N-Heterocyclic Dronic Acids: Applications and Synthesis

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Abstract: Substituted hydroxymethylenebisphosphonic acid derivatives – either as dronic acids or their dronate sodium salts, are important pharmaceuticals in the treatment of diseases arising from excessive bone-resorption. Potential has also been identified in areas ranging from parasite-growth inhibition to immunological and cancer therapeutics. Representative clinically relevant *N*-heterocyclic derivatives include zoledronic and risedronic acids. The biochemical background and mechanism of action of these drugs are discussed, along with trends in structural development and future prospects. Synthetic routes to dronates are then summarized. The most popular route to valuable dronic acids involves the 3-component condensation of a substituted acetic acid, phosphorous acid, and phosphorus trichloride. However, the protocols recorded in the literature are very diverse. This review gives a critical account of reported methods, explores the contradictions and suggests a practical synthetic procedure after clarifying the inconsistencies described. Possible mechanisms of the reaction are also discussed.

Keywords: Zoledronic acid, risedronic acid, dronates, bone disease, anti-cancer, anti-parasitic, practical synthesis, reaction mechanism.

1. INTRODUCTION

1-Substituted-1-hydroxy-1,1-bisphosphonic acid derivatives (as dronates or dronic acids) have made an enormous contribution to the global pharmaceutical market in treatments of various bone diseases, such as osteoporosis, osteolytic metastases, tumour-induced hypercalcaemia and Paget′s disease [1-12]. They exhibit antiparasitic activity towards trypanosomatids [13] such as *Trypanosoma cruzi* (causative agent of Chagas′s disease) [14-18], *Trypanosoma brucei rhodesiense* (sleeping sickness) [19,20], *Leishmania donovani* and *Leishmania mexicana* (visceral and cutaneous leishmaniasis respectively) [21-23], and apicocomplexans [13] such as *Toxoplasma gondii* (toxoplasmosis) [17,18,22,24] and *Plasmodium falciparum* (malaria) [25,26]. Their spectrum of activity also encompasses inhibitory action towards the growth of simple amoebic eukaryotes such as *Dictyostelium discoideum* [27,28] and *Entamoeba histolytica* [25], herbicidal activity [29], antibacterial [30] and anticancer properties [31-39], and stimulation of γδ-T cells of the immune system [40,41] - introducing potential for an immunological approach to anti-bacterial and cancer therapeutics [42-44] complementary to classical chemotherapeutics [45,46]. Interest is also being expressed with respect to the therapeutic potential of bisphosphonates towards cardiovascular disease [47].

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Geminal-bisphosphonates (BPs, **1**, Fig. **1**) constitute catabolically and hydrolytically stable analogues of pyrophosphate (PP_i, **2**), substituting a P-C-P backbone for the P-O-P arrangement of PP_i. The methylene function therein presents a basis for tailoring biological and physiological properties through appendage of R^1- and R^2-side-chain functions, and the titled compound class is characterised by an R^1-hydroxyl substituent. The resulting 1-hydroxy-1,1-bisphosphonate arrangement exhibits tridentate functionality towards binding Ca^{2+} ions, and an affinity for the species that directs accumulation at the hydroxyapatite mineral $\{Ca_{10}(PO_4)_6(OH)_2\}$ in bone tissue [1-6,48-51] and, it is thought, acidocalcisome organelles of equivalent composition in parasitic protozoa [52,53]. In conjunction with the efficacy of intracellular transport, this directs the cellular sites of action of dronates, with R^2-substituents instrumental in determining molecular effects at the cell target.

Maintenance of bone structure and endocrine function requires the anabolic and catabolic functions respectively of osteoblasts (bone forming) and osteoclasts (bone resorbing) acting in concert in basic multicellular units (BMU) on the bone surface. Conditions of deteriorating bone mineral density (BMD) are characterised by disruption of this balanced bone-turnover through excessive osteoclast activity [54], which is addressed when the drug is internalised by the osteoclast cell-target through its natural catabolic function. Meanwhile, it is thought likely that drug uptake to protozoa occurs through pinocytosis, their mode of nutrient ingestion.

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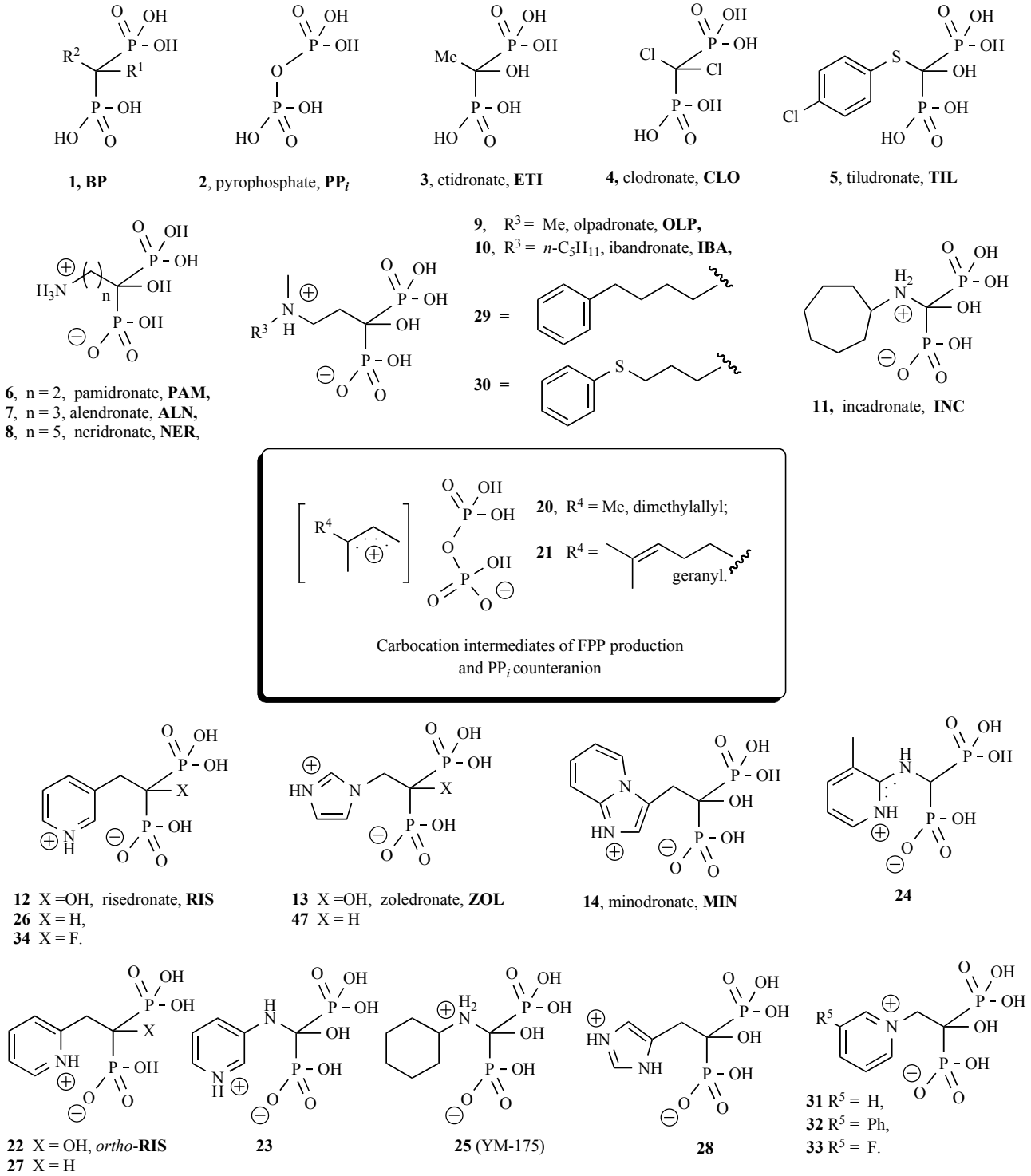


Fig. (1). Clinically relevant and interesting N-BPs, and intermediates of FPP biosynthesis.

The anti-resorptive properties of BPs towards bone were first identified in etidronate (ETI, **3**), clodronate (CLO, **4**) and tiludronate (TIL, **5**, Fig. **1**), their mode of action involving metabolic incorporation into non-hydrolysable analogues of ATP [1-6,48-51] which accumulate in the cell cytoplasm, inhibiting mitochondrial ADP/ATP translocase and leading ultimately to apoptosis [55,56]. These first generation agents were only moderately potent, exhibiting a narrow therapeutic window between desired inhibition and impairment of bone mineralisation. Hence they have been superseded in clinical use by second generation (*N*-aliphatic, *e.g.* **6-11**, Fig. **1**) [57-60] and third generation (*N*-heterocyclic, *e.g.* **12-14**) [61-64] nitrogen-bearing bisphosphonates (N-BPs) with superior pharmacological profiles. The main molecular target of N-BPs has been established as farnesyl pyrophosphate synthase (FPPS), a key enzyme in the cellular

mevalonate pathway. Inhibition prevents biosynthesis of the C_{15}-isoprenoid farnesyl pyrophosphate (FPP, **15**) and other downstream products (*e.g.* the C_{20}-species geranylgeranyl pyrophosphate; GGPP, **16**) required for the synthesis of essential sterols, and the post-translational prenylation of GTPase signalling proteins such as Ras, Rac, Rap1, Rho and Cdc42 (Scheme **1**) [4,5]. This prevents their membrane association and impedes signalling processes essential for cell function, ultimately leading to apoptosis. FPPS inhibition can also lead to formation of the highly apoptotic isopentenyl ester of ATP (ApppI) [56]. While these signalling pathways are critical to all cells, the observed site-selectivity of inhibitory action stems from the aforementioned Ca^{2+}-chelating abilities of N-BPs (and BPs in general) leading to exposure of the cell target to high drug concentrations.

At this point, seven BPs (five of them N-BPs) are FDA-approved for clinical use: etidronate (ETI, **3**, Didronel®), tiludronate (TIL, **5**, Skelid®), pamidronate (PAM, **6**, Aredia®), alendronate (ALN, **7**, Fosamax®), ibandronate (IBA, **10**, Boniva®), risedronate (RIS, **12**, Actonel®) and zoledronate (ZOL, **13**; granted indications as Zometa® and Reclast® formulations). Of these, Actonel® and Reclast® are indicated for treatment and prevention of osteoporosis in postmenopausal women, treatment of osteoporosis in men, glucocorticoid-induced osteoporosis and Paget′s disease of bone. Meanwhile, Zometa® is indicated for hypercalcaemia of malignancy and multiple myeloma, and bone metastases of solid tumours [65]. In addition to these, minodronate (MIN, **14**, Recalbon®) has recently been granted a Japanese marketing approval for the treatment of osteoporosis [66].

Perhaps the easiest synthesis of 1-hydroxy-1,1-bisphosphonic acids can be performed by the reaction of carboxylic acids and tetraphosphorus hexoxide (P_4O_6) [67]. Their industrial synthesis involves the condensation of the substituted acetic acid, phosphorous acid and phosphorus trichloride under harsh conditions using a variety of inert solvents [6,68-82]. There are also other possibilities for their preparation. One method comprises the addition of dialkyl phosphites to the carbonyl group of α-oxophosphonates. However, this reaction has long been the subject of controversy, as it was published erroneously that the products were hydroxymethylenebisphosphonates, although they were, in fact, the rearranged phosphonate-phosphates [83]. The situation was then clarified, recognising that the hydroxy-methylenebisphosphonates can only be synthesized under 80 °C and by avoiding distillation of the products [84]. Subsequently, a reproducible procedure in the presence of dibutylamine catalyst was elaborated [85] and this procedure was then extended [86]. Keglevich *et al*. tried to make use of the microwave (MW) and solventless techniques in the above synthesis [87,88]. Substituted methylenebisphosphonates and methylene(bisphosphine oxides) may be synthesized by the alkylation of the parent CH acidic substrates. The phase transfer catalytic and the MW techniques allowed selective monoalkylation [89,90]. A three-component condensation of primary amines, orthoformates and dialkyl phosphites affords aminomethylenebisphosphonic acids after hydrolysis [91,92]. A MW-promoted variation was also elaborated [93].

