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FORMULARIO ÚNICO DE OPOSICIÓN
2000-12

1	OPOSICIÓN A:	
☒	Patente de Invención	☐ Marca Colectiva
☐	Patente de Modelo de Utilidad	☐ Marca de Certificación
☐	Diseño Industrial	☐ Lema Comercial
☐	Esquema de Trazado para Circuitos Integrados	
	Marcas de Productos o Servicios	
2	SOLICITUD PUBLICADA Número del expediente: 10-063-351 Solicitante: NOVARTIS AG. Apoderado: CARLOS REYNALDO OLARTE GARCIA. Título de la nueva creación o denominación del signo: "BIFOSFATOS DE ALQUILO DE 2 A 5 ATOMOS DE CARBONOIMIDAZOL" Gaceta: 628 de 20-05-2011, Número de Publicación 421	
3	OPOSICIÓN PRESENTADA POR:	
OPOSITOR	Nombre: Lafrancol S.A. Dirección: Carrera 1 No. 46-84 Teléfono: (092) 449.30.51 Fax: Domicilio: Cali - Valle	IDENTIFICACIÓN C.C. ☐ NIT ☒ C.E. ☐ Otro ☐ Número: 890 301 463 -8
REPRESENTANTE O APODERADO (74)	Nombre: José Luis Reyes Villamizar Dirección: Cra. 17 No. 88-23, Of. 205 Teléfono: 621.26.31 E-mail: info@reyes-abogados.com Fax: 621.25.42 Domicilio: Bogotá, D.C.	IDENTIFICACIÓN C.C. ☒ NIT ☐ C.E. ☐ Otro ☐ Número: 79'152.473 de Usaquén TP 44655
4	FUNDAMENTOS DE LA OPOSICION: Causal: Incumplimiento de requisitos de patentabilidad (Título II, Capítulo I, Decisión 486 de 2000). Justificación: VER ANEXO.	
5	Comprobante de pago No. 11-0080125 (Oposición) Fecha Agosto 19/2011 (Prórroga) Fecha	

6

Anexos:

- ☒ Comprobante de pago de la tasa
- ☒ Poderes, si fuere el caso
- ☒ Pruebas de los fundamentos de la oposición
- ☒ Certificado de existencia y representación legal de las partes, cuando sean personas jurídicas
- ☐ Documentos que acrediten el legítimo interés, si fuere el caso
- ☒ Copia de la oposición para el traslado al solicitante.

7

NOMBRE: JOSÉ LUIS REYES VILLAMIZAR

FIRMA:

C.C. No. 79152.473 T.P. 44655

Señor

SUPERINTENDENTE DE INDUSTRIA Y COMERCIO

E.

S.

D.

REF:

Expediente 10-063-351

TÍTULO SOLICITADO: BIFOSFATOS DE ALQUILO DE 2 A 5 ATOMOS DE CARBONOIMIDAZOL.

PUBLICACIÓN: Gaceta 628 de 20/05/2011, N° de publicación 421.

SOLICITANTE: NOVARTIS AG.

APODERADO: CARLOS REYNALDO OLARTE GARCIA.

TRÁMITE: PRESENTACIÓN DE OPOSICION

JOSÉ LUIS REYES VILLAMIZAR, mayor de edad, identificado como aparece al pie de mi firma, abogado en ejercicio, titular de la tarjeta profesional número 44655 del Consejo Superior de la Judicatura, obrando en calidad de apoderado de la sociedad **LAFRANCOL S.A.**, sociedad constituida y vigente de conformidad con las leyes colombianas, con domicilio en Cali, Valle, en ejercicio de lo dispuesto por el artículo 42 de la Decisión 486 de 2000, muy respetuosamente me permito formular la siguiente

I. PETICIÓN

Sírvase negar el beneficio patentario a la solicitud contenida en el expediente **10-063-351**, por no cumplir con lo dispuesto por los artículos 14, 18 y 20 literal d) de la Decisión 486 de 2000, con base en los fundamentos que se expondrán a continuación.

II. LEGÍTIMO INTERÉS

Asiste a **LAFRANCOL S.A.**, legítimo interés para presentar esta oposición, pues la eventual concesión de la solicitud restringiría, en forma contraria al Régimen Común Andino de Propiedad Industrial, el empleo de ciertas sustancias y composiciones farmacéuticas, en detrimento de sus legítimos intereses comerciales y de la libre competencia.

III. FUNDAMENTO DE LA OPOSICIÓN

La solicitud colombiana en trámite incumple con los requisitos indispensables para otorgársele el privilegio de patente de invención, tal y como se demuestra con los siguientes argumentos:

En primer lugar, y ante la reivindicación textual de un diagnóstico o tratamiento de una resorción ósea excesiva o inapropiada, específicamente en las reivindicaciones 6 y 7, es importante indicar que esto contraviene lo dispuesto en la Decisión 486 de 2000 que literalmente refiere a que *"No serán patentables (...) d) los métodos terapéuticos o quirúrgicos para el tratamiento humano o animal, así como los métodos de diagnóstico aplicados a los seres humanos o a animales."*

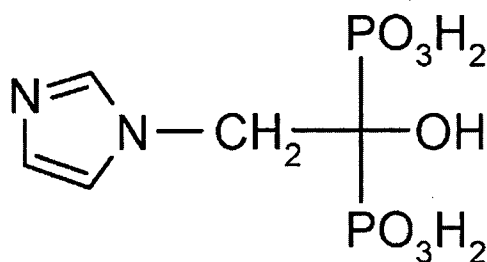
En otro aspecto, y esta vez refiriéndonos a la ausencia de nivel inventivo de la solicitud colombiana en cuestión, podemos citar algunos documentos que revelaban con anterioridad los mismos compuestos reivindicados por dicha solicitud en trámite, veamos:

La publicación científica ***"Highly Potent Geminal Bisphosphonates. From Pamidronate Disodium (Aredia) To Zoledronic Acid (Zometa)"***¹ revela:

Abstract: *Bisphosphonates (BPs) are pyrophosphate analogues in which the oxygen in P-O-P has been replaced by a carbon, resulting in a metabolically stable P-C-P structure. Pamidronate (1b, Novartis), a second-generation BP, was the starting point for extensive SAR studies. Small changes of the structure of pamidronate lead to marked improvements of the inhibition of osteoclastic resorption potency. Alendronate (1c, MSD), with an extra methylene group in the N-alkyl chain, and olpadronate (1h, Gador), the N,N-*

¹ Leo Widler, * Knut A. Jaeggi, Markus Glatt, Klaus Müller, Rolf Bachmann, Michael Bisping, Anne-Ruth Born, Reto Cortesi, Gabriela Guiglia, Heidi Jeker, Rémy Klein, Ueli Ramseier, Johann Schmid, Gerard Schreiber, Yves Seltenmeyer, and Jonathan R. Green. J. Med. Chem., 2002, 45 (17), pp 3721-3738

dimethyl analogue, are about 10 times more potent than pamidronate. Extending one of the N-methyl groups of olpadronate to a pentyl substituent leads to ibandronate (1k, Roche, Boehringer-Mannheim), which is the most potent close analogue of pamidronate. Even slightly better antiresorptive potency is achieved with derivatives having a phenyl group linked via a short aliphatic tether of three to four atoms to nitrogen, the second substituent being preferentially a methyl group (e.g., 4g, 4j, 5d, or 5r). The most potent BPs are found in the series containing a heteroaromatic moiety (with at least one nitrogen atom), which is linked via a single methylene group to the geminal bisphosphonate unit. Zoledronic acid (6i), the most potent derivative, has an ED₅₀ of 0.07 mg/kg in the TPTX in vivo assay after sc administration. It not only shows by far the highest therapeutic ratio when comparing resorption inhibition with undesired inhibition of bone mineralization but also exhibits superior renal tolerability. Zoledronic acid (6i) has thus been selected for clinical development under the registered trade name Zometa. The results of the clinical trials indicate that low doses are both efficacious and safe for the treatment of tumor-induced hypercalcemia, Paget's disease of bone, osteolytic metastases, and postmenopausal osteoporosis.



Por su parte la publicación Científica “A Quantitative Structure-Activity Relationship And Pharmacophore Modeling Investigation Of Aryl-X And Heterocyclic Bisphosphonates As Bone Resorption Agents²” revela:

Abstract: We have used quantitative structure-activity relationship (QSAR) techniques, together with pharmacophore modeling, to investigate the relationships between the structures of a wide variety of geminal bisphosphonates and their activity in inhibiting osteoclastic bone resorption. For aryl-X (X = alkyl, oxyalkyl, and sulfanyalkyl) derivatives of pamidronate and one alendronate, a molecular field analysis (MFA) yielded an R(2) value of 0.900 and an F-test of 54 for a training set of 29 compounds. Using reduced training sets, the activities of 20 such compounds were predicted with an average error of 2.1 over a 4000x range in activity. Such good results were only obtained when using the X-ray crystallographic structure of farnesyl pyrophosphate (FPP) bound to the target enzyme, farnesyl pyrophosphate synthase (FPP synthase), to guide the initial molecular alignment. For a series of heterocyclic bisphosphonates, use of the MFA method yielded an R(2) of 0.873 and an F-test of 36 for a training set of 26 compounds. Using a reduced training set, the activities of 20 compounds were predicted with an average error of 2.5 over a 2000x range in activity. With the heterocyclic compounds, test calculations indicated the importance of correct choice of protonation of the heterocyclic rings. For example, thiazoles, pyrazoles, and triazoles have low (approximately 2-3) pK(a) values and the derived bisphosphonates are inactive in bone resorption since they cannot readily be side chain protonated

² Evangelia Kotsikourou and Eric Oldfield. Journal of Medicinal Chemistry, 2003, Vol. 46, No. 14.

and are thus poor carbocation reactive intermediate analogues. On the other hand, aminothiazoles, imidazoles, pyridyl, and aminopyridyl species typically have $pK(a)$ values in the range approximately 5-9 and, in the absence of unfavorable steric interactions, the corresponding bisphosphonates are generally good inhibitors. However, aminoimidazole bisphosphonates are generally less active, since their $pK(a)$ s (approximately 11) are so high, due to guanidinium-like resonance, that they cannot readily be deprotonated, which we propose results in poor cellular uptake. The results of pharmacophore modeling using the Catalyst program revealed the importance of two negative ionizable and one positive charge feature for both aryl-X and heterocyclic pharmacophores, together with the presence of a distal hydrophobic feature in the aryl bisphosphonate and a more proximal aromatic feature in the heterocyclic bisphosphonate pharmacophores. When taken together, these results show that it is now possible to predict the activity, within a factor of about 2.3, of a wide range of aryl-X and heterocyclic bisphosphonates. The results emphasize the importance of utilizing crystallographic structural information to guide the initial alignment of extended bisphosphonates, and in the case of heterocyclic bisphosphonates, the importance of side chain protonation state. These simple ideas may facilitate the design of other, novel bisphosphonates, of use in bone resorption therapy, and as antiparasitic and immunotherapeutic agents.

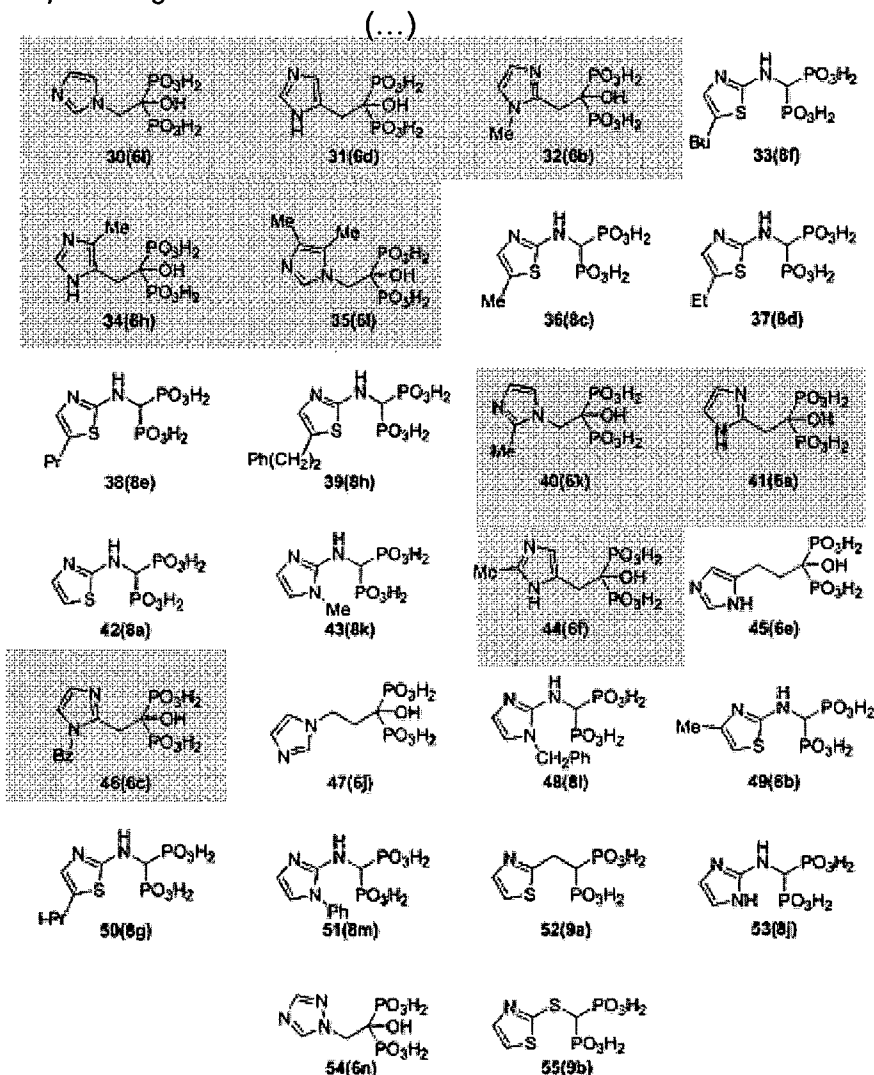


Figure 5. Structures of the 26 heterocyclic bisphosphonates investigated. The designations in parentheses are those used by Widler et al. (ref 1).

En el mismo sentido la publicación científica “**Quantitative Structure–Activity Relationship Study Of Bisphosphonates³**” también revela:

Abstract

Motivation. Bisphosphonates (BPs) are most widely used as agents for treating osteoporosis. They have also been used for other purposes such as herbicides, anticancer agents, and antiparasitics. Here we report QSAR models of four BPs datasets based on the 118 structural and biological data we have collected from various literature sources.

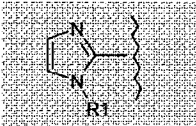
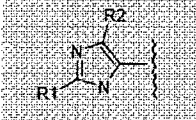
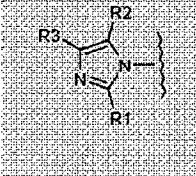
Method. The structures were energy minimized with the MMFF94 force field, then 181 2D and 3D descriptors were calculated with MOE (Molecular Operating Environment). The step by step multiple regression and principle analysis were used to generate QSAR models. The predictive power of each QSAR model was estimated with the leave-one-out (LOO) cross-validation method.

Results. The QSAR model for a dataset of 28 GGPPSase inhibitors ($r^2 = 0.86$, $r^2_{LOO} = 0.82$, $s = 0.45$, $s_{LOO} = 0.51$, $F = 77.56$) is made up of two descriptors. Another dataset of 28 compounds with bioactivities against the growth of *T. Brucei rhodesiense* was studied using PCA and reached a model ($r^2 = 0.85$, $r^2_{LOO} = 0.79$, $s = 0.30$, $s_{LOO} = 0.35$, $F = 32.03$) with four principle components (PCs). Both the above models have comparable predictive ability with CoMFA model reported by Szabo *et al.* The 86 BPs provided by Novartis with *in vivo* bio-data of TPTX rats were divided into two datasets. A six PCs model ($r^2 = 0.80$, $r^2_{LOO} = 0.72$, $s = 0.44$, $s_{LOO} = 0.53$, $F = 24.83$) elucidated the dataset of 44 compounds in which containing aliphatic linked nitrogen atoms. The other dataset includes 42 BPs containing a heterocyclic moiety with at least one nitrogen atom. Its PCA model ($r^2 = 0.80$, $r^2_{LOO} = 0.71$, $s = 0.46$, $s_{LOO} = 0.57$, $F = 19.99$) consists of seven PCs.

Conclusions. A leave-four-out test procedure shows that though the QSAR models based on *in vivo* bone resorption pED₅₀ values cannot provide explicit indications for drug design, their predictive ability for related compounds is quite good.

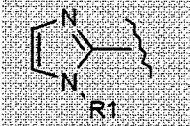
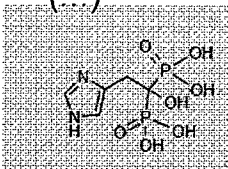
Keywords. Bisphosphonate; principal component analysis (PCA); GGPPSase inhibitor; quantitative structure–activity relationships (QSAR); IC₅₀; ED₅₀.

(...)

58	Novartis 6a		H			1	5
59	Novartis 6b		Me			1	0.6
60	Novartis 6c		Bz			1	25
61	Novartis 6d		H	H		1	0.3
62	Novartis 6e		H	H		2	20
63	Novartis 6f		Me	H		1	15
64	Novartis 6h		H	Me		1	1.5
65	Zoledronate		H	H	H	1	0.07
66	Novartis 6j		H	H	H	2	45
67	Novartis 6k		Me	H	H	1	3
68	Novartis 6l		H	Me	Me	1	1.5

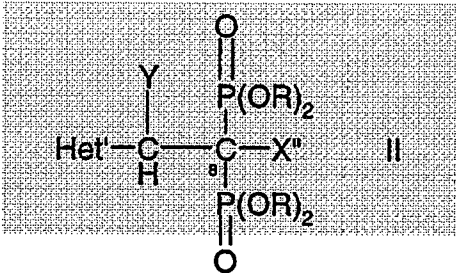
(...)

³ A. Xie, C. Liao, Z. Li, Z. Ning, W. Hu, X. Lu, L. Shi, and J. Zhou, Quantitative Structure–Activity Relationship Study of Bisphosphonates, Internet Electron. J. Mol. Des. 2004, 3, 622–650, <http://www.biochempress.com>.

81	Novartis 8j		H	500
82	Novartis 8k		Me	5
83	Novartis 8l		PhCH ₂	75
84	Novartis 8m		Ph	200
(...)				
107	T.B. 012		8.6	220.0

Finalmente la publicación internacional WO/2002/043738 publicada el 06 de junio de 2002 revela:

In a further embodiment a particularly preferred bisphosphonate for use in the invention comprises a compound of Formula II



wherein

Het' is a substituted or unsubstituted heteroaromatic five-membered ring selected from the group consisting of imidazolyl, imidazoliny, isoxazolyl, oxazolyl, oxazoliny, thiazolyl, thiazoliny, triazolyl, oxadiazolyl and thiadiazolyl wherein said ring can be partly hydrogenated and wherein said substituents are selected from at least one of the group consisting of C₁-C₄ alkyl, C₁-C₄ alkoxy, phenyl, cyclohexyl, cyclohexylmethyl, halogen and amino and wherein two adjacent alkyl substituents of Het can together form a second ring; Y is hydrogen or C₁-C₄ alkyl; X'' is hydrogen, hydroxyl, amino, or an amino group substituted by C₁-C₄ alkyl, and R is hydrogen or C₁-C₄ alkyl;

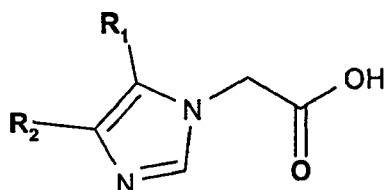
(...)

2-(4,5-Dimethylimidazol-1-yl)-1-hydroxyethane-1,1-diphosphonic acid, and

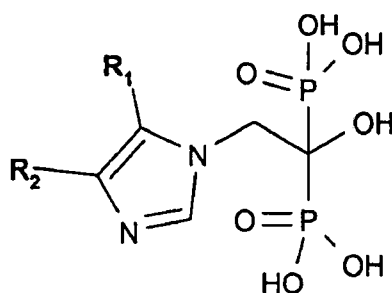
Como se puede apreciar, en todas estas anterioridades no sólo se revelan compuestos relacionados a los reivindicados en la solicitud colombiana en trámite, sino que además se obtienen diversos compuestos para estudios de estructura

actividad, en donde se realizan diversas modificaciones estructurales sobre dichos compuestos bisfosfonatos, lo que deja en evidencia también la versatilidad en la obtención de dichos compuestos.

En complemento a lo anterior, podemos demostrar también que el proceso de obtención específico reivindicado en la solicitud colombiana en trámite, que comprende la reacción de un compuesto de ácido carboxílico de la fórmula II:



Con un oxihaluro de fósforo para dar un compuesto de fórmula I:



Tal y como se menciona en la reivindicación 9, carece de nivel inventivo si se tiene en cuenta el contenido, por ejemplo, de la publicación científica denominada **“Bisfosfonatos: Síntese, Análises Químicas E Aplicações Farmacológicas⁴”** que revela:

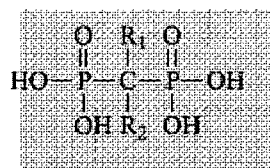
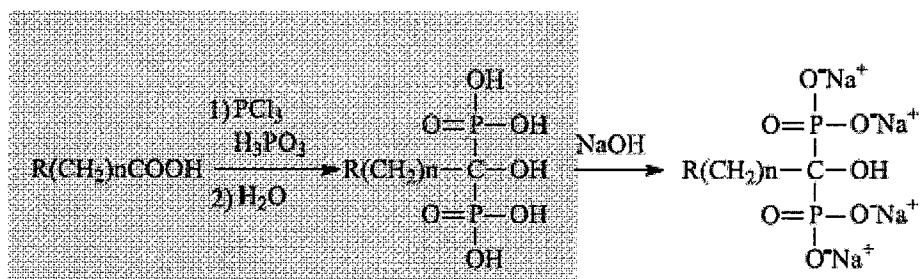


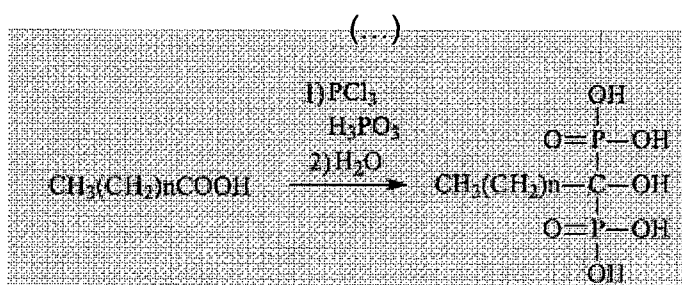
Figura 2. Estrutura química geral dos BP's

(...)

⁴ Christian Fernandes, Rodrigo Souza Leite e Fernando Mauro Lanças. Quim. Nova, Vol. 28, No. 2, 274-280, 2005.



Esquema 2. Reacción entre ácido carboxílico e ácido fosforoso na presença de tricloreto de fósforo



Esquema 3. Síntese de BP's a partir de ácidos graxos de cadeia pequena (n = 1, 2, 3 e 4)

(...)

Y teniendo en cuenta lo descrito además en la patente española ES 2174364 publicada el 10/03/1999:

El oxiclórico de fósforo se fabrica industrialmente a partir de tricloruro de fósforo mediante oxidación con oxígeno del aire. En este caso se obtiene un producto muy puro, apenas impurificado con productos orgánicos.

Como se puede apreciar en el estado de la técnica ya se reconocía la reacción entre oxihaluros de fósforo (preparado in situ a partir de trihaluros de fósforo y ácido fosforoso como agente oxidante) para la obtención de bisfosfonatos.

En conclusión, la solicitud colombiana en trámite carece de los requisitos fundamentales para otorgársele el privilegio de patente de invención, en primer lugar, porque incurre en una improcedencia directa de patentabilidad al reivindicar un método de diagnóstico y/o tratamiento, lo cual es claramente inaceptable bajo la Decisión 486 de 2000, y en segundo lugar, porque carece de nivel inventivo al reivindicar compuestos descritos con anterioridad en el estado de la técnica,

obtenidos a través de un proceso que involucra reactivos e intermediarios igualmente reconocidos en el estado de la técnica.

Así las cosas, y con fundamento en los argumentos expuestos a lo largo del presente escrito, respetuosamente reitero la solicitud para que su despacho se abstenga de conceder el privilegio de patente solicitado, y ordene en consecuencia el archivo del expediente respectivo.

IV. FUNDAMENTOS DE DERECHO

Son fundamentos de derecho el Capítulo I, artículos 14, 18 y 20 literal d) de (Requisitos de Patentabilidad) y el artículo 42 (Oposiciones) de la Decisión 486 de 2000.

V. PRUEBAS

Ruego que se tengan como tales, sin perjuicio de las que puedan presentarse al vencimiento de la prórroga solicitada, y de aquellas que el Despacho considere oficiosamente, las siguientes:

- Copia de todos los documentos citados en el acápite de Fundamentos de la Oposición.

VI. ANEXOS

Para efectos del trámite correspondiente, me permito anexar al presente escrito los siguientes anexos

1. Certificado de existencia y representación legal de Lafrancol S.A.
2. Poder para actuar.
3. Constancia de pago de las tasas de tramitación de oposición.
4. Copia para correr traslado al solicitante.
5. El mencionado en el acápite de pruebas.

VII. NOTIFICACIONES

El suscrito recibirá notificaciones en la secretaría de su Despacho o en mi oficina localizada en la Carrera 17 No. 88-23, Oficina 205, Fax (1) 621.25.42 de esta ciudad. Lafrancol S.A., las recibirá en sus oficinas localizadas en la Carrera 1 N° 46-84 de Cali.

Señor Superintendente,



JOSÉ LUIS REYES VILLAMIZAR
C.C. 79'152.473 de Usaquén
T.P.A. 44.655 del C.S.J.

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 - Aliphatic Compounds
 - Alkaloids
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 - Heterocyclic Compounds (One Hetero Atom)
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Leo Widler,* Knut A. Jaeggi, Markus Glatt, Klaus Müller, Rolf Bachmann, Michael Bisping, Anne-Ruth Born, Reto Cortesi, Gabriela Guiglia, Heidi Jeker, Rémy Klein, Ueli Ramseier, Johann Schmid, Gerard Schreiber, Yves Seltenmeyer, and Jonathan R. Green
Novartis Pharma Research, Arthritis and Bone Metabolism Therapeutic Area, CH-4002 Basel, Switzerland
J. Med. Chem., 2002, 45 (17), pp 3721–3738

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Abstract

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Bisphosphonates (BPs) are pyrophosphate analogues in which the oxygen in P–O–P has been replaced by a carbon, resulting in a metabolically stable P–C–P structure. Pamidronate (**1b**, Novartis), a second-generation BP, was the starting point for extensive SAR studies. Small changes of the structure of pamidronate lead to marked improvements of the inhibition of osteoclastic resorption potency. Alendronate (**1c**, MSD), with an extra methylene group in the *N*-alkyl chain, and olpadronate (**1h**, Gador), the *N,N*-dimethyl analogue, are about 10 times more potent than pamidronate. Extending one of the *N*-methyl groups of olpadronate to a pentyl substituent leads to ibandronate (**1k**, Roche, Boehringer-Mannheim), which is the most

- potent close analogue of pamidronate. Even slightly better antiresorptive potency is achieved with derivatives having a phenyl group linked via a short aliphatic tether of three to four atoms to nitrogen, the second substituent being preferentially a methyl group (e.g., **4g**, **4j**, **5d**, or **5r**). The most potent BPs are found in the series containing a heteroaromatic moiety (with at least one nitrogen atom), which is linked via a single methylene group to the geminal bisphosphonate unit. Zoledronic acid (**6i**), the most potent derivative, has an ED₅₀ of 0.07 mg/kg in the TPTX in vivo assay after sc administration. It not only shows by far the highest therapeutic ratio when comparing resorption inhibition with undesired inhibition of bone mineralization but also exhibits superior renal tolerability. Zoledronic acid (**6i**) has thus been selected for clinical development under the registered trade name Zometa. The results of the clinical trials indicate that low doses are both efficacious and safe for the treatment of tumor-induced hypercalcemia, Paget's disease of bone, osteolytic metastases, and postmenopausal osteoporosis.

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- Design, Synthesis, and Biological Evaluation of Novel

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*Journal of Medicinal Chemistry***2008** 51 (21), 6800-6807

A small series of aminobisphosphonates (N-BPs) structurally related to zoledronic acid was synthesized with the aim of improving activity toward activation of human $\gamma\delta$ T cells and in turn their in vivo antitumor activity. The absence of the 1-OH moiety, ...

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The reaction of Zoledronate with β -tricalcium phosphate (β -TCP) was probed using ^{23}Na magic-angle-spinning (MAS) NMR, ^{31}P homonuclear through-space double quantum–single quantum correlation MAS, and ^{31}P – ^1H {FSLG} HETCOR MAS NMR experiments. The nature and ...

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We investigated three series of sulfonium bisphosphonates for their activity in inhibiting the growth of three human tumor cell lines. The first series consisted of 6 cyclic sulfonium bisphosphonates, the most active species having an (average) IC₅₀ of 89 ...

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Deng-Ke Cao, Yi-Zhi Li, and Li-Min Zheng

Inorganic Chemistry **2007** 46 (18), 7571-7578

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Inorganic Chemistry **2007** 46 (18), 7571-7578

Reactions of 2-(1-imidazole)-1-hydroxyl-1,1'-ethylidenediphosphonic acid (ImhedpH₄) and cobalt or nickel salts under hydrothermal conditions lead to four new metal phosphonates with two types of structures: M₃(ImhedpH)₂(H₂O)₄·2H₂O [M = Co(II) (1) and Ni(...

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Journal of Medicinal Chemistry **2007** 50 (17), 4147-4161

A series of ring-substituted analogues of imidazole-4-acetic acid (IAA, 4), a partial agonist at both GABAA and GABAC receptors (GABA = γ -aminobutyric acid), have been synthesized. The synthesized compounds 8a–l have been evaluated as ligands for the $\alpha 1\beta 2$...

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Abstract: We report the inhibition of a human recombinant geranylgeranyl diphosphate synthase (GGPPSase) by 23 bisphosphonates and six azaprenyl diphosphates. The IC₅₀ values range from 140 nM to 690 μM. None of the nitrogen-containing bisphosphonates that inhibit ...

