Comparative Molecular Field Analysis of Some Clodronic Acid Esters

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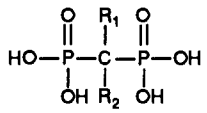
Comparative molecular field analysis (CoMFA) has been used as a three-dimensional quantitative structure–activity relationship (QSAR) method to correlate three different types of biological activity data with physicochemical properties of some clodronate ester analogues, which act as bone-resorption regulators in cell cultures and rats. The QSAR studies show the importance of the steric properties of these new bisphosphonate derivatives for the inhibition of bone resorption in bone cell cultures and for their bioavailability in rats. This information will be used in predicting the structure of new more potent bisphosphonic compounds.

Introduction

Bisphosphonates are compounds characterized by two C–P bonds:



These compounds have widely been used in drugs in the treatment of various bone and tooth diseases and as regulators in calcium metabolism. The mechanism of action of these drugs in detail is still unknown. Bisphosphonates seem to bind to the surface of bone, but some cellular mechanisms are also involved.[1] The high bone affinity of these drugs is due to their high affinity for calcium phosphate.[2] Bisphosphonates inhibit both the formation[3,4] and dissolution[5] of this mineral. Bisphosphonates are also very powerful inhibitors of bone resorption, both in vivo and in vitro.[1]

There are still some major practical problems with currently clinically used bisphosphonates. The therapeutic index and the absorption of these highly ionized drugs (in physiological pH) is poor. There is extensive work carried out in the pharmaceutical industry to develop new and better bisphosphonate analogues for the treatment of various bone diseases, from which some like osteoporosis is becoming more and more common in our society nowadays.

We have used various theoretical drug-design techniques in order to understand the mechanism of action of bisphosphonates and to guide the development of new analogues. In this work comparative molecular field analysis (CoMFA) developed by Cramer et al.[6,7] was used to model various (dichloromethylene)bisphosphonic acid (clodronic acid) esters and to correlate three different types of biological activities, the relative bioavailability and inhibition of bone resorption in vitro and in vivo, with ligand molecules' three-dimensional steric and and electrostatic fields. Measured log P values have also been correlated with these biological activities.

Materials and Methods

The molecular modeling and the comparative molecular field analyses were carried out in the molecular modeling project of the University of Joensuu chemistry department. We have used CoMFA to map three-dimensional quantitative structure activity relationships between the measured biological data and molecules' steric and electrostatic properties. This fairly new three-dimensional QSAR technique is based on the analysis of the steric and electrostatic fields of molecules mapped by a probe atom in a molecular mechanics force field. The resulting field values on a regularly spaced lattice around the molecules are then correlated with the biological activity data by a very efficient statistical method, partial least squares, PLS.[8] Modern statistical sampling techniques, bootstrapping and cross-validation,[9] are used to determine the quality of the correlations. The resulting QSAR equation with its hundreds of terms is then visualized in a graphical form on a molecular modeling workstation's screen. This maplike presentation of the QSAR equation can then easily be used both qualitatively and quantitatively in predicting the activities of new analogues and in drug-design work. However, the results of these QSAR studies obtained from the analyses of rather specific set of compounds do not alone necessarily give very general information of the mechanisms of molecular interactions, but they can be used as very precise drug-development tools.

The bisphosphonate esters (Table I) were synthesized[10] and their bioactivities and log P values were measured at the research center of Huhtamäki Oy Leiras.

The biological activities used in the CoMFA analysis of these bisphosphonate esters were the percent bone resorption inhibition in vitro (inhibn res), the percent bone resorption inhibition in vivo (Δ Ca), and the relative bioavailability of the compounds (rel bioaval). These measurements are described briefly below. Both relative bioavailability and bone resportion inhibition in vitro data were originally reported as "response at a standard concentration" form, and they were converted to "concentration needed to produce a standard response" form by using eq 1, the "logit transformation",[11] where

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(1) Fleisch, H. In *Handbook of Experimental Pharmacology*; Baker, P. F., Ed.; Springer: Berlin/Heidelberg, 1988; Vol. 83, pp 441–465.
(2) Jung, A.; Bisaz, S.; Bartholdi, P.; Fleisch, H. *Calcif. Tissue Res.* **1973**, *13*, 27.
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(7) Clark, M.; Cramer, R. D.; Jones, D. M.; Patterson, D. E.; Simerath, P. E. *Tetrahedron Comput. Methodol.* **1990**, *3*, 47.
(8) Wold, S.; Ruhe, A.; Wold, H.; Dunn, W. J., III *SIAM J. Sci. Stat. Comput.* **1984**, *5*, 735.
(9) Cramer, R. D., III; Bunce, J. D.; Patterson, D. E.; Frank, I. E. *Quant. Struct.-Act. Relat.* **1988**, *7*, 18.
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(11) Franke, R. *Theoretical Drug Design Methods*; Elsevier: Amsterdam, 1984; p 21.