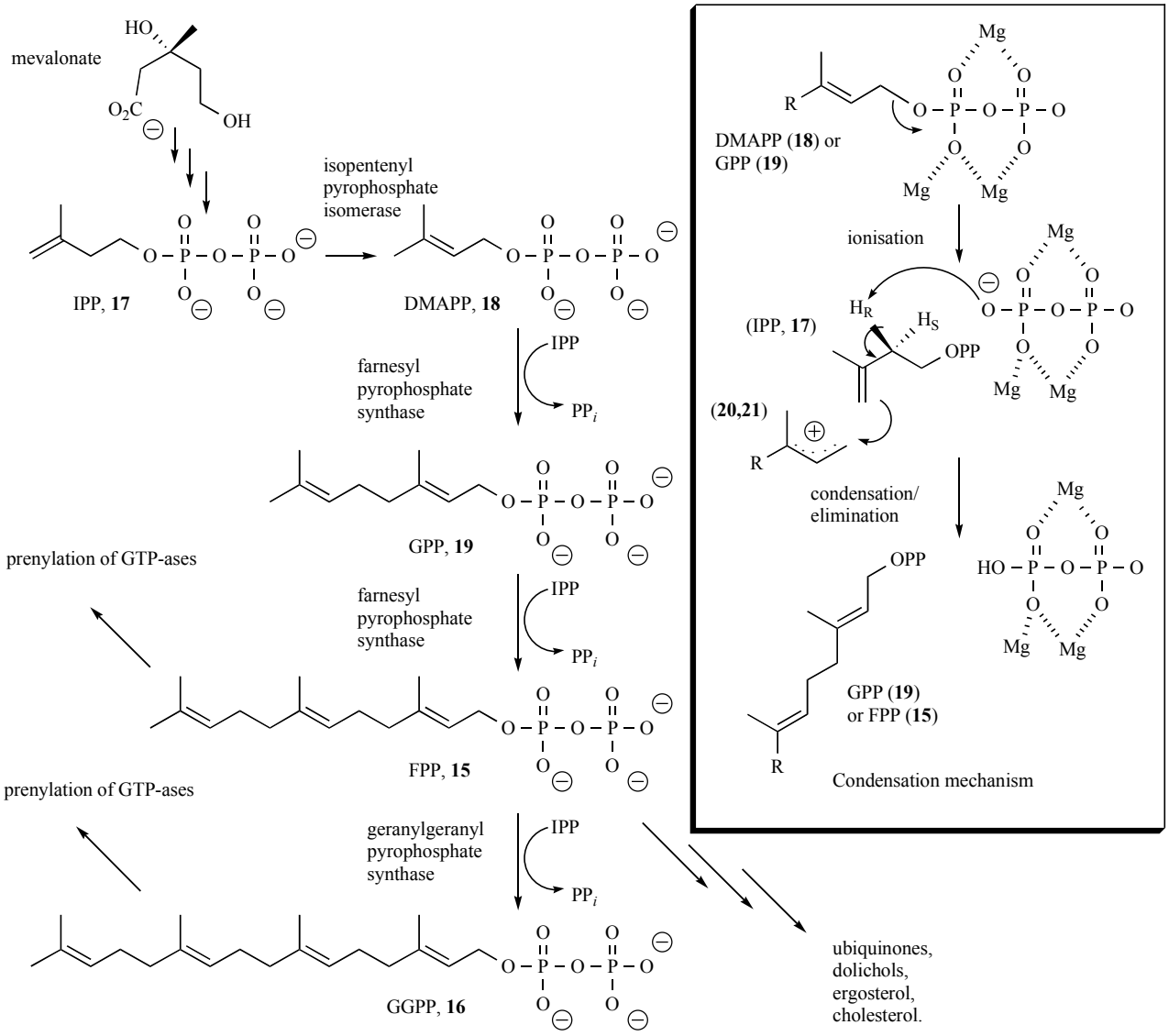
In this paper, the biochemical background and mechanism of action of N-BP drugs are discussed, along with current trends in structural development and future prospects. Also summarized are reported synthetic routes to dronates and associated reaction mechanisms.

2. BIOCHEMISTRY OF *N*-HETEROCYCLIC DRONIC ACIDS AND DERIVATIVES

2.1. Farnesyl Pyrophosphate Synthase – General Mechanism of Action and Inhibition

Farnesyl pyrophosphate synthase (FPPS) catalyses the synthesis of farnesyl pyrophosphate (FPP, **15**) in two condensation steps from isopentenyl pyrophosphate (IPP, **17**) and dimethylallyl pyrophosphate (DMAPP, **18**), *via* C_{10}-isoprenoid geranyl pyrophosphate (GPP, **19**, Scheme **1**). Each condensation involves an ionisation-condensation-elimination mechanism in which the double bond of IPP attacks the C1 atom of an allylic carbocation intermediate {dimethylallyl (**20**) or geranyl (**21**); Fig. **1**} derived from Mg^{2+}-initiated ionisation in the active site (Scheme **1**). Stabilisation of these carbocation species occurs through electrostatic interactions with the trinuclear-Mg^{2+}-cluster-bound PP_i leaving group and with the active-site residues Lys200 and Thr201 (in human FPPS; huFPPS), and N-BPs are now considered to act as azacarbocation analogues of these intermediates [94]. This contention was supported at an early stage both by computational studies using an *ab initio* Hartree-Fock approach, comparing electrostatic potentials of the molecular surface of N-BPs with those of putative allylic carbocation intermediates [94], and by docking studies using the ordinates of FPPS residues when complexed with GPP [95].

Subsequently, clinically relevant N-BPs were assessed for inhibitory activity towards recombinant huFPPS, giving a relative order of inhibitory potency as ≥3nM; ZOL≈MIN> RIS>IBA>INC>ALN>PAM, closely matching the order of anti-resorptive potency [9]. The first detailed 3D-QSAR investigation in this area employed Comparative Molecular Similarity Indices Analysis (CoMSIA) and Comparative Molecular Field Analysis (CoMFA) approaches [96] to identify the structural prerequisites for potent inhibition of the FPPS of *Leishmania major*. The need for both a positive charge in the R^2-side-chain and a hydrophobic feature was highlighted, along with the importance of the position of *N*-atom in the R^2-heterocycle and nature of additional ring substituents. A correlation of potency with pK_a of the heterocyclic base was also derived (*cf.* Section **2.2**). The most active compounds in the study were ZOL (**13**), pyridyl-1-ethane-1,1-BPs RIS (**12**) and **22** respectively, pyridyl/picolyl-aminomethylene-BPs **23** and **24** respectively, and the cyclohexylaminomethylene-BP YM-175 (**25**, Fig. **1**). Good theory-vs-experiment correlation was generated *via* both CoMSIA and CoMFA approaches and the study was predictive to a degree [97]. Other studies have highlighted the contribution of the 2-aminopyridyl motif (*e.g.* in **24**) towards potency in corresponding anti-resorption agents [19,98], and the structural characteristics arising from resonance stabilisation; a planar amidinium-type structure and delocalised positive charge.



Scheme 1. Pathway to protein prenylation, and mechanism of GPP/FPP biosynthesis.

Recent structural determinations of N-BP′s complexed to human [99,100], trypanosomal [101] and bacterial [102] FPPS′s have revealed inhibitor binding modes. Analysis of complexes of RIS and ZOL with huFPPS have confirmed binding of these N-BP′s in the GPP/DMAPP binding site, with the phosphonate groups coordinated *via* Mg^{2+} ions with enzyme active-site DDXXD motifs normally concerned with binding PP_i. The 1-OH function forms a water-mediated interaction with Gln240 and an H-bond interaction with Asp243, without participating directly in chelation with Mg^{2+} ions. Interactions linking the R^2-heterocyclic nitrogens of ZOL and RIS respectively with Lys200 and Thr201 further support the hypothesis that N-BPs derive inhibitory potency from the positioning of nitrogen in the proposed carbocation-binding site. However, while binding effectively with the enzyme (albeit with reduced potency), *N*-aliphatic N-BPs such as PAM (**6**) and IBA (**10**) do not form interactions with Lys200 and Thr201 through their *N*-functions, implicating interaction with alternative residues [99,100].

Enzyme action involves structural rearrangement on binding the allylic DMAPP/GPP substrate (**18** or **19**), decreasing the size of internal cavity while forming the IPP binding site. Binding of IPP (**17**) then initiates a second isomerisation which promotes active-site closure, constituting a ternary-complex metabolic mechanism. N-BPs are competitive inhibitors with respect to allylic substrate but non-competitive with respect to IPP and have been revealed to be slow, tight-binding inhibitors [100,103]. A study of Dunford *et al.* [103] quantified a time-dependent inhibitory profile for clinically relevant N-BPs and RIS derivatives consistent with the above isomerisation model. Initial inhibition constants in the range K_i = 35-400 nM were observed in the test set, followed by time-dependent enhancement in inhibitory potency, with final K_i values reflecting the respective stabilities of the closed huFPPS/IPP/N-BP ternary complexes. Isomerisation constants (K_{isom}) based on the ratio of initial and final K_i′s indicated an order of ability to stabilise the isomerised enzyme state in clinically relevant N-BPs (Table **1**). RIS

derivatives were also analysed in this way. Unsurprisingly, the -P-C-P- backbone was found to be critical to potency and K_{isom} values, while nitrogen-position was found to influence potency in the initial competitive phase and in stabilising the final isomerised ternary-complex state. On the other hand, the absence of 1-OH function in deshydroxy derivatives (*e.g.* **26** and **27**, Fig. **1**) was actually found to facilitate competitive inhibition initially; however, OH-bearing N-BP counterparts such as RIS (**12**) and **22** (Fig. **1**) were invariably stronger final inhibitors (*i.e.* greater K_{isom}), suggesting further stabilisation of the isomerised state *via* OH-mediated interactions. The study also illustrated that K_{isom} and final K_i values of N-BPs towards huFPPS are closely correlated with anti-resorptive efficacy.

Active-site and aspartate-rich DDXXD domains are highly conserved between different types of FPPS, and there is broad (if not total) confluence with respect to structural requirements for inhibition. For example, RIS is a potent inhibitor of FPPSs from humans [9], *T. cruzi* [101,104], *T. brucei* [105], *L. major* [97] and *E. Coli* [102], and it has been possible to predict binding modes of clinically relevant N-BPs with an Autodock modelling programme for FPPS/IPP/N-BP ternary complexes in eukaryote and prokaryote cases [106]. However, shared sequence identities can be minimal overall (25-35% for huFPPS with respect to the above FPPSs), with eukaryotic and prokaryotic FPPSs expressing particularly divergent sequences towards their C-terminii, which mediate IPP-binding.

