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Review

The Potential of Indole Alkaloids in Bone Health and Osteoporosis Management

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Abstract: Indole alkaloids, a class of plant-derived nitrogen-containing compounds, have emerged as promising candidates for osteoporosis treatment. Their favorable biocompatibility profile demonstrated efficacy in preclinical models, and low reported toxicity make them attractive alternatives to existing therapies. This review focuses on the therapeutic potential of specific indole alkaloids, including vindoline, rutaecarpine, harmine, and its derivatives, in promoting bone health and managing osteoporosis.

Keywords: indole alkaloids; vindoline; rutaecarpine; harmine; osteoporosis

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1. Introduction

Osteoporosis is a skeletal disease characterized by low bone mass and the microarchitectural deterioration of bone tissue, leading to increased bone fragility and susceptibility to fracture [1]. Essentially, it is a condition where bones become weak and brittle, making them more prone to breaking. This weakening occurs gradually over time and often goes unnoticed until a fracture happens, usually from a minor fall or even just bending over. Osteoporosis is a significant global health issue affecting millions of people all over the world, particularly postmenopausal women and the elderly. Its silent progression often leads to a diagnosis only after a fracture, highlighting the urgent need for effective and well-tolerated treatments [2,3]. While current therapies, such as anti-resorptive and anabolic agents, are available, they can be associated with adverse effects like bisphosphonate-induced esophagitis. Effective and better-tolerated treatments are therefore desirable. It is well known that alkaloids are a wide class of nitrogen-containing phytochemicals found in medicinal plants. Because of their biopotential, efficacy, and low toxicity, natural alkaloids have been used in experimental studies as anti-osteoporosis drugs and some have subsequently been used clinically. Among the diverse alkaloid classes with anti-osteoporotic properties (including isoquinoline, quinolizidine, piperidine, pyrrolizidine, and steroid alkaloids), indole alkaloids have emerged as particularly promising candidates. This review focuses on studies of natural indole alkaloids that are important in the treatment of osteoporosis.

2. Osteoporosis

Osteoporosis is a disease characterized by a reduction in bone mass and a deterioration in the microarchitecture of bone tissue, leading to increased bone fragility and an increased risk of fracture. In other words, osteoporosis occurs when bone loss exceeds bone formation. Several factors contribute to this imbalance such as aging, where as we age, our bones naturally become thinner and weaker. Also, menopause in women and decreased

testosterone in men can accelerate bone loss. Inadequate calcium and vitamin D intake, lack of weight-bearing exercise, and smoking can increase the risk. Certain medications, such as corticosteroids and some cancer treatments, can weaken bones. Conditions like rheumatoid arthritis, hyperthyroidism, and celiac disease can contribute to osteoporosis. Bone tissue, like muscle, skin, and blood, is not a static and inert structure, but a living and highly metabolically active tissue that renews itself under physiological conditions through a metabolic process called bone remodeling [1,2]. Unlike static tissues, bone is a dynamic organ undergoing constant remodeling through a balanced process of bone resorption and formation. A decrease in the size of the thoracic and abdominal cavities as well as an increase in thoracic kyphosis can result from vertebral fractures, which can also cause discomfort and postural abnormalities that can lower quality of life overall. Up until a fracture occurs, the condition is clinically asymptomatic [3,4]. Osteoporosis is a health and social problem; it affects in Italy 14% of men over 60 and 23% of women over 40. Its frequency is rising, but the majority of cases are still undiagnosed and untreated [5]. Due to a complex pathophysiology, resorption outweighs new production in the two stages of bone remodeling, resulting in an imbalance [6]. The primary causes include a shortage of calcium and vitamin D, loss of sex hormones, and neglect. The decrease in sex hormones is exponentially correlated with bone loss. The typical loss of lumbar spine bone density in the first year following menopause is 8%; this decreases to half in the subsequent years and reaches a plateau approximately five years later. Postmenopausal bone loss occurs at such a fast pace that it causes trabecular bone perforation and irreversible skeletal tissue loss [7,8]. The thinning and perforation of the trabeculae lead to a progressive loss of connectivity between the trabeculae, which is the source of the change in skeletal microarchitecture seen after menopause and during aging. The porotic bone has the characteristic 'palisade' radiographic look because the horizontal trabeculae, which are not under direct load, are the first to weaken and disintegrate. An exponential rise in bone fragility occurs when the horizontal trabeculae vanish [9–11].

Because there is no physical foundation for new bone production, trabecular perforation causes irreversible bone loss. In other words, the resorbed bone cannot be replaced by new bone. In actuality, the physiological function of osteoblasts is to fill the trabecular pits that they create with the bone matrix, which will subsequently calcify. Even if the osteoblastic activity is elevated, no bone is created because trabecular perforation leaves no cavity for the osteoblasts to deposit the matrix on [12–22].

Bone growth, maintenance of skeletal mass, and subsequent bone loss occur as a result of continuous resorption and new formation processes, known as bone remodeling (Figure 1). This is the process in which old bone is replaced by new bone.

