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Nanotechnologies and controlled release formulations for the administration of bisphosphonates and their potential in radiation protection

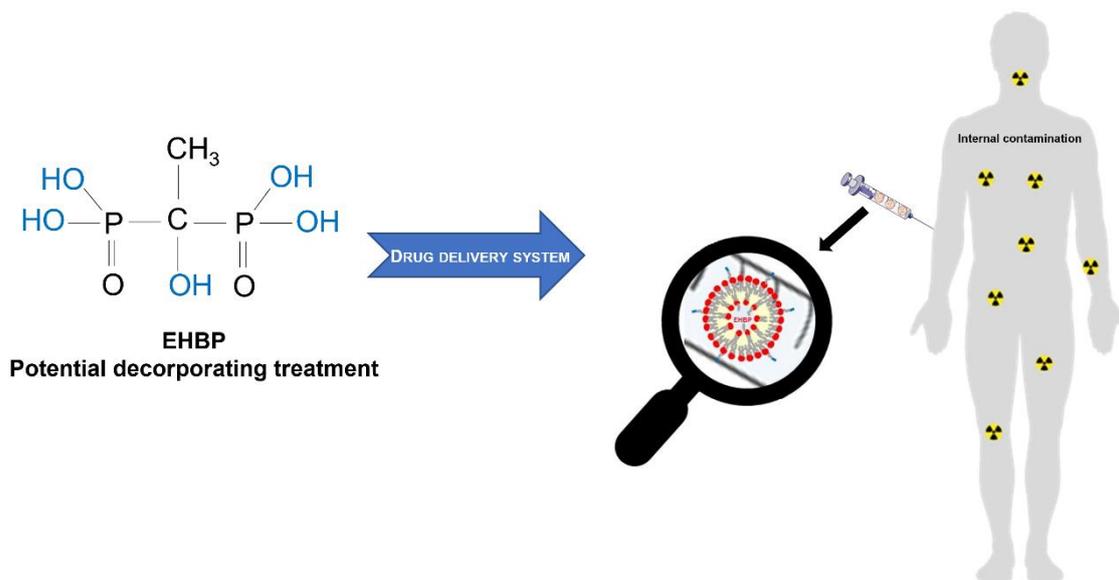
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Abstract

In the event of internal contamination with radionuclides, decorporating treatment is crucial. It must be initiated as soon as possible to limit tissue retention of radiocontaminants and accelerate their excretion. However, the existing therapies for such contaminations often lack effectiveness. The conventional research strategy is based on selecting or synthesizing new chelating agents, a lengthy and costly process. Another approach is drug repurposing, such as molecules belonging to the biphosphonate series. This primary therapeutic class is mainly used to treat bone metabolism disorders. However, for decades, biphosphonates have offered a wide range of applications in research, including macrophage depletion or bone regeneration, but also in more unexpected areas such as skin decontamination and decorporation. In addition, controlled drug delivery and nanotechnologies have offered a promising approach for significantly improving the delivery and efficacy of these active substances. They increase their distribution to target organs and bypass issues related to their

26 toxicity or poor pharmacokinetic performance. After outlining the pharmaceutical properties
27 of bisphosphonates, the present review aims to present the central innovative systems for their
28 delivery and targeting in the field of radiation protection.

29

30 **Keywords**

31 Bisphosphonate, internal contamination, radionuclide, nanotechnologies, controlled drug
32 delivery, repurposing drug

33 **1 Introduction**

34 Bisphosphonates (BPs) have been initially used as corrosion inhibitors or complexing
35 agents in the textile or petroleum industries. Etidronate (ethane-1-hydroxy-1,1-
36 bisphosphonate, EHBP, or 1-Hydroxyethylidene-1,1-diphosphonate, HEDP) was the first
37 clinically applied in the 1960s [1]. Other molecules (clodronate, pamidronate, risedronate,
38 alendronate, tiludronate, zoledronate, and ibandronate) were then successively available on
39 the market. As bone resorption inhibitors, they were employed as first-line treatment of
40 metabolic bone diseases such as osteoporosis and Paget's disease. Prescription of these drugs
41 has been growing extensively due to population aging, with the global prevalence of
42 osteoporosis estimated at 18.3% [2]. BPs are also widely used in therapy to treat
43 hypercalcemia and as an adjuvant in bone metastasis.

44 Besides their known therapeutic indications, BPs have been particularly interesting in
45 radioprotection due to their mechanism of action and bone tropism. Since one of the target
46 tissue after radiocontamination is the bone [3, 4], applications have included decontamination
47 and decorporation [5, 6]. These studies demonstrated that BPs could be effective medical
48 countermeasures for the population and workers exposed accidentally or intentionally to
49 radiation.

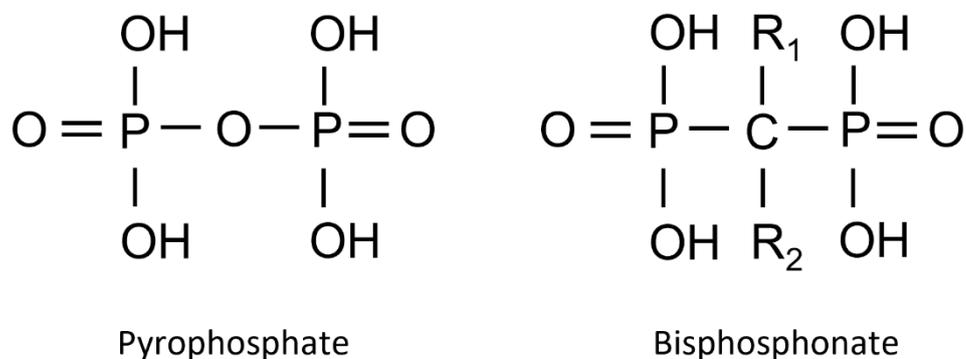
50 The mechanism of action of BP molecules relies on their binding to hydroxyapatite (HA)
51 crystals related to their strong affinity for calcium (Ca) [7]. A few conditional stability constants
52 ($\log K$) for Ca-BP complexes are available. For instance, for the CaL^{2-} complex, the values for
53 clodronate, pamidronate, and EHBP are 5.77 ± 0.01 (0.1 M $(\text{CH}_3)_4\text{NCl}$, 25°C), 6.13 ± 0.1 (0.1 M
54 KCl, 25°C), and 6.18 ± 0.03 (0.1 M $(\text{CH}_3)_4\text{N}(\text{NO}_3)$, 25°C), respectively [8-10]. Regarding the
55 EHBP-Ca complex, the overall stability constant was determined as $\log \beta$ equal to 20.20 ± 0.06
56 [11]. Other elements, such as divalent cations like copper (Cu^{2+}), zinc (Zn^{2+}), or iron (Fe^{2+}), have

57 also been investigated for complexation and exhibit comparable affinities to BPs [12].
 58 Recently, a detailed analysis of cobalt (Co²⁺) complexation with EHBP was published and
 59 provided several overall stability constants up to 27.11 ± 0.1 [13]. Trivalent cations (Al³⁺, Cr³⁺,
 60 Eu³⁺) were also investigated and displayed high values for the overall stability constants (log β
 61 > 25) [14, 15]. Moreover, a few studies have reported that BPs have a good affinity with
 62 hexavalent cations like uranium [16, 17]. Among all these elements, we can note that the
 63 recommended medical treatment for cobalt contamination has minimal effectiveness. The
 64 recent observations suggest that clinical indications of BPs could be extended to other
 65 radionuclides and increase the therapeutic arsenal in the event of internal contamination.

