519.

(63) Grzywa, R.; Oleksyszyn, J. First Synthesis of α-Aminoalkyl-(N-substituted)-thiocarbamoylphosphinates. Inhibitors of Aminopeptidase N (APN/CD13) with the new Zinc-Binding Group. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3734–3736.

(64) Grzywa, R.; Oleksyszyn, J.; Salvesen, G. S.; Drąg, M. Identification of Very Potent Inhibitor of Human Aminopeptidase N (CD13). *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2497–2499.

(65) Zitka, O.; Kukacka, J.; Krizkov, S.; Huska, D.; Adam, V.; Masarik, M.; Prusa, R.; Kizek, R. Matrix Metalloproteinases. *Curr. Med. Chem.* **2010**, *17*, 3751–3768.

(66) Kessenbrock, K.; Plaks, V.; Werb, Z. Matrix Metalloproteinases: Regulators of the Tumor Microenvironment. *Cell* **2010**, *141*, 52–67.

(67) Li, N.-G.; Shi, Z.-H.; Tang, Y.-P.; Duan, J.-A. Selective Matrix Metalloproteinase Inhibitors for Cancer. *Curr. Med. Chem.* **2009**, *16*, 3805–3827.

(68) Konstantinopoulos, P. A.; Karamouzis, M. V.; Papatsoris, A. G.; Papavassiliou, A. G. Matrix Metalloproteinase Inhibitors As Anticancer Agents. *Int. J. Biochem. Cell Biol.* **2008**, *40*, 1156–1168.

(69) Coussens, L. M.; Fingleton, B.; Matrisian, L. M. Matrix Metalloproteinases and Cancer: Trials and Tribulations. *Science* **2002**, *295*, 2387–2392.

(70) Devel, L.; Czarny, B.; Beau, F.; Georgiadis, D.; Stura, E.; Dive, V. Third Generation of Matrix Metalloprotease Inhibitors: Gain in Selectivity by Targeting the Depth of the S1′ Cavity. *Biochimie* **2010**, *92*, 1501–1508.

(71) Nuti, E.; Tuccinardi, T.; Rossello, A. Matrix Metalloproteinase Inhibitors: New Challenges in the Era of Post Broad-Spectrum Inhibitors. *Curr. Pharm. Des.* **2007**, *13*, 2087–2100.

(72) Veerendhar, A.; Reich, R.; Breuer, E. Phosphorus Based Inhibitors of Matrix Metalloproteinases. *C. R. Chim.* **2010**, *13*, 1191–1202.

(73) Dive, V.; Georgiadis, D.; Matziari, M.; Makaritis, A.; Beau, F.; Cuniasse, P.; Yiotakis, A. Phosphinic Peptides As Zinc Metalloproteinase Inhibitors. *Cell. Mol. Life Sci.* **2004**, *61*, 2010–2019.

(74) Yiotakis, A.; Vassiliou, S.; Jiráček, J.; Dive, V. Protection of the Hydroxyphosphinyl Function of Phosphinic Dipeptides by Adamantyl. Application to the Solid-Phase Synthesis of Phosphinic Peptides. *J. Org. Chem.* **1996**, *61*, 6601–6605.

(75) Yiotakis, A.; Georgiadis, D.; Matziari, M.; Makaritis, A.; Dive, V. Phosphinic Peptides: Synthetic Approaches and Biochemical Evaluation as Zn-Metalloprotease Inhibitors. *Curr. Org. Chem.* **2004**, *8*, 1135–1158.

(76) Vassiliou, S.; Mucha, A.; Cuniasse, P.; Georgiadis, D.; Lucet-Levannier, K.; Beau, F.; Kannan, R.; Murphy, G.; Knäuper, V.; Rio, M.-C.; Basset, P.; Yiotakis, A.; Dive, V. Phosphinic Pseudo-Tripeptides as Potent Inhibitors of Matrix Metalloproteinases: A Structure−Activity Study. *J. Med. Chem.* **1999**, *42*, 2610–2620.

(77) Matziari, M.; Dive, V.; Yiotakis, A. Matrix Metalloproteinase 11 (MMP-11; Stromelysin-3) and Synthetic Inhibitors. *Med. Res. Rev.* **2007**, *27*, 528–552.

(78) Cuniasse, P.; Devel, L.; Makaritis, A.; Beau, F.; Georgiadis, D.; Matziari, M.; Yiotakis, A.; Dive, V. Future Challenges Facing the Development of Specific Active-Site-Directed Synthetic Inhibitors of MMPs. *Biochimie* **2005**, *87*, 393–402.

(79) Matziari, M.; Beau, F.; Cuniasse, P.; Dive, V.; Yiotakis, A. Evaluation of P-1′-Diversified Phosphinic Peptides Leads to the Development of Highly Selective Inhibitors of MMP-11. *J. Med. Chem.* **2004**, *47*, 325–336.

(80) Matziari, M.; Georgiadis, D.; Dive, V.; Yiotakis, A. Convenient Synthesis and Diversification of Dehydroalaninyl Phosphinic Peptide Analogues. *Org. Lett.* **2001**, *3*, 659–662.

(81) Dive, V.; Andarawewa, K. L.; Boulay, A.; Matziari, M.; Beau, F.; Guerin, E.; Rousseau, B.; Yiotakis, A.; Rio, M.-C. Dosing and Scheduling Influence the Antitumor Efficacy of a Phosphinic Peptide Inhibitor of Matrix Metalloproteinases. *Int. J. Cancer* **2005**, *113*, 775–781.

(82) Gall, A. L.; Ruff, M.; Kannan, R.; Cuniasse, P.; Yiotakis, A.; Dive, V.; Rio, M. C.; Basset, P.; Moras, D. Crystal Structure of the Stromelysin-3 (MMP-11) Catalytic Domain Complexed with a Phosphinic Inhibitor Mimicking the Transition-State. *J. Mol. Biol.* **2001**, *307*, 577–586.

(83) Bernstein, K. E.; Shen, X. Z.; Gonzalez-Villalobos, R. A.; Billet, S.; Okwan-Duodu, D.; Ong, F. S.; Fuchs, S. Different in Vivo Functions of the Two Catalytic Domains of Angiotensin-Converting Enzyme (ACE). *Curr. Opin. Pharmacol.* **2011**, *11*, 105–111.

(84) Dive, V.; Cotton, J.; Yiotakis, A.; Michaud, A.; Vassiliou, S.; Jiráček, J.; Vazeux, G.; Chauvet, M.-T.; Cuniasse, P.; Corvol, P. RXP 407, a Phosphinic Peptide, Is a Potent Inhibitor of Angiotensin I Converting Enzyme Able To Differentiate between Its Two Active Sites. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 4330–4335.