There are two main types of cells responsible for bone remodeling: osteoclasts, which are responsible for bone resorption, and osteoblasts, which are responsible for bone formation. Bone remodeling takes place in the trabecular, endosteal, and cortical parts of the skeleton at well-defined anatomical structures called BMUs [23].

Mononuclear precursors of the monocytic–macrophage type, which are produced in the bone marrow and freely circulate throughout the bloodstream to the location where their activity is needed, fuse to generate osteoclasts. Numerous systemic and local cues from the supporting stromal cells, which are intimately linked to the osteoblasts, lead to the recruitment of osteoclastic precursors and their development into mature osteoclasts. Numerous systemic and local variables that operate by regulating RANKL production or osteoclast apoptosis might have a positive or negative impact on osteoclastic differentiation [24–29]. In particular, OPG (osteoprotegerin)/RANK (receptor activator of nuclear factor κ B)/RANKL (receptor activator of nuclear factor κ B ligand) is a cytokine network important for the osteoclast differentiation and activation as a main regulator of the equilibrium between bone formation (osteoblasts) and bone resorption (osteoclasts) (Figure 2).

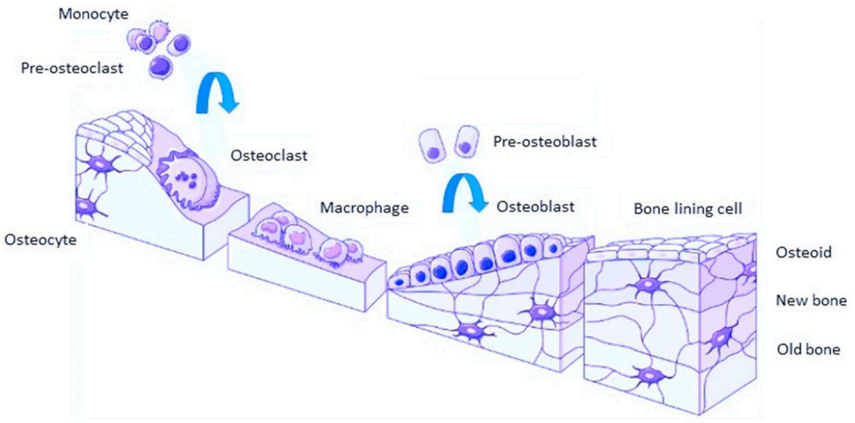

Figure 1. Phases of bone remodeling. The process of bone remodeling takes place in cycles involving the resorption of bone by osteoclasts and its remodeling by osteoblasts. Osteoclasts and osteoblasts carrying out a remodeling cycle form a Bone Multicellular Unit (BMU). The process involves numerous BMUs working in specific areas through a precise sequence of steps: activation, resorption, reversal, ossification.

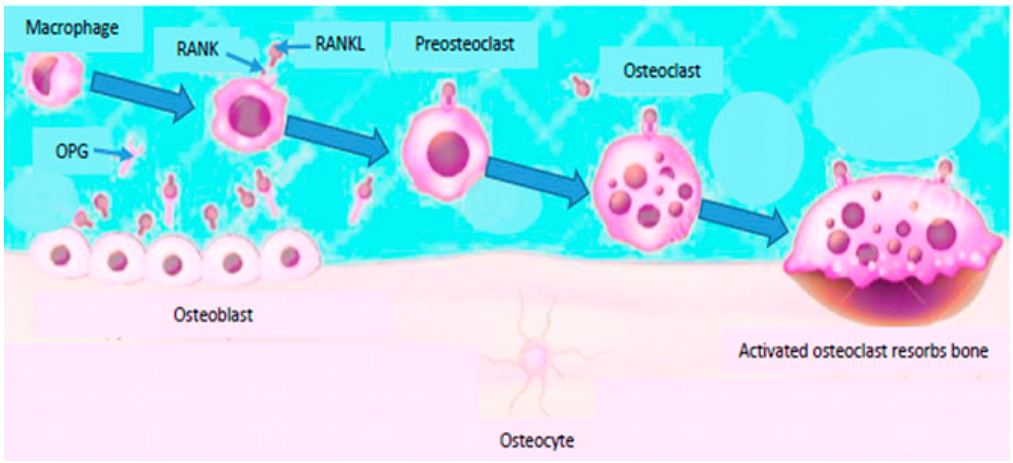

Figure 2. Bone biology. Role of RANK, RANKL, and OPG. Bone remodeling bone is broken down by osteoclasts and rebuilt by osteoblasts. The RANKL activating receptor is the mediator of bone resorption. Osteoprotegerin, OPG; paracranial; and endocrine actions of bone functions of proteins derived from osteoblasts, osteocytes, and osteoclasts.

Osteoblasts (OBs) originate from mesenchymal progenitors, and multiple local bone growth factors, such as bone morphogenetic proteins (BMPs), certain insulin-like growth factors (IGFs), and transforming growth factor beta (TGF beta), are necessary for their development [24–29].