66 This review aims to update the knowledge of this significant class of drugs. After reviewing
 67 the pharmacological properties and mechanism of action of BPs, we will discuss the
 68 applications of BPs in the field of radiation protection.

69 2 Bisphosphonates: structure-activity relations

70 Also known as diphosphonates, BPs are a family of synthetic pharmaceutical molecules
 71 characterized by two phosphonate groups, PO(OH)₂, in geminal position, *i.e.* linked to the
 72 same carbon. BPs have a strong structural analogy with inorganic pyrophosphate, a
 73 degradation product of ATP that inhibits calcium phosphate precipitation by binding to HA
 74 crystals, which has a P-O-P bond (Fig. 1) [18-20]. However, replacing oxygen with a carbon
 75 atom in the center of the P-C-P bridge allows BPs to resist enzymatic degradation and are,
 76 therefore, non-hydrolyzable and, consequently, very stable molecules [21]. The differences
 77 between each BP are the two residues (R₁ and R₂), allowing the creation of multiple structures
 78 with different pharmacological properties.



79

80 Fig. 1: General chemical structure of pyrophosphoric acid and bisphosphonic acid

81 BP molecules available on the market include clodronate, risedronate, ibandronate,
 82 pamidronate, alendronate, and zoledronate. EHBP is the reference BP. The chemical structure
 83 of these seven compounds is given in Fig. 2. These BPs share the P-C-P bridge and two
 84 phosphonate groups essential for bone resorption inhibition and targeting the bone mineral
 85 fraction [22]. Indeed, chemical modifications, such as adding carbon in the P-C-P bridge or
 86 replacing hydroxyl with methyl in the phosphonate groups, lead to a loss of bone affinity and
 87 resorption capacity [21, 23].

88 The short R_1 chain provides BP an affinity for bone tissue, notably *via* the presence of the
 89 hydroxyl group. In contrast, the function and the potential to inhibit bone resorption are
 90 essentially linked to the R_2 chain [24]. BPs can be classified into two categories according to
 91 the presence or absence of a nitrogen atom in the R_2 chain. The first category includes the first
 92 generation of non-amino BP (EHBP, clodronate, tiludronate) with mono-atomic or hydroxyl
 93 groups. The second category consists of a second generation of amino BP with a primary
 94 amine group in R_2 , including pamidronate and alendronate, and a third generation of amino
 95 BP, which has a more complex structure and includes a tertiary nitrogen atom (ibandronate)
 96 or an aromatic N-heterocycle (risedronate, zoledronate).

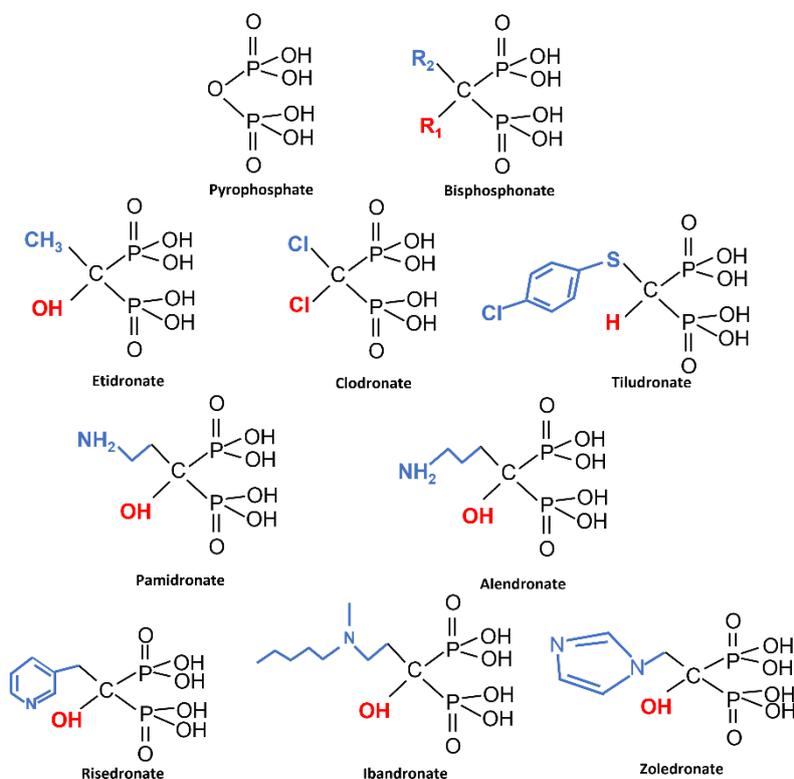


Fig. 2: Chemical structure of bisphosphonic acid family members

113 The presence of a pyridine-type heterocycle or the R₂ chain length dramatically influences
 114 the antiresorptive potency of the BPs molecules [18, 22]. The minimum dose of each BP
 115 required to induce osteopetrosis (a hereditary disease characterized by osteoclast dysfunction
 116 resulting in a defect in bone resorption) in young rats was determined to assess the potency
 117 of the BPs molecules. The doses were then compared to EHBP as a reference [25]. Table 1
 118 shows the structural characteristics of BPs and their relative potency [26].

119

120

Table 1: Chemical structure and relative potency of bisphosphonates

Molecule	R ₁ residue	R ₂ residue	Generation	Potency
Etidronate	OH	CH ₃	1 st	1
Clodronate	Cl	Cl		10
Tiludronate	H	4-chlorothiophenyl		10
Pamidronate	OH	(CH ₂) ₂ NH ₂	2 nd	100
Alendronate	OH	(CH ₂) ₃ NH ₂		1,000
Risedronate	OH	Pyridine	3 rd	5,000
Ibandronate	OH	(CH ₂) ₂ -N $\begin{matrix} \diagup \text{CH}_3 \\ \diagdown \text{(CH}_2\text{)}_4\text{-CH}_3 \end{matrix}$		10,000
Zoledronate	OH	Imidazole		20,000

121

122 3 Pharmacokinetic properties of BPs

123 The administration of BPs usually occurs by the oral or intravenous route. Intramuscular
 124 and subcutaneous routes of administration are not considered because they induce irritation
 125 and tissue damage at the injection site [27, 28]. In humans, the oral absorption of BPs is
 126 minimal. The amount absorbed ranges between 2 and 2.5% for non-amino BPs and is about
 127 0.7% for amino BPs [29]. Furthermore, in the presence of food and drink containing calcium
 128 and magnesium, the bioavailability of BPs collapses dramatically.