Table 1. Inhibition and Isomerisation Constants of Selected N-BPs for huFPPS [103]

Compound	Initial K_i (nM)	Final K_i (nM)	K_{isom}
PP_i (**2**)	40.5±3.9 μM	29.8±3.2 μM	0.4
PAM (**6**)	331.4±33.8	55.9±6.1	4.9
ALN (**7**)	393.1±24.7	44.2±3.4	7.9
IBA (**10**)	195±13.8	3.6±0.3	52.9
ZOL (**13**)	85.9±3	0.07±0.01	1244
RIS (**12**)	82.2±2.2	0.36±0.06	226
o-RIS (**22**)	59.3±3.6	0.74±0.12	78.9
(**26**)	34.76±2.1	7.4±0.2	3.7
(**27**)	38.95±2.0	4.45±0.18	7.8

2.2. Inhibition of Bone Resorption

Early QSAR studies on second and third generation N-BPs highlighted a high dependence of anti-resorption potency on the position of the R^2-substituent *N*-atom with respect to the 1-hydroxy-1,1-bisphosphonate ("bone hook") function {ED_{50} (μg/kg); ZOL = 0.07, **28** = 0.3, IBA = 1.1, ALN = 8, OLP = 12, NER = 60, PAM = 61 in rat}. Also illustrated were the potencies of imidazolyl derivatives ZOL (**13**) and **28** (Fig. **1**), and the superior therapeutic index (TI) of ZOL, as a precursor to clinical development [6]. Constraints on the length and nature of *N*-substitutents in PAM derivatives were also revealed, correlating enhanced hydrophobic stabilisation with extension up to an optimum length commensurate with that of FPP (*cf.* **29** and **30**, Fig. **1**). After this point further extension diminished potency, implicating adverse steric interactions with enzyme residues [6,95]. Comparative Molecular Field Analysis studies (CoMFA) conducted on Aryl-X derivatives of PAM were consistent with this, achieving high correlation between experimental and computed activities (R^2 = 0.90) [107], using an initial alignment model based on the crystal structure of FPP complexed with avian FPPS [95], and uniformly protonated *N*-atoms throughout the test set. It is noteworthy that **29** is also the most potent inhibitor of *E. Coli* cell-growth to date, while N-BPs such as RIS (**12**), ZOL (**13**) and MIN (**14**) exhibit no measurable activity [30]. Given the affinity of RIS for the FPPS of *E. Coli* [102], this suggests poor cellular transport.

A similar CoMFA approach adopted towards *N*-heterocyclic-BPs used the crystal structure of RIS (**12**) [108] docked onto GPP (**19**) for initial alignment [107]. However, good correlation (R^2 = 0.87) between experimental and theoretical activities was only achieved in a model employing appropriate protonation states for the ring *N*-atoms, commensurate with the pK_as of the parent heterocycle. N-BPs developed from poor bases (pK_a < 3; *i.e.* thiazole, pyrazole and triazole) incapable of supporting a protonated state, and thus poor carbocation analogues, were all found to be inactive. Meanwhile, those developed from species with pK_as ~ 5-9 (i.e. pyridine, aminopyridine, imidazole and aminothiazole) were generally good inhibitors in the absence of adverse steric interactions. However, aminoimidazole-based derivatives, not readily deprotonated (pK_a ~ 11), were less active and poor cellular transport was mooted in explanation [107].

This and other 3D-QSAR studies [40,97,107] have investigated the influence of charge distribution in the R^2-side-chain of N-BPs, in light of the delocalised nature of positive charge in the intermediates of FPP biosynthesis (*i.e.* **20** and **21**, Fig. **1**). In this context, quantum chemical calculations of Merz-Singh-Kollman charges were carried out on candidate structures likely to exhibit charge localised at the *N*-site, close to the bisphosphonate backbone. A library of pyridinium-1-yl derivatives based on **31** (Fig. **1**) was thereby evaluated with respect to *in vitro* anti-resorptive behaviour and activity towards targets such as *L. major* FPPS and *D. discoideum*, and towards γδ-T cell activation [109]. Compounds **31** and **32** were of comparable anti-resorptive potency to N-BPs RIS and ALN (**7**), while 3-fluoro analogue **33** (Fig. **1**) was more potent still, comparable to ZOL. Good correlation of activity between all targets was achieved, identifying leads for the development of anti-resorptive, anti-infective and anti-cancer drugs. As an aside, the theme of varying the nature of cation centre in BP compounds has been further extended ($N^+HMe \rightarrow S^+Me$) with some success in sulfonium bisphosphonates inhibiting three cancer cell lines *in vitro* [110].

As the 1-OH function does not participate in chelation with Mg^{2+} in RIS-FPPS complexes [99-102], it has been speculated that bisphosphonate interactions may be influenced indirectly through e^--withdrawing effects, leaving

scope for modification through introduction of alternative e⁻-withdrawing groups. Hence 1-halogenated RIS derivatives were investigated for hydroxyapatite affinity (based on chromatographic retention times), huFPPS inhibition and inhibition of Rap1-prenylation in J774 cells. While all halogenated derivatives exhibited diminished affinity for hydroxyapatite with respect to RIS itself, 1-fluorinated derivative **34** (Fig. **1**) retained significant activity towards huFPPS (IC_{50} **34** = 16.4 nM; RIS = 5.7 nM) and Rap1-prenylation, characteristics of therapeutic interest where reduced drug retention in bone is desirable [111].

Alternative design approaches have introduced estrogen-mimetic features, reflecting the role of estrogen in down-regulating osteoclast numbers and activity through inhibition of RANKL (receptor activator of nuclear factor κB ligand) expression [54]. Appending a 2-phenylindole scaffold to alendronate generated bisphosphonates capable of inducing enhanced osteoclast apoptosis at 100 nM (**35**) and 10 pM (**36**, Fig. **2**) concentrations respectively, compared with the activity of ALN (**7**) itself at 100 μM, and reduced osteoclastogenesis. Presentation of the phosphonate ester functions was thought to enhance the lipophilicity of the structure. Growth inhibition of *L. donovani* was also observed [23]. Catechol derivative **37** can also inhibit osteoclast activity and induce proliferation of osteoblasts, exploiting the discovery of CBMID (**38**, Fig. **2**) as a compound lead for enhancing osteoid volume [112].

Introduction of NO-donor functions to BPs has sought to exploit their affinity for bone tissue and arterial walls in order to target the broad spectrum pharmacological action of NO as a secondary chemical messenger. The NO-BPs so formed are relevant to vasodilation and inhibition of RANKL-induced osteoclastogenesis from cells of the monocyte/macrophage (M/M) lineage [113,114]. Aliphatic nitrates **39** and **40** [113] and derivatives **41-43** (Fig. **2**) based on 1,2,5-oxadiazole-2-oxide (furoxan; known to release NO through thiol-cofactor activity) were capable of inhibiting differentiation in a RAW 264.7 M/M (pre-osteoclastic) cell line. All structures demonstrated the requisite affinity for hydroxyapatite and vasodilation capability, but the furoxan ring system exhibited no *N*-protonation characteristics, and anti-osteoclastogenesis activity was unmodified with respect to their non-NO-releasing furazan counterparts. Closer investigation of **41** and **42** revealed no contribution through any activity towards FPPS, identifying geranylgeranyl pyrophosphate synthase (GGPPS) or squalene synthase (SQS) as potential molecular targets, better able to accommodate the sterically demanding furoxan function [114].

2.3. Activation of γδ-T Cells

3-D QSAR studies have been instrumental in delineating the mechanisms by which different structures activate γδ-T cells of the immune system. Direct interaction with γδ-T-cell receptors is proposed for phosphoantigens such as the metabolite (*E*)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP, **44**, Fig. **3**), through which bacteria and protozoa are recognised, and the synthetic antigen phosphatim (**45**) [115]. Meanwhile, activation by N-BPs is thought to be an indirect result of FPPS inhibition, through elevated levels of IPP (**17**).

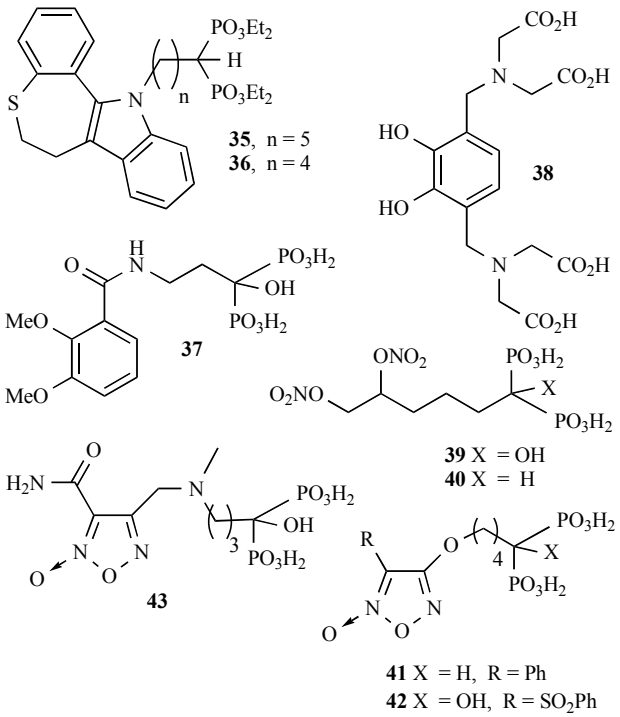


Fig. (2). Bisphosphonate derivatives **35-43**.

Excellent correlation exists comparing γδ-T cell activation (*i.e.* indicated by TNF-α release) with inhibition of huFPPS (R^2 = 0.88) and *L. major* FPPS (R^2 = 0.92) respectively for a data set of nine N-BPs including clinically relevant examples [40]. CoMSIA analysis has also generated similar fields for γδ-T cell activation, anti-resorptive activity and inhibition of *L. major* FPPS [97] in respective sets of clinically relevant N-BPs [40,97] and pyridinium-1-yl-BPs [109]. Meanwhile, pharmacophore modelling produces similar hypotheses for N-BPs with respect to FPPS inhibition and γδ-T cell activation; two negative ionisable groups, one cationic feature and a hydrophobic function, but a divergent hypothesis for phosphoantigens [116], which lacks positive charge functionality, possessing instead an H-bond donor feature.

The ability of γδ-T cells to recognise tumours through cancer antigens and stress-induced molecules conveys potential applications towards many cancer-types, identifying a role for N-BPs in anti-proliferative strategies. For example, co-administration of PAM (**6**) with interleukin-2 (IL-2) has achieved γδ-T cell activation in non-Hodgkin lymphoma patients, exhibiting an anti-lymphoma effect in some cases [42]. ZOL/IL-2 co-administration regimens have also demonstrated efficacy of γδ-T cell activation comparable to IPP (**17**) [43], and utility in phase 1 clinical trials in cases of metastatic, hormone-refractory prostate cancer [44]. Recently a series of deshydroxy-N-BPs was developed, commensurate with the physicochemical requirements for anti-tumour activity, namely reduced bone-retention with enhanced exposure to soft tissue and peripheral blood, and enhanced cell-membrane permeability [41]. The activating ability of purine derivatives **46** (Fig. **3**) was comparable to that of ZOL (**13**) in peripheral blood

mononuclear cells (PBMC's) *in vitro*, while deshydroxy-ZOL (**47**, Fig. **1**) was more potent by two orders of magnitude. Anti-proliferative and pro-apoptotic profiles of **47** *in vitro* were superior to those of ZOL, and synergistic activity in co-administration regimens with the leukaemia drug imatinib was greater. The potential utility of **47** *in vivo* was also illustrated in mice inoculated with BT549 (breast cancer) cells, with improved survival times resulting from N-BP/IL-2/$\gamma\delta$-T cell administration.