(85) Junot, C.; Gonzales, M.-F.; Ezan, E.; Cotton, J.; Vazeux, G.; Michaud, A.; Azizi, M.; Vassiliou, S.; Yiotakis, A.; Corvol, P.; Dive, V. RXP 407, a Selective Inhibitor of the N-Domain of Angiotensin I-Converting Enzyme, Blocks in Vivo the Degradation of Hemoregulatory Peptide Acetyl-Ser-Asp-Lys-Pro with No Effect on Angiotensin I Hydrolysis. *J. Pharmacol. Exp. Ther.* **2001**, *297*, 606–611.

(86) Georgiadis, D.; Beau, F.; Czarny, B.; Cotton, J.; Yiotakis, A.; Dive, V. Roles of the Two Active Sites of Somatic Angiotensin-Converting Enzyme in the Cleavage of Angiotensin I and Bradykinin Insights from Selective Inhibitors. *Circ. Res.* **2003**, *93*, 148–154.

(87) Anthony, C. S.; Corradi, H. R.; Schwager, S. L. U.; Redelinghuys, P.; Georgiadis, D.; Dive, V.; Acharya, K. R.; Sturrock, E. D. The N Domain of Human Angiotensin-I-Converting Enzyme. The Role of N-Glycosylation and the Crystal Structure in Complex with an N Domain-Specific Phosphinic Inhibitor, RXP407. *J. Biol. Chem.* **2010**, *285*, 35685–35693.

(88) Georgiadis, D.; Cuniasse, P.; Cotton, J.; Yiotakis, A.; Dive, V. Structural Determinants of RXPA380, a Potent and Highly Selective Inhibitor of the Angiotensin-Converting Enzyme C-Domain. *Biochemistry* **2004**, *43*, 8048–8054.

(89) Corradi, H. R.; Chitapi, I.; Sewell, B. T.; Georgiadis, D.; Dive, V.; Sturrock, E. D.; Acharya, K. R. The Structure of Testis Angiotensin-Converting Enzyme in Complex with the C Domain-Specific Inhibitor RXPA380. *Biochemistry* **2007**, *46*, 5473–5478.

(90) Kröger, W. L.; Douglas, R. G.; O'Neill, H. G.; Dive, V.; Sturrock, E. D. Investigating the Domain Specificity of Phosphinic Inhibitors RXPA380 and RXP407 in Angiotensin-Converting Enzyme. *Biochemistry* **2009**, *48*, 8405–8412.

(91) Jullien, N.; Makritis, A.; Georgiadis, D.; Beau, F.; Yiotakis, A.; Dive, V. Phosphinic Tripeptides as Dual Angiotensin-Converting Enzyme C-Domain and Endothelin-Converting Enzyme-1 Inhibitors. *J. Med. Chem.* **2010**, *53*, 208–220.

(92) Akif, M.; Georgiadis, D.; Mahajan, A.; Dive, V.; Sturrock, E. D.; Isaac, R. E.; Acharya, K. R. High-Resolution Crystal Structures of Drosophila Melanogaster Angiotensin-Converting Enzyme in Complex with Novel Inhibitors and Antihypertensive Drugs. *J. Mol. Biol.* **2010**, *400*, 502–517.

(93) Krapcho, J.; Turk, C.; Cushman, D. W.; Powell, J. R.; DeForrest, J. M.; Spitzmiller, E. R.; Karanewsky, D. S.; Duggan, M. E.; Rovnyak, G. C.; Schwartz, J.; Natarajan, S.; Godfrey, J. D.; Ryono, D. E.; Neubeck, R.; Atwal, K. S.; Petrillo, E. W. Angiotensin-Converting Enzyme Inhibitors. Mercaptan, Carboxyalkyl Dipeptide, and Phosphinic Acid Inhibitors Incorporating 4-Substituted Prolines. *J. Med. Chem.* **1988**, *31*, 1148–1160.

(94) Murdoch, D.; McTavish, D. Fosinopril. A Review of Its Pharmacodynamic and Pharmacokinetic Properties, and Therapeutic Potential in Essential Hypertension. *Drugs* **1992**, *43*, 123–140.

(95) Ding, P. Y.; Chu, K. M.; Hu, O. Y.; Huang, G. M.; Jeng, J. J.; Chang, A.; Delaney, C. L.; MacAskill, M.; Yang, B. C.; Jemal, M.; Smith, R.; Liao, W. C. Fosinopril: Pharmacokinetics and Pharmacodynamics in Chinese Subjects. *J. Clin. Pharmacol.* **1999**, *39*, 155–160.

(96) Krajewska, B.; Ureases, I. Functional, Catalytic and Kinetic Properties: A Review. *J. Mol. Catal. B* **2009**, *59*, 9–21.

(97) Mobley, H. L. T.; Island, M. D.; Hausinger, R. P. Molecular Biology of Microbial Ureases. *Microbiol. Rev.* **1995**, *59*, 451–480.

(98) Burne, R. A.; Chen, Y. M. Bacterial Ureases in Infectious Diseases. *Microbes Infect.* **2000**, *2*, 533–542.

(99) Follmer, C. Ureases as a Target for the Treatment of Gastric and Urinary Infections. *J. Clin. Pathol.* **2010**, *63*, 424–430.

(100) Kosikowska, P.; Berlicki, Ł. Urease Inhibitors as Potential Drugs for Gastric and Urinary Tract Infections: A Patent Review. *Expert Opin. Ther. Pat.* **2011**, *21*, 945–957.

(101) Amtul, Z.; Atta-ur-Rahman; Siddiqui, R. A.; Choudhary, M. I. Chemistry and Mechanism of Urease Inhibition. *Curr. Med. Chem.* **2002**, *9*, 1323–1348.

(102) Pope, A. J.; Toseland, C. D.; Rushant, B.; Richardson, S.; McVey, M.; Hills, J. Effect of Potent Urease Inhibitor, Fluorofamide, on *Helicobacter* sp. in Vivo and in Vitro. *Dig. Dis. Sci.* **1998**, *43*, 109–119.

(103) Vassiliou, S.; Grabowiecka, A.; Kosikowska, P.; Yiotakis, A.; Kafarski, P.; Berlicki, Ł. Design, Synthesis and Evaluation of Novel Organophosphorus Inhibitors of Bacterial Ureases. *J. Med. Chem.* **2008**, *51*, 5736–5744.

(104) Berlicki, Ł.; Bochno, M.; Grabowiecka, A.; Białas, A.; Kosikowska, P.; Kafarski, P. N-Substituted Aminomethanephosphonic and Aminomethane-*P*-methylphosphinic Acids as Inhibitors of Ureases. *Amino Acids* **2011**, DOI: 10.1007/s00726-011-0920-4.

(105) Vassiliou, S.; Kosikowska, P.; Grabowiecka, A.; Yiotakis, A.; Kafarski, P.; Berlicki, Ł. Computer-Aided Optimization of Phosphinic Inhibitors of Bacterial Ureases. *J. Med. Chem.* **2010**, *53*, 5597–5606.

(106) Bailie, N. C.; Osborne, C. A.; Leininger, J. R.; Fletcher, T. F.; Johnston, S. D.; Ogburn, P. N.; Griffith, D. P. Teratogenic Effect of Acetohydroxamic Acid in Clinically Normal Beagles. *Am. J. Vet. Res.* **1986**, *47*, 2604–2611.