129 Once in the bloodstream, at physiological pH (7.4), BPs are partially ionized in the
 130 bloodstream, which explains their ability to bind to proteins present in plasma, mainly
 131 albumin. In humans, however, protein binding varies between BPs: for instance, on average,
 132 the binding fraction is 32% for zoledronate, 26% for ibandronate, 36% for clodronate [30, 31],
 133 and 78% for alendronate [32]. After a plasma half-life of around two hours, BPs disappear
 134 from the blood compartment and are retained in organs or undergo urinary excretion [33].
 135 BPs are found throughout the human body in calcified (approximately 50-80%) and non-
 136 calcified tissues [34].

137 The distribution of BPs within calcified tissues is heterogeneous and complex [35]. Indeed,
138 BPs will preferentially localize in trabecular bones where bone turnover is high. According to
139 Lin's team, the concentrations of radiolabeled alendronate with carbon-14 (¹⁴C) are two to
140 three times higher in these areas [36, 37]. In humans, the available data support the above
141 results. According to the work of Carnevale *et al.*, carried out on pre-and post-menopausal
142 women using a technetium-99m-labeled methylene diphosphonate molecule, the distribution
143 is predominantly in areas of high bone remodeling [38]. However, recent work by Roelofs'
144 team suggests a more profound localization of BP. Indeed, 24 hours after administering
145 fluorescent risedronate analogs to 3-month-old mice, the labeled molecule was found in
146 osteocyte lacunae near vascular channels and in monocytes in the bone marrow [39].
147 The elimination half-life of BPs in skeletal tissue varies according to the molecule considered
148 and depends on factors such as the rate of bone turnover and renal function [40]. In the case
149 of alendronate, it is estimated to be more than ten years in healthy humans [41, 42].

150 The distribution of BPs in non-calcified tissues has not been investigated as much as in
151 skeletal tissues. However, it appears to be relatively homogeneous with a preference for the
152 liver, kidney, and spleen, but their retention decreases rapidly over time. After intravenous
153 (IV) administration of 1 mg.kg⁻¹ of ¹⁴C-alendronate in rats, the retention of the molecule in
154 these tissues ranges from 63% of the dose at 5 min to 5% at 1 h. At the same time, retention
155 in bone tissue increases to a peak at 1 hour after injection [37].

156 The characteristic of BPs structure based on a P-C-P bridge makes enzymatic hydrolysis
157 difficult. However, in contrast to nitrogen containing bisphosphonate (N-BP), *in vitro* studies
158 in mammalian cells (murine macrophage cells J774 and human osteosarcoma cells MG63)
159 suggest that clodronate and EHBP are metabolized to non-hydrolyzable cytotoxic analogs of
160 ATP identified by chromatographic analysis. No data on the percentage of the dose that would
161 be metabolized is available. In contrast, for alendronate, no metabolites were detected [43],
162 and N-BP was eliminated in an unchanged form [35].

163 The elimination route of BPs is almost exclusively urinary via glomerular filtration [35].
164 Some BPs are undergoing tubular secretion, as shown by Troehler's team on rodents [44]. The
165 molecules used were EHBP and dichloromethylene diphosphonate labeled with ¹⁴C. According
166 to Ruggiero *et al.*, 50% of the absorbed BPs is excreted unmetabolized in the urine [24]. The
167 removal of BPs is complex as it is dependent on bone turnover. Once integrated into the bone,
168 BPs will only be mobilized during a bone remodeling phase. This slow removal kinetics explains

169 the continuous urinary and blood concentrations over long periods. A minor elimination route
170 occurs via bile excretion (less than 0.5%) [37].

171 **4 Mechanism of action of BPs**

172 The principal therapeutic action of BPs is the inhibition of bone resorption. Bone tissue is,
173 therefore, the main target of this class of drugs. Before detailing the mechanism of action, we
174 will discuss the chelation process essential to this mechanism.

175 **4.1 Chelation**

176 The high affinity of BPs for calcium is necessary to inhibit bone resorption. Indeed, the
177 binding intensity of BPs to calcium present in the mineral fraction of bone by adsorption forms
178 the basis of their mechanism of action [7]. Chromatographic studies suggest that the
179 phosphonate groups and the R₁ and R₂ residues of BPs significantly bind to HA crystals. These
180 chemical differences explain the variability observed between BPs. The results suggest an
181 increasing affinity in the following order: clodronate < EHBP < risedronate < ibandronate
182 < alendronate < zoledronate [45]. More recently, DFT (Density Functional Theory) analyses
183 based on quantum calculations of molecular structures have confirmed these results [46].
184 They support the stronger affinity to the mineral matrix of BPs with a nitrogen atom, attesting
185 to a more consistent anti-resorption effect [47].

186 To better understand the chelation process, which is essential for the biological action
187 of BPs, the Ca-BP bond has been studied extensively, particularly by Claessens and Van der
188 Linden [8]. Among the ligands (L), EHBP and methylene diphosphonate were studied.
189 According to the authors, the complexes would only exist at pH above 4. The predominant
190 chemical complex at pH 5-7 would be a monoprotonated calcium one (CaHL⁻) with a formation
191 constant log K at 3.12 ± 0.06. Then, by increasing the pH, the unprotonated dicalcium species
192 (Ca₂L) would be predominant with log K about 4.63 ± 0.06. The studies of Foti support these
193 values [11]. Indeed, for the CaHL⁻ complex, log K and log β (overall stability constant) are 2.87
194 and 14.31 ± 0.05, respectively. For the Ca₂L complex, log K and log β are 4.70 and 11.22 ± 0.06,
195 respectively. Furthermore, it was shown that the bond between the BP and the calcium was
196 bidentate or even tridentate when the R₁ residue contained a hydroxyl group [48].

197 In addition to calcium, the complexation between BP and especially EHBP (the head of
198 BPs series) and other divalent elements has also been investigated, such as Cu²⁺, Zn²⁺, Fe²⁺,
199 nickel (Ni²⁺), cadmium (Cd²⁺), magnesium (Mg²⁺), Co²⁺ and strontium (Sr²⁺) [11-13, 49].

200 Complex formation with trivalent cations was also studied, especially with aluminium (Al^{3+}),
 201 chromium (Cr^{3+}) and europium (Eu^{3+}) [14, 15]. The stability constants of these cations with
 202 EHBP are listed in Table 2.