OBs have three possible outcomes: they can undergo apoptosis or develop into mesenchymal or osteocyte cells, both of which are crucial for skeletal metabolism. OB apoptosis is suppressed by PTH and a few growth hormones, including TGF-beta and IL-6. The cycle of bone remodeling requires the simultaneous existence of osteoblasts and osteoclasts, and normally, bone creation only takes place at the locations where bone has been resorbed.

The close links between the vasculature and the areas of bone remodeling are highlighted by the fact that osteoclasts are derived from hematopoietic progenitor cells. Circulating monocytic cells are signaled by endothelial cells at the extremities of capillaries close to bone marrow units (BMUs) to exit the circulation and transform into adult osteoclasts. The capillary network encircles the resorption gap, where osteoblasts must be recruited to create new bone, as bone resorption advances [24–29].

It is plausible that mesenchymal precursor cells initiate the process of osteoblastic differentiation, propelled by biochemical cues of an endothelial origin. Young osteoclasts with a high level of metabolic activity are seen at the deepest region of the bone remodeling site. Less active osteoclasts and eventually apoptotic osteoclasts occupy the sidewalls of the resorption chamber as one advances away from the apex of resorption. The osteoblasts have a more specialized fate: between 20 and 50 percent develop into osteocytes or lining cells, while the remaining 50 to 80 percent suffer apoptosis [24–29].

3. Diagnosis

The BMD (bone mineral density) test is used to determine the quantity of minerals in one square centimeter of bone, or bone mineral density. The findings are contrasted with the bone mineral density of a reference value that has been established, namely the bone mineral density of a young adult in good health, between the ages of 25 and 30, who has attained a bone mass, to which a T-score is awarded. A 1994 technical study from the World Health Organization (WHO) determined the T-score values of BMD within which an individual can be classified as normal, with reduced bone mass (osteopenia), or with osteoporosis. The WHO established the subsequent diagnostic standards based on comparisons with the average peak bone mass in young adults:

- Normal bone mineral density: BMD within 1 standard deviation (SD) of the average peak bone mass in young adults.
- Osteopenia: BMD between −1 and −2.5 SD below the average peak bone mass in young adults.
- Osteoporosis: BMD more than 2.5 SD below the average peak bone mass in young adults.
- Severe osteoporosis: BMD more than 2.5 SD below the average peak bone mass in young adults, along with one or more fragility fractures.

These standards, which pertain to postmenopausal women, were established by utilizing dual-energy X-ray absorptiometry (DXA) data from bone density assessments. It is estimated that at least 30% of postmenopausal Caucasian women have osteoporosis at risk of fracture using a cut-off of −2.5 SD of bone mass [3,7,30–34].

4. Treatment

The main goal of osteoporosis treatment is to prevent fractures. Some common medication classes are the following:

Anti-resorptive agents: They inhibit bone loss by reducing osteoclast activity and they include

- Bisphosphonates: These are the most commonly prescribed medications for osteoporosis. They slow bone loss and increase bone density. Examples include alendronate (**1**), ibandronate (**2**), risedronate (**3**), and zoledronic acid (**4**) (Figure 3).
- Monoclonal antibodies such as the RANK-L inhibitor (denosumab) and the sclerostin inhibitor (romozumab) [35,36].
- Parathyroid hormone (PTH) analogs: These medications stimulate new bone formation. Examples include abaloparatide, and teriparatide (teriparatide is a recombinant protein consisting of the first 34 amino acids of the parathyroid hormone).
- Selective estrogen receptor modulators (SERMs): These medications mimic some of the effects of estrogen on bone. Raloxifene (**5**) is a common example.
- Drugs with dual mechanisms of action (anti-resorptive and anabolic), such as strontium ranelate (**6**).
- Calcitonin: This medication slows bone loss but is less commonly used.

These treatments are systemic and have a lot of negative effects. Specifically, the most frequent adverse effects linked to bisphosphonates are a fever, gastrointestinal issues, discomfort in the muscles and bones, and necrosis of the jaw. For instance, osteonecrosis and atypical fractures can be brought on by denosumab and bisphosphonates. However, romosozumab can also result in joint pain and swelling, as well as a heart attack or stroke

in those who are more susceptible [37–39]. After the first year of treatment, 50% of patients elect to discontinue, primarily due to the adverse effects that outweigh the benefits of the medications. This is the reason why a lot of individuals are searching for a medication-free approach to treat the illness. Indole alkaloids may be a potential treatment.