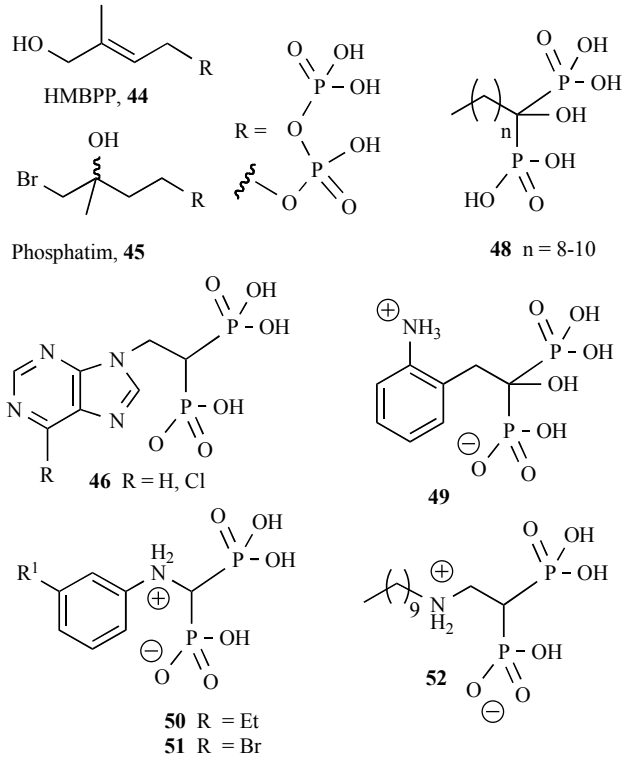


Fig. (3). $\delta\gamma$ T-cell activators **44-46** and anti-parasitic agents **48-52**.

2.4. Anti-Parasitic Activity

Trypanosomatids

Correlations between FPPS inhibition and anti-parasitic activity were derived in early studies demonstrating inhibition of sterol biosynthesis by RIS (**12**) in *T. cruzi* and *L. major* [13] and explicit inhibition of *T. cruzi* FPPS by this and other N-BPs [104]. Also, rescue experiments reversing RIS-induced growth inhibition of *T. brucei rhodesiense* on addition of FPP (**15**) identified FPPS as the molecular target, and it was possible to construct a predictive 3D-QSAR (CoMFA) model, correlating well with experimental values (R^2 = 0.87) when *N*-protonation states commensurate with pK_a values were used [19]. Particularly potent growth inhibitors of *T. brucei in vitro* were *ortho*-RIS (**22**; IC_{50} = 0.22 μM), **24** (0.70 μM), IBA (**10**; 0.96 μM) and RIS itself (8.6 μM, Fig. **1**) [13,19], while the most potent towards *T. cruzi in vitro* were the ZOL isomer **28** (IC_{50} = 35 μM), **22** (105 μM), RIS (123 μM), and PAM (**5**; 60 μM) [13]. General activity of N-BPs *in vitro* was greater towards *T. brucei* than *T. cruzi,* due to the relative cellular transport challenges presented in each case.

n-Alkyl-BPs lacking *N*- and aryl functionalities also exhibit significant growth inhibition of *T. brucei* [13,19], whereby potency increased with increasing chain length to a 10/11-carbon optimum (*cf* **48**, Fig. **3**; IC_{50} = 8.0 μM and 2.0 μM respectively), similar in length to FPP. This trend is identical to that reported for apicocomplexan parasites *T. gondii* [24] and *P. falciparum* [25], and also *E. histolytica* [25]. Similar observations relating to *T. cruzi* growth inhibition [14,15] led to investigation of 1-amino-1,1-BPs derived from fatty acids [16] and 2-alkylaminoethyl-1,1-BPs [17,18].

The antiparasitic activity of PAM [52] and RIS [117] in murine models of Chagas' disease has demonstrated the therapeutic potential of N-BPs *in vivo* towards *T. cruzi*. However, a relatively diminished inhibitory activity *in vivo* towards *T. brucei* growth [105] has led to examination of other molecular targets. A vacuolar pyrophosphatase (TbVSP1), critical to parasite growth in the bloodstream, was identified as such a candidate, and 3D-QSAR analyses (CoMSIA and CoMFA) were conducted over a set of 81 compounds, identifying the need for aryl and H-bond R^2-functions [118]. The most potent inhibitors were aniline derivative **49** and a series of compounds exhibiting α-NH functionality (*e.g.* **50**, **51**, Fig. **3**).

Apicocomplexans

Third generation N-BPs RIS [13], ZOL [24] and MIN [24] all exhibit great inhibitory potency (IC_{50} = 0.79-2.40 μM) towards *T. gondii* cell growth [13,24]; however, this is matched by the potency of C_9- and C_{10}-*n*-alkyl-BPs (**48**), which have exhibited far superior therapeutic indices *in vitro* (KB cells) and significantly improved "protection against death" in infected mice [24]. FPPS was identified as the molecular target for both N-BP and *n*-alkyl-BP sets in studies using over-expressing *T. gondii* strains. Investigation of 2-alkylaminoethyl-1,1-BPs [17,18] has subsequently revealed **52** as a lead anti-parasitic agent. None of the N-BPs in clinical use exhibit any measurable activity against *P. falciparum*, *n*-alkyl-BPs being the most potent growth inhibitors [13,25,26]. Growth inhibition of *P. falciparum* is essentially uncorrelated with inhibition of the representative FPPS from *Plasmodium vivax*; however, models incorporating two descriptors derived from a combinatorial descriptor search have achieved good predictability [26]. Cellular transport appears key to a description of activity in this case.

3. SYNTHESIS OF *N*-HETEROCYCLIC DRONIC ACIDS; MECHANISTIC ASPECTS

The practical synthesis of dronic acids involves the 3-component reaction of a substituted acetic acid, phosphorous acid or sometimes phosphoric acid and a *P*-chloride, which is in most cases phosphorus trichloride and rarely phosphorus oxychloride [6,68,72-80]. The solvents applied embrace a wide spectrum including chlorobenzene, toluene, 1,4-dioxane, 1,2-dimethoxyethane, PEG-400, *n*-octane and the molar ratio of the components varies widely.

Regarding the synthesis of zoledronic acid, the ratio of imidazolylacetic acid (IAA), phosphorous acid and phosphorus trichloride varies in the range of 1 : 1–5 : 2–4.6,

in different combinations [72-80]. 1 : 1.3 : 2.4, 1 : 3 : 3 and 1 : 4.6 : 4.6 can be mentioned as typical ratios. In a few instances, phosphorous acid was generated *in situ* by the partial hydrolysis of phosphorus trichloride used in a 6-equivalents quantity [81,82]. The yields reported are rather controversial and vary in the range 24–86%. The yields may refer to crude mixtures consisting of the dronic acid and its mono sodium salt. The realistic yields for pure dronic acid may be around 30–40%. The ratio and the excess of the *P*-reactants have never been explained or commented on in the literature. In most cases, the components were used in tentative and inadequate ratios and in unnecessary excesses. This resulted in a "black-box" procedure in respect of the synthesis of dronic acids by the method under discussion. The role of the tervalent P-components has not been clarified; the optimum conditions regarding especially the relative quantity of the reactants and the reaction time remained unclear, and hence the reaction cannot be efficient. Moreover, a large quantity of hydrochloric acid is formed by the hydrolysis of the excess of phosphorus trichloride during work-up.

We studied the synthesis of zoledronic and risedronic acids in detail to establish the optimum choice and ratio of the reactants, optimum concentrations and reaction times [119,120]. We also wished to get insight into the mechanism of the formation of the hydroxy-methylenebisphosphonic acid.

The reaction of imidazolylacetic acid (IAA), phosphorous acid and phosphorus trichloride leading to zoledronic acid (ZOL, **13**) was studied in methanesulfonic acid (MSA) at 80 °C in detail (Scheme **2**) under different conditions as shown in Table **2**. After the reaction of the components, the mixture was hydrolyzed, the hydrogen chloride liberated was neutralized, and the precipitated crude product containing the mixture of ZOL and its mono sodium salt (ZOL-Na) was recrystallized from aqueous hydrochloric acid to furnish pure ZOL. The sodium chloride formed and the impurities remained in the MSA-H_2O mixture.

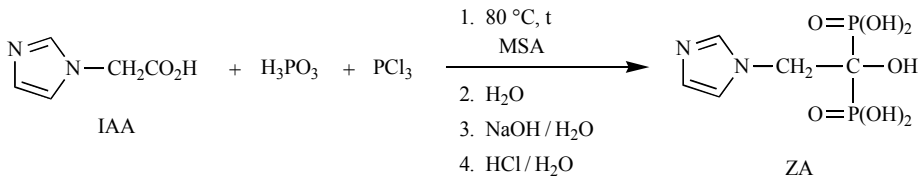
It can be seen from the data of Table **1** that the real reagent is phosphorus trichloride and that it should be used in a 3.1 equivalent quantity. It is also important that a shorter reaction time of 3 h is sufficient and, due to the dense consistency of the reaction mixture, the use of 3.33 mL MSA/g IAA instead of 2.22 mL MSA/g IAA is advantageous as it makes possible a more efficient stirring.

It is worthy of note that recrystallization of the crude mixture of ZOL and ZOL-Na is not only a recrystallization, but also a transformation of a part of ZOL-Na to ZOL. According to our experience, some of the yields reported in the patent literature seem to be unrealistic in respect of pure ZOL [74,77,78,80]. No criteria of purity were provided in these cases.

Similar experiments were carried out on the synthesis of risedronic acid (RIS) from pyridylacetic acid (PAA), phosphorous acid and phosphorus trichloride (Scheme **3**, Table **3**). In these cases, there was no need for recrystallization.

Similar trends could be observed as in the synthesis of zoledronic acid: the use of 3.1 equivalents of phosphorus trichloride (in the absence of phosphorous acid) led to the best yield of RIS.

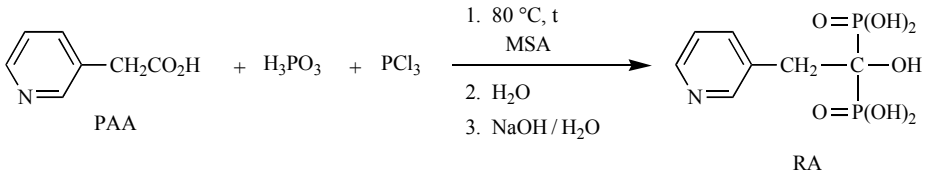
From the above experiments it is clear that in the preparation of dronic acids there is no need to use phosphorous acid. At the same time, efficient syntheses require the use of 3.1 equivalents of phosphorus trichloride. According to this, phosphorous acid was added entirely in vain in earlier syntheses, and the quantities of phosphorus trichloride beyond 3.1 were unnecessary. The use of



Scheme 2.

Table 2. The Effect of the Molar Ratio of the Components, the Reaction Time and the Concentration on the Formation of Zoledronic Acid at 80 °C

H_3PO_3 (equiv.)	PCl_3 (equiv.)	MSA (mL/g IAA)	t (h)	ZOL + ZOL-Na in the crude mixture		ZOL after recrystallization		Entry
				Yield (%)	Acidic content (%)	Yield (%)	Acidic content (%)	
1	2.1	2.22	5	42	11	26	97	1
2	1.1	2.22	5	15	-	7	96	2
3.1	0	2.22	5	0	-	0	–	3
0	3.1	2.22	3	68	23	49	98	4
0	3.1	3.33	3	71	26	53	99	5



Scheme 3.

