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First synthesis of α-aminoalkyl-(N-substituted)thiocarbamoyl-phosphinates: Inhibitors of aminopeptidase N (APN/CD13) with the new zinc-binding group

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ABSTRACT

OO-Di-trimethylsilyl esters of α-*N*-benzyloxycarbonylaminoalkylphosphinates (**III**) undergo triethylamine catalyzed addition to isothiocyanates to give after hydrolysis, a series of new α-aminoalkyl-(*N*-substituted)thiocarbamoyl-phosphinates. Thiocarbamoyl-phosphinate moiety can be included in the structures of the metalloproteinase inhibitors as the zinc-binding group and the new compounds reported here are good inhibitors of important aminopeptidase N(CD13) with IC_{50} in range of 10.56–0.25 μM.

The proteolytic degradation of the extracellular matrix is important in many biological processes. Structurally related proteinases that utilize a zinc (**II**) metal for catalysis of the proteolysis, matrix metalloproteinases (MMPs), are at least partially responsible for these functions. MMPs mediated degradation of the extracellular matrix and the basement-membrane are crucial steps in the early development of several diseases, such as arthritis, osteoporosis, periodontal disease, cancer growth, and metastasis.[1] The inhibition of such degradation offers a potential for new therapeutics, and several of the MMPs inhibitors have entered clinical trials for cancer treatment. However, clinical trials for hydroxamate-based MMPs inhibitors have been disappointing, due to lack of specificities, low oral availability, poor in vivo stability, and unacceptable side effects associated with hydroxamates.[2] Therefore, there is a serious effort to replace the hydroxamic acid with a group having more pharmaceutically acceptable properties. In general, metalloproteinase inhibitors molecules consist of two parts. The first part is similar to the peptide sequence around the hydrolyzed peptide bond, including the overall shape and the distribution of electron density. The second part is the zinc-binding group (ZBG) essential for binding to the active site of the zinc (**II**) metal. While the first part is subjected to the classical structure–activity based improvement, the second one is more difficult to design. The ZBGs described in literature may be grouped into two classes: those which are transition state analogs (TSA) of tetrahedral gem-diol of carbonyl carbon of amide bound intermediate, undergoing hydrolysis at the enzyme active site (these include phosphonate and phosphinate types of inhibitors) and simple zinc chelating group (these include thiols, carboxylates, mercaptoalcohols and hydroxamates). Hydroxamates act as a bidente ligand of zincs active site and despite the small resemblance to the TS, at least for MMPs, they are usually superior to other ZBGs, thus offering more potent inhibitors.

Recently, we have proposed several new ZBGs (Fig. 1, **II**–**IV**), which are combinations of TSA and hydroxamic acid-related phosphinates with α_1-substituent. These are able to form, together with P-OH, a bidente coordination to the zinc atom.[3]

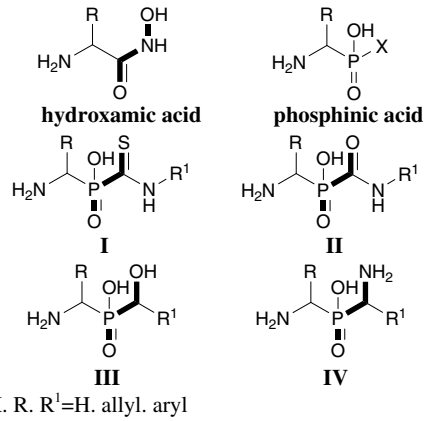

Figure 1. Comparison of the general structures of hydroxamic acid and hydroxamic acid-related phosphinates.