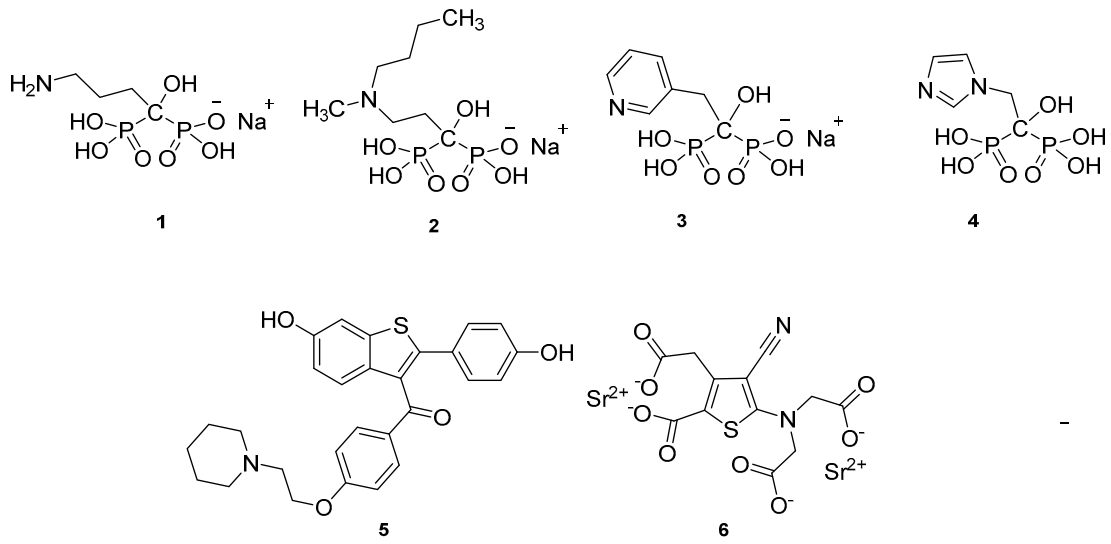

Figure 3. Structures of bisphosphonates (**1**–**4**); raloxifene (**5**); and strontium ranelate (**6**).

The mechanism of action, limitations, and adverse effects of the main drugs used in conventional therapies are shown in Table 1 [22,24,32,35,37–39].

Table 1. The mechanism of action, limitations, and adverse effects of the main drugs used in conventional therapies.

Drugs	Mechanism Of Action	Limitations	Adverse Effects
Bisphosphonates	Inhibit bone resorption by targeting osteoclasts.	Highly effective in reducing fracture risk but primarily prevent bone loss rather than building new bone. Long-term use can lead to medication-related osteonecrosis of the jaw (MRONJ) and atypical femoral fractures.	Gastrointestinal upset, esophagitis, and in rare cases, bone pain.
Denosumab	Monoclonal antibody that inhibits osteoclast formation and function.	Effective in reducing fracture risk but similar to bisphosphonates, it primarily prevents bone loss. Long-term safety data are limited.	Increased risk of osteonecrosis of the jaw, atypical femoral fractures, and potentially increased risk of infections.
Teriparatide	Synthetic form of parathyroid hormone that stimulates bone formation.	Effective in increasing bone mass and reducing fracture risk but its use is limited to two years due to the risk of bone cancer. Expensive.	Nausea, leg cramps, and increased calcium levels.
Selective Estrogen Receptor Modulators (SERMs)	Mimic estrogen's effects on bone but without affecting the uterus.	Less effective than other options in preventing fractures.	Hot flashes, blood clots, and increased risk of uterine cancer.
Calcitonin	Reduces bone resorption by inhibiting osteoclast activity.	Less effective than other options in preventing fractures.	Nasal irritation (nasal spray), flushing, and increased risk of bone pain.

Thus, the target of most conventional medications is primarily preventing bone loss rather than stimulating bone formation. Many medications are associated with serious side effects, limiting their long-term use. In fact, some therapies, such as teriparatide, have restricted treatment periods due to safety concerns [40]. Moreover, the high cost of certain medications, especially newer biologics, poses a significant barrier to treatment. These limitations and the adverse effects associated with current osteoporosis therapies underscore the need for innovative approaches. Research is focused on developing drugs that stimulate bone formation, have a reduced risk of adverse effects, can be used long-term, and are more cost-effective.

Natural alkaloids have been studied for osteoporosis treatment. Natural alkaloids are nitrogen-containing, polycyclic compounds that are derived from plants. Isoquinoline (**7**), quinolizidine (**8**), pyrrolizidine (**9**), steroidal (**10**), piperidine (**11**), and indole (**12**) (Figure 4) are structural variation examples of anti-osteoporosis alkaloids [41].

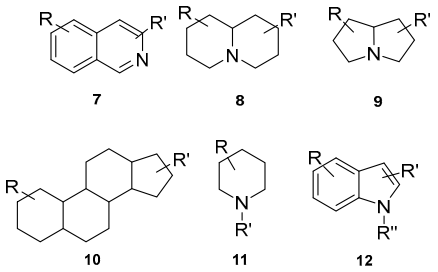

Figure 4. General structures of isoquinoline molecules (**7**), quinolizidine molecules (**8**), pyrrolizidine molecules (**9**), steroidal molecules (**10**), piperidine molecules (**11**), and indole molecules (**12**).

