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Synthesis, characterization and biological properties of Vanadyl(IV) complexes of Diclofenac and Indomethacin: an experimental and theoretical study

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Two new vanadyl(IV) complexes with the non-steroidal antiinflammatory drugs Indomethacin and Diclofenac were synthesized and characterized by elemental analysis, electronic, diffuse reflectance and FTIR spectroscopies and thermal behavior. The structures of the oxo-vanadium(IV) complexes were obtained by carrying out *ab initio* calculations (B3LY/3–21G) owing to the difficulties of obtaining single crystals of good quality for X-ray studies. Indomethacin and Diclofenac did not cause any effect when tested on cellular proliferation in two osteoblast-cell lines in culture (MC3T3E1 and UMR106). The biological effect of the complexes depends on the cellular type and on the nature of the coordinated ligands. Copyright © 2005 John Wiley & Sons, Ltd.**

INTRODUCTION

Indomethacin [1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indole-3-acetic acid = IndoH] and Diclofenac [2-(2,6-dichloroamino)phenyl]acetic acid = DiclofH are potent non-steroidal antiinflammatory drugs (NSAIDs). Non-selective cyclooxigenase (COX) inhibitors of the general arylalkanoic acid formula ArCRHCOOH make up the largest group of NSAIDs, e.g. salicylate, indoles, propionic acids and fenamates. Their action as antiinflammatory agents in the treatment of musculoskeletal and painful diseases is the major clinical application.[1] Inflammation is an important response to tissue injury due to noxious stimulus. There are a number of recently published papers on the effects of NSAIDs in bone-related tissues under different inflammatory conditions.[2–4] The importance of this multifaceted process is better appreciated as the beginning of the tissue repair. An antiinflammatory agent that facilitates the repair process would be expected to reestablish normal function. It is known that many diseases that are not generally recognized as inflammatory diseases do have an inflammatory component that requires tissue repair to attain normal function. Appreciation of this point allows one to understand why antiinflammatory agents able to promote tissue repair are also effective in treating or preventing these other diseases, such as cancer, seizures, etc. The recognition that other diseases are in part inflammatory may account for other therapeutical applications of antiinflammatory agents that promote tissue repair processes.[5]

The major mediators of inflammation are prostaglandins. Prostaglandins that contribute to inflammation are derived from COX-2 (cyclooxygenase-2), whereas prostaglandins that are involved in physiological processes are derived from the constitutively expressed isoform COX-1.[6] COX-1 is constitutively expressed as a 'housekeeping' enzyme in most tissues. In contrast, COX-2 can be upregulated by different proinflammatory agents. Recently, COX-2 was also shown to be expressed under basal conditions in various organs (bone tissue included), suggesting that this isoenzyme may play a more complex physiological role than was expected.[7] The view that all NSAIDs act by inhibiting the production of prostaglandins has been challenged by the discovery that they also affect a wide variety of cellular processes that are important for their therapeutic actions and side effects.[8] In this context it is known that bone metabolism is regulated by several mediators, such as interleukins, growth factors, prostaglandins, etc. These mediators play a role in the balance between bone formation and resorption by

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different mechanisms.[9] For instance, it has been shown that the induction of COX-2 in mouse osteoblasts is involved in IL-6 (interleukine 6)-induced osteoclast formation, with increased levels of PGE2. These effects were totally abolished by the addition of NSAIDs to the culture medium.[10] On the other hand, it has been shown that vanadate also inhibited the stimulated bone resorption in neonatal mouse calvaria. The inhibition occurred in a dose–response manner, vanadate being effective against several resorption stimulators like PGE2, PTH, VitD.[11]

Since it is well known that the interaction of metal ions with different ligands may influence the biological activity of drugs administered for therapeutic reasons,[12–20] we have initiated studies on the coordination chemistry of NSAIDs with vanadyl(IV) cations[21] in an attempt to examine their mode of binding and their biological effects on osteoblast-like cells in culture. It is well known that vanadium is retained mainly in bones. For this reason it is very interesting to study the biological effects of vanadium compounds with NSAIDs in bone-related cells. In this study we synthesized and characterized two vanadyl(IV) complexes with Indomethacin and Diclofenac. It was not possible to obtain single crystals of good quality for structural determinations. This fact has been observed for other systems, like complexes of vanadyl(IV) cations with ligands such as carbohydrate derivatives and monodentate carboxylates.[22–24] For this reason their structures have been derived by *ab initio* calculations. Moreover, we tested their effects on the proliferation of normal and tumoral osteoblasts in culture.