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Evangelia Kotsikorou and Eric Oldfield
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A Quantitative Structure–Activity Relationship and Pharmacophore Modeling Investigation of Aryl-X and Heterocyclic Bisphosphonates as Bone Resorption Agents

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Received February 3, 2003

We have used quantitative structure–activity relationship (QSAR) techniques, together with pharmacophore modeling, to investigate the relationships between the structures of a wide variety of geminal bisphosphonates and their activity in inhibiting osteoclastic bone resorption. For aryl-X (X = alkyl, oxyalkyl, and sulfanylalkyl) derivatives of pamidronate and one alendronate, a molecular field analysis (MFA) yielded an R^2 value of 0.900 and an F -test of 54 for a training set of 29 compounds. Using reduced training sets, the activities of 20 such compounds were predicted with an average error of 2.1 over a 4000 $\times$ range in activity. Such good results were only obtained when using the X-ray crystallographic structure of farnesyl pyrophosphate (FPP) bound to the target enzyme, farnesyl pyrophosphate synthase (FPP synthase), to guide the initial molecular alignment. For a series of heterocyclic bisphosphonates, use of the MFA method yielded an R^2 of 0.873 and an F -test of 36 for a training set of 26 compounds. Using a reduced training set, the activities of 20 compounds were predicted with an average error of 2.5 over a 2000 $\times$ range in activity. With the heterocyclic compounds, test calculations indicated the importance of correct choice of protonation of the heterocyclic rings. For example, thiazoles, pyrazoles, and triazoles have low (~ 2 – 3) pK_a values and the derived bisphosphonates are inactive in bone resorption since they cannot readily be side chain protonated and are thus poor carbocation reactive intermediate analogues. On the other hand, aminothiazoles, imidazoles, pyridyl, and aminopyridyl species typically have pK_a values in the range ~ 5 – 9 and, in the absence of unfavorable steric interactions, the corresponding bisphosphonates are generally good inhibitors. However, aminoimidazole bisphosphonates are generally less active, since their pK_a s (~ 11) are so high, due to guanidinium-like resonance, that they cannot readily be deprotonated, which we propose results in poor cellular uptake. The results of pharmacophore modeling using the Catalyst program revealed the importance of two negative ionizable and one positive charge feature for both aryl-X and heterocyclic pharmacophores, together with the presence of a distal hydrophobic feature in the aryl bisphosphonate and a more proximal aromatic feature in the heterocyclic bisphosphonate pharmacophores. When taken together, these results show that it is now possible to predict the activity, within a factor of about 2.3, of a wide range of aryl-X and heterocyclic bisphosphonates. The results emphasize the importance of utilizing crystallographic structural information to guide the initial alignment of extended bisphosphonates, and in the case of heterocyclic bisphosphonates, the importance of side chain protonation state. These simple ideas may facilitate the design of other, novel bisphosphonates, of use in bone resorption therapy, and as antiparasitic and immunotherapeutic agents.

Introduction

There is currently considerable interest in the development of geminal bisphosphonates for the treatment of tumor-induced hypercalcemia, Paget's disease of bone, osteolytic metastases, and postmenopausal osteoporosis,¹ with bisphosphonates making an $\sim \$4$ – 5 billion contribution to the global pharmaceutical market.² In addition to treating these bone related disorders, bisphosphonates have also been found to have considerable antiparasitic activity³ and to be immunomodulators.^{4–6} For example, bisphosphonates inhibit the growth of many trypanosomatid and apicomplexan parasites,³ and parasitological cures of cutaneous leishmaniasis (caused by the protozoan *Leishmania mexicana*) and visceral leishmaniasis (caused by *Leishmania donovani*) have

been obtained in mice using the bisphosphonates pamidronate (Aredia) and risedronate (Actonel), respectively.^{7,8} The bisphosphonates pamidronate, alendronate, ibandronate, risedronate, and zoledronate have also been shown to be potent stimulators of $\gamma\delta$ T cells,^{4–6,9} and it has been proposed that this T cell activation may represent a new approach to the development of antibiotic compounds⁹ and potentially the development of novel anticancer drugs.^{6,10,11} There is, therefore, considerable interest in understanding the mode of action of these compounds.

In early work, it was thought that bisphosphonates acted by directly modulating bone mineral dissolution.¹² However, more recently, it has been shown that bisphosphonates act in the mevalonate pathway,¹³ and very recently it has been demonstrated that the most potent, nitrogen-containing bisphosphonates (such as alendronate, risedronate, and zoledronate) act by in-

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hibiting the mevalonate/isoprene pathway enzyme farnesyl pyrophosphate synthase.^{14–18} In so doing, they prevent the post-translation modification (prenylation) of proteins such as rho, in osteoclasts, which leads to decreases in osteoclastic bone resorption.¹⁹ Of particular importance here has been the observation that there is an excellent correlation between the activity of a given bisphosphonate in inhibiting bone resorption and its activity in inhibiting the farnesyl pyrophosphate synthase enzyme.¹⁸ On the basis of the likely mode of action of farnesyl pyrophosphate synthase, it appears that the most potent, nitrogen-containing bisphosphonates act as aza-analogues of one or more putative isoprene pyrophosphate carbocation reactive intermediates,²⁰ a view supported by several recent molecular modeling studies.^{21,22}

In our group, we have been interested in using quantitative techniques to investigate the relationships between the three-dimensional structures of bisphosphonates (and related systems) and their activities in inhibiting farnesyl pyrophosphate synthases,²³ in inhibiting the growth of parasitic protozoa,²⁴ in $\gamma\delta$ T cell stimulation,²⁵ and in bone resorption.²⁶ In this paper, we focus on predicting the activities of a wide variety of novel aryl-X (X = alkyl, oxyalkyl, and sulfanylalkyl) substituted pamidronates, one alendronate, and a series of five-membered heterocyclic aromatic ring-containing bisphosphonates, whose activities in inhibiting bone resorption have recently been reported by the Novartis group.¹

Results and Discussion

In recent work, Widler et al. reported the activities of a large number of aryl-X and heterocyclic bisphosphonates.¹ However, there have been no published, quantitative analyses of these results. In earlier work, we used QSAR techniques to investigate the activities of variety of bisphosphonate bone resorption drugs developed, primarily, by the Procter and Gamble Company, and we found that it was possible to predict their activities within about a factor of 3.²⁶ Here, we report an analysis of the bone resorption activity of a larger range of compounds, consisting of arylalkyl, aryloxyalkyl, arylsulfanylalkyl, and heterocyclic (imidazole, thiazole, triazole, and pyrazole) bisphosphonates, which display a broad range of structural diversity and activity.

Initially, we carried out a quantitative structure–activity relationship analysis of 86 compounds reported by Widler et al.¹ to have specific (i.e., not greater than) activities, using the molecular field analysis (MFA) method.²⁷ For the alignment of the side chains of these compounds, we used the crystal structure of geranyl pyrophosphate (GPP) bound to farnesyl pyrophosphate synthase,²⁸ and for the positions of the nitrogens we used the crystal structure of risedronate, aligned to geranyl pyrophosphate. After some adjustments to achieve the best alignment for all compounds, we obtained an R^2 of 0.558, a cross-validation R^2_{cv} of 0.491 and an F -test of 25.6. These results indicated a poor correlation between the experimental activity and that predicted using the MFA method. Consequently, we decided to focus our study on the two major groups of compounds investigated by Widler et al.: the aryl-X

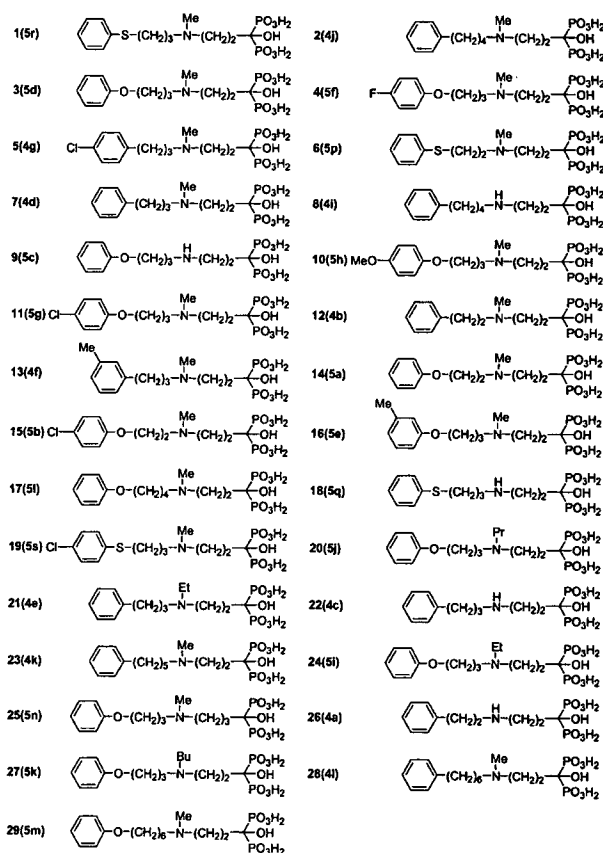
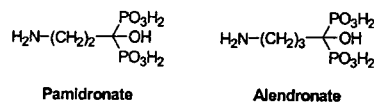


Figure 1. Structures of the 29 aryl-X bisphosphonates investigated. The designations in parentheses are those used by Widler et al. (ref 1).

derivatives of pamidronate (and one derivative of alendronate) and the heterocyclic bisphosphonates, to try to isolate possible deficiencies in our initial approach.

Aryl-X Bisphosphonates. We show in Figure 1 the structures of the 29 aryl bisphosphonates whose activities in inhibiting bone resorption were reported by Widler et al.¹ The structures shown in Figure 1 are listed (left to right and top to bottom) in order of decreasing activity. Only compounds having discrete activities are presented. To facilitate comparison with the published results, the code designations used by Widler et al. are shown in parentheses in Figure 1. All of these compounds are analogues of pamidronate or alendronate.



The experimentally determined activities of compounds 1–29 are shown in Table 1 and cover a very wide range in activity (ED_{50} values from 0.33 to 7500 $\mu\text{g/kg}$). The ED_{50} values are taken directly from Widler et al. and are expressed in $\mu\text{g/kg}$, while the pIC_{50} values are those we have estimated assuming a rat density $\rho = 1$, using the relation $pIC_{50} = -\log(IC_{50}/M)$. This approximation is necessary for accurate QSAR investigations since the molecular weights of the compounds investigated differ considerably. The compounds of interest contain a broad range of nitrogen substitutions,

Table 1. Experimental (ED₅₀ and pIC₅₀) and MFA Predicted (pIC₅₀) Values for Bisphosphonates against Bone Resorption and Statistical Results of 3D-QSAR MFA Models

compound ^a	experimental activity		training set	predicted pIC ₅₀ ^b				
	ED ₅₀ (μg/kg)	pIC ₅₀		four-compound test sets				
1 (5r)	0.33	9.209	8.635	8.632	8.669	8.691	8.624	8.620
2 (4j)	0.4	8.979	8.494	8.491	8.512	8.550	8.487	8.473
3 (5d)	0.5	8.885	8.716	8.714	8.754	8.773	8.708	8.700
4 (5f)	0.6	8.835	8.306	8.298	8.333	8.354	8.292	8.309
5 (4 g)	0.7	8.778	8.489	8.482	8.525	8.539	8.475	8.480
6 (5p)	0.7	8.741	8.566	8.559	8.601	8.618	8.553	8.541
7 (4d)	1	8.586	8.637	8.633	8.671	8.692	8.627	8.616
8 (4i)	1.0	8.565	8.368	8.360	8.393	8.417	8.355	8.356
9 (5c)	1.2	8.488	8.383	8.378	8.401	8.435	8.374	8.373
10 (5h)	1.2	8.546	8.626	8.622	8.667	8.680	8.616	8.621
11 (5 g)	1.3	8.506	8.472	8.466	8.503	8.524	8.461	8.467
12 (4b)	1.4	8.407	8.395	8.387	8.417	8.446	8.382	8.370
13 (4f)	1.5	8.405	8.651	8.649	8.686	8.708	8.643	8.638
14 (5a)	1.5	8.402	8.450	8.443	8.470	8.502	8.438	8.416
15 (5b)	1.7	8.385	8.303	8.293	8.326	8.350	8.287	8.281
16 (5e)	1.7	8.369	8.731	8.729	8.771	8.789	8.723	8.715
17 (5l)	4	7.997	8.432	8.428	8.444	8.487	8.425	8.408
18 (5q)	7	7.741	8.439	8.434	8.465	8.491	8.429	8.434
19 (5s)	7.8	7.745	8.425	8.419	8.453	8.476	8.413	8.420
20 (5j)	10	7.624	7.416	7.365	7.511	7.412	7.348	7.423
21 (4e)	15	7.405	7.293	7.241	7.376	7.287	7.225	7.299
22 (4c)	20	7.253	6.580	6.581	6.574	6.580	6.581	6.586
23 (4k)	20	7.306	7.418	7.367	7.513	7.413	7.350	7.427
24 (5i)	20	7.142	7.416	7.365	7.511	7.411	7.348	7.423
25 (5n)	100	6.609	6.544	6.499	6.570	6.536	6.490	6.637
26 (4a)	300	6.053	6.721	6.721	6.728	6.721	6.720	6.716
27 (5k)	500	5.930	5.943	5.937	5.956	5.948	5.936	6.442
28 (4l)	1500	5.436	5.188	5.166	4.946	5.209	5.188	5.173
29 (5m)	7500	4.754	5.047	5.021	4.808	5.063	5.043	5.044
<i>R</i> ² _c			0.900	0.902	0.901	0.919	0.906	0.886
<i>F</i> _{test} ^d			54.275	46.272	45.278	56.734	48.277	39.946
<i>R</i> ² _{cv} ^e			0.799	0.758	0.713	0.809	0.802	0.766
<i>R</i> ² _{bs} ^f			0.900	0.902	0.901	0.919	0.906	0.886
<i>N</i> _{opt} ^g			5	5	5	5	5	5
<i>N</i> ^h			29	25	25	25	25	25

^a See Figure 1 for compound structures. Structures in parentheses are from ref 1. ^b Bold values represent predicted activities of compounds that were not included in the training set. ^c Correlation coefficient. ^d Ratio of *R*² explained to unexplained = *R*²/(1 - *R*²). ^e Cross-validated correlation coefficient after leave-one-out procedure. ^f Average squared correlation coefficient calculated during the validation procedure. ^g Optimal number of principal components. ^h Number of compounds.

as shown in Figure 1. The most active compounds (1–6) are all analogues of pamidronate containing one N-Me group, but the aryl-X side chains differ in overall length and chemical composition. It is also clear from even a cursory inspection of Figure 1 that those compounds having the longest side chains (28, 29) are the ones that are the least active.

We used the molecular field analysis (MFA) approach²⁷ to develop QSAR models for the compounds shown in Figure 1 using basically the techniques described in detail previously.^{23,24,26} To guide the molecular alignment, we utilized the X-ray crystallographic structure of farnesyl pyrophosphate bound to an avian farnesyl pyrophosphate synthase.²⁸ This facilitated positioning of the extended side chains and the alignment so obtained is shown in Figure 2. We also utilized the crystallographic structure of risedronate,²⁹ docked onto geranyl pyrophosphate, to localize the positions of the nitrogen atoms in the aryl bisphosphonates. A second and less successful approach, in which the alignment was guided by the crystallographic structure of geranyl pyrophosphate bound to FPP synthase, was also investigated, and is described in more detail below. In all cases, the nitrogens in the bisphosphonate side chains were protonated, while the bisphosphonate phos-

phonate groups each carried a single negative charge. This is the pattern of protonation which has been found in several single-crystal X-ray diffraction studies of bisphosphonates crystallized in the pH range ~4–10,^{23,26,29} and is also what we used previously.^{23,24,26} Bisphosphonate structures were generated and geometry optimized by using a three-step protocol consisting of steepest descents,³⁰ conjugate gradients,³¹ and Newton–Raphson minimization,³² with no constraints on the internal geometries of the molecules, using the minimizer function of the OFF methods module in Cerius² 4.6.³³ Each molecule was aligned to the shape reference compound, farnesyl pyrophosphate, using the shape reference alignment function of the QSAR module of Cerius² 4.6. A second set of alignments was made by using a set of fully extended aryl side chains.

MFA 3-D QSAR methodology was then applied to the bone resorption inhibition data, to help identify the structural features required for optimum bone resorption activity. We applied a regression analysis to the data, leading to eq 1:

$$\text{pIC}_{50} = 5.137 - 0.021(\text{H}^+/326) + 0.052(\text{CH}_3/335) - 0.073(\text{H}^+/514) - 0.041(\text{H}^+/564) \quad (1)$$

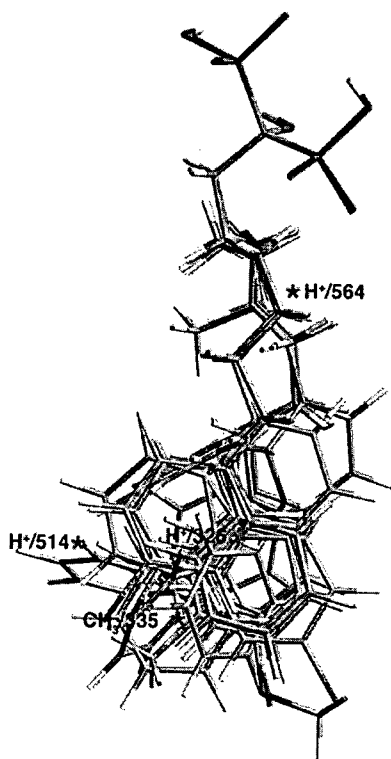


Figure 2. Maximum common subgroup alignment of the 29 aryl-X bisphosphonates that had quantitatively measurable activities, together with the locations of the four descriptors given in eq 1.

where the H^+ descriptors define the corresponding interaction energies between a proton probe and the molecule at the specified (numbered) grid points; see Figure 2 for their locations. The CH_3 descriptor defines the corresponding interaction energy between a hydrophobic probe and the molecule at the specified (numbered) grid point, as described by a Lennard-Jones 6–12 potential. The descriptor positions and coefficients in the 3D-QSAR/MFA equation were chosen by using a genetic function approximation (GFA) algorithm.³⁴ The genetic function approximation involves starting with an initial population of random equations, which are then allowed to evolve by application of crossover operations such that terms from models with the best fit will most likely occur in the “offspring” models. Best-fit models are retained, while poor-fit models are replaced with subsequent generations. The optimal number of components in the final GFA model was determined by cross validated R^2 values (R^2_{cv}), as obtained from the leave-one-out cross validation technique,³⁵ standard error prediction values, and also by F -test values.³⁶ An MFA model containing the five components shown above afforded a correlation coefficient $R^2 = 0.900$ and a cross-validation R^2_{cv} of 0.80. These statistical parameters are given in Table 1, together with the experimental and predicted pIC_{50} values. Training set results for the 29 compounds are shown graphically in Figure 3A, and a confidence limit of 95% was established by using the data randomization procedure in Cerius² 4.6.

Next, we investigated to what extent it might be possible to predict the activities of such aryl-X bispho-

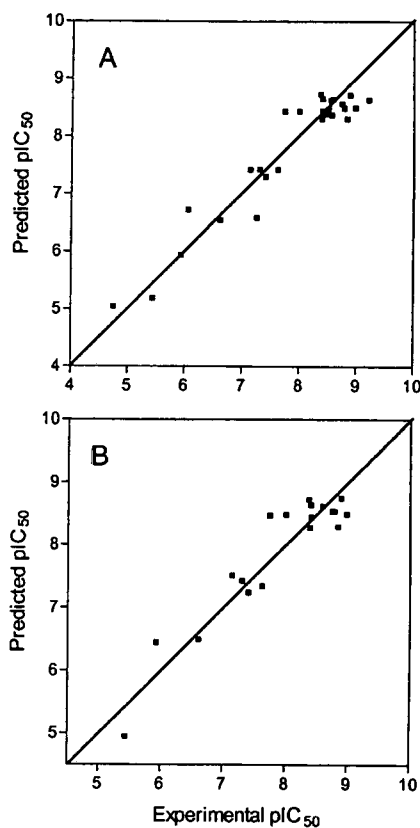


Figure 3. Graphical comparisons between experimental and predicted pIC_{50} values for bone resorption inhibition. A, 29-compound training set. B, superposition of five, four-compound test sets. For the test set, $R^2 = 0.854$.

sphonates by using the MFA approach. We followed basically the same approach as that reported previously,^{24,26} in which we deleted four compounds from the overall training set of 29 compounds, then we used the reduced training set of 25 compounds to compute new MFA equations, which were then used to predict the activities of the four test set compounds. This procedure was repeated four more times using different training set compounds, resulting in five sets of four compound predictions ($N = 20$). The five sets of results are shown in Table 1, with the predicted activities shown in bold. The five new QSAR equations utilized were very similar, and were as follows:

$$pIC_{50} = 5.123 - 0.022(H^+/326) + 0.052(CH_3/335) - 0.073(H^+/514) - 0.040(H^+/564) \quad (2)$$

$$pIC_{50} = 5.074 - 0.021(H^+/326) + 0.053(CH_3/335) - 0.079(H^+/514) - 0.043(H^+/564) \quad (3)$$

$$pIC_{50} = 5.109 - 0.023(H^+/326) + 0.054(CH_3/335) - 0.074(H^+/514) - 0.041(H^+/564) \quad (4)$$

$$pIC_{50} = 5.129 - 0.023(H^+/326) + 0.052(CH_3/335) - 0.073(H^+/514) - 0.039(H^+/564) \quad (5)$$

$$pIC_{50} = 5.224 - 0.021(H^+/326) + 0.052(CH_3/335) - 0.073(H^+/514) - 0.027(H^+/564) \quad (6)$$

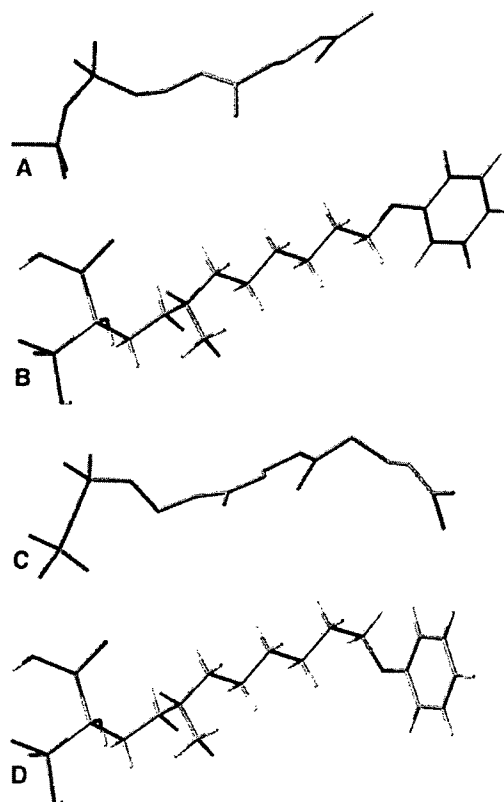
Table 2. Comparison of Statistics of Bent Aryl-X versus Linear Bisphosphonates

statistics	bent (FPP-like)	linear
R^2 ^a	0.900	0.853
F_{test} ^b	54.275	34.904
R^2_{cv} ^c	0.799	0.757
R^2_{bs} ^d	0.900	0.853
N^e	5	5
N^f	29	29

^a Correlation coefficient. ^b Ratio of R^2 explained to unexplained = $R^2/(1 - R^2)$. ^c Cross-validated correlation coefficient after leave-one-out procedure. ^d Average squared correlation coefficient calculated during the validation procedure. ^e Optimal number of principal components. ^f Number of compounds.

The predicted activities for the 20 test set compounds are shown graphically in Figure 3B, plotted as a function of the experimental pIC_{50} values. For these 20 compounds, the average absolute error in pIC_{50} prediction is 0.319, which corresponds to about a $2.1\times$ error, in this case covering a $4000\times$ range in experimental activity. This is similar to the $2.8\times$ error found previously (over a similar range in activity) for a series of aminomethylene and 1-hydroxybisphosphonates which lack the long aryl-X side chains.²⁶

We also investigated a second alignment of molecules 1–29, in which the alignments of the side chains of the compounds were guided by the X-ray crystal structure of geranyl pyrophosphate bound to FPP synthase.²⁸ This alignment performed well for the shorter chain compounds, but not for the longest compounds (28 and 29). Their side chains adopted an essentially all-trans conformation, since this is the expected lowest energy conformation. With this alignment there was a noticeable degradation in each of the statistical parameters computed, as shown in Table 2. Specifically, the R^2 value decreased from 0.900 to 0.853, the F -test value decreased from 54.3 to 34.9, and the R^2_{cv} decreased from 0.799 to 0.757. These results strongly suggest that the longer arylalkyl and related side chains bind in the "bent" or "curved" manner exhibited by farnesyl pyrophosphate when bound in the FPP synthase active site.²⁸ We show in Figure 4 an illustration of the conformations of geranyl and farnesyl pyrophosphate in the active site of farnesyl pyrophosphate synthase (Figures 4A, 4C), together with both extended (Figure 4B) and bent (Figure 4D) structures of 29. The activities of the least active compounds are quite well described using this bent structure (4D), as shown in Table 1, and as noted above, all the training set statistics are improved with this alignment. These results imply that fully extended long aryl-X side chains will encounter strong repulsive interactions with the protein. The longer chain inhibitors thus conform to the "bent" FPP structure in the FPP synthase binding site. However, even in this bent conformation, 28 and 29 are still very poor inhibitors of bone resorption, which strongly suggests that there is still a repulsive or steric interaction with the protein, resulting in relatively weak binding, rather than an enhanced hydrophobic stabilization, due to the increased chain length. This can, however, be readily understood based on the results of several mutagenesis studies. For example, it has been shown that there are two phenyl groups in FPP synthase which when converted to the smaller Ala and

**Figure 4.** Conformations of A, GPP (PDB File 1UBW); B, 29, extended alignment; C, FPP (PDB File 1UBX); and D, 29, alignment based on C.

Ser groups, permit chain elongation from C_{15} to C_{20} .²⁸ These two Phe groups appear to provide a steric block to chain elongation, and it seems likely that they may also have a repulsive interaction with the long arylalkyl side chains of 28 and 29, resulting in the high (1500, 7500 $\mu\text{g/kg}$) ED_{50} values, Table 1. A related effect also appears to be operative in geranylgeranyl pyrophosphate synthase (GGPP synthase), where engineering an MS $\rightarrow$ YF mutation in the same region results in conversion of GGPP to FPP synthase activity.³⁷

As can be seen in the data of Widler et al.,¹ the shorter chain aryl-X compounds having 5–6 heavy (i.e., carbon, nitrogen, oxygen, or sulfur) atoms in the side chain linker (and containing an N-Me substituent) typically have somewhat less activity than do slightly longer chain compounds, and maximal activity (0.33–0.5 $\mu\text{g/kg}$) is seen with 1–3, which all have seven heavy atoms (plus a phenyl) in their side chain. This corresponds to a chain length longer than that which would be found in the GPP substrate or reactive intermediate. That is, optimum activity is found with species having chain lengths close to that of FPP, but not much longer, where repulsive interactions occur. Shorter chains are less active, presumably due to decreased hydrophobic stabilization in the active site.

Heterocyclic Bisphosphonates. Next, we describe our QSAR results for a series of heterocyclic bisphosphonates. The structures of the 26 compounds considered (30–55) are shown in Figure 5, together with the designations (in parentheses) used by Widler et al.¹ The activities of the compounds are, once again, taken

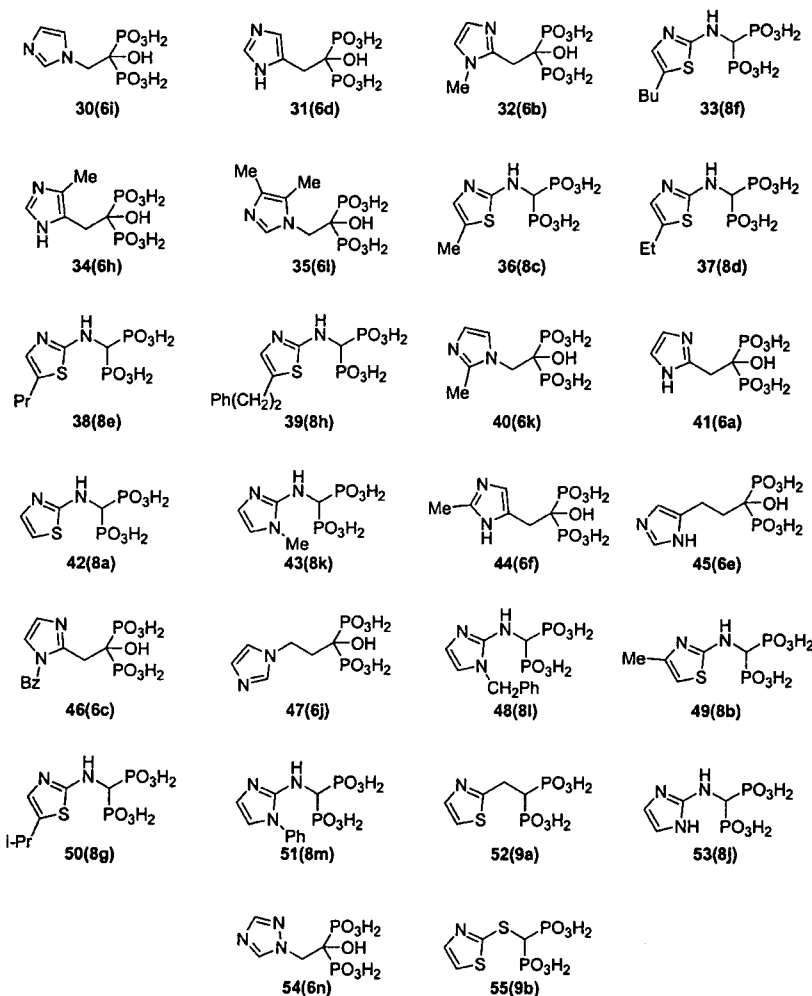


Figure 5. Structures of the 26 heterocyclic bisphosphonates investigated. The designations in parentheses are those used by Widler et al. (ref 1).

directly from the paper of Widler et al. and are reproduced as ED_{50} ($\mu\text{g/kg}$) values and as transformed pIC_{50} values, in Table 3. The compounds investigated have quite broad structural diversity in that activities for both aminomethylene bisphosphonate and 1-hydroxy-1,1-bisphosphonate backbones, together with imidazole, thiazole, triazole, and pyrazole side chains have been reported, with activities covering a $10\,000\times$ range (or $>100\,000\times$ if the "inactive" benzannulated species are considered).

We began our investigation of these compounds using the structures of geranyl pyrophosphate and risedronate to guide the alignment. The phosphonate moieties each contained one protonated and two nonprotonated oxygens (the same as with the aryl-X bisphosphonates), and the ring nitrogens were all protonated. This basic motif has been used successfully by us in the past²⁶ to predict the activities of a small set of, primarily, pyridyl bisphosphonates, but with this more diverse set of compounds, the initial training set results were poor. While it might be that our alignment procedure was inadequate, such problems had not been observed previously, so we hypothesized that this poor agreement between experiment and prediction might in fact be due to an inappropriate choice of protonation states

for these new heterocycles. For example, it is known that thiazoles³⁸ and triazoles³⁹ are very weak bases ($pK_a \sim 3$), unlike imidazoles ($pK_a \sim 7.2$), so it seemed likely that the low activity of compounds such as **52**, **54**, and **55** might be related to their weak basicity. However, some thiazoles, for example **33**, **36**, **37**, **38**, and **39**, have quite pronounced activity. The difference between the active thiazoles and the most inactive species, at least to first order, would thus appear to be due to the presence of either α -NH protonation (or amidinium-like structures) in the most active thiazoles, which would be absent in the least active compounds, although other factors, such as the presence of an unbranched 4'-substituent, also appear to contribute to high activity. In any case, to test the protonation state hypothesis, we next computed QSAR equations using structures in which imidazolium groups were ring-protonated, aminothiazoles were protonated on the α -N, while the triazole and non-amino-substituted thiazoles were not protonated.