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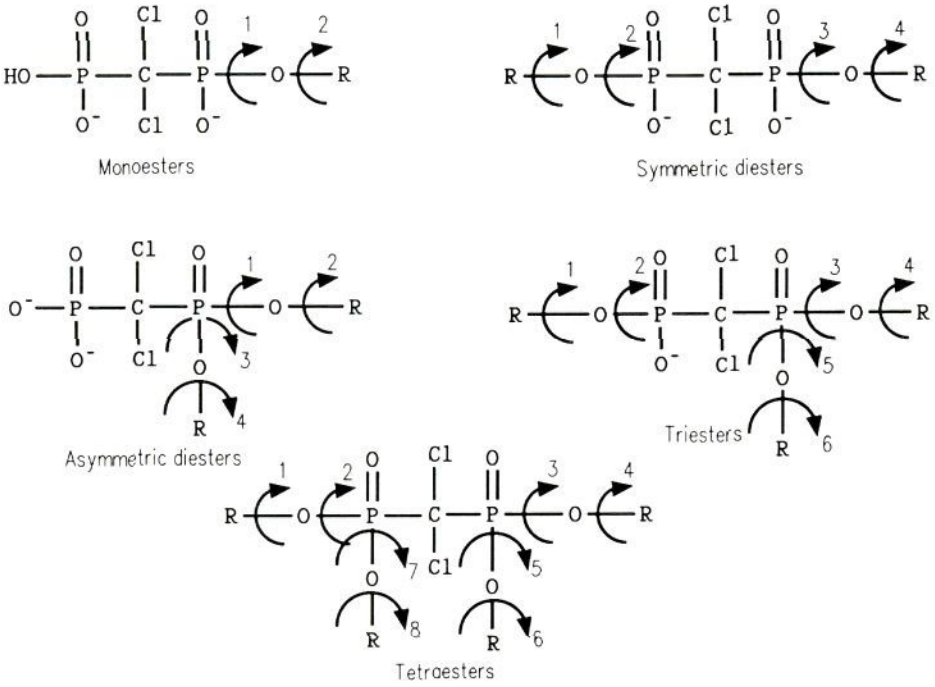

Figure 1. The rotatable bonds in the conformation analysis.

value A is used as a correction factor to the logarithm of the standard concentration.

$$A = \log\left(\frac{\%\ \text{activity of standard}}{(100\% - \%\ \text{activity of standard})}\right) \quad (1)$$

Experimental Section

1. Comparative Molecular Field Analysis. The comparative molecular field analysis and all the molecular modeling calculations were performed with Sybyl 5.2 molecular modeling program.[12] Log P (buffer 7.4) values were used in the calculations. The steric (van der Waals$^{6-12}$) and electrostatic (Coulombic, with a $1/r$ dielectric) interaction between each of the compounds and a probe atom placed at the various intersections of a regularly spaced three-dimensional lattice were calculated automatically by the program. The interaction energies were calculated by the standard Tripos force field,[13] and the atomic charges were calculated by the method of Gasteiger and Marsili.[14] The probe atom had the van der Waals (vdW) properties of calcium atom and a charge of +2 in the CoMFA of bone resorption in vivo and in vitro data, and vdW properties of sp^3 carbon atom and a charge of +1.0 in the CoMFA of bioavailability data. The CoMFA grid spacing was 2 Å in all three dimensions in the region definition. The region was large enough to surround all the molecules in the analysis.