(107) Bajgar, J. Organophosphates/Nerve Agent Poisoning: Mechanism of Action, Diagnosis, Prophylaxis, and Treatment. *Adv. Clin. Chem.* **2004**, *38*, 151–216.

(108) Oleksyszyn, J.; Powers, J. C. Amino Acid and Peptide Phosphonate as Specific Inhibitors of Serine Peptidases. In *Methods in Enzymology*; Barrett, A. J., Ed.; Academic Press: San Diego, CA, 1994; Vol. 244, pp 423−441.

(109) Oleksyszyn, J. Aminophosphonic and Aminophosphinic Acid Derivatives in the Design of Transition State Analogue Inhibitors: Biomedical Opportunities and Limitations. In *Aminophosphonic and Aminophosphinic Acids. Chemistry and Biological Activity*; Kukhar, V. P., Hudson, H. R., Eds.; John Wiley & Sons: Chichester, U.K., 2000; pp 537−557.

(110) Sieńczyk, M.; Oleksyszyn, J. Irreversible Inhibition of Serine Proteases: Design and in Vivo Activity of Diaryl α-Aminophosphonate Derivatives. *Curr. Med. Chem.* **2009**, *16*, 1673–1687.

(111) Bertrand, J. A.; Oleksyszyn, J.; Kam, C. M.; Boduszek, B.; Presnell, S.; Plaskon, R. R.; Suddath, F. L.; Powers, J. C.; Williams, L. D. Inhibition of Trypsin and Thrombin by Amino(4-amidinophenyl)-methanephosphonate Diphenyl Ester Derivatives: X-ray Structures and Molecular Models. *Biochemistry* **1996**, *35*, 3147–3155.

(112) Oleksyszyn, J.; Powers, J. C. Irreversible Inhibition of Serine Proteases by Peptide Derivatives of (alpha-Aminoalkyl)phosphonate diphenyl esters. *Biochemistry* **1991**, *30*, 485–493.

(113) Bartlett, P. A.; Lamden, L. A. Inhibition of Chymotrypsin by Phosphonate and Phosphonamidate Peptide Analogs. *Bioorg. Chem.* **1986**, *14*, 356–377.

(114) Sampson, N. S.; Bartlett, P. A. Peptidic Phosphonylating Agents as Irreversible Inhibitors of Serine Proteases and Models of the Tetrahedral Intermediates. *Biochemistry* **1991**, *30*, 2255–2263.

(115) Bone, R.; Sampson, N. S.; Bartlett, P. A.; Agard, D. A. Crystal Structures of alpha-Lytic Protease Complexes with Irreversibly Bound Phosphonate Esters. *Biochemistry* **1991**, *30*, 2263–2272.

(116) Skordalakes, E.; Dodson, G. G.; Green, D. S.; Goodwin, C. A.; Scully, M. F.; Hudson, H. R.; Kakkar, V. V.; Deadman, J. J. Inhibition of Human alpha-Thrombin by a Phosphonate Tripeptide Proceeds via a Metastable Pentacoordinated Phosphorus Intermediate. *J. Mol. Biol.* **2001**, *311*, 549–555.

(117) Rahil, J.; Pratt, R. F. Mechanism of Inhibition of the Class C beta-Lactamase of *Enterobacter cloacae* P99 by Phosphonate Monoesters *Biochemistry* **1992**, *31*, 5869–5878.

(118) Lobkovsky, E.; Billings, E. M.; Moews, P. C.; Rahil, J.; Pratt, R. F.; Knox, J. R. Crystallographic Structure of a Phosphonate Derivative of the *Enterobacter cloacae* P99 Cephalosporinase: Mechanistic Interpretation of a beta-Lactamase Transition-State Analog. *Biochemistry* **1994**, *33*, 6762–6772.

(119) Ulisse, S.; Baldini, E.; Sorrenti, S.; D'Armiento, M. The Urokinase Plasminogen Activator System: A Target for Anti-Cancer Therapy. *Curr. Cancer Drug Targets* **2009**, *9*, 32–71.

(120) Dass, K.; Ahmad, A.; Azmi, A. S.; Sarkar, S. H.; Sarkar, F. H. Evolving Role of uPA/uPAR System in Human Cancers. *Cancer Treat. Rev.* **2008**, *34*, 122–136.

(121) Mekkawy, A. H.; Morris, D. L.; Pourgholami, M. H. Urokinase Plasminogen Activator System as a Potential Target for Cancer Therapy. *Future Oncol.* **2009**, *5*, 1487–1499.

(122) Hildenbrand, R.; Allgayer, H.; Marx, A.; Stroebel, P. Modulators of the Urokinase-Type Plasminogen Activation System for Cancer. *Expert Opin. Invest. Drugs* **2010**, *19*, 641–652.

(123) Joossens, J.; Van der Veken, P.; Lambeir, A.-M.; Augustyns, K.; Haemers, A. Development of Irreversible Diphenyl Phosphonate Inhibitors for Urokinase Plasminogen Activator. *J. Med. Chem.* **2004**, *47*, 2411–2413.

(124) Joossens, J.; Ali, O. M.; El-Sayed, I.; Surpateanu, G.; Van der Veken, P.; Lambeir, A.-M.; Setyono-Han, B.; Foekens, J. A.; Schneider, A.; Schmalix, W.; Haemers, A.; Augustyns, K. Small, Potent, and Selective Diaryl Phosphonate Inhibitors for Urokinase-Type Plasminogen Activator with in Vivo Antimetastatic Properties. *J. Med. Chem.* **2007**, *50*, 6638–6646.

(125) Sieńczyk, M.; Oleksyszyn, J. Inhibition of Trypsin and Urokinase by Cbz-amino(4-guanidinophenyl)methanephosphonate Aromatic Ester Derivatives: The Influence of the Ester Group on Their Biological Activity. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2886–2890.

(126) Havale, S. H.; Pal, M. Medicinal Chemistry Approaches to the Inhibition of Dipeptidyl Peptidase-4 for the Treatment of Type 2 Diabetes. *Bioorg. Med. Chem.* **2009**, *17*, 1783–1802.

(127) Gwaltney, S. L. Medicinal Chemistry Approaches to the Inhibition of Dipeptidyl Peptidase IV. *Curr. Top. Med. Chem.* **2008**, *8*, 1545–1552.

(128) Gupta, R.; Walunj, S. S.; Tokala, R. K.; Parsa, K. V.; Singh, S. K.; Pal, M. Emerging Drug Candidates of Dipeptidyl Peptidase IV (DPP IV) Inhibitor Class for the Treatment of Type 2 Diabetes. *Curr. Drug Targets* **2009**, *10*, 71–87.

(129) Doupis, J.; Veves, A. DPP4 Inhibitors: A New Approach in Diabetes Treatment. *Adv. Ther.* **2008**, *25*, 627–643.

