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A patent review on cathepsin K inhibitors to treat osteoporosis (2011 – 2021)

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Abstract

Introduction: Cathepsin K (CatK) is a lysosomal cysteine protease and the predominant cathepsin expressed in osteoclasts, where it degrades the bone matrix. Hence, CatK is an attractive therapeutic target related to diseases characterized by bone resorption, like osteoporosis.

Areas covered: This review summarizes the patent literature from 2011 to 2021 on CatK inhibitors and their potential use as new treatments for osteoporosis. The inhibitors were classified by their warheads, with the most explored nitrile-based inhibitors. Promising in vivo results have also been disclosed.

Expert opinion: As one of the most potent lysosomal proteins whose primary function is to mediate bone resorption, cathepsin K remains an excellent target for therapeutic intervention. Nevertheless, there is no record of any approved drug that targets CatK. The most notable cases of drug candidates targeting CatK were balicatib and odanacatib, which reached Phase II and III clinical trials, respectively, but did not enter the market. Further developments include exploring new chemical entities beyond the nitrile-based chemical space, with improved ADME and safety profiles. In addition, CatK's role in cancer immunoexpression and its involvement in the pathophysiology of osteo- and rheumatoid arthritis have raised the race to develop activity-based probes with excellent potency and selectivity.

Keywords: cathepsin K inhibitors, cysteine protease inhibitors, osteoporosis, odanacatib, nitriles, activity-based probes

Article highlights:

- Cathepsin K is one of the most attractive targets associated with bone-related diseases.
- Selectivity over other cathepsins can be a problem and needs further attention in developing new inhibitors.
- Derivatives of odanacatib and balicatib are still of interest to inhibit CatK.
- The chemical diversity has increased through the last decade by introducing new classes of warheads to achieve high potency and selectivity for CatK.
- The use of a new activity-based probe as a CatK substrate is described for monitoring abnormal bone resorption.

1. Introduction

Cathepsin K (CatK) is a lysosomal cysteine protease that belongs to the human cathepsin family; an eleven-membered family categorized according to their structures and catalytic mechanisms. The cysteine cathepsin family possesses a standard papain-like structure with a conserved active site region with a Cys-His-Asn catalytic triad (Figure 1a) [1]. Cathepsins exist in several living organisms, such as bacteria, viruses, plants, and animals. They are known to be involved in a variety of diseases, such as many types of cancer [2–4], autoimmune disorders [5], and bone-related diseases [6].

The active site of CatK consists of 4-four well-characterized subsites (S1', S1, S2, S3 Figure 1b), which are explored to design new ligands. These inhibitors may or may not present selectivity since the subsites share a high degree of structural homology with other cathepsins [7]. The S1 subsite contains the catalytic triad (Cys25, His162, and Asn182) responsible for the mechanism of action of the enzyme. The S2 pocket of CatK is the smallest among the enzymes of the C1 family, mainly because of the Tyr67 and Leu209 residues. The S3 subsite is often used to achieve selectivity over other cathepsins and is shaped by the Asp61 residue [7].

Consequently, inhibitors are designed to interact with CatK subsites to achieve high potency and selectivity over other cathepsins. The use of peptidomimetic compounds is a common approach, in which a reactive group, usually called warhead, is commonly linked to it (Figure 1c) [8,9]. The warhead is a highly electrophilic moiety that participates in a nucleophilic attack promoted by the thiolate from the Cys25 residue, forming a covalent bond between the inhibitor and the enzyme. The other moieties of the inhibitors labelled as P1, P2, and P3 will participate in noncovalent interactions with the respective residues present in the S1, S2, and S3 subsites.

Figure1

Interestingly, CatK is the only cathepsin highly expressed in osteoclasts, where the enzyme is present in the lysosome and cytoplasmic vesicles [10]. Activated osteoclasts widely secrete it to degrade the bone matrix, primarily type I collagen protein, constituting approximately 90% of the organic bone matrix [6]. The enzyme can also degrade type II collagen, the main matrix protein in cartilage [11]. Research

with murine models reinforces the critical role of CatK in bone resorption[12–14]. Studies showed that mice with CatK deficiency could develop osteopetrosis of the long bones, in which inefficient osteoclasts activity was observed [13]. Additionally, a recent study showed that osteocytes could also express and secrete CatK, required for lactation-induced peri-lacunar resorption, to assure the right amounts of calcium in milk and aid skeletal development in offspring [15]. Therefore, the enzyme has become an attractive and essential biological target for treating bone-related diseases, primarily osteoporosis [8,16,17], which will be discussed throughout this review. Furthermore, it is essential to mention that despite CatK's role in osteoporosis, its implication goes beyond as the enzyme is also expressed in other cell types [16], which makes the protein a promising target for many diseases, such as diabetes [18], obesity [19], and some types of cancer [20,21]. Additionally, pycnodysostosis [22], that is a rare autosomal recessive disorder, which is caused by inactivating mutations in CatK expressed in a wide range of non-bone cells, to which more research needs to be devoted.

Osteoporosis is an age-related systemic skeletal disease characterized by decreased bone mass and microarchitectural deterioration of bone tissue, resulting in fragile bones and susceptibility to fracture [23]. The disease is caused by an imbalance between bone resorption and bone formation, affecting about 200 million people worldwide, mainly postmenopausal women [24,25]. To date, there are multiple classes of drugs to treat osteoporosis, such as hormone replacement therapy [26], calcitonin [27], Denosumab (RANKL inhibitor) [28], Romosozumab (a new monoclonal antibody that binds sclerostin) [29], and bisphosphonate drugs (BPs) [30]. However, the use of these drugs can lead to several side effects; for example, the use of BPs and Romosozumab is associated with hypocalcemia, impairment of renal function, flu-like symptoms, and ocular inflammation, and severe side effects such as osteonecrosis of the jaw, which is more common in immunosuppressed patients [31–35]. It is, nevertheless, a matter of concern that the medical-related osteonecrosis of the jaw (MRONJ) condition, caused by powerful BPs direct toxicity to bone and soft tissue cells, is fostered by treatment duration and concomitant oral surgery [36]. Fortunately, there is evidence of the use of intra-oral formulation of low potency BPs (lpBP) to dramatically reduce the osteocyte necrosis area when locally administered in Zoledronate-pretreated mice [37]. Therefore, beyond the need for new drugs to treat osteoporosis where CatK

inhibitors can play a central role as new therapies for this disease, its role in patients with MRONJ needs to receive further dedicated studies [38].

An analysis of the Google Scholar database showed that research involving the treatment of osteoporosis by targeting CatK has a yearly average of 469 papers published between 2000 and 2010. This value increased to an average of 922.8 articles per year in the following decade (2011 – 2021), indicating a compelling research topic. CatK has 65 crystal structures published in the Protein Data Bank (RCSB PDB). All structures containing inhibitors are with covalent ones, in which a wide range of warheads can be found. In a survey of the ChEMBL database [39], we have found 1799 compounds tested against human CatK (ChEMBL268), with 1450 of them having a pIC_{50} or pK_i equal to or higher than six. A search in the Google Patents database showed that approximately 5000 patents related to CatK inhibition were submitted during the last twenty years

Despite all the effort made in the subject, there are no CatK inhibitors approved for the treatment of osteoporosis, mainly due to harsh side effects and selectivity issues related to other cathepsins. There are up to eight clinical trials focusing on CatK inhibition, of which four are related to the treatment of osteoporosis with the compounds Odanacatib, Balicatib, ONO-5334, and Relacatib [7]. Promising therapeutics such as ONO-5334 (Figure 2a) developed by ONO Pharmaceutical Co., Ltd. (Japan) reached phase II clinical trials. The drug is a non-lysosomotropic hydrazine-based inhibitor with high potency against CatK and moderate selectivity towards Cathepsins B, L, and S. ONO-5334 was able to significantly increase bone mineral density (BMD) and reduced urine collagen degradation markers in postmenopausal women, with a similar effect seen in BPs [40,41]. Despite the lack of clinically relevant safety concerns, the drug was discontinued due to competitiveness-related problems. Balicatib (AAE581) (Figure 2b), developed by Novartis Pharmaceuticals S.A. (Switzerland), is a lysosomotropic reversible covalent peptidomimetic compound that also reached phase II clinical trials. The compound exhibits significant efficacy in *in vitro* and *in vivo* assays of bone resorption. A study with ovariectomized monkeys demonstrated that periosteal bone formation rates were increased during treatment with Balicatib despite a significant decrease in bone turnover [42]. Unfortunately, during phase II clinical trials, patients developed morphea-like skin lesions when a dose of 50 mg/day was administered [43], leading to the discontinuation of the studies.

Odanacatib (ODN, MK-0822) (Figure 2c) is the only CatK inhibitor to achieve phase III clinical trials. ODN is a selective and reversible nitrile-based CatK inhibitor developed by Merck & Co. (USA). In a phase III study with postmenopausal women, called Long-term Odanacatib Fracture Trial (LOFT), the drug was able to reduce serum (CTx) and urine markers (NTx) of bone resorption by more than 50% 24 hours after administration of the drug. Hence, ODN significantly reduced the risk of vertebral and hip fracture due to a possible increase in the BMD [44]. Despite the excellent results, ODN treatment presented severe side effects, such as the increased risk of strokes and atrial fibrillation, which led to the discontinuation of the trial.

Figure2

In 2011, a review of patents describing CatK inhibitors (2004 – 2010) was published [45] in which all the inhibitors found were derived from low molecular weight peptides that displayed reversible binding. Most of them carried a nitrile as a warhead, probably influenced by Balicatib and Odanacatib studies since, at that time, the two compounds were entering phase II and III clinical trials, respectively. This article aims to review patents that address novel cathepsin K inhibitors developed in the last ten years focusing on potential new treatments for osteoporosis. Patents filed between January 2011 and August 2021 were analyzed, following preceding articles on the subject [45,46].

2. Covalent Inhibitors

Covalent inhibitors (CIs) are frequently small molecules that bind to a target enzyme by forming a covalent bond [47]. CIs can act through a reversible or irreversible mechanism depending on their electrophilic functional group [48]. Due to the stronger character of covalent bonds, drugs that display a covalent inhibition possess many advantages over traditional noncovalent drugs, such as higher efficiency, longer residence times, drug resistance rate, and less-frequent dosing [47,49]. Despite being avoided by the pharmaceutical companies until the past decade due to concerns of side effects, the scenario has changed a lot since then, with over 50 covalent drugs currently approved by the FDA [49].

To inhibit CatK, the relevance of covalent ligands that display a reversible inhibition mechanism is well known. This can be seen in patents filed in the last ten years, of which the majority presents reversible inhibitors with a nitrile as a warhead, most of them being ODN derivatives. Interestingly, most of these patents belong to Merck & Co. (USA), the assignees of the ODN patent, and Asian pharmaceutical companies.

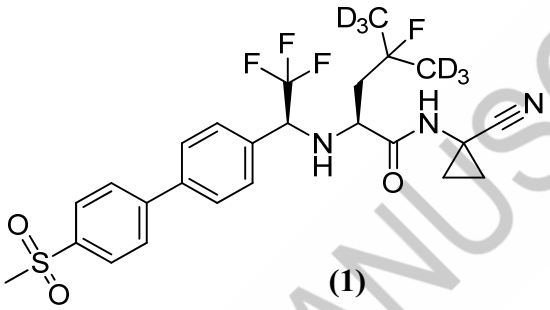
2.1. Nitrile-Based Inhibitors

Nitriles are widely used as warheads in the medicinal chemistry field. The chemical group is considered a bioisostere of carbonyl, hydroxyl, and carboxyl groups, due to the capability of the nitrogen atom to act as a hydrogen bond acceptor [50]. In addition, nitriles have a linear shape that can enable a better fit into the protein-binding site [51].

Inhibitors such as ODN provide an indispensable guide for developing and designing novel anti-resorptive drugs and studies related to new formulations. Mahjour et al. from Merck & Co. (USA) filed a patent describing new formulations comprising the use of ODN in combination with vitamin D [52]. Vitamin D possesses two main forms: vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol). The two forms are inactive precursors of the hydroxylated biologically active metabolites of vitamin D, which are essential to regulate calcium homeostasis when serum calcium decreases, responsible for the effects of vitamin D on calcium and bone metabolism [53]. Thus, its combination with a CatK inhibitor could potentialize its effects. Moreover, the invention describes different types of formulations comprising the combination of ODN and Vitamin D but does not show any results related to these formulations. Prior to the patent publication, clinical trials regarding ODN in combination with vitamin D in postmenopausal women sponsored by Merck & Co. (USA) were performed under the identifier NCT00729183. The clinical trials reached phase III, in which results showed that the combination decreased bone resorption, maintained bone formation, and increased areal and volumetric BMD [54,55].

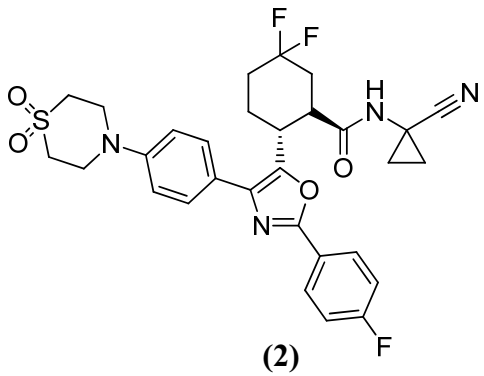
Kassahun et al. [56] from Merck & Co. (USA) developed an ODN derivative, with the replacement of hydrogen atoms by deuterium in the 4-fluoro-L-leucine group at the P2 position (**1**). The authors stated that the replacement improved metabolic and pharmacokinetic profiles by reducing the cytochrome P450 (CYP3A4) mediated

clearance compared to non-deuterated analogues. An *in vivo* study in male rhesus monkeys (n = 4) via intravenous route was performed with a mixture of deuterated (D_6-ODN) and non-deuterated Odanacatib (ODN). It was determined that D_6-ODN has a 3-fold higher exposure, 3-fold lower clearance, and 4-fold longer half-life when compared to ODN. In addition, a pharmacokinetic evaluation was carried out following oral administration to monkeys using different formulations. The study showed that a combination of deuterium substitution and spray drying provided a more significant boost in exposure (6-fold increase). Furthermore, deuterium substitution increased the plasma elimination half-life of ODN by about 2.5-fold.

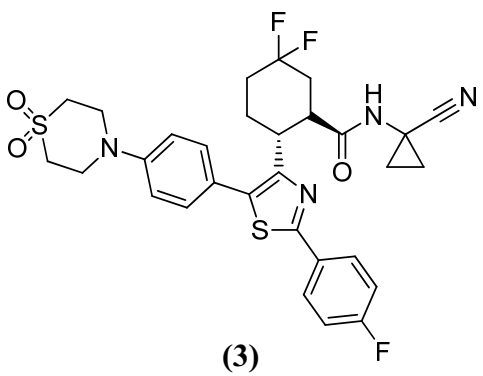


(1)

Stachel et al. from Merck & Co. (USA) published two patents using the same class of nitrile compounds. In one of the patents [57], a library of 159 compounds was studied, and immunoprecipitation (IP) values were specified for CatK and Cathepsin F (CatF). The best inhibitor presented was compound **2**, with an IP value of 0.28 nM for CatK and 71 nM for CatF. In the second patent [58], the authors claimed 248 nitrile compounds, which were structurally similar to the first one [57]. Values of IP were also provided for CatK and CatF, with compound **3** presenting IP values of 0.3 nM for CatK and 35 nM for CatF. Interestingly, the only structural change between compounds **2** and **3** is the replacement of an oxazole for a thiazole. The changes between the compounds demonstrate that the groups are equivalent regarding CatK, but not against CatF, once compound **3** performed better. In both patents, the authors mentioned kinetic assays against cathepsins K, L, S, B, and F and in vivo experiments using male Sprague



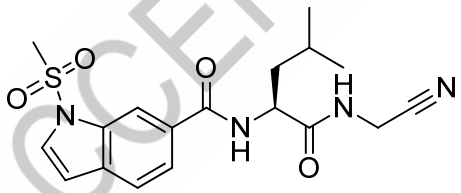
(2)



(3)

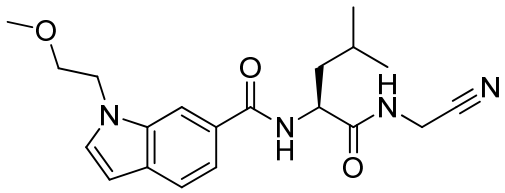
Dawley rat line, but no results were displayed.

Nagaraj and co-workers from Alkem Laboratories LTD. (Bangalore, IN) published two patents regarding the use of nitrile-based inhibitors targeting CatK. In one of the patents [59], 72 inhibitors substituted bicyclic heteroaryl amide derivatives were presented. The inhibitors were tested against human cathepsins K, L, S, V, and rat CatK. Several compounds displayed high affinity and selectivity for human CatK over the other cathepsins, such as compounds **4** and **5**, with an IC_{50} value of ≤ 10 nM for human CatK (IC_{50} < 500 nM for rat CatK) and IC_{50} values in the range of 100 to 1000 nM for the other cathepsins. The authors conducted a bone resorption inhibition activity assay and a pharmacokinetic study using the Sprague Dawley rat line, but they did not specify which inhibitor was used. A known CatK inhibitor (not specified by the authors) was used for comparative purposes. Results showed that C_{max} levels in rats were 3-fold higher for the invention compound, yielding a C_{max}/CatK that is approximately 2-fold higher than that for the known inhibitor. The compound displayed a superior pharmacokinetic profile, stability, and selectivity, demonstrating a clear advantage over known CatK inhibitors. In an efficacy study in ovariectomized rat osteoporosis model, no toxicity was observed during compound administration for three weeks and no gross pathological observation of abnormality in the treated animals. Moreover, the invention compound showed a dose-dependent reduction level of the known osteoporosis marker carboxy-terminal cross-linked telopeptide of type I collagen (CTx-1). Furthermore, no apparent accumulation or decrease in steady-state concentration related to repeat dosing was observed in the experiment.



(4)

CatK IC_{50} ≤ 10 nM

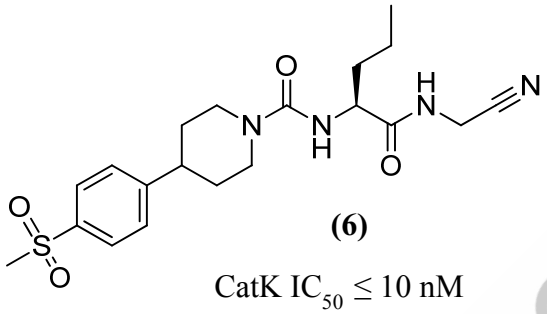


(5)

CatK IC_{50} ≤ 10 nM

The second patent describes potent nitrile-based CatK inhibitors that are substituted piperidine urea derivatives [60]. The work comprised the synthesis of 151 compounds, all of them tested against human cathepsin K, and S, and rat CatK. Compound **6** exhibited an IC_{50} ≤ 10 nM for CatK and CatS, not showing a significant

selectivity between the cathepsins. For rat CatK, an IC_{50} of < 500 nM was obtained. An efficacy study using the Sprague Dawley rat line was conducted for three weeks using a non-specified compound of the invention. A dose-dependent reduction in CTx-1 levels was observed during this period, but the pharmacokinetics study did not show any apparent accumulation or decrease in steady-state concentration on repeated dosing.



(6)

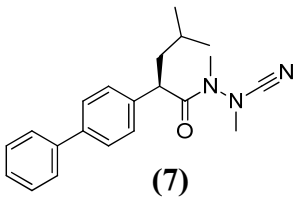
CatK $IC_{50} \leq 10$ nM

2.1.1 Hydrazinonitriles

Researchers from Jilin University (China) published two patents describing cathepsin K inhibitors containing the hydrazinonitrile moiety, known as azanitriles. These inhibitors are similar to the widely used structures containing a nitrile warhead; however, their reactivity increases since the hydrazinonitrile is more electrophilic than the carbonitrile. Despite that, the covalent bond formation with the cysteine thiolate is still reversible, which decreases the likelihood of side effects and general toxicity. In addition, the authors state that, with modulation of the P1-P3 substituents, high selectivity was achieved when tested against cathepsins B, L and S.

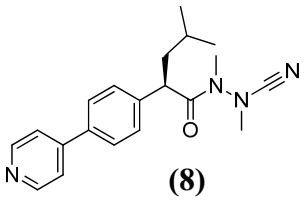
These authors published two patents: (i) Hongwei et al. [61] described a series of non-peptidic CatK inhibitors (**7-10**), demonstrating improved selectivity by removing the P2–P3 amide linker and exploiting different substituents at P3; (ii) Busch et al. [62] described two novel inhibitors (**11,12**) by changing the P3 group to better match the S3 pocket of cathepsin K. In addition to an improved selectivity profile, these compounds did not present toxicity (in murine muscle cells, murine chondrocytes, and human osteosarcoma cells) and showed intracellular CatK activity in a murine primary chondrocyte model.

The use of azanitriles as cathepsin K inhibitors had been described in the non-patent literature before this patent publication [63,64]. Further research on this scaffold by some of the authors of the patents has also been reported in the academic literature [65,66].



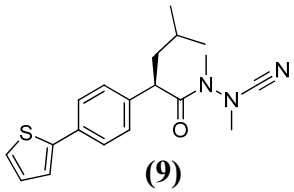
(7)

CatK IC_{50} = 1.93 nM



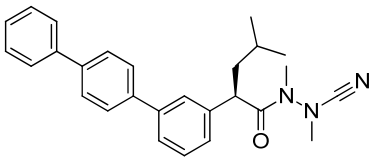
(8)

CatK IC_{50} = 1.03 nM



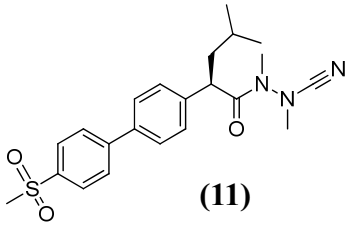
(9)

CatK IC_{50} = 0.79 nM



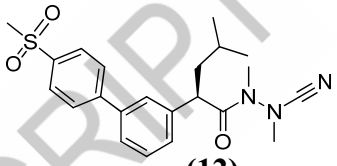
(10)

CatK IC_{50} = 0.29 nM



(11)

CatK IC_{50} = 1.14 nM



(12)

CatK IC_{50} = 7.21 nM

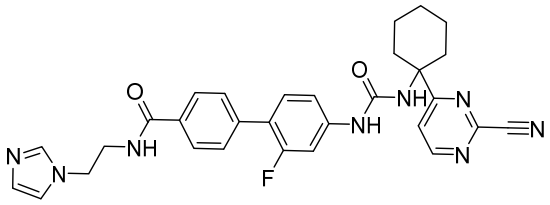
2.1.2. 2-Cyanopyrimidin-4-yl Carbamate

Kim et al. from Hanlim Pharmaceutical Co., LTD. (Republic of Korea) claimed a novel series of 191 compounds using the moiety 2-cyanopyrimidin-4-yl carbamate with a nitrile as the reactivity group [67]. Herein, the pyrimidine group acts as an electron-withdrawing group (EWG) to increase the warhead's reactivity to a nucleophilic attack. In addition, they also presented a urea moiety, right after the cyclohexyl group, to retain the hydrogen bonds with the Gly66 of CatK.

In general, the compounds displayed good inhibitory activity against CatK. For instance, compounds **13-16** show an IC_{50} of 0.1 nM for CatK, more than 10-fold higher than Balicatib (despite the scaffold similarity) and 2-fold higher than ODN. This improvement in activity is likely due to the higher reactivity of the warhead. It is also possible to rationalize that the hydrogen bond acceptor group after the double ring at the P3 position in all four compounds participates in a hydrogen bond interaction with a structural water molecule, like ODN [68].

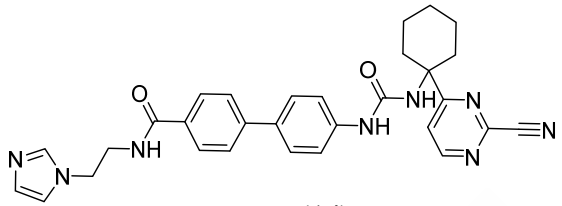
Interestingly, the authors also synthesized a compound with the methylsulfonyl group at P3 (**17**) with an IC_{50} = 2.1 nM against CatK and more than 5000-fold selectivity against the cathepsins B, L, and S. They also published an article with a formulated self-micro emulsifying drug delivery system (SMEDDS) based on compound (**17**) to enhance its oral bioavailability [69]. In addition, they suggest that

the SMEDDS can be used with other CatK inhibitors to treat osteoporosis if the compounds present poor solubility in water and consequently low oral bioavailability.



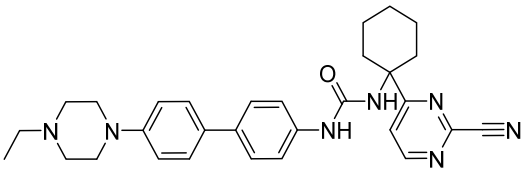
(13)

CatK IC_{50} = 0.10 nM



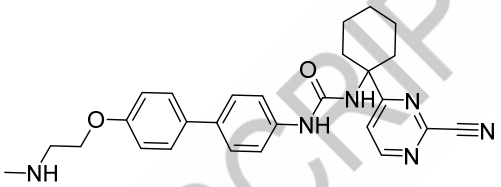
(14)

CatK IC_{50} = 0.10 nM



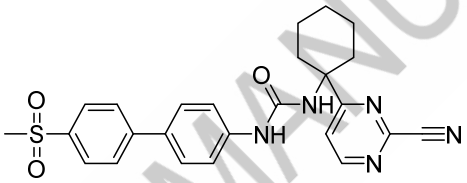
(15)

CatK IC_{50} = 0.10 nM



(16)

CatK IC_{50} = 0.10 nM



(17)

CatK IC_{50} = 2.10 nM

2.2. Other classes

2.2.1. α-oxoacyl amino-caprolactam

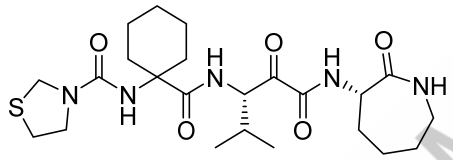
Kobayashi and co-workers from the Japanese pharmaceutical company Seikagaku Corporation published two patents describing α-oxoacyl amino-caprolactam derivatives [70,71]. These chemicals bear a seven-membered cyclic amide moiety in which the α-carbon is bonded to the nitrogen atom of an α-ketoamide. The chemical structure also contains two amino acid-related groups (valine and cyclohexane derivatives in all cases) and a terminal group (R). According to the patent under the scope, the terminal group (R) can be a five or six-membered ring.

It is claimed that this type of chemical scaffold displays low inhibitory activity for CYP3A4. This is a desirable property, once drugs to treat osteoporosis would be mainly administered to older people, who are probably taking other medications. As

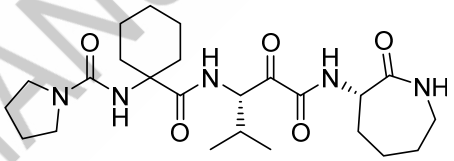
mentioned in the patent [70], the use of α-ketoamides in combination with cyclic amides had been previously described [72]. However, the authors stated that the preceding patent did not cover the R moieties explored in these new series of compounds, thereby justifying the novelty of the works.

Moreover, three novel compounds (**18-20**) with IC_{50} values of > 40 μM for CYP3A4 showed a selectivity of over 40000 times for CatK over CYP3A [70], which is a 100-folder improvement when compared with 15 chemicals from reference [72] with CYP3A4 IC_{50} < 10 μM.

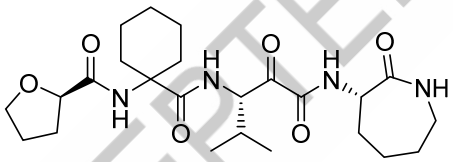
The series with α-oxoacyl amino-caprolactam motif was expanded by Kobayashi and co-workers [71] in a second patent. This time, only two novel derivatives have their CatK and CYP3A4 activity described (**21,22**). CatK inhibitory potencies are around 0.5 nM, while the selectivity profile was improved by keeping CYP3A4 inhibition low. The most selective compound could achieve a CYP/CatK inhibition ratio higher than 140000. In both works, the selectivity among cathepsins was not discussed.



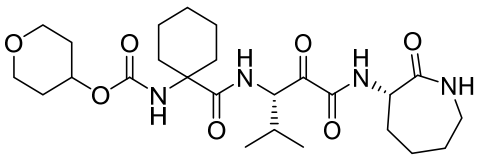
(18)
CatK IC_{50} = 0.79 nM



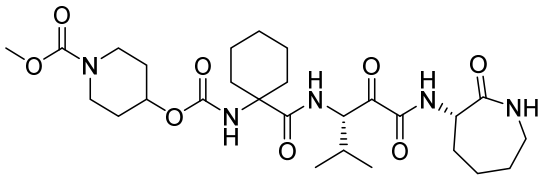
(19)
CatK IC_{50} = 1.4 nM



(20)
CatK IC_{50} = 3.4 nM



(21)
CatK IC_{50} = 0.56 nM



(22)
CatK IC_{50} = 0.52 nM

2.2.2. Vitamin K and related compounds

In 2021, Chao et al. from the Shanghai Center for Disease Control and

Prevention (China) [73] described CatK inhibition by some vitamin K subtypes and β-lapachone, which are compounds based on naphthoquinone and phenanthrenequinone scaffolds (Figure 3).

Figure3

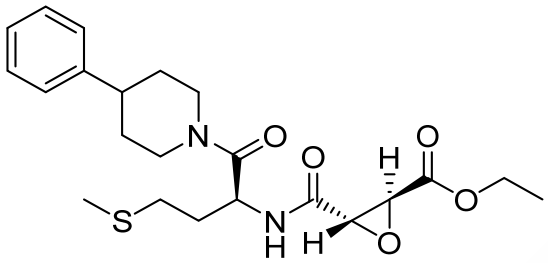
CatK inhibition assays were only reported for β-lapachone and vitamin K subtypes menaquinone-4 and menaquinone-7, with all three presenting an IC_{50} = 1.3 ± 0.2, 21.8 ± 5.5, and 52.8 ± 5.5 μM, respectively. In addition, β-lapachone can decrease the degradation of collagen (90.3 %), elastin (65.4 %), and thyroglobulin (74.3 %), comparable to ODN. Other *in vivo* experiments were performed in murine models using β-lapachone and menaquinone-4 to evaluate bone health, such as increased bone density and decreased bone resorption.

As stated by the authors based on the literature [74], vitamin K deficiency has been associated with bone degradation and the development of osteoporosis, although its precise role has not been determined. β-lapachone has also been shown to inhibit osteoclastogenesis [75], independently from CatK modulation.

2.2.3. Epoxysuccinyl derivatives

Epoxysuccinic acid derivatives compose another class of compounds widely used to inhibit cysteine proteases. E-64 is a classic example of a pan-cysteine protease inhibitor containing the trans-epoxysuccinic acid moiety [7]. Aiming to circumvent the selectivity issue while maintaining the electrophilic group, Li and co-workers [76] developed a series of 2,3-epoxysuccinyl derivatives to exploit the noncovalent interactions.

Compound **23** was designed with a phenyl-piperidinyl group at one of its extremities in the molecular structure. This group probably mimics the P3 group of the Balicatib and Odanacatib, with the presence of the double-ring. Thus, this moiety of the compound can fit similarly at the S3 subpocket of the enzyme. This compound showed an IC_{50} of 164 nM against CatK with good selectivity over CatL (38-fold) and CatS (10-fold). The biggest drawback was observed for CatB, once compound **23** was 40-fold more potent against this enzyme than CatK.



(23)

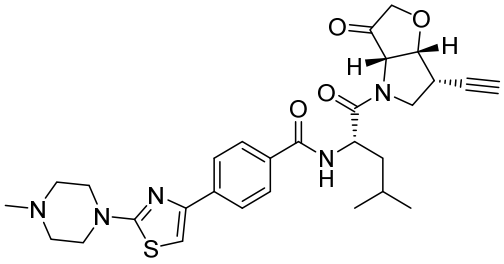
CatK IC_{50} = 164 nM

2.2.4. Tetrahydrofuro[3,2-b] pyrrol-3-one

The tetrahydrofuro[3,2-b]pyrrol-3-one warhead is being studied to obtain highly potent and selective inhibitors for CatK [77]. Recently, Oden *et al*. [78] have exploited analogues bearing this warhead, wherein changes were made in position 6 of the 5,5-bicyclic ring, coupled to a peptidomimetic scaffold in position 4. The most promising compounds present an acetylene group in position 6, acting as an EWG group.

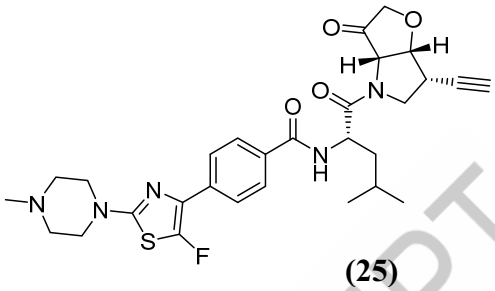
In addition, the authors modified the side chains of leucine to test other substituents as P1 groups, such as cyclopentylglicine. At the P3 position, the authors decided to use three adjacent and connected distinct rings, starting with benzamide, followed by thiazole, and finishing with piperazine. While in the second ring (thiazole), they tested the presence of a fluorine atom in position 5, as showed in the chemical structures of compounds **24** and **25**. The compounds displayed a K_i = 1.6 nM **24** and 1.1 nM against CatK and are highly selective (1250-fold) over the cathepsins S and L.

Pharmacokinetic parameters were also evaluated for compound **24**. According to the authors, it presents good metabolic stability through the human liver cytosol pool assay, with a calculated whole blood half-life approaching 4 hours. It also shows better liver metabolism stability than an analogue with a methyl group in position six instead of the acetylene. Compound **25** was also subject to an experiment using the human gastroenteric canal cells (Caco-2) to quantify the permeability coefficient (P_{app}) of $9.1x10^{-6}$ cm/s, almost three times better than the reference compound.



(24)

CatK K_i = 1.60 nM



(25)

CatK K_i = 1.10 nM

2.3. Activity-Based Probes

The use of activity-based probes (ABPs) as substrates of CatK has been described in the literature for measuring CatK activity [79–81]. A recent patent [82] presents a series of "osteoadsorptive fluorogenic sentinel" (OFS-1) probes synthesized at the University of Southern California by McKenna CE and shown by Nishimura I (University of California, Los Angeles) to act as substrates of CatK released by osteoclasts. The probe consists of a bone-anchoring bisphosphonate moiety connected to a FRET quencher in a CatK peptide substrate sequence (Figure 4). The fluorogenic substrates are adsorbed by hydroxyapatite (HAp) and bone mineral surfaces, where an external fluorescent signal is generated in response to the osteoclast-secreted CatK. Thus, it is a promising new imaging tool to detect abnormal bone resorption. The resorption can be monitored throughout extended periods with a single dose, differently from existing probes cleared within a few days *in vivo*.

An *in vivo* assay was performed to confirm the hypothesis, and the OSF-1 probe could label multiple myeloma-induced aberrant osteoclastogeneses in NSG/BLT humanized mice injected with human RPMI-8226-Luc cells. Micro-computed tomography analysis indicated that the OSF-1 signal was in the relatively early multiple myeloma lesion without detrimental osteolysis. Additionally, the authors stated that the OSF-1 probe could be used *in vitro* to determine whether osteoclasts are secreting CatK to track their migration on mineral substrates. An article detailing their findings was recently published [83].

Figure4

3. Conclusion

Cathepsin K is a papain-like cysteine protease highly expressed in osteoclasts and is considered a biological target for bone-related diseases, such as osteoporosis. Thus, new chemical entities have been developed in the last decade as potential new treatments for osteoporosis *via* inhibition of CatK. This review summarizes findings in the patent literature filed between 2011-2021, showing the most promising compounds. In this period, new classes of warheads were introduced, and derivatives of the clinical candidates Odanacatib and Balicatib are still being exploited. Encouraging results were achieved with the design of highly potent and selective CatK inhibitors coupled to *in vitro* and *in vivo* improved properties. Furthermore, a new activity-based probe that acts as a substrate of CatK was filed. The probe could track down abnormal bone resorption by targeting CatK activity and might be a valuable tool for future research.

4. Expert Opinion

As one of the most potent lysosomal proteins whose primary function is to mediate bone resorption, cathepsin K remains an excellent target for therapeutic intervention. Recently, a great interest in this target can be observed by the surmount publications and patents filed.

Unfortunately, there is no CatK inhibitor currently in clinical trials. Balicatib and Odanacatib, the most advanced compounds, were discontinued after reaching phase II and III clinical trials, respectively. Despite failing in clinical trials, there is still interest in new formulations with Odanacatib and the design of novel derivatives. However, the patents prioritize the design of covalent reversible inhibitors; among them, the nitriles constitute the preferred warhead to target CatK.

Current treatments for osteoporosis include BPs and, more recently, Romosozumab, a monoclonal antibody (MAB). BPs drugs Alendronate and Zoledronate are the most used and effective treatments for osteoporosis. Unfortunately, BPs showed

several side effects in osteoporotic patients, making them undesirable for long-term use. Romosozumab, currently approved to treat osteoporosis, is shown to be more effective than BPs, but the MAB could increase the cardiovascular risk (a subject that still needs further investigation) [22]. MAB drugs have a very high cost, which makes the replacement of BPs difficult. Thus, developing a new class of small molecules is still of much interest, especially when considering the enormous impact on the elderly population worldwide, which are prone to develop osteoporosis.

This review has shown that many of the patents filed in the last ten years are built upon previously published molecular scaffolds, mostly peptidomimetics compounds bearing an electrophilic warhead. The most promising compounds have similar scaffolds, but the molecular diversity of the novel chemicals could overcome the limitations presented by the compounds that failed the clinical trial. Computational methods, such as molecular dynamics simulations with the determination of absolute or relative binding free energy and machine learning-based approaches or even the combination of both methods [84–86], are likely to impact the discovery of novel CatK inhibitors positively.

According to biochemical and cell-based assays, the analysed patents have described compounds with excellent in vitro activity, and some works even described promising *in vivo* assays on murine and other animal models. CatK selectivity is not a significant issue for many inhibitors, with several compound classes presenting more than 100-fold selectivity. Therefore, ADME properties, safety, and *in vivo* potency constitute the most relevant topics for improvement in the following years. The failed clinical trial outcomes from Balicatib and Odanacatib should guide the future development of these novel chemicals. The mechanism of action behind the increased risk of stroke in patients treated with ODN was not further investigated. Therefore, new CatK inhibitors are likely designed not to avoid this specific side effect, although it would be advisable to test for cardiovascular side effects in *in vivo* assays. Strikingly, the patents did not describe any known methods to justify this side effect or even how to avoid it. Consequently, a further body of investigation is wanted to comprehend the nature of any relationship between CatK inhibition and increased risk of cardiovascular events such as stroke.

From a structural point of view in the development of new inhibitors, there are still many molecules with a basic moiety, such as a nitrogen atom, at the P3 position, which, despite being associated as a characteristic of CatK inhibitors, may lead to

harmful properties, such as hERG inhibition and lysosomotropism [87]. Thus, new moieties at the P3 position should be considered when designing new inhibitors without toxicity concerns. The balance between reactivity and unwanted off-target effects is another cornerstone parameter for selecting the electrophilic warhead that forms the covalent bond with the catalytic cysteine.

The first patent in which the use of ABPs is described as CatK substrate was filed earlier this year (2021). The probe showed excellent results regarding its use in detecting abnormal bone resorption, displaying high potency and selectivity. CatK is also a promising target for different types of cancer [20,21,88]; thus, the probe can monitor osteoclast's activity to detect and diagnose neoplasia accurately. New ABPs should be designed bearing in mind their valuable contribution as a detection tool to impact the patient's therapeutic response positively.

Worthy of note is the successful application of cysteine protease inhibitors to treat viral diseases. Recently, the Food and Drug Administration has approved Paxlovid, a combination drug that has the inhibitors Nirmatrelvir and Ritonavir, for emergency use for COVID-19 (https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-authorizes-first-oral-antiviral-treatment-covid-19). Nirmatrelvir is a SARS-CoV-2 main protease inhibitor that bears a nitrile group as 'warhead' [89], which demonstrate that active-site-directed compounds based on nitriles are still a good strategy as therapeutic cysteine proteases inhibitors. However, for CatK inhibitors, although nitrile is a good choice for the warhead, selectivity issues must be addressed in order to reduce the risk of off-target activity. Thus, the requirements for a minor side effect-prone CatK inhibitor may be reached on modulation of warhead electrophilicity that controls the reactivity and modifications in P2 and P3. Therefore, we expect that novel cysteine protease inhibitors will enter the clinical trials in the foreseeable future due to the successful development of chemicals coupling favourable pharmacodynamic and pharmacokinetic properties.

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Declaration of interests

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or

materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

References

Interesting papers have been highlighted *

[1] Turk V, Stoka V, Vasiljeva O, et al. Cysteine cathepsins: From structure, function and regulation to new frontiers. Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics. 2012;1824:68–88.

[2] Aggarwal N, Sloane BF. Cathepsin B: Multiple roles in cancer. PROTEOMICS - Clinical Applications. 2014;8:427–437.

[3] Olson OC, Joyce JA. Cysteine cathepsin proteases: regulators of cancer progression and therapeutic response. Nature Reviews Cancer. 2015;15:712–729.

[4] Jean D, Rousselet N, Frade R. Expression of cathepsin L in human tumor cells is under the control of distinct regulatory mechanisms. Oncogene. 2006;25:1474–1484.

[5] Ainscough JS, Macleod T, McGonagle D, et al. Cathepsin S is the major activator of the psoriasis-associated proinflammatory cytokine IL-36γ. Proceedings of the National Academy of Sciences. 2017;114:E2748–E2757.

[6] Drake MT, Clarke BL, Oursler MJ, et al. Cathepsin K Inhibitors for Osteoporosis: Biology, Potential Clinical Utility, and Lessons Learned. Endocrine Reviews. 2017;38:325–350.

[7] Cianni L, Feldmann CW, Gilberg E, et al. Can Cysteine Protease Cross-Class Inhibitors Achieve Selectivity? Journal of Medicinal Chemistry. 2019;62:10497–10525.

[8] Lu J, Wang M, Wang Z, et al. Advances in the discovery of cathepsin K inhibitors on bone resorption. Journal of Enzyme Inhibition and Medicinal Chemistry. 2018;33:890–904.

[9] Gehringer M, Laufer SA. Emerging and Re-Emerging Warheads for Targeted Covalent Inhibitors: Applications in Medicinal Chemistry and Chemical Biology. J Med Chem. 2019;62:5673–5724.

[10] Littlewood-Evans A, Kokubo T, Ishibashi O, et al. Localization of cathepsin K in human osteoclasts by in situ hybridization and immunohistochemistry. Bone. 1997;20:81–86.

[11] Kafienah W, Brömme D, Buttle DJ, et al. Human cathepsin K cleaves native type I and II collagens at the N-terminal end of the triple helix. Biochemical Journal. 1998;331:727–732.

*** Provides an overview about cathepsin K mouse model studies**

[12] Li CY, Jepsen KJ, Majeska RJ, et al. Mice Lacking Cathepsin K Maintain Bone Remodeling but Develop Bone Fragility Despite High Bone Mass. J Bone Miner Res. 2006;21:865–875.

[13] Saftig P, Hunziker E, Wehmeyer O, et al. Impaired osteoclastic bone resorption leads to osteopetrosis in cathepsin-K-deficient mice. Proceedings of the National Academy of Sciences. 1998;95:13453–13458.

[14] Pennypacker B, Shea M, Liu Q, et al. Bone density, strength, and formation in adult cathepsin K (−/−) mice. Bone. 2009;44:199–207.

[15] Lotinun S, Ishihara Y, Nagano K, et al. Cathepsin K–deficient osteocytes prevent lactation-induced bone loss and parathyroid hormone suppression. Journal of Clinical Investigation. 2019;129:3058–3071.

[16] Dai R, Wu Z, Chu HY, et al. Cathepsin K: The Action in and Beyond Bone. Front Cell Dev Biol. 2020;8:433.

[17] Garber K. Two pioneering osteoporosis drugs finally approach approval. Nat Rev Drug Discov. 2016;15:445–446.

*** Provides an overview about cathepsin K's role in different diseases**

[18] Nurullina GM, Akhmadullina GI. The study of new biomarkers of bone metabolism of sclerostin and cathepsin K in patients with type 2 diabetes mellitus. Probl Endokrinol (Mosk). 2019;64:363–370.

[19] Vashum Y, Khashim Z. Obesity and Cathepsin K: A Complex Pathophysiological Relationship in Breast Cancer Metastases. EMIDDT. 2020;20:1227–1231.

[20] Elevated Cathepsin K potentiates metastasis of epithelial ovarian cancer. Histol Histopathol. 2018;33:673–680.

[21] Liang W, Wang F, Chen Q, et al. Targeting cathepsin K diminishes prostate cancer establishment and growth in murine bone. J Cancer Res Clin Oncol. 2019;145:1999–2012.

[22] Turan S. Current research on pycnodysostosis. Intractable & Rara Diseases Research. 2014;3:91–93.

[23] Sinaki M. Osteoporosis. Braddom's Physical Medicine and Rehabilitation, Elsevier; 2021, p. 690-714.e3.

[24] Dawson-Hughes B, Tosteson ANA, Melton LJ, et al. Implications of absolute fracture risk assessment for osteoporosis practice guidelines in the USA. Osteoporos Int. 2008;19:449–458.

[25] Pesce V, Speciale D, Sammarco G, et al. Surgical approach to bone healing in osteoporosis. Clin Cases Miner Bone Metab. 2009;6:131–135.

*** Provides an overview about drugs that are currently been used for the treatment of osteoporosis.**

[26] Gambacciani M, Levancini M. Featured Editorial Hormone replacement therapy and the prevention of postmenopausal osteoporosis. Pm. 2014;4:213–220.

[27] Mehta N, Malootian A, Gilligan J. Calcitonin for Osteoporosis and Bone Pain. CPD. 2003;9:2659–2676.

[28] Zaheer S, LeBoff M, Lewiecki EM. Denosumab for the treatment of osteoporosis. Expert Opinion on Drug Metabolism & Toxicology. 2015;11:461–470.

[29] Shakeri A, Adanty C. Romosozumab (sclerostin monoclonal antibody) for the treatment of osteoporosis in postmenopausal women: A review. Jptcp. 2020;27:e25–e31.

[30] Fleisch HA. Bisphosphonates: Preclinical Aspects and Use in Osteoporosis. Annals of Medicine. 1997;29:55–62.

[31] Oryan A, Sahvieh S. Effects of bisphosphonates on osteoporosis: Focus on zoledronate. Life Sciences. 2021;264:118681.

[32] Kuźnik A, Październiok-Holewa A, Jewula P, et al. Bisphosphonates—much more than only drugs for bone diseases. European Journal of Pharmacology. 2020;866:172773.

[33] Cosman F, Crittenden DB, Adachi JD, et al. Romosozumab Treatment in Postmenopausal Women with Osteoporosis. N Engl J Med. 2016;375:1532–1543.

[34] Pimolbutr K, Porter S, Fedele S. Osteonecrosis of the Jaw Associated with Antiangiogenics in Antiresorptive-Naïve Patient: A Comprehensive Review of the Literature. BioMed Research International. 2018;2018:1–14.

[35] McKenna CE, Haratipour P, Duro MVV, et al. Chemistry of Bisphosphonates. Encyclopedia of Bone Biology, Elsevier; 2020, p. 551–564.

[36] Reid IR. Osteonecrosis of the jaw — Who gets it, and why? Bone. 2009;44:4–10.

[37] Hokugo A, Kanayama K, Sun S, et al. Rescue bisphosphonate treatment of alveolar bone improves extraction socket healing and reduces osteonecrosis in zoledronate-treated mice. Bone. 2019;123:115–128.

[38] Yang G, Kim Y-N, Kim H, et al. Effect of Human Umbilical Cord Matrix-Derived Mesenchymal Stem Cells on Bisphosphonate-Related Osteonecrosis of the Jaw. Tissue Eng Regen Med. 2021;18:975–988.
[39] Gaulton A, Hersey A, Nowotka M, et al. The ChEMBL database in 2017. Nucleic Acids Research. 2017;45:D945–D954.

*** Provides clinical detail data of the inhibitors ONO-5334, Balicatib, and ODN.**

[40] Eastell R, Nagase S, Small M, et al. Effect of ONO-5334 on Bone Mineral Density and Biochemical Markers of Bone Turnover in Postmenopausal Osteoporosis: 2-Year Results From the OCEAN Study: 2-YEAR TREATMENT OF OSTEOPOROSIS WITH ONO-5334. J Bone Miner Res. 2014;29:458–466.
[41] Tanaka M, Hashimoto Y, Hasegawa C, et al. Antiresorptive effect of a cathepsin K inhibitor ONO-5334 and its relationship to BMD increase in a phase II trial for postmenopausal osteoporosis. BMC Musculoskelet Disord. 2017;18:267.
[42] Jerome C, Missbach M, Gamse R. Balicatib, a cathepsin K inhibitor, stimulates periosteal bone formation in monkeys. Osteoporos Int. 2011;22:3001–3011.
[43] Rünger TM, Adami S, Benhamou C-L, et al. Morphea-like skin reactions in patients treated with the cathepsin K inhibitor balicatib. Journal of the American Academy of Dermatology. 2012;66:e89–e96.
[44] McClung MR, O'Donoghue ML, Papapoulos SE, et al. Odanacatib for the treatment of postmenopausal osteoporosis: results of the LOFT multicentre, randomised, double-blind, placebo-controlled trial and LOFT Extension study. The Lancet Diabetes & Endocrinology. 2019;7:899–911.

*** Previous review on Cathepsin K patents.**

[45] Wijkmans J, Gossen J. Inhibitors of cathepsin K: a patent review (2004 – 2010). Expert Opinion on Therapeutic Patents. 2011;21:1611–1629.
[46] Cai J, Jamieson C, Moir J, et al. Cathepsin K inhibitors, 2000 – 2004. Expert Opinion on Therapeutic Patents. 2005;15:33–48.

*** Provides an overview about covalent inhibitors, focusing on nitriles warheads.**

[47] Du H, Gao J, Weng G, et al. CovalentInDB: a comprehensive database facilitating the discovery of covalent inhibitors. Nucleic Acids Research. 2021;49:D1122–D1129.
[48] Martin JS, MacKenzie CJ, Fletcher D, et al. Characterising covalent warhead reactivity. Bioorganic & Medicinal Chemistry. 2019;27:2066–2074.
[49] Sutanto F, Konstantinidou M, Dömling A. Covalent inhibitors: a rational approach to drug discovery. RSC Med Chem. 2020;11:876–884.
[50] Wang Y, Du Y, Huang N. A survey of the role of nitrile groups in protein–ligand interactions. Future Medicinal Chemistry. 2018;10:2713–2728.
[51] Fleming FF, Yao L, Ravikumar PC, et al. Nitrile-Containing Pharmaceuticals: Efficacious Roles of the Nitrile Pharmacophore. Journal of Medicinal Chemistry. 2010;53:7902–7917.

*** Provides data about Merck & CO. USA efforts on finding new formulations with ODN, as for new nitriles inhibitors of CatK.**

[52] Mahjour M, Ma D, Bacci CM, et al. inventor; Merck Sharp and Dohme Corp., assignee. Formulations for Cathepsin K inhibitors with Vitamin D. United States Patent US2016/0166529A1, 2016 June 16.
[53] Fleet JC. The role of vitamin D in the endocrinology controlling calcium homeostasis. Molecular and Cellular Endocrinology. 2017;453:36–45.
[54] Brixen K, Chapurlat R, Cheung AM, et al. Bone Density, Turnover, and Estimated Strength in Postmenopausal Women Treated With Odanacatib: A Randomized Trial. The Journal of Clinical Endocrinology & Metabolism. 2013;98:571–580.

[55] Engelke K, Fuerst T, Dardzinski B, et al. Odanacatib Treatment Affects Trabecular and Cortical Bone in the Femur of Postmenopausal Women: Results of a Two-Year Placebo-Controlled Trial: ODANACATIB TREATMENT AFFECTS TRABECULAR AND CORTICAL BONE IN THE FEMUR. J Bone Miner Res. 2015;30:30–38.
[56] Kassahun K, Fandozzi C, Gauthier, Jr DR, et al. inventor; Merck Sharp and Dohme Corp., assignee. Cathepsin cysteine protease inhibitors. United States Patent US 8791162 B2, 2014 July 29.
[57] Stachel S, Paone DV, Li J, et al. inventor; Merck Sharp and Dohme Corp., assignee. Cathepsin cysteine protease inhibitors. Australia Patent AU2014332250B2, 2015 April 16.
[58] Stachel S, Paone DV, Li J, et al. inventor; Merck Sharp and Dohme Corp., assignee. Cathepsin cysteine protease inhibitors. United States Patent US9573913B2, 2017 February 21.
[59] Nagaraj HKM, Bandodkar BS, Ravilla L, et al. inventor; Alkem Laboratories LTD., assignee. Bicyclic heteroaryl amides as cathepsin cysteine protease inhibitors. WO Patent WO2016027285, 2016 February 25.
[60] Nagaraj HKM, Bandodkar BS, Yellapu S, et al. inventor; Alkem Laboratories LTD., assignee. Piperidine ureas as cathepsin cysteine protease inhibitors. WO Patent WO2016027284, 2016 February 25.
[61] Hongwei L, Yuqing W, Xiaoyu Y. inventor; Jilin University, assignee. Hydrazine cathepsin K inhibitor and application thereof in treating osteoporosis. China Patent CN103086923A CN103086923B, 2013 May 08.
[62] Busch R, Merrick S, Yuqing W, et al. inventor; Jilin University, assignee. Hydrazinonitrile cathepsin K inhibitor and application thereof in preparation of osteoarthritis treatment drugs. China Patent CN105837479A, 2016 August 10.
[63] Frizler M, Lohr F, Furtmann N, et al. Structural Optimization of Azadipeptide Nitriles Strongly Increases Association Rates and Allows the Development of Selective Cathepsin Inhibitors. Journal of Medicinal Chemistry. 2011;54:396–400.
[64] Löser R, Frizler M, Schilling K, et al. Azadipeptide Nitriles: Highly Potent and Proteolytically Stable Inhibitors of Papain-Like Cysteine Proteases. Angew Chem Int Ed. 2008;47:4331–4334.
[65] Ren Z-Y, Machuca-Gayet I, Domenget C, et al. Azanitrile Cathepsin K Inhibitors: Effects on Cell Toxicity, Osteoblast-Induced Mineralization and Osteoclast-Mediated Bone Resorption. PLoS ONE. 2015;10:e0132513.
[66] Yuan X-Y, Fu D-Y, Ren X-F, et al. Highly selective aza-nitrile inhibitors for cathepsin K, structural optimization and molecular modeling. Org Biomol Chem. 2013;11:5847.
[67] Kim K-H, Chin J-W, Lee J-H, et al. inventor; Hanlim Pharmaceutical Co., LTD. assignee. 2-Cyanopyrimidin-4-yl carbamate or urea derivative or salt thereof, and pharmaceutical composition including same. United States Patent US20210047301, 2021 February 18.
[68] Law S, Andrault P-M, Aguda AH, et al. Identification of mouse cathepsin K structural elements that regulate the potency of odanacatib. Biochemical Journal. 2017;474:851–864.
[69] Visetvichaporn V, Kim K-H, Jung K, et al. Formulation of self-microemulsifying drug delivery system (SMEDDS) by D-optimal mixture design to enhance the oral bioavailability of a new cathepsin K inhibitor (HL235). International Journal of Pharmaceutics. 2020;573:118772.

[70] Kobayashi N, Koji T, Kunii H, et al. inventor; Seikagaku Corp., assignee. Alpha-oxoacyl amino-caprolactam. United States Patent US9562042B2, 2017 February 07.
[71] Kobayashi N, Koji T, Kunii H, et al. inventor; Seikagaku Corp., assignee. Alpha-oxoacyl amino-caprolactam derivative. United States Patent US20160137691, 2016 December 29.
[72] Akihiko H, Nobuo K, Naoko T, et al. inventor; Cyclic amide derivatve. JP2000204071, 2000.
[73] Chao F, Yiping J, Yu'E J, et al. inventor; Shanghai Municipal Center for Disease Control & Prevention, assignee. Cathepsin K inhibitor containing naphthoquinone and phenanthrenequinone structures, composition and application thereof. China Patent CN112294958A, 2021 February 02.
[74] Palermo A, Tuccinardi D, D'Onofrio L, et al. Vitamin K and osteoporosis: Myth or reality? Metabolism. 2017;70:57–71.
[75] Gu DR, Lee JN, Oh G-S, et al. The inhibitory effect of beta-lapachone on RANKL-induced osteoclastogenesis. Biochemical and Biophysical Research Communications. 2017;482:1073–1079.
[76] Li S, Xiao J, Zhang X, et al. inventor; Institute of Pharmacology and Toxicology Academy of Military Medical Sciences P.L.A China, assignee. 2,3-Epoxy succninyl derivative, preparation method and use thereof. United States Patent US20190062290, 2019 February 28.
[77] Quibell M, Benn A, Flinn N, et al. Bicyclic peptidomimetic tetrahydrofuro[3,2-b]pyrrol-3-one and hexahydrofuro[3,2-b]pyridine-3-one based scaffolds: synthesis and cysteinyl proteinase inhibition. Bioorganic & Medicinal Chemistry. 2004;12:5689–5710.
[78] Oden LS, Nilsson M, Kahnberg P, et al. inventor; Medivir AB, assignee. Protease Inhibitors. United States Patent US20210017140, 2021 January 21.

*** Provides an overview about the field of activity-based probes targeting Cat K detection.**

[79] Lemke C, Benýšek J, Brajtenbach D, et al. An Activity-Based Probe for Cathepsin K Imaging with Excellent Potency and Selectivity. J Med Chem. 2021;64:13793–13806.
[80] Kozloff KM, Quinti L, Patntirapong S, et al. Non-invasive optical detection of cathepsin K-mediated fluorescence reveals osteoclast activity in vitro and in vivo. Bone. 2009;44:190–198.
[81] Jaffer FA, Kim D-E, Quinti L, et al. Optical Visualization of Cathepsin K Activity in Atherosclerosis With a Novel, Protease-Activatable Fluorescence Sensor. Circulation. 2007;115:2292–2298.
[82] Nishimura I, Hokugo A, Morinaga K, et al. inventor; Univeristy of California and University of Southern California. An osteoadsorptive fluorogenic substrate of cathepsin K for imaging osteoclast activity and migration. United States Patent US20200215205A1, 2020 July 09.
[83] Richard ET, Morinaga K, Zheng Y, et al. Design and Synthesis of Cathepsin-K-Activated Osteoadsorptive Fluorogenic Sentinel (OFS) Probes for Detecting Early Osteoclastic Bone Resorption in a Multiple Myeloma Mouse Model. Bioconjugate Chem. 2021;32:916–927.
[84] Cournia Z, Allen BK, Beuming T, et al. Rigorous Free Energy Simulations in Virtual Screening. J Chem Inf Model. 2020;60:4153–4169.
[85] Muratov EN, Bajorath J, Sheridan RP, et al. QSAR without borders. Chem Soc Rev. 2020;49:3525–3564.

[86] Albanese SK, Chodera JD, Volkamer A, et al. Is Structure-Based Drug Design Ready for Selectivity Optimization? J Chem Inf Model. 2020;60:6211–6227.
[87] Brömme D, Lecaille F. Cathepsin K inhibitors for osteoporosis and potential off-target effects. Expert Opinion on Investigational Drugs. 2009;18:585–600.
[88] Gu X, Peng Y, Zhao Y, et al. A novel derivative of artemisinin inhibits cell proliferation and metastasis via down-regulation of cathepsin K in breast cancer. European Journal of Pharmacology. 2019;858:172382.
[89] Owen DR, Allerton CMN, Anderson AS, et al. An oral SARS-CoV-2 M^{pro} inhibitor clinical candidate for the treatment of COVID-19. Science. 2021:eabl4784.