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Novel Matrix Metallo-Proteinase (MMP-2) Phosphonoboronate Inhibitors

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Abstract—A series of novel phosphonoboronates consisting of PC_1B, PC_nB, $PC(X)C_nB$, and PCC=CB derivatives were evaluated as MMP-2 inhibitors. Structure–activity relationships (SARs) data for the compounds were discovered and are discussed.

Introduction

Matrix metalloproteinases are a family of zinc-dependent, calcium endopeptidases that play a significant role in tissue remodeling both, in normal physiological and pathological processes. Over production or activity of MMPs has been implicated in a number of pathological processes, including rheumatoid arthritis, osteoarthritis, tumor growth and metastasis, degradation of aortic wall in aneurysms and other diseases.[1] MMP-2 is type IV collagenase (gelatinase) which is a key enzyme involved in basement membrane degradation. There is increasing evidence for a positive correlation between MMP-2 activity and tumor cell invasion.[2] Therefore, there is a growing number of attempts to design and develop active inhibitors that may restore the balance of MMPs in these pathological processes.[3]

The requirement for a molecule to be an effective inhibitor of the MMP is a functional group (e.g., carboxylic acid, hydroxamic acid, phosphonic acid, etc.) capable of chelating the active-site zinc(II) ion.[4] There are a number of problems with hydroxamate inhibitors: poor bioavailability and pharmacokinetics, highly toxicity. Modification of physical properties, particularly by introduction of non-peptide structures and replacement of the hydroxamate by other zinc ligands, for example by phosphonate, improved pharmacokinetics without a reduction in metabolism.[5]

The use of both boronates and phosphonates as transition state analogue inhibitors is well known.[6] In addition, boronates were slow to bind zinc to certain aminopeptidases by X-ray crystallography.[7] Phosphonates are known as a MMP inhibitors.[8] In the current communication, we tested for possible synergistic inhibition of MMP-2 with compounds that incorporate both boronate and phosphonate groups in the same molecule.

Materials and Methods

Biology

Cell culture. Human fibrosarcoma cells from ATCC were maintained in Minimal Essential Medium, supplemented with calf serum, 5%. Glutamine, piruvate, non-essential amino acids, vitamines and antibiotics (Biological Industries, Kibbutz Beth HaEmek, Israel) are added as additional supplements.

Basement membrane invasion. Boyden chamber chemoinvasion assays were performed as previously described. Matrigel (25 μg) was dried on a polycarbonated filter (PVP free, Nucleopore). Fibroplast conditioned medium (obtained from confluent NIH-3T3 cells

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cultured in serum free DMEM) was used as the chemoattractant. Cells were harvested by brief exposure to 1 mM EDTA, washed with DMEM containing 0.1% bovine serum albumin and added to the Boyden chamber (200,000 cells). The chamber was incubated in a humidified incubator at 37 °C in 5% CO_2/95% air atmosphere for 6 h. The cells which traversed the Matrigel layer and attached to the lower surface of the filter, were stained with Diff Quick (American Scientific Products) and counted.[2,9,10]

Chemotaxis. Chemotaxis evaluation were performed in a similar way to basement membrane invasion, with the exception that the filters were coated with 5 μg collagen IV instead of Matrigel. This amount of collagen does not form a barrier to the migrating cells but rather a attachment substratum.

Chemistry

Four groups of phosphonoboronate inhibitors were tested in this study (Fig. 1): (A) C1-bridged phosphonoboronates PC_1B **1–6**; (B) C_n-bridged phosphonoboronates PC_nB **7–8**; (C) Heteroatom substituted phosphonoboronates $PC(X)C_nB$ **9–11** and (D) unsaturated phosphonoboronates PCC=CB **12–13**. These newly prepared compounds contain pentacoordinated phosphorus and trigonal boron linked by a carbon or by chain. Since they contain oxygenated phosphorus and boron moieties they are more stable than their nonoxygen derivatives.

Synthesis

C1 bridged phosphonoboronates **1–6** were prepared from primary or secondary iodoboronates by Arbuzov reaction with trimethylphosphite.[11] Hydrolysis of the phosphonate esters by Me_3SiBr followed by treatment with methanol gave the free acid derivatives. C_n bridged phosphonoboronates **7–11** were prepared by hydroboration of alkenyl phosphonates.[12]

Alkenylphosphonoboronate **12** was prepared by hydroboration of propargyl bromide followed by Becker reaction (Scheme 1), in good overall yield. Compound **13** was obtained by the hydrolysis of the phosphonate ester moiety as described above.[13]

Control compounds used in this study are shown in Figure 3.