EXPERIMENTAL

Materials and methods

Diclofenac sodium salt and Indomethacin (Sigma) and $VOSO_4$ (Merck) were used as supplied. Corning or Falcon provided tissue culture materials. Dulbecco's modified Eagle's medium (DMEM), and trypsin–EDTA were purchased from Gibco (Gaithersburg, MD, USA) and fetal bovine serum (FBS) from GibcoBRL (Life Technologies, Germany). All other chemicals used were of analytical grade.

The electronic UV–vis and diffuse reflectance spectra were recorded on a Hewlett-Packard 8453 diode-array spectrophotometer and a Shimadzu UV-300 spectrophotometer respectively. IR spectra were recorded on a Bruker IFS 66 FTIR-spectrophotometer from 4000 to 400 cm^{-1} using the KBr pellet technique. Elemental analyses for carbon, hydrogen and nitrogen were performed using a Carlo Erba EA 1108 analyzer. Sodium content was determined by flame photometry. Vanadium contents were determined by the tungsto-phosphovanadic method. Thermogravimetric (TG) and differential thermal analysis (DTA) were performed on a Shimadzu system (models TG-50 and DTA-50 respectively) working in an oxygen flow (50 ml min^{-1}) and at a heating rate of 10 °C min^{-1}. Sample quantities ranged between 5–10 mg. Al_2O_3 was used as a DTA standard.

Synthesis

$Na_2[VO(Diclof)(BuO)_3]$ (I)

A solution of Diclofenac sodium salt (2 mmol) in hot butanol (10 ml) was prepared. Then, $VO(acac)_2$ (1 mmol) dissolved in 10 ml of hot butanol was added under nitrogen atmosphere. Under these conditions, the solution was concentrated to eliminate Hacac by evaporation. In this case, acac acted as a base to form butoxide. The driving force for this reaction was the excess of butanol and the elimination of Hacac.[26] A green precipitate was formed. It was filtered, washed successively with warm butanol and dried in air. Anal. Found: C, 49.7; H, 5.8; N, 2.3; Na, 7.1; V, 7.9. Calc. for $C_{26}H_{37}O_6Cl_2NNa_2V$: C, 49.8; H, 5.9; N, 2.2; Na, 7.3; V, 8.1%. MW_{calc}: 627.

For comparative purposes in the IR studies the Diclofenac acid was prepared as follows: Diclofenac sodium salt (3 mmol) was dissolved in methanol (30 ml). To this solution a dilution of 1/1 hydrochloric acid was added dropwise until a white precipitate was obtained. This was filtered, washed with distilled water and dried under vaccum. HDiclof was characterized by IR spectroscopy.

$Na_2[VO(Indo)(BuO)_3]$ (II)

The Indomethacin complex was prepared by the method described for (**I**) using Indomethacin sodium salt, NaIndo. Anal. Found: C, 53.6; H, 5.8; N, 2.0; Na, 6.5; V, 7.3. Calc. for $C_{27}H_{33}O_7ClNNaV$: C, 54.0; H, 6.1; N, 2.0; Na, 6.7; V, 7.4%. MW_{calc}: 688. The NaIndo salt was synthesized by dissolving Indomethacin (3 mmol) in methanol (30 ml) and adding sodium methoxide up to pH 8. A yellow precipitate was obtained by water addition and keeping it at 0 °C overnight. This was filtered and washed with cold water and dried under vacuum. The final product was characterized by IR spectroscopy.

Stability studies

The rates of the decomposition reaction of both complexes were determined by measuring the variation of the UV–vis spectra with time. The $b_2 \rightarrow e$ electronic absorption bands were monitored at 810 nm and 37 °C. The compounds are sparingly soluble in water. The dissolution of the complexes (0.025 mmol) was performed by successive additions of ethanol up to 1 ml and then adding water to a final volume of 5 ml. In order to prevent the contact of the sample with atmospheric oxygen, the measurements were carried out directly in the cell of the spectrophotometer, with the corresponding stoppers and parafilm. Under these conditions, no significant amount of vanadium(V) can be observed, as can be seen from the position of the electronic absorption bands.