All the clodronate esters were built from clodronic acid calcium salt by adding the parent groups to the crystal structure.[15] The compounds were built in ionized forms by using measured pK_a values (our unpublished results), i.e. all the mono- and diesters had a negative charge of −2, and the triesters a charge of −1. These charged molecules were used throughout all the analyses. Since no general assumptions are made about the mechanism of action, both diesters and triesters are used in the same analyses despite the possible different mode of binding. The resulting structures were minimized with the standard Tripos force field without the electrostatic term. The conformational space was mapped by a rigid bond rotation algorithm. The rotatable bonds were chosen to be the bonds on both sides of the ester sp^3 oxygen (Figure 1).

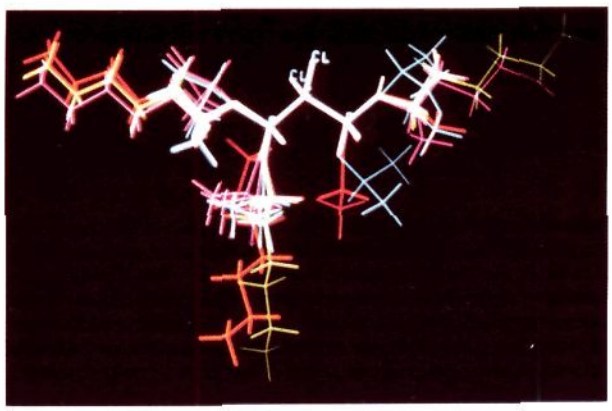

Figure 2. The superimposed structures of all the compounds used in the CoMFA, "the orientation rule".

In the conformational analysis the bonds were rotated from 0° to 360° with an increment of 10° for monoesters, 20° for diesters, 30° for triesters, and 40° for tetraesters. In the isopropyl group the torsion angles of the methyl groups were not scanned. In aliphatic chains (ethyl and hexyl) the chain was built in the extended conformation (the conformation between carbon atoms is trans).

The rotations of the bisphosphonate C–P–C bonds were not scanned, because it was assumed that the active conformation has to have the ionized oxygen groups in the eclipsed conformation in Ca^{2+} binding as in the crystal structure of clodronate.[15] The torsion barrier of the C–P bond in monoisopropyl ester was 16.7 kJ/mol. This energy value is small if compared with interaction energies between charged species, so the Ca^{2+} ion is able to force the torsion angles of C–P–C bonds into the eclipsed conformation whenever it is interacting with bisphosphonates.

The resulting minimum-energy structures from the conformational analysis were minimized once more with the Tripos force field with a very tight convergence criteria. This was necessary due to the relatively large torsional increment in bond rotations with larger esters. The resulting geometries were used in further analysis.

The orientation rule was chosen to be the superimposition of the central carbon and phosphorus atoms of bisphosphonates (Figure 2), and it was used in all of the analyses. This is the most

(12) Sybyl Version 5.2, Tripos Associates, Inc., St. Louis, MO, running on Digital Microvax-II with Evans&Sutherland PS390 workstation.
(13) Vinter, J. G.; Davis, A.; Sauder, M. R. *J. Comput.-Aided Mol. Des.* **1987**, *1*, 31.
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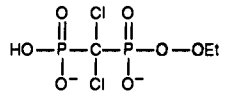
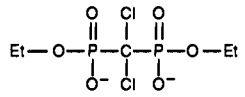
(15) Nardelli, M.; Pelizzi, G. *Inorg. Chim. Acta* **1983**, *80*, 259.

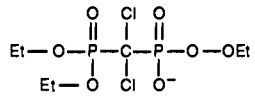
Table I. The Structures of Clodronate Esters Analyzed in This Work



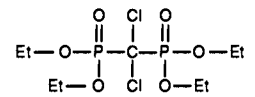
1, (dichloromethylene)bisphosphonic acid, monoethyl ester



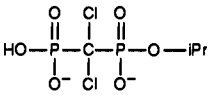
2, (dichloromethylene)bisphosphonic acid, symmetric diethyl ester



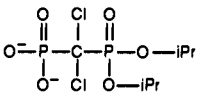
3, (dichloromethylene)bisphosphonic acid, triethyl ester



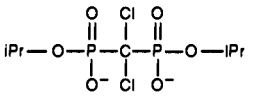
4, (dichloromethylene)bisphosphonic acid, tetraethyl ester



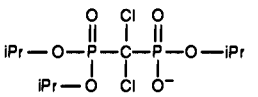
5, (dichloromethylene)bisphosphonic acid, monoisopropyl ester



