Bioorganic & Medicinal Chemistry Letters 24 (2014) 2617–2620

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Dual carbonic anhydrase/matrix metalloproteinase inhibitors incorporating bisphosphonic acid moieties targeting bone tumors

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ARTICLE INFO

Article history:
Received 31 January 2014
Revised 15 April 2014
Accepted 21 April 2014
Available online 30 April 2014

Keywords:
Carbonic anhydrase
Enzyme inhibitor
Bisphosphonate
Bone resorption inhibitor
Matrix metalloproteinase inhibitor

ABSTRACT

A set of bisphosphonate matrix metalloproteinase (MMP) inhibitors was investigated for inhibitory activity against several carbonic anhydrase (CA, EC 4.2.1.1) isozymes, some of which are overexpressed in hypoxic tumors. Some of the bisphosphonate revealed to be very potent inhibitors (in the low nanomolar range) of the cytosolic isoform CA II and the membrane-bound CA IX, XII and XIV isozymes, a feature useful for considering them as interesting compounds for bone resorption inhibition applications. We suggest here that it is possible to develop dual enzyme inhibitors bearing bisphosphonate moieties that may target both MMPs and CAs, two families of enzymes involved in tumor formation, growth, and metastasis.

Every year, about 100,000 Americans with prostate or breast primary cancer realize that the malignancy has spread to their bones. Bone metastasis present different features compared to primary bone cancer, and it is actually much more common, especially in adults. Once they have reached the bone, cancer cells undergo through more changes to avoid the attack of the immune system. This means the new tumor becomes different from the primary tumor and this can make it more difficult to treat.

Bone remodeling is a delicate balance between bone matrix synthesizing osteoblasts and bone resorbing osteoclasts activities, and the presence of active bone metastases typically subvert this process to generate lesions that include extensive areas of pathological osteogenesis and osteolysis.[1] Osteoclasts have developed efficient and unique machinery for dissolving mineral and degrading organic bone matrix. The osteoclastic bone resorption cycle consists in a first step of adhesion to bone surface, followed by secretion of protons into an extracellular compartment formed between osteoclast and bone surface, bone mineral solubilization, digestion of organic bone matrix by acid proteases and rejection of waste products. Inorganic mineral dissolution requires intense acidification of the resorption site between the ruffled border and the bone surface; an acidic pH is also necessary for the digestion of the organic bone matrix by proteolytic enzymes secreted by osteoclasts.

Carbonic anhydrases (CAs, EC 4.2.1.1) and matrix metalloproteinases (MMPs) both represent key regulators of this process.[2,3]

CAs are a family of 16 enzymes that catalyze the inter-conversion of carbon dioxide and bicarbonate in order to maintain the required pH in biological fluids. CAs are involved in many physiological and pathological processes, including respiration and transport of CO2 and bicarbonate between metabolizing tissues and lungs, pH and CO2 homeostasis, electrolyte secretion in various tissues and organs, biosynthetic reactions, bone resorption, calcification and carcinogenesis.[4] CA II generates the primary source of protons for the vacuolar proton pump in osteoclast cytoplasm and there is clear evidence that it is involved in bone resorption. CA II is also critically involved in osteoclast differentiation and individuals with CA II deficiency, a rare genetic condition, show a decreased bone resorption rate and clinical osteopetrosis.[5] Membrane-bound carbonic anhydrase isoenzymes (CA IV, CA IX, CA XII and CA XIV) may compensate for the lack of cytoplasmic carbonic anhydrase II, and they also could have significant effects on osteoclast physiology.[2] Many of the CA isoforms are important therapeutic targets, whose inhibition is a possibility to treat a range of disorders, including osteoporosis, through reduction of osteoclast activity and subsequently bone resorption.[6,7]

Matrix metalloproteinases play a critical role in bone remodeling and tumor invasion.[8,9] Pre-clinical experiments have reported the efficacy of broad spectrum matrix metalloproteinase inhibitors

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http://dx.doi.org/10.1016/j.bmcl.2014.04.077
0960-894X/

(MMPIs) in preventing the skeletal related events secondary to prostate and breast tumors evolution.[3,8]

MMPs are often expressed in the rapidly remodeling metastatic tumor-bone microenvironment and can be derived from multiple cellular sources. In addition to their roles in the extra cellular matrix remodeling, MMPs should be considered as key for cell–cell communication in the tumor-bone microenvironment given their ability to regulate the bioactivity and bioavailability of factors such as PTHrP, RANKL and TGFβ that are traditionally associated with driving the 'vicious cycle' occurring between tumor metastases growth and bone remodeling.[3]

In particular, MMP-2 processes the proform of IL-1β into a soluble active form and this form mediates osteoclastogenesis in an IL-6 dependent manner,[10] the same enzyme mediates the release of TGFβ.[11] Moreover, MMP-2 has been described as generating functional endothelin-1 in the tumor-bone microenvironment[12] leading to the formation of osteogenic metastases.

