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Effects of non-steroid anti-inflammatory drugs in membrane bilayers

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Abstract

The thermal effects of non-steroidal anti-inflammatory drugs (NSAIDs) meloxicam, tenoxicam, piroxicam and lornoxicam have been studied in dipalmitoylphosphatidylcholine (DPPC) membrane bilayers using neutral and acidic environments (pH 2.5). The strength of the perturbing effect of the drugs is summarized to a lowering of the main phase transition temperature and a broadening of the phase transition temperature as well as broadening or abolishment of the pretransition of DPPC bilayers. The thermal profiles in the two environments were very similar. Among the NSAIDs studied meloxicam showed the least perturbing effect. The differential scanning calorimetry results (DSC) in combination with molecular modeling studies point out that NSAIDs are characterized by amphoteric interactions and are extended between the polar and hydrophobic segments of lipid bilayers. The effects of NSAIDs in membrane bilayers were also investigated using Raman spectroscopy. Meloxicam showed a *gauche*:*trans* profile similar to DPPC bilayers while the other NSAIDs increased significantly the *gauche*:*trans* ratio. In conclusion, both techniques show that in spite of the close structural similarity of the NSAIDs studied, meloxicam appears to have the lowest membrane perturbing effects probably attributed to its highest lipophilicity.

Keywords: NSAIDs; Lipid bilayers; Molecular modeling; Differential scanning calorimetry; Raman spectroscopy

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are the initial therapy for common inflammation, but they can also be used to treat a high fever. They are called non-steroidal because their chemical structure

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declines from steroidal and are used to treat inflammation by suppressing the immune system (inflammation is one of the body's healing responses to trauma). All NSAIDs treat inflammation in a way similar to the mechanism of aspirin, the most well-known and oldest member of the class.

NSAIDs mainly inhibit the body's ability to synthesize prostaglandins. Prostaglandins are a family of hormone-like chemicals, some of which are made in response to cell injury. Although the clinical efficacy of NSAIDs was well-known in the 19th century, their mechanism of action was elucidated in 1971. Sir John Vane demonstrated that the enzymatic production of prostaglandins could be attenuated by aspirin and indomethacin. It had been known that enzymes, including COX, and the 5-, 12-, and 15-lipoxygenases act on arachidonic acid and result in the production of inflammatory prostaglandins E_2, I_2, and thromboxane. Vane further established that inhibition of COX by aspirin was responsible for its effects, and he shared the 1982 Nobel Prize in medicine for that discover. The common mechanism of action for all NSAIDs is the inhibition of the enzyme cyclooxgenase (COX). COX is necessary in the formation of prostaglandins. This enzyme exists in two forms, COX-1 which protects the stomach lining and intestine, and COX-2 that is involved in making the prostaglandins and is important in the process of inflammation (Vane, 1971, 1998; Simon, 1998; Herschman, 1996; Vane and Boring, 1995; Lane, 1997).

Most NSAIDs currently available inhibit both COX-1 and 2. The stomach irritation and ulcers that can occur with the use of these drugs is due to the COX-1 inhibition. COX-2 inhibitors stop the formation of prostaglandins responsible for pain, fever and inflammation. Recently, newer drugs (available in the US by prescription only) that inhibit only COX-2 have been approved by the FDA for acute pain, rheumatoid arthritis, osteoarthritis, and dysmenorrhoea. Most people tolerate the non-specific NSAIDs but a group of people with gastric complaints will benefit from the new drugs. COX inhibitors have been reported to have a protective effect against colon cancer and Alzheimer's disease. Metal complexes of NSAIDs with interesting structures and pharmacological profile have been reported (Cini, 2000; Kovala-Demertzi, 2000).

Several additional effects also contribute to the efficacy of NSAIDs. Within the cell membrane, NSAIDs affect many processes, such as the oxidation of nicotinamide adenine dinucleotide phosphate in neutrophils and macrophage-based phospholipase C. Neutrophil function can be directly inhibited by all NSAIDs to some degree, but indomethacin, piroxicam, ibuprofen, and all of the salicylates have the most pronounced effect. Additionally, high anti-inflammatory doses of NSAIDs have been found to interfere with the synthesis of proteoglycans by chondrocytes, trans-membrane ion fluxes, and cell-to-cell binding. NSAIDs also have demonstrated the ability to unmask T-cell suppressor activity that may lead to a reduction in the rheumatoid factor. While it has been demonstrated that NSAIDs may differ in their ability to influence the activities listed above in vitro, the clinical differences are less distinct (Brune, 1986; Tonnessen et al., 1989; Herchuelz et al., 1989).

The membrane effects of NSAIDs drugs indomethacin, difflunisal and flurbiprofen have been studied. However, no similar studies for the newer NSAIDs oxicams have been appeared in the literature. For this reason we showed interest to study the thermal changes that a small group of NSAIDs oxicams can cause in membrane bilayers (Fig. 1). Such information may be relevant with their pharmacological profile. DPPC bilayers were used for these studies because their thermal effects and dynamic properties are well studied (Janiak et al., 1976). In addition, the incorporation of various drugs in the spontaneously phospholipid bilayers formed after hydration have been studied extensively in an attempt to understand the molecular interactions between drugs with phospholipids. The presence of an additive in membrane bilayers affects the thermodynamic parameters that govern a thermogram such as the maximum of the main phase transition or the pretransition (T_m), the heat capacity of the peaks (C_p) and the half-width ($T_{m_{1/2}}$). The nature of the DSC thermograms can be understood if the total intermolecular effects (i.e. interfacial, hydrogen bonds and non-specific hydrophobic and electrostatic interactions) between the additive and the phospholipid bilayers are considered (Mavromoustakos and Theodoropoulou, 1998; Mavromoustakos et al., 1997, 1996).

In addition, Raman spectroscopy was used in order to compliment the information derived from DSC study. Raman spectroscopy was used extensively to study the *gauche*:*trans* ratio in lipid bilayers without and with the presence of bioactive additives (Yellin and Levin, 1977; Wolfgang, 1986).