Theoretical studies

The conformational spaces for the molecules of the DiclofH and IndoH were studied using the molecular dynamics (MD) module of the HyperChem package.[27] Several simulations were accomplished with the aid of the MM+ force field also

available in that package. The starting geometries were those characterized by the gauche, cis and trans conformations around the NH or C═O atoms between two rings. The starting geometries were heated from 0 to 600 K in 0.1 ps. Then, the temperature was kept constant by coupling the system to a simulated thermal bath with a bath relaxation time of 0.5 ps. The simulation time step was 0.5 fs. After an equilibration period of 1 ps, a 500 ps simulation was run and the coordinates saved every 1 ps. Those geometries were then optimized to an energy gradient less than 0.001 kcal mol^{-1} $Å^{-1}$ using the MM+ force field. The lowest energy conformers of the molecules obtained according to the above methodology were further studied using the density functional theory as implemented in the Gaussian 98 package.[28] Geometry optimizations were performed using the Becke' three-parameter hybrid functional[29] with the Lee–Yang–Parr correlation functional,[30] a combination that gives rise to the well-known B3LYP method. The 6–31G** basis set is used for all the atoms.

The basis set used in the determination of the optimized geometries of the vanadium(IV) complexes was 3–21G**. First, the structure of the 'naked' oxo-vanadium(IV) cation bound to the four neighboring oxygen atoms, $VO(O_4)$, was determined. Once the geometry of $VO(O_4)$ was established, the calculation of the geometry of the species was obtained by stepwise addition of CH_2 and CH_3 groups (for butoxide). Finally, the optimized ligand moieties were added.

Cell culture

Rat osteosarcoma UMR106 and osteoblastic non-transformed mouse-calvaria-derived MC3T3E1 cells were grown in DMEM supplemented with 10% (v/v) FBS and antibiotics (100 U ml^{-1} penicillin and 100 mg ml^{-1} streptomycin) in a humidified atmosphere of 95% air/5% CO_2. Cells were grown at near-confluence (70–80%) and were subcultured using 0.1% trypsin–1 mM EDTA in Ca^{2+}–Mg^{2+}-free phosphate-buffered saline (PBS). For experiments, about 5.5×10^4 cells/well (UMR106) and 3.3×10^4 cells/well (MC3T3E1) were plated into 24-well plates. After the culture reached 70% confluence, the cells were washed twice with DMEM. Cells were incubated in 0.5 ml DMEM overnight with vanadium compounds at different doses in serum-free DMEM.

Cell proliferation assay

For the mitogenic bioassay, the method described by Okajima *et al.*[31] was used with some modifications. The cells in 24-well plates were washed with PBS and fixed with 5% glutaraldehyde–PBS at room temperature for 10 min. Cells were then stained with 0.5% crystal violet/25% methanol for 10 min and the dye solution was discarded. After that, the plate was washed with water and dried. The crystal violet fixed by the cells was quantified at 540 nm after an extraction procedure. The dye in the cells was extracted using 0.5 ml/well 0.1 M glycine–HCl buffer, pH 3.0/30% methanol and transferred to test tubes. The correlation between cell number/well and the absorbance at 540 nm of diluted extraction sample after crystal violet staining has been established previously.[32] Data are expressed as the mean plus/minus standard error of the mean (SEM). Statistical differences were analyzed using Student's *t*-test; *t*-tests were done to compare treated and untreated cultures. Fresh solutions of vanadyl(IV) complexes were added to the culture dishes. The studies were performed in the vanadium concentration range 2.5–100 μM. Higher concentrations of vanadium compounds proved to be toxic and caused osteoblast death after several hours of incubation.[33] In order to prepare the stock solution for these studies, the same dissolution procedure described in the Stability studies section was followed. The effect of alcoholic solutions on the cells has been checked. The results showed that the maximum alcohol concentration used in the wells of the culture (0.43%) did not produce any damage to the osteoblasts.

RESULTS AND DISCUSSION

Electronic Properties

The electronic spectra of the complexes in methanolic solution (L/M = 2/1) and their diffuse reflectance spectra are shown in Table 1. The band patterns were similar to those previously reported for other vanadium(IV) complexes with NSAIDs.[21] Briefly, the shift of the $b_2 \rightarrow e$ and $b_2 \rightarrow b_1$ bands to the red and blue respectively, compared with that of $[VO(H_2O)_5]^{2+}$, suggested that the carboxylate anion was coordinated to the vanadyl center. The band pattern of a (1/5) ethanol/H_2O solution of the complexes are also reported in Table 1. The same procedure was used to prepare solutions for stability and biological studies.