Table 3. The Effect of the Molar Ratio of the Components and the Reaction Time on the Formation of Risedronic Acid at 80 °C

H_3PO_3 (equiv.)	PCl_3 (equiv.)	t (h)	Yield of RIS (%)	Acidic content of RIS (%)	Entry
1	2.1	<10	44	78	1
2.1	1	<10	7	–	2
3.1	0	<10	0	–	3
0	3.1	3	74	92	4

phosphorous acid without any reason and the unnecessary excess of phosphorus trichloride caused extra costs and environmental burdens (after the hydrolysis of the excess of PCl_3 to H_3PO_3 and HCl). On the other hand, when phosphorus trichloride was used in a quantity less than 3 equivalents, the conversions were incomplete, causing again extra costs in the production of dronic acids/dronates. Moreover, after the optimization, the reaction time was found to be 3 h, which is much shorter than that suggested in most of the procedures described.

The inefficiency of phosphorous acid may be explained by its very low nucleophilicity. At the same time, phosphorus trichloride is of satisfactory reactivity. Scheme **4** shows a possible mechanism for the formation of dronic acids in the reaction of a heteroarylacetic acid **53** with phosphorus trichloride. In the first step, the heteroarylacetic acid (**53**) is transformed to the corresponding acid chloride (**54**). In reaction with a second molecule of phosphorus trichloride, the acyl chloride is converted to an acyl phosphonium salt (**55**) whose carbonyl group may be attacked by a third molecule of phosphorus trichloride. For bisphosphonium intermediate **56**, the semi-pentavalent forms **56a** and **56b** may bring some relief regarding energy content. Hydrolysis of the intermediate (**56**) so formed affords the hydroxy-bisphosphonic acid (**57**) and, as a by-product, its mono sodium salt. The latter species is not shown in Scheme **4**.

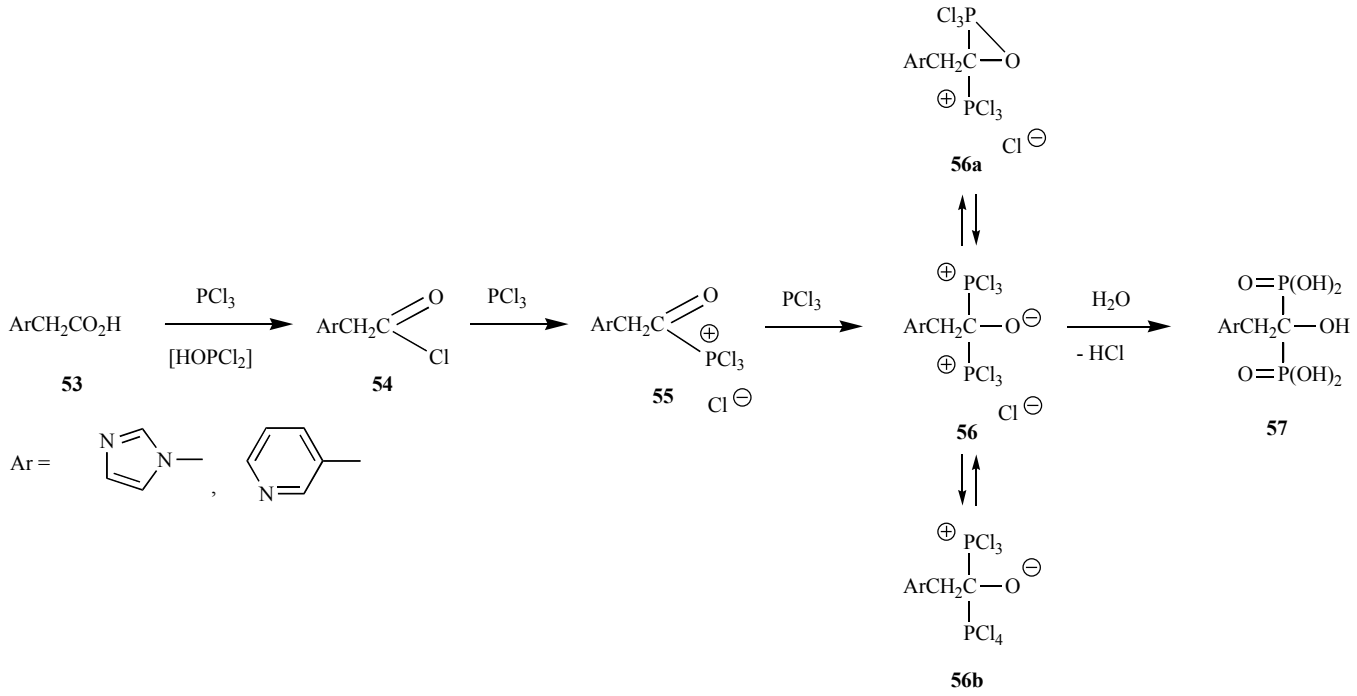
The involvement of heteroarylacetyl chloride intermediate **54** was proved by an experiment when the acid chloride (**54**) was formed separately by the reaction of carboxylic acid **53** with triphosgene. The subsequent reaction of the intermediate so formed (**54**) with 2.1 equivalents of phosphorus trichloride resulted in pure ZOL (**13**) in a yield of 50%. In a related study, Nicholson *et al*. prepared "condensates" that were claimed to be possible intermediates during the formation of ethane-1-hydroxy-1,1-diphosphonic acid in the reaction of acetylating agents and P(III) sources, such as H_3PO_3 and PCl_3 [71]. The major condensate was prepared by heating a mixture of phosphorous acid and 8.5 eq. of acetic anhydride at 50 °C for 15 min. In the light of our experience, heteroarylacetic acid and phosphorous acid do not form a condensate under these conditions.. At the same time, the interaction of the acid chloride (**54**) deriving from the heteroaryl acid (**53**) and PCl_3 may result in the formation of adduct **56** *via* intermediate **55**.

4. CONCLUSIONS

The therapeutic potential of N-BPs in the areas of cancer disease states, immunomodulation and diseases arising from excessive bone-resorption have been appreciated for many years and development pursued to the point of clinical application. In these areas, 3-D QSAR analytical methods have been instrumental in identifying the molecular sites of action of dronates, and in describing desirable pharmacophore arrangements for drug development. There is every reason to believe that such methods can be used to predict structures of interest for development and to predict activities of existing drugs at novel molecular targets with increasing accuracy. However, the established applications outlined above are in no small part derived from the innate ability of dronates to bind hydroxyapatite mineral, with the attendant accumulation in bone tissue, coupled with their ready uptake to target through osteoclast catabolic function. Development towards application in the area of antiparasitic treatments, on the other hand, remains a challenge, requiring accumulation of drug to therapeutic concentrations in the bloodstream. This constitutes a considerable obstacle to progress at present, given the bioavailability profiles of N-BP's observed in non-calcified tissues [121].

However, developments in the area of drug delivery, *e.g.* through encapsulation in liposomes [122] are broadening the scope of N-BP therapeutic application, and novel applications with respect to bone-directed drug delivery are also emerging, exemplified by cytidine-substituted aminoalkyl (hydroxy)methylenebisphosphonates developed recently [123].

Synthetic routes for N-BPs have been summarised and critically discussed. The best method involves reaction of the corresponding heteroarylacetic acid with 3.1 equivalents of phosphorus trichloride in methanesulfonic acid at 80 °C for



Scheme 4.

3h. After hydrolysis, pH adjustment and recrystallization (or even without a recrystallization), the dronic acids may be obtained after short reaction times, in reasonable yields and purities. The Hungarian authors have proved that, contrary to widespread practice, there is no need to use phosphorous acid in the synthesis, as this reagent does not take part in the reaction due to its low nucleophilicity. Mechanistic details have also been provided and the method elaborated may be of value in promoting the rational synthesis of dronic acids in general.

CONFLICT OF INTEREST

Declared none.

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REFERENCES

[1] Russell, R.G.G. Bisphosphonates: The first 40 years. *Bone* **2011**, *49*, 2-19.

[2] Breuer, E. The development of bisphosphonates as drugs, In *Analogue-based drug discovery*; Fischer, J.; Ganelli, C.R., Eds.; Wiley-VCH, Weinheim, **2006**; Ch. 15.

[3] Russell, R.G.G. Bisphosphonates: Mode of action and pharmacology. *Pediatrics* **2007**, *119*, S150-S162.

[4] Reszka, A.A.; Rodan, G.A. Nitrogen-containing bisphosphonate mechanism of action. *Mini-Rev. Med. Chem.* **2004**, *4*, 711-719.

[5] Reszka, A.A.; Rodan, G.A. Mechanism of action of bisphosphonates. *Curr. Osteoporos. Rep.* **2003**, *1*, 45-52.

[6] Widler, L.; Jaeggi, K.A.; Glatt, M.; Müller, K.; Bahmann, R.; Bisping, M.; Born, A.-R.; Cortesi, R.; Guiglia, G.; Jeker, H.; Klein, R.; Ramseier, U.; Schmid, J.; Schreiber, G.; Seltenmeyer, Y.; Green, J.R. Highly potent geminal bisphosphonates. From pamidronate disodium (Aredia) to zoledronic acid (Zometa). *J. Med. Chem.* **2002**, *45*, 3721-3738.

[7] van Beek, E.; Pieterman, E.; Cohen, L.; Löwik, C.; Papapolous, S. Nitrogen-containing bisphosphonates inhibit isopentenyl pyrophosphate isomerase/farnesyl pyrophosphate synthase activity with relative potencies corresponding to their antiresorptive potencies *in vitro* and *in vivo*. *Biochem. Biophys. Res. Commun.* **1999**, *255*, 491-494.

[8] van Beek, E.; Pieterman, E.; Cohen, L.; Löwik, C.; Papapolous, S. Farnesyl pyrophosphate synthase is the molecular target of nitrogen-containing bisphosphonates. *Biochem. Biophys. Res. Commun.* **1999**, *264*, 108-111.

[9] Dunford, J.E.; Thompson, K.; Coxon, F.P.; Luckman, S.P.; Hahn, F.M.; Poulter, C.D.; Ebetino, F.H.; Rogers, M.J. Structure-activity relationships for inhibition of farnesyl diphosphate synthase *in vitro* and inhibition of bone resorption *in vivo* by nitrogen-containing bisphosphonates. *J. Pharmacol. Exp. Ther.* **2001**, *296*, 235-242.

[10] Bergstrom, J.D.; Bostedor, R.G.; Masarachia, P.J.; Reszka, A.A.; Rodan, G. Alendronate is a specific, nanomolar inhibitor of farnesyl diphosphate synthase. *Arch. Biochem. Biophys.* **2000**, *373*, 231-241.

[11] Luckman, S.P.; Hughes, D.E.; Coxon, F.P.; Russell, R.G.G.; Rogers, M.J. Nitrogen-containing bisphosphonates inhibit the mevalonate pathway and prevent post-translational prenylation of GTP-binding proteins, including Ras. *J. Bone. Miner. Res.* **1998**, *13*, 581-589.

[12] Dunford, J.E.; Rogers, M.J.; Ebetino, F.H.; Phipps, R.J.; Coxon, F.P. Inhibition of protein prenylation by bisphosphonates causes sustained activation of Rac, Cdc42 and Rho GTPases. *J. Bone. Miner. Res.* **2006**, *21*, 684-694.