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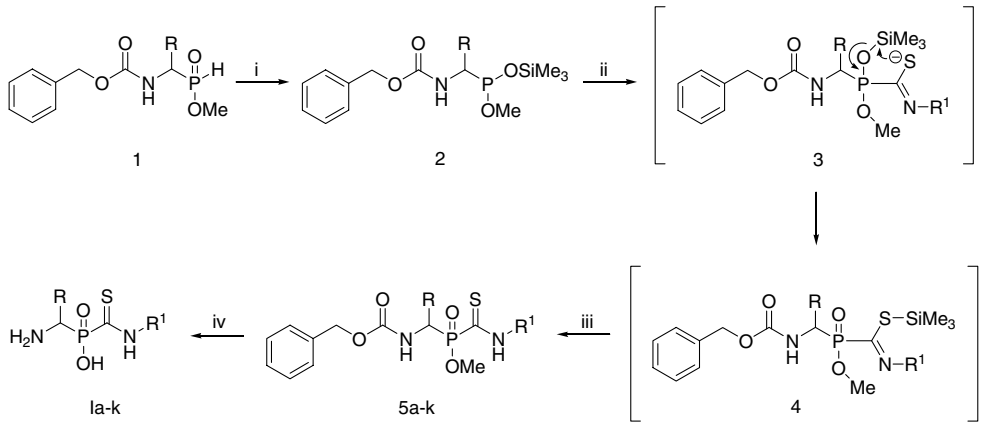


Scheme 1. Preparation of compounds **Ia–k** with the proposed reaction mechanism. Reagents and conditions: (i) TMSCl, TEA, DCM, Ar atm, rt; (ii) isothiocyanate; (iii) H^+/H_2O; (iv) 1—HBr/AcOH, 2—propylene oxide.

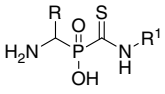
Here, we report the synthesis of a new member of such a family of compounds, the α-aminoalkyl-(N-substituted)thiocarbamoyl-phosphinic acids (**I**).[4] Our first target is aminopeptidase N, identical to CD13 (APN/EC 3.4.11.2) a type II membrane-bound metalloproteinase present on various cell types.[5] APN is a new emerging target for anti-cancer therapy, and recent studies suggest that inhibition of APN/CD13 by APN inhibitors or siRNA leads to suppressed progressive potential in ovarian carcinoma cells.[6] Bestatin (ubenimex) and curcumin, known inhibitors of APN, are compounds with well-established anti-cancer properties.[7] Curcumin now considered by oncologists as a potential cancer chemopreventive agent[8] is an irreversible inhibitor of APN.[9]

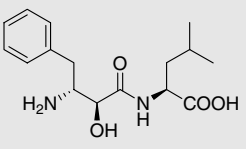
Nucleophilic addition of phosphites to isothiocyanates, to produce phosphonothiocarbamoyl derivatives (phosphonothioformic acid amides) is a well-known reaction.[10] A similar reaction to synthesize aryl-(N-substituted)thiocarbamoyl-phosphinates was reported recently.[11] Due to the low electrophilicity of the isothiocynates, we have applied an additional activation of phosphorus III nucleophile by silylation.

The synthetic route shown in Scheme 1 starts with α-*N*-benzyloxycarbonylamino-alkylphosphinates(H) **1**, which are esterificated with trimethylchlorosilane.[12]

Crude esters **2** undergo addition reaction with isothiocyanates to give an intermediate product which, after hydrolysis, gives the desired final product **I**. The proposed mechanism of addition could involve a S-sililated iminothioether intermediate **4**. The final α-aminoalkyl-(N-substituted)thiocarbamoyl-phosphinic acids **I** are quite stable because they survive the acidic removal of *N*-benzyloxycarbonyl moiety, as well as the selective basic hydrolysis of the alkyl phosphinic ester bond (data not shown for the latter). However, due to low yields for the overall synthesis process, some of the decomposition cannot be excluded. Nevertheless, compounds **Ij**, incubated in the water (pH 2.0; 4.0; 7.2 and 9.15, 20% DMSO-d_6) show no change in ^{31}P NMR spectrum after 72 h. The IC_{50} values for inhibition of APN[13] are shown in Table 1. The new α-aminoalkyl-(N-substituted)thiocarbamoyl-phosphinic acids **Ia–Ik** are moderate to good inhibitors of aminopeptidase N. Even the short preliminary series of compounds show a clear structure–activity relationship, as demonstrated by the data in Table 1. The preferred R group, probably interacting with S1 subsite of APN within this series of derivatives is a long aliphatic chain corresponding to the norleucine side chain (e.g., **Ig** and **Ih**). The

Table 1
Structures and IC_{50} values for the inhibition of aminopeptidase N by thiocarbamoyl-phosphinic acid derivatives **I** and bestatin