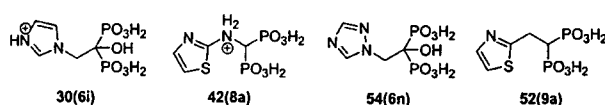


Table 3. Experimental (ED₅₀ and pIC₅₀) and MFA Predicted (pIC₅₀) Values for Bisphosphonates against Bone Resorption and Statistical Results of 3D-QSAR MFA Models

compound ^a	experimental activity		predicted pIC ₅₀ ^b					
	ED ₅₀ (μg/kg)	pIC ₅₀	training set	four-compound test sets				
30 (6i)	0.07	9.617	9.162	9.135	9.238	9.060	9.129	9.277
31 (6d)	0.3	8.958	9.281	9.253	9.359	9.177	9.242	9.399
32 (6b)	0.6	8.705	8.134	8.080	8.211	8.026	8.156	8.226
33 (8f)	0.9	8.565	8.378	8.452	8.365	8.384	8.355	8.424
34 (6h)	1.5	8.287	7.958	7.977	7.915	8.105	7.835	7.984
35 (6l)	1.5	8.301	7.746	7.799	7.735	7.744	7.758	7.780
36 (8c)	1.5	8.290	8.370	8.446	8.355	8.376	8.347	8.415
37 (8d)	1.5	8.317	8.376	8.450	8.362	8.382	8.353	8.421
38 (8e)	2	8.211	8.377	8.451	8.363	8.383	8.354	8.422
39 (8h)	2.7	8.146	8.378	8.452	8.364	8.384	8.355	8.424
40 (6k)	3	7.979	7.982	7.995	7.944	8.123	7.860	8.012
41 (6a)	5	7.750	8.266	8.208	8.349	8.151	8.283	8.365
42 (8a)	5	7.739	7.126	7.054	7.197	7.019	7.200	7.193
43 (8k)	5	7.734	7.210	7.342	7.245	7.077	7.234	7.235
44 (6f)	15	7.294	7.828	7.885	7.817	7.827	7.835	7.863
45 (6e)	20	7.176	6.940	6.895	6.925	7.046	6.881	6.959
46 (6c)	25	7.187	7.150	7.299	7.171	7.033	7.172	7.165
47 (6j)	45	6.803	6.844	6.797	6.830	6.949	6.791	6.862
48 (8l)	75	6.732	7.198	7.329	7.234	7.064	7.223	7.223
49 (8b)	100	6.460	7.112	7.041	7.182	7.006	7.187	7.178
50 (8 g)	200	6.199	5.931	5.925	5.931	5.907	6.043	5.930
51 (8m)	200	6.222	5.938	6.099	5.865	6.039	5.880	5.879
52 (9a)	200	6.135	6.320	6.242	6.381	6.222	6.434	6.363
53 (8j)	~500	5.711	6.505	6.558	6.462	6.610	6.445	6.488
54 (6n)	600	5.658	5.583	5.506	5.566	5.683	5.596	5.571
55 (9b)	700	5.619	5.705	5.622	5.697	5.797	5.715	5.701
<i>R</i> ² _c			0.873	0.902	0.879	0.899	0.865	0.876
<i>F</i> _{test} ^d			35.991	39.158	30.814	37.803	27.267	30.034
<i>R</i> ² _{cv} ^e			0.809	0.846	0.802	0.827	0.775	0.799
<i>R</i> ² _{bs} ^f			0.873	0.902	0.879	0.899	0.865	0.876
<i>N</i> ^g			5	5	5	5	5	5
<i>N</i> ^h			26	26	26	26	26	26

^a See Figure 5 for compound structures. Structures in parentheses are from ref 1. ^b Bold values represent predicted activities of compounds that were not included in the training set. ^c Correlation coefficient. ^d Ratio of *R*² explained to unexplained = *R*²/(1 - *R*²). ^e Cross-validated correlation coefficient after leave-one-out procedure. ^f Average squared correlation coefficient calculated during the validation procedure. ^g Optimal number of principal components. ^h Number of compounds.

We then computed QSAR equations using the MFA technique and the alignment shown in Figure 6. We obtained the following MFA QSAR equation:

$$\text{pIC}_{50} = 7.781 - 0.052(\text{CH}_3/156) + 0.050(\text{H}^+/227) + 0.021(\text{H}^+/294) - 0.032(\text{CH}_3/365) \quad (7)$$

The spatial locations of the grid points are shown in Figure 6 and the detailed training set prediction results are shown in Table 3. The statistical results so obtained are compared with those obtained by using uniformly protonated rings in Table 4, where it can be seen that there is a marked improvement using the revised protonation scheme. For example, the *R*² value increases from 0.710 to 0.873, the cross-validated *R*²_{cv} increases from 0.629 to 0.809, and the *F*-test result increases from 18 to 36.

The new training set predictions are shown plotted as a function of the experimental pIC₅₀ values in Figure 7A. These results are somewhat worse than those obtained with the aryl bisphosphonates, but they nevertheless do suggest predictive utility. To test this idea further, we again carried out five training (*N* = 22)/test (*N* = 4) set calculations for a total of 20 activity predictions, shown in bold in Table 3 and graphically

in Figure 7B. The MFA equations so derived were the following:

$$\text{pIC}_{50} = 7.737 - 0.049(\text{CH}_3/156) + 0.052(\text{H}^+/227) + 0.020(\text{H}^+/294) - 0.025(\text{CH}_3/365) \quad (8)$$

$$\text{pIC}_{50} = 7.815 - 0.055(\text{CH}_3/156) + 0.050(\text{H}^+/227) + 0.023(\text{H}^+/294) - 0.034(\text{CH}_3/365) \quad (9)$$

$$\text{pIC}_{50} = 7.776 - 0.048(\text{CH}_3/156) + 0.051(\text{H}^+/227) + 0.017(\text{H}^+/294) - 0.032(\text{CH}_3/365) \quad (10)$$

$$\text{pIC}_{50} = 7.750 - 0.050(\text{CH}_3/156) + 0.047(\text{H}^+/227) + 0.022(\text{H}^+/294) - 0.032(\text{CH}_3/365) \quad (11)$$

$$\text{pIC}_{50} = 7.844 - 0.055(\text{CH}_3/156) + 0.051(\text{H}^+/227) + 0.022(\text{H}^+/294) - 0.034(\text{CH}_3/365) \quad (12)$$

and show considerable similarity. For the *N* = 20 test set prediction, we found using these MFA equations an average absolute error of 0.39 in the pIC₅₀ predictions, which corresponds to about a factor of 2.5× average error over the 2000× range in predicted activity. This, once again, indicates that the MFA technique appears to have utility in predicting ED₅₀ values even in as

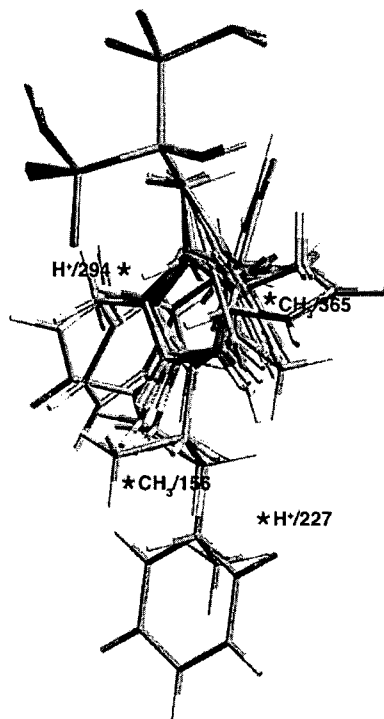


Figure 6. Maximum common subgroup alignment of the 26 heterocyclic bisphosphonates that had quantitatively measurable activities, together with the locations of the four descriptors given in eq 7.

Table 4. Comparison of Ring Protonated versus Selectively Protonated Heterocyclic Bisphosphonate MFA Statistics

statistics	ring protonated	selectively protonated
R^2 ^a	0.710	0.873
F_{test} ^b	17.991	35.991
R^2_{cv} ^c	0.629	0.809
R^2_{bs} ^d	0.710	0.873
N^e	5	5
N^f	26	26

^a Correlation coefficient. ^b Ratio of R^2 explained to unexplained = $R^2/(1 - R^2)$. ^c Cross-validated correlation coefficient after leave-one-out procedure. ^d Average squared correlation coefficient calculated during the validation procedure. ^e Optimal number of principal components. ^f Number of compounds.

complex a system as bone resorption, although it is equally clear that additional information on protein crystal structures will be desirable to clarify more precisely how these inhibitors function. For example, while it seems very likely that the long aryl-X compounds are GPP mimics, the small heterocycles might also inhibit at the isopentenyl diphosphate (IPP) site (or both GPP and IPP sites), as suggested recently for risedronate.^{21,22} Nevertheless, when both the aryl-X and heterocyclic predictions are combined, Figure 7C, it is clear that quite accurate activity predictions (2.3× average error) can now be made.

pK_a -Activity Correlations. The results described above suggest an important relationship between the pK_a of the heterocycle ring and activity. While this is of course not wholly unexpected, the relationship appears not to have been previously reported with bisphosphonate drugs and in fact only becomes apparent when data on a wide range of compounds, such as the thiazoles, pyrazoles, etc., is available, as exemplified in the recent

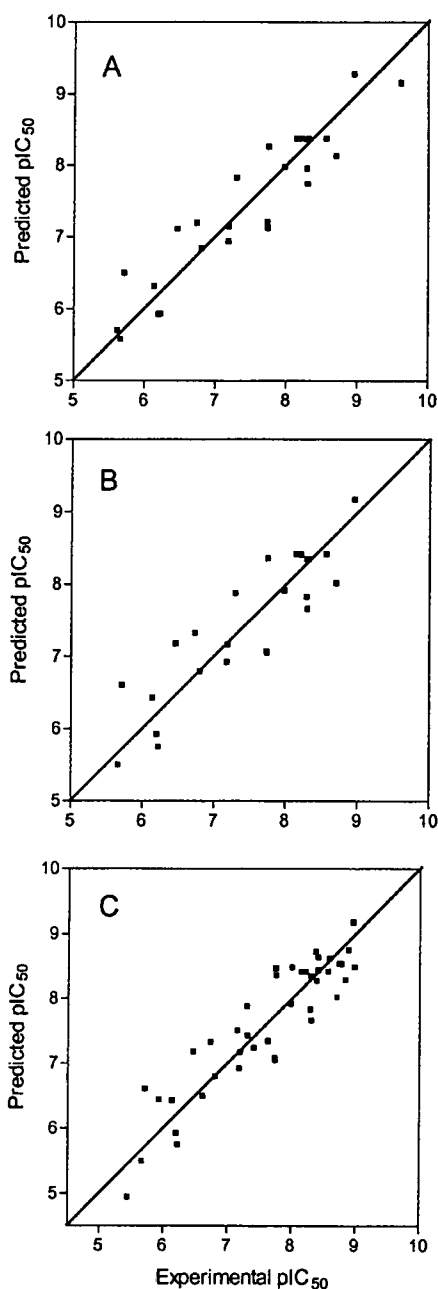


Figure 7. Graphical comparisons between experimental and predicted pIC_{50} values for bone resorption inhibition. A, 26-compound heterocyclic training set. B, Superposition of five, four-compound heterocyclic test set predictions. C, Superposition of the five, four-compound test sets for both aryl-X and heterocyclic bisphosphonates ($N = 40$). For the heterocyclic test set B, $R^2 = 0.784$. For the combined test sets, $R^2 = 0.824$.

work of Widler et al. Here, then, we explore this topic in more detail and try to put on a somewhat more firm foundation some of the very interesting (and at first sight, puzzling) recent results. Moreover, we extend the investigation to bisphosphonates investigated by other groups to further test some of these ideas, which if correct, may have important implications for bisphosphonate drug design.

We show in Figure 8 the structures and pK_a values computed by using SciFinder Scholar/ACD software⁴⁰ of nine heterocycles which form the side chains of

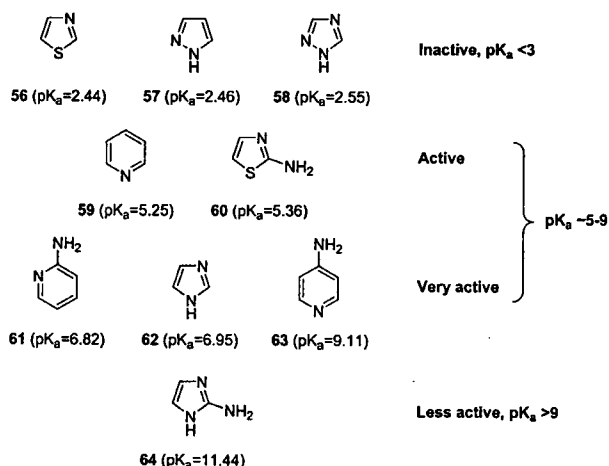
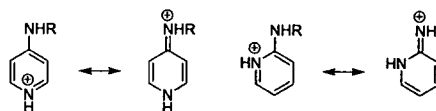


Figure 8. Structures of nine heterocycles with their computed pK_a values and an indication of the activities of the derived bisphosphonates.

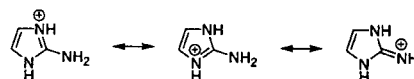
bisphosphonates having a wide range of activity in bone resorption, and in some cases as herbicides.⁴¹ The free base compounds are ordered by increasing basicity. Comparison of these computed heterocycle pK_a values with the corresponding bisphosphonate activity results reported by Widler et al.¹ immediately helps explain many of the rather puzzling activity results obtained. For example, Widler et al. note that the 2-aminothiazolyl derivative **42** (their **8a**) has a potency comparable to that of alendronate, or at least 10 times that of pamidronate, but unexpectedly, replacement of the nitrogen linker atom of the thiazole by a methylene or sulfur bridge led to species (**52**, **55**) which were much less potent. We propose this is related to the basicity of the heterocycle rings. Specifically, the pK_a of free thiazole (**56**) is 2.44,^{38,40} so S- and CH₂-linked bisphosphonates will be unprotonated, lacking the key positive charge feature required for carbocation-mimicry. On the other hand, the bisphosphonate (**42**) derived from 2-aminothiazole (**60**), whose parent base has a pK_a of 5.36, would likely be protonated on the α -N, with some resonance stabilization, and would consequently be active. Similarly, low pK_a values are found with the free pyrazole **57** (basic $pK_a = 2.46$) and the 1,2,4-triazole **58** (basic $pK_a = 2.55$), and the corresponding bisphosphonates are inactive.¹ Specifically, the ED₅₀ for a pyrazole bisphosphonate (**6m** in ref 1) is $>300 \mu\text{g/kg}$ while that for the triazole bisphosphonate **54** is $600 \mu\text{g/kg}$, since in the absence of a linker nitrogen there is unlikely to be any positive charge feature. The low basicity of such triazoles is also confirmed in studies of phospholipase A₂ and in lysozyme in which 1,2,4-triazole alanine was substituted for histidine.^{42,43} On the other hand, **59–62** all have pK_a values in the range $\sim 5-7$, and the derived bisphosphonates are generally all very active and include species such as risedronate and zoledronate (**30**). These results strongly support the idea that a pK_a for the heterocyclic ring in the range $\sim 5-7$ is likely to support high activity, due to carbocation formation, although certainly it is necessary to have the protonatable group in a suitable location. For example, the para pyridyl analogue of risedronate has generally low activity.⁴⁴ However, the presence of a para nitrogen

in a phenyl ring, as opposed to an ortho or meta nitrogen, does not in and of itself guarantee low activity. For example, **63** (Figure 8) has a para nitrogen but is very basic, with a pK_a of 9.11, and the corresponding aminomethylene bisphosphonate was found by Nissan⁴¹ to be one of the most potent herbicidal bisphosphonates investigated, many of which are known low nM inhibitors of FPP synthase (such as analogues of **61**). This activity is likely to be due to the large charge delocalization which can be expected in the quinonoid-like structure, shown on the left below, in much the same way that charge delocalization can occur in the amidinium-like species which can be formed for **61** with the



charge being highly delocalized over the entire ring in these quinonoid-like species, as shown above.

Paradoxically, having an exceptionally basic nitrogen may contribute to decreased activity. Specifically, Widler et al.¹ note that most of the aminomethylene bisphosphonates containing imidazolyl groups are "surprisingly less interesting" with **53** (the unsubstituted species) having an activity of $\sim 500 \mu\text{g/kg}$, to be compared with the $0.07 \mu\text{g/kg}$ of zoledronate (**30**). One possible explanation for this extremely low activity may lie in the fact that the predicted pK_a of the parent compound (**64**) is ~ 11.4 . In this case, the side chain will readily protonate and form a guanidinium-like species. While the charge



here will be delocalized, it may result in bisphosphonate species which are poorly transported into the cell, since they may be incapable of deprotonation. Addition of an N-Me group increases activity, as found in all of the N-Me pamidronate aryl-X species, and may contribute to hydrophobic stabilization, although the N-benzyl and N-phenyl species (**48**, **51**) are less active, due perhaps to steric repulsion in the active site.

While admittedly qualitative, we nevertheless believe that the major patterns of activity seen by Widler et al. for heterocyclic bisphosphonates can now be correlated with well-known pK_a values. The poor bases ($pK_a < 3$): thiazoles, pyrazoles, and triazoles, are all inactive. However, species with pK_a values in the range $\approx 5-9$ can be very active. This is well illustrated by the conversion **52** $\rightarrow$ **42**, where a $40\times$ increase in activity can be correlated with a pK_a change from 2.44 (**56**) to 5.36 (**60**), for the parent bases. However, species which cannot deprotonate (**43**, **48**, **51**, and **53**) are clearly in the less active category, although it is likely that if transported, they will be good inhibitors of FPP synthase, something which can be tested in future work. With benzannulated thiophenes and imidazoles, activity is very low (>3000 and $10\,000 \mu\text{g/kg}$, respectively). While activity might be expected with the benzthiazol-2-yl aminomethylene bisphosphonate, the C2-substitution most likely causes a steric repulsion with the

protein, as is found in the isosteric minodronate derivative.⁴⁵ In the benzimidazol-2-yl aminomethylene bisphosphonate (**80** in ref 1), this steric effect is compounded with the proposed guanidinium fixed-charge effect, resulting in an ED₅₀ of >10 000 µg/kg.

Pharmacophore Modeling. In addition to these molecular field analysis investigations, we used pharmacophore modeling to investigate structure–activity relationships for bisphosphonates in inhibiting bone resorption. We used the Catalyst approach,⁴⁶ in which up to 255 conformations of each molecule can be investigated, to develop a series of pharmacophore hypotheses. We used the structure/protonation files developed for the MFA analysis to generate a series of Catalyst pharmacophore hypotheses for both the aryl-X and heterocyclic bisphosphonates. The top scoring Catalyst hypotheses all contained two negative ionizable groups and a positive charge feature. For the aryl bisphosphonates, we added a custom feature, an aromatic ring attached to an alkyl chain via a sulfur or oxygen atom, while for the heterocyclic bisphosphonates, we added a custom ring feature, generating the pharmacophore hypotheses shown in Figures 9A and 9B, superimposed on **12**(A) and **30**(B). The two pharmacophore hypotheses are shown superimposed in Figure 9C. The two pharmacophores are clearly quite similar with respect to the location of the negative ionizable and positive charge features but, of course, the locations of the ring features (shown as green spheres) necessarily differ in the two set of compounds.

The training set activity predictions for both sets of molecules (**1–29**; **30–55**) are shown in Tables 5 and 6 and are somewhat worse than those obtained by using the MFA method, with *R*² values in the range 0.83–0.85, to be compared with 0.87–0.90 (Tables 1, 3). Likewise, the test set results (on the same two sets of 20 compounds as used in the molecular field analysis, Tables 1 and 3) shown in Tables 5 and 6 were less well correlated with experiment, with error factors of ~2.9–3.4 being found.

And finally, in an effort to make these basic QSAR approaches more synthetically useful, we have begun to explore the possible utility of a comparative molecular similarity indices analysis (CoMSIA)⁴⁷ of the results since it has been argued that CoMSIA contour maps may provide a useful graphical approach to drug design. We followed the MFA alignment protocol described above but used the Tripos Sybyl 6.8 program⁴⁸ for the CoMSIA analysis. The training set results were relatively predictive (average errors ~4.3–5.9) but more importantly yielded the CoMSIA contour maps shown in Figure 10 for the steric (A,D), electrostatic (B,E) and hydrophobic (C,F) interactions for the aryl-X (A,B,C) and heterocyclic (D,E,F) compounds. For the steric interaction, green represents a favorable interaction, yellow unfavorable. For the electrostatic interaction, blue is favorable for more positive charge density in the ligand while red is favorable for more negative charge density in the ligand. For the hydrophobic interaction, orange represents a favorable hydrophobic interaction; white denotes an area of unfavorable interaction.

For the aryl-X compounds, the hydrophobic interaction (C) dominates (59%) followed (A) by the steric contribution (31%), and the location of these features clearly correlate with the (favorable) presence of the

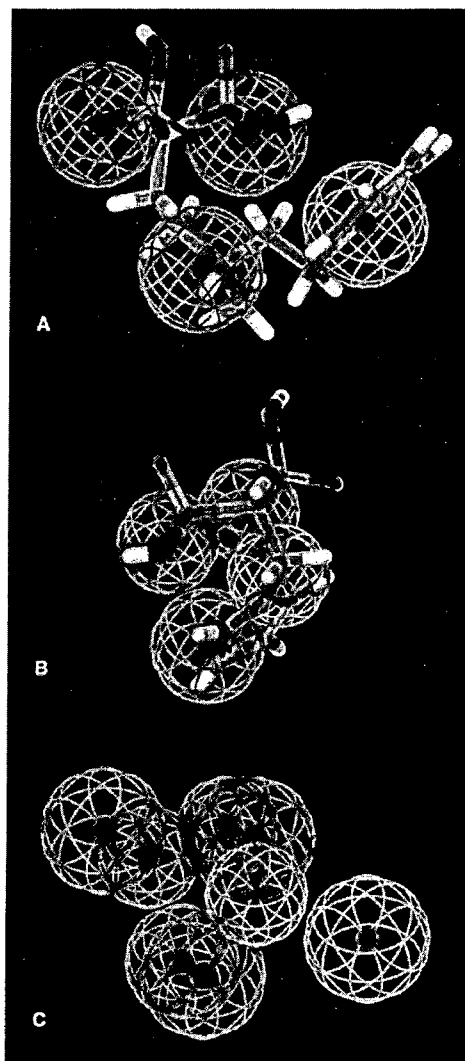


Figure 9. Catalyst pharmacophore hypotheses for bone resorption inhibition by aryl-X and heterocyclic bisphosphonates. The blue spheres represent negative ionizable features, the red spheres positive charge features, the green spheres custom hydrophobic features. In A, the green sphere represents an aromatic ring attached to a hydrophobic chain, in B, it represents an aromatic ring carbon. The pharmacophores are superimposed in A on compound **12**(4b), in B, on compound **30**(6i), zoledronate. C is the superposition of the two pharmacophores.

aromatic residues in the medium chain length compounds, Figure 10A,C. The red feature in Figure 10B indicates a favorable negative electrostatic feature in the region where the sulfur or oxygen atoms of the compounds are located, consistent with the observation that the thio analogue **1** is the most active species seen in this series.

For the heterocyclic bisphosphonates, the hydrophobic interaction (F) is still large (41%) but the electrostatic interaction has increased considerably, to 32%. The corresponding CoMSIA contour maps are shown in Figures 10E,F. Quite clearly, the hydrophobic (E) and steric (D) contour maps suggest that ring substitution might be beneficial. The yellow feature in Figure 10D represents a repulsive interaction found with some side chains (e.g., in **46**, **48**).

Table 5. Experimental (ED₅₀ and pIC₅₀) and Catalyst Predicted (pIC₅₀) Values for Bisphosphonates against Bone Resorption and Statistical Results

compound	experimental activity		predicted pIC ₅₀ ^a					
	ED ₅₀ (μg/kg)	pIC ₅₀	training set	four-compound test sets				
1 (5r)	0.33	9.209	8.489	8.763	8.497	8.648	8.502	8.686
2 (4j)	0.4	8.979	8.474	8.581	8.326	8.502	8.542	8.540
3 (5d)	0.5	8.885	8.192	8.584	8.121	8.542	8.291	8.407
4 (5f)	0.6	8.835	8.355	8.358	8.291	8.291	8.477	8.409
5 (4 g)	0.7	8.778	8.351	8.368	8.301	8.368	8.410	8.393
6 (5p)	0.7	8.741	8.183	8.307	8.355	8.264	8.264	8.188
7 (4d)	1	8.586	8.322	8.331	8.331	8.206	8.243	8.285
8 (4i)	1.0	8.565	7.963	8.286	8.060	8.310	8.363	8.310
9 (5c)	1.2	8.488	7.908	8.225	8.266	8.120	8.370	8.337
10 (5h)	1.2	8.546	8.280	8.395	8.450	8.395	8.541	8.480
11 (5 g)	1.3	8.506	8.382	8.240	8.341	8.319	8.377	8.444
12 (4b)	1.4	8.407	7.944	7.702	7.650	7.512	7.540	7.696
13 (4f)	1.5	8.405	8.293	8.326	8.183	8.326	8.277	8.259
14 (5a)	1.5	8.402	8.036	8.499	8.256	8.374	8.502	8.536
15 (5b)	1.7	8.385	8.241	8.273	8.293	8.315	8.257	8.360
16 (5e)	1.7	8.369	8.485	8.237	8.520	8.184	8.298	8.423
17 (5l)	4	7.997	8.334	8.081	8.201	8.108	8.243	8.081
18 (5q)	7	7.741	8.115	8.171	8.307	8.081	8.132	8.109
19 (5s)	7.8	7.745	7.935	8.407	8.523	8.359	8.407	8.491
20 (5j)	10	7.624	7.719	8.624	8.544	8.544	8.581	8.679
21 (4e)	15	7.405	8.022	8.351	8.303	8.201	8.323	8.377
22 (4c)	20	7.253	7.843	8.173	8.211	8.106	8.351	8.139
23 (4k)	20	7.306	7.873	8.227	8.528	8.115	8.239	8.493
24 (5i)	20	7.142	8.214	8.239	8.188	8.267	8.433	8.297
25 (5n)	100	6.609	7.247	7.752	7.495	7.640	7.451	7.405
26 (4a)	300	6.053	6.705	7.489	7.557	7.300	7.166	7.384
27 (5k)	500	5.930	5.694	5.689	5.633	5.656	5.653	5.656
28 (4l)	1500	5.436	5.798	5.639	5.621	5.625	5.665	5.639
29 (5m)	7500	4.754	5.683	5.670	5.638	5.638	5.660	5.629
R ² ^b			0.85	0.73	0.66	0.74	0.72	0.71
N ^c			29	25	25	25	25	25

^a Bold values represent predicted activities of compounds that were not included in the training set. ^b Correlation coefficient for training set compounds. ^c Number of compounds.

Table 6. Experimental (ED₅₀ and pIC₅₀) and Catalyst Predicted (pIC₅₀) Values for Bisphosphonates against Bone Resorption and Statistical Results

compound	experimental activity		predicted pIC ₅₀ ^a					
	ED ₅₀ (μg/kg)	pIC ₅₀	training set	four-compound test sets				
30 (6i)	0.07	9.617	8.533	8.538	8.630	8.421	8.349	8.489
31 (6d)	0.3	8.958	8.393	8.393	7.972	8.745	8.443	8.571
32 (6b)	0.6	8.705	8.161	8.161	7.759	8.121	7.965	8.253
33 (8f)	0.9	8.565	8.343	8.440	8.000	8.565	8.288	8.477
34 (6h)	1.5	8.287	8.477	8.463	8.162	8.872	8.500	8.644
35 (6l)	1.5	8.301	8.155	8.398	7.864	8.199	8.199	8.155
36 (8c)	1.5	8.290	7.922	8.019	7.891	8.144	7.935	8.019
37 (8d)	1.5	8.317	7.949	8.002	8.477	7.962	7.962	8.031
38 (8e)	2	8.211	8.007	8.211	7.968	8.282	8.211	8.336
39 (8h)	2.7	8.146	7.814	8.347	7.965	8.323	8.323	8.464
40 (6k)	3	7.979	8.507	8.512	8.415	8.377	8.252	8.457
41 (6a)	5	7.750	7.795	7.858	7.836	7.592	7.716	7.733
42 (8a)	5	7.739	7.905	7.991	7.610	8.116	7.906	7.991
43 (8k)	5	7.734	6.902	6.820	6.685	6.800	6.865	6.717
44 (6f)	15	7.294	7.993	8.128	7.857	7.926	7.868	8.039
45 (6e)	20	7.176	7.221	7.508	7.744	7.272	7.513	7.435
46 (6c)	25	7.187	7.635	8.284	8.041	7.895	7.922	8.017
47 (6j)	45	6.803	7.226	7.377	7.156	7.201	7.415	7.280
48 (8l)	75	6.732	6.984	7.566	7.227	7.566	7.643	7.781
49 (8b)	100	6.460	6.778	6.380	6.590	6.608	6.735	6.515
50 (8 g)	200	6.199	6.737	7.199	5.746	6.857	6.819	6.995
51 (8m)	200	6.222	6.807	7.018	6.867	6.703	6.869	6.979
52 (9a)	200	6.135	5.783	5.931	7.018	6.005	5.704	6.021
53 (8j)	~500	5.711	6.437	6.632	6.854	6.471	6.618	6.578
54 (6n)	600	5.658	5.591	5.729	5.755	5.696	5.585	5.746
55 (9b)	700	5.619	5.774	5.884	5.732	5.831	5.740	5.908
R ² ^b			0.83	0.77	0.80	0.81	0.74	0.73
N ^c			26	26	26	26	26	26

^a Bold values represent predicted activities of compounds that were not included in the training set. ^b Correlation coefficient for training set compounds. ^c Number of compounds.