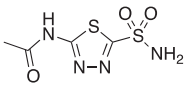
Therefore, the selective inhibition of MMP-2 should allow for the successful treatment of patients with debilitating bone metastases without the side effects noted with broad spectrum inhibitors.

In recent papers we reported a series of matrix metalloproteinases inhibitors (MMPIs) characterized by a bisphosphonic moiety (BP-MMPIs), a well known bone seeking moiety, as zinc binding group (ZBG).[13–15] The benefits deriving from the introduction of this ZBG are twofold. Firstly, it realizes an efficient interaction with the catalytic zinc ion, inhibiting the proteolytic activity of matrix metalloproteinases involved in several pathological conditions. Secondly, the molecules are more effective towards MMPs involved in the remodeling cycle, since bone targeting concentrates the pharmacological agents at the desired active site allowing a more potent effect without increasing the administered doses.[16]

Both CAs and MMPs are zinc enzymes possessing isoforms in the extracellular environment; therefore, it is not surprising that they may be inhibited by similar compounds with zinc-binding functionalities.[17–23] It follows that the anti-resorptive activity that we have reported recently for some BP analogs[15] could be originated from the simultaneous inhibition of both CA and MMP enzymatic systems. For this reason it is of interest to examine in detail such BP derivatives as CA inhibitors. Increasingly, it has been recognized that a balanced modulation of several targets can provide superior therapeutic and side effect profiles compared to the action of a selective activity.[24,25]

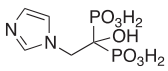
Inhibitors with a combined action against MMPs and CAs were reported by several authors as anti-metastatic agents, but they incorporated hydroxamate,[26–28] hydroxypyrimidinone[29] or carbamoylphosphonate[30] moieties as ZBG. At the best of our knowledge, only methylene diphosphonic acid (MDPA)[31] and aniline bisphosphonates[32] have been reported so far as inhibitors of CAs bearing a bisphosphonic core as ZBG (BP-CAIs).

In this Letter, we describe the inhibitory properties of zoledronate, currently the most potent BP in the treatment of bone diseases, and of a series of previously reported BP-MMPIs (**1**–**14**),[15] against the cytosolic carbonic anhydrase CA II and three membrane-bound CAs (CA IX, XII and XIV) due to their involvement in the osteoclasts activity, and on the cytosolic CA I (Table 1).[32] The standard, clinically used, CA inhibitor (CAI) acetazolamide (AAZ) has been used as reference compound.

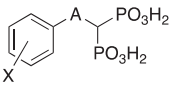


Acetazolamide

MDPA



Zoledronate



1-14

As compared with the reference inhibitor, our best compounds reveal similar activities against CA II, IX, XII and XIV, but result quite inactive against the ubiquitous isozyme CA I not involved in the osteoclasts activity. Furthermore, they result at least 1000-fold more potent than MDPA.[31] Zoledronate shows a high inhibitory activity against CA II, XII and XIV, with IC_{50} in nanomolar range (62–316 nM), whereas it results less active against CA IX and I (IC_{50} = 5 and >10 μM, respectively).

The following should be noted regarding the inhibition data of Table 1.

Table 1
Inhibition data for bisphosphonates **1**–**14** and zoledronate against MMP-2[15] and human CA isoforms I, II, IX, XII and XIV by a stopped-flow CO_2 hydrase assay[32]

Compd	X	A	IC_{50}* (μm)					
			CA I	CA II	CA IX	CA XII	CA XIV	MMP-2
	AAZ		0.299	0.023	0.903	0.088	0.224	
	MDPA		A[31]	1250[31]	860[31]	—	—	49
	Zoledronate		>10	0.062	5.323	0.316	0.092	7.0
1	H	SO_2NH	>10	>100	0.397	0.855	3.31	15.8
2	4-Cl	SO_2NH	>10	6.16	0.095	0.464	6.59	9.5
3	4-Br	SO_2NH	>10	>10	0.234	0.062	0.898	4.8
4	4-Br	CONH	>10	>10	>10	0.278	6.05	30
5	4-Br	SO_2	>10	>10	>10	0.225	6.05	52
6	4-CH_3	SO_2NH	>10	>100	0.200	0.461	0.541	18
7	4-CH_3O	SO_2NH	>10	>10	0.090	0.376	0.516	19
8	4-NO_2	SO_2NH	>10	1.83	0.078	0.092	0.092	4.9
9	3-NO_2	SO_2NH	>10	3.82	2.670	0.794	>10	16
10	4-C_6H_5	SO_2NH	6.80	1.41	0.347	0.386	0.729	0.14
11	4-(2-Thienyl)	SO_2NH	6.17	0.504	0.601	0.087	0.593	0.23
12	4-(4-Cl-C_6H_4)	SO_2NH	1.75	2.66	6.131	0.476	2.44	0.037
13	4-(4-CH_3O-C_6H_4)	SO_2NH	0.041	0.070	0.084	0.076	0.070	0.11
14	4-(4-NO_2-C_6H_4)	SO_2NH	9.03	0.071	0.596	0.065	0.082	0.192

A = activator.
* Errors in the range of 5–10% of the reported value (from three different assays).