Results and Discussion

Compounds **1–13** and control compounds **14–17**, were tested in vitro for inhibition of MMP-2 protease. The results are summarized in Table 1 and Figure 2A–D. The activity of the phosphonoboronates was compared with control non-boronate containing compounds (Fig. 3). Generally, phosphonoboronates showed promising activity as MMP-2 inhibitors. Since MMP-2 is a zinc-containing protein, we believe that the boronate group can act as a zinc chelator and increase the activity. In the C1 bridged compounds, only the free phosphonic acids were active. Neither compound **3** (free acid) nor **1** (with phosphonate and boronate esters) showed activity. We also observed a similar trend for the C_n bridged compounds (**7**, **8**, Fig. 2A).

Increasing the chain length of the carbon chain between boronate and phosphonate (**7**, **8**, Fig. 2A) did not result

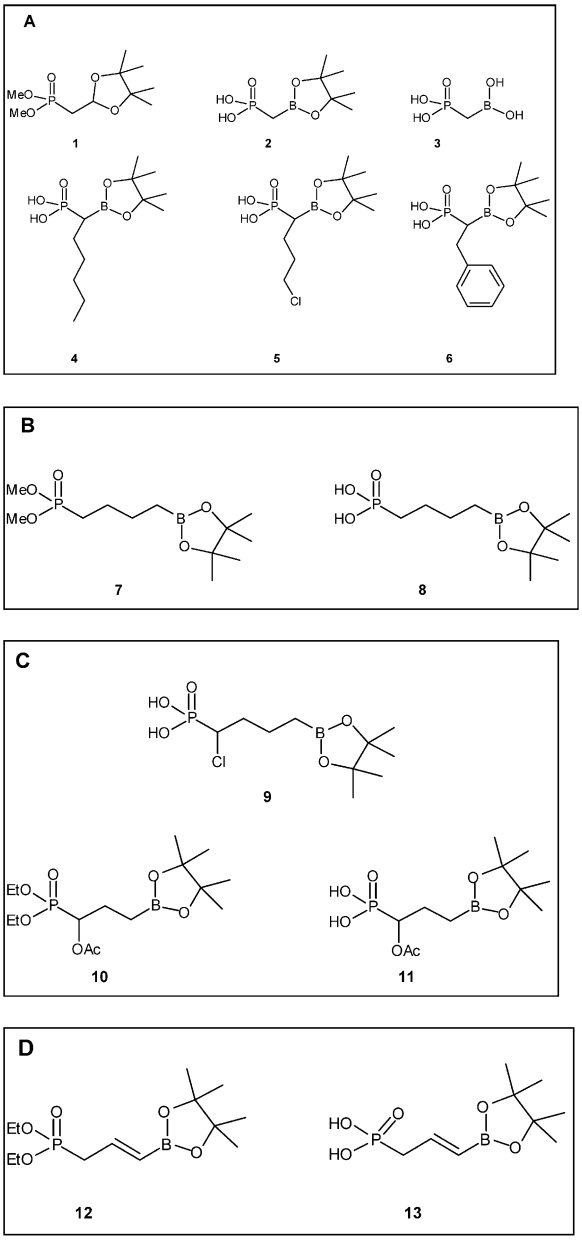
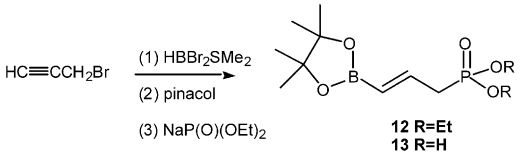


Figure 1. Structures of phosphonoboronate inhibitors: (A) PC_1B inhibitors; (B) PC_nB inhibitors; (C) $PC(X)C_nB$ inhibitors; (D) PCC=CB inhibitors.



Scheme 1. Synthesis of alkenylphosphonoboronates **12**, **13**.

in an increase of activity compared to PC_1B compounds (Table 1). The C_n bridged phosphonoboronate **11**, which contains an acetate group α to the phosphonate was more active than the chloride derivative, **10** (Fig. 2B).