These natural agents have generated a great deal of interest because of their diverse biological and pharmacological effects. They enhance osteoblast proliferation, encourage osteoblast autophagy, inhibit osteoclast formation, and support mesenchymal stem cell differentiation. The wingless and int-1 pathway in mesenchymal stem cells, the p38 mitogen-activated protein kinase pathway in osteoblasts, the inhibition of the nuclear factor kappa B pathway in osteoclasts, and the disruption of the tumor necrosis factor receptor-associated factor 6-receptor activator of nuclear factor kappa B interaction are just a few of the signaling pathways that these naturally occurring alkaloids can regulate.

This review offers proof and backing for new medications and the therapeutic use of indole natural alkaloids to treat osteoporosis [42,43].

5. Indole Alkaloids

Indole alkaloids are nitrogen-containing heterocycles present in the families *Apocynaceae*, *Loganiaceae*, *Rubiaceae*, and *Nyssaceae*. Among them are significant bioactive substances, including ellipticine, which was extracted from *Ochrosia elliptica* Labill's leaves [44–50]. The pharmacological actions of indole derivatives are diverse and include antiviral, antioxidant, anticancer, anti-inflammatory, antihistaminic, antibiotic, and anti-Alzheimer properties [51–54].

Indole alkaloids primarily exert their effects by interacting with specific molecular targets, often proteins, within cells [55]. These interactions can lead to a variety of cellular responses, including:

- Modulation of receptor activity: indole alkaloids can act as agonists or antagonists of various receptors, including G protein-coupled receptors, ion channels, and nuclear receptors.
- Enzyme inhibition or activation: some indole alkaloids inhibit or activate specific enzymes involved in metabolic pathways or signaling cascades.
- Interaction with DNA: certain indole alkaloids can bind to DNA, affecting gene expression.
- Antioxidant activity: many indole alkaloids possess antioxidant properties, protecting cells from oxidative damage.

While the specific mechanisms vary widely among different indole alkaloids, some common pathways have been implicated in their actions:

1. Neurotransmitter Systems

- Serotonin: some indole alkaloids, such as those found in ergot alkaloids, interact with serotonin receptors, leading to effects on mood, sleep, and cognition.
- Dopamine: certain indole alkaloids can influence dopamine signaling, contributing to their potential therapeutic applications in conditions like Parkinson's disease.
- Acetylcholine: some indole alkaloids have been shown to modulate acetylcholine receptors, affecting neuromuscular transmission and cognitive function.

2. Signal Transduction Pathways

- MAPK pathway: several indole alkaloids have been shown to activate or inhibit mitogen-activated protein kinase (MAPK) pathways, which are involved in cell proliferation, differentiation, and apoptosis.
- Phosphoinositide 3-kinase (PI3K)/AKT pathway: this pathway is crucial for cell survival and growth, and some indole alkaloids have been reported to modulate its activity.
- NF-κB pathway: indole alkaloids can influence the nuclear factor kappa B (NF-κB) pathway, which regulates inflammatory responses and immune function.

3. Cell Cycle Regulation

- Some indole alkaloids can arrest the cell cycle at specific phases, leading to apoptosis or senescence. This mechanism underlies their potential anticancer activity.

4. Oxidative Stress and Inflammation

- Many indole alkaloids possess antioxidant properties, scavenging free radicals and protecting cells from oxidative damage.
- They can also modulate inflammatory responses by inhibiting the production of pro-inflammatory cytokines.

Indole alkaloids as a class have a reputation for being potent compounds with potential toxicity. Their safety profile varies widely depending on the specific alkaloid and the dosage. Common side effects include gastrointestinal disturbances; neurological symptoms; bone marrow suppression; and cardiovascular effects. It is essential to weigh the potential benefits of indole alkaloid therapy against the risks for each individual patient.

They have been used for centuries in traditional medicine and have gained significant attention in modern drug discovery; for example, vincristine and vinblastine have gained FDA approval for anti-tumor activity; ajmaline for anti-arrhythmic activity; and physostigmine for glaucoma and Alzheimer's disease.

Some indole derivatives that possess anti-osteoclastogenic and/or anti-resorptive qualities have emerged in recent years as possible treatments for osteoporosis. Specifically, research has been performed on naturally occurring indole chemicals such as vindoline (**13**), rutaecarpine (**14**), harmine (**15**), and its derivatives (**16**–**19**) [41,56] and also on vinblastine, vincristine, yohimbine, and strychnine, but this research is limited and their effects on bone health are unclear.

This mini-review presents intriguing findings about the management of osteoporosis and the pathways connected with the mechanisms of action of the examined compounds.

5.1. Mechanisms of Action of Indole Alkaloids in Osteoporosis

While research is still ongoing, several potential mechanisms have been proposed for the anti-osteoporotic effects of indole alkaloids:

1. Modulation of bone turnover [57]

- Influence on osteoblast activity:
    - Indole alkaloids can stimulate osteoblast proliferation and differentiation, leading to increased bone formation.

- They might regulate the expression of bone-specific genes involved in matrix synthesis and mineralization.

- Inhibition of osteoclast activity:
  - Some indole alkaloids can suppress osteoclast differentiation and function, reducing bone resorption.
  - They may interfere with osteoclast-mediated bone resorption by inhibiting key enzymes or signaling pathways.