203 Table 2: Complexation constants for MH_2L complex

	$\log K$	$\log \beta$
0.1 M KCl (20°C) ^a		
Co^{2+}	ND	8.52 ± 0.04
0.1 M KNO_3 (25°C) ^{b,c,d}		
Sr^{2+}	1.52	ND
Co^{2+}	2.78	ND
Cu^{2+}	3.0 ± 0.4	20.1 ± 0.5
Zn^{2+}	3.8 ± 0.3	20.2 ± 0.5
Ni^{2+}	4.8 ± 0.1	20.3 ± 0.2
Cd^{2+}	4.5 ± 0.2	20.7 ± 0.3
Fe^{2+}	3.3 ± 0.3	21.0 ± 0.4
Cr^{3+}	4.0 ± 0.1	28.9 ± 0.1
Al^{3+}	1.8 ± 0.3	29.1 ± 0.3
0.1 M NaCl (25°C) ^e		
Mg^{2+}	1.54	19.91 ± 0.2
Ca^{2+}	1.83	20.20 ± 0.06
0 M ^f		
Eu^{3+}	5.6 ± 0.4	25.2 ± 0.4

221 M is metal and L is ligand (EHBP), ND means
 222 not determined.

223 ^a[13], ^b[49], ^c[12], ^d[15], ^e[11], ^f[14].

224 All the elements examined form stable complexes with EHBP molecules, and the
 225 results are pretty similar. Concerning Cu^{2+} , the complex M_2L_2 is predominant in a strongly
 226 alkaline solution. $\log \beta$ was determined at 30.42 in 0.3 M NaOH solution at 25°C [50].
 227 Differences can be observed and are function, among other things, of the ligand coordination
 228 sites, the charge of the complex, or the steric hindrance generated [49]. The use of BPs has
 229 also been considered for the decorporation of actinides such as uranium (U). A conditional
 230 constant for the complex UO_2^{2+} -HEDP (1:1 complex stoichiometry) was determined at

231 7.7 ± 0.3 by using a 0.1 M sodium perchlorate solution (at 20°C), and the stability constant was
232 established at 19.1 [16].

233 In addition to their anti-bone resorption property, BPs are used for imaging through
234 bone scintigraphy. They can be coupled to radiotracers, which are beta/gamma emitters such
235 as ^{99m}Tc, ¹⁵³Sm, ¹⁸⁶Re, ¹⁸⁸Re, depending on the purpose for diagnostic purposes. The
236 radiotracers can be coupled due to the chelation property of BPs towards these radionuclides
237 [51, 52]. In summary, the powerful capacity of BPs, especially EHBP, to complex such cations
238 is well documented and thus paving the way potentially for other elements.

239 4.2 Tissue level

240 At the tissue level, all BPs derivatives have a similar action. They bind preferentially to
241 HA crystals, replacing inorganic pyrophosphate and inhibiting their degradation, thus
242 preventing bone resorption. Employing a histomorphometric evaluation which allows a
243 qualitative and quantitative analysis of the bone structure, it appears that in ovariectomized
244 baboons treated with alendronate (0.05 or 0.25 mg.kg⁻¹ by IV every 2 weeks for 2 years), there
245 is a decrease in resorption gaps and then in a second phase (4 to 6 months after the start of
246 treatment), an overall reduction in bone formation without any impact on osteoblast activity
247 [53]. Consequently, a decrease in bone fragility and an increase in bone mass occur.
248 Furthermore, as the newly formed bone is less prone to resorption, the mineralization phase
249 is more complete, as it has been observed in women with post-menopausal osteoporosis
250 treated with alendronate (5 to 10 mg.day⁻¹ for 3 years or 20 mg.day⁻¹ for 2 years followed by
251 5 mg for 1 year) [54]. From a densitometric point of view, the decrease in resorption is more
252 significant than that of bone formation, resulting in a positive calcium balance.

253 4.3 Cellular level

254 Since the pharmacological action of BPs is an anti-bone resorption effect, their preferred
255 target is the osteoclast cell. Different results with a direct or indirect action on the target have
256 been reported [55]. Depending on the model used, experimental results regarding the effect
257 of BPs on the osteoclasts provide additional information. Indeed, *in vitro* studies using
258 osteoclast-like cells and mouse bone marrow have shown osteoclastogenesis inhibition after
259 alendronate treatment [56]. On the other hand, *in vivo* studies in rats have shown an increase
260 in non-active osteoclasts and then, after chronic administration of BP (EHBP), a decrease in
261 their number [57]. Several studies, especially those carried out in mice, have demonstrated

262 the penetration of certain radiolabeled BPs, such as tiludronate, into osteoclasts and the
263 dysfunctions caused, including modification of the actin structure [58]. The team of Hughes *et*
264 *al.* revealed, through *in vitro* and *in vivo* studies in mice, that treatment with different BPs
265 (risedronate, pamidronate, and clodronate) promoted the apoptotic induction of osteoclasts
266 [59]. In the presence of BPs, mouse osteoblasts in marrow cultures would indirectly inhibit
267 osteoclast recruitment (the last step in osteoclastogenesis) or their activity via inhibitory
268 factors present in the culture medium [60]. Concentrations as low as 10^{-11} mol.L⁻¹ and an
269 exposure time of 5 min would be sufficient to cause this effect [22]. However, the exact
270 mechanism of action has not been elucidated. Finally, at the cellular level, the mode of action
271 of BPs will differ according to their chemical structure [61]. They can block osteoclastogenesis
272 by inhibiting precursor osteoclast proliferation and recruitment, promoting osteoclast
273 apoptosis, and inhibiting osteoclast activity [55].

274 **4.4 Molecular level**

275 The molecular mechanism of action of BPs has been elucidated in the late 1990s. During
276 bone resorption, when the environment becomes acidic through the H⁺/Cl⁻ transporter at the
277 osteoclast membrane, the BPs will be protonated, making the binding with calcium less
278 favorable, thus allowing their internalization by an endocytosis mechanism [62-64].
279 BPs will not have the same molecular target depending on their chemical structure. Indeed,
280 once internalized within osteoclasts, non-nitrogenous first-generation BPs will be integrated
281 into the ATP molecules pool. The newly formed non-hydrolyzable ATP cytotoxic compounds
282 will accumulate intracellularly, inhibiting essential ATP-dependent functions of the osteoclasts
283 (disruption of the mitochondrial machinery and cell death), leading to their apoptosis [65].
284 The principal mechanism of action of nitrogenous BPs (second and third generations) is quite
285 different. After entering the cell, BPs will play a key role in inhibiting mevalonate/cholesterol
286 cycle enzymes (Fig. 3, [66]) and particularly farnesyl pyrophosphate synthase (FPP-synthase)
287 [67, 68].