(130) Ohnuma, K.; Takahashi, N.; Yamochi, T.; Hosono, O.; Dang, N. H.; Morimoto, C. Role of CD26/Dipeptidyl Peptidase IV in Human T Cell Activation and Function. *Front. Biosci.* **2008**, *13*, 2299–2310.

(131) Thompson, M. A.; Ohnuma, K.; Abe, M.; Morimoto, C.; Dang, N. H. CD26/Dipeptidyl Peptidase IV as a Novel Therapeutic Target for Cancer and Immune Disorders. *Mini-Rev. Med. Chem.* **2007**, *7*, 253–273.

(132) Boduszek, B.; Oleksyszyn, J.; Kam, C. M.; Selzler, J.; Smith, R. E.; Powers, J. C. Dipeptide Phosphonates as Inhibitors of Dipeptidyl Peptidase IV. *J. Med. Chem.* **1994**, *37*, 3969–3976.

(133) Belyaev, A.; Zhang, X.; Augustyns, K.; Lambeir, A.-M.; De Meester, I.; Vedernikova, I.; Scharpe, S.; Haemers, A. Structure−Activity Relationship of Diaryl Phosphonate Esters as Potent Irreversible Dipeptidyl Peptidase IV Inhibitors. *J. Med. Chem.* **1999**, *42*, 1041–1052.

(134) Augustyns, K.; Van der Veken, P.; Senten, K.; Haemers, A. The Therapeutic Potential of Inhibitors of Dipeptidyl Peptidase IV (DPP IV) and Related Proline-Specific Dipeptidyl Aminopeptidases. *Curr. Med. Chem.* **2005**, *12*, 971–998.

(135) Van der Veken, P.; Haemers, A.; Augustyns, K. Prolyl Peptidases Related to Dipeptidyl Peptidase IV: Potential of Specific Inhibitors in Drug Discovery. *Curr. Top. Med. Chem.* **2007**, *7*, 621–635.

(136) Jung, F. J.; Yang, L.; De Meester, I.; Augustyns, K.; Cardell, M.; Hillinger, S.; Vogt, P.; Lardinois, D.; Scharpé, S.; Weder, W.; Korom, S. CD26/Dipeptidylpeptidase IV-Targeted Therapy of Acute Lung Rejection in Rats. *J. Heart Lung Transplant.* **2006**, *25*, 1109–1116.

(137) Zhai, W.; Cardell, M.; De Meester, I.; Augustyns, K.; Hillinger, S.; Inci, I.; Arni, S.; Jungraithmayr, W.; Scharpé, S.; Weder, W.; Korom, S. Intragraft DPP IV Inhibition Attenuates Post-Transplant Pulmonary Ischemia/Reperfusion Injury after Extended Ischemia. *J. Heart Lung Transplant.* **2007**, *26*, 174–180.

(138) Gilmore, B. F.; Lynas, J. F.; Scott, C. J.; McGoohan, C.; Martin, L.; Walker, B. Dipeptide Proline Diphenyl Phosphonates Are Potent, Irreversible Inhibitors of Seprase (FAPα). *Biochem. Biophys. Res. Commun.* **2006**, *346*, 436–446.

(139) Van der Veken, P.; Soroka, A.; Brandt, I.; Chen, Y.-S.; Maes, M.-B.; Lambeir, A.-M.; Chen, X.; Haemers, A.; Scharpé, S.; Augustyns, K.; De Meester, I. Irreversible Inhibition of Dipeptidyl Peptidase 8 by Dipeptide-Derived Diaryl Phosphonates. *J. Med. Chem.* **2007**, *50*, 5568–5570.

(140) Juillerat-Jeanneret, L.; Gerber-Lemaire., S. The Prolyl-aminodipeptidases and Their Inhibitors as Therapeutic Targets for Fibrogenic Disorders. *Mini-Rev. Med. Chem.* **2009**, *9*, 215–226.

(141) Singh, J.; Petter, R. C.; Baillie, T. A.; Whitty, A. The Resurgence of Covalent Drugs. *Nat. Rev. Drug Discovery* **2011**, *10*, 307–317.

(142) Kiss, T.; Lázár, I. Structure and Stability Constants of Metal Complexes in Solution. In *Aminophosphonic and Aminophosphinic Acids. Chemistry and Biological Activity*; Kukhar, V. P., Hudson, H. R., Eds.; John Wiley & Sons: Chichester, U.K., 2000; pp 285−325.

(143) Fleisch, H.; Russel, R. G. G.; Staruman, F. Effect of Pyrophosphate and Hydroxyapatite and Its Implications in Calcium Homeostasis. *Nature* **1966**, *212*, 901–903.

(144) Russell, R. G. G. Bisphosphonates: From Bench to Bedside. *Ann. N.Y. Acad. Sci.* **2006**, *1068*, 367–401.

(145) Shaw, N. J.; Bishop, N. J. Bisphosphonate Treatment of Bone Disease. *Arch. Dis. Child.* **2005**, *90*, 494–499.

(146) Wu, S.; Dahut, W. L.; Gulley, J. L. The Use of Bisphosphonates in Cancer Patients. *Acta Oncol.* **2007**, *46*, 581–591.

(147) Mukherjee, S.; Song, Y.; Oldfield, E. NMR Investigations of the Static and Dynamic Structures of Bisphosphonates on Human Bone: A Molecular Model. *J. Am. Chem. Soc.* **2008**, *130*, 1264–1273.

(148) Mönkkönen, H.; Kuokkanen, J.; Holen, I.; Evans, A.; Lefley, D. V.; Jauhiainen, M.; Auriola, S.; Mönkkönen, J. Bisphosphonate-Induced ATP Analog Formation and Its Effect on Inhibition of Cancer Cell Growth. *Anti-Cancer Drugs* **2008**, *19*, 391–399.

(149) Clezardin, P.; Ebetino, F. H.; Fournier, P. G. Bisphosphonates and Cancer-Induced Bone Disease: Beyond Their Antiresorptive Activity. *Cancer Res.* **2005**, *65*, 4971–4974.

(150) Usui, T.; Oiso, Y.; Tomito, A.; Ogata, E.; Uchida, T.; Ikeda, K. Pharmacokinetics of Incadronate, a New Bisphosphonate, in Healty Volunteers and Patients with Malignancy-Associated Hypercalcemia. *Int. J. Clin. Pharmacol. Ther.* **1997**, *35*, 239–244.

(151) Kim, S.; Lee, J.; Kim, H.; Im, Y.; Kim, T.; Ryoo, B.; Park, Y.; Park, J.; Park, K.; Katoh, H. Clinical Evaluation of Incadronate in Korean Patients with Malignancy-Associated Hypercalcemia: An Open-Label, Multicenter Study. *Curr. Ther. Res.* **2007**, *68*, 193–204.

(152) Shipman, C. M.; Croucher, P. I.; Russell, R. G. G.; Helfrich, M. H.; Rogers, M. J. The Bisphosphonate Incadronate (YM175) Causes Apoptosis of Human Myeloma Cells in Vitro by Inhibiting Mevalonate Pathway. *Cancer Res.* **1998**, *58*, 5294–5297.