2. Anti-inflammatory effects [58]

- Reduction in inflammatory cytokines:
  - Chronic inflammation is linked to bone loss. Indole alkaloids can downregulate the production of pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) and interleukin-1 (IL-1), which contribute to osteoclastogenesis.
- Inhibition of NF-κB Pathway:
  - Indole alkaloids may inhibit the NF-κB signaling pathway, a key regulator of inflammation and bone resorption.

3. Antioxidant properties [42]

- Protection against oxidative stress:
  - Oxidative stress contributes to bone loss by damaging bone cells. Indole alkaloids with antioxidant properties can neutralize reactive oxygen species, protecting bone tissue.

4. Hormonal modulation [41]

- Estrogen-like effects:
  - Some indole alkaloids exhibit estrogenic activity, which can be beneficial in postmenopausal osteoporosis, as estrogen deficiency is a major risk factor.
- Vitamin D-like effects:
  - Certain indole alkaloids may influence vitamin D metabolism or receptor signaling, contributing to bone health.

5. Other potential mechanisms [41]

- Regulation of bone-related signaling pathways:
  - Indole alkaloids might interact with various signaling pathways involved in bone metabolism, such as Wnt, BMP, and Notch pathways.
- Influence on bone microenvironment:
  - These compounds could affect the composition and function of bone cells and the extracellular matrix.

5.2. Vindoline

The indole alkaloid called vindoline (methyl 3β,4β-dihydroxy-16-methoxy-1-methyl-6,7-didehydro-2β,5α,12β,19α-aspirosepsin-3α-carboxylate) (**13**) (Figure 5) was isolated from the medicinal plant *Catharanthus roseus* and has been shown to have anti-tumor, antidiabetic, antioxidant, and anti-inflammatory properties [59,60]. Vindoline can decrease osteoclast development from bone marrow macrophage (BMM) progenitor cells as well as mature osteoclastic bone resorption, as Zhan et al. [61] were the first to demonstrate.

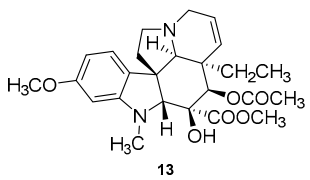

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Figure 5. Structure of vindoline (**13**).

Additionally, they have shown that the molecular mechanism of action of vindoline is based on its inhibitory effect against the production of intracellular reactive oxygen species (ROS) and the activation of MAPK, including p38, JNK, and ERK. This effect suppresses the induction of c-Fos and NFATc1, which in turn downregulates the expression of the genes required for the formation of osteoclasts and bone resorption.

Also, their in vivo investigation on vindoline-treated mice revealed protection against trabecular bone degradation and bone loss brought on by ovariectomies (OVXs). According to their research, vindoline reduced the in vitro bone resorption capacity of mature osteoclasts and the generation of osteoclasts derived from BMM [61]. Vindoline specifically inhibits the activation of all three MAPK signaling pathways as well as the generation of intracellular ROS, which in turn decreases the expression of c-Fos and NFATc1.

Moreover, they found that the increase in NFATc1 expression was postponed during the differentiation of osteoclasts, which led to the downregulation of several NFAT-responsive genes implicated in the fusion of osteoclasts, such as CTSK, MMP9, and TRAP. The same scientists then looked at the possible advantages of vindoline treatment in OVX mice model-induced bone loss *in vivo*, and they found that vindoline treatment decreased the quantity of TRAP-positive osteoclasts in bone. They demonstrated that vindoline has anti-osteoclastogenic and anti-resorptive qualities that can be used to treat or control osteoporosis and other osteoclast-mediated osteolytic disorders.

5.3. Rutaecarpine

Rutaecarpine (8,13-hydroindolo [2′,3′:3,4]pyrido[2,1-b]quinazolin-5(7*H*)-one) (**14**) (Figure 6) [62] is a bioactive alkaloid that was isolated from the adaptable medicinal herb *Evodia rutaecarpa*. In China, this herb is used clinically to treat amenorrhea, headache, stomach pain, diarrhea, and postpartum hemorrhage.

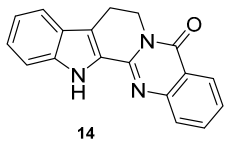

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Figure 6. Structure of rutaecarpine (**14**).

Rutaecarpine, one of *Evodia* rutaecarpa's most characteristic indolopyridoquinazoline alkaloids, has a wide range of pharmacological applications in the treatment of metabolic, cerebrovascular, and cardiovascular illnesses.

In 2024 [63], Ali et al. reported on the effects of rutaecarpine on promoting osteoblast development in hBMSCs. Specifically, rutaecarpine induced the expression of genes linked to osteogenesis, including ALP, OC, ON, and RUNX2, which are crucial for the maturation of osteogenic processes, matrix mineralization, and the control of transcription factors that are essential for the development of bones and osteogenesis [64]. Their rutaecarpine therapies resulted in an upregulation of COMP, another osteogenic gene marker. Through the activation of BMP2 and ALP activity, COMP has been demonstrated in an ectopic bone formation rat model to improve osteogenesis [65].