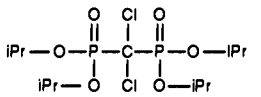
6, (dichloromethylene)bisphosphonic acid, asymmetric diisopropyl ester



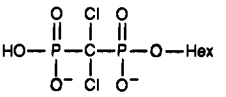
7, (dichloromethylene)bisphosphonic acid, symmetric diisopropyl ester



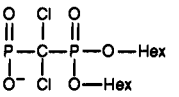
8, (dichloromethylene)bisphosphonic acid, triisopropyl ester



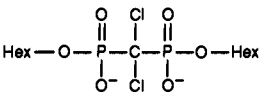
9, (dichloromethylene)bisphosphonic acid, tetraisopropyl ester



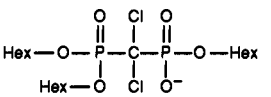
10, (dichloromethylene)bisphosphonic acid, monohexyl ester



11, (dichloromethylene)bisphosphonic acid, asymmetric dihexyl ester



12, (dichloromethylene)bisphosphonic acid, symmetric dihexyl ester



13, (dichloromethylene)bisphosphonic acid, trihexyl ester

crucial point of the whole methodology; the orientation rule determines the orientation of the corresponding molecular fields, and so the whole CoMFA and the results depend on it. Here our unpublished studies on the Ca^{2+} binding properties suggest the importance of the basic bisphosphonic backbone, and if the Ca^{2+} binding is chosen to be the basic model mechanism of action, the superimposition of the backbones is the best way to compare the binding properties.

In modeling the bioavailability the situation is not so straightforward. In the QSAR analysis the "plane" log P parameter takes care of the hydrophobic effect, which is the major parameter adjusting the passive permeation processes. For that reason the expectations with steric and electrostatic parameters in this data set were not very high; these effects are often negligible in absorption processes.

The PLS methodology was used in the QSAR calculations. The cross-validation was used to obtain the optimum number of the components extracted in PLS. The final models were computed with the conventional r^2. The confidence intervals of each statistical parameter were computed with the bootstrapping procedure.

The QSAR analysis was performed separately for each measured biological activity type, i.e. three separate analysis and data sets were prepared. The first conventional QSAR studies (not presented here because of their small informational value) suggested that the reference compound clodronate with its pretty high activity influenced the results too much, so clodronate was not used in the following QSAR analyses as a standard data set compound.

2. Relative Bioavailability. Relative bioavailability of the alkyl ester derivatives of clodronate disodium salt was determined from urinary excretion data in rats. The ratio (F) of the total amount of unchanged drug excreted in the urine after oral administration to that following intravenous (iv) administration is a measure of the absorption of the drug, where $[X^{\infty}_u]$ is the cumulative amount of drug eliminated unchanged in the urine and D the dose.[16]

$$F = [X^{\infty}_u]_{oral} D_{iv} / [X^{\infty}_u]_{iv} D_{oral} \quad (2)$$

The compounds were administered to male rats (Sprague–Dawley) orally and intravenously at the dose level of 100 μmol/kg. Three animals were used for each dose level and administration route. Urine was collected in metabolism cages at 0–1, 1–3, 3–6, 6–12, 12–24, 24–48, and 48–72 h after dosing. The volumes for the urine samples were measured, and the analysis of the compounds was performed by a gas chromatographic method using a nitrogen–phosphorus sensitive detector and a capillary column.

3. Inhibition of Bone Resorption in Vitro. Bone resorption in vitro was tested in a mouse calvarial resportion assay by measuring the release of ^{45}Ca from labeled bones.[17-19] Calvarial pieces were cultured in the presence of parathyroid hormone (PTH, 10 nmol/l) and clodronate esters at the concentrations of 100 μmol/l for 72 h. Results were calculated as a percentage of control values (PTH-stimulated bones without bisphosphonates).

4. Inhibition of Retinoid-Induced Bone Resorption in Vivo. The inhibitory effect of the test compound was assessed with a model of retinoid-induced bone resorption in thyroparathyroidectomized (TPTX) rats[20,21] with slight modifications. The retinoid induced an increase in bone resorption and in the number of vertebral subperiosteal osteoclasts. In this model, plasma calcium (Ca) level is used to monitor changes in bone resorption.