IR spectroscopy

The IR spectra of the free ligands, their sodium salts and the complexes with VO(IV) are shown in Tables 2 and 3. The assignments of the main bands for Diclofenac[12,17] and Indomethacin[15,18,34] were taken from published results. In the

Table 1. UV–vis of methanolic solution and ethanolic/water (1/5) dissolutions and diffuse reflectance (brackets) bands (nm) of $Na_2[VO(Diclof)(BuO)_3]$ and $Na_2[VO(Indo)(BuO)_3]$. Molar extinction coefficients (l mol^{-1} cm^{-1}) in parentheses

	$b_2 \rightarrow e$	$b_2 \rightarrow b_1$
Diclof–VO	845[a] (28.4)	552[a] (15.8)
	810[b] (54.2)	560[b] (31.3)
	[830]	[580]
Indo–VO	826[a] (34.2)	570[a] (16.5)
	810[b] (52.9)	560[b] (42.6)
	[830]	[580]

[a] Methanolic solution.
[b] Ethanolic/water solution.

Table 2. Characteristic IR bands of Diclofenac derivatives[a]

DiclofenacH	DiclofenacNa	DiclofenacVO	Assignments
3325 s	3380 m	3268 m	ν(NH)
	3254 m		
1694 vs			ν(C═O), carboxylic
1580 sh	1605 m	1621 m	δ_{ip}(NH)
1571 m	1577 s	1577 sh	
	1556 s	1594 s	$\nu_{as}(COO^-)$
1501 m	1508 s	1517 s	ν(ring)
1449 s	1453 s	1453 m	
	1390 s	1389 s/1370 sh	$\nu_s(COO^-)$
1318 m	1306 m	1311 m	ν(aryl–O)
1300 m	1282 m	1277 m	
1276 m			
		955 m	ν(V═O)

[a] vs: very strong; s: strong; m: medium; sh: shoulder

Table 3. Characteristic IR bands of Indomethacin derivatives[a]

IndoH	IndoNa	IndoVO	Assignments
1717 s			ν(C═O), carboxylic
1692 s	1678 sh	1686 m	ν(C═O), amide
1609 sh		1643 m	
1591 m	1590 s	1594 s	
	1562 s	1578 m	$\nu_{as}(COO^-)$
		1517 s	
	1400 s	1370 sh	$\nu_s(COO^-)$
1362 s	1353 s	1382 sh	
1227 s	1217 m	1223 m	ν(aryl–O)
1027 m	1017 m	1023 m	ν(O–CH_3)
		944 s	ν(V═O)
592 m	592 m	591 m	δ_{ip}(NCO)

[a] s: strong; m: medium; sh: shoulder.

case of Diclofenac, the single band corresponding to ν(NH) in the free ligand splits into two bands in the sodium salt. The band at the lower frequency is due to intramolecular hydrogen bonding, NH···O.[17] The vanadyl(IV) complex also presents this band, showing that the NH group remains protonated and participates in hydrogen bonding like in the sodium salt. The lack of shifts for the ν(C═O) of the amide group of Indomethacin indicates that there is no interaction between this group and the metal center. The major characteristic of the IR spectra is the frequency of the $\nu_{as}(COO^-)$ and $\nu_s(COO^-)$ stretching vibrations. The frequency of these bands depends on the coordination mode and/or deprotonation of the carboxylate ligand. The parameter that determines the coordination mode of the carboxylate group is the value of $\Delta\nu$ ($\Delta\nu = \nu_{as}(COO^-) - \nu_s(COO^-)$).[35] This difference changes from 166 cm^{-1} in the Diclofenac sodium salt to 188 cm^{-1} in DiclofenacVO, indicating that the deprotonation of the carboxylate group in the first case and the complexation of the carboxylate anion as monodentate mode in the vanadyl(IV) compound. In the complex of copper(II) with Diclofenac the carboxylate group binds the metal in a bidentate bridging manner,[12] whereas other Diclofenac complexes with transition metals have been poorly characterized.[17]