Introduction of a double bond dramatically increased inhibition of MMP-2 protease, either as the free phosphonic acid or the phosphonate ester (**12**, **13**, Fig. 2C). Among the tested compounds, alkenylphosphonate **13** was identified as a most potent inhibitor of MMP-2. While the phosphonate group was not necessary for activity, compounds without the boronate group were inactive. Thus, allylphosphonate, **16** (Fig. 2D) was not active, but butenyl boronate, **17** (Fig. 2D) showed much better inhibitor activity.

It is known that boronic acids and their esters possess similar serine protease inhibitory activity.[14] We observed that the pinacol phosphonoboronates are more active than the free acids. This can possibly be explained by oligomer formation by association between the OH groups on boron and the OH groups of the free phosphonic acid. Boron readily forms association complexes with hydroxyl groups, particulary with polyols.[15] We investigated this phenomenon by ^{11}B NMR.[16] At −40 °C, compound **3** showed a broad singlet at +31 ppm. Raising the temperature to 25 °C caused collapse of the singlet into two peaks at +31.9 and 30.3. The reaction is reversible. Thus the decreased activity we observed with **3** may be due to association. In this connection, we have observed that free

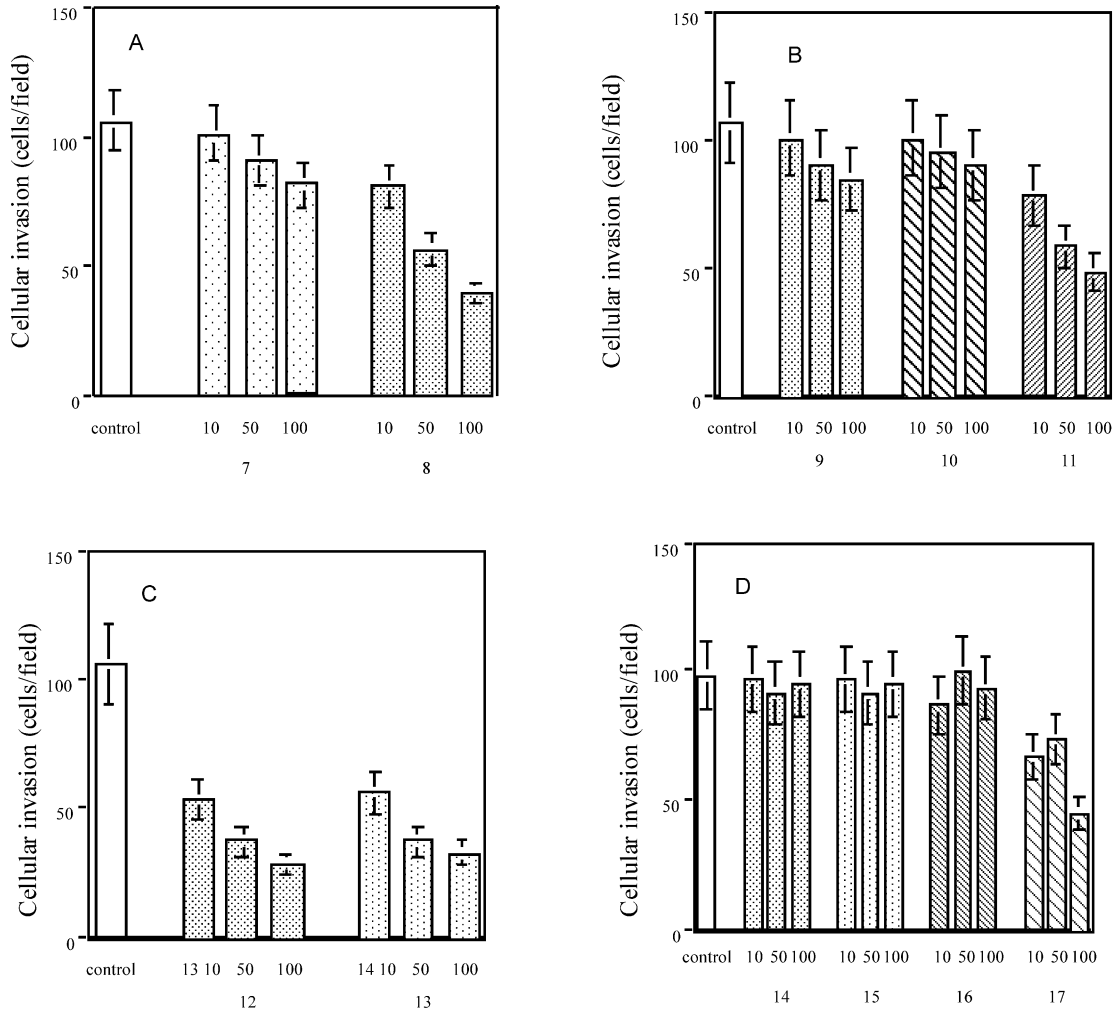


Figure 2. The effect of phosphonoboronates on cellular invation of HT-1080 human fibrisarcoma cells: (A) PC_nB inhibitors; (B) $PC(X)C_nB$ inhibitors; (C) PCC = CB inhibitors; (D) controls. All concentrations in μM.

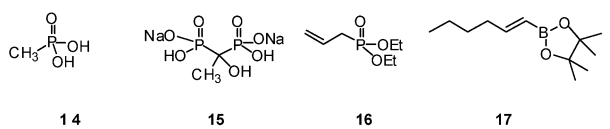


Figure 3. Control compounds.

Table 1. Inhibition activity of PC_1B phosphonoboronates

Compd	% inhibition at 50 μM
1	0
2	50
3	0
4	50
5	55
6	40

butylboronic acid is as active as pinacolbutylboronate (~50% inhibition at 50 μM), which indicates that the OH groups of free phosphonic acids decrease the activity of the boronic acid derivatives.[16] Alternatively, the fact that the boronates in this study were more active than the free boronic acids could also be explained by the hydrophobic influence of the pinacol moiety. SAR data for MMP-2 and MMP-9 gelatinases showed that these enzymes tolerate hydropohobic side chains of the inhibitor at the active site.[17]

Conclusions