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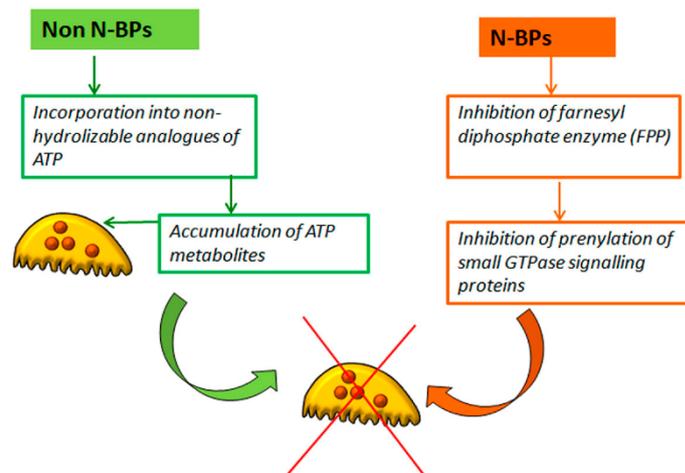


Fig. 3: Summary of the mechanism of action of BPs

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By interfering with FPP-synthase, the prenylation (*i.e.*, the addition of an isoprenoid lipid farnesyl at C15 or geranylgeranyl at C20, via a thioether linkage to proteins at or near the C-terminal amino acid cysteine position) of numerous small proteins such as Ras, Rho, Rac will no longer take place. Rho/Rac proteins constitute a subgroup from the Ras superfamily of small GTPases. The biological functions of these proteins that regulate cellular processes essential to osteoclasts, such as the organization of the cytoskeleton, the modification of the cell membrane, or the transduction of the cellular signal, will no longer be ensured and will then lead to the loss of the anti-resorption activity of osteoclasts and their apoptosis.

The class of BPs could also have a beneficial role on osteoblastic cells by having an anti-apoptotic action. Indeed, all BPs activate a kinase enzyme called Extracellular Signal-Regulated Kinase (ERK). By increasing the phosphorylation of ERK enzymes, BPs would thus prevent the apoptosis of osteoblasts [69].

Due to their bone tropism and role as inhibitors of bone resorption, BP molecules are mainly used in treating bone pathologies, including osteoporosis and Paget's disease [24, 70]. In addition, they are prescribed for preventing bone complications in adult patients with advanced malignant disease with bone involvement and treating tumor-induced hypercalcemia [71, 72]. BPs are also used in nuclear medicine for diagnostic, metabolic imaging, or therapeutic purposes [73, 74]. Finally, BPs seem to have a potential antitumoral effect, especially by inhibiting tumor migration and angiogenesis, inducing tumor cell apoptosis, or impairing tumor cell adhesion to bone [64].

319 Generally, BPs are well tolerated if the recommendations for usage are respected (to
320 take the treatment on an empty stomach, to remain in a sitting or standing position, and to
321 respect a 30-minute delay before taking a meal). However, some patients experience
322 potentially significant side effects that can cause great discomfort. Among the side effects
323 noted are gastrointestinal symptoms (such as esophagitis, nausea, vomiting, diarrhea), kidney
324 disorders, inflammation of certain membranes of the eye (such as uveitis, scleritis), metabolic
325 troubles (hypocalcemia) [75-78]. Effects on the skeleton are also to be listed, such as atypical
326 femoral fracture and osteonecrosis of the jaw [79-81]. Finally, minor cutaneous effects
327 (eczema, urticaria, or Stevens-Johnson syndrome, which is more serious) and controversial
328 effects (including atrial fibrillation and bone pain) have been described [82, 83].

329 Due to significant strengths (bone tropism, inhibitor of bone resorption, stable complex
330 with various divalent cations), BPs, widely studied in research, have interested researchers in
331 a more unexpected field: radiation protection. In this latter case, adverse effects seen in
332 chronic treatments can be excluded since it becomes an emergency treatment.

333

334 **5 Dosage forms and route of administration for the delivery of BPs**

335 **5.1 Nanotechnologies for targeted delivery**

336 Among the formulations considered for the delivery of BPs are liposomes. They are small
337 biodegradable vesicles composed of a phospholipid bilayer delimiting an aqueous
338 compartment. This form allows the encapsulation of hydrophilic molecules in its core and
339 lipophilic molecules within the bilayer. In oncology, clodronate liposomes are widely used to
340 study the role of macrophages by inducing their apoptosis [84]. In addition to conventional
341 liposomes, second-generation liposomes called stealth liposomes have also been applied to
342 the delivery of BPs. They differ from the former by the addition, of hydrophilic polymers such
343 as polyethylene glycol (PEG), allowing them to avoid opsonization (the process by which
344 opsonins adsorb to the surface and promote phagocytosis) and thus to circulate longer in the
345 body. These pegylated alendronate liposomes have been tested in mice to investigate the
346 potential anti-tumor role of BPs [85]. In cancer-related research, pegylated nanoparticles of
347 BP calcium salts were also tested in a model of mice with 4T1 tumors [86]. The results showed
348 that M2 macrophages are depleted in large quantities and a drastic decrease in interleukin-10

349 secreted by M2 macrophages. This formulation has been responsible for a tumor
350 microenvironment modification that allows for better radiotherapy efficacy.

351 BPs are also used as ligands to target bone in different diseases. One study describes
352 micelles loaded with curcumin (potential anti-tumor effect) and functionalized with
353 alendronate conjugated to a polymer (hyaluronan-octadecanoic acid) [87]. After injections
354 every 2 days for 20 days in a mouse model with osteosarcoma, the results showed that the
355 formulation of curcumin-loaded micelles in the presence of alendronate demonstrated a high
356 affinity for HA and delayed tumor growth compared to the free molecule ($p < 0.05$).

357 The IV route, one of the routes of BP administration in the clinic, allows for the
358 distribution of various attractive targeting forms. Although it is invasive and requires a medical
359 procedure, it will enable rapid delivery of the drug, in its totality, into the vascular
360 compartment.

361 **5.2 Dosage forms to improve BPs bioavailability.**

362 5.2.1 Oral route

363 The oral route is one of the routes of administration of BPs in clinical practice. The main
364 objective is thus to enhance the gastrointestinal absorption rate of the molecules. The use of
365 ibandronate-based gastro-resistant reservoirs has been studied in rodents [88]. This
366 formulation is composed of nanoparticles with citrus pectin as the raft former, calcium
367 carbonate to enhance the raft strength, sodium bicarbonate as an effervescent mixture and
368 PEG as the permeability enhancer. Pharmacokinetics studies showed a 2-fold increase in
369 bioavailability of the gastro-resistant reservoir compared to the reference formulation
370 (calculated area under the curve (AUC) of 6899 ± 3.5 ng/ml.h and 3708 ± 3.4 ng/ml.h). The
371 resistance to stomach acidic pH was also explored in rabbits with gastro-resistant liposomes
372 of alendronate [89]. Bioavailability was increased by 12 between the gastro-resistant
373 formulation and the tablet form. Since 2012, alendronate is commercially available in Japan
374 in a weekly gel form [90]. This interesting dosage form was shown to be more suitable for
375 aging populations. It has the advantage of not requiring water and is easier to swallow.
376 Although no difference was found with the tablet form, the results are significantly more
377 favorable for oral-related adverse events (heartburn and epigastralgia). The effervescent
378 solution of alendronate (taken weekly) has also been clinically assessed compared to the
379 tablet form [91]. The results indicated a better follow-up of the treatment and a better

380 tolerance with the effervescent solution due to the dissolution of alendronate in a buffered
381 appetent solution, limiting the risk of gastrointestinal irritation.