In the case of Indomethacin sodium salt, $\Delta\nu = 190$ cm^{-1} changed to 224 cm^{-1} upon complexation. This value again shows the participation of the monodentate carboxylate anion in the coordination sphere of vanadyl(IV). Recently, the copper(II) complex of Indomethacin has been introduced as a veterinary antiinflammatory drug. Its structure is again dimeric. On the contrary, the Indomethacin zinc complexes presented different structures, with the bonding of the carboxylate group in dimeric or monomeric (mono or bidentate) forms.[34]

Another important feature of the IR spectra is the presence of new bands corresponding to the stretching V═O. The position of these bands is in agreement with the values previously reported for vanadyl cation in an oxygenated environment, as in the case of other NSAIDs complexes.[21]

In the present case the oxygenated coordination sphere around the vanadium atom consists of one carboxylate group and three butoxide anions.

Thermal decomposition

Figure 1 shows the thermal behavior (TG and DTA) under oxygen atmosphere.

DiclofenacVO

In a first stage, the loss of three BuO^- anions ($\Delta\omega_{exp} = 35\%$, $\Delta\omega_{calc} = 34.9\%$) occurred with a very strong exothermic DTA peak at 230 °C (Fig. 1a). Upon further heating the sample slowly loses mass up to about 350 °C, then the decomposition speeds up suddenly because of the ligand degradation. The total decomposition reaction is

$$Na_2[VO(Diclof)(BuO)_3] \xrightarrow{\phi,O_2} NaVO_3 + \tfrac{1}{2}\,Na_2O + \text{volatile products}$$

with a total weight loss of $\Delta\omega_{exp} = 76\%$. This is in good agreement with the calculated value, $\Delta\omega_{calc} = 75.6\%$. The residue has been characterized by IR spectroscopy.

IndomethacinVO

The first three stages (Fig. 1b) correspond to the loss of the three BuO^- anions ($\Delta\omega_{exp} = 31.6\%$, $\Delta\omega_{calc} = 31.8\%$). The degradation of the ligand takes place in two successive steps together with the formation of $NaVO_3$ and Na_2O as the solid residue, determined by IR measurements. A very strong exothermic peak can be observed in the DTA at 446 °C.

The final equation for the whole decomposition is

$$Na_2[VO(Indo)(BuO)_3] \xrightarrow{\phi,O_2} NaVO_3 + \tfrac{1}{2}\,Na_2O + \text{volatile products}$$

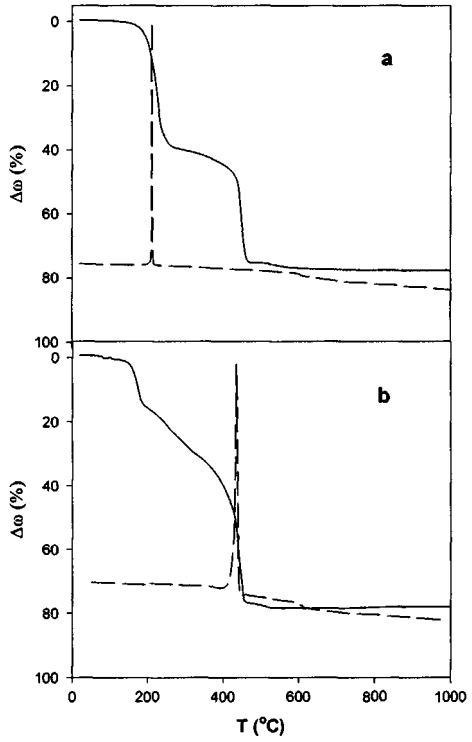

Figure 1. TG and DTA curves of the thermal decomposition of (a) $Na_2[VO(Diclof)(BuO)_3]$ (**I**) and (b) $Na_2[VO(Indo)(BuO)_3]$ (**II**). Oxygen flow: 50 ml min^{-1}; rate: 10 °C min^{-1}.

The experimental residue of 22.0% is in agreement with the calculated value (22.2%).

Stability studies

The decomposition reactions were measured at the temperature of the biological assays (37 °C). Plots of ln $A(t)$ versus t were linear at least for a half reaction period and were first order in the concentration of both complexes. The rate constant for the decompositions of Diclof–VO and Indo–VO were 6.88×10^{-4} min^{-1} and 2.68×10^{-4} min^{-1} respectively. These results suggest that the compounds were stable during the period of sample preparation and addition to the culture media. Nevertheless, these values are only an approximation of the phenomenon that takes place in the culture media for various reasons: (i) there are differences in the atmosphere of the stability studies (air) and the atmosphere of the cultures (a mix of CO_2 and air); (ii) the culture medium is a complex solution of salts and different nutrients; (iii) since the cells are living systems, it can be supposed that the interaction with the dissolved compounds was performed immediately after their addition. The compounds interact with the cells and induce different metabolic reactions that also produce environment modifications.