[13] Martin, M.B.; Grimley, J.S.; Lewis, J.C.; Heath III., H.T.; Bailey, B.N.; Kendrick, H.; Yardley, V.; Caldera, A.; Lira, R.; Urbina, J.A.; Moreno, S.N.J.; Docampo, R.; Croft, S.L.; Oldfield, E. Bisphosphonates inhibit the growth of *Trypanosoma brucei*, *Trypanosoma cruzi*, *Leishmania donovani*, *Toxoplasma gondii* and *Plasmodium falciparum*: A potential route to chemotherapy. *J. Med. Chem.* **2001**, *44*, 909-916.

[14] Szajnman, S.H.; Bailey, B.N.; Docampo, R.; Rodriguez, J.B. Bisphosphonates derived from fatty acids are potent growth inhibitors of *Trypanosoma cruzi. Bioorg. Med. Chem. Lett.* **2001**, *11*, 789-792.

[15] Szajnman, S.H.; Montalvetti, A.; Wang, Y.; Docampo, R.; Rodriguez, J.B. Bisphosphonates derived from fatty acids are potent inhibitors of *Trypanosoma cruzi* farnesyl pyrophosphate synthase. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3231-3235.

[16] Szajnman, S.H.; Ravaschino, E.L.; Docampo, R.; Rodriguez, J.B. Synthesis and biological evaluation of 1-amino-1,1-bisphosphonates derived from fatty acids against *Trypanosoma cruzi* targeting farnesyl pyrophosphate synthase. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4685-4690.

[17] Szajnman, S.H.; Linãres, G.E.G.; Li, Z.-H.; Jiang, C.; Galizzi, M.; Bontempi, E.J.; Ferella, M.; Moreno, S.N.J.; Docampo, R.; Rodriguez, J.B. Synthesis and biological evaluation of 2-alkylaminoethyl-1,1-bisphosphonic acids against *Trypanosoma cruzi* and *Toxoplasma gondii* targeting farnesyl pyrophosphate synthase. *Bioorg. Med. Chem.* **2008**, *16*, 3283-3290.

[18] Rosso, V.S.; Szajnman, S.H.; Malayil, L.; Galizzi, M.; Moreno, S.N.J.; Docampo, R.; Rodriguez, J.B. Synthesis and biological evaluation of new 2-alkylaminoethyl-1,1-bisphosphonic acids against *Trypanosoma cruzi* and *Toxoplasma gondii* targeting farnesyl diphosphate synthase. *Bioorg. Med. Chem. Lett.* **2011**, *19*, 2211-2217.

[19] Martin, M.B.; Sanders, J.M.; Kendrick, H.; de Luca-Fradley, K.; Lewis, J.C.; Grimley, J.S.; Van Brussel, E.M.; Olsen, J.R.; Meints, G.A.; Burzynska, A.; Kafarski, P.; Croft, S.L.; Oldfield, E. Activity of bisphosphonates against *Trypanosoma brucei rhodesiense*. *J. Med. Chem.* **2002**, *45*, 2904-2914.

[20] Kotsikorou, E.; Song, Y.; Chan, J.M.W.; Faelens, S.; Tovian, Z.; Broderick, E.; Bakalara, N.; Docampo, R.; Oldfield, E. Bisphosphonate inhibition of the exopolyphosphatase activity of the *Trypanosoma brucei* soluble vacuolar pyrophosphatase. *J. Med. Chem.* **2005**, *48*, 6218-6139.

[21] Rodriguez, N.; Bailey, B.N.; Martin, M.B.; Oldfield, E.; Urbina, J.A.; Docampo, R. Radical cure of experimental cutaneous leishmaniasis by the bisphosphonate pamidronate. *J. Infect. Diseases.* **2002**, *186*, 138-140.

[22] Yardley, V.; Khan, A.A.; Martin, M.B.; Slifer, T.R.; Araujo, F.G.; Moreno, S.N.J.; Docampo, R.; Croft, S.; Oldfield, E. *In vivo* activities of farnesyl pyrophosphate synthase inhibitors against *Leishmania donovani* and *Toxoplasma gondii. Antimicrob. Agents Chemother.* **2002**, *46*, 929-931.

[23] Singh, U.S.; Shankar, R.; Kumar, A.; Trivedi, R.; Chattopadhyay, N.; Shakya, N.; Palne, S.; Gupta, S.; Hajela, K. Synthesis and biological evaluation of indolyl bisphosphonates as anti-bone resorptive and anti-leishmanial agents. *Bioorg. Med. Chem.* **2008**, *16*, 8482-8491.

[24] Ling, Y.; Sahota, G.; Odeh, S.; Chan, J.W.M.; Araujo, F.G.; Moreno, S.N.J.; Oldfield, E. Bisphosphonate inhibitors of *Toxoplasma gondi* Growth: *In vitro*, QSAR, and *in vivo* investigations. *J. Med. Chem.* **2005**, *48*, 3130-3140.

[25] Ghosh, S.; Chan, J.M.W.; Lea, C.R.; Meints, G.A.; Lewis, J.C.; Tovian, Z.S.; Flessner, R.M.; Loftus, T.C.; Bruchhaus, I.; Kendrick, H.; Croft, S.L.; Kemp, R.G.; Kobayashi, S.; Nozaki, T.; Oldfield, E. Effects of bisphosphonates on the growth of *Entamoeba histolytica* and *Plasmodium* species *in vitro* and *in vivo*. *J. Med. Chem.* **2004**, *47*, 175-187.

[26] Mukkamala, D.; No, J. H.; Cass, L.M.; Chang, T.-K.; Oldfield, E. Bisphosphonate inhibition of a *Plasmodium* farnesyl diphosphate synthase and a general method for predicting cell-based activity from enzyme data. *J. Med. Chem.* **2008**, *51*, 7827-7833.

[27] Szabo, C.M.; Martin, M.B.; Oldfield, E. An investigation of bone resorption and *Dictyostelium discoideum* growth inhibition by bisphosphonate drugs. *J. Med. Chem.* **2002**, *45*, 2894-2903.

[28] Hounslow, A.M.; Carran, J.; Brown, R.J.; Rejman, D.; Blackburn, G.M.; Watts, D.J. Determination of the microscopic equilibrium dissociation constants for risedronate and its analogues reveals two distinct roles for the nitrogen atom in nitrogen-containing bisphosphonate drugs. *J. Med. Chem.* **2008**, *51*, 4170-4178.

[29] Cromartie, T.H.; Fisher, K.J.; Grossman, J.N. The discovery of a novel site of action for herbicidal bisphosphonates. *Pestic. Biochem. Physiol.* **1999**, *63*, 114-126.

[30] Leon, A.; Liu, L.; Yang, Y.; Hudock, M.P.; Hall, P.; Yin, F.; Studer, D.; Puan, K.-J.; Morita, C.T.; Oldfield, E. Isoprenoid biosynthesis as a drug target: Bisphosphonate inhibition of *Escherichia coli*, K12 growth and synergistic effects of fosmidomycin. *J. Med. Chem.* **2006**, *49*, 7331-7341.

[31] Clèzardin, P.; Ebetino, F.H.; Fournier, P.G.J. Bisphosphonates and cancer-induced bone disease: beyond their antiresorptive activity. *Cancer Res.* **2005**, *65*, 4971-4974.

[32] Green, J.R. Antitumor effects of bisphosphonates. *Cancer* **2003**, *97 (Suppl. 3)*, 840-847.

[33] Coleman, R.E. Bisphosphonates in breast cancer. *Ann. Oncol.* **2005**, *16*, 687-695.

[34] Chuah, C.; Barnes, D.J.; Kwok, M.; Corbin, A.; Deininger, M.W.N.; Druker, B.J.; Melo, J.V. Zoledronate inhibits proliferation and induces apoptosis of imatinib-resistant chronic myeloid leukaemia cells. *Leukemia* **2005**, *19*, 1896-1904.

[35] Yuasa, T.; Nogawa, M.; Kimura, S.; Yokota, A.; Sato, K.; Segawa, H.; Kuroda, J.; Maekawa, T. A third generation bisphosphonate, mindronic acid (YM529), augments the interferon α/β-mediated inhibition of renal cell cancer cell growth both *in vitro* and *in vivo*. *Clin. Cancer Res.* **2005**, *11*, 853-859.

[36] Segawa, H.; Kimura, S.; Kuroda, J.; Sato, K.; Nogawa, M.; Yuasa, T.; Yokota, A.; Hodohara, K.; Fujiyama, Y.; Maekawa, T. The anti-leukemic efficacy of the third generation bisphosphonate ONO5920/YM529. *Leukemia Res.* **2005**, *29*, 451-457.

[37] Yonou, H,; Ochiai, A.; Ashimine, S.; Maeda, H.; Horiguchi, Y.; Yoshioka, K.; Ogawa, Y.; Hatano, T.; Tachibana, M. The bisphosphonate YM529 inhibits osteoblastic bone tumor proliferation of prostate cancer. *Prostate* **2007**, *67*, 999-1009.

[38] Miwa, S.; Mizokami, A.; Keller, E.T.; Taichman, R.; Zhang, J.; Namiki, M. The bisphosphonate YM529 inhibits osteolytic and osteoblastic changes and CXCR-4-induced invasion in prostate cancer. *Cancer Res.* **2005**, *65*, 8818-8825.

[39] Sato, K.; Yuasa, T.; Nogawa, M.; Kimura, S.; Segawa, H.; Yokota, A.; Maekawa, T. A third-generation bisphosphonate, minodronic acid (YM529), successfully prevented the growth of bladder cancer *in vitro* and *in vivo*. *Br. J. Cancer* **2006**, *95*, 1354-1361.

[40] Sanders, J.M.; Ghosh, S.; Chan, J.M.W.; Meints, G.; Wang, H.; Raker, A.M.; Song, Y.; Colantino, A.; Burzynska, A.; Kafarski, P.; Morita, C.T.; Oldfield, E. Quantitative structure-activity relationships for γδ T cell activation by bisphosphonates. *J. Med. Chem.* **2004**, *47*, 375-384.

[41] Simoni, D.; Gebbia, N.; Invidiata, F.P.; Eleopra, M.; Marchetti, P.; Rondanin, R.; Baruchello, R.; Provera, S.; Marchioro, C.; Tolomeo, M.; Marinelli, L.; Limongelli, V.; Novellino E.; Kwaasi, A.; Dunford, J.; Buccheri, S.; Caccamo, N.; Dieli, F. Design, synthesis, and biological evaluation of novel aminobisphosphonates possessing an *in vivo* antitumor activity through a γδ-T lymphocytes-mediated activation mechanism. *J. Med. Chem.* **2008**, *51*, 6800-6807.

[42] Wilhelm, M.; Kunzmann, V.; Eckstein, S.; Reiner, P.; Weissinger, F.; Ruediger, T.; Tony, H.-P. γδT cells for immune therapy of patients with lymphoid malignancies. *Blood* **2003**, *102*, 200-206.

[43] Dieli, F.; Gebbia, N.; Poccia, F.; Caccamo, N.; Montesano, C.; Fulfaro, F.; Arcara, C.; Valerio, M.R.; Meraviglia, S.; Di Sano, C.; Sireci, G.; Salerno, A. Induction of γδT-lymphocyte effector functions by bisphosphonate zoledronic acid in cancer patients *in vivo*. *Blood* **2003**, *102*, 2310-2311.

[44] Dieli, F.; Vermijlen, D.; Fulfaro, F.; Caccamo, C.; Meraviglia, S.; Cicero, G.; Roberts, A; Buccheri, S.; D′Asaro, M.; Gebbia, N.; Salerno, A.; Eberl, M.; Hayday, A.C. Targeting human γδT cells with zoledronate and interleukin-2 for immunotherapy of hormone-refractory prostate cancer. *Cancer Res.* **2007**, *67*, 7450-7457.