(153) Tanvetyanon, T.; Stiff, P. J. Management of the Adverse Efects Associated with Intravenous Bisphosphonates. *Ann. Oncol.* **2006**, *17*, 897–907.

(154) Drake, M. T.; Clarke, B. L.; Khosla, S. Bisphosphonates: Mechanism of Action and Role in Clinical Practice. *Mayo Clin. Proc.* **2008**, *83*, 1032–1045.

(155) Dunford, J. E.; Thompson, K.; Coxon, F. P.; Luckman, S. P.; Hahn, F. M.; Poulter, C. D.; Ebetino, F. H.; Rogers, M. J. Structure−Activity Relationships for Inhibition of Farnesyl Diphosphate Synthase in Vitro and Inhibition of Bone Resorption in Vivo by Nitrogen-Containing Bisphosphonates. *J. Pharmacol. Exp. Ther.* **2001**, *296*, 235–242.

(156) Russell, R. G.; Xia, Z.; Dunford, J. E.; Oppermann, U.; Kwaasi, A.; Hulley, P. A.; Kavanagh, K. L.; Triffitt, J. T.; Lundy, M. W.; Phipps, R. J.; Barnett, B. L.; Coxon, F. P.; Rogers, M. J.; Watts, N. B.; Ebetino, F. H. Bisphosphonates: An Update on Mechanisms of Action and How These Relate to Clinical Efficacy. *Ann. N.Y. Acad. Sci.* **2007**, *1117*, 209–257.

(157) Frith, J. C.; Mönkkönen, J.; Auriola, S.; Mönkkönen, H.; Rogers, M. J. The Molecular Mechanism of Action of the Antiresorptive and Antiinflammatory Drug Clodronate: Evidence for the Formation in Vivo of a Metabolite That Inhibits Bone Resorption and Causes Osteoclast and Macrophage Apoptosis. *Arthritis Rheum.* **2001**, *44*, 2201–2210.

(158) Frith, J. C.; Mönkkönen, J.; Blackburn, G. M.; Russell, R. G.; Rogers, M. J. Clodronate and Liposome-Encapsulated Clodronate Are

Metabolized to a Toxic ATP Analog, Adenosine 5′-(beta,gamma-Dichloromethylene) Triphosphate, by Mammalian Cells in Vitro. *J. Bone Miner. Res.* **1997**, *12*, 1358–1367.

(159) van Beek, E.; Pieterman, E.; Cohen, L.; Löwik, C.; Papapoulos, S. Farnesyl Pyrophosphate Synthase Is the Molecular Target of Nitrogen-Containing Bisphosphonates. *Biochem. Biophys. Res. Commun.* **1999**, *264*, 108–111.

(160) Grove, J. E.; Brown, R. J.; Watts, D. J. The Intracellular Target for the Antiresorptive Aminobisphosphonate Drugs in *Dictyostelium discoideum* is the Enzyme Farnesyl Diphosphate Synthase. *J. Bone Miner. Res.* **2000**, *15*, 971–981.

(161) Bergstrom, J. D.; Bostedor, R. G.; Masarachia, P. J.; Reszka, A. A.; Rodan, G. Alendronate is a Specific, Nanomolar Inhibitor of Farnesyl Diphosphate Synthase. *Arch. Biochem. Biophys.* **2000**, *373*, 231–241.

(162) van Beek, E.; Pieterman, E.; Cohen, L.; Löwik, C.; Papapoulos, S. Nitrogen-Containing Bisphosphonates Inhibit Isopentenyl Pyrophosphate Isomerase/Farnesyl Pyrophosphate Synthase Activity with Relative Potencies Corresponding to Their Antiresorptive Potencies in Vitro and in Vivo. *Biochem. Biophys. Res. Commun.* **1999**, *255*, 491–494.

(163) Martin, M. B.; Arnold, W.; Heath, H. T., III; Urbina, J. A.; Oldfield, E. Nitrogen-Containing Bisphosphonates as Carbocation Transition State Analogs for Isoprenoid Biosynthesis. *Biochem. Biophys. Res. Commun.* **1999**, *263*, 754–758.

(164) Hosfield, D. J.; Zhang, Y.; Dougan, D. R.; Broun, A.; Tari, L. W.; Swanson, R. V.; Finn, J. Structural Basis for Bisphosphonate-Mediated Inhibition of Isoprenoid Biosynthesis. *J. Biol. Chem.* **2004**, *279*, 8526–8529.

(165) Rondeau, J. M.; Bitsch, F.; Bourgier, E.; Geiser, M.; Hemmig, R.; Kroemer, M.; Lehmann, S.; Ramage, P.; Rieffel, S.; Strauss, A.; Green, J. R.; Jahnke, W. Structural Basis for the Exceptional in Vivo Efficacy of Bisphosphonate Drugs. *ChemMedChem* **2006**, *1*, 267–273.

(166) Kavanagh, K. L.; Guo, K.; Dunford, J. E.; Wu, X.; Knapp, S.; Ebetino, F. H.; Rogers, M. J.; Russell, R. G.; Oppermann, U. The Molecular Mechanism of Nitrogen-Containing Bisphosphonates as Anti-osteoporosis Drugs. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 7829–7834.

(167) Martin, M. B.; Grimley, J. S.; Lewis, J. C.; Heath, H. T., III; Bailey, B. N.; Kendrick, H.; Yardley, V.; Caldera, A.; Lira, R.; Urbina, J. A.; Moreno, S. N. J.; Docampo, R.; Croft, S. L.; Oldfield, E. Bisphosphonates Inhibit the Growth of *Trypanosoma brucei*, *Trypanosoma cruzi*, *Leishmania donovani*, *Toxoplasma gondii*, and *Plasmodium falciparum*: A Potential Route to Chemotherapy. *J. Med. Chem.* **2001**, *44*, 909–916.

(168) Ling, Y.; Sahota, G.; Odeh, S.; Chan, J. M. W.; Araujo, F. G.; Moreno, S. N. J.; Oldfield, E. Bisphosphonate Inhibitors of *Toxoplasma gondi* Growth: In Vitro, QSAR, and in Vivo Investigations. *J. Med. Chem.* **2005**, *48*, 3130–3140.

(169) Yardley, V.; Khan, A. A.; Martin, M. B.; Slifer, T. R.; Araujo, F. G.; Moreno, S. N.; Docampo, R.; Croft, S. L.; Oldfield, E. In Vivo Activities of Farnesyl Pyrophosphate Synthase Inhibitors against *Leishmania donovani* and *Toxoplasma gondii*. *Antimicrob. Agents Chemother.* **2002**, *46*, 929–931.

(170) Martin, M. B.; Sanders, J. M.; Kendrick, H.; de Luca-Fradley, K.; Lewis, J. C.; Grimley, J. S.; Van Brussel, E. M.; Olsen, J. R.; Meints, G. A.; Burzynska, A.; Kafarski, P.; Croft, S. L.; Oldfield, E. Activity of Bisphosphonates against *Trypanosoma brucei rhodesiense*. *J. Med. Chem.* **2002**, *45*, 2904–2914.