Male Spraque–Dawley rats (Han-SPRD/Le) weighing 130–160 g were used. They were anesthetized and surgically TPTX. The arotinoid Ro 13-6298 (ethyl *p*-[(*E*)-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)-1-propenyl]benzoate), a gift from Roche Oy, Helsinki, Finland, was administered at a dose of 25 μg/rat subcutaneously for three consecutive days. Plasma Ca (mmol/l) was measured before the first and 24 h after the last injection of retinoid (day 0 and 3).

The test compound was administered intravenously (iv) on day 0 after which the first retinoid injection was given. The inhibitory effect of the test compound against retinoid-induced bone resorption was expressed as a change in plasma Ca in TPTX rats.

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(17) Reynolds, J. J.; Dingle, J. T. *Calcif. Tissue Res.* **1970**, *4*, 339.
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(20) Stutzer, A.; Fleisch, H.; Trechsel, U. *Calcif. Tissue. Int.* **1988**, *43*, 294.
(21) Trechsel, U.; Stutzer, A.; Fleisch, H. *J. Clin. Invest.* **1987**, *80*, 1679.

Table II. The Data Table of the Biological Activity Inhibition of Bone Resorption in Vitro[a]

compound	% inhibn res	logit (inhibn res)	log $P_{7.4}$	(log $P_{7.4}$)^2	CoMFA
1, monoethyl	15.4	3.26	−5.44	29.59	195.00
2, symmetric diethyl	12.3	3.15	−3.96	15.68	209.00
3, triethyl	14.7	3.23	−2.37	5.62	138.00
4, tetraethyl	18.8	3.36	1.31	1.72	120.00
5, monoisopropyl	11.8	3.12	−3.24	10.50	200.00
6, asymmetric diisopropyl	0.8	1.30	−3.56	12.67	226.00
7, symmetric diisopropyl	8.0	2.93	−2.96	8.76	218.00
8, triisopropyl	7.4	2.90	−0.95	0.90	152.00
9, tetraisopropyl	17.5	3.32	2.89	8.35	148.00
10, monohexyl	21.1	3.42	−2.75	7.56	227.00
11, asymmetric dihexyl	15.1	3.25	−0.15	0.02	268.00
12, symmetric dihexyl	20.5	3.41	−0.64	0.41	266.00
13, trihexyl	58.0	4.31	4.89	23.91	218.00

[a] The number in the CoMFA column indicates the lattice intersections located "inside" each individual molecule, which is also a very crude volume estimate. The standard concentration of the activity measurement is 100 μM.

5. log *P* Measurements. Each clodronate ester (200 mg) was dissolved in 4 mL of phosphate buffer, pH 7.4, or 1-octanol (saturated with the opposite phase), depending on the properties of the molecule. 1-Octanol or buffer (4 mL) was added and the tube was shaken for 1 h and allowed to stand overnight. The phases were finally separated by centrifugation.

The determination of the analyte in the organic phase as well as in the water phase was carried out with the capillary gas chromatographic method and nitrogen–phosphorus sensitive detection.

Results

1. CoMFA of Inhibition of Bone Resorption in Vitro. The data table used in the analysis is presented in Table II.

The initial analyses were done with four PLS components and five cross-validation groups. This information was used to determine the optimum number of the components and to find the possible outliers. The analyses were then repeated without cross-validation and with bootstrapping procedures.

The conventional QSAR analyses of log *P* variables gave only poor correlations with almost no predictive value due to small correlation coefficients and high standard deviations.

The analysis of the CoMFA variables:

cross-validation groups =
5, components = 4; coefficients not listed for CoMFA run: $r^2_{cross} = 0.419$, s = 0.315, $F_{4,7} = 1.261$, $n = 12$ (3)

The final CoMFA equation used for predictions and CoMFA coefficient contour map calculations:

cross-validation groups =
0, components = 4; coefficients not listed for CoMFA run: $r^2 = 0.993$, $s = 0.034$, $F_{4,7} = 241.570$, $n = 12$ (4)

Bootstrapping eq 4 gives the following:

cross-validation groups =
0, bootstrap samples = 6, components =
4; coefficients not listed for the CoMFArun, $r^2 =$
0.985 ± 0.022, $s = 0.024 \pm 0.018$, $n = 12$ (5)

Predicted activities and residuals using eq 4 are in Table III.