Compound	R	R^1	IC_{50},[11] (μM)
Ia	$-CH_3$	$-CH_2CH_2C_6H_5$	4.20
Ib	$-CH_3$	$-CH_2(p\text{-}OCH_3\text{-}C_6H_4)$	1.12
Ic	$-CH_2CH_2CH_3$	$-CH_2CH_2C_6H_5$	2.28
Id	$-CH_2CH_2CH_3$	$-CH_2(p\text{-}OCH_3\text{-}C_6H_4)$	0.68
Ie	$-CH(CH_3)_2$	$-CH_2CH(CH_3)_2$	1.90
If	$-CH(CH_3)_2$	$-CH_2(CH_2)_4CH_3$	1.18
Ig	$-CH_2(CH_2)_2CH_3$	$-CH_2CH_2C_6H_5$	0.73
Ih	$-CH_2(CH_2)_2CH_3$	$-CH_2(p\text{-}OCH_3\text{-}C_6H_4)$	0.25
Ii	$-CH_2CH(CH_3)_2$	$-CH_2(CH_2)_4CH_3$	0.50
Ij	$-CH_2CH(CH_3)_2$	$-CH_3$	10.56
Ik	$-CH_2CH_2C_6H_5$	$-CH_2(p\text{-}OCH_3\text{-}C_6H_4)$	0.38
7			2.10

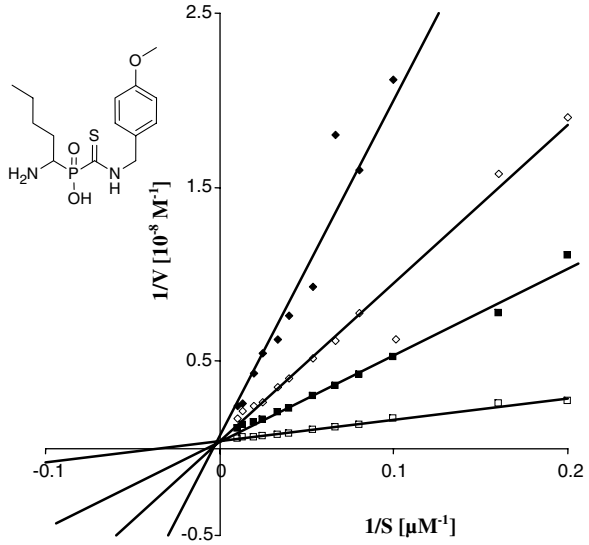


Figure 2. Lineweaver–Burk plot for compound **Ih** (K_i = 0.143 μM).

best moiety in the R^1 position, interacting probably with the S1′ subsite of the enzyme, is *p*-methoxybenzyl, for example, **Ih** and **Ik**. The combination of the two gives the best inhibitor, **Ih**, with IC_{50} = 0.25 μM (K_i = 143 nM, see Fig. 2) as the racemic mixture. It is interesting to see the clear additive effect of both optimal substitutions at the S1 and S1′ subsite of the enzyme. This could suggest an easier development of future inhibitors. The thiocarbamoyl-phosphinates **Ia–Ik** are comparable to the previously reported,[3] corresponding oxo analogs, derivatives of carbamoyl phosphinates. Competitive inhibition of APN by **Ih** proves the binding to the active site of enzyme and the competition with substrate (Fig. 2). As reported here (as well as earlier),[3] the compound **Ih**, with K_i = 143 nM (Fig. 2), is the best inhibitor with the new thiocarbamoyl-phosphinate ZBG. Bestatin **7** has IC_{50} = 2.1 μM under the conditions of our assay. The compound **Ih**, even as racemic mixture, is almost ten times better an inhibitor than bestatin, an accepted anti-cancer drug, active in vivo. Therefore, it is a good candidate for the cell culture or in vivo studies to establish the role of APN activity in cancer development.