The electrostatic field result, Figure 10E, shows an interesting feature (in addition to the expected favored positive charge feature, on the imidazole ring shown),

a negative charge favored region (red) encompassing the bisphosphonate 1-OH group. This feature is not seen in the aryl-X CoMSIA contour maps, Figure 10B, since

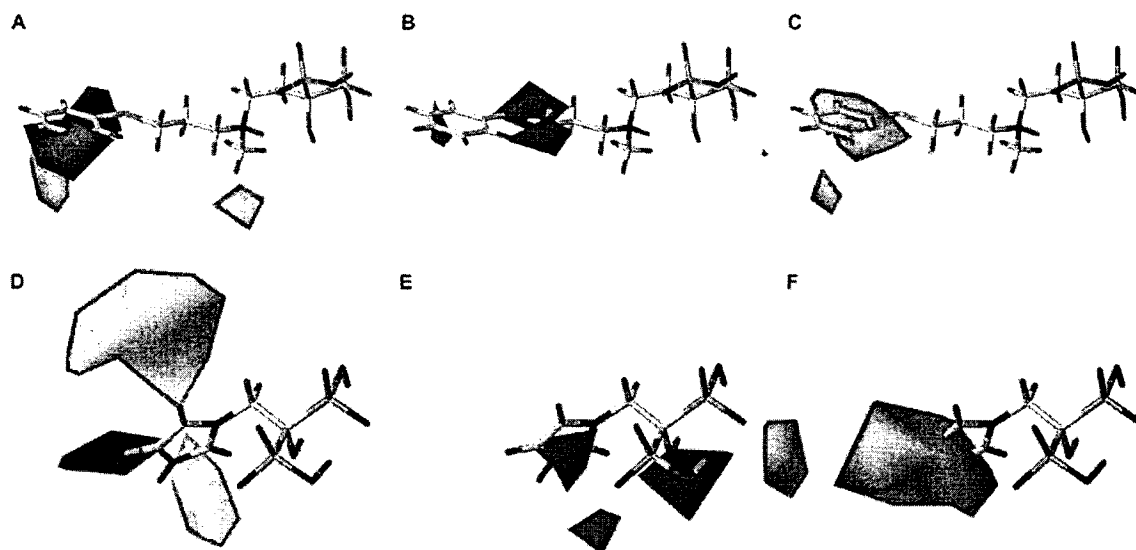


Figure 10. CoMSIA field analysis contours for A–C: aryl-X bisphosphonates and D–F, heterocyclic bisphosphonates. A, D, steric field (green, favored; yellow, disfavored). B, E, electrostatic field (blue, favored; red, disfavored). C, F, hydrophobic interactions (orange, favored; white, disfavored). The CoMSIA field contours are superimposed on **1(5r)** in A–C and zoledronate **30(6i)** in D–F.

all compounds in the aryl-X series have this feature in common. Since this feature is not seen in the CoMSIA field for FPP synthase itself (unpublished results) it seems likely that this group is indeed important in bisphosphonate binding to bone (the “bone hook”). These CoMSIA contour maps thus augment our pK_a and other QSAR data and may help guide the design of additional novel species.

Conclusions

The results we have obtained above are of interest for a number of reasons. First, we have used 3D-QSAR/MFA techniques to predict the activities of a wide range of aryl-X (X = alkyl, oxyalkyl, and sulfanyalkyl) substituted bisphosphonates (pamidronates and one alendronate). Activities can be predicted with an average error of $2.1\times$ over an overall range in activity of $4000\times$, when using crystallographic structural information to guide the overall molecular alignment. Second, we have used MFA techniques to predict the activities of a wide range of heterocyclic bisphosphonates. Activities can be predicted within a factor of $2.5\times$ over a $2000\times$ range in activity. Third, we propose that, in general, the activities of heterocyclic bisphosphonates are strongly related to the pK_a values of the (parent) heterocycle. Thiazole, triazole, and pyrazole-based bisphosphonates have low activity since the pK_a values of the parent bases are all low, typically <3 . On the other hand, pyridyl-, imidazolyl-, and aminothiazole species are all active since they have (free base) pK_a values in the range ~ 5 – 9 and can thus be protonated and therefore act as carbocation reactive-intermediate analogue FPP synthase inhibitors. For aminoimidazole bisphosphonates, activity is surprisingly low, but this may be correlated with an extremely high pK_a (~ 11) which could result in diminished transport. Fourth, we have used the Catalyst pharmacophore modeling approach to develop pharmacophore hypotheses for bone resorption by bisphosphonates. The hypotheses consist of two negative ionizable groups, a positive charge feature and an aromatic

feature, but with different spatial dispositions of the aromatic feature in the two classes of compounds. Activity predictions using this approach are slightly worse than when using the MFA approach, but are still quite promising. Fifth, we used a CoMSIA analysis to investigate the various contributions to activity in both the aryl-X and heterocyclic bisphosphonates, finding at least initial evidence for the importance of the 1-OH “bone hook”.

These results represent the most comprehensive quantitative structure–activity relationship investigation of bisphosphonates as bone resorption agents to date and demonstrate that activities can be predicted within about a factor of 2.3 (over an overall range in activity of $\sim 4000\times$). In addition, for heterocyclic bisphosphonates, the results are of considerable interest since they provide the first insights into structure–activity relationships at the level of protonation state or pK_a values. This can be expected to facilitate the further development of novel bisphosphonates and related systems for a wide variety of therapeutic uses.

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Part 5

Quantitative Structure–Activity Relationship Study of Bisphosphonates

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Quantitative Structure–Activity Relationship Study of Bisphosphonates[#]

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Abstract

Motivation. Bisphosphonates (BPs) are most widely used as agents for treating osteoporosis. They have also been used for other purposes such as herbicides, anticancer agents, and antiparasitics. Here we report QSAR models of four BPs datasets based on the 118 structural and biological data we have collected from various literature sources.

Method. The structures were energy minimized with the MMFF94 force field, then 181 2D and 3D descriptors were calculated with MOE (Molecular Operating Environment). The step by step multiple regression and principle analysis were used to generate QSAR models. The predictive power of each QSAR model was estimated with the leave-one-out (LOO) cross-validation method.

Results. The QSAR model for a dataset of 28 GGPPSase inhibitors ($r^2 = 0.86$, $r^2_{\text{LOO}} = 0.82$, $s = 0.45$, $s_{\text{LOO}} = 0.51$, $F = 77.56$) is made up of two descriptors. Another dataset of 28 compounds with bioactivities against the growth of *T. Brucei rhodesiense* was studied using PCA and reached a model ($r^2 = 0.85$, $r^2_{\text{LOO}} = 0.79$, $s = 0.30$, $s_{\text{LOO}} = 0.35$, $F = 32.03$) with four principle components (PCs). Both the above models have comparable predictive ability with CoMFA model reported by Szabo *et al.* The 86 BPs provided by Novartis with *in vivo* bio-data of TPTX rats were divided into two datasets. A six PCs model ($r^2 = 0.80$, $r^2_{\text{LOO}} = 0.72$, $s = 0.44$, $s_{\text{LOO}} = 0.53$, $F = 24.83$) elucidated the dataset of 44 compounds in which containing aliphatic linked nitrogen atoms. The other dataset includes 42 BPs containing a heterocyclic moiety with at least one nitrogen atom. Its PCA model ($r^2 = 0.80$, $r^2_{\text{LOO}} = 0.71$, $s = 0.46$, $s_{\text{LOO}} = 0.57$, $F = 19.99$) consists of seven PCs.

Conclusions. A leave-four-out test procedure shows that though the QSAR models based on *in vivo* bone resorption pED₅₀ values cannot provide explicit indications for drug design, their predictive ability for related compounds is quite good.

Keywords. Bisphosphonate; principal component analysis (PCA); GGPPSase inhibitor; quantitative structure–activity relationships (QSAR); IC₅₀; ED₅₀.

Abbreviations and notations

Bps, bisphosphonates

r^2_{LOO} , leave-one-out cross validated correlation

ED₅₀, the dose of compound administrated sc, which
results in a 50% reduction of the hypercalcemia

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induced in TPTX rats by 1,25–dihydroxyvitamin D ₃	coefficient. $r_{LOO}^2 = 1 - \frac{\sum (y_{pred} - y_{act})^2}{\sum (y_{act} - y_{mean})^2}$
FPPSase, farnesyl pyrophosphate synthase	
GGPPSase, geranylgeranyl diphosphate synthase	
IC ₅₀ , experimental concentration required to reduce activity/proliferation of enzymes/cells/parasites by 50%	s , root mean square error. $s = \sqrt{\frac{\sum (y_{calc} - y_{act})^2}{n - k - 1}}$, n , number of observations; k , number of descriptors
PCA, principal component analysis	TPTX, thyroparathyroidectomy
PRED, leave–one–out cross validated prediction	s_{LOO} , leave–one–out cross validated correlation root mean square error. $s_{LOO} = \sqrt{\frac{\sum (y_{pred} - y_{act})^2}{n - k - 1}}$, n , number of objects; k , number of descriptors
QSAR, quantitative structure–activity relationships	
r^2 , correlation coefficient. $r^2 = 1 - \frac{\sum (y_{calc} - y_{act})^2}{\sum (y_{act} - y_{mean})^2}$	

1 INTRODUCTION

Bisphosphonates (BPs) are the most widely used inhibitors of bone resorption. They all contain two phosphonate groups attached to a single carbon atom, forming a P–C–P structure. Bisphosphonates are stable analogs of naturally occurring pyrophosphate–containing compounds, which now helps to explain their intracellular as well as their extracellular modes of action. Several bisphosphonates, *e.g.*, etidronate, clodronate, pamidronate, alendronate, tiludronate, risedronate, and ibandronate, have been established as effective treatments in clinical disorders such as Paget’s disease of bone, tumour–associated bone disease, and osteoporosis [1]. Bisphosphonates have also been repeated for uses as herbicides [2], anticancer agents [3], and antiparasitics [4,5].

Recent studies suggest that bisphosphonates inhibit bone resorption by cellular effects on bone–resorbing osteoclasts, rather than by purely physicochemical mechanisms. It is likely that BPs are internalized by osteoclasts and interfere with specific biochemical process and induce apoptosis [6]. In recent work, the site of action has been narrowed down to the mevalonate pathway and the isoprene pathway.

The exact enzymes of the mevalonate pathway that are inhibited by BPs have not yet been fully identified. However, incadronate and ibandronate are known inhibitors of squalene synthase, an enzyme in the mevalonate pathway required for cholesterol biosynthesis [6]. Alendronate and pamidronate are less potent inhibitors of squalene synthase but can also inhibit sterol biosynthesis, suggesting that these bisphosphonates may inhibit up stream enzymes of the mevalonate pathway other than squalene synthase [7].

Several enzymes of the mevalonate pathway such as isoprenoid diphosphate isomerase (IPP isomerase), farnesyl diphosphate synthase (FPPSase), geranylgeranyl diphosphate synthase (GGPPSase), and squalene synthase, utilize an isoprenoid diphosphate as a substrate and thus are likely to have similar substrate binding sites. Thus if nitrogen–containing BPs act as substrate analogs of an isoprenoid diphosphate, it is likely that these BPs actually inhibit several enzymes of the mevalonate pathway. FPPSase are the most reported target for many BPs. For example,

Cromartie and Fisher demonstrated that herbicidal bisphosphonates were potent, low-nanomolar inhibitors of a daffodil FPPSase [2,8], and Grove *et al.* reported that BPs were growth and FPPSase inhibitors of the primitive eukaryote *Dictyostelium discoideum* [9].

Several groups [1,3,10,11] have reported that FPPSase was the target of the nitrogen-containing bisphosphonates in bone, leading to the apoptosis of osteoclasts. The group of Eric Oldfield, which did a lot of jobs on chemotherapy of parasitic protozoa diseases, reported that bisphosphonates were in vitro inhibitors of the growth of the causative agents of Chagas' disease, human East African trypanosomiasis, visceral leishmaniasis, toxoplasmosis, malaria, and cryptosporidiosis, *T. Cruzi*, *Trypanosoma brucei rhodesiense*, *Leishmania donovani*, *Toxoplasma gondii*, *Plasmodium falciparum*, and *Cryptosporidium parvum* [4,5]. They also showed that in some of the parasites, such as *T. b. rhodesiense* and *D. discoideum*, the molecular target of some bisphosphonates such as risedronate is FPPSase and reported 3D-QSAR/CoMFA investigation of bisphosphonate drugs, in the inhibition of bone resorption as well as the growth of *D. discoideum* and the bloodstream form of *T. b. rhodesiense* [5,12]. Though there are fewer reports, other enzymes, *e.g.* IPP isomerase, GGPP synthase, and squalene synthase of the mevalonate pathway, may be also potential targets for different bisphosphonates.

Eric Oldfield group has investigated the inhibition of a human recombinant GGPPSase by 23 bisphosphonates and six azaptenyl diphosphates. In addition to CoMFA analysis of structure-activity relationship, the pharmacophore of these GGPPSase inhibitors obtained from Catalyst was also provided [13].

Though the actual conformations of the bisphosphonates in the FPPSase and GGPPSase active sites are not yet known, good predictive CoMFA models were obtained using the molecular mechanics-derived lowest-energy conformers [5,12,13].

Widler *et al.* reported an extensive structure-activity relationship (SAR) study of bisphosphonates [14]. Small changes of the structure of pamidronate (compound 2) lead to marked improvements of the inhibition of osteoclastic resorption potency. Alendronate (compound 3 in Table 1), with an extra methylene group in the *N*-alkyl chain, and olpadronate (compound 7), the *N,N*-dimethyl analogue, are about 10 times more potent than pamidronate (compound 2).

Extending one of the *N*-methyl groups of olpadronate to a pentyl substituent leads to ibandronate (compound 10), which is the most potent close analogue of pamidronate. Even slightly better antiresorptive potency is achieved with derivatives having a phenyl group linked via a short aliphatic tether of three to four atoms to nitrogen, the second substituent being preferentially a methyl group. The most potent bisphosphonate, zoledronate (compound 65), is found in the series containing a heteroaromatic moiety with at least one nitrogen atom, which is linked via a single methylene group to the geminal bisphosphonate unit [14].

Table 1. Stucture and bioactivity of bisphosphonates

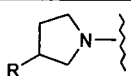
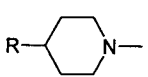
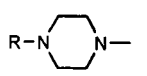
$\begin{array}{c} \text{R1} \\ \diagdown \\ \text{N} - (\text{CH}_2)_n - \text{C} \begin{array}{l} \text{PO}_3\text{H}_2 \\ \\ \text{X} \\ \\ \text{PO}_3\text{H}_2 \end{array} \\ \diagup \\ \text{R2} \end{array}$								
No	Cmpd code	R ₁	R ₂	X	n	ED ₅₀ (μg/kg) ^a	IC ₅₀ (μM) ^b	IC ₅₀ (μM) ^c
1	Novartis 1a	H	H	OH	1	150		
2	Pamidronate	H	H	OH	2	61	177	180
3	Alendronate	H	H	OH	3	8		440
4	Novartis 1d	H	H	OH	4	20		
5	Neridronate	H	H	OH	5	60	31.7	690
6	Novartis 1g	Me	H	OH	2	15		
7	Olpadronate	Me	Me	OH	2	12	5.4	
8	T.B. 009	propyl	Me	OH	2	3	7.8	330
9	Novartis 1j	Et	Et	OH	2	3		
10	Ibandronate	pentyl	Me	OH	2	1.1	0.96	83
11	Novartis 1l	Me	Me	H	2	100		
$\begin{array}{c} \text{R1} \\ \diagdown \\ \text{N} - \text{CH}(\text{R}) - \text{CH}_2 - \text{C} \begin{array}{l} \text{PO}_3\text{H}_2 \\ \\ \text{OH} \\ \\ \text{PO}_3\text{H}_2 \end{array} \\ \diagup \\ \text{R2} \end{array}$								
No	Cmpd code	R ₁	R ₂	R	ED ₅₀ (μg /kg) ^a	IC ₅₀ (μM) ^b	IC ₅₀ (μM) ^c	
12	Novartis 1n	H	H	Me	3.4			
13	Novartis 1o	Me	Me	Me	18			
14	Novartis 1p	pentyl	Me	Me	65			
$\begin{array}{c} \text{PO}_3\text{H}_2 \\ \\ \text{N} - (\text{CH}_2)_n - \text{C} \begin{array}{l} \text{PO}_3\text{H}_2 \\ \\ \text{OH} \\ \\ \text{PO}_3\text{H}_2 \end{array} \end{array}$								
No	Cmpd code			R	n	ED ₅₀ (μg /kg) ^a		
15	Novartis 2a			H	2	10		
16	Novartis 2b			H	3	25		
17	Novartis 2c			H	5	250		
18	Novartis 2d			Ph	2	70		
19	Novartis 2e			4-Cl-Ph	2	3.5		
20	Novartis 2f			H	2	5.6		
21	Novartis 2g			Ph	2	11		
22	Novartis 2h			Ph	3	100		
23	Novartis 2j			3-F-Ph	2	30		
24	Novartis 2k				2	25		
25	Novartis 2m			Me	2	400		

Table 1. (Continued)

Table IV (Continued)

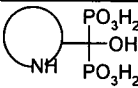
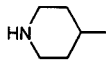
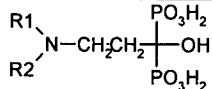
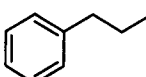
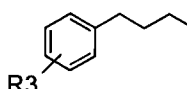
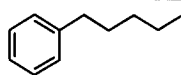
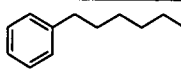
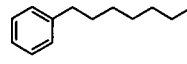
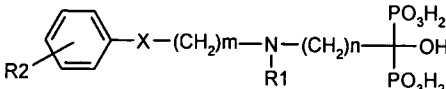
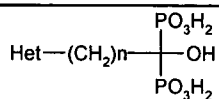
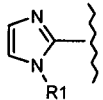
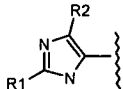
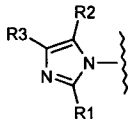
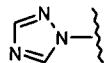
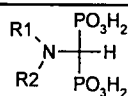
Serial	Cmpd code		ED ₅₀ (μg /kg) ^a					
26	Novartis 3a		50					
27	Novartis 3b		250					
28	Novartis 3c		2500					
Serial	Cmpd code		R ₁	R ₂	R ₃	ED ₅₀ (μg /kg) ^a		
29	Novartis 4a			H		300		
30	Novartis 4b			Me		1.4		
31	Novartis 4c			H	H	20		
32	Novartis 4d			Me	H	1		
33	Novartis 4e			Et	H	15		
34	Novartis 4f			Me	3-Me	1.5		
35	Novartis 4g			Me	4-Cl	0.7		
36	Novartis 4i			H		1.0		
37	Novartis 4j			Me		0.4		
38	Novartis 4k			Me		20		
39	Novartis 4l			Me		1500		
Serial	Cmpd code		X	R ₁	R ₂	m	n	ED ₅₀ (μg /kg) ^a
40	Novartis 5a		O	Me	H	2	2	1.5
41	Novartis 5b		O	Me	4-Cl	2	2	1.7
42	Novartis 5c		O	H	H	3	2	1.2
43	Novartis 5d		O	Me	H	3	2	0.5
44	Novartis 5e		O	Me	3-Me	3	2	1.7
45	Novartis 5f		O	Me	4-F	3	2	0.6
46	Novartis 5g		O	Me	4-Cl	3	2	1.3
47	Novartis 5h		O	Et	4-MeO	3	2	1.2
48	Novartis 5i		O	propyl	H	3	2	20
49	Novartis 5j		O	butyl	H	3	2	10
50	Novartis 5k		O	Me	H	4	2	500

Table 1. (Continued)

Serial	Cmpd code	X	R ₁	R ₂	m	n	ED ₅₀ (μg/kg) ^a
51	Novartis 5l	O	Me	H	6	2	4
52	Novartis 5m	O	Me	H	3	2	7500
53	Novartis 5n	O	Me	H	2	3	100
54	Novartis 5p	S	Me	H	2	2	0.7
55	Novartis 5q	S	H	H	3	2	7
56	Novartis 5r	S	Me	H	3	2	0.33
57	Novartis 5s	S	Me	4-Cl	3	2	7.8



Serial	Cmpd code	Het	R ₁	R ₂	R ₃	n	ED ₅₀ (μg/kg) ^a
58	Novartis 6a		H			1	5
59	Novartis 6b		Me			1	0.6
60	Novartis 6c		Bz			1	25
61	Novartis 6d		H	H		1	0.3
62	Novartis 6e		H	H		2	20
63	Novartis 6f		Me	H		1	15
64	Novartis 6h		H	Me		1	1.5
65	Zoledronate		H	H	H	1	0.07
66	Novartis 6j		H	H	H	2	45
67	Novartis 6k		Me	H	H	1	3
68	Novartis 6l		H	Me	Me	1	1.5
69	Novartis 6n					1	600



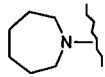
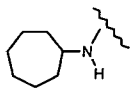
Serial	Cmpd code	R ₁ R ₂ N	ED ₅₀ (μg/kg) ^a
70	Novartis 7c		800
71	Novartis 7d		40
72	Novartis 7e		7

Table 1. (Continued)

Serial	Cmpd code	Het	R ₁	R ₂	ED ₅₀ (μg /kg) ^a
73	Novartis 8a		H	H	5
74	Novartis 8b		H	Me	100
75	Novartis 8c		Me	H	1.5
76	Novartis 8d		Et	H	1.5
77	Novartis 8e		Pr	H	2
78	Novartis 8f		Bu	H	0.9
79	Novartis 8g		Pr	H	200
80	Novartis 8h		PhCH ₂ CH ₂	H	2.7
81	Novartis 8j		H		500
82	Novartis 8k		Me		5
83	Novartis 8l		PhCH ₂		75
84	Novartis 8m		Ph		200
Serial	Cmpd code	X	R	ED ₅₀ (μg /kg) ^a	
85	Novartis 9a	CH ₂	OH	200	
86	Novartis 9b	S	OH	700	
Serial	Cmpd code	R	n	IC ₅₀ (μM) ^c	
87	3-azaGGPP		2	0.14	
88	3-azaFPP		2	0.74	
89	3-azaGPP	Me	2	240	
90	3-azahomoGGPP		3	0.37	
91	3-azahomoFPP		3	0.31	
92	15-azaGGPP			>>100	
Serial	Cmpd code	n	IC ₅₀ (μM) ^b	IC ₅₀ (μM) ^c	
93	T.B. 024	2	92.0	620	
94	T.B. 025	3	99.8	200	
95	T.B. 023	4	62.4	53	
96	GGPP018	5		11.0	

Table 1. (Continued)

Serial	Cmpd code	n	IC ₅₀ (μM) ^b	IC ₅₀ (μM) ^c
97	GGPP017	6		4.3
98	T.B. 014	8	20.5	0.72
99	T.B. 010	9	8.0	1.4
100	T.B. 007	10	2.0	0.92

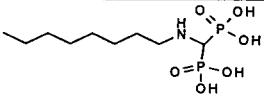
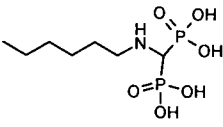
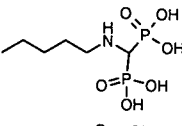
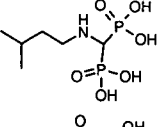
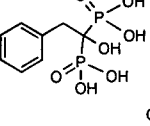
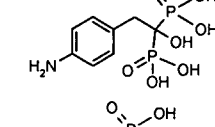
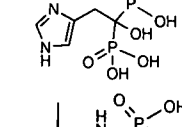
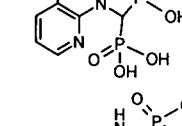
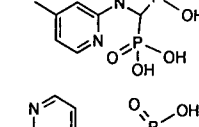
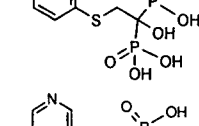
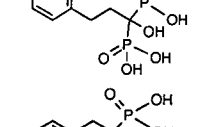
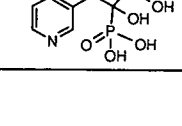
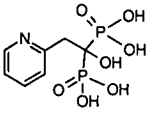
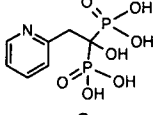
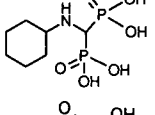
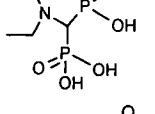
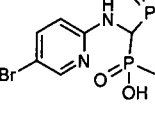
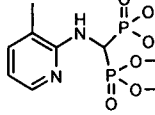
Serial	Cmpd code	Structure	IC ₅₀ (μM) ^b	IC ₅₀ (μM) ^c
101	T.B. 006		1.7	2.2
102	GGPP031			19.0
103	T.B. 021		50.6	
104	T. B. 026		102	
105	T.B. 016		21.3	220.0
106	T.B. 020		40.0	180.0
107	T.B. 012		8.6	220.0
108	NE97220		0.7	220.0
109	N-(2-(4-picolyl))ADMP		0.61	260.0
110	T.B. 013		19.8	550.0
111	Homorisedronate		1.7	410.0
112	Risedronate		8.6	350.0

Table 1. (Continued)

Serial	Cmpd code	Structure	IC ₅₀ (μM) ^b	IC ₅₀ (μM) ^c
113	NE58018		0.22	
114	N-(2-(5-chloro)-pyridyl)AMDP		53.3	
115	T.B. 015		20.9	
116	T.B. 018		34.4	
117	T.B. 019		39.5	
118	T.B. 2-13		27.9	

^a the dose of compound administered sc, which results in a 50% reduction of the hypercalcemia induced in TPTX rats by 1,25-dihydroxyvitamin D₃ [14]

^b experimental concentration required to reduce proliferation of *T. Brucei rhodesiense* by 50% [4,5]

^c experimental concentration required to reduce activity of GGPPSase by 50% [13].

The comprehension of BPs mechanism gives us indications to investigate the quantitative structure–activity relationship of the bisphosphonates provided by Novartis [14] with *in vivo* ED₅₀ against hypercalcemia induced in thyroparathyroidectomy (TPTX) rats. The total of 86 compounds were divided into two datasets, one for the series containing a heterocyclic moiety, which contains at least one nitrogen atom; the other for bisphosphonates that contains a nitrogen atom in aliphatic link and do not possess a heterocyclic substitute. The two datasets were analyzed using QSAR module of MOE and achieved two predictive models through principal component analysis.

We also investigated the BPs with IC₅₀ against *T. Brucei Trypomastigotes* [4,5] and BPs with IC₅₀ for GGPPSase inhibition [13] using the molecular modeling package MOE respectively, and achieved more simple and lightening models.

2 MATERIALS AND METHODS

2.1 Chemical Data

The structures we have collected here are listed in the Table 1 along with compound code and bioactivities. Some of the compound codes were assigned following their traditional name such as

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(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LT, LU, LV, MA, MD, MK, MN, MX, NO, NZ, OM, PH, PL, PT, RO, RU, SE, SG, SI, SK, TJ, TM, TR, TT, UA, US, UZ, VN, YU, ZA, ZW.

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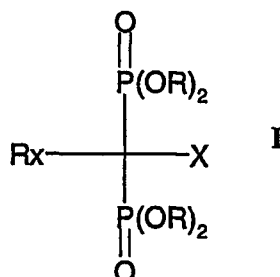
For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



WO 02/43738 A2

- (54) Title: USE OF BISPHOSPHONATES FOR PAIN TREATMENT
- (57) Abstract: A method for the treatment of pain, in particular antinociceptive or anti-allodynic treatment of pain, in a patient in need of such treatment, e.g. a patient with osteoporosis or osteopenia, a tumour patient or a patient suffering from an inflammatory disease, which comprises administering an effective amount of a bisphosphonate, e.g. zoledronic acid or salts or hydrates thereof, to the patient.

bisphosphonates. For the purposes of the present description a nitrogen containing bisphosphonate is a compound which in addition to the characteristic geminal bisphosphate (P-C-P) moiety comprises a nitrogen containing side chain, e.g. a compound of formula I



wherein

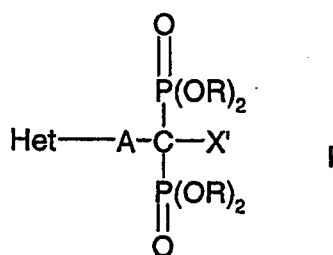
X is hydrogen, hydroxyl, amino, alkanoyl, or an amino group substituted by C₁-C₄ alkyl, or alkanoyl;

R is hydrogen or C₁-C₄ alkyl and

Rx is a side chain which contains an optionally substituted amino group, or a nitrogen containing heterocycle (including aromatic nitrogen-containing heterocycles), and pharmaceutically acceptable salts thereof or any hydrate thereof.

Particularly preferred nitrogen containing bisphosphonates are those having side chains containing nitrogen-containing heterocycles, most especially containing aromatic nitrogen-containing heterocycles.

Thus in one embodiment a particularly preferred bisphosphonate for use in the invention comprises a compound of Formula I'



wherein

Het is an imidazole, oxazole, isoxazole, oxadiazole, thiazole, thiadiazole, pyridine, 1,2,3-triazole, 1,2,4-triazole or benzimidazole radical, which is optionally substituted by alkyl,

alkoxy, halogen, hydroxyl, carboxyl, an amino group optionally substituted by alkyl or alkanoyl radicals or a benzyl radical optionally substituted by alkyl, nitro, amino or aminoalkyl;

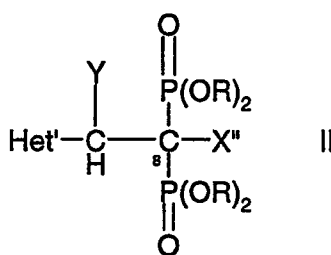
A is a straight-chained or branched, saturated or unsaturated hydrocarbon moiety containing from 1 to 8 carbon atoms;

X is a hydrogen atom, optionally substituted by alkanoyl, or an amino group optionally substituted by alkyl or alkanoyl radicals, and

R is a hydrogen atom or a C₁-C₄ alkyl radical,

and the pharmacologically acceptable salts thereof.

In a further embodiment a particularly preferred bisphosphonate for use in the invention comprises a compound of Formula II



wherein

Het' is a substituted or unsubstituted heteroaromatic five-membered ring selected from the group consisting of imidazolyl, imidazoliny, isoxazolyl, oxazolyl, oxazoliny, thiazolyl, thiazoliny, triazolyl, oxadiazolyl and thiadiazolyl wherein said ring can be partly hydrogenated and wherein said substituents are selected from at least one of the group consisting of C₁-C₄ alkyl, C₁-C₄ alkoxy, phenyl, cyclohexyl, cyclohexylmethyl, halogen and amino and wherein two adjacent alkyl substituents of Het can together form a second ring;

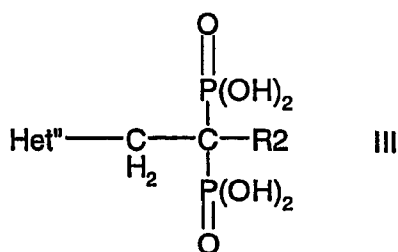
Y is hydrogen or C₁-C₄ alkyl;

X'' is hydrogen, hydroxyl, amino, or an amino group substituted by C₁-C₄ alkyl, and

R is hydrogen or C₁-C₄ alkyl;

as well as the pharmacologically acceptable salts and isomers thereof.

In a yet further embodiment a particularly preferred bisphosphonate for use in the invention comprises a compound of Formula III



wherein

Het'' is an imidazolyl, 2H-1,2,3-, 1H-1,2,4- or 4H-1,2,4-triazolyl, tetrazolyl, oxazolyl, isoxazolyl, oxadiazolyl, thiazolyl or thiadiazolyl radical which is unsubstituted or C-mono- or di-substituted by lower alkyl, by lower alkoxy, by phenyl which may in turn be mono- or disubstituted by lower alkyl, lower alkoxy and/or halogen, by hydroxy, by di-lower alkylamino, by lower alkylthio and/or by halogen and is N-substituted at a substitutable N-atom by lower alkyl or by phenyl-lower alkyl which may in turn be mono- or di-substituted in the phenyl moiety by lower alkyl, lower alkoxy and/or halogen, and

R₂ is hydrogen, hydroxy, amino, lower alkylthio or halogen,

lower radicals having up to and including 7 C-atoms,

or a pharmacologically acceptable salt thereof.