Equations with CoMFA columns (3–5) cannot be printed out with coefficients; instead, statistical and graphical analyses of CoMFA results are possible. The field-distribution histograms show the distribution of the CoMFA field points around the significant QSAR coefficients values and also the field range. Histograms can be used to determine the contour levels for QSAR maps and to determine whether the particular field values are high enough to make the field significant in the QSAR. In Figure 3 is the field distribution histogram for the steric CoMFA field of inhibition of bone resorption in vitro data (eq 4).

Table III. Predicted Activities and Residuals from Equation 4 of the Data Set of Inhibition of Bone Resorption in Vitro

compd	logit (inhibn res)	predicted inhibn res	residual value
1	3.26	3.22	0.04
2	3.15	3.11	0.04
3	3.23	3.24	−0.01
4	3.36	3.36	0.00
5	3.12	3.15	−0.03
7	2.93	2.93	0.00
8	2.90	2.91	−0.01
9	3.32	3.32	0.00
10	3.42	3.48	−0.06
11	3.25	3.25	0.00
12	3.41	3.41	0.00
13	4.14	4.12	0.02

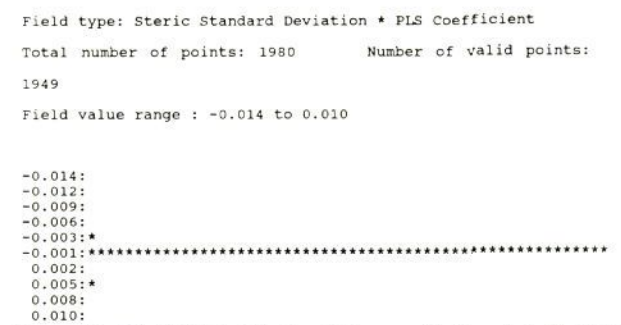

Figure 3. The field distribution histogram for the steric CoMFA of the inhibition of bone resorption in vitro data.

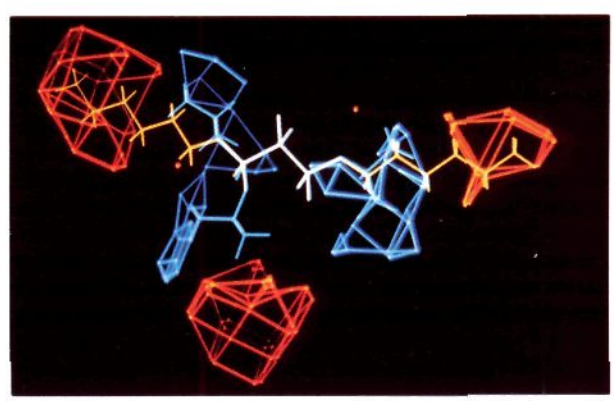

Figure 4. The major steric features of the QSAR for inhibition of bone resorption in vitro. Red contours surround regions where a higher steric interaction would increase the activity, and blue regions show where lower steric interaction would increase the activity. The contour levels are −0.006 for blue and 0.006 for red.

The QSAR produced by a CoMFA is represented as a 3-D coefficient contour map. Figure 4 shows this map, for the steric aspect only, for the inhibition of bone resorption in vitro data set using eq 4. The electrostatic maps are almost featureless for this data set. In Figure 4 the orange molecule, asymmetric dihexyl ester, is a "good", active compound, and the light blue triisopropyl ester is a less active compound. The colored polyhedra surround all lattice points where the QSAR associates changes in molecule field values with changes in biological activity. The polyhedra surround lattice points where the scalar products of the associated QSAR coefficient and the

Table IV. The Data Table of the Biological Activity of Relative Bioavailability[a]

compd	% rel bioaval	logit bioaval	log $P_{7.4}$	(log $P_{7.4}$)^2	CoMFA
1	37.9	3.79	−5.44	29.59	109.00
2	34.0	3.71	−3.96	15.68	129.00
3	7.0	2.88	−2.37	5.62	127.00
4	5.0	2.72	1.31	1.72	134.00
5	3.6	2.57	−3.24	10.50	115.00
6	13.6	3.19	−3.56	12.67	149.00
7	1.9	2.30	−2.96	8.76	142.00
8	1.9	2.28	−0.95	0.90	145.00
9	32.1	3.67	2.89	8.35	174.00
10	17.9	3.40	−2.75	7.56	149.00
11	7.6	2.91	−0.15	0.02	199.00
12	31.0	3.65	−0.64	0.41	201.00

[a] See Table II for the CoMFA column explanation. The standard dose was 100 μmol/kg.

standard deviation of all values in the corresponding column of the data table are higher or lower than a specified value. In Figure 4 the blue polyhedra surround regions where less bulk is "good" (the steric column-variance-weighted QSAR coefficients are less than −0.006, so activity is expected to decrease with steric bulk), while red polyhedra surround regions where more bulk is "good" (the steric QSAR coefficients are greater than +0.006 in Figure 4).