Acknowledgments

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12. General procedure for synthesis of compounds **5** (**a**–**k**). 1.6 mM of *N*-benzyloxycarbonyl-α-aminoalkylphosphinate methyl ester was dissolved in 5 ml of dry dichloromethane. In rt and Ar atm 2.4 mM TEA and 1.6 mM of TMSCl were added. After 30 min of stirring 3.2 mM of appropriate isothiocyanate was added. Reaction was stired overnight. 5 ml of saturated NH_4Cl was added. After 10 min mixture was dissolved in 20 ml of AcOEt and washed with saturated NH_4Cl, saturated $NaHCO_3$ and brine. The organic layer was dried and evaporated. The crude oil was purified by silica gel column chromatography using chloroform/ethyl acetate (2:1) as an eluent.
General procedure for synthesis of compound **I** (**a**–**k**). 0.5 mM of compound **5** (**a**–**k**) was dissolved in 5 ml 2% HBr/AcOH. After 2 h mixture was evaporated, dissolved in 1 ml MeOH and propylene oxide was added to pH 6. Compounds were crystallized as a white solid.
Compound **Ia**: yield 25.6%; $C_{11}H_{17}N_2O_2PS$; MW: 272.301/mol; LC–MS 273.2 (M+1); ^{31}P NMR (D_2O): 28.10; ^{1}H NMR (D_2O): 0.77 (dd, 3 H, *J* = 14.7; 7.2 Hz, CH_3), 2.65–2.70 (t, 2H, CH_2CH_2Ph) 2.79–2.84 (m, 1H, NH_2CHP), 3.49–3.64 (m, 2H, CH_2CSNH), 6.39–7.06 (m, 5H, Ar–H).
Compound **1b**: yield 55.6%; $C_{11}H_{17}N_2O_3PS$; MW: 288.31/mol; LC–MS 289.3 (M+1); ^{31}P NMR (D_2O): 28.16; ^{1}H NMR (D_2O): 0.90 (dd, 3H, *J* = 14.7; 7.5 Hz, CH_3), 3.00–3.05 (m, 1H, NH_2CHP), 3.54 (s, 3H, OCH_3), 4,45–4,50 (m, 2H, CH_2CSNH), 6.70–7.04 (m, 4H, Ar–H).
Compound **Ic**: yield 21.3%; $C_{13}H_{21}N_2O_2PS$; MW: 300.351/mol; LC–MS 301.3 (M+1); ^{31}P NMR (D_2O): 27.81; ^{1}H NMR (D_2O): 0.45–0.50 (t, 3H, CH_3), 0.78–0.92 (m, 2H, CH_2CH_3), 1.06–1.18 (m, 2H, CH_2CHP), 2.59–2.66 (m, 3H, NHCHP, CH_2Ph), 3.43–3.56 (m, 2H, CH_2CSNH), 6.88–7.00 (m, 5H, Ar–H).
Compound **Id**: yield 49.0%; $C_{13}H_{21}N_2O_3PS$; MW: 316.351/mol; LC–MS: 317.3 (M+1); ^{31}P NMR (D_2O): 27.89; ^{1}H NMR (D_2O): 0.62 (d, 3H, *J* = 7.2 Hz, CH_3), 1.06–1.08 (m, 2H, CH_2CH_3), 1.26–1.36 (m, 2H, CH_2CHP), 2.88–2.94 (m, 1H, NH_2CHP), 3.56 (s, 3H, $PhOCH_3$), 4.50 (d, 2H, *J* = 2.70 Hz, CH_2CSNH), 6.71–7.17 (m, 4H, Ar–H).
Compound **Ie**: yield 43.5%; $C_9H_{21}N_2O_2PS$; MW: 252.311/mol; LC–MS 253.2 (M+1); ^{31}P NMR (D_2O): 29.22; ^{1}H NMR (D_2O): 0.99–1.07 (m, 12 H, 4 × CH_3), 2.10–2.18 (m, 2H, 2 × CH), 3.23 (dd, *J* = 9.6; 4.2 Hz, 1H, NH_2CHP), 3.51–3.63 (m, 2H, CH_2NHCS).