Theoretical studies

The relevant optimized calculated parameters for the most stable conformers are compiled in Table 4, together with reported theoretical[36] and X-ray diffraction values for the Diclofenac sodium salt. The reported values were calculated at the B3LYP level with 3–21G** and 6–31G** basis sets. Calculated parameters of the DiclofH are compared against the Diclofenac sodium salt theoretical (calculated with B3LYP/6–31G*) and experimental values.

The optimized geometries of the complexes and the isolated ligands in their acidic form are shown in Fig. 2a–d. On these bases, the geometry of both complexes around the vanadium atom may be described by a square-pyramidal arrangement of the five oxygen atoms. The vanadium is placed close to the center of gravity of the pyramid. Table 4 summarizes the most relevant geometrical parameters for those structures. The vanadyl V=O distances are 1.60 Å for the two complexes. These distances are somewhat longer than that observed in most oxoV(IV) compounds[37,38] due to the strong σ-bonding of the equatorial coordinated (butoxide and carboxylate) ligands. These bond distances fit the observed values of the V=O stretchings (see Tables 2 and 3). These vibrations are placed at lower frequencies than those observed for other five-coordinated vanadyl(IV) complexes.[39] The butoxide–vanadium distances are in accordance with other reported values for this type of bond.[40] The length of the C=O bond of the carboxylic acids of the free ligands increased upon complexation. On the contrary, the C–O(–H) bond suffered a decrease when it is coordinated to the vanadium center. This bond is stronger upon deprotonation, as can be observed for the Diclofenac sodium salt. The calculated bond distances in the complexes are similar to those of other carboxylate complexes with vanadyl(IV) cation.[41] Variations in the carboxylate bond angles are seen upon the formation of the complex. Considering the DiclofH and IndoH ligands, the optimization geometries at B3LYP/3–21G** show that the dihedral angles between the planes that contain the rings are 53° and 41.3° respectively, and these change to 49.3° and 59.5° when bonded to the $VO(O4)(BuO)_3$ moiety. The theoretical value of the dihedral angle between the two phenyl rings of the Diclofenac acid calculated as 62.3° reproduces quite well the experimental one of 69°.[42] The orientation of the methoxy group in the Indomethacin complex is rotated in 180° probably due to favoring intramolecular interactions with the oxygen atom of the vanadyl(IV) cation in the complex. This rotation is not observed for the chelating monomeric complex of Indomethacin with zinc(II).[18]

Biological assays

To determine whether Indomethacin and Diclofenac modulated MC3T3E1 and UMR106 cells, their effect on cellular proliferation was studied by the crystal violet bioassay. The free ligands were added to cell culture in a concentration range of 2.5–100 μM. No significant difference from the basal condition can be observed with these drugs. The effects of the vanadyl(IV) complexes of these two NSAIDs on MC3T3E1 osteoblast-like cells are shown in Fig. 3. As can be seen, DiclofenacVO has no effect on the whole range of concentrations, whereas IndomethacinVO caused inhibition of

Table 4. Selected bond lengths (r, Å) and bond angles (α, °) of $VO(O_4)$ group and proximal bondings of optimized geometries of complexes