The activation of the FAK pathway by rutaecarpine is essential for the stimulation of osteogenesis and bone formation. Another route that is increased when rutaecarpine is exposed to hBMSCs is TGFβ. Through the SMAD signaling pathway, TGFβ recruits stromal stem cells to the bone resorption process and controls the maintenance of postnatal bone and cartilage. Osteoblasts' production of new bone and osteoclastogenesis's induction of bone degradation are linked by TGFβ [66].

A key signaling role for TGFb isoforms and their receptors, such as TGFβR2, is played in the development of bones. In their investigation, the downregulation of rutaecarpine's osteogenic induction effects was caused by the inhibition of the TGFβ pathway. Rutaecarpine was found to also upregulate the Toll-like receptor signaling pathway. Following rutaecarpine treatments, oxidative stress and selenium pathways are also activated in

hBMSCs, suggesting rutaecarpine's preventive antioxidant effect against age-related bone loss. These findings suggest that rutaecarpine may have a therapeutic benefit by lowering senescent cells and preventing age-related bone loss.

Furthermore, ex vivo organotypic cultures of embryonic chick femurs treated with rutaecarpine demonstrated the beneficial effects of this chemical on metrics related to bone, such as cortical thickness and BV/TV. The potential of rutaecarpine to shield rats against OVX-induced bone loss has also been studied. Rutaecarpine treatments of OVX mice for three months resulted in improved bone density, probably as a result of mechanisms related to osteoprotegerin induction [67].

Anti-osteoporotic medication use may raise the risk of cardiovascular illnesses, myocardial infarction, and stroke [68]. Both men and women with cardiovascular disorders typically have lower bone mass densities. Rutaecarpine has been studied previously for its beneficial effects as an antioxidant and an inflammatory modulator that lower the risk of heart disorders [69]. In conclusion, their research shows that rutaecarpine plays protective roles in promoting bone formation by raising the osteoblast differentiation potential of hBMSCs and lowering oxidative stress and senescent cell load.

5.4. Harmine

As an alkaloid of β-carbolines with a tricyclic pyrido[3,4-b] indole structure, harmine (7-methoxy-1-methyl-9*H*-pyrido[3,4-b]indole) (**15**) (Figure 7) has been shown to have a variety of pharmacological effects in vitro and *in vivo*, including vasorelaxant and antidepressant effects as well as improved insulin sensitivity [70,71]. Harmine has several traditional medicinal uses and pharmacological activities such as anti-inflammatory, antiparasitic, anti-tumor, and anti-diabetes properties. Uygur medicine in China uses different preparations of harmine to treat rheumatoid arthritis [72].

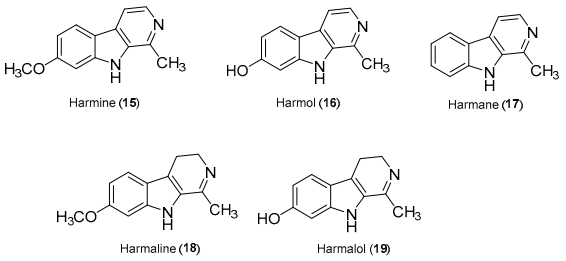

Figure 7. Structure of harmine (**15**) and its derivatives (**16**–**19**).

A mini library of indole derivatives was the focus of research by Yonezawa et al. (2011) [73]. These derivatives included harmine (**15**), harmol (1-methyl-2,9-dihydropyrido [3,4-b]indol-7-one) (**13**), harmane (1-methyl-9*H*-pyrido[3,4-b]indole) (**14**), harmaline (7-methoxy-1-methyl-4,9-dihydro-3*H*-pyrido[3,4-b]indole) (**15**), and harmalol (1-methyl-4,9-dihydro-3*H*-pyrido[4,3-b]indol-7-ol) (**16**), among others.

Researchers have looked at how harmine and its derivatives (Figure 7) affect osteoclastogenesis in order to evaluate the relationship between the chemical structure and action of harmine. They found that harmol has similar effects to harmine in inhibiting RANKL-induced TRAP activity. After analyzing harmine and its derivatives, researchers found that harmine reduces osteoclastogenesis in RAW264.7 murine macrophage-like cells (Figure 7). According to these researchers, harmine prevented osteoclast differentiation in RAW 264.7 cells while preserving cell viability. It also prevented progenitor cells from differentiating into mononuclear osteoclasts and from fusing into multinucleated osteoclasts. The study employed primary BMMs and co-culture systems to confirm the inhibitory effects of harmine on osteoclastogenesis. Given that harmine was able to decrease osteoclastogenesis in single cultures of RAW264.7 cells and BMMs without osteoblasts, their findings imply that harmine primarily works on osteoclast precursors rather than osteoblasts. Using both in vitro and ex vivo models, harmine was demonstrated to decrease bone resorption in

investigations. An analysis of mice with ovariectomies revealed that harmine inhibits bone loss in vivo in models of osteoporosis. An analysis of harmine and its derivatives' structure–activity relationship (SAR) on osteoclastogenesis revealed that a double bond between C3 and C4 and a methoxy or hydroxy group at position 7 in β-carboline structures are critical. Therefore, harmine inhibits the mRNA and protein expressions of c-Fos and NFATc1, crucial transcription factors for osteoclastogenesis, that are increased by RANKL. Specifically, TRAP, c-Src, Atp6v0d2, and cathepsin K are among the osteoclast-specific genes whose expressions are regulated by NFATc1 and which modulate osteoclast fusion, activation, and function [74].