(171) Ghosh, S.; Chan, J. M. W.; Lea, C. R.; Meints, G. A.; Lewis, J. C.; Tovian, Z. S.; Flessner, R. M.; Loftus, T. C.; Bruchhaus, I.; Kendrick, H.; Croft, S. L.; Kemp, R. G.; Kobayashi, S.; Nozaki, T.; Oldfield, E. Effects of Bisphosphonates on the Growth of *Entamoeba histolytica* and *Plasmodium* Species in Vitro and in Vivo. *J. Med. Chem.* **2004**, *47*, 175–187.

(172) Sanders, J. M.; Gomez, A. O.; Mao, J.; Meints, G. A.; Van Brussel, E. M.; Burzynska, A.; Kafarski, P.; Gonzalez-Pacanowska, D.; Oldfield, E. 3-D QSAR Investigations of the Inhibition of *Leishmania major* Farnesyl Pyrophosphate Synthase by Bisphosphonates. *J. Med. Chem.* **2003**, *46*, 5171–5183.

(173) Kotsikorou, E.; Song, Y.; Chan, J. M.; Faelens, S.; Tovian, Z.; Broderick, E.; Bakalara, N.; Docampo, R.; Oldfield, E. Bisphosphonate Inhibition of the Exopolyphosphatase Activity of the *Trypanosoma brucei* Soluble Vacuolar Pyrophosphatase. *J. Med. Chem.* **2005**, *48*, 6128–6139.

(174) Hudock, M. P.; Sanz-Rodríguez, C. E.; Song, Y.; Chan, J. M.; Zhang, Y.; Odeh, S.; Kosztowski, T.; Leon-Rossell, A.; Concepción, J. L.; Yardley, V.; Croft, S. L.; Urbina, J. A.; Oldfield, E. Inhibition of *Trypanosoma cruzi* Hexokinase by Bisphosphonates. *J. Med. Chem.* **2006**, *49*, 215–223.

(175) Sanz-Rodríguez, C. E.; Concepción, J. L.; Pekerar, S.; Oldfield, E.; Urbina, J. A. Bisphosphonates as Inhibitors of *Trypanosoma cruzi* Hexokinase: Kinetic and Metabolic Studies. *J. Biol. Chem.* **2007**, *282*, 12377–12387.

(176) Yajima, S.; Hara, K.; Sanders, J. M.; Yin, F.; Ohsawa, K.; Wiesner, J.; Jomaa, H.; Oldfield, E. Crystallographic Structures of Two Bisphosphonate:1-deoxyxylulose-5-phosphate Reductoisomerase Complexes. *J. Am. Chem. Soc.* **2004**, *126*, 10824–10825.

(177) Szabo, C. M.; Matsumura, Y.; Fukura, S.; Martin, M. B.; Sanders, J. M.; Sengupta, S.; Cieslak, J. A.; Loftus, T. C.; Lea, C. R.; Lee, H.-J.; Koohang, A.; Coates, R. M.; Sagami, H.; Oldfield, E. Inhibition of Geranylgeranyl Diphosphate Synthase by Bisphosphonates and Diphosphates: A Potential Route to New Bone Antiresorption and Antiparasitic Agents. *J. Med. Chem.* **2002**, *45*, 2185–2196.

(178) Oldfield, E. Targeting Isoprenoid Biosynthesis for Drug Discovery: Bench to Bedside. *Acc. Chem. Res.* **2010**, *43*, 1216–1226.

(179) Derenne, S.; Amiot, M.; Barille, S.; Collette, M.; Robillard, N.; Berthaud, P.; Harousseau, J. L.; Bataille, R. Zoledronate Is a Potent Inhibitor of Myeloma Cell Growth and Secretion of IL-6 and MMP-1 by the Tumoral Environment. *J. Bone Miner. Res.* **1999**, *14*, 2048–2056.

(180) Wilhelm, M.; Kunzmann, V.; Eckstein, S.; Reimer, P.; Weissinger, F.; Ruediger, T.; Tony, H. P. $\gamma\delta$ T Cells for Immune Therapy of Patients with Lymphoid Malignancies. *Blood* **2003**, *102*, 200–206.

(181) Dieli, F.; Vermijlen, D.; Fulfaro, F.; Caccamo, N.; Meraviglia, S.; Cicero, G.; Roberts, A.; Buccheri, S.; D'Asaro, M.; Gebbia, N.; Salerno, A.; Eberl, M.; Hayday, A. C. Targeting Human $\gamma\delta$ T Cells with Zoledronate and Interleukin-2 for Immunotherapy of Hormone-Refractory Prostate Cancer. *Cancer Res.* **2007**, *67*, 7450–7457.

(182) Gnant, M.; Mlineritsch, B.; Schippinger, W.; Luschin-Ebengreuth, G.; Postlberger, S.; Menzel, C.; Jakesz, R.; Seifert, M.; Hubalek, M.; Bjelic-Radisic, V.; Samonigg, H.; Tausch, C.; Eidtmann, H.; Steger, G.; Kwasny, W.; Dubsky, P.; Fridrik, M.; Fitzal, F.; Stierer, M.; Rucklinger, E.; Greil, R.; Marth, C. Endocrine Therapy plus Zoledronic Acid in Premenopausal Breast Cancer. *N. Engl. J. Med.* **2009**, *360*, 679–691.

(183) Gao, Y. G.; Robinson, H.; Song, Y.; No, J. H.; Bergan, K.; Leon, A.; Cass, L.; Goddard, A.; Chang, T. K.; Lin, F. Y.; Van Beek, E.; Papapoulos, S.; Wang, A. H.; Kubo, T.; Ochi, M.; Mukkamala, D.; Oldfield, E. Lipophilic Bisphosphonates as Dual Farnesyl/Geranylgeranyl Diphosphate Synthase Inhibitors: An X-ray and NMR Investigation. *J. Am. Chem. Soc.* **2009**, *131*, 5153–5162.

(184) Sanders, J. M.; Ghosh, S.; Chan, J. M. W.; Meints, G.; Wang, H.; Raker, A. M.; Song, Y.; Colantino, A.; Burzynska, A.; Kafarski, P.; Morita, C. T.; Oldfield, E. Quantitative Structure−Activity Relationships for gamma-delta-T Cell Activation by Bisphosphonates. *J. Med. Chem.* **2004**, *47*, 375–384.

(185) Zhang, Y.; Cao, R.; Yin, F.; Lin, F. Y.; Wang, H.; Krysiak, K.; No, J. H.; Mukkamala, D.; Houlihan, K.; Li, J.; Morita, C. T.; Oldfield, E. Lipophilic Pyridinium Bisphosphonates: Potent gammadelta T Cell Stimulators. *Angew. Chem., Int. Ed.* **2010**, *49*, 1136–1138.