		DiclofenacH		DiclofenacNa ref			IndoH	
Parameter	DiclofenacV-O	3–21G**	6–31G**	Calc.	Exp.	IndomethacinVO	3–21G**	6–31G**
$r(V_1-O_2)$	1.60					1.60		
$r(V_1-O_4)$	1.90					1.85		
$r(V_1-O_5)$	2.07					1.91		
$r(V_1-O_3)$	1.90					1.85		
$r(V_1-O_6)$	1.91					1.84		
$r(O_5-C_7)$	1.32	1.39	1.36	1.28	1.28	1.33	1.38	1.35
$r(C_7-O_8)$	1.26	1.23	1.21	1.24	1.24	1.25	1.23	1.21
$r(C_7-C_9)$	1.53	1.52	1.52	1.53	1.52	1.54	1.52	1.52
$r(C_9-C_{10})$	1.49	1.52	1.52			1.51	1.51	1.51
$r(C_9-H_{11})$	1.09	1.10	1.10			1.00	1.08	1.09
$r(C_9-H_{12})$	1.10	1.09	1.09			1.09	1.09	1.09
$\alpha(O_2-V_1-O_4)$	111.0					112.0		
$\alpha(O_2-V_1-O_5)$	101.7					102.3		
$\alpha(O_2-V_1-O_3)$	114.0					108.1		
$\alpha(O_2-V_1-O_6)$	103.6					98.7		
$\alpha(O_4-V_1-O_5)$	82.2					86.0		
$\alpha(O_5-V_1-O_3)$	85.3					86.3		
$\alpha(O_3-V_1-O_6)$	86.8					88.1		
$\alpha(O_4-V_1-O_6)$	86.6					85.3		
$\alpha(O_5-C_7-C_9)$	110.5	108.2	110.1	114.6	113.8	114.9	110.5	111.7
$\alpha(O_8-C_7-C_9)$	124.4	130.1	128.0	121.7	122.4	121.8	126.7	125.5
$\alpha(O_5-C_7-O_8)$	125.2	121.7	121.8	123.5	123.7	123.4	122.9	122.8
$\alpha(C_7-C_9-C_{10})$	122.6	117.8	117.7	112.9	113.1	113.2	110.5	111.9
$\alpha(H_{11}-C_9-H_{12})$	102.6	105.5	105.7			110.2	110.0	108.5

cell proliferation at the higher concentrations (75–100 μM). In a previous paper the action of other vanadyl(IV) complexes with NSAIDs (Ibuprofen and Naproxen) has been reported.[21] Those complexes produced cytotoxic effects similar to IndomethacinVO at 75 and 100 μM, but they were different from that of vanadyl(IV) cation, which inhibited cell growth in a dose–response manner.

Figure 4 shows the action of the two new complexes upon the development of the osteosarcoma-derived cell line (UMR106). Both complexes produced a stimulatory effect on cell proliferation. On the contrary, the only previously reported vanadyl(IV) complex[21] that caused stimulation on cell proliferation was IbuprofenVO in the low range of concentrations (2.5–10 μM). In this context, the two new complexes reported herein are stimulatory agents on the whole range of tested concentrations. Even though the addition of vanadyl(IV) to the culture media induced a stimulation of cell proliferation in the range 2.5–75 μM, this effect was weaker than that of the new complexes in this cell line.[21]

This study, as well as previous reports,[21] suggests that vanadium derivatives can modulate cell growth. These complexes can be used as useful tools for its differential action upon normal and transformed osteoblasts to search different aspects of cell development.

CONCLUSIONS

In the series previously reported on the vanadyl(IV) compounds with Tolmetin, Naproxen and Ibuprofen, they were prepared in methanol and neutral complexes were obtained. In the present series, attempts to prepare the solid compounds using the same experimental techniques failed. Methanol was then changed to butanol, leading to anionic compounds with different stoichiometries. Since it was not possible to obtain single crystals for these complexes in order to determine their structure, theoretical studies have been undertaken. A square-pyramidal geometry around the vanadium atom was determined for both complexes. Even though the coordination sphere and the oxidation state of the vanadium atom is the same for the two complexes, the different behavior on normal osteoblasts indicates that the bioactivity of these compounds is a complex phenomenon related to different factors, such as the cellular type and the ligand nature.