Examples of particularly preferred bisphosphonates for use in the invention are:

2-(1-Methylimidazol-2-yl)-1-hydroxyethane-1,1-diphosphonic acid;

2-(1-Benzylimidazol-2-yl)-1-hydroxyethane-1,1-diphosphonic acid;

2-(1-Methylimidazol-4-yl)-1-hydroxyethane-1,1-diphosphonic acid;

1-Amino-2-(1-methylimidazol-4-yl)ethane-1,1-diphosphonic acid;

1-Amino-2-(1-benzylimidazol-4-yl)ethane-1,1-diphosphonic acid;

2-(1-Methylimidazol-2-yl)ethane-1,1-diphosphonic acid;

2-(1-Benzylimidazol-2-yl)ethane-1,1-diphosphonic acid;

2-(Imidazol-1-yl)-1-hydroxyethane-1,1-diphosphonic acid;

2-(Imidazol-1-yl)ethane-1,1-diphosphonic acid;

2-(4H-1,2,4-triazol-4-yl)-1-hydroxyethane-1,1-diphosphonic acid;

2-(Thiazol-2-yl)ethane-1,1-diphosphonic acid;

2-(Imidazol-2-yl)ethane-1,1-diphosphonic acid;

2-(2-Methylimidazol-4(5)-yl)ethane-1,1-diphosphonic acid;

2-(2-Phenylimidazol-4(5)-yl)ethane-1,1-diphosphonic acid;
2-(4,5-Dimethylimidazol-1-yl)-1-hydroxyethane-1,1-diphosphonic acid, and
2-(2-Methylimidazol-4(5)-yl)-1-hydroxyethane-1,1-diphosphonic acid,
and pharmacologically acceptable salts thereof.

The most preferred bisphosphonate for use in the invention is 2-(imidazol-1-yl)-1-hydroxyethane-1,1-diphosphonic acid (zoledronic acid) or a pharmacologically acceptable salt thereof or any hydrate thereof.

Pharmacologically acceptable salts are preferably salts with bases, conveniently metal salts derived from groups Ia, Ib, IIa and IIb of the Periodic Table of the Elements, including alkali metal salts, e.g. potassium and especially sodium salts, or alkaline earth metal salts, preferably calcium or magnesium salts, and also ammonium salts with ammonia or organic amines.

Especially preferred pharmaceutically acceptable salts are those where one, two, three or four, in particular one or two, of the acidic hydrogens of the bisphosphonic acid are replaced by a pharmaceutically acceptable cation, in particular sodium, potassium or ammonium, in first instance sodium.

A very preferred group of pharmaceutically acceptable salts is characterized by having one acidic hydrogen and one pharmaceutically acceptable cation, especially sodium, in each of the phosphonic acid groups.

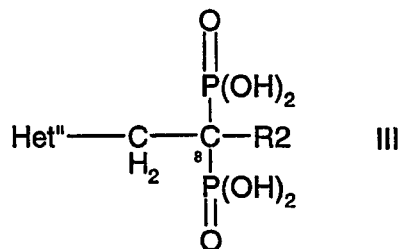
All the bisphosphonic acid derivatives mentioned above are well known from the literature. This includes their manufacture (see e.g. EP-A-513760, pp. 13-48). For example, 3-amino-1-hydroxypropane-1,1-diphosphonic acid is prepared as described e.g. in US patent 3,962,432 as well as the disodium salt as in US patents 4,639,338 and 4,711,880, and 1-hydroxy-2-(imidazol-1-yl)ethane-1,1-diphosphonic acid is prepared as described e.g. in US patent 4,939,130. See also US patents 4,777,163 and 4,687,767.

CLAIMS

1. A method for the treatment of pain in a patient in need of such treatment which comprises administering an effective amount of a bisphosphonate to the patient.
2. Use of a bisphosphonate in the preparation of a medicament for the treatment of pain.
3. Use of a bisphosphonate to treat pain associated with diseases or pathological conditions in mammals.
4. A method for the anti-nociceptive or anti-allodynic treatment of pain in a patient in need of such treatment which comprises administering an effective amount of a bisphosphonate to the patient;
use of a bisphosphonate in the preparation of a medicament for the anti-nociceptive or anti-allodynic treatment of pain; or
use of a bisphosphonate as an anti-nociceptive or anti-allodynic agent.
5. A method for the treatment of bone pain in a patient in need of such treatment which comprises administering an effective amount of a bisphosphonate to the patient;
use of a bisphosphonate in the preparation of a medicament for the treatment of bone pain; or
use of a bisphosphonate as an agent for treatment of bone pain.
6. A method according to claim 1 or a use according to claim 2 or 3 for the treatment of pain associated with osteoporosis, rheumatoid arthritis, osteoarthritis and tumour formation, e.g. tumour growth, invasion or metastasis.
7. A method according to claim 1 or a use according to claim 2 or 3, in which the bisphosphonate is selected from the following compounds or a pharmaceutically acceptable

salt thereof, or any hydrate thereof: 3-amino-1-hydroxypropane-1,1-diphosphonic acid (pamidronic acid), e.g. pamidronate (APD); 3-(N,N-dimethylamino)-1-hydroxypropane-1,1-diphosphonic acid, e.g. dimethyl-APD; 4-amino-1-hydroxybutane-1,1-diphosphonic acid (alendronic acid), e.g. alendronate; 1-hydroxy-ethidene-bisphosphonic acid, e.g. etidronate; 1-hydroxy-3-(methylpentylamino)-propylidene-bisphosphonic acid, ibandronic acid, e.g. ibandronate; 6-amino-1-hydroxyhexane-1,1-diphosphonic acid, e.g. amino-hexyl-BP; 3-(N-methyl-N-n-pentylamino)-1-hydroxypropane-1,1-diphosphonic acid, e.g. methyl-pentyl-APD (= BM 21.0955); 1-hydroxy-2-(imidazol-1-yl)ethane-1,1-diphosphonic acid; 1-hydroxy-2-(3-pyridyl)ethane-1,1-diphosphonic acid (risedronic acid), e.g. risedronate, including N-methyl pyridinium salts thereof, for example N-methyl pyridinium iodides such as NE-10244 or NE-10446; 1-(4-chlorophenylthio)methane-1,1-diphosphonic acid (tiludronic acid), e.g. tiludronate; 3-[N-(2-phenylthioethyl)-N-methylamino]-1-hydroxypropane-1,1-diphosphonic acid; 1-hydroxy-3-(pyrrolidin-1-yl)propane-1,1-diphosphonic acid, e.g. EB 1053 (Leo); 1-(N-phenylaminothiocarbonyl)methane-1,1-diphosphonic acid, e.g. FR 78844 (Fujisawa); 5-benzoyl-3,4-dihydro-2H-pyrazole-3,3-diphosphonic acid tetraethyl ester, e.g. U-81581 (Upjohn); 1-hydroxy-2-(imidazo[1,2-a]pyridin-3-yl)ethane-1,1-diphosphonic acid, e.g. YM 529; and 1,1-dichloromethane-1,1-diphosphonic acid (clodronic acid), e.g. clodronate..

8. A method according to claim 1 or a use according to claim 2 or 3, in which the bisphosphonate is a compound of Formula III



wherein

Het'' is an imidazolyl, 2H-1,2,3-, 1H-1,2,4- or 4H-1,2,4-triazolyl, tetrazolyl, oxazolyl, isoxazolyl, oxadiazolyl, thiazolyl or thiadiazolyl radical which is unsubstituted or C-mono- or di-substituted by lower alkyl, by lower alkoxy, by phenyl which may in turn be mono- or disubstituted by lower alkyl, lower alkoxy and/or halogen, by hydroxy, by di-lower

alkylamino, by lower alkylthio and/or by halogen and is N-substituted at a substitutable N-atom by lower alkyl or by phenyl-lower alkyl which may in turn be mono- or di-substituted in the phenyl moiety by lower alkyl, lower alkoxy and/or halogen, and R₂ is hydrogen, hydroxy, amino, lower alkylthio or halogen, lower radicals having up to and including 7 C-atoms, or a pharmacologically acceptable salt thereof.

9. A method according to claim 1 or a use according to claim 2 or 3, in which the bisphosphonate is zoledronic acid, or a pharmaceutically acceptable salt thereof, or any hydrate thereof.
10. All novel compounds, processes, methods and uses substantially as hereinbefore described with particular reference to the Examples.

BISFOSFONATOS: SÍNTESE, ANÁLISES QUÍMICAS E APLICAÇÕES FARMACOLÓGICAS

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BISPHOSPHONATES: SYNTHESIS, CHEMICAL ANALYSIS AND PHARMACOLOGICAL APPLICATIONS. Bisphosphonates are drugs that have been widely used in different bone diseases, and have recently been used successfully against many parasites. Various synthetic routes to prepare different types of bisphosphonates have been described, with distinct potency and pharmacological activity. A number of analytical techniques are currently being used to analyze these drugs; among these, the high performance liquid chromatography (HPLC), with different systems of detection, is worth highlighting. However, the development of more sensitive methods is still necessary, once they are essential for bioavailability and bioequivalence studies. This paper reports the major synthesis routes, chemical analysis methodologies and pharmacological applications of bisphosphonates.

Keywords: bisphosphonates; bone diseases; high performance liquid chromatography.

INTRODUÇÃO

Os bisfosfonatos (BP's) formam uma classe de substâncias químicas que apresenta uma ligação P-C-P em sua estrutura, e agem como inibidores da reabsorção óssea, mediada pelos osteoclastos. Estes compostos são extensivamente utilizados no tratamento de várias doenças ósseas, destacando-se a doença de Paget, a hipercalcemia maligna, a osteoporose e a doença metastática e osteolítica.

São análogos químicos da substância endógena denominada ácido pirofosfórico (Figura 1), que no organismo se encontra como pirofosfato, um inibidor natural da reabsorção óssea. No entanto, essa substância não pode ser utilizada como agente terapêutico no tratamento de doenças ósseas, pois sofre uma rápida hidrólise enzimática. Os bisfosfonatos são seus análogos sintéticos, onde o átomo central de oxigênio é substituído por um de carbono. Essa modificação faz com que os BP's sejam mais resistentes à degradação enzimática, e possuam uma meia-vida biológica maior, suficiente para influenciar o metabolismo ósseo¹.

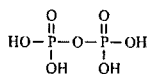


Figura 1. Estrutura química do ácido pirofosfórico

Diferentes substituintes ligados ao carbono central dão características únicas para cada fármaco (Figura 2). O grupo R1 fornece a afinidade dos BP's pelos cristais ósseos, enquanto o grupo R2 é responsável pela potência e atividade farmacológicas². Segundo Shinoda³, a adição de um grupo hidroxila ao átomo de carbono na posição 1 aumenta a potência anti-reabsortiva. Derivados com um grupo amino no final da cadeia lateral são extremamente ativos. Além disso, o comprimento da cadeia lateral é também muito importante, sendo a maior atividade encontrada em compostos com quatro carbonos, como o alendronato. Mais recentemente tem sido demonstrado que o grupo amino não tem que estar localizado necessariamente

no fim da cadeia. No entanto, ainda não está bem clara a relação existente entre estrutura e atividade, podendo-se dizer apenas que a estrutura P-C-P é fundamental para a atividade e que a intensidade do efeito é intensamente dependente da cadeia lateral.

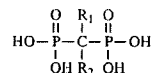


Figura 2. Estrutura química geral dos BP's

Os BP's são sintetizados por meio de uma grande variedade de rotas sintéticas. Estas levam à produção de BP's com diferentes estruturas químicas e rendimentos.

Os BP's podem ser analisados quantitativa e qualitativamente por um conjunto de técnicas analíticas eficientes. As mais usuais são a cromatografia líquida de alta eficiência (HPLC), cromatografia gasosa (GC) e eletroforese capilar (CE), utilizando diferentes detectores, tais como espectrômetro de massas (MS), índice de refração, espectrofotômetro de ultravioleta e eletroquímicos.

O objetivo do presente trabalho é mostrar a importância dos BP's no tratamento de várias doenças ósseas e suas novas aplicações. Propõe-se também descrever as rotas de síntese mais usuais, bem como os diferentes métodos utilizados na análise destes fármacos.

FARMACOLOGIA

Farmacodinâmica

O tecido ósseo contém três tipos de células: osteoblastos, osteoclastos e osteócitos. Os osteoblastos formam os ossos por deposição de materiais protéicos na matriz, levando à mineralização. A função dos osteoclastos é a reabsorção óssea. Essas células destroem a matriz sempre que o osso precisa ser remodelado. O terceiro tipo de células, os osteócitos, encontram-se localizados profundamente nas fibras protéicas da matriz óssea estando, provavelmente, envolvidos na homeostase do cálcio plasmático⁴.

O mecanismo molecular pelo qual os BP's inibem a reabsorção óssea ainda não é completamente entendido. Inicialmente, acredita-

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va-se que apenas um mecanismo físico-químico, após a adsorção óssea, era suficiente para sua ação⁵. No entanto, ultimamente, tem-se tornado claro que efeitos celulares também estão envolvidos⁵. Como os osteoclastos produzem endocitose, os BP's presentes no espaço onde ocorre a reabsorção podem penetrar nestas células, afetando seu metabolismo intracelular e induzindo apoptose⁵. Recentes estudos têm demonstrado que parte da ação inibitória dos BP's sobre os osteoclastos é mediada por uma atividade dos osteoblastos⁶.

Green⁷ demonstrou em estudos *in vitro* que os BP's também inibem a proliferação, reduzem a viabilidade e induzem apoptose em muitas células tumorais humanas.

As propriedades físico-químicas dos BP's são bem similares entre os membros da série. No entanto, suas atividades anti-reabsortivas diferem grandemente. Por causa dessa grande diferença de potência entre os BP's, suas doses clínicas também diferem acentuadamente⁸. Os principais BP's estudados estão apresentados na Tabela 1.

Tabela 1. BP's e suas respectivas potências anti-reabsortivas relativas em modelos *in vivo*

Bisfosfonato	R1	R2	Potência
Clodronato	Cl	Cl	~10
Etidronato	OH	CH ₃	~1
Pamidronato	OH	(CH ₂) ₂ NH ₂	~100
Alendronato	OH	(CH ₂) ₃ NH ₂	>100-<1000
Neridronato	OH	(CH ₂) ₅ NH ₂	~100
Olpadronato	OH	(CH ₂) ₂ N(CH ₃) ₂	>100-<1000
Ibandronato	OH	(CH ₂) ₂ N(CH ₃)(CH ₂) ₄ CH ₃	>1000-<10000
Risedronato	OH	CH ₂ -3-piridina	>1000-<10000
Zoledronato	OH	CH ₂ -imidazol	>10000

Os BP's tornaram-se uma alternativa para o tratamento de uma variedade de doenças ósseas onde há uma excessiva atividade dos osteoclastos, incluindo doença de Paget, hipercalcemia maligna, osteoporose e doença metastática e osteolítica⁹. São também úteis em muitas formas de osteoporose secundária, causadas por utilização de glicocorticóides, gravidez, transplante de órgãos e paraplegia¹.

Altas doses de muitos BP's podem dificultar a mineralização normal dos ossos¹⁰. Dessa forma, busca-se atualmente o desenvolvimento de BP's com alta atividade anti-reabsortiva, que tenham uma ampla faixa de segurança para a mineralização normal.

O *Trypanosoma cruzi* (protozoário causador da doença de Chagas) contém importantes depósitos de pirofosfato e polifosfatos em organelas denominadas acidocalcisomas. Estudos recentes mostram que os BP's inibem o crescimento do protozoário *T. cruzi* *in vitro* e *in vivo*, por meio da competição com o pirofosfato em sua via metabólica, sem causar toxicidade nas células hospedeiras¹¹.

Martin e colaboradores¹² verificaram a ação dos BP's que possuem nitrogênio na cadeia lateral, sobre o crescimento dos parasitas *Trypanosoma brucei*, *Trypanosoma cruzi*, *Leishmania donovani*, *Toxoplasma gondii* e *Plasmodium falciparum*. O mecanismo de ação dos BP's consiste, basicamente, na inibição da formação de proteínas de importância vital para os parasitas.

A artrite reumatóide está relacionada com uma erosão óssea nas articulações. Alguns estudos clínicos têm sido realizados para avaliar a eficiência de agentes anti-artríticos; recentemente, a família dos BP's vem obtendo destaque no tratamento dessa doença¹³. Os ésteres dos BP's têm conseguido diminuir, significativamente, as inflamações associadas com a formação do granuloma cutâneo e com a artrite erosiva, inibindo a erosão óssea nas articulações^{13,14}.

Devido à sua capacidade quelante, os bisfosfonatos também têm sido estudados para elaboração de novos medicamentos capazes de controlar a quantidade de íons ferro e alumínio no organismo huma-

no¹⁵. Recentemente novos BP's foram sintetizados por meio das reações de Michaelis-Arbuzov, Michaelis-Becker e transesterificação do fosfonoacetato de trietila. Estas metodologias foram satisfatórias, tendo em vista o bom rendimento obtido e a facilidade de execução. Estes bisfosfonatos mostraram-se eficientes agentes quelantes frente a diferentes metais¹⁶.

Estudos realizados com etidronato e clodronato demonstraram que estes fármacos apresentam baixa toxicidade. Testes realizados em diferentes espécies animais indicaram não serem teratogênicos, mitogênicos e carcinogênicos¹⁷.

De maneira geral, os BP's apresentam poucos efeitos adversos. O etidronato pode causar inibição na mineralização normal dos ossos, originando fraturas¹⁸. Distúrbios gastrintestinais e alterações no paladar também podem ocorrer¹⁹. Para o clodronato, apenas alguns poucos casos de diarreia branda foram relatados²⁰. Pamidronato pode causar distúrbios gastrintestinais tais como náusea, vômito e diarreia. Após a administração oral ou intravenosa, pode induzir pirexia de 1 a 2 °C²¹.

Farmacocinética

A biodisponibilidade oral, fração de uma dose que alcança a circulação sistêmica, é determinada pela comparação da área total sob a curva de concentração no plasma *versus* tempo, após a administração oral e intravenosa²².

Os BP's apresentam baixa absorção intestinal em humanos, com biodisponibilidade de cerca de 0,7% para alendronato, 0,3% para pamidronato, 3-7% para etidronato e 1-2% para clodronato⁸. Esta baixa absorção ocorre porque estes fármacos são pouco lipofílicos, dificultando o transporte através da barreira epitelial²³. Além do mais, são moléculas relativamente grandes, negativamente carregadas no pH intestinal e complexam facilmente com o cálcio, prejudicando ainda mais a sua absorção²⁴. Os alimentos causam uma acentuada diminuição na absorção dos BP's, devendo estes serem administrados pelo menos 30 min antes da primeira alimentação diária²⁵.

Estudos realizados em ratos, com o alendronato, demonstraram que ele possui forte interação com as proteínas plasmáticas, principalmente a albumina sérica. O aumento do pH e da concentração de íons cálcio acentua ainda mais essa interação²⁶.

Após a administração intravenosa, o alendronato é amplamente distribuído por todo o corpo, incluindo os tecidos calcificados e não calcificados. Os BP's ligam-se preferencialmente em ossos com alta taxa de remodelagem, devido à grande exposição da hidroxiapatita nesses sítios⁸.

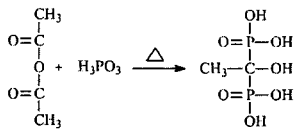
Não foram encontradas evidências de metabólitos dos BP's nos estudos realizados, indicando serem muito estáveis. Essa característica minimiza a toxicidade do tratamento, devido à ausência de intermediários reativos e metabólitos tóxicos²⁷.

A excreção renal é a única rota de eliminação dos BP's. Estudos realizados em animais e humanos indicam que os BP's administrados sistemicamente são parcialmente ligados aos tecidos ósseos e o restante é excretado pelos rins. Após a administração intravenosa, os BP's desaparecem do plasma rapidamente, com uma meia-vida de 1-2 h. Por outro lado, uma vez ligado ao osso, eles só são liberados quando o tecido ósseo, ao qual estão ligados, sofre reabsorção⁸.

SÍNTESE

O primeiro bisfosfonato utilizado no tratamento de doenças ósseas foi o etidronato, o qual pode ser facilmente sintetizado por meio de reação entre anidrido acético e ácido fosforoso (Esquema 1)²⁸.

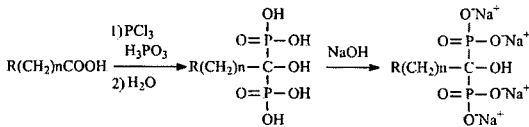
Estudos posteriores demonstraram que os BP's que possuem um átomo de nitrogênio em sua cadeia lateral possuem potência supe-



Esquema 1. Síntese do etidronato

rrior ao etidronato²⁹. Mais recentemente, foi demonstrado que o grupo amino não deve estar, necessariamente, no final da cadeia. A atividade é aumentada quando um grupo metil e um pentil são ligados ao nitrogênio³⁰.

A reação mais comum utilizada na preparação desses BP's é entre um ácido carboxílico e o ácido fosforoso, na presença de tricloreto de fósforo (Esquema 2)³⁰. Vários tipos de BP's são obtidos a partir de diversos ácidos carboxílicos, com bons rendimentos (Tabela 2). Os reagentes são deixados sob agitação a 65 °C por aproximadamente 18 h, utilizando como solvente o ácido metanossulfônico ou benzenossulfônico. Logo após, é adicionado hidróxido de sódio para ocorrer a precipitação dos sais de BP's.



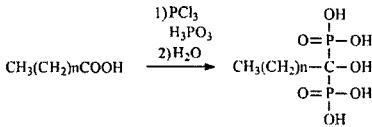
Esquema 2. Reação entre ácido carboxílico e ácido fosforoso na presença de tricloreto de fósforo

Tabela 2. Valores típicos de rendimento da síntese dos BP's para diferentes ácidos carboxílicos

R	n	R%
NH ₂	2	57
NH ₂	3	89
NH ₂	4	78
NH ₂	5	89
CH ₃	10	95
3-imidazol	1	31
3-piridil	1	38
4-aminofenil	1	26
Cl	3	30

Esta síntese também pode ser realizada utilizando-se ácidos graxos de cadeia pequena e que não contenham nitrogênio (Esquema 3). Os BP's assim formados mostraram-se ativos contra o crescimento do *Trypanosoma cruzi*¹¹.

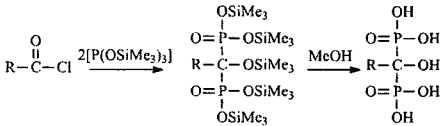
Outra rota de síntese mais moderna é realizada pela reação entre um cloreto de acila e o tris(trimetilsilil)fosfito, com posterior metanólise³¹.



Esquema 3. Síntese de BP's a partir de ácidos graxos de cadeia pequena (n = 1, 2, 3 e 4)

A reação do cloreto de acila com 2 equivalentes de tris(trimetilsilil)fosfito leva, em poucos minutos e com temperatura

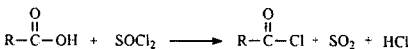
de 25 °C, à formação do éster tetrakis(trimetilsilil) do ácido 1-trimetilsiloxi-1,1-bifosfônico. Logo após, ocorre a metanólise durante 1 h à temperatura de 25 °C. Os BP's são obtidos com a evaporação da fração volátil (Esquema 4).



Esquema 4. Síntese de BP's por meio da reação entre o cloreto de acila e o tris(trimetilsilil)fosfito

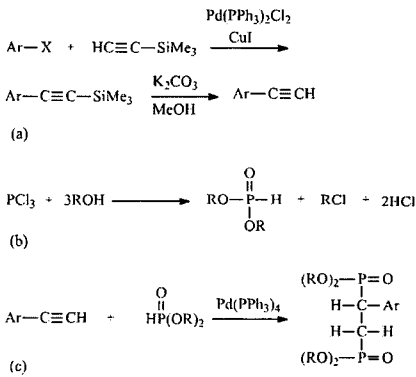
Esta reação possui inúmeras vantagens quando comparada à reação mencionada anteriormente, destacando-se: condições mais brandas do sistema reacional, maiores rendimentos obtidos e menores tempos de reação. Por outro lado, observam-se algumas desvantagens: alto preço do reagente tris(trimetilsilil)fosfito e dificuldade de se encontrar comercialmente diferentes cloretos de acila (sendo necessária a preparação dos mesmos)³¹.

A preparação de cloretos de acila é bastante conhecida na literatura e consiste na reação entre um ácido carboxílico e o cloreto de tionila (Esquema 5)³². Entretanto, isso representa uma nova etapa na síntese dos BP's, acarretando, principalmente, redução do rendimento total.



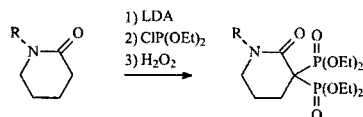
Esquema 5. Síntese de cloretos de acila

Outro método de preparação dos BP's consiste na reação de bis-hidrofosforilação de alcinos terminais por fosfitos de dialquila, catalisada por paládio³³. Os alcinos terminais são sintetizados por uma reação de Heck entre um haleto de arila e o trimetilsililacetileno, com a remoção do grupo trimetilsilil em condições básicas (Esquema 6a). Os fosfitos de dialquila, por sua vez, são sintetizados pela reação entre um equivalente de tricloreto de fósforo e três equivalentes do álcool correspondente (Esquema 6b)³⁴. Refluxando a mistura contendo um equivalente do alcino terminal e três equivalentes de fosfito de dialquila, na presença de Pd(PPh₃)₄ e tolueno (solvente), obtém-se uma grande variedade de BP's vicinais com ótimos rendimentos (Esquema 6c)³³.



Esquema 6. Síntese de BP's através de uma reação de bis-hidrofosforilação catalisada por paládio: (a) síntese do alcino terminal; (b) síntese do fosfito de dialquila; (c) reação do alcino terminal com fosfito de dialquila

Outros tipos de BP's podem ser preparados utilizando os íons enolato como intermediários. O tratamento de compostos carbonílicos com uma base forte e posterior adição de excesso de grupos eletrofílicos [CIP(OEt)₂] resulta na formação de duas ligações P-C no carbono α. Estes BP's são obtidos após oxidação com peróxido de hidrogênio³⁵. O Esquema 7 ilustra uma reação realizada nestas condições.



Esquema 7. Síntese de BP's utilizando íons enolato como intermediários

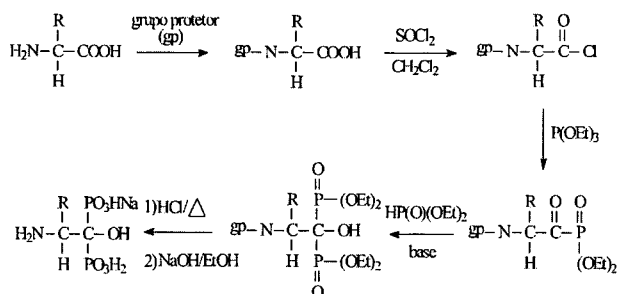
Na Tabela 3 são mostrados outros exemplos de produtos formados a partir de diferentes substratos³⁵.

Tabela 3. BP's obtidos a partir de diferentes lactamas e compostos carbonílicos

Reagentes	Produtos	Rendimento (%)
		77
		67
		72

R =

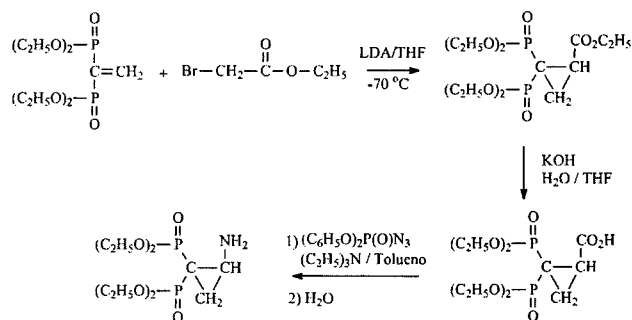
BP's derivados de α-aminoácidos foram sintetizados, demonstrando também atividade anti-reabsortiva³⁶. Nesta reação é feita, inicialmente, a proteção do grupo amina do aminoácido, seguida de substituição do grupo hidroxila pelo cloro. Posteriormente, é feita a inserção dos átomos de fósforo em duas etapas. Primeiramente, ocorre reação com um reagente adequado de fósforo, formando um fosfonato de acila e, em seguida, ocorre ataque de um segundo átomo de fósforo, formando o bisfosfonato. Finalmente, o grupo protetor é retirado, os grupos ésteres são hidrolisados e os BP's são obtidos como sais de sódio (Esquema 8)³⁶.



Esquema 8. Síntese de BP's derivados de α-aminoácidos

Como descrito anteriormente, a estrutura da cadeia lateral, bem como a presença ou não de heteroátomo fornece características bem distintas aos BP's. O 2-aminociclopropilideno-1,1-bisfosfonato de tetraetila, ainda não avaliado clinicamente, possui anel de 3 membros em sua cadeia lateral, podendo proporcionar uma atividade farmacológica ainda não encontrada em outros BP's³⁷.

A síntese deste composto é realizada em três passos (Esquema 9). Primeiramente, o lítio-carbânion do 2-bromoacetato de etila é adicionado, via adição de Michael, ao vinilideno 1,1-bisfosfonato de tetraetila. Essa adição é seguida de uma ciclicização intramolecular levando à formação de um derivado ciclopropil. Posteriormente, este derivado é saponificado, e o ácido obtido é convertido em uma amina³⁷.



Esquema 9. Síntese do 2-aminociclopropilideno-1,1-bisfosfonato de tetraetila

ANÁLISE

Os BP's podem ser analisados por várias técnicas analíticas, destacando-se a cromatografia gasosa, a cromatografia líquida de alta eficiência e a eletroforese capilar. Estas técnicas são utilizadas para a determinação de BP's na matéria-prima, no produto acabado, e em fluidos biológicos, sendo neste caso necessário o desenvolvimento de metodologias analíticas com alta sensibilidade, tendo em vista a baixa concentração destes fármacos neste meio.

A análise em meio biológico requer, muitas vezes, a utilização de técnicas de pré-concentração, tais como microextração em fase sólida (SPME) e extração em fase sólida (SPE), sendo esta última a mais comum.

O desenvolvimento de métodos sensíveis é dificultado pelas características das moléculas dos BP's mais utilizados. Estes não possuem grupos cromóforos e não podem ser determinados pelos detectores mais usuais (ultravioleta e fluorescência). Para contornar esta dificuldade têm sido desenvolvidos métodos de derivação e detecção indireta. Entretanto, estes métodos requerem maior tempo para sua execução, além de serem mais passíveis de erros.

Nos últimos anos tem-se observado um aumento no desenvolvimento de métodos que utilizam a espectrometria de massas. Este detector possui a grande vantagem de ser seletivo e universal, além de possuir boa sensibilidade e robustez. Além disso, permite a análise direta, sem necessidade de derivação. Por outro lado, é uma técnica mais cara e que exige maior experiência por parte dos analistas.

Cromatografia Líquida de Alta Eficiência (HPLC)

Esta técnica é a mais utilizada para análise dos BP's, sendo empregados diferentes detectores, fases móveis e mecanismos de retenção.