Equation 4 has been used to predict the activity for two molecules. Firstly, clodronate has been evaluated by a similar CoMFA, and the parameters have been substituted into eq 4. The predicted activity value for logit (inhibn res) was 3.110, which is much lower than the measured value, 4.00. This may be due to the fact that the Ca^{2+} binding is not the only effect in biological action of bisphosphonates. The second compound, dihexyl pentyl triester, was also predicted. The predicted activity was 3.551. This suggests that it may not be necessary to use as long as six-carbon chains in aliphatic esters, and the activity should still remain high.

2. CoMFA of Bioavailability. The data table used in the analysis is presented in Table IV.

The bioavailability data was first correlated with log P terms. No significant equations could be found.

The CoMFA of the bioavailability data set was performed separately. The optimum number of components in the initial CoMFA run after five cross-validation groups was four (eq 6). Compound **9** (tetraisopropyl ester) was an outlier, and it was dropped out from the conventional CoMFA run (eq 7).

The results of CoMFA of the bioavailability data set:

cross-validation groups = 5; coefficients not listed for the CoMFA run: optimum number of components = 4, r^2_{cross} = 0.145, s = 0.673, $F_{4,7}$ = 0.296, n = 12 (6)

The final conventional CoMFA equation which has been used for predictions and coefficient contour map calculations:

cross-validation groups = 0, components = 4, dropped **9** (tetraisopropyl ester); coefficients not listed for the CoMFA run: r^2 = 0.944, s = 0.168, $F_{4,7}$ = 25.384, n = 11 (7)

Bootstrapping eq 7:

cross-validation groups = 0, bootstrap samples = 6, components = 4, dropped **9** (tetraisopropyl ester); coefficients not listed for the CoMFA run: r^2 = 0.980 ± 0.015, s = 0.0960 ± 0.085, n = 11 (8)

Table V. Predicted Activities and Residuals from Equation 7 for the Data Set of Relative Bioavailability

compd	logit bioaval	predicted bioaval	residual value
1	3.79	3.67	0.12
2	3.71	3.69	0.02
3	2.88	3.93	−0.05
4	2.72	3.68	0.04
5	2.57	2.90	−0.33
6	3.19	3.24	−0.05
7	2.30	2.20	0.10
8	2.28	2.30	−0.02
10	3.40	3.33	0.07
11	2.91	2.77	0.14
12	3.65	3.70	−0.05

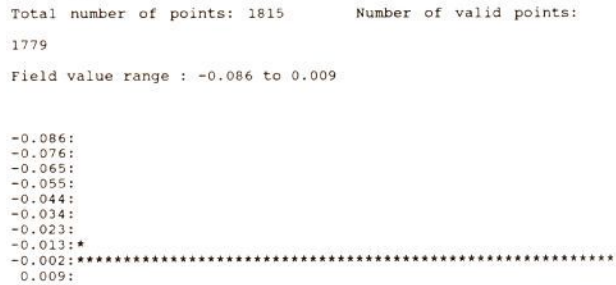

Figure 5. The field distribution histogram for the steric CoMFA of the relative bioavailability data.

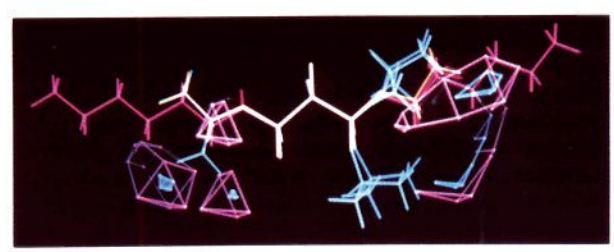

Figure 6. The steric coefficient contour plot of CoMFA of the relative bioavailability data set. The contour levels are −0.02 for violet and −0.05 for blue. Light blue molecules have lower activity than the purple, red, and orange molecules.