382 5.2.2 Pulmonary route

383 BP delivery through the pulmonary route was poorly studied. A dried powder
384 formulation was proposed based on alendronate, leucine, and ammonium bicarbonate [92].
385 Administration of the formulation (with particle size less than 12 μm) was performed by the
386 intra-tracheal route. The bioavailability of alendronate using this formulation ($6.23 \pm 0.83\%$)
387 was increased by a factor of 3.5 compared to the oral route. Another study focused on
388 risedronate sodium which was formulated into poly(lactide-co-glycolic acid) microspheres
389 (with particle size mean of $3.68 \pm 0.69 \mu\text{m}$) to test the possibility for risedronate to be
390 delivered through an alternative route instead of oral administration [93]. *In vivo* results
391 showed high bone deposition compared to controls. The pulmonary route has many
392 advantages: a large surface area of absorption, close vascularization (subject to a small
393 aerodynamic diameter), no hepatic first-pass effect, and few notable adverse effects, unlike
394 the oral route.

395 5.2.3 Transdermal route

396 A microemulsion (water/oil) formulation of alendronate has been tested in rat model
397 [94]. At an equivalent dose ($30 \text{ mg}\cdot\text{kg}^{-1}$), statistically significant differences were reported
398 between the oral and transdermal routes. The bioavailability of the microemulsion is twice
399 greater than the solution. Another dosage form using the transdermal route was examined. It
400 is a protransfersome gel loaded with risedronate [95]. Unlike liposomes, which have limited
401 elastic properties and will primarily accumulate on the skin surface, protransfersomes are
402 ultra-flexible lipid vesicles that can deform by absorbing water from the skin. Skin penetration
403 is then increased without any sign of toxicity. Compared to the oral form, the results showed
404 a statistically significant improvement in bone architecture and a strengthening of trabecular
405 bone connectivity. Through these two examples, bioavailability was improved owing to the
406 dosage form. The transdermal route displays real advantages such as no first hepatic passage,
407 easy administration, the comfort of application, rapid withdrawal if necessary, and an absence
408 of adverse effects related to oral administration.
409

410 **6 BPs application in research in the field of radiation protection**

411 As previously mentioned, BPs are a historical class of drugs and have been the subject of
412 research for many years due to their attractive (tissue of interest and pharmacological
413 property) and promising (affinity and different speciation with divalent cations)
414 characteristics. The therapeutic molecules recommended in case of internal contamination by
415 radionuclides are few and not entirely adequate. Using BPs as a decorporation treatment is a
416 judicious strategy because, in the case of uranium, the retention organs include the kidneys
417 and bone tissue [4]. Unlike other therapeutics, a decorporating agent must meet specific
418 essential criteria [96]: (i) to be non-toxic; (ii) to have high affinity for the molecule of interest;
419 (iii) to form stable M-L complexes; (iv) to form soluble and urinary excretable M-L complexes
420 (v) to be orally administrable. Designing a decorporation treatment based on BP molecule
421 could be hazardous due to the low gastrointestinal absorption. However, relying on new
422 technologies and considering an alternative route of administration is a challenging but
423 promising approach.

424 **6.1 Skin decontamination**

425 Research applications using BP molecules have included skin decontamination after
426 exposure to actinides, particularly to uranium. Modes of exposure are either poorly soluble
427 form (U(IV)) or soluble form (U(VI)), which is the most stable form in biological and
428 environmental media [97, 98]. One of the first studies worth mentioning is the one from
429 Houpert *et al.* dealing with early skin decontamination after exposure to industrial U
430 compounds [6]. The treatment uses a paste or a dressing based on hydrocolloid and
431 carboxymethylcellulose (with high absorbency) associated or not with potential chelating
432 agents such as EHBP. The authors conducted *ex vivo* studies on bovine muscle and *in vivo*
433 experiments carried out on two different models in rats. One with intermuscular U deposition
434 to mimic a blunt object injury and the other with IM U deposition to mimic a sharp object
435 injury. The results are reported in Table 3. Adding the BP molecules did not improve treatment
436 efficacy in all these experiments. The reason is probably due to the insoluble character of the
437 uranium oxides being unfavorable to chemical chelation.

438 In a second study, BP molecules incorporated into hydrogels were also considered for U
439 decontamination [99]. Different types of hydrogels were formulated: pamidronate-based
440 hydrogel (I), diethylene triamine pentaacetic acid (DTPA)-based hydrogel (II), pamidronate-

441 based polymeric hydrogel (III) and hydrogel in which BP molecule is linked to the motif of
442 naphthalene phenylalanine-phenylalanine (NapFF) or NapFFP hydrogel (IV). For *in vivo* studies
443 conducted on mice, a 1*1 cm² wound was created using a razor blade on the back of the
444 animals. Different parameters were monitored, such as survival rate at 10 days, change in
445 body weight over 10 days, and the amount of U in the organs, particularly the kidneys (target
446 organ) (Table 3). All 4 treatments demonstrated significant efficacy compared to controls, but
447 the pamidronate-based hydrogel (I) yielded better results.

448 The hydrogel-based treatment offers attractive results and combines interesting
449 properties (absorptivity, stability, biocompatibility, biodegradability). However, the
450 improvement of the effectiveness of these treatments by adding chelators to the formulas has
451 not been demonstrated. Furthermore, the results obtained after using the dressing and paste
452 forms are promising regarding reducing U in the kidneys. However, the results highlight the
453 lack of efficacy of chelating agents within these dosage forms since they did not further retain
454 the radionuclide. Nonetheless, in contrast to a simple EHBP solution, the dosage forms
455 proposed (paste, dressing, hydrogel) are easier to use, ensure that the active molecule
456 remains at the site of administration, and control the release of the drug by providing the
457 carrier with a remanent effect. Effective emergency treatment after skin contamination
458 appears essential to prevent the penetration of U in the body as much as possible and thus
459 avoid long-term retention.