(186) Cremers, S. C.; Pillai, G.; Papapoulos, S. E. Pharmacokinetics/Pharmacodynamics of Bisphosphonates: Use for Optimisation of Intermittent Therapy for Osteoporosis. *Clin. Pharmacokinet.* **2005**, *44*, 551–570.

(187) Kimmel, D. B. Mechanism of Action, Pharmacokinetic and Pharmacodynamic Profile, and Clinical Applications of Nitrogen-Containing Bisphosphonates. *J. Dent. Res.* **2007**, *86*, 1022–1033.

(188) Chebbi, I.; Migianu-Griffoni, E.; Sainte-Catherine, O.; Lecouvey, M.; Seksek, O. In Vitro Assessment of Liposomal Neridronate on MDA-MB-231 Human Breast Cancer Cells. *Int. J. Pharm.* **2010**, *383*, 116–122.

(189) Pignatello, R.; Cenni, E.; Micieli, D.; Fotia, C.; Salerno, M.; Granchi, D.; Avnet, S.; Sarpirtro, M. G.; Castelli, F.; Baldini, N. A Novel Biomaterial for Osteotropic Drug Nanocarriers: Synthesis and

Biocompatibility Evaluation of a PLGA-ALE Conjugate. *Nanomedicine* **2009**, *4*, 161–175.

(190) Morioka, M.; Kazimono, A.; Takikawa, H.; Mori, A.; Ueno, H.; Kadowaki, S.; Nakao, Y.; Kato, K.; Umezawa, K. Design, Synthesis, and Biological Evaluation of Novel Estradiol−Bisphosphonate Conjugates as Bone-Specific Estrogens. *Bioorg. Med. Chem.* **2010**, *18*, 1142–1148.

(191) Wang, J.; Yang, C.; Ding, H.; Yan, X.; Wu, X.; Xie, Y. Synthesis of Novel Bone-Targeted Agents for Treatment of Osteoporosis. *Indian J. Chem.* **2006**, *45*, 318–321.

(192) Tanaka, K. S. E.; Dietrich, E.; Ciblat, S.; Métayer, C.; Arhin, F. F.; Sarmiento, I.; Moeck, G.; Parr, T. R., Jr.; Far, A. R. Synthesis and in Vitro Evaluation of Bisphosphonated Glycopeptide Prodrugs for the Treatment of Osteomyelitis. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1355–1359.

(193) Doschak, M. R.; Kucharski, C. M.; Wright, J. E. I.; Zernicke, D. F.; Uluda, H. Improved Bone Delivery of Osteoprotegerin by Bisphosphonate Conjugation in a Rat Model of Osteoarthritis. *Mol. Pharmaceutics* **2009**, *6*, 634–640.

(194) Gittens, S. A.; Bansal, G.; Kucharski, C.; Borden, M.; Uluda, H. Imparting Mineral Affinity to Fetuin by Bisphosphonate Conjugation: A Comparison of Three Bisphosphonate Conjugation Schemes. *Mol. Pharmaceutics* **2005**, *2*, 392–406.

(195) Davis, M. P.; Walsh, D. Epidemiology of Cancer Pain and Factors Influencing Poor Pain Control. *Am. J. Hospice Palliative Med.* **2004**, *21*, 137–142.

(196) Bauman, G.; Charette, M.; Reid, R.; Sathya, J. Radiopharmaceuticals for the Palliation of Painful Bone-Metastasis: A Systematic Review. *Radiother. Oncol.* **2005**, *75*, 258–270.

(197) Kubíček., V.; Rudovský, J.; Kotek, J.; Hermann, P.; Van der Elst, L.; Muller, R. N.; Kolar, Z. I.; Wolterbeek, H. T.; Peters, J. A.; Lukeš, I. A Bisphosphonate Monoamide Analogue of DOTA: A Potential Agent for Bone Targeting. *J. Am. Chem. Soc.* **2005**, *127*, 16477–16485.

(198) Notni, J.; Hermann, P.; Havlíčková, J.; Kotek, J.; Kubíček, V.; Plutnar, J.; Loktionova, N.; Riss, P. J.; Rösch, F.; Lukeš, I. A Triazacyclononane-Based Bifunctional Phosphinate Ligand for the Preparation of Multimeric ^{68}Ga Tracers for Positron Emission Tomography. *Chem.—Eur. J.* **2010**, *16*, 7174–7185.

(199) Tanwar, J.; Datta, A.; Tiwari, A. K.; Thirumal, M.; Chuttani, K.; Mishra, A. K. Preclinical Evaluation of DOP3P-AME-DOP3P: A Polyazamacrocyclic Methylene Phosphonate for Diagnosis and Therapy of Skeletal Matastases. *Bioconjugate Chem.* **2010**, *22*, 244–255.

(200) Lacerda, S.; Marques, F.; Campello, P.; Gano, L.; Kubíček, V.; Hermann, P.; Santos, I. Chemical, Radiochemical and Biological Studies of Sm and Ho Complexes of H4dota Analogues Containing One Methylphosphonic/Phosphinic Acid Pedant Arm. *J. Labelled Compd. Radiopharm.* **2010**, *53*, 36–43.

(201) Boswell, C. A.; Regino, C. A.; Baidoo, K. E.; Wong, K. J.; Milenic, D. E.; Kelley, J. A.; Lai, C. C.; Brechbiel, M. W. A Novel Side-Bridged Hybrid Phosphonate/Acetate Pendant Cyclam: Synthesis, Characterization, and ^{64}Cu Small Animal PET Imaging. *Bioorg. Med. Chem.* **2009**, *17*, 548–552.

(202) Fellner, M.; Baum, R. P.; Kubíček, V.; Hermann, P.; Lukeš, I.; Prasad, V.; Rösch, F. PET/CT Imaging of Osteoblastic Bone Metastases with ^{68}Ga-Bisphosphonates: First Human Study. *Eur. J. Nucl. Med. Mol. Imaging* **2010**, *37*, 834–834.

(203) Murphy, G. The ADAMs: Signalling Scissors in the Tumour Microenvironment. *Nat. Rev. Cancer* **2008**, *8*, 929–941.

(204) Rocks, N.; Paulissen, G.; El Hour, M.; Quesada, F.; Crahay, C.; Gueders, M.; Foidart, J.-M.; Noel, A.; Cataldo, D. Emerging Roles of ADAM and ADAMTS Metalloproteinases in Cancer. *Biochimie* **2008**, *90*, 369–379.

(205) Wagstaff, L.; Kelwick, R.; Decock, J.; Edwards, D. R. The Roles of ADAMTS Metalloproteinases in Tumorigenesis and Metastasis. *Front. Biosci.* **2011**, *16*, 1861–1872.

(206) Huber, T.; Manzenrieder, F.; Kuttruff, C. A.; Dorner-Ciossek, C.; Kessler, H. Prolonged Stability by Cyclization: Macrocyclic Phosphino Dipeptide Isostere Inhibitors of β-Secretase (BACE1). *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4427–4431.