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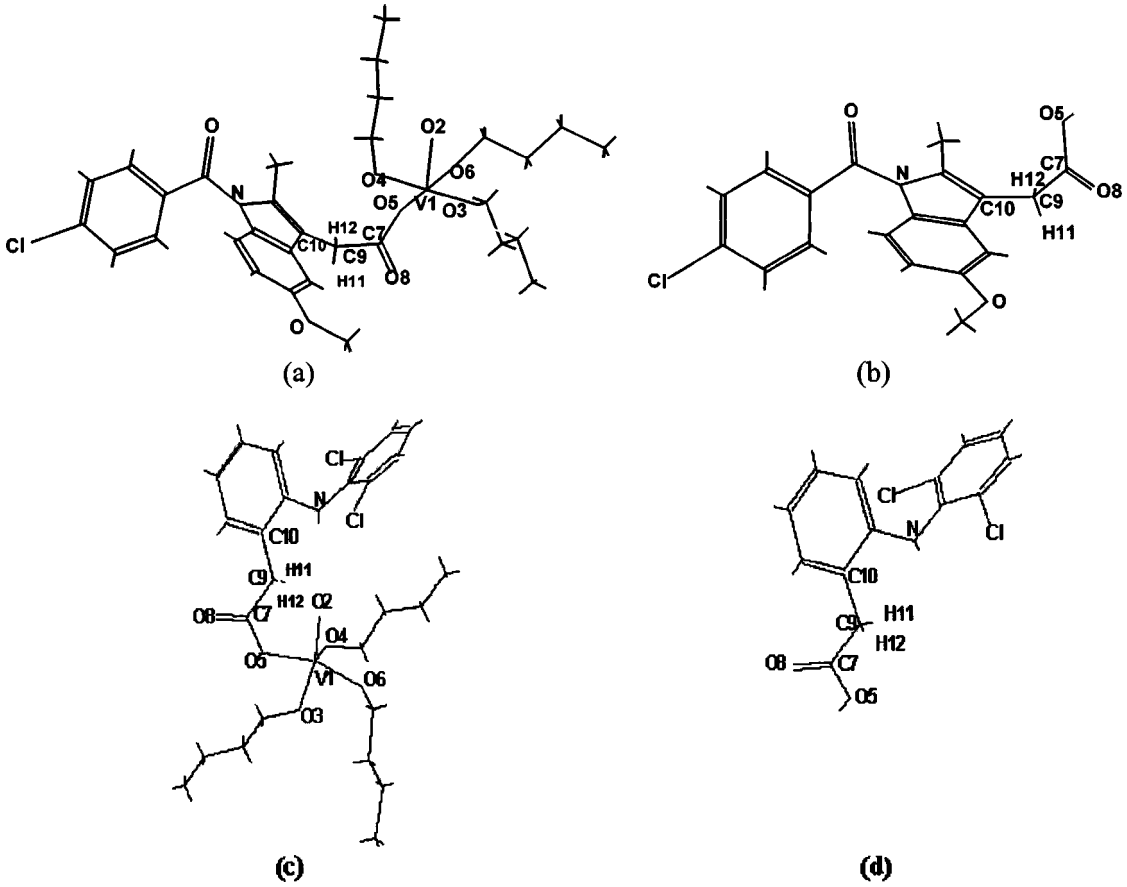

Figure 2. Optimized geometry of (a) Indomethacin–VO, (b) Indomethacin, (c) Diclofenac–VO, (d) DiclofenacH.

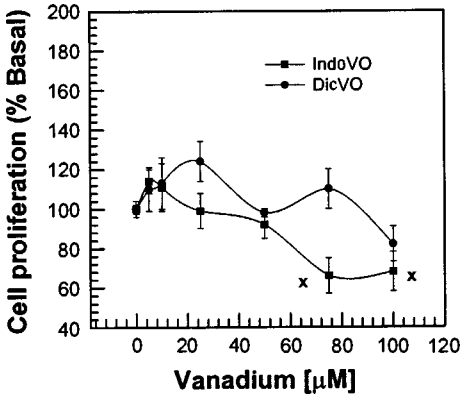

Figure 3. Effects of vanadium complexes on MC3T3E1 cell proliferation. Osteoblasts were cultured in the presence of vanadium compounds over 16 h at 37 °C. Results are expressed as mean ± SEM. $\times p < 0.02$.

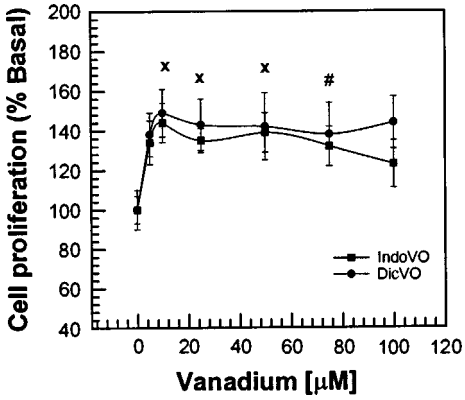

Figure 4. Effects of vanadium complexes on UMR106 osteosarcoma cell proliferation. Cells were cultured in the presence of vanadium compounds over 16 h at 37 °C. Results are expressed as mean ± SEM. # $p < 0.05$; $\times p < 0.02$.

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