Compound **If**: yield 46.2%; $C_{11}H_{25}N_2O_2PS$; MW: 280.361/mol; LC–MS 281.3 (M+1); ^{31}P NMR (D_2O): 26.61; ^{1}H NMR (D_2O): 0.78–0.81 (t, 3H, CH_2CH_3), 0.92 (dd, *J* = 29.7; 6.6 Hz, 6H, 2 × CH_3), 1.21–1.25 (m, 2H, $CH_2(CH_2)_2CH_3$), 1.28–1.31 (m, 4H, 2 × CH_2), 1.60–1.65 (m, 2H, CH_2CH_2NH), 2.01–2.04 (m, 1H, $CH(CH_3)_2$), 3.10 (dd, *J* = 9.0; 4.2 Hz 1H, NHCHP), 3.56–3.65 (m, 2H, CH_2NHCS).
Compound **Ig**: yield 63.3%; $C_{14}H_{23}N_2O_2PS$; MW: 314.411/mol; LC–MS: 315.3 (M+1); ^{31}P NMR (D_2O): 27.31; ^{1}H NMR (D_2O): 0.51–0.55 (t, 3H, CH_3), 0.85–1.28 (m, 6H, 3 × CH_2), 2.32 (d, 1H, *J* = 1.5 Hz, NH_2CHP), 2.59–2.67 (m, 2H, CH_2Ph), 3.44–3.47 (m, 2H, CH_2NHCS), 6.89–7.00 (m, 5H, Ar–H).
Compound **Ih**: yield 66.8%; $C_{14}H_{23}N_2O_3PS$; MW: 330.381/mol; LC–MS: 331.4 (M+1); ^{31}P NMR (D_2O): 27.90; ^{1}H NMR (D_2O): 0.48−0.52 (t, 3H, CH_3), 0.84–1.01 (m, 4H, 2 × CH_2), 1.08–1.16, (m, 2H, CH_2CHP), 1.26–1.35 (m, 2H, CH_2CHP), 2.76–2.81 (m, 1H, NH_2CHP), 3.48 (s, 3H, $PhOCH_3$), 4.40 (d, 2H, *J* = 2.70 Hz, CH_2CSNH), 6.62–7.00 (m, 4H, Ar–H).
Compound **Ii**: yield 43.7%; $C_{12}H_{27}N_2O_2PS$; MW: 294.391/mol; LC–MS 395.3 (M+1); ^{31}P NMR (D_2O): 27.23. ^{1}H NMR (D_2O): 0.77–0.78 (m, 9H, 3 × CH_3), 0.84 (d, *J* = 6.61 Hz, 6H, 2 × CH_3), 1.21–1.30 (m, 8H, 4 × CH_2), 1.59–1.64 (m, 2H, $CH_2CH(CH_3)_2$), 1.71–1.74 (m, 1H, $CH(CH_3)_2$), 3.14–3.18 (q, 1H, NH_2CHP), 3.57–3.65 (m, 2H, CH_2NHCS).
Compound **Ij**: yield 55%; $C_7H_{17}N_2O_2PS$; MW: 224.261/mol; LC–MS 225.1 (M+1); ^{31}P NMR (D_2O): 27.73; ^{1}H NMR (D_2O): 0.81 (dd, *J* = 35.8; 6.6 Hz, 6H, 2 × CH_3), 1.25–1.32 (m, 2H, CH_2), 1.69–1.74 (m, 1H, $CH(CH_3)_2$), 3.09 (d, 3H, *J* = 1.8 Hz, CH_3NHCS), 3.17–3.26 (m, 1H, NH_2CHP).
Compound **Ik**: yield 64.9%; $C_{18}H_{23}N_2O_3PS$; MW: 378.431/mol; LC–MS 379.5 (M+1); ^{31}P NMR (D_2O): 27.02; ^{1}H NMR (D_2O): 1.24–1.33 (m, 2H, CH_2CHP), 1.59–1.68 (m, 2H, CH_2CHP), 2.23–2.32 (m, 2H, CH_2CH_2Ph), 2.52–2.61 (m, 2H, CH_2CH_2Ph), 2.82–2.89 (m, 1H, NH_2CHP), 3.47–3.52 (t, 3H, $PhOCH_3$), 4.43–4.51 (t, 2H, CH_2CSNH), 6.58–7.06 (m, 9H, Ar–H).
13. *Aminopeptidase N inhibition studies.* The inhibitory effect of compounds **Ia–k** and bestatin towards aminopeptidase N (from porcine kidney, Sigma–Aldrich) was evaluated using Leu-AMC (Sigma–Aldrich) as a fluorogenic substrate. For the assay, sodium phosphate buffer (pH 7.2) was used. The final concentrations were 0.2 μg/ml for APN and 12.5 μM for substrate. All inhibitors was measured for 10 min at 25 °C without preliminary incubation, final DMSO concentration was 2%. All IC_{50} values presented in Table 1 are means of two experiments, and standard deviation is ±20%. All compounds are racemic mixture.