[45] Feldman, E.J.; Brandwein, J.; Stone, R.; Kalaycio, M.; Moore, J.; O′Connor, J.; Wedel, N.; Roboz, G.J.; Miller, C.; Chopra, R.; Jurcic, J.C.; Brown, R.; Ehmann, W.C.; Schulman, P.; Frankel, S.R.; De Angelo, D.; Scheinberg, D. Phase III randomized multicenter study of a humanized anti-CD33 monoclonal antibody lintuzumab, in combination with chemotherapy, versus chemotherapy alone in patients with refractory or first-relapsed acute myeloid leukemia. *J. Clin. Oncol.* **2005**, *23*, 4110-4116.

[46] Linck, D.; Lentini, G.; Tiemann, M.; Fauser, A.A.; Parwaresch, R.; Basara, N. Sequential application of chemotherapy and monoclonal CD20 antibody: successful treatment of advanced composite-lymphoma. *Leuk. Lymphoma* **2005**, *46*, 285-288.

[47] McFarlane, S.I.; Muniyappa, R.; Shin, J.J.; Bahtiyar, G.; Sowers, J.R. Osteoporosis and cardiovscular disease. Brittle bones and boned arteries, Is there a link? *Endocrine* **2004**, *23*, 1-10.

[48] Rogers, M.J.; Frith, J.C.; Luckman, S.P.; Coxon, E.P.; Benford, H.L.; Mönkkönen, J.; Auriola, S.; Chilton, K.M.; Russell, R.G.G. Molecular mechanisms of action of bisphosphonates. *Bone* **1999**, *24 (Suppl.)*, 73S-79S.

[49] Russell, R.G.G.; Croucher, P.I.; Rogers, M.J. Bisphosphonates: pharmacology, mechanism of action and clinical uses. *Osteoporosis Int.* **1999**, *9*, S66-80.

[50] Russell, R.G.G.; Rogers, M.J.; Bisphosphonates: From the laboratory to the clinic and back again. *Bone* **1999**, *25*, 97-106.

[51] Roelofs, A.J.; Thompson, K.; Gordon, S.; Rogers, M.J. Molecular mechanism of action of bisphosphonates: Current status. *Clin. Cancer Res.* **2006**, *12*, 6222-6230.

[52] Urbina, J.A.; Moreno, B.; Vierkotter, S.; Oldfield, E.; Payares, G.; Sanoja, C.; Bailey, B.N.; Yan, W.; Scott, D.A.; Moreno, S.N.; Docampo, R. *Trypanosoma cruzi* contains major pyrophosphate stores, and its growth *in vitro* and *in vivo* is blocked by pyrophosphate analogues. *J. Biol. Chem.* **1999**, *274*, 33609-33615.

[53] Docampo, R.; Moreno, S.N. Acidocalcisome: A novel Ca^{2+} storage compartment in trypanosomatids and apicomplexan parasites. *Parasitol. Today* **1999**, *15*, 443-448.

[54] Allen, J.G.; Fotsch, C.; Babij, P. Emerging targets in osteoporosis disease modification. *J. Med. Chem.* **2010**, *53*, 4332-4353.

[55] Lehenkari, P.P.; Kellinsalmi, M.; Napankangas, J.P.; Ylitalo, K.V.; Mönkkönen, J.; Rogers, M.J.; Azhayev, A.; Vaananen, H.K.; Hassinen, I.E. Further insight into mechanism of action of clodronate: Inhibition of mitochondrial ADP/ATP translocase by a nonhydrolyzable, adenine-containing metabolite. *Mol. Pharmacol.* **2002**, *61*, 1255-1262.

[56] Mönkkönen, H.; Auriola, S.; Lehenkari, P.; Kellinsalmi, M.; Hassinen, I.E.; Vepsalainen, J.; Mönkkönen, J. A new endogenous ATP analog (AppplI) inhibits the mitochondrial adenine nucleotide translocase (ANT) and is responsible for the apoptosis induced by nitrogen-containing bisphosphonates. *Br. J. Pharmacol.* **2006**, *147*, 437-445.

[57] Coutkell, A.J.; Markham, A. Pamidronate. A review of its use in the management of osteolytic bone metastases, tumour-induced hypercalcaemia and Paget′s disease of bone. *Drugs Aging* **1998**, *12*, 149-168.

[58] Sharpe, M.; Noble, S.; Spencer, C.M. Alendronate: An update of its use in osteoporosis. *Drugs* **2001**, *61*, 999-1039.

[59] Chesnut III, C.H. Treating osteoporosis with bisphosphonates and addressing adherence: A review of oral ibandronate. *Drugs* **2006**, *66*, 1351-1359.

[60] Compston, J. Intravenous ibandronate in the treatment of osteoporosis: A viewpoint by Juliet Compston. *Drugs* **2006**, *66*, 1602-1603.

[61] Dunn, C.J.; Goa, K.L. Risedronate: A review of its pharmacological properties and clinical use in resorptive bone disease. *Drugs* **2001**, *61*, 685-712.

[62] Cheer, S.M.; Noble, S. Zoledronic acid. *Drugs* **2001**, *61*, 799-805.

[63] Perry, C.M.; Figgitt, D.P. Zoledronic acid. A review of its use in patients with advanced cancer. *Drugs* **2004**, *64*, 1197-1211.

[64] Lyseng-Williamson, K.A. Zoledronic acid. A review of its use in breast cancer. *Drugs* **2008**, *68*, 2661-2682.

[65] http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatients-andProviders/DrugSafetyInformationforHeathcareProfessionals/ucm070303.htm

[66] http://www.astellas.com/en/corporate/news/detail/approval-for-recalbon-tablets.html

[67] Schülke, U. Phosphonylation by tetraphosphorus hexoxide. *Phosphorus, Sulfur, Silicon* **1990**, *51/52*, 153-156.

[68] Kieczykowski, G.R.; Jobson, R.B.; Melillo, D.G.; Reinhold, D.F.; Grenda, V.J.; Shinkai, I. Preparation of (4-amino-1-hydroxybutylidene)bisphosphonic acid sodium salt, MK-217 (Alendronate sodium). An improved procedure for the preparation of 1-hydroxy-1,1-bisphosphonic acids. *J. Org. Chem.* **1995**, *60*, 8310-8312.

[69] Abdou, W.M.; Shaddy A.A. The development of bisphosphonates for therapeutic uses, and bisphosphonate structure-activity consideration. *ARKIVOC* **2009**, 143-182.

[70] Lecouvey, M.; Leroux, Y. Synthesis of 1-hydroxy-1,1-bisphosphonates. *Heteroatom Chem.* **2000**, *11*, 556-561.

[71] Prentice, J B.; Quimby, O.T.; Grabenstetter, R.J.; Allan Nicholson, D. Interaction of acylating agents and phosphorus(III) sources. I. Intermediacy of condensed species in the formation of (1-hydrocyethylidene) diphosphonic acid. *J. Am. Chem. Soc.* **1972**, *94*, 6119-6124.

[72] Bosies, E.; Gall, R. Diphosphonic acid derivatives, process for their preparation and medicines containing those compounds. *EP0258618*, **1988**; *Chem. Abstr.* **1988**, *108*, 186996.

[73] De Ferra, L.; Turchetta, S.; Massardo, P.; Casellato, P. Preparation of biphosphonic acids and salts thereof. *WO03093282*, **2003**; *Chem. Abstr.* **2003**, *139*, 365070.

[74] Aronhime, J.; Lifshitz-Liron R. Zoledronic acid crystal forms, Zoledronate sodium salt crystal forms, amorphous Zoledronate sodium salt, and processes for their preparation. *WO2005005447*, **2005**; *Chem. Abstr.* **2005**, *142*, 162610.

[75] Patel, V.M.; Chitturi T.R.; Thennati R. A process for preparation of bisphosphonic acid compounds. *WO2005044831*, **2005**; *Chem. Abstr.* **2005**, *142*, 463876.

[76] Patel, V.M.; Chitturi T.R.; Thennati R. A process for the preparation of 2-(imidazol-1-yl)-1-hydroxyethane-1, 1-diphosphonic acid. *WO2005066188*, **2005**; *Chem. Abstr.* **2005**, *143*, 153514.

[77] Pandey, S.C.; Haider, H.; Saxena, S.; Singh, M.K.; Thaper, R.K.; Dubey, S.K. Process for producing bisphosphonic acids and forms thereof. *WO2006134603*, **2006**; *Chem. Abstr.* **2007**, *146*, 62922.

[78] Yadav, R.P.; Shaikh, Z.G.; Mukarram, S.M.J.; Kumar, Y. Processes for the preparation of pure zoledronic acid. WO2007069049, **2007**; *Chem. Abstr.* **2007**, *147*, 72885.

[79] Baptista, J.; Mendes, Z. Process for the preparation of biphosphonic acids and salts thereof. *WO2008056129*, **2008**; *Chem. Abstr.* **2008**, *148*, 517833.

[80] Kas, M.; Benes, M.; Pis, J. Process for making zoledronic acid. *WO2010060619*, **2010**; *Chem. Abstr.* **2010**, *152*, 592167.

[81] Labriola, R.A.; Tombari, D.G.; Vecchioli, A. A crystalline form of the zoledronic acid, a process to obtain it and the pharmaceutical composition comprising it. *WO2007016982*, **2007**; *Chem. Abstr.* **2007**, *146*, 229463.

[82] Dembkowski, L.; Krzyzanowski, M.; Rynkiewicz, R.; Szramka, R.; Roznerski, Z.; Zyla, D.; Rachon, J.; Makowiec, S. Process for the preparation of [1-hydroxy-2-(1h-imidazol-1-yl)- ethylidene] bisphosphonic acid. *WO2010050830*, **2010**; *Chem. Abstr.* **2010**, *152*, 526005.

[83] McConnel, R.L.; Coover, H.W. Preparation of 1-hydroxyalkylidenediphosphonates. *J. Am. Chem. Soc.* **1956**, *78*, 4450-4452.

[84] Fitch, S. J.; Moedritzer, K. N.m.r. Study of the P-C(OH)-P to P-C-O-P rearrangement: Tetraethyl 1-hydroxyalkylidenediphosphonates. *J. Am. Chem. Soc.* **1962**, *84*, 1876-1879.

[85] Nicholson, D. A.; Vaughn, H. A general method of preparation of tetramethyl alkyl-1-hydroxy-1,1-diphosphonates. *J. Org. Chem.* **1971**, *36*, 3843-3845.

[86] Turhanen, P.A.; Ahlgren, M.J.; Järvinen, T.; Vepsäläinen, J.J. Bisphosphonate prodrugs. Synthesis and identification of (1-hydroxyethylidene)-1,1-bisphosphonic acid tetraesters by mass spectrometry, NMR spectroscopy and X-ray crystallography. *Phosphorus, Sulfur, Silicon* **2001**, *170*, 115-133.

[87] Grün, A.; Molnár, I. G.; Bertók, B.; Greiner, I.; Keglevich, G. Synthesis of α-hydroxy-methylenebisphosphonates by the microwave-assisted reaction of α-oxophosphonates and dialkyl phosphites under solventless conditions. *Heteroatom Chem.* **2009**, *20*, 350-354.

[88] Keglevich, G.; Grün, A.; Molnár, I. G.; Greiner, I. Phenyl-, benzyl- and unsymmetrical hydroxy-methylenebisphosphonates as dronic acid ester analogues from α-oxophosphonates by microwave-assisted synthesis. *Heteroatom Chem.* **2011**, *22*, 640-648.