Predicted activities and residuals using eq 7 are in Table V.

The final conventional correlation coefficient (r^2) in the CoMFA run (eq 7) was 0.944. This is a very high value, but the first cross-validation run (eq 6) gave r^2_{cross} = 0.145. Here it seems to be possible chance correlation in the conventional analysis, and the predictions using this equation may not be very good. The steric CoMFA coefficient plot was still computed. The field distribution histogram for the steric field is presented in Figure 5. Here, too, the electrostatic map is featureless. In Figure 6 is the column-variance-weighted CoMFA steric coefficient contour map with two contour levels from eq 7 for the bioavailability data set. The molecules in Figure 6 are triisopropyl and asymmetric diisopropyl ester (light blue), which have low activity, and monoethyl ester (red), symmetric diethyl ester (orange), and symmetric dihexyl ester (purple), which have higher activity.

The results are somewhat similar to those obtained for the inhibition of bone resorption in vitro data set; i.e. the small bulky groups like isopropyl are not good for the activity. These results must be used with care due to the possible chance correlation in the conventional CoMFA run.

3. CoMFA of Inhibition of Bone Resorption in Vivo. The data table used in the analysis is presented in Table VI.

Table VI. Data Table of the Biological Activity of Inhibition of Bone Resorption in Vivo at the Dose of 150 μmol/kg (iv)[a]

compd	ΔCa, mmol/l	logit of 1/ΔCa	log $P_{7.4}$	(log $P_{7.4}$)^2	CoMFA
1	0.77	3.11	-5.44	29.59	195.00
2	1.05	2.98	-3.96	15.68	209.00
3	0.70	3.15	-2.37	5.62	138.00
4	1.52	2.81	1.31	1.72	120.00
5	1.27	2.90	-3.24	10.50	200.00
6	1.25	2.90	-3.56	12.67	226.00
7	1.09	2.96	-2.96	8.76	218.00
8	1.51	2.82	-0.95	0.90	152.00
9	1.54	2.81	2.89	8.35	148.00
11	0.93	3.03	-0.15	0.02	268.00
12	1.43	2.84	-0.64	0.41	266.00

[a] See Table II for the CoMFA column explanation.

The analysis of log P variables did not yield to any significant equations. Also, the CoMFA of the inhibition of bone resorption in vivo data gave only very poor correlation with a negative cross-validated r^2, which suggests no QSAR equation. Even in the conventional PLS run the r^2 was only 0.235, so no QSAR were found with log P or CoMFA parameters in the inhibition of bone resorption in vivo data set.

Discussion

It may be very difficult to predict and model the activity measured in whole animal tests in QSAR work. It is often impossible to differentiate absorption, distribution, metabolism, and excretion processes from each other and from the binding of the drug to a proposed receptor. All these processes are still important for the therapeutic value of a drug. This was also true here, and the best results were obtained in the analysis of biological data of bone resorption in vitro. The conventional QSAR analyses of hydrophobicity variables resulted to equations with very small coefficients and large deviations, so they had no predictive value. The CoMFA of the inhibition of bone resorption in vitro and bioavailability data sets showed the importance of the steric factors in both cases, but there may be some chance correlation with the bioavailability data set. According to this study the aliphatic straight-chained esters are predicted to be the best analogues in this series of compounds. Small bulky ester groups like isopropyl should be avoided near both phosphonate groups.

In the CoMFA analyses high correlations were found where both diesters and triesters fit the model. This suggests that the bidentate binding mode seems not to be the only prerequisite for the bisphosphonate activity, but this needs further investigations.

A very important thing to remember is that even if these CoMFA contour maps look like receptor maps, they are not comparable with them. The reason is that each of these maps is computed just from a small set of compounds with a certain specific biological activity. They can be used in certain specific analogue groups as predicting tools, but they do not provide any general information about the proposed receptor.

Conclusions

The QSAR, and especially the CoMFA methodology, has been used successfully in this study. The CoMFA QSAR coefficient 3-D maps can help to visualize the steric and electrostatic properties of the compounds important to the biological activity. In this work correlations were found between two biological data types and CoMFA steric structural parameters and this information will be used in designing new analogues.

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