In 2018, Huang et al. [75] created an oil-in-water harmine emulsion and found that the oral administration of harmine in an emulsion form reduced harmine accumulation in the mouse brain and that the intragastric administration of a harmine emulsion provoked bone-sparing effects in OVX-induced osteoporotic mice. These findings were made in the light of harmine's low bioavailability and potential side effects (an intraperitoneal injection of harmine can cause side effects in the central nervous system) [76–78]. Moreover, quantities of harmine were found in mouse bone marrow following oral treatment, which may account for the bone-protective effects observed in OVX mice. Consequently, their research validates that the intragastric delivery of a harmine emulsion promotes the development of type H blood vessels and bone in osteoporotic mice while inhibiting the creation of osteoclasts and enhancing preosteoclast PDGF-BB-induced angiogenesis. Harmine may be a viable medication for the treatment and prevention of osteoporosis, according to their research.

The same researchers [79] reported in 2021 that activator protein-1 (AP-1) and inhibitor of DNA binding-2 (Id2) are involved in harmine-enhanced preosteoclast PDGF-BB production. Primary murine bone marrow macrophages (BMMs) were obtained from the tibiae and femora of 6-week-old male mice. Specifically, harmine-upregulated Id2 inhibited preosteoclasts' ability to fuse into multinucleated osteoclasts, and harmine's effects were reversed by Id2 knockdown. The dimeric transcription factor AP-1, which is made up of the proteins Jun and Fos, is an important positive regulator of osteoclast development that is first triggered by the RANKL signal [80]. This study indicated that in RANKL-induced BMMs, harmine significantly increases the expression of the AP-1 factors, c-Fos and c-Jun. The study conducted by the authors provides mechanistic insight into the regulation of PDGF-BB synthesis during osteoclast formation. Additionally, the study has enabled the discovery of a novel therapeutic target for the management and prevention of metabolic bone disorders. In 2011, Egusa et al. [81] demonstrated that harmine also had an impact on DC-STAMP expression, which is necessary for cell–cell fusion in osteoclasts [82].

5.5. Other Indole Derivatives That Influence Bone Metabolism

Other classes of indole alkaloids that influence bone metabolism are:

- Vinca alkaloids such as vinblastine and vincristine: Primarily known for anticancer properties, these alkaloids also influence bone metabolism and they might have side effects related to bone health [83].
- Yohimbine-type alkaloids: Some members of this class have shown potential for bone health, though research is limited. Yohimbine, found in yohimbe bark, has been studied for various conditions, including erectile dysfunction, but its effects on bone health are unclear [84].
- Strychnine-type alkaloids: While primarily known for neurotoxicity, certain compounds in this class such as strychnine might have unexpected effects on bone [85].

6. Conclusions

Osteoporosis, a silent disease characterized by bone weakening, presents a significant health challenge. While conventional therapies form the cornerstone of osteoporosis management, their limitations and adverse effects necessitate the exploration of novel therapeutic strategies. Indole alkaloids, a diverse group of plant-derived compounds, exhibit a broad spectrum of pharmacological activities. The complex structures of these

compounds and their multifaceted interactions with biological targets hinder the elucidation of their precise mechanisms of action. This review compiles studies on naturally occurring indole compounds, including vindoline (**13**), rutaecarpine (**14**), harmine (**15**), and its derivatives (**16**–**19**), with a particular emphasis on the potential and underlying mechanisms of harmine (**15**).

The activation of the nuclear factor of activated T cells c1 (NFATc1) by the receptor activator of the nuclear factor-κB ligand (RANKL) stimulates the production of osteoclasts. This study examined the data indicating that harmine prevents RAW264.7 cells from becoming osteoclasts when stimulated by RANKL. It does this by inhibiting the expression of NFATc1 and c-Fos. Furthermore, harmine stops bone loss in a mouse model of osteoporosis that has been subjected to ovariectomy. It is observed to decrease osteoclast production and increase preosteoclast formation in both ovariectomy-induced osteoporotic animals and RANKL-stimulated RAW264.7 cells. This impact is probably caused by harmine-promoting platelet-derived growth factor-BB-induced type H vessel development prior to osteoclast formation [72].

Importantly, further studies, such as structure–activity relationship studies, are needed to provide further evidence for the development of drugs for osteoporosis.

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