Chester³⁸ desenvolveu um detector seletivo para fósforo, baseado na emissão molecular de luz pelo HPO, para HPLC, analisando

diversos ácidos fosfônicos. Posteriormente, o mesmo autor utilizou esse detector para análise do clodronato em fluidos biológicos. O mecanismo de separação empregado foi de troca aniônica, obtendo-se um limite de quantificação de 2 µmol/L³⁹.

Foram relatados vários métodos utilizando detecção por índice de refração. Um método para determinação do ácido metileno-bisfosfônico, utilizando pareamento iônico, foi desenvolvido por Yeh⁴⁰. Foi utilizada uma coluna convencional C₁₈ e o composto tri-*n*-octilamina como agente de pareamento iônico. Wong e colaboradores⁴¹ desenvolveram um método empregando coluna de troca aniônica, para análise de diferentes polifosfonatos, obtendo limites de detecção de 2,4 a 15,2 ppm. Han e colaboradores⁴² analisaram comprimidos de alendronato sódico utilizando coluna de troca aniônica. Este método mostrou-se exato, específico, robusto e com limite de detecção adequado para análises de rotina de comprimidos.

A análise de BP's utilizando detectores de fluorescência também é muito utilizada. Como estas moléculas, em geral, não possuem grupos que fluorescem, são necessárias algumas estratégias para a utilização de tais detectores. Derivatizações pré ou pós-coluna e detecção indireta são as mais utilizadas. No primeiro caso, uma molécula fluorescente é ligada ao BP, enquanto na detecção indireta o composto fluorescente encontra-se presente na fase móvel.

Métodos de determinação indireta de BP's foram desenvolvidos utilizando o complexo de alta fluorescência formado entre o alumínio e o morin (2',3',4',5,7-pentidroxiflavona). Na presença dos BP's o alumínio liga-se a estes compostos formando um complexo mais estável e que não fluoresce. A passagem do complexo alumínio-morin pelo detector de fluorescência gera absorção ao passo que o complexo BP-alumínio, ao passar pelo detector, causa diminuição da absorção, registrando um pico negativo^{43,44}. A detecção por fluorescência com derivatização pós-coluna foi descrita por Kwong e colaboradores⁴⁵ para a análise de comprimidos de alendronato. Foram utilizados os compostos *o*-ftalaldeído e mercaptoetanol (OPA-MERC) como reagente pós-coluna, sendo avaliada a eficiência da reação de derivatização, variando-se os seguintes parâmetros: pH, fluxo da fase móvel, fluxo do reagente pós-coluna, temperatura da reação, tamanho e tipo do reator. Foi utilizada uma coluna de fase reversa com pareamento iônico.

Vários compostos podem ser utilizados para derivatização pré-coluna em detecção por fluorescência. O pamidronato foi determinado em urina, empregando a fluorecamina como reagente de fluorescência e coluna de fase reversa (C₁₈). O limite de quantificação obtido foi de 1 µmol/L de urina e o limite de detecção foi de 50 nmol/L de urina⁴⁶. Posteriormente, foram alcançados melhores limites de quantificação e detecção (0,7 µmol/L de urina e 10 nmol/L de urina, respectivamente), aprimorando-se o processo de extração dos analitos⁴⁷.

O composto 2,3-naftaleno dicarboxaldeído também é utilizado em derivatizações pré-coluna. Kline e colaboradores⁴⁸ determinaram o alendronato em urina utilizando este reagente, na presença do íon cianeto, e coluna de fase reversa polimérica. Foi obtido limite de quantificação de 5 ng/mL. Este método foi melhorado substituindo-se o íon cianeto pelo composto N-acetil-D-penicilamina, obtendo-se um limite de quantificação de 1 ng/mL⁴⁹.

Outra opção para a derivatização pré-coluna é a utilização do reagente 1-naftilisotiocianato, para a determinação do pamidronato em fluidos biológicos. Para tanto, a separação foi feita por meio de pareamento iônico⁵⁰⁻⁵².

Para a determinação de BP's utilizando detectores de ultravioleta (UV) são também necessárias estratégias, devido à ausência de grupos cromóforos nestes fármacos. Sparidans e colaboradores⁵³ desenvolveram um método para a análise de oito diferentes BP's, onde estes são complexados "in-line" com íons cobre(II), absorvendo no UV em 245 nm. Utilizou-se coluna de troca aniônica e fase móvel

constituída de ácido nítrico 1,5 mmol/L e nitrato de cobre(II) 0,5 mmol/L. O limite de detecção obtido foi de 0,4 µg/mL⁵³.

Foram também desenvolvidos métodos utilizando derivatizações pré-coluna. De Marco e colaboradores⁵⁴ determinaram o alendronato empregando o composto cloroformato de 9-fluorenilmetil (FMOC), na presença de citrato de sódio, como reagente de derivatização. Utilizou-se coluna de fase reversa polimérica, com detecção em 266 nm. Outro método foi desenvolvido utilizando o isotiocianato de fenila seguido de tratamento com peróxido de hidrogênio, antes da injeção. Os limites de detecção e quantificação obtidos foram de 0,1 µg/mL⁵⁵.

A análise de BP's com detecção no UV-Visível e derivação pós-coluna é muito utilizada. Daley-Yates e colaboradores⁵⁶ desenvolveram um método para análise em plasma e urina. Os BP's foram oxidados a ortofosfato com persulfato de amônio e posterior reação com molibdênio-ascorbato, formando o cromóforo fosfomolibdato, detectado em 820 nm. Obteve-se limite de detecção de 10 ng/mL.

Foi desenvolvido outro método para análise de clodronato, empregando coluna de poliestireno-divinilbenzeno. O analito foi detectado em 300 nm após reação com solução ácida de ferro (III)⁵⁷. Foram propostos métodos utilizando o complexo tório-EDTA-laranja de xilenol. Os BP's reagem quantitativamente com este complexo em condições levemente ácidas^{58,59}.

Outra alternativa para a detecção no UV são os métodos indiretos. Tsai e colaboradores⁶⁰ propuseram um método para análise de comprimidos de etidronato utilizando ácido nítrico (absorção máxima em 220 nm) como eluente. Os cromatogramas obtidos apresentam uma queda no sinal quando ocorre a passagem dos BP's pela célula do detector.

Detectores eletroquímicos também podem ser utilizados na determinação de BP's, com a vantagem de não haver necessidade de derivação do analito. Entretanto, são muito sensíveis a variações externas, o que compromete a reprodução dos resultados. Usui e colaboradores⁶¹ propuseram um método para análise de um novo BP, extraído de plasma, urina e osso, empregando detector eletroquímico operando no modo oxidativo. Foram obtidos limites de quantificação de 0,5 ng/mL no plasma, 1 ng/mL na urina e 25 ng/g no osso.

Detectores condutimétricos são utilizados na determinação de diferentes BP's, também sem a necessidade de derivatização do analito. São empregadas colunas de troca aniônica⁶²⁻⁶⁴.

Qin e colaboradores⁶⁵ desenvolveram método para a determinação de alendronato (altamente polar e termolábil) em comprimidos utilizando cromatografia iônica acoplada a detector de massas, empregando interface do tipo íon-spray, operando no modo negativo. Observou-se neste estudo que a fragmentação dominante deste composto é a clivagem das ligações C-P.

O clodronato e BP's relacionados foram analisados por espectrometria de massas com fonte de ionização por eletrospray no modo negativo, em um equipamento do tipo triplo quadrupolo. Foram propostos diferentes caminhos para a fragmentação destes compostos e avaliada a influência do pH no grau de desprotonação⁶⁶.

Cromatografia Gasosa

Os BP's possuem natureza polar e baixa volatilidade. Desta forma, para análise por cromatografia gasosa, é necessário o processo de derivatização para a formação de compostos voláteis e estáveis. Comprimidos de etidronato foram analisados por meio da derivatização com bis(trimetilsilil)-trifluoroacetamida. Utilizou-se detector de ionização de chama para análise e detector de massas para controle do derivado formado. O método mostrou-se rápido e específico, porém sem sensibilidade suficiente para análise deste fármaco em fluidos biológicos⁶⁷.

Auriola e colaboradores⁶⁸ descreveram um método para análise

de clodronato em urina. O analito extraído reagiu com um derivado trimetilsilil e foi analisado utilizando detector de massas.

Foi desenvolvida metodologia para análise de diferentes BP's nitrogenados, utilizando o composto cloroformato de isobutila como reagente de derivatização. Utilizou-se detector fotométrico de chama, obtendo-se limite de detecção no plasma de 200 ng/mL. Realizou-se também análise com espectrômetro de massas para confirmação estrutural dos derivados formados⁶⁹.

Eletroforese Capilar

Isotacoforese capilar foi utilizada para análise de pamidronato e impurezas de síntese e degradação presentes. Foram comparados dois sistemas com diferentes condições e estes mostraram-se adequados para análises de rotina⁷⁰.

Um método para análise de alendronato em formulações farmacêuticas foi descrito por Tsai e colaboradores⁷¹. Utilizou-se detecção direta no UV baseada na formação "on-line" de complexo cromóforo entre este fármaco e os íons cobre(II) presentes na solução eletrolítica. No entanto, este método não pode ser utilizado para análise de soluções intravenosas devido à presença dos ânions citrato (forte agente quelante) em suas formulações.

Sirén e colaboradores⁷² desenvolveram método para análise de BP's, com detecção indireta no UV, utilizando corantes nitrosonaftóis sulfonados na solução eletrolítica. O limite de detecção obtido foi de 1 µg/mL em 254 nm.

Peng e colaboradores⁷³ determinaram um BP, 2-tioetano-1,1-ácido bisfosfônico, utilizando método com determinação direta no UV, coluna revestida com glicerol, fosfato em alta concentração como tampão e injeção eletrocínica sob condições de "sample stacking". O limite de detecção foi de 50 ng/mL.

Um método para determinação de clodronato e suas impurezas, utilizando detecção de massas por eletrospray e modo negativo, foi desenvolvido por Huikko e colaboradores⁷⁴. Este mostrou alta capacidade de separação e especificidade para análise de BP's.

Um método com detecção direta no UV em baixo comprimento de onda e polaridade invertida foi aplicado para separação e quantificação de impurezas em clodronato. Utilizou-se capilar de poliácridamida e avaliou-se a influência do pH e força iônica do eletrólito sobre a separação⁷⁵.

Outros métodos

Reed e colaboradores⁷⁶ descreveram um método direto para análise de alendronato em comprimidos. As amostras foram dissolvidas em água e o conteúdo de fósforo foi analisado por plasma acoplado indutivamente (ICP). O método apresentou boa precisão e exatidão.

A formação de complexo entre alendronato e cloreto férrico em solução de ácido perclórico foi estudada e analisada por espectrofotometria. Este método apresentou limite de detecção de 2 µg/mL, suficiente para análise nas formulações farmacêuticas⁷⁷.

CONCLUSÃO

Os BP's são fármacos de escolha para uma variedade de doenças ósseas, destacando-se dentre estas a osteoporose, que tem se tornado nos últimos anos um importante problema de saúde pública, principalmente devido ao aumento da expectativa de vida da população.

Além disso, outras aplicações relevantes têm sido relatadas com o uso dos BP's. Estes fármacos têm se mostrado ativos sobre o metabolismo de vários protozoários, podendo ser utilizados no tratamento da doença de Chagas, leishmaniose visceral, malária e toxoplasmose, doenças que acometem milhões de indivíduos em todo o mundo.

Um grande número de rotas sintéticas tem sido relatado para a preparação de diferentes BP's, objetivando-se melhorar as condições experimentais, os rendimentos e os custos, bem como encontrar estruturas com maior potência e atividade farmacológica.

Os BP's, devido à sua baixa absorção, são encontrados em concentrações muito pequenas nos fluidos biológicos. A grande maioria dos métodos analíticos descritos mostraram-se adequados para a determinação destes fármacos na matéria-prima e nas formulações farmacêuticas, entretanto, ainda há a necessidade do desenvolvimento de métodos mais sensíveis, de fácil execução e de baixo custo, para determinações em meio biológico.

Métodos adequados de análise em fluidos biológicos são fundamentais para a realização de estudos de biodisponibilidade e bioequivalência. Este último constitui uma das etapas na obtenção de um medicamento genérico, que é atualmente considerada como política de prioridade do Ministério da Saúde do Brasil.

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DESCRIPCION

Procedimiento para la purificación de oxiclورو de fósforo.

El oxiclورو de fósforo se fabrica industrialmente a partir de tricloruro de fósforo mediante oxidación con oxígeno del aire. En este caso se obtiene un producto muy puro, apenas impurificado con productos orgánicos.

En los últimos años se han dado a conocer procedimientos de cloración para la fabricación de heterociclos clorados, preferentemente de heterociclos nitrogenados, a partir de hidroxicompuestos con oxiclورو de fósforo y subsiguiente o simultánea regeneración de POCl_3 con PCl_5 , que también puede formarse in situ durante la reacción de cloración a partir de PCl_3 y de cloro.

El POCl_3 , recuperado mediante eliminación por destilación, está impurificado, la mayoría de las veces, por compuestos orgánicos, en gran parte clorados, que dificultan o imposibilitan una reutilización. Los orígenes están constituidos por productos orgánicos con un punto de ebullición similar, tal como el POCl_3 , de manera que solo es difícil o en todo caso imposible una separación por destilación de los productos perjudiciales presentes en el intervalo de ppm. La tarea de la invención consistía en separar estos productos, parcialmente muy reactivos, de manera eficaz, del oxiclورو de fósforo.

Esta tarea pudo resolverse ahora de manera inesperada mediante la adición de aminas terciarias orgánicas, tales como trialquilaminas, piridinas, anilinas y con una proporción de reciclo especial durante la destilación fraccionada.

El objeto de la presente invención consiste, por lo tanto, en un procedimiento para la purificación de oxiclورو de fósforo recuperado o regenerado, impurificado por compuestos orgánicos reactivos, con un 0,1 hasta un 5 % en peso de una amina orgánica con 1 ó 2 átomos de N-terciarios y con un punto de ebullición por encima del punto de ebullición del oxiclورو de fósforo y destilación fraccionada a presión normal, caracterizado porque se establece durante la destilación fraccionada, hasta alcanzarse el punto de ebullición del oxiclورو de fósforo puro en la cabeza de la columna de destilación, una proporción de reciclo A:R de 1:1 hasta 1:4 y, as continuación, se retira oxiclورو de fósforo puro con una relación de reciclo A:R de 1:1 hasta 4:1.

En el procedimiento según la invención se purifica oxiclورو de fósforo (POCl_3), que se recupera o que se regenera después de las reacciones de cloración, de compuestos orgánicos reactivos. Por reacciones de cloración deben entenderse en este caso procedimientos para la obtención de heterociclos clorados, especialmente de heterociclos nitrogenados clorados tales como por ejemplo cloropirimidinas.

Las impurezas perjudiciales son en este caso, por ejemplo, diversos cloruros de carbonilo, tales como por ejemplo ácidos mono-, di- y triclorocarboxílicos, así como nitrilos de ácidos carboxílicos.

El procedimiento según la invención es especialmente adecuado para la eliminación de derivados de ácidos carboxílicos con 2 a 4 átomos

de carbono, especialmente de derivados del ácido acético, que proceden de diversos productos de partida o de las impurezas de los mismos. Para la eliminación de las impurezas perjudiciales se combina el POCl_3 , a ser purificado, con aminas orgánicas, que tienen 1 ó 2 átomos de N terciarios.

Las aminas adecuadas con 1 ó 2 átomos de N terciarios son, en este caso, aquellas que presenten un punto de ebullición mayor que el del POCl_3 . Preferentemente su punto de ebullición se encuentra por encima de los 150°C , de manera especialmente preferente por encima de los 200°C .

Ejemplos de aminas adecuadas son trialquilaminas con 4 hasta 12 átomos de carbono en las partes alquilo. Las partes alquilo pueden ser en este caso iguales o diferentes, así como lineales o ramificadas. Preferentemente se emplearán trialquilaminas con 4 a 8 átomos de carbono en la parte alquilo, siendo a su vez preferentemente iguales las partes alquilo.

Las trialquilaminas adecuadas son, por lo tanto, por ejemplo n-tributilamina, n-trihexilamina, n-trioctilamina, n-tridecilamina etc.

Otras aminas, que pueden emplearse en el procedimiento según la invención son piridinas, que presentan de 1 hasta 3 substituyentes. Los substituyentes adecuados son, por ejemplo, grupos alquilo con 1 a 4 átomos de carbono, grupos arilo o cloro. Ejemplos de piridinas adecuadas son 2-, 3- ó 4-metilpiridina, 2,4-dimetilpiridina, 2,4,6-trimetilpiridina, 5-etil-2-metilpiridina, etc. Las piridinas, que pueden estar substituidas por grupos arilo, son en este caso, por ejemplo, 2-fenilpiridina y similares, así como también sistemas anulares benzocondensados, tales como quinolina e isoquinolina.

Como aminas terciarias adecuadas entran en consideración además anilinas o toluidinas N,N-substituidas, tales como por ejemplo N,N-di-alquilanilinas, o bien -toluidinas con 1 a 4 átomos de carbono, por ejemplo N,N-dimetilanilina, etc.

La cantidad de la amina, a ser añadida, depende en este caso de la cantidad presente de impurezas. Preferentemente se empleará desde un 0,1 hasta un 5 % en peso, referido al POCl_3 , de amina. De forma especialmente preferente se añadirá desde un 0,2 hasta un 2 % en peso de amina.

Tras adición de la amina se calienta la mezcla de la reacción, tratada de este modo, hasta la temperatura de reflujo y se deja reaccionar aproximadamente durante 10 hasta 60 minutos a esta temperatura. A continuación se aísla el POCl_3 , purificado, por medio de destilación fraccionada a partir de la mezcla de la reacción. Para ello se emplea preferentemente una columna de destilación con 5 a 10 platos teóricos.

En la primera fase de la destilación se establece una proporción de reciclo A:R de 1:1 hasta 1:4, de forma especialmente preferente de 1:2 hasta 1:3 y en primer lugar se separan los compuestos fácilmente volátiles. Cuando se alcanza en la cabeza de la columna el punto de ebullición del POCl_3 puro, se retira el POCl_3 puro a continuación con una proporción de reciclo A:R de 1:1 hasta 4:1 preferentemente de 3:1.

El residuo de la destilación puede permanecer en el recipiente de destilación y puede someterse

de nuevo a una destilación junto con la carga siguiente o con los productos de cabeza reunidos.

El POCl₃ purificado, obtenido mediante el procedimiento según la invención, corresponde a los criterios importantes para una reutilización, que se verifican mediante la medida del punto de solidificación (>0,8°C), del contenido en carbono orgánico (TOC <200 ppm), del contenido en Fe, Cr, Ni (<5 ppm), así como mediante el ensayo de coloración con piridina y por medio de ensayos de cromatografía gaseosa.

Ejemplo 1

Se combinaron, en un matraz de 1 litro, con termómetro, cestillo de calefacción, con una columna de cuerpos de relleno con una longitud de 1 metro, cargada con anillos Raschig de 3 mm, parte de reflujo, termómetro y refrigerante de reflujo, 500 ml de oxiclورو de fósforo que contienen 400 ppm de TOC (recuperadas a partir de una reacción de cloración para la obtención de 4,6-dicloropirimidina), con 5,0 g de n-tributilamina y se calientan a ebullición bajo reflujo. Al cabo de 30 minutos de reflujo total se estableció una temperatura en la cabeza de la columna de 101,8°C. A continuación se ajustó una proporción de reflujo de 1:3. Tras una eliminación por destilación de 10 ml se alcanzaron 103,1°C, tras 25 ml, 105,3°C, la temperatura de la cabeza permaneció entonces constante a esta temperatura. Una vez que estaban presentes 40 ml de destilado al cabo de 20 minutos de tiempo de destilación, se conmutó a la fracción principal y la proporción de reflujo se reajustó a 3:1. Al cabo de 80 minutos de tiempo de destilación y con una temperatura de transición constante de 105,3°C, se retiraron 420 ml de fracción principal. El producto era incoloro, contenía 50 ppm de TOC y no tenía metales pesados a escala de ppm, el punto de solidificación era de 1,1°.

Se añadieron al residuo líquido, coloreado de negro, otros 500 ml de POCl₃ y se llevó a cabo la destilación como se ha descrito anteriormente. Además de 40 ml de cabeza se retiraron de nuevo 420 ml de fracción principal, que correspondía de nuevo a las exigencias de las normas establecidas. Se encontraron 60 ppm de TOC.

Ahora se recargaron de nuevo 500 ml de POCl₃ y de nuevo se llevó a cabo la destilación. Los 420 ml de la fracción principal, contenían 100 ppm de TOC y en el ensayo a la piridina se encontraba en el límite con respecto a la coloración. Todos los demás criterios eran conformes.

Al cabo de otra adición de 500 ml de POCl₃ y realización de la destilación se retiraron 480 ml de destilado como fracción principal, que contenía 110 ppm de TOC pero que ya no correspondían al ensayo a la piridina. La serie de ensayos se interrumpió con los resultados siguientes:

Carga 2.000 ml de POCl ₃ obtenido a partir de reacciones de cloración		
160 ml de cabeza	=	8 %
1740 ml de corriente principal	=	87 %
100 ml de reciclo	=	5 %.

Con empleo de 5 g de tributilamina se obtuvieron 1260 ml ó 2100 g de POCl₃ puro que corresponde a un gasto de tributilamina del 0,24 % refe-

rido al POCl₃ puro o del 0,2% referido al POCl₃ empleado, impurificado.

Ejemplo 2

Se combinaron en un matraz de 2 litros, con columna y con distribuidor de reflujo, como se ha descrito en el ejemplo 1, 1.500 ml de POCl₃ recuperado y que no corresponde a las exigencias de las normas establecidas, que contienen 400 ppm de TOC con 25 g de trioctilamina y a continuación se elaboraron por destilación como se ha descrito en el ejemplo 1. Al cabo de 1/2 horas de ebullición a reflujo permaneció constante la temperatura de la cabeza a 99,8°C. Tras retirada de 55 ml de destilado con una proporción de reflujo de 1:3 se estableció una temperatura de cabeza de 105,4°C, al cabo de otros 50 ml de destilado, la temperatura permaneció constante a 105,7°C. Se necesitaron 27 minutos para la eliminación por destilación de 105 ml de producto de cabeza. Ahora se conmutó a la fracción principal con una proporción de reciclo de 3:1 y se retiraron 1300 ml de destilado (2 x 500 ml, 1 x 200 ml, 1 x 100 ml). La temperatura de la cabeza permaneció constante a 105,7°C. El conjunto de los 1300 ml de las fracciones principales correspondía a los requisitos de pureza.

Se añadieron a la cola remanente otros 1500 ml de POCl₃ impurificado y se repitió el proceso de destilación. Al cabo de 110 ml de producto de cabeza se retiraron 1370 ml de corriente principal que correspondía a todas las exigencias de las normas establecidas. A partir de 3000 ml de POCl₃ bruto se obtuvieron 215 ml de producto de cabeza, lo que corresponde a un 7,2% y 2670 ml de producto puro, lo que corresponde a un rendimiento del 89%.

Ejemplo 3

Se combinaron 200 g de POCl₃ impurificado con 2 g de 5-etil-2-metilpiridina y se sometieron a destilación fraccionada a través de una columna. Se obtuvieron 17 g (8,5%) de producto de cabeza y 160 g (80%) de fracción principal, que correspondía a todas las exigencias de las normas establecidas.

Ejemplo 4

Se cargaron en una cuba esmaltada de 2,5 m³, con calefacción por vapor, con la configuración de utillaje, descrita en el ejemplo 1, con una potencia de separación equivalente a la de la columna de vidrio, 2500 kg de POCl₃, que contienen 400 ppm de TOC procedente de un proceso de cloración y se combinaron con 20 litros de n-tributilamina, a continuación se hirvió en el refrigerador a reflujo durante 30 minutos, seguidamente se retiraron de la cabeza de la columna 150 kg de producto de cabeza con una proporción de reflujo de 1:3, hasta un punto de ebullición constante en la cabeza, seguidamente se conmutó a la fracción principal y se eliminaron por destilación 1700 kg de POCl₃ con una proporción de reflujo de 3:1. La destilación se interrumpió, se recargaron en la cuba 1850 kg de POCl₃ impurificado, obtenido en el proceso de cloración, y se añadieron 15 litros de n-tributilamina, a continuación se destiló de nuevo como se ha descrito anteriormente, obteniéndose en este caso nuevamente 150 kg de producto de cabeza y 1470 kg de destilado puro. Se llevaron a cabo otras cuatro cargas ulteriores de 7380 kg en

total de POCl₃ y 60 litros de n-tributilamina. A partir de 11630 kg de POCl₃ en bruto se obtuvieron 9,9 toneladas de POCl₃ puro, de acuerdo con las normas establecidas, aproximadamente 0,9 toneladas de producto de cabeza, que se recicló de nuevo al proceso de cloración, y 760 kg de residuo de destilación, que contenía todavía aproximadamente un 70% de POCl₃ elaborable por destilación. La cantidad total de POCl₃ recuperado y reutilizable fue, por lo tanto, aproximadamente de 11430 kg, es decir un 97,4% de la cantidad de POCl₃ empleada.

Ejemplo 5

Se dispusieron en un aparato de destilación, como el que se ha descrito en el ejemplo 1, en un matraz de 1 litro, 440 ml ó 660 g de residuo de destilación procedente de la destilación descrita en el ejemplo 4 y se recuperaron 280 ml de POCl₃ puro hasta un punto de ebullición de la mezcla de la reacción de 118°C, con una proporción de reflujo de 2:1.

El residuo negro remanente, viscoso a temperatura ambiente, puede eliminarse mediante combustión en una instalación adecuada.

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REIVINDICACIONES

1. Procedimiento para la purificación de oxiclورو de fósforo recuperado o regenerado, impurificado por compuestos orgánicos reactivos, con 0,1 hasta 5 % en peso de una amina orgánica con 1 ó 2 átomos de N terciarios y con un punto de ebullición mayor que el punto de ebullición del oxiclورو de fósforo y destilación fraccionada a presión normal, **caracterizado** porque en la destilación fraccionada se establece una proporción de reciclo en la cabeza de la columna de destilación, hasta alcanzarse el punto de ebullición del oxiclورو de fósforo puro, A:R de 1:1 hasta 1:4 y

a continuación se retira el oxiclورو de fósforo puro con una proporción de reciclo A:R de 1:1 hasta 4:1.

2. Procedimiento según la reivindicación 1, **caracterizado** porque la destilación fraccionada se lleva a cabo por medio de una columna de destilación con 5 hasta 10 platos teóricos.

3. Procedimiento según la reivindicación 1, **caracterizado** porque el residuo de la destilación permanece en el matraz de destilación y se somete a otra destilación fraccionada junto con la siguiente carga o con los productos de cabeza reunidos.

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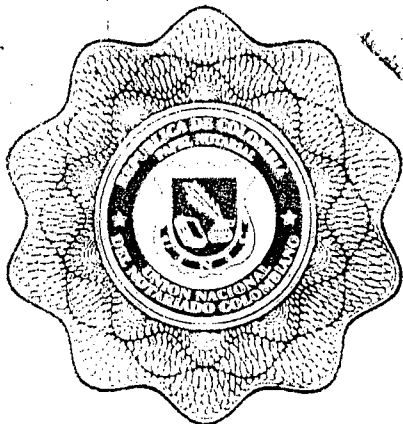
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REPUBLICA DE COLOMBIA

NOTARIA 33 DE BOGOTA D.C.

ESCRITURA PUBLICA NUMERO: 1 6 5 8

- - MIL SEISCIENTOS CINCUENTA Y OCHO - -

FECHA DE OTORGAMIENTO: CUATRO (4) DE

JULIO - - - - -

DEL AÑO DOS MIL DOS (2002). -----

ACTO: -----

1.- PODER ESPECIAL. -----

OTORGADO POR: LAFRANCOL S.A., con Nit. No. - - - -

890.301.463-8 - - - -

a favor de: JOSE LUIS REYES VILLAMIZAR, con C.C.No.

79.152.473 de Usaquén - Bogotá. -----

DIANA BEATRIZ LOPEZ

NOTARIA TREINTA Y TRES (33) DE BOGOTA D.C.

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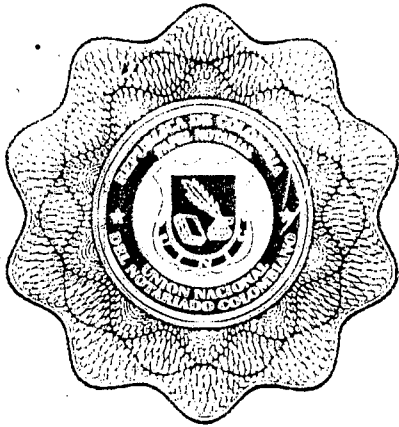
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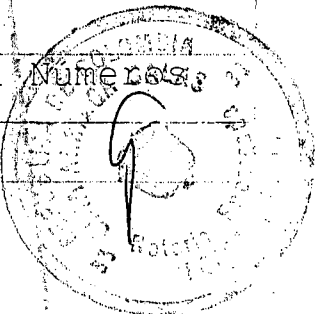
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JUAN MARIA RENDON

C.C. No. 17.125.100 de Bogotá

LAFRANCOL S.A.

Nit. No. 890.301.463-8

TEL: 622-04-66.

ACEPTO :

JOSE LUIS REYES VILLAMIZAR

C.C. No. 29.152.473

TEL: 621-2631

DIANA BEATRIZ LOPEZ
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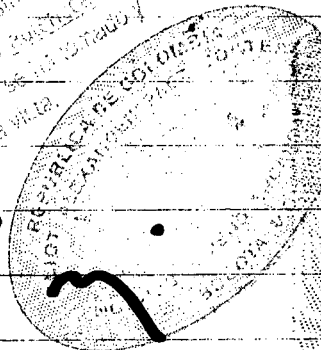
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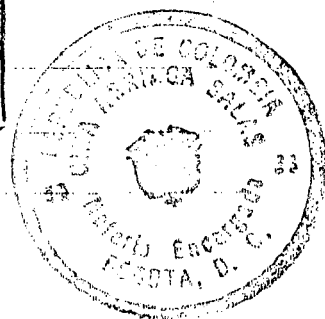
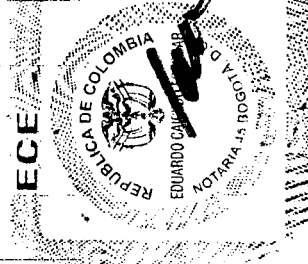
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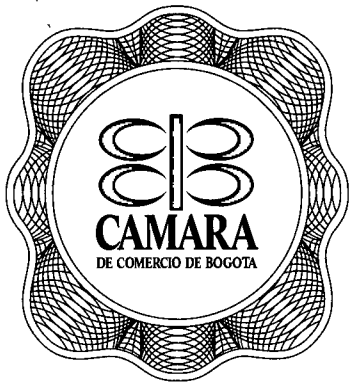
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HORA: 09:50:29

OPERACION: 01NJJ0810075

PAGINA: 1

CERTIFICA:

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SIGLA : LAFRANCOL S.A.