[89] Greiner, I.; Grün, A.; Ludányi, K.; Keglevich, G. Solid–liquid two-phase alkylation of tetraethyl methylenebisphosphonate under microwave irradiation. *Heteroatom Chem.* **2011**, *22*, 11-14.

[90] Keglevich, G.; Grün, A.; Blastik, Z.; Greiner, I. Solid–liquid phase alkylation of P=O-functionalized CH acidic compounds utilizing phase transfer catalysis and microwave irradiation. *Heteroatom Chem.* **2011**, *22*, 174-179.

[91] Krutikov, V.I.; Erkin, A.V.; Pautov, P.A.; Zolotukhina, M.M. Heteryl- and arylaminomethylenebisphosphonates: synthesis and biologic activity. *Russ. J. Gen. Chem.* **2003**, *73*, 187-191.

[92] Dąbrowska, E.; Burzyńska, A.; Mucha, A.; Matczak-Jon, E.; Sawka-Dobrowolska, W.; Berlicki, L.; Kafarski, P. Insight into the mechanism of three component condensation leading to aminomethylenebisphosphonates. *J. Organomet. Chem.* **2009**, *694*, 3806-3813.

[93] Chandra Sekhar Reddy, G.; Veera Narayana Reddy, M.; Bakthavatchala Reddy, N.; Suresh Reddy, C. Green synthesis of amonobisphosphonates under microwave irradiation. *Phosphorus, Sulfur, Silicon* **2011**, *186*, 74-80.

[94] Martin, M.B.; Arnold, W.; Heath III, H.T.; Urbina, J.A.; Oldfield, E. Nitrogen-containing bisphosphonates as carbocation transition state analogs for isoprenoid biosynthesis. *Biochem. Biophys. Res. Commun.* **1999**, *263*, 754-758.

[95] Tarshis, L.C.; Proteau, P.J.; Kellogg, B.A.; Sacchettini, J.C.; Poulter, C.D. Regulation of product chain length by isoprenyl diphosphate synthases. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 15018-15023.

[96] Berlicki, Ł.; Kafarski, P. Computer-aided analysis and design of phosphonic and phosphinic enzyme inhibitors as potential drugs and agrochemicals. *Curr. Org. Chem.* **2005**, *9*, 1829-1850.

[97] Sanders, J.M.; Gómez, A.O.; Mao, J.; Meints, G.A.; van Brussel, E.M.; Burzynska, A.; Kafarski, P.; González-Pacanowska, D.; Oldfield, E. 3-D QSAR investigations of the inhibition of *Leishmania major* farnesyl pyrophosphate synthase by bisphosphonates. *J. Med. Chem.* **2003**, *46*, 5171-5183.

[98] Szabo, C.M.; Matusmura, Y.; Fukura, S.; Martin, M.B.; Sanders, J.M.; Sengupta, R.; Cieslak, J.; Loftus, T.C.; Lea, C.R.; Lee, H.-J.; Koohang, A.; Coates, R.; Sagami, H.; Oldfield, E. Inhibition of geranylgeranyl diphosphate synthase by bisphosphonates and diphosphates: A potential route to new bone antiresorption and antiparasitic agents. *J. Med. Chem.* **2002**, *45*, 2185-2196.

[99] Rondeau, J-M.; Bitsch, F.; Bourgier, E.; Geiser, M.; Hemmig, R.; Kroemer, M.; Lehmann, S.; Ramage, P.; Rieffel, S.; Strauss, A.; Green, J.R.; Jahnke, W. Structural basis for the exceptional *in vivo* efficacy of bisphosphonate drugs. *ChemMedChem* **2006**, *1*, 267-273.

[100] Kavanagh, K.L.; Guo, K.; Dunford, J.E.; Wu, X.; Knapp, S.; Ebetino, F.H.; Rogers, M.J.; Russell, R.G.G.; Oppermann, U. The molecular mechanism of nitrogen-containing bisphosphonates as antiosteoporosis drugs. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 7829-7834.

[101] Gabelli, S.B.; McLellan, J.S.; Montalvetti, A.; Oldfield, E.; Docampo, R.; Amzel, L.M. Structure and mechanism of the farnesyl diphosphate synthase from *Trypanosoma cruzi*: Implications for drug design. *Proteins* **2006**, *62*, 80-88.

[102] Hosfield, D.J.; Zhang, Y.; Dougan, D.R.; Broun, A.; Tari, L.W.; Swanson, R.V.; Finn, J. Structural basis for bisphosphonate-mediated inhibition of isoprenoid biosynthesis. *J. Biol. Chem.* **2004**, *279*, 8526-8529.

[103] Dunford, J.E.; Kwaasi, A.A.; Rogers, M.J.; Barnett, B.L.; Ebetino, F.H.; Russell, R.G.G.; Oppermann, U.; Kavanagh, K.L. Structure-activity relationships among the nitrogen containing bisphosphonates in clinical use and other analogues: Time-dependent inhibition of human farnesyl pyrophosphate synthase. *J. Med. Chem.* **2008**, *51*, 2187-2195.

[104] Montalvetti, A.; Bailey, B.N.; Martin, M.B.; Severin, G.W.; Oldfield, E.; Docampo, R.; Bisphosphonates are potent inhibitors of *Trypanosoma cruzi* farnesyl pyrophosphate synthase. *J. Biol. Chem.* **2001**, *276*, 33930-33937.

[105] Montalvetti, A.; Fernandez, A.; Sanders, J.M.; Ghosh, S.; van Brussel, E.; Oldfield, E.; Docampo, R. Farnesyl pyrophosphate synthase is an essential enzyme in *Trypanosoma brucei. In vitro* RNA interference and *in vivo* inhibition studies. *J. Biol. Chem.* **2003**, *278*, 17075-17083.

[106] Cheng, F.; Oldfield, E. Inhibition of isoprene biosynthesis pathway enzymes by phosphonates bisphosphonates and diphosphates. *J. Med. Chem.* **2004**, *47*, 5149-5158.

[107] Kotsikorou E.; Oldfield, E. A quantitative structure-activity relationship and pharmacophore modeling investigation of Aryl-X and heterocyclic bisphosphonates as bone resorption agents. *J. Med. Chem.* **2003**, *46*, 2932-2944.

[108] Gossman, W.L.; Wilson, S.R.; Oldfield, E. Three hydrates of 1-hydroxy-2-(3-pyridinyl)ethylidene-1,1-bisphosphonate (Risedronate) consisting of one molecular and two ionic structures. *Acta Crystallogr.* **2003**, *C59*, m33-m35.

[109] Sanders, J.M.; Song, Y.; Chan, J.M.W.; Zhang, Y.; Jennings, S.; Kosztowski, T.; Odeh, S.; Flessner, R.; Schwerdtfeger, C.; Kotsikorou, E.; Meints, G.A.; Gómez, A.O.; González-Pacanowska, D.; Raker, A.M.; Wang, H.; van Beek, E.R.; Papapoulos, S.E.; Morita, C.T.; Oldfield, E. Pyridinium-1-yl bisphosphonates are potent inhibitors of farnesyl diphosphate synthase and bone resorption. *J. Med. Chem.* **2005**, *48*, 2957-2963.

[110] Zhang, Y.; Hudock, M.P.; Krysiak, K.; Cao, R.; Bergan, K.; Yin, F.; Leon, A.; Oldfield, E. Activity of sulfonium bisphosphonates on tumor cell lines. *J. Med. Chem.* **2007**, *50*, 6067-6079.

[111] Marma, M.S.; Xia, Z.; Stewart, C.; Coxon, F.; Dunford, J.E.; Baron, R.; Kashemirov, B.A.; Ebetino, F.H.; Triffitt, J.T.; Russell, R.G.G.; McKenna, C.E. Synthesis and biological evaluation of α-halogenated bisphosphonate and phosphonocarboxylate analogues of risedronate. *J. Med. Chem.* **2007**, *50*, 5967-5975.

[112] Xie, Y.; Ding, H.; Qian, L.; Yan, X.; Yang, C.; Xie, Y. Synthesis and biological evaluation of novel bisphosphonates with dual activities on bone *in vitro*. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3267-3270.

[113] Lazzarato, L.; Rolando, B.; Lolli, M.L.; Tron, G.C.; Fruttero, R.; Gasco, A.; Deleide, G.; Guenther, H.L. Synthesis of NO-donor bisphosphonates and their in-vitro action on bone resorption.. *J. Med. Chem.* **2005**, *48*, 1322-1329.

[114] Lolli, M.L.; Rolando, B.; Tosco, P.; Chaurasia, S.; Di Stilo, A.; Lazzarato, L.; Gorassini, E.; Ferracini, R.; Oliaro-Bosso, S.; Fruttero, R.; Gasco, A. Synthesis and preliminary pharmacological characterisation of a new class of nitrogen-containing bisphosphonates (N-BPs). *Bioorg. Med. Chem.* **2010**, *18*, 2428-2438.

[115] Espinosa, E.; Belmant, C.; Pont, F.; Luciani, B.; Poupot, R.; Romagne, F.; Brailly, H.; Bonneville, M.; Fournié, J.J. Chemical synthesis and biological activity of bromohydrin pyrophosphate, a potent stimulator of human γδ T cells. *J. Biol. Chem.* **2001**, *276*, 18337-18344.

[116] Gossman, W.; Oldfield, E. Quantitative structure-activity relations for γδT cell activation by phosphoantigens. *J. Med. Chem.* **2002**, *45*, 4868-4874.

[117] Garzoni, L.R.; Waghabi, M.C.; Baptista, M.M.; de Castro, S.L.; Meirelles, M.N.L.; Britto, C.C.; Docampo, R.; Oldfield, E.; Urbina, J.A., *Int. J. Antimicrob. Agents* **2004**, *23*, 286-290.

[118] Kotsikorou, E.; Song, Y.; Chan, J.W.M.; Faelens, S.; Tovian, Z.; Broderick, E.; Bakalara, N.; Docampo, R.; Oldfield, E. Bisphosphonate inhibition of the exopolyphosphatase activity of the *Trypanosoma brucei* soluble vacuolar pyrophosphatase. *J. Med. Chem.* **2005**, *48*, 6128-6139.

[119] Keglevich, G.; Grün, A.; Aradi, K.; Garadnay, S.; Greiner, I. Optimized synthesis of *N*-heterocyclic dronic acids; closing a black-box era. *Tetrahedron Lett.* **2011**, *52*, 2744-2746.

[120] Keglevich, G.; Grün, A.; Garadnay, S.; Neu, J. HUP1100071 Patent Application, **2011**, filing date 8th February.

[121] Veerendhar, A.; Reich, R.; Breuer, E. Phosphorus based inhibitors of matrix metalloproteinases. *C. R. Chimie* **2010**, *13*, 1191-1202.

[122] Epstein-Barash, H.; Gutman, D.; Markovsky, E.; Mishan-Eisenberg, G.; Koroukhov, N.; Szebeni, J.; Golomb, G. Physicochemical parameters affecting liposomal bisphosphonates bioactivity for restenosis therapy: Internalisation, cell inhibition, activation of cytokines and complement, and mechanism of cell death. *J. Controlled Release* **2010**, *146*, 182-195.

[123] Schott, H.; Goltz, D.; Schott, T.C.; Jauch, C.; Schwendener, R. A. N^4-[Alkyl-(hydroxyphosphono)phosphonate]-cytidine–New drugs covalently linking antimetabolites (5-FdU, araU or AZT) with bone-targeting bisphosphonates (alendronate or pamidronate). *Bioorg. Med. Chem.* **2011**, *19*, 3520-3526.

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