(207) Manzenrieder, F.; Frank, A.; Huber, T.; Dorner-Ciossek, C.; Kessler, H. Synthesis and Biological Evaluation of Phosphino Dipeptide Isostere Inhibitor of Human β-Secretase (BACE1). *Bioorg. Med. Chem.* **2007**, *15*, 4136–4143.

(208) Gąsowska, B.; Wojtasek, H.; Hurek, J.; Drąg, M.; Nowak, K.; Kafarski, P. Redox reaction between Amino-(3,4-dihydroxyphenyl)-methylphosphonic Acid and Dopaquinone Is Responsible for the Apparent Inhibitory Effect on Tyrosinase. *Eur. J. Biochem.* **2002**, *269*, 4098–4104.

(209) Coudray, L.; Pennebaker, A. F.; Montchamp, J.-L. Synthesis and in Vitro Evaluation of Aspartate Transcarbamoylase Inhibitors. *Bioorg. Med. Chem.* **2009**, *17*, 7680–7689.

(210) Flohr, A.; Aemissegger, A.; Hilvert, D. alpha-Functionalized Phosphonylphosphinates: Synthesis and Evaluation as Transcarbamoylase Inhibitors. *J. Med. Chem.* **1999**, *42*, 2633–2640.

(211) Collinsová, M.; Castro, C.; Garrow, T. A.; Yiotakis, A.; Dive, V.; Jiráček, J. Combining Combinatorial Chemistry and Affinity Chromatography: Highly Selective Inhibitors of Human Betaine: Homocysteine *S*-Methyltransferase. *Chem. Biol.* **2003**, *10*, 113–122.

(212) Jiráček, J.; Collinsová, M.; Rosenberg, I.; Budesinsky, M.; Protivinska, E.; Netusilova, H.; Garrow, T. A. S-Alkylated Homocysteine Derivatives: New Inhibitors of Human Betaine-Homocysteine *S*-Methyltransferase. *J. Med. Chem.* **2006**, *49*, 3982–3989.

(213) Makhaeva, G. F.; Aksinenko, A. Y.; Sokolov, V. B.; Baskin, I. I.; Palyulin, V. A.; Zefirov, N. S.; Hein, N. D.; Kampf, J. W.; Wijeyesakere, S. J.; Richardson, R. J. Kinetics and Mechanizm od Inhibition of Serie Esterases by Fluorinated Aminophosphonates. *Chem.-Biol. Interact.* **2010**, *187*, 177–194.

(214) Steere, J. A.; Sampson, P. B.; Honek, J. F. Synthesis of an alpha-Aminophosphonate Nucleoside as an Inhibitor of *S*-Adenosyl-L-Homocysteine Hydrolase. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 457–460.

(215) Khomutov, R. M.; Shchipanova, A. I.; Khurs, E. N. Phosphoanalogues of Amino Acids Involved in Methionine Metabolism as a New Source of Antiviral Compounds. *Dokl. Biochem. Biophys.* **2009**, *425*, 84–86.

(216) Sheng, X. C.; Pyun, H.-J.; Chaudhary, K.; Wang, J.; Doerffler, E.; Fleury, M.; McMurtrie, D.; Chen, X.; Delaney, W. E., IV; Kim, C. U. Discovery of Novel Phosphonate Derivatives as Hepatitis C Virus NS3 Protease Inhibitors. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3453–3457.

(217) Pyun, H.-J.; Chaudhary, K.; Somoza, J. R.; Sheng, X. C.; Kim, C. U. Synthesis and Resolution of Diethyl (1*S*,2*S*)-1-Amino-2-vinylcyclopropane-1-phosphonate for HCV NS3 Protease Inhibitors. *Tetrahedron Lett.* **2009**, *50*, 3833–3835.

(218) Cherenok, S.; Vovk, A.; Muravyova, I.; Shivanyuk, A.; Kukhar, V.; Lipkowski, J.; Kalchenko, V. Calix[4]arene alpha-Aminophosphonic Acids: Asymmetric Synthesis and Enantioselective Inhibition of an Alkaline Phosphatase. *Org. Lett.* **2006**, *8*, 549–552.

(219) Morrison, M. J.; Li, N.; Pratt, R. F. Inverse Acyl Phosph(on)ates: Substrates or Inhibitors of β-Lactam-Recognizing Enzymes? *Bioorg. Chem.* **2001**, *29*, 271–281.

(220) Liu, W.; Rogers, C. J.; Fisher, A. J.; Toney, M. D. Aminophosphonate Inhibitors of Dialkylglycine Decarboxylase: Structural Basis for Slow Binding Inhibition. *Biochemistry* **2002**, *41*, 12320–12328.

(221) Asojo, O.; Friedman, J.; Adir, N.; Belakhov, V.; Shoham, Y.; Baasov, T. Crystal Structures of KDOP Synthase in Its Binary Complex with the Substrate Phosphoenolpyruvate and with Mechanism-Base Inhibitor. *Biochemistry* **2001**, *40*, 6326–6334.

(222) Cressina, E.; Lloyd, A. J.; De Pascale, G.; Mok, B. J.; Caddick, S.; Roper, D. I.; Dowson, C. G.; Bugg, T. D. H. Inhibition of tRNA-Dependent Ligase MurM from *Straptococcus pneumoniae* by Phosphonate and Sulfonamide Inhibitors. *Bioorg. Med. Chem.* **2009**, *17*, 3443–3455.

(223) Berlicki, Ł.; Obojska, A.; Forlani, G.; Kafarski, P. Design, Synthesis, and Activity of Analogues of Phosphinothricin as Inhibitors of Glutamine Synthetase. *J. Med. Chem.* **2005**, *48*, 6340–6349.

(224) Hiratake, J.; Kato, H.; Oda, J. Mechanism-Based Inactivation of Glutathione Synthetase by Phosphinic Acid Transition-State Analog. *J. Am. Chem. Soc.* **1994**, *116*, 12059–12060.

(225) Chen, S.; Coward, J. K. Investigations on New Strategies for the Facile Synthesis of Polyfunctionalized Phosphinates: Phosphinopeptide Analogues of Glutathionylspermidine. *J. Org. Chem.* **1998**, *63*, 502–509.

(226) Tanner, M. E.; Vaganay, S.; van Heijenoort, J.; Blanot, D. Phosphinate Inhibitors of the D-Glutamic Acid-Adding Enzyme of Peptidoglycan Biosynthesis. *J. Org. Chem.* **1996**, *61*, 1756–1760.

(227) Štrancar, K.; Blanot, D.; Gobec, S. Design, Synthesis and Structure−Activity Relationships of New Phosphinate Inhibitors of MurD. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 343–348.

(228) Miller, D. J.; Hammond, S. M.; Anderluzzi, D.; Bugg, T. D. H. Aminoalkylphosphinate Inhibitors of D-Ala-D-Ala Adding Enzyme. *J. Chem. Soc., Perkin Trans. 1* **1998**, 131–142.