DOMICILIO: CALI VALLE

DIRECCION COMERCIAL : CRA. 1 NRO. 46 84

DIRECCION NOTIFICACION JUDICIAL: CRA. 1 NRO. 46 84

CIUDAD: CALI

MATRICULA MERCANTIL NRO. 5233-4 FECHA MATRICULA : 02 DE OCTUBRE DE 1958

DIRECCION ELECTRONICA : lafrancolsa@lafrancol.co

DIRECCION WEB : www.lafrancol.com

AFILIADO

NIT : 890301463-8

CERTIFICA:

CERTIFICA:

QUE POR ESCRITURA NRO. 1434 DEL 29 DE SEPTIEMBRE DE 1958 NOTARIA CUARTA DE CALI , INSCRITA EN LA CAMARA DE COMERCIO EL 02 DE OCTUBRE DE 1958 BAJO EL NRO. 18319 DEL LIBRO IX , SE CONSTITUYO LA SOCIEDAD DENOMINADA LABORATORIO FRANCO COLOMBIANO LAFRANCOL S.A.

CERTIFICA:

QUE POR ESCRITURA NRO. 3672 DEL 24 DE AGOSTO DE 2009 NOTARIA TERCERA DE CALI , INSCRITA EN LA CAMARA DE COMERCIO EL 27 DE AGOSTO DE 2009 BAJO EL NRO. 9876 DEL LIBRO IX , CAMBIO SU NOMBRE DE LABORATORIO FRANCO COLOMBIANO LAFRANCOL S.A. . POR EL DE LABORATORIO FRANCO COLOMBIANO LAFRANCOL S.A. SIGLA: LAFRANCOL S. A. .

CERTIFICA:

REFORMAS

DOCUMENTO	FECHA.DOC	ORIGEN	FECHA.INS	NRO.
INS LIBRO				
E.P. 1104	30/06/1959	NOTARIA CUARTA DE CALI	07/07/1959	
19467				
E.P. 2309	26/12/1959	NOTARIA CUARTA DE CALI	29/12/1959	
20089				
E.P. 1725	24/07/1962	NOTARIA CUARTA DE CALI	26/07/1962	
24528				
E.P. 1824	31/07/1963	NOTARIA CUARTA DE CALI	14/08/1963	

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26360

E.P. 2422 25/10/1963 NOTARIA CUARTA DE CALI 28/10/1963

26717

E.P. 1720 16/09/1964 NOTARIA CUARTA DE CALI 22/09/1964

28406

E.P. 101 31/01/1966 NOTARIA CUARTA DE CALI 01/02/1966

31039

E.P. 552 21/03/1968 NOTARIA CUARTA DE CALI 01/04/1968

35711

E.P. 4964 12/12/1972 NOTARIA CUARTA DE CALI 16/01/1973

2975 IX

E.P. 1956 30/05/1973 NOTARIA CUARTA DE CALI 13/06/1973

4337 IX

E.P. 4964 12/12/1972 NOTARIA CUARTA DE CALI 28/11/1973

5994 IX

E.P. 5579 12/11/1973 NOTARIA CUARTA DE CALI 28/11/1973

5995 IX

E.P. 4406 30/09/1974 NOTARIA CUARTA DE CALI 28/11/1974

10887 IX

E.P. 192 03/02/1978 NOTARIA CUARTA DE CALI 14/02/1978

25355 IX

E.P. 3479 07/09/1979 NOTARIA CUARTA DE CALI 19/09/1979

34204 IX

E.P. 4274 12/12/1984 NOTARIA NOVENA DE CALI 19/12/1984

73088 IX

E.P. 1155 21/05/1985 NOTARIA SEPTIMA DE CALI 03/06/1985

76917 IX

E.P. 1553 20/06/1986 NOTARIA SEPTIMA DE CALI 08/07/1986

85997 IX

E.P. 1458 09/06/1987 NOTARIA SEPTIMA DE CALI 24/12/1987

3508 IX

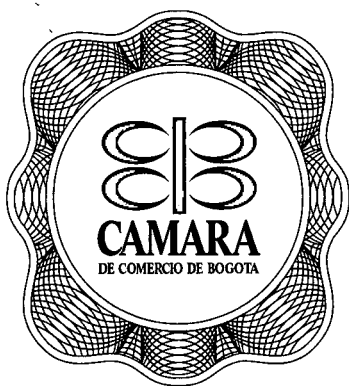
E.P. 7222 14/12/1988 NOTARIA TERCERA DE CALI 19/12/1988

13847 IX

E.P. 4530 04/09/1989 NOTARIA TERCERA DE CALI 07/09/1989

21508 IX

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E.P. 3865 31708 IX	02/08/1990	NOTARIA TERCERA DE CALI	13/08/1990
E.P. 8305 557 IX	13/12/1995	NOTARIA TERCERA DE CALI	23/01/1996
E.P. 4559 7911 IX	16/11/1999	NOTARIA TERCERA DE CALI	26/11/1999
E.P. 6.004 13371 IX	02/12/2004	NOTARIA TERCERA DE CALI	14/12/2004
E.P. 6241 625 IX	27/12/2006	NOTARIA TERCERA DE CALI	19/01/2007
E.P. 389 1880 IX	07/02/2008	NOTARIA TERCERA DE CALI	19/02/2008
E.P. 1907 5950 IX	11/05/2009	NOTARIA TERCERA DE CALI	26/05/2009
E.P. 3672 9876 IX	24/08/2009	NOTARIA TERCERA DE CALI	27/08/2009
ACT 116 5470 IX	04/03/2010	ASAMBLEA GRAL ORDINARIA	11/05/2010
E.P. 4421 12622 IX	21/10/2010	NOTARIA TERCERA DE CALI	26/10/2010
E.P. 1786 6318 IX	16/05/2011	NOTARIA TERCERA DE CALI	24/05/2011

CERTIFICA:

QUE POR ACTA NRO. 116 DEL 04 DE MARZO DE 2010 ASAMBLEA GRAL ORDINARIA, INSCRITA EN LA CAMARA DE COMERCIO EL 11 DE MAYO DE 2010 BAJO EL NRO. 5470 DE LIBRO IX, SE TRANSFORMO DE LABORATORIO FRANCO COLOMBIANO LAFRANCOL S.A. SIGLA: LAFRANCOL S.A. POR EL DE LABORATORIO FRANCO COLOMBIANO LAFRANCOL S.A.S. SIGLA: LAFRANCOL S.A.S.

CERTIFICA

QUE POR ESCRITURA NRO. 2339 DEL 10 DE JUNIO DE 2010 NOTAIRA TERCERA DE CALI, INSCRITA EN LA CAMARA DE COMERCIO EL 15 DE JUNIO DE 2010 BAJO EL NRO. 7070 DEL LIBRO IX, SE INSCRIBIO LA RESOLUCION DE LA TRANSFORMACION DE SOCIEDAD POR ACCIONES SIMPLIFICADA A SOCIEDAD ANONIMA.

CERTIFICA:

VIGENCIA: 29 DE SEPTIEMBRE DEL AÑO 2057

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CERTIFICA:

OBJETO SOCIAL. LA SOCIEDAD EN DESARROLLO DE SU OBJETO SOCIAL PODRÁ REALIZAR, ENTRE OTROS, LAS SIGUIENTES ACTIVIDADES COMERCIALES Y EMPRESARIALES: 1) FABRICACIÓN, DISTRIBUCIÓN Y VENTA EN EL TERRITORIO DE LA REPÚBLICA DE COLOMBIA O EN EL EXTRANJERO DE CUALESQUIERA TIPOS DE VACUNAS, PRODUCTOS QUÍMICOS, FARMACÉUTICOS, ALIMENTICIOS FUNCIONALES, COSMÉTICOS Y FISIOLÓGICOS REACTIVOS, ANTIBIÓTICOS PARA USO HUMANO Y VETERINARIO, PRODUCTOS DIETÉTICOS, COLORANTES, DE PERFUMERÍA, DISPOSITIVOS MÉDICOS Y AFINES. 2) LA REPRESENTACIÓN DE CASAS FABRICANTES NACIONALES O EXTRANJERAS DE ESTOS MISMOS PRODUCTOS. 3) FABRICACIÓN, IMPORTACIÓN, DISTRIBUCIÓN Y VENTA DE CUALESQUIERA TIPO DE PRODUCTOS VETERINARIOS. 4) LA REALIZACIÓN DE TODOS LOS ACTOS, CONTRATOS, CONVENIOS, DIRECTAMENTE RELACIONADOS CON LOS FINES ANTES INDICADOS. 5) IMPORTAR CUALESQUIERA TIPOS DE VACUNAS, PRODUCTOS QUÍMICOS, FARMACÉUTICOS, ALIMENTICIOS FUNCIONALES, COSMÉTICOS Y FISIOLÓGICOS, REACTIVOS, ANTIBIÓTICOS PARA USO HUMANO Y VETERINARIO, PRODUCTOS DIETÉTICOS, COLORANTES, DE PERFUMERÍA, DISPOSITIVOS MÉDICOS Y AFINES Y/O PRODUCTOS INTERMEDIOS O A GRANEL. 6) EN DESARROLLO DE SU OBJETO SOCIAL PODRÁ LA SOCIEDAD ADQUIRIR Y VENDER BIENES RAÍCES Y MUEBLES, DARLOS Y TOMARLOS EN ARRENDAMIENTO, GARANTIZAR CON HIPOTECA SOBRE LOS BIENES RAÍCES O CON PRENDA SOBRE LOS BIENES MUEBLES, OBLIGACIONES A CARGO DE LA SOCIEDAD; DAR Y RECIBIR A MUTUO COMERCIAL DINERO Y CELEBRAR LOS CONTRATOS COMERCIALES DE CAMBIO EN TODAS SUS MANIFESTACIONES, FUNDAR EMPRESAS QUE TENGAN O NO LA MISMA FINALIDAD SOCIAL O ASOCIARSE CON ELLAS. 7) ADEMÁS DE LAS ACTIVIDADES REFERIDAS, PODRÁ REALIZAR CUALQUIER ACTIVIDAD, DE GESTIÓN DE LICENCIAS, REGISTROS DE EXPORTACIÓN O IMPORTACIÓN, CONVENIOS RELATIVOS A ESAS ACTIVIDADES Y LOS ACTOS RELACIONADOS CON EL MISMO.

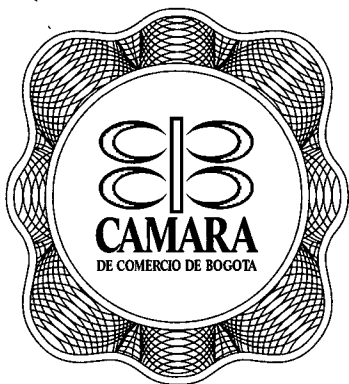
PARÁGRAFO: LA SOCIEDAD NO PODRÁ CONSTITUIRSE GARANTE, NI FIADORA DE OBLIGACIONES DISTINTAS DE LAS PROPIAS, NI GARANTIZAR CON SUS BIENES OBLIGACIONES DE TERCEROS, A MENOS QUE LO AUTORICE EN CADA CASO LA JUNTA DIRECTIVA CON EL VOTO DE LA TOTALIDAD DE LOS MIEMBROS PRESENTES EN LA REUNIÓN CORRESPONDIENTE.

CERTIFICA:

ÓRGANOS DE ADMINISTRACIÓN DE LA SOCIEDAD.

LA DIRECCIÓN Y ADMINISTRACIÓN DE LA SOCIEDAD ESTARÁN A CARGO DE: 1. LA ASAMBLEA GENERAL DE ACCIONISTAS; 2. LA JUNTA DIRECTIVA; 3. LOS REPRESENTANTES LEGALES.

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FUNCIONES DE LA ASAMBLEA GENERAL DE ACCIONISTAS, ADEMÁS DE LAS ESTABLECIDAS EN LA LEY, ESTE ÓRGANO TENDRÁ LAS SIGUIENTES FUNCIONES, ENTRE OTRAS: 5) DELEGAR EN LA JUNTA DIRECTIVA, CUANDO LO ESTIME OPORTUNO, ALGUNA O ALGUNAS DE SUS FUNCIONES CUYA DELEGACIÓN NO ESTÉ PROHIBIDA POR LA LEY O POR LOS ESTATUTOS; 9) NOMBRAR Y REMOVER EL LIQUIDADOR DE LA SOCIEDAD Y SU SUPLENTE, FIJAR SU REMUNERACIÓN Y CONSIDERAR, APROBAR O IMPROBAR SUS CUENTAS; 10) DECLARAR ORDINARIA O EXTRAORDINARIAMENTE DISUELTA Y EN ESTADO DE LIQUIDACIÓN A LA SOCIEDAD; 11) AUTORIZAR LA VENTA, TRANSFERENCIA, ACTOS DISPOSITIVOS, DE ADMINISTRACIÓN, O ARRIENDO, HIPOTECAR, GRAVAR, TOTAL O PARCIALMENTE LOS BIENES QUE COMPONEN SU PATRIMONIO; 12) DECRETAR EL AUMENTO DE CAPITAL AUTORIZADO; 13) DECRETAR LA PRÓRROGA DEL TÉRMINO DE DURACIÓN DE LA SOCIEDAD; 14) REMOVER EN CUALQUIER MOMENTO AL PRESIDENTE EJECUTIVO, A LOS MIEMBROS DE LA JUNTA DIRECTIVA O AL REVISOR FISCAL Y SUS RESPECTIVOS SUPLENTE; 15) REALIZAR CUALQUIER MODIFICACIÓN ESTATUTARIA; 16) DISPONER LA ESCISIÓN, FUSIÓN O TRANSFORMACIÓN DE LA SOCIEDAD; 17) NOMBRAR A LOS MIEMBROS DE LA JUNTA DIRECTIVA Y EL REVISOR FISCAL DE LA SOCIEDAD Y SUS RESPECTIVOS SUPLENTE; 19) ADOPTAR TODAS LAS MEDIDAS QUE RECLAMEN EL CUMPLIMIENTO DE LOS ESTATUTOS Y EL INTERÉS DE LOS ACCIONISTAS Y DE LA SOCIEDAD. 20) LAS QUE POR LEY Y POR SU CONDICIÓN DE SUPREMO ÓRGANO SOCIAL NATURALMENTE LE CORRESPONDAN Y NO ESTÉN ASIGNADAS A OTRO ÓRGANO SOCIETARIO.

LA JUNTA DIRECTIVA. SE COMPONE DE TRES (3) MIEMBROS QUE SERÁN ELEGIDOS POR LA ASAMBLEA GENERAL DE ACCIONISTAS... LAS SUPLENCIAS SERÁN NUMÉRICAS...

FUNCIONES DE LA JUNTA DIRECTIVA, ADEMÁS DE LAS FUNCIONES DE LEY, ENTRE OTRAS:

2) CREAR LOS CARGOS QUE JUZGUE NECESARIOS PARA LA BUENA MARCHA DE LA EMPRESA, NOMBRAR A QUIEN DEBA EJERCERLOS, SEÑALAR SUS FUNCIONES, FIJAR SUS ASIGNACIONES O LA FORMA DE RETRIBUCIÓN, NOMBRAR Y REMOVER LIBREMENTE A LOS EMPLEADOS DE LA SOCIEDAD, EXCEPTO AQUELLOS CUYO NOMBRAMIENTO O REMOCIÓN CORRESPONDA A LA ASAMBLEA GENERAL DE ACCIONISTAS. 3) NOMBRAR, REMOVER Y FIJAR ASIGNACIONES AL

PRESIDENTE EJECUTIVO, VICEPRESIDENTES Y ADMINISTRADORES QUE REPORTEN DIRECTAMENTE A LA PRESIDENCIA EJECUTIVA. 9) AUTORIZAR AL PRESIDENTE

EJECUTIVO, PARA QUE SOLICITE, DE ACUERDO CON LA LEY VIGENTE, LLEGADO EL CASO, QUE SE ADMITA A LA COMPAÑÍA EN LA CELEBRACIÓN DE PROCESO CONCURSAL CON SUS ACREEDORES, O REESTRUCTURACIÓN EMPRESARIAL. 17) AUTORIZAR AL TERCER

REPRESENTANTE LEGAL QUE ES EL PRESIDENTE EJECUTIVO, AL CUARTO Y QUINTO REPRESENTANTES LEGALES PARA COMPROMETER A LA SOCIEDAD O A LOS NEGOCIOS

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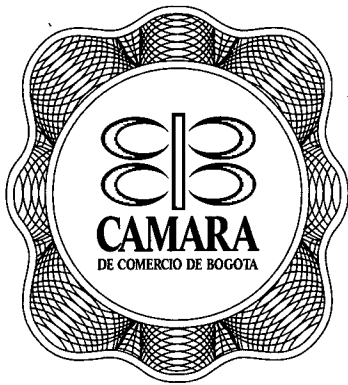
SOCIALES EN CUANTÍA SUPERIOR A SEIS MIL SALARIOS MÍNIMOS MENSUALES LEGALES VIGENTES (6.000 SMMLV) PARA CELEBRAR CONTRATOS COMERCIALES DE COMPRAVENTA CON LA NACIÓN (MINISTERIOS Y DEPARTAMENTOS ADMINISTRATIVOS), ENTIDADES DESCENTRALIZADAS (ESTABLECIMIENTOS PÚBLICOS, EMPRESAS INDUSTRIALES Y COMERCIALES DEL ESTADO, SOCIEDADES DE ECONOMÍA MIXTA), EMPRESAS SOCIALES CIVILES Y COMERCIALES, NACIONALES O EXTRANJERAS, CUALQUIERA QUE SEA SU NATURALEZA, GOBIERNOS O ENTIDADES EXTRANJEROS, CON FACULTADES PARA EXIGIR O SUSCRIBIR LOS DOCUMENTOS Y GARANTÍAS NECESARIOS HASTA LA CUANTÍA ANTES MENCIONADA Y REPRESENTAR A LA COMPAÑÍA EN CUALQUIER TRÁMITE O PROCESO JUDICIAL QUE SE ADELANTE ANTE LAS JURISDICCIONES CIVIL, LABORAL, COMERCIAL, PENAL Y CONTENCIOSO ADMINISTRATIVA EN CALIDAD DE DEMANDANTES O DEMANDADOS Y CUALQUIER OTRO TRÁMITE EN EL QUE SE REQUIERA REPRESENTAR A LA COMPAÑÍA ANTE ENTIDADES Y ORGANISMOS DEL ESTADO DE CUALQUIER ORDEN O ENTIDADES DE NATURALEZA PRIVADA.

18) AUTORIZAR AL TERCER Y CUARTO REPRESENTANTE LEGAL PARA ADQUIRIR ACTIVOS FIJOS SUPERIOR A DOSCIENTOS UN SALARIOS MÍNIMOS MENSUALES LEGALES VIGENTES (201 SMMLV), CRÉDITOS CON ENTIDADES FINANCIERAS SUPERIORES A UN MILLÓN QUINIENTOS MIL DÓLARES AMERICANOS (USD\$1.500.0000) Y DEFINIR LAS ATRIBUCIONES AL TERCER Y CUARTO REPRESENTANTE LEGAL CORRESPONDIENTE A OTRAS COMPRAS POR CONCEPTO DE ASESORÍAS Y GASTOS DIFERENTES A LOS ANTERIORES. 19) AUTORIZAR AL TERCER Y CUARTO REPRESENTANTE LEGAL DE LA COMPAÑÍA PARA QUE CELEBREN CONTRATOS DE SOCIEDAD O PARTICIPEN EN OTRAS COMPAÑÍAS COMERCIALES, NACIONALES O EXTRANJERAS. 20) AUTORIZAR AL TERCER Y CUARTO REPRESENTANTE LEGAL PARA QUE SUSCRIBAN TODA CLASE DE DOCUMENTOS PÚBLICOS O PRIVADOS Y CON EL PROPÓSITO DE INSTRUMENTAR CUALQUIER TIPO DE ACTOS, NEGOCIOS JURÍDICOS, OPERACIONES DE CUALQUIER ÍNDOLE, Y SIEMPRE Y CUANDO SUPERE LA CUANTÍA PREVISTA PARA LA REALIZACIÓN DE ACTOS POR PARTE DEL REPRESENTANTE LEGAL DE LA COMPAÑÍA. 22) AUTORIZAR LA CONSTITUCIÓN O SUPRESIÓN DE SUCURSALES O AGENCIAS. 26) AUTORIZAR LA NOVACIÓN O CUALQUIER FORMA EXTINTIVA DE OBLIGACIONES. 27) AUTORIZAR LA ENAJENACIÓN A CUALQUIER TÍTULO, RELACIONADA CON MAQUINARIA INDUSTRIAL, TERRENOS, EDIFICIOS E INTANGIBLES Y APROBAR DONACIONES. IGUALMENTE AUTORIZARÁ LA CONSTITUCIÓN DE GARANTÍAS HIPOTECARIAS, PRENDARIAS O DE CUALQUIER OTRA ÍNDOLE SOBRE DICHOS ACTIVOS. 29) LAS DEMÁS QUE LE ASIGNEN LOS ESTATUTOS Y LAS LEYES SOBRE LA MATERIA.

REPRESENTACIÓN LEGAL. ADMINISTRACIÓN.

LA ADMINISTRACIÓN DE LA SOCIEDAD CORRESPONDE AL PRESIDENTE EJECUTIVO, TERCER

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REPRESENTANTE LEGAL DE LA COMPAÑÍA, ASÍ COMO LA GESTIÓN Y EJECUCIÓN DE LOS NEGOCIOS Y ASUNTOS SOCIALES. EL PRESIDENTE EJECUTIVO DE LA SOCIEDAD, SERÁ DESIGNADO POR LA JUNTA DIRECTIVA PARA PERÍODOS DE UN (1) AÑO, SIN PERJUICIO DE SU LIBRE REMOCIÓN Y REELECCIÓN INDEFINIDA.

EL PRESIDENTE Y VICEPRESIDENTE DE LA JUNTA DIRECTIVA, SERÁ TAMBIÉN REPRESENTANTE LEGAL PRIMERO Y SEGUNDO DE LA SOCIEDAD.

LAS ATRIBUCIONES INHERENTES A LA CALIDAD DE REPRESENTANTES LEGALES, DE ESTOS CARGOS Y EN ESPECIAL LAS DISPOSITIVAS, SERÁN EJERCIDAS SIN LAS LIMITACIONES ESTABLECIDAS EN ÉSTOS ESTATUTOS PARA OTROS REPRESENTANTES LEGALES.

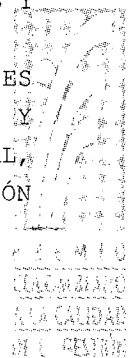
EL CUARTO Y QUINTO REPRESENTANTE LEGAL, SERÁN REPRESENTANTES LEGALES DE LA SOCIEDAD QUIENES SON ELEGIDOS POR LA JUNTA DIRECTIVA CON SUS LIMITACIONES EN SUS FUNCIONES.

LAS ATRIBUCIONES INHERENTES A LA CALIDAD DEL CUARTO REPRESENTANTE LEGAL, Y EN ESPECIAL LAS DISPOSITIVAS, SERÁN EJERCIDAS CON SUJECCIÓN A LAS LIMITACIONES ESTABLECIDAS EN ÉSTOS ESTATUTOS PARA EL PRESIDENTE EJECUTIVO QUIEN ES EL TERCER REPRESENTANTE LEGAL.

EXISTIRÁ UN QUINTO REPRESENTANTE LEGAL DE LA SOCIEDAD QUE TENDRÁ LIMITACIÓN EN SUS FUNCIONES CUANDO SE TRATE DE COMPROMETER A LA SOCIEDAD O A LOS NEGOCIOS SOCIALES EN CUANTÍA NO SUPERIOR A SEIS MIL SALARIOS MÍNIMOS MENSUALES LEGALES VIGENTES (6.000 SMMLV) PARA CELEBRAR CONTRATOS COMERCIALES DE COMPRAVENTA CON LA NACIÓN (MINISTERIOS Y DEPARTAMENTOS ADMINISTRATIVOS), ENTIDADES DESCENTRALIZADAS (ESTABLECIMIENTOS PÚBLICOS, EMPRESAS INDUSTRIALES Y COMERCIALES DEL ESTADO, SOCIEDADES DE ECONOMÍA MIXTA), EMPRESAS SOCIALES CIVILES Y COMERCIALES, NACIONALES O EXTRANJERAS, CUALQUIERA QUE SEA SU NATURALEZA, GOBIERNOS Y ENTIDADES DEL EXTERIOR, CON FACULTADES PARA EXIGIR O SUSCRIBIR LOS DOCUMENTOS Y GARANTÍAS NECESARIOS HASTA LA CUANTÍA ANTES MENCIONADA Y REPRESENTAR A LA COMPAÑÍA EN CUALQUIER TRÁMITE O PROCESO JUDICIAL QUE SE ADELANTE ANTE LAS JURISDICCIONES CIVIL, LABORAL, COMERCIAL, PENAL Y CONTENCIOSO ADMINISTRATIVA EN CALIDAD DE DEMANDANTES O DEMANDADOS Y CUALQUIER OTRO TRÁMITE EN EL QUE SE REQUIERA REPRESENTAR A LA COMPAÑÍA ANTE ENTIDADES Y ORGANISMOS DEL ESTADO DE CUALQUIER ORDEN O ENTIDADES DE NATURALEZA PRIVADA.

FUNCIONES DEL PRESIDENTE EJECUTIVO. EL PRESIDENTE EJECUTIVO DE LA SOCIEDAD ES UN MANDATARIO CON REPRESENTACIÓN, INVESTIDO DE FACULTADES EJECUTIVAS Y ADMINISTRATIVAS, Y COMO TAL, TIENE A SU CARGO LA TERCERA REPRESENTACIÓN LEGAL, LA GESTIÓN COMERCIAL Y FINANCIERA, LA RESPONSABILIDAD DE LA ACCIÓN

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ADMINISTRATIVA, LA COORDINACIÓN Y SUPERVISIÓN GENERAL DE LA EMPRESA, LAS CUALES CUMPLIRÁ CON ARREGLO A LAS NORMAS DE ESTOS ESTATUTOS Y LAS DISPOSICIONES LEGALES, Y CON SUJECCIÓN A LAS ÓRDENES E INSTRUCCIONES DE LA JUNTA DIRECTIVA. ADEMÁS DE LAS FUNCIONES ANTES INDICADAS, CORRESPONDE AL PRESIDENTE EJECUTIVO DE LA SOCIEDAD: 1) COMPROMETER A LA SOCIEDAD O A LOS NEGOCIOS SOCIALES EN CUANTÍA NO SUPERIOR A SEIS MIL SALARIOS MÍNIMOS MENSUALES LEGALES VIGENTES (6.000 SMMLV) PARA CELEBRAR CONTRATOS COMERCIALES DE COMPRAVENTA CON LA NACIÓN (MINISTERIOS Y DEPARTAMENTOS ADMINISTRATIVOS), ENTIDADES DESCENTRALIZADAS (ESTABLECIMIENTOS PÚBLICOS, EMPRESAS INDUSTRIALES Y COMERCIALES DEL ESTADO, SOCIEDADES DE ECONOMÍA MIXTA), EMPRESAS SOCIALES CIVILES Y COMERCIALES, NACIONALES O EXTRANJERAS, CUALQUIERA QUE SEA SU NATURALEZA, CON GOBIERNOS O ENTIDADES EXTRANJERAS, CON FACULTADES PARA EXIGIR O SUSCRIBIR LOS DOCUMENTOS Y GARANTÍAS NECESARIOS HASTA LA CUANTÍA ANTES MENCIONADA Y REPRESENTAR A LA COMPAÑÍA EN CUALQUIER TRÁMITE O PROCESO JUDICIAL QUE SE ADELANTE ANTE LAS JURISDICCIONES CIVIL, LABORAL, COMERCIAL, PENAL Y CONTENCIOSO ADMINISTRATIVA EN CALIDAD DE DEMANDANTES O DEMANDADOS Y CUALQUIER OTRO TRÁMITE EN EL QUE SE REQUIERA REPRESENTAR A LA COMPAÑÍA ANTE ENTIDADES Y ORGANISMOS DEL ESTADO DE CUALQUIER ORDEN O ENTIDADES DE NATURALEZA PRIVADA; ADQUISICIÓN DE ACTIVOS FIJOS NO SUPERIOR A DOSCIENTOS UNO SALARIOS MÍNIMOS MENSUALES LEGALES VIGENTES (201 SMMLV); OBTENER CRÉDITOS CON ENTIDADES FINANCIERAS NO SUPERIORES A UN MILLÓN QUINIENTOS MIL DÓLARES AMERICANOS (US\$1.500.0000) Y CONTRATAR OTRAS COMPRAS POR CONCEPTO DE ASESORÍAS Y GASTOS DIFERENTES A LOS ANTERIORES PREVIA APROBACIÓN DE LA JUNTA DIRECTIVA. 2) CUMPLIR Y HACER CUMPLIR LAS DECISIONES DE LA ASAMBLEA GENERAL DE ACCIONISTAS Y DE LA JUNTA DIRECTIVA Y HACER USO DE LA RAZÓN SOCIAL. C) REPRESENTAR LEGALMENTE A LA SOCIEDAD CONFORME A LA LEY ANTE TODAS LAS AUTORIDADES CIVILES, JUDICIALES, MILITARES, SANITARIAS, DE POLICÍAS ADUANALES, POSTALES, ETC. 3) ORGANIZAR LOS SERVICIOS Y LAS OFICINAS DE LA SOCIEDAD, LA CONTABILIDAD Y APLICAR LOS ESTATUTOS Y REGLAMENTOS. 4) LLEVAR A CABO LA GESTIÓN COMERCIAL Y FINANCIERA. 5) TENDRÁ LA PLENA RESPONSABILIDAD DE LA ACCIÓN ADMINISTRATIVA, LA COORDINACIÓN Y LA SUPERVISIÓN GENERAL DE LA SOCIEDAD, LAS CUALES CUMPLIRÁ CON ARREGLO A LAS NORMAS DE ESTOS ESTATUTOS. 6) COMPARECER EN LOS JUICIOS, TRANSIGIR Y COMPROMETER LOS NEGOCIOS SOCIALES DE CUALESQUIERA NATURALEZA QUE FUEREN, INTERPONER TODO GÉNERO DE RECURSOS Y CONSTITUIR APODERADOS QUE LO REPRESENTEN JUDICIAL O EXTRAJUDICIALMENTE. 7) HACER DEPÓSITOS EN BANCOS Y

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HORA: 09:50:29

OPERACION: 01NJJ0810075

PAGINA: 10

FUE(RON) NOMBRADO(S) :

PRESIDENTE DE LA JUNTA DIRECTIVA Y REPRESENTANTE LEGAL

ESTHER ISABEL VENTURA DE RENDON

C.C.38979831

VICEPRESIDENTE DE LA JUNTA DIRECTIVA Y SEGUNDO REPRESENTANTE LEGAL

JUAN MARIA RENDON GUTIERREZ

C.C.17125100

PRESIDENTE EJECUTIVO - TERCER REPRESENTANTE LEGAL

JUAN CAMILO PALACIO RAMIREZ

C.C.19455622

CUARTO REPRESENTANTE LEGAL

ROBERTO MAURICE VENTURA CRISPINO

C.C.9086003

QUINTO REPRESENTANTE LEGAL

JUAN PABLO REYES VILLAMIZAR

C.C.80419709

CERTIFICA:

DOCUMENTO: ACTA No. 116 DEL 04 DE MARZO DE 2010

ORIGEN: ASAMBLEA GRAL ORDINARIA

INSCRIPCION: 11 DE MAYO DE 2010 No. 5471 DEL LIBRO IX

FUE(RON) _NOMBRADO(S)

JUNTA DIRECTIVA

PRINCIPALES

PRIMER RENGLON

ESTHER ISABEL VENTURA DE RENDON

C.C.38979831

SEGUNDO RENGLON

JUAN MARIA RENDON GUTIERREZ

C.C.17125100

TERCER RENGLON

LUIS ALFONSO MORA TEJADA

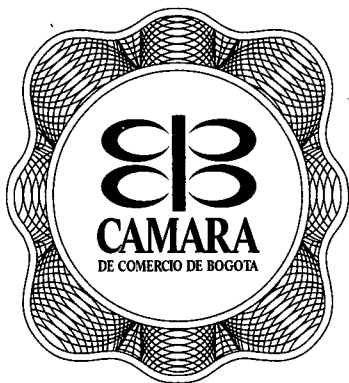
C.C.4610260

SUPLENTES

PRIMER RENGLON

JUAN MANUEL RENDON VENTURA

*** CONTINUA ***



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CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE CALI

FECHA: 2011/08/10

HORA: 09:50:29

OPERACION: 01NJJ0810075

PAGINA: 11

C.C.79939921

SEGUNDO RENGLON

ADRIANA RENDON DE ANTORCHA

C.C.66901857

TERCER RENGLON

CARLOS ANTONIO ESPINOSA PEREZ

C.C.16684716

CERTIFICA:

DOCUMENTO: ACTA No. 118 DEL 29 DE JUNIO DE 2010

ORIGEN: ASAMBLEA DE ACCIONISTAS

INSCRIPCION: 12 DE AGOSTO DE 2010 No. 9529 DEL LIBRO IX

FUE (RON) NOMBRADO(S):

REVISOR FISCAL FIRMA

DELOITTE & TOUCHE LTDA D & T LTDA

NIT.860005813-4

CERTIFICA:

DOCUMENTO: DOCUMENTO PRIVADO DEL 02 DE AGOSTO DE 2010

ORIGEN: DELOITTE & TOUCHE LTDA D & T LTDA

INSCRIPCION: 12 DE AGOSTO DE 2010 No. 9530 DEL LIBRO IX

FUE (RON) NOMBRADO(S):

REVISOR FISCAL PRINCIPAL

LEANDRO FELIPE PAZ AGELVIS

C.C.14700811

REVISOR FISCAL SUPLENTE

HEYMAN ANTONIO GIL BALCAZAR

C.C.16796791

CERTIFICA:

CAPITAL AUTORIZADO: \$40,000,000,000

NUMERO DE ACCIONES: 4,000,000,000

VALOR NOMINAL: \$10

CAPITAL SUSCRITO: \$20,150,975,220

NUMERO DE ACCIONES: 2,015,097,522

VALOR NOMINAL: \$10

CAPITAL PAGADO: \$20,150,975,220

NUMERO DE ACCIONES: 2,015,097,522

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CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE CALI

FECHA: 2011/08/10

HORA: 09:50:29

OPERACION: 01NJJ0810075

PAGINA: 12

VALOR NOMINAL: \$10

CERTIFICA:

DOCUMENTO: DOCUMENTO PRIVADO DEL 03 DE DICIEMBRE DE 2009
INSCRIPCIÓN: 28 DE DICIEMBRE DE 2009 BAJO EL NRO. 14869 DEL LIBRO IX
CONSTA LA SITUACIÓN DE CONTROL:
CONTROLANTE: DORAL INVESTMENTS INTERNATIONAL INC
DOMICILIO: ISLAS VIRGENES BRITÁNICAS
ACTIVIDAD: OTRAS ACTIVIDADES EMPRESARIALES
CONTROLADA: LABORATORIO FRANCO COLOMBIANO LAFRANCOL S.A.
NIT: 890301463-8

DOMICILIO: CALI VALLE

NACIONALIDAD: COLOMBIANA

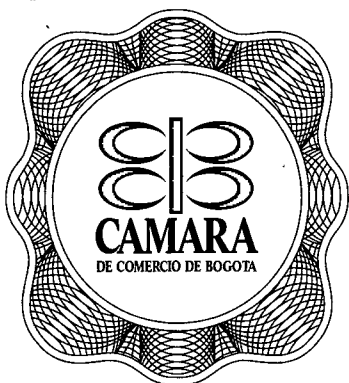
ACTIVIDAD: LA FABRICACIÓN, DISTRIBUCIÓN Y VENTA EN EL TERRITORIO DE LA
REPÚBLICA DE COLOMBIA O EN EL EXTRANJERO DE CUALESQUIERA TIPOS DE VACUNAS,
PRODUCTOS QUÍMICOS, FARMACÉUTICOS, ALIMENTOS FUNCIONALES, COSMÉTICOS Y
FISIOLÓGICOS, REACTIVOS, ANTIBIÓTICOS PARA USO HUMANO Y VETERINARIO, PRODUCTOS
DIETETICOS, COLORANTES DE PERFUMERÍA Y AFINES.

PRESUPUESTO DE CONTROL: POSEE PARTICIPACIÓN ACCIONARIA MAYOR AL 50% A PARTIR
DEL 10 DE NOVIEMBRE DE 2009.

CERTIFICA:

DOCUMENTO: DOCUMENTO PRIVADO DEL 30 DE ABRIL DE 2004
INSCRIPCIÓN: 03 DE MAYO DE 2004 No. 4859 DEL LIBRO IX
DOCUMENTO: DOCUMENTO PRIVADO DEL 29 DE ABRIL DE 2005
INSCRIPCIÓN: 06 DE MAYO DE 2005 No. 4983 DEL LIBRO IX
DOCUMENTO: DOCUMENTO PRIVADO DEL 15 DE JUNIO DE 2006
INSCRIPCIÓN: 21 DE JUNIO DE 2006 No. 7526 DEL LIBRO IX
DOCUMENTO: DOCUMENTO PRIVADO DEL 01 DE NOVIEMBRE DE 2006
INSCRIPCIÓN: 28 DE NOVIEMBRE DE 2006 No. 13380 DEL LIBRO IX
DOCUMENTO: DOCUMENTO PRIVADO DEL 19 DE DICIEMBRE DE 2006
INSCRIPCIÓN: 21 DE DICIEMBRE DE 2006 No. 14301 DEL LIBRO IX
DOCUMENTO: DOCUMENTO PRIVADO DEL 19 DE DICIEMBRE DE 2007
INSCRIPCIÓN: 21 DE DICIEMBRE DE 2007 No. 13604 DEL LIBRO IX
DOCUMENTO: DOCUMENTO PRIVADO DEL 19 DE OCTUBRE DE 2009
INSCRIPCIÓN: 22 DE OCTUBRE DE 2009 No. 12216 DEL LIBRO IX
DOCUMENTO: DOCUMENTO PRIVADO DEL 23 DE FEBRERO DE 2010

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CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE CALI

FECHA: 2011/08/10

HORA: 09:50:29

OPERACION: 01NJJ0810075

PAGINA: 13

INSCRIPCIÓN: 25 DE FEBRERO DE 2010 No. 2203 DEL LIBRO IX

DOCUMENTO: DOCUMENTO PRIVADO DEL 27 DE ABRIL DE 2010

INSCRIPCIÓN: 28 DE ABRIL DE 2010 NRO. 4911 DEL LIBRO IX

DOCUMENTO: DOCUMENTO PRIVADO DEL 10 DE JUNIO DE 2010

INSCRIPCION: 17 DE JUNIO DE 2010 NRO. 7195 DEL LIBRO IX

DOCUMENTO: DOCUMENTO PRIVADO DEL 25 DE AGOSTO DE 2010

INSCRIPCION: 07 DE SEPTIEMBRE DE 2010 BAJO EL NRO. 10541 DEL LIBRO IX

DOCUMENTO: DOCUMENTO PRIVADO DEL 14 DE JUNIO DEL 2011

INSCRIPCION: 22 DE JUNIO DE 2011 BAJO EL NRO. 7772 LIBRO IX

CONSTA EL SIGUIENTE GRUPO EMPRESARIAL:

CONTROLANTE

LABORATORIO FRANCO COLOMBIANO LAFRANCOL S.A.

NIT. 890301463-8

DOMICILIO: CALI VALLE

NACIONALIDAD: COLOMBIA

ACTIVIDAD: A) LA FABRICACIÓN, DISTRIBUCIÓN Y VENTA EN EL TERRITORIO DE LA REPÚBLICA DE COLOMBIA O EN EL EXTRANJERO DE CUALESQUIERA TIPOS DE VACUNAS, PRODUCTOS QUÍMICOS, FARMACÉUTICOS, ALIMENTOS FUNCIONALES, COSMÉTICOS Y FISIOLÓGICOS, REACTIVOS, ANTIBIÓTICOS PARA USO HUMANO Y VETERINARIO, PRODUCTOS DIETÉTICOS, COLORANTES DE PERFUMERÍA Y AFINES. B) LA REPRESENTACIÓN DE CASAS FABRICANTES NACIONALES O EXTRANJERAS DE ESTOS MISMOS PRODUCTOS. C) FABRICACIÓN, IMPORTACIÓN, DISTRIBUCIÓN Y VENTA DE CUALESQUIERA TIPOS DE PRODUCTOS VETERINARIOS. D) LA REALIZACIÓN DE TODOS LOS ACTOS, CONTRATOS, CONVENIOS, DIRECTAMENTE RELACIONADOS CON LOS FINES ANTES INDICADOS. EN DESARROLLO DE SU OBJETO SOCIAL PUEDE LA SOCIEDAD ADQUIRIR Y VENDER BIENES RAÍCES Y MUEBLES, DARLOS Y TOMARLOS EN ARRENDAMIENTO, GARANTIZAR CON HIPOTECA SOBRE LOS BIENES RAÍCES O CON PRENDA SOBRE LOS BIENES MUEBLES, OBLIGACIONES A CARGO DE LA SOCIEDAD, DAR Y RECIBIR A MUTUO COMERCIAL DINERO Y CELEBRAR LOS CONTRATOS COMERCIALES DE CAMBIO EN TODAS SUS MANIFESTACIONES, FUNDAR EMPRESAS QUE TENGAN LA MISMA FINALIDAD SOCIAL O ASOCIARSE CON ELLAS.

SUBORDINADA

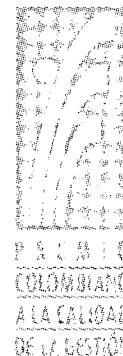
AMERICAN GENERICS S A S

NIT. 800223214-9

DOMICILIO: CALI VALLE

NACIONALIDAD: COLOMBIA

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CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE CALI

FECHA: 2011/08/10

HORA: 09:50:29

OPERACION: 01NJJ0810075

PAGINA: 14

PRESUPUESTO DE CONTROL: NUMERAL 1, ARTÍCULO 27 DE LA LEY 222 DE 1995.

ACTIVIDAD: DISTRIBUCIÓN Y COMERCIALIZACIÓN DE PRODUCTOS FARMACÉUTICOS DE USO HUMANO.

SUBORDINADA

LAFRANCOL INTERNACIONAL S.A.S.

NIT. 815003912-2

DOMICILIO: PALMIRA VALLE

NACIONALIDAD: COLOMBIA

PRESUPUESTO DE CONTROL: NUMERAL 3, ARTICULO 27 DE LA LEY 222 DE 1995.

ACTIVIDAD: REALIZACIÓN DE ACTIVIDADES DESDE LA ZONA FRANCA, LAS CUALES CONSISTEN EN LA PRESTACIÓN DE TODO TIPO DE SERVICIOS ASÍ COMO DISTRIBUIR, VENDER, COMPRAR, IMPORTAR, EXPORTAR Y ALMACENAR TODO TIPO DE MATERIAS PRIMAS, PRODUCTOS TERMINADOS Y SEMITERMINADOS RELACIONADOS CON LA INDUSTRIA DE ALIMENTOS, COSMÉTICA Y FARMACÉUTICA.

SUBORDINADA

LAFRANCOL PERÚ S.A

DOMICILIO: LIMA

NACIONALIDAD: PERU

PRESUPUESTO DE CONTROL: NUMERAL 1, ARTICULO 27 DE LA LEY 222 DE 1995.

ACTIVIDAD: DISTRIBUCIÓN Y COMERCIALIZACIÓN DE PRODUCTOS FARMACÉUTICOS DE USO HUMANO.

SUBORDINADA

LABORATORIOS PAULY PHARMACEUTICAL S.A.S.

NIT. 800097402-6

DOMICILIO: CALI VALLE

NACIONALIDAD: COLOMBIA

PRESUPUESTO DE CONTROL: NUMERAL 1, ARTÍCULO 27 DE LA LEY 222 DE 1995.

ACTIVIDAD: DISTRIBUCIÓN Y COMERCIALIZACIÓN DE PRODUCTOS FARMACÉUTICOS DE USO HUMANO.

SUBORDINADA

LAFRANCOL (USA) CORP

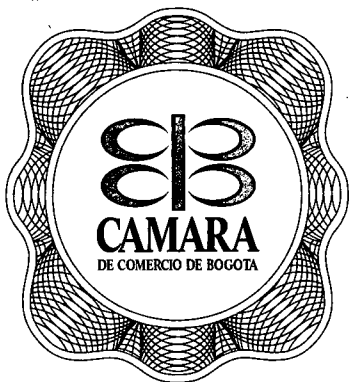
DOMICILIO: ESTADOS UNIDOS

NACIONALIDAD: ESTADOS UNIDOS

PRESUPUESTO DE CONTROL: NUMERAL 1, ARTICULO 27 DE LA LEY 222 DE 1995.

ACTIVIDAD: DISTRIBUCIÓN Y COMERCIALIZACIÓN DE PRODUCTOS FARMACÉUTICOS DE USO

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CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE CALI

FECHA: 2011/08/10

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OPERACION: 01NJ0810075

PAGINA: 15

HUMANO.

SUBORDINADA

GAMAR SOCIEDAD S A S EN LIQUIDACION

NIT. 890312050-7

DOMICILIO: CALI VALLE

NACIONALIDAD: COLOMBIA

PRESUPUESTO DE CONTROL: NUMERAL 1, ARTICULO 27 DE LA LEY 222 DE 1995.

ACTIVIDAD: TODAS LAS ACTIVIDADES RELACIONADAS CON LAS ARTES GRÁFICAS.

SUBORDINADA

LAFRANCOL GUATEMALA S.A.

NIT. 4088707

DOMICILIO: GUATEMALA

NACIONALIDAD: GUATEMALA

PRESUPUESTO DE CONTROL: NUMERAL 1, ARTICULO 27 DE LA LEY 222 DE 1995.

ACTIVIDAD: PRODUCCIÓN, FABRICACIÓN, EXPORTACIÓN Y DISTRIBUCIÓN DE PRODUCTOS FARMACÉUTICOS Y/O ALIMENTICIOS Y NATURALES DE CONSUMO HUMANO Y LA COMERCIALIZACIÓN DE TODA CLASE DE MERCANCÍAS POR CUENTA PROPIA O EN NOMBRE DE TERCEROS.

SUBORDINADA

DISTRIBUCIONES UQUIFA S A S

NIT. 800182021-7

DOMICILIO: CALI VALLE

NACIONALIDAD: COLOMBIA

PRESUPUESTO DE CONTROL: NUMERAL 1, ARTICULO 27 DE LA LEY 222 DE 1995.

ACTIVIDAD: DISTRIBUCIÓN Y COMERCIALIZACIÓN DE PRODUCTOS FARMACÉUTICOS Y ALIMENTOS FUNCIONALES DE CONSUMO HUMANO.

SUBORDINADA

LABORATORIOS NATURMEDIK S A S

NIT. 800226363-1

DOMICILIO: CALI VALLE

NACIONALIDAD: COLOMBIA

PRESUPUESTO DE CONTROL: NUMERAL 1, ARTICULO 27 DE LA LEY 222 DE 1995.

ACTIVIDAD: DISTRIBUCIÓN Y COMERCIALIZACIÓN DE PRODUCTOS FARMACÉUTICOS DE USO HUMANO.

SUBORDINADA

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CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE CALI

FECHA: 2011/08/10

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OPERACION: 01NJJ0810075

PAGINA: 16

LAFRANCOL DOMINICANA S.A.

DOMICILIO:SANTO DOMINGO REPÚBLICA DOMINICANA

NACIONALIDAD:REPÚBLICA DOMINICANA

PRESUPUESTO DE CONTROL:NUMERAL 1, ARTÍCULO 27 DE LA LEY 222.

ACTIVIDAD:FABRICACIÓN DE PRODUCTOS FARMACÉUTICOS.

SUBORDINADA

LABORATORIO FRANCO-COLOMBIANO DEL ECUADOR S.A.

DOMICILIO: GUAYAQUIL, REPÚBLICA DEL ECUADOR

NACIONALIDAD: ECUATORIANA

PRESUPUESTO DE CONTROL: NUMERAL 1, ARTICULO 27 DE LA LEY 222 DE 1995. LA FECHA DE LA PRIMERA TRANSACCION COMERCIAL FUE EN JUNIO DE 2009 Y LA CAPITALIZACION SE HIZO EN OCTUBRE DE 2009.

ACTIVIDAD: DEDICARSE A LA FABRICACIÓN, PRODUCCIÓN, EMPAQUE, COMERCIALIZACIÓN, DISTRIBUCIÓN, COMPRAVENTA, IMPORTACIÓN, EXPORTACIÓN Y/O REPRESENTACIÓN DE TODO TIPO DE PRODUCTOS FARMACÉUTICOS, HIGIÉNICOS, COSMÉTICOS, ALIMENTICIOS, DIETETICOS Y BAJOS EN CALORÍAS, VETERINARIOS, APARATOS E INSTRUMENTOS QUIRÚRGICOS, MÉDICOS, DENTALES Y VETERINARIOS. PODRÁ ADQUIRIR ACCIONES, PARTICIPACIONES O CUOTAS SOCIALES DE CUALQUIER CLASE DE COMPAÑÍA, SEA NACIONAL O EXTRANJERA, PUDIENDO INTERVENIR EN LA FUNDACIÓN O AUMENTO DE CAPITAL DE OTRAS SOCIEDADES QUE TENGAN OBJETIVOS SOCIALES RELACIONADOS CON EL SUYO. PODRÁ CONSTRUIR, ADQUIRIR EN PROPIEDAD, ARRENDAMIENTO O EN ASOCIACIÓN TODA CLASE DE PLANTAS INDUSTRIALES PARA PRODUCCIÓN, EMPAQUE, PROCESAMIENTO O CUALQUIER OTRA FORMA PARA LA COMERCIALIZACIÓN EN EL MERCADO INTERNO Y EXTERNO DE PRODUCTOS FARMACÉUTICOS YA SEA PARA EL CONSUMO HUMANO, VEGETAL O ANIMAL.

SUBORDINADA

FOCUS PHARMACEUTICAL S.A.S.

NIT: 900.104.555-8

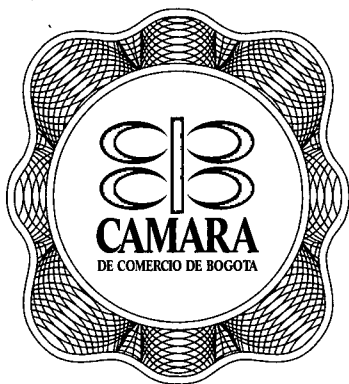
DOMICILIO: BOGOTÁ D.C.

NACIONALIDAD: COLOMBIANA

PRESUPUESTO DE CONTROL: NUMERAL 1, ARTICULO 27 DE LA LEY 222 DE 1995. LA FECHA DE LA PRIMERA TRANSACCION COMERCIAL FUE EN ENERO DE 2010 Y LA CAPITALIZACION SE HIZO EN ENERO DE 2010.

ACTIVIDAD: EL OBJETO PRINCIPAL DE LA SOCIEDAD ES EL DESARROLLO, FABRICACIÓN O PRODUCCIÓN, DIRECTAMENTE O A TRAVÉS DE TERCEROS EN CUALQUIER MODALIDAD NEGOCIAL, LA COMERCIALIZACIÓN Y DISTRIBUCIÓN, NACIONAL O INTERNACIONAL, DE

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CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE CALI

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OPERACION: 01NJJ0810075

PAGINA: 17

TODA CLASE DE ARTÍCULOS DE GÉNERO FARMACÉUTICO, ESPECÍFICAMENTE DE MEDICAMENTOS ESA ACTIVIDAD PRINCIPAL CONLLEVA LA HABILITACIÓN PARA DESARROLLAR TODA OTRA CONEXA O NECESARIA, INCLUYENDO DE MANERA ESPECIAL PERO NO LIMITATIVA, LA INVESTIGACIÓN FARMACÉUTICA, EL DESARROLLO DE PRODUCTOS MÉDICOS Y FARMACÉUTICOS, EQUIPOS MÉDICOS, TRATAMIENTOS ALTERNATIVOS Y EN GENERAL, TODO AQUELLO VINCULADO A LA PROTECCIÓN DE LA SALUD HUMANA.

PODRÁ EFECTUAR LA INVERSIÓN EN BIENES INMUEBLES, URBANOS O RURALES Y LA ADQUISICIÓN, ADMINISTRACIÓN, ARRENDAMIENTO, GRAVAMEN Y ENAJENACIÓN DE LOS MISMOS.

LA INVERSIÓN DE FONDOS PROPIOS, EN BIENES INMUEBLES, BONOS, VALORES BURSÁTILES Y PARTES DE INTERÉS EN SOCIEDADES COMERCIALES, ASÍ COMO LA NEGOCIACIÓN DE TODA CLASE DE DERECHOS DE CRÉDITO.

CERTIFICA:

QUE A NOMBRE DE LA SOCIEDAD FIGURA MATRICULADO EN LA CAMARA DE COMERCIO BAJO EL NRO.5234- 2 ESTABLECIMIENTO DE COMERCIO: LABORATORIO FRANCO COLOMBIANO LAFRANCOL

UBICADO EN: ---K 1 46 84 DE CALI
RENOVO : POR EL AÑO 2011

CERTIFICA:

QUE A NOMBRE DE LA SOCIEDAD FIGURA MATRICULADO EN LA CAMARA DE COMERCIO BAJO EL NRO.369809- 2 ESTABLECIMIENTO DE COMERCIO: LABORATORIO FRANCO COLOMBIANO LAFRANCOL

UBICADO EN: --K 1 46A 70 DE CALI
FECHA MATRICULA : 29 DE ABRIL DE 1994
RENOVO : POR EL AÑO 2011

CERTIFICA:

QUE A NOMBRE DE LA SOCIEDAD FIGURA MATRICULADO EN LA CAMARA DE COMERCIO BAJO EL NRO.369810- 2 ESTABLECIMIENTO DE COMERCIO: LABORATORIO FRANCO COLOMBIANO LAFRANCOL

UBICADO EN: ---K 1A 46 A 32 DE CALI
FECHA MATRICULA : 29 DE ABRIL DE 1994
RENOVO : POR EL AÑO 2011

CERTIFICA:

QUE A NOMBRE DE LA SOCIEDAD FIGURA MATRICULADO EN LA CAMARA DE COMERCIO BAJO EL NRO.734908- 2 ESTABLECIMIENTO DE COMERCIO: LABORATORIO FRANCO COLOMBIANO

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CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE CALI

FECHA: 2011/08/10

HORA: 09:50:29

OPERACION: 01NJJ0810075

PAGINA: 18

LAFRANCOL

UBICADO EN: CRA. 1A NRO. 46A 28 DE CALI

FECHA MATRICULA : 28 DE MARZO DE 2008

RENOVO : POR EL AÑO 2011

CERTIFICA:

QUE A NOMBRE DE LA SOCIEDAD FIGURA MATRICULADO EN LA CAMARA DE COMERCIO BAJO EL NRO.734911- 2 ESTABLECIMIENTO DE COMERCIO: LABORATORIO FRANCO COLOMBIANO LAFRANCOL

UBICADO EN: CRA. 1B NRO. 46A 121 DE CALI

FECHA MATRICULA : 28 DE MARZO DE 2008

RENOVO : POR EL AÑO 2011

CERTIFICA:

QUE A NOMBRE DE LA SOCIEDAD FIGURA MATRICULADO EN LA CAMARA DE COMERCIO BAJO EL NRO.734913- 2 ESTABLECIMIENTO DE COMERCIO: LABORATORIO FRANCO COLOMBIANO LAFRANCOL

UBICADO EN: CRA. 3 NRO. 47 25 DE CALI

FECHA MATRICULA : 28 DE MARZO DE 2008

RENOVO : POR EL AÑO 2011

CERTIFICA:

QUE LA SOCIEDAD EFECTUO LA RENOVACION DE SU MATRICULA MERCANTIL EL 30 DE MARZO DE 2011 .

CERTIFICA:

QUE HACIENDA CALI FUE INFORMADO(A) EL 03 DE ABRIL DE 2008 DE LA APERTURA DEL ESTABLECIMIENTO DE COMERCIO. 734908-2 LABORATORIO FRANCO COLOMBIANO LAFRANCOL

QUE LA SECRETARIA DE SALUD FUE INFORMADO (A) EL 03 DE ABRIL DE 2008 DE LA APERTURA DEL ESTABLECIMIENTO DE COMERCIO. 734908- 2 LABORATORIO FRANCO COLOMBIANO LAFRANCOL

QUE PLANEACION FUE INFORMADO (A) EL 03 DE ABRIL DE 2008 DE LA APERTURA DEL ESTABLECIMIENTO DE COMERCIO. 734908-2 LABORATORIO FRANCO COLOMBIANO LAFRANCOL

QUE HACIENDA CALI FUE INFORMADO(A) EL 03 DE ABRIL DE 2008 DE LA APERTURA DEL ESTABLECIMIENTO DE COMERCIO. 734913-2 LABORATORIO FRANCO COLOMBIANO LAFRANCOL

QUE LA SECRETARIA DE SALUD FUE INFORMADO (A) EL 03 DE ABRIL DE 2008 DE LA APERTURA DEL ESTABLECIMIENTO DE COMERCIO. 734913- 2 LABORATORIO FRANCO COLOMBIANO LAFRANCOL

QUE PLANEACION FUE INFORMADO (A) EL 03 DE ABRIL DE 2008 DE LA APERTURA DEL

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CERTIFICADO NACIONAL

CAMARA DE COMERCIO DE CALI

FECHA: 2011/08/10

HORA: 09:50:29

OPERACION: 01NJ0810075

PAGINA: 19

ESTABLECIMIENTO DE COMERCIO. 734913-2 LABORATORIO FRANCO COLOMBIANO LAFRANCOL
QUE HACIENDA CALI FUE INFORMADO(A) EL 03 DE ABRIL DE 2008 DE LA APERTURA DEL
ESTABLECIMIENTO DE COMERCIO. 734911-2 LABORATORIO FRANCO COLOMBIANO LAFRANCOL
QUE LA SECRETARIA DE SALUD FUE INFORMADO (A) EL 03 DE ABRIL DE 2008 DE LA
APERTURA DEL ESTABLECIMIENTO DE COMERCIO. 734911- 2 LABORATORIO FRANCO
COLOMBIANO LAFRANCOL

QUE PLANEACION FUE INFORMADO (A) EL 03 DE ABRIL DE 2008 DE LA APERTURA DEL ESTABLECIMIENTO DE COMERCIO. 734911-2 LABORATORIO FRANCO COLOMBIANO LAFRANCOL

CERTIFICA:

QUE NO FIGURAN OTRAS INSCRIPCIONES QUE MODIFIQUEN TOTAL O PARCIALMENTE EL PRESENTE CERTIFICADO.

LOS ACTOS Y DOCUMENTOS REGISTRADOS QUEDAN EN FIRME CINCO (5) DIAS HABILES
DESPUES DE LA FECHA DE SU INSCRIPCION, SIEMPRE Y CUANDO DENTRO DE DICHO
TERMINO NO SEAN OBJETO DE RECURSOS EN LA VIA GUBERNATIVA.

DE CONFORMIDAD CON EL DECRETO 2150 DE 1.995 Y LA AUTORIZACION IMPARTIDA POR LA SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO LA FIRMA MECANICA QUE APARECE A CONTINUACION TIENE PLENA VALIDEZ PARA TODOS LOS EFECTOS LEGALES.

DADO EN CALI A LOS 10 DIAS DEL MES DE AGOSTO DEL AÑO 2011 HORA: 09:53:53 AM

Charles E. Felt



THE
FEDERAL
BUREAU
OF
INVESTIGATION
OF
THE
DEPARTMENT
OF
JUSTICE
WASHINGTON, D. C.

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 800.176.089-2

- / -



Industria y Comercio
SUPERINTENDENCIA

RECIBO DE CAJA

No. 11 - 0080125

Bogotá D.C., Agosto 19 de 2011 - 11:53:39

RECIBIDO DE : REYES Y REYES ABOGADOS

NI 900.048.328

*** Soporte del Pago ***

TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	VR. PAGO
CONSIGNACION	BANCO DE BOGOTA	062754387	135712861	19/08/2011	304.000.00

*** Conceptos Pagados ***

CANT.	RENTISTICO	CONCEPTO	Vr. UNDITARIO	Vr. CONCEPTO
1 50005-01-04	PRESENTACION DE OBSERVACIONES A SOLICITUDES	12 MARCAS Y LEMAS COMERCIALES	304.000.00	304.000.00
				\$304.000.00

=====

SON: **TRESCIENTOS CUATRO MIL PESOS MONEDA CORRIENTE**

Responsable: _____

Recibo de Caja Aplicado al Expediente No. _____

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 10-063351- -00003-0000

Fecha: 2011-08-19 11:59:54 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 400 OPOSIOBSERVTER Folios: 79

Sede Centro: Carrera 13 No. 27 - 00 Pisos 3,4,5 y 10 Bogotá, D.C.- Colombia

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Agosto 19 de 2011 - 11:53:39

2020
Bogotá, D.C.,

Doctor:
Carlos Reinaldo Olarte García
Apoderado

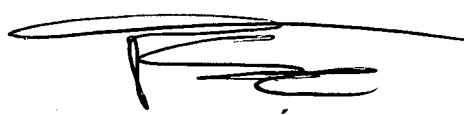
Oficio N°:
E 15001

ASUNTO:	Radicación PCT N°	10-63351
	Trámite	011
	Evento	378
	Actuación	440
	Folios	066

Con fundamento en lo previsto por el artículo 43 de la Decisión 486 de la Comisión de la Comunidad Andina, se le comunica que la oposición presentada por el doctor José Luis Reyes Villamizar, en su calidad de apoderado del Laboratorio Franco Colombiano Lafrancol S.A., reúne los requisitos formales exigidos por la ley al momento de su presentación.

En cumplimiento de lo dispuesto en la citada norma, se le concede el término de sesenta (60) días hábiles siguientes a la fecha de notificación del presente oficio, para que haga valer sus argumentaciones y presente pruebas.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única N° 10 de 2001.


JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (C).

El anterior oficio fue notificado por fijación en lista de fecha, _____.

2020 