Novel non-toxic phosphonoboronates were evaluated for their inhibitor activity of MMP-2 protease. Most of these compounds showed moderate activity ~50% inhibition at 50 μM. Alkenyl phosphonoboronate **13** showed significant activity against MMP-2 (~75% inhibition at 50 μM). The exact mechanism of action of phosphonoboronates as MMP-2 inhibitors has not been determined. A zinc chelation mechanism and/or a transition state analogue mechanism could be proposed. The novel inhibitors may provide new effective approaches for treatment of tumor growth and malignant dissemination of cancer cell.

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13. **Preparation of 12**: A 1 M solution of $HBBrSMe_2$ in dichloromethane (20 mL, 20 mmol) was added to a solution of propargyl bromide (3 mL, 10 mmol) in dry dichloromethane (20 mL) at 0 °C and the reaction mixture was stirred at room temperature for 6 h. Pinacol (1.2 g, 10 mmol) dissolved in dry dichloromethane was added at 0 °C and the reaction stirred at room temperature for an additional 1 h. Dichloromethane was then removed under reduced pressure and water (10 mL) was added to the residue. Diethyl ether (50 mL) was added to extract the product. The organic layer was dried on sodium sulfate, evaporated and the residue was distilled to yield the product of hydroboration, 1-bromo-2-propenyl pinacol boronate as colored liquid (77% yield).

Sodium (230 mg, 10 mmol) was added to a solution of diethylphosphite (1.38 g, 10 mmol) in THF (1 mL). When reaction was complete, it was cooled to −70 °C and a solution of 1-bromo-2-propenyl pinacol boronate (2.5 g, 10 mmol) in THF (2 mL) was added in one portion. The mixture was warmed to room temperature and stirred for 5 h. Then water (20 mL) was added followed by ethyl acetate (2×50 mL). The organic layer was dried on sodium sulfate and evaporated to obtain **12** as colorless oil (85% yield). NMR data of **12**: ^{1}H NMR δ, ppm: 1.29 (s, 12H), 1.29 (t, 6H), 2.79–2.81 (dd, 2H), 4.08 (q, 4H), 5.75 (d, 1H), 6.88 (m, 1H). ^{13}C NMR ($CDCl_3$) 16.43, 24.93, 33.73, 61.50, 83.24, 123.72 (br), 146.91. ^{31}P NMR: 17.51, ^{11}B NMR: 31.66.

Preparation of 13: $BrSiMe_3$ (5 equiv) was added in one portion to a solution of **12** in dried CH_2Cl_2. The reaction mixture was stirred for 1 h and methylenedichloride was then removed in vacuo. The residue was treated with MeOH and the solution was stirred about 10 min followed by evaporation to give **13** as a colored oil (89% yield).
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