Table 3: Treatments for the decontamination of uranium using BPs

Model	CONTAMINATION			TREATMENT						R _{Ligand} / RN	RESULTS	Bibliographical references
	U compound	Route	Single or repeated	Time before treatment	Molecule	Galenic form	Route	Single or repeated	Duration			
Bovine muscle	UO ₂ , UO ₄ or U ₃ O ₈	Deposition on incisions	Single	5 min	Carboxymethyl-cellulose-based hydrocolloids associated or not with EHBP	Paste	Application on incisions	Single	15 min (only for UO ₄) or 1 h	90 (UO ₂)	U retention in the galenic form: 15 min: 9% (6% with EHBP) 1h: 75-84% (no increase with EHBP)	[6]
						Dressing				189 (UO ₄) 602 (U ₃ O ₈)		
Rat	UO ₄	Inter muscular deposition	Single	2 min	Carboxymethyl-cellulose-based hydrocolloids associated (paste) or not with EHBP (paste/dressing)	Dressing or paste	Application on the inter or intra muscular pocket	Single	1 h	28	Tissue retention of U at T0 + 1 h: Dressing: ⊆ 63% (wound site), ⊆ 68% (kidneys) Paste: ⊆ 39% (wound site), ⊆ 65% (kidneys). Paste + EHBP: ⊆ 35% (wound site), ⊆ 70% (kidneys)	[6]
		IM deposition	Single		/	Dressing			15 min or 1 h			
Mice	UO ₂ (NO ₃) ₂	3 drops on the wound	Single	15 min	Pamidronate-based hydrogel (I), DTPA-based hydrogel (II), Pamidronate-based polymeric hydrogel (III), NapFFP hydrogel (BP molecule linked to a naphthalene phenylalanine unit) (IV)	Hydrogel	Application on the wound site (0.125 cm ³)	Repeated (3 times)	3 min	NA	Survival rate at T0 + 10 days: 92% (I), 100% (II), 83% (III), and 58% (IV), 42% (control) Body weight during 10 days: Significantly gain for all treated groups (from day 5 to day 10 for I; at day 10 for II, III and IV) vs control group. U quantity in kidneys: ⊆ 93% (II), ⊆ 97% (III), ⊆ 89% (IV) < DL for hydrogel (I)	[99]

U, uranium; RN, radionuclide; BP, bisphosphonate; NA, not available ; R_{Ligand/RN} means molar ratio ligand to radionuclide.

462 **6.2 Decorporation**

463 In the nuclear industry or following a nuclear incident/accident, the risk of internal
464 contamination by inhalation, ingestion, or skin injury is real. It requires appropriate medical
465 countermeasures using a decorporating agent. It removes radioactive elements from the body
466 using a chelating agent or administering another pharmaceutical agent [100]. For some
467 radionuclides (cesium-137 or iodine-131), treatments exist. However, for U, the proposed
468 treatment is based on sodium bicarbonate, a non-specific molecule that lacks effectiveness.
469 Until recently, various publications outlined the treatment strategies envisaged for the
470 decorporation of this actinide [101-106]. Among the ligand families listed are
471 polyaminocarboxylic acids, siderophores and bisphosphonates.

472 Some research groups are considering BP as a potential chelator of U. Wang *et al.* developed
473 a conjugate of dopamine and BP that binds to iron oxide with a high partitioning constant at
474 pH 7 [107]. Another team has synthesized different ligands formed by several BP compounds
475 (EHBP) anchored on a calix[4]arene structure [98], including one with conditional stability
476 constants with the uranyl ion UO_2^{2+} greater than 14 (at pH values ranging from 5.5 to 9.0 and
477 hypothesizing a 1:1 ligand/uranyl complex formation), which is favorable to strong ligand-
478 radionuclide complexations. Furthermore, the affinity of EHBP with U has also been
479 demonstrated in rat serum using X-ray absorption spectroscopy (XAS) [108]. These *in vitro*
480 data are promising clues for considering the application of this formulation to *in vivo*
481 experiments.

482 Studies with EHBP have also been conducted in the rodent model. The results are listed
483 in Table 4. Firstly, decorporation studies were considered after IM radiocontamination. Wistar
484 rats received an IP injection with EHBP 1 h after being contaminated by IM injection of uranyl
485 nitrate [109]. The treatment was pursued for 28 days, with one injection per day. The
486 administration of EHBP resulted in a significant decrease in U concentrations between 40 and
487 50% in organs (kidney, liver, and femur) compared to controls. These encouraging data
488 concerning the reduction of U retention in the kidneys had also been observed by Henge-
489 Napoli's team, who carried out a study in the rodent model [5]. The treatment used was EHBP.
490 Henge-Napoli *et al.* considered several protocols depending on the time elapsed between
491 contamination and treatment (immediate, 5, or 30 min), the route (IM, IP), or the type of
492 administration (single or repeated). The significant results were similar regardless of the ratio

493 ligand/RN or route of administration. Indeed, during early administration of EHBP, the
494 retention of U in the kidneys and the whole body declined by around 80% and 30% at
495 T0 + 24 h, respectively, compared to controls. After a delayed treatment (30 min), U retention
496 decreased by 45% in kidneys compared to controls. After repeated treatment, at T0 + 4 days,
497 EHBP reduced the U retention in the kidneys by 72% and
498

Table 4: Treatments studied for the decorporation of uranium using BPs

Model	CONTAMINATION			TREATMENT						R _{Ligand/RN}	Results	Bibliographical references
	U compound	Route	Single or repeated	Time before treatment	Molecule	Galenic form	Route	Single or repeated	Duration			
Rat	UO ₂ (NO ₃) ₂	IM	Single	1 h	EHPB	Solution	IP	Repeated (1/day)	28 days	8	U retention at T 28 days ∽ ~ 50% (kidneys), ∽ ~ 40% (liver), ∽ ~ 43% (femur) Survival rate at T 28 days 20% (control group), 50% (treated group)	[109]
Rat	²³³ UO ₂ (NO ₃) ₂	IM	Single	5 min	EHPB	Solution	IM	Single		5000	After early administration, U retention (at T 24 h): IM injection: ∽ 76% (kidneys), ∽ 30% (whole-body) IP injection: ∽ 83% (kidneys), ∽ 32% (whole-body)	[5]
				30 min								
				At the same time								
				30 min								
				At the same time			IP	Repeated (1/day)	3 days	2500	After delayed administration, U retention (at T 24 h): IM injection: ∽ 45% (kidneys) IP injection: ∽ 45% (kidneys)	
				At the same time			IP	Repeated (1/day)	3 days	2500	U retention at T 4 days: ∽ 72% (kidneys), ∽ 23% (whole-body)	
Rat	²³⁸ UO ₂ (NO ₃) ₂	IP	Single	At the same time	EHPB	Solution	IP	Single		7.5	For animals exposed and treated: bone healing did not differ from the non-exposed control group.	[110]
Rat	²³⁸ UO ₂ (NO ₃) ₂	IP	Single	At the same time	EHPB	Solution	IP	Single		8	For animals exposed and treated: - Survival rate: 100% over 60-day period - Body weight: no difference with control groups not exposed (untreated or treated with EHPB) at T 8, 30, and 60 days - Kidney tissue: no difference with control groups not exposed (untreated or treated with EHPB) on the 60 th day	[111]
Rat	²³⁸ UO ₂ (NO ₃) ₂ ·6H ₂ O	IP	Single	At the same time, 24 h or 48 h	EHPB	Solution	IP or SC	Single		10	Survival rate on the 60th day of the experiment: 100% with EHPB (IP or SC) given immediately or 24 h after exposure (IP) Biometric parameters of the mandibles 60 days after the beginning of the experiment: better result with EHPB administered immediately by SC route	[112]
Rat	²³³ U	IP	Single	5 min	BP ligands or EHPB alone	Solution	IP	Single		100	For 5 most promising ligands at T 5 days: U retention: ∽ 53-58% (kidneys) for ligands n°3C, 5C and 7B, ∽ 18-30% (bone) for ligands n°5C, 6A and 7B U excretion: ∽ 31% for ligand n°3C only For EHPB alone, at T 5 days: ∽ 31% (kidneys), ∽ 23% urinary excretion	[17]
Mice	UO ₂ (NO ₃) ₂ ·6H ₂ O	Oral	Single	20 min	EHPB	Solution	Oral	Single		2 to 4	For oral route (R_{L/RN} = 3) and SC route (R_{L/RN} = 0.3): - Survival rate for at least 14 days: 50% - Urea and creatinine serum levels similar to control group at T 14 days - Kidney lesions less severe at T 48 h and signs of tissue recovery at T 14 days - Chelating effect of EHPB may prevent U from reaching acquiescent cells in the growth cartilage, allow normal formation of bone trabeculae.	[113 – 115]
							SC			0.3		

500 U, uranium ; RN, radionuclide ; BP, bisphosphonate; R_{Ligand/RN} means molar ratio ligand to radionuclide.

501 in the whole body by 23%. Under favorable conditions, EHBP has been shown to be
502 significantly more effective if administered earlier.

503 Decorporation studies have also been performed after radiocontamination with IP
504 injection. Research was carried out on an alveolar bone healing model to study bone
505 formation inhibition following uranium exposure [110]. After dental extraction, the rats were
506 exposed to U and, at the same time, received a solution of EHBP at a ratio of 7.5 by the IP
507 route. After an observation period of 14 days, unlike the exposed group in which bone healing
508 did not occur, the exposed and treated group showed results similar to those of the control
509 group. In another study, the researchers focused on renal function, another target of U [111].
510 A solution of EHBP was administered without delay to rats by IP at a molar ratio ($R_{L/RN}$) of 10.
511 At the end of the 60-day experiment, the exposed and treated animals showed a 100% survival
512 rate, body weight, and histological analysis of the kidneys similar to the control groups [111].
513 The Ubios team also confirmed a 100% survival rate after late treatment administration
514 (24 hours after contamination) and by SC route without delay after exposure to U [112]. The
515 results also highlighted that a single SC injection of EHBP immediately after contamination
516 was more effective in preventing undesirable changes in mandibular growth (compared with
517 IP). Other chemical entities have been proposed, dipodal or tripodal analogs bearing BP
518 functions [17]. Almost all of these compounds showed high complexation constants (between
519 10^{15} and $10^{19.5}$) at pH 7.4 but also at pH 5.5 (pH of the kidney medium). An *in vivo* study was
520 performed with the 5 most promising ligands (Table 4). At T0 + 5 days, for 3 ligands (n°3C, 5C,
521 and 7B), U retention decreased by around 55% in kidneys, and for 3 ligands (n°5C, 6A, and 7B)
522 18-30% in bone, but only one ligand (dipod n°3C) showed a significant increase of 31% in U
523 excretion compared to controls. For EHBP alone, at T0 + 5 days, urinary excretion of the
524 radionuclide was increased by 10% compared to controls.

525 Finally, uranium decorporation has also been studied after oral exposure, a potential
526 route of intake for the radionuclide. A solution of EHBP was administered 20 min after
527 contamination by the oral route ($R_{L/RN}$ between 2 and 4) or by the SC route ($R_{L/RN}$ of 0.3) [113].
528 A survival rate of 50% for at least 14 days post-contamination was obtained after
529 administration of BP by the oral route (at an $R_{L/RN}$ of 3) and by the SC route. The studies were
530 pursued using the latter experimental conditions. In these groups of animals, the kidney
531 lesions observed at 48 h were less severe than in the untreated exposed group and showed
532 signs of healing at 14 days [114]. Finally, under these contamination/treatment conditions,

533 Bozal *et al* showed that EHBP allowed to improve the adverse effects regarding the endothelial
534 ossification [115].

535 As mentioned above, the free form of EHBP has shown interesting efficacy in terms of
536 clinical parameters (survival rate, body weight), histology, tissue retention of uranium, and
537 excretion. However, using targeting systems such as colloidal nanoparticles could significantly
538 improve the efficacy of these pharmaceutical molecules. Indeed, these dosage forms
539 undoubtedly provide them with the following benefits: (i) to protect them from the
540 environment by reducing the potential enzymatic degradation, (ii) to avoid the premature
541 binding with endogenous compounds, (iii) to improve the ligand distribution and target tissues
542 [116]. Combining free and encapsulated forms would ultimately act on circulating uranium
543 and prevent U retention in the organs of interest.

544

545 **7 Conclusion and perspectives**

546 As discussed in this review, BPs represent a historical and significant class of drugs in the
547 pharmaceutical field. Due to their macrophage inhibitory properties and their distribution in
548 bone tissue, BPs have opened several avenues of research, and various future applications are
549 proposed. The results of studies using BP molecules are attractive due to innovative dosage
550 forms that improve their bioavailability or allow the targeting of the proper tissue. Drug
551 delivery sciences appear essential in designing new treatments by allowing better delivery of
552 BPs, limiting collateral effects, and ultimately allowing a benefit for the patient.

553 Among the applications mentioned, those related to skin decontamination and decorporation
554 are promising, as evidenced by uranium studies. In addition, the global formation constants
555 from the literature are attractive for both U and other divalent cations. For radionuclides such
556 as radium, cobalt, or strontium, the efficacy of the therapy recommended by nuclear safety
557 authorities worldwide is unsatisfactory, and the set-up of efficient medical countermeasures
558 is essential for the population (civilian, responders, and nuclear workers). Thus, it appears
559 worthwhile to continue the research with EHBP. Moving forward to nanotechnologies in the
560 field of pharmacology must be considered for further investigations to maximize the efficiency
561 of EHBP by focusing its action to decorporate the radiocontaminant.

562 Through this review, potential cases of drug repurposing are numerous. Even though drug
563 repositioning remains a minority path compared to traditional research, this is a valuable

564 strategy that gives us early access to strong knowledge, particularly regarding toxicological
565 and pharmacokinetic aspects. In addition to this precious time saving, there is a financial gain
566 by avoiding lengthy and costly screening processes. Although there are many challenges to
567 overcome, because of the abundant literature on the subject, drug repurposing of BPs is
568 becoming feasible.

569

570 **8 Declaration of interest**

571 The authors have no conflicts of interest to declare.

572

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576

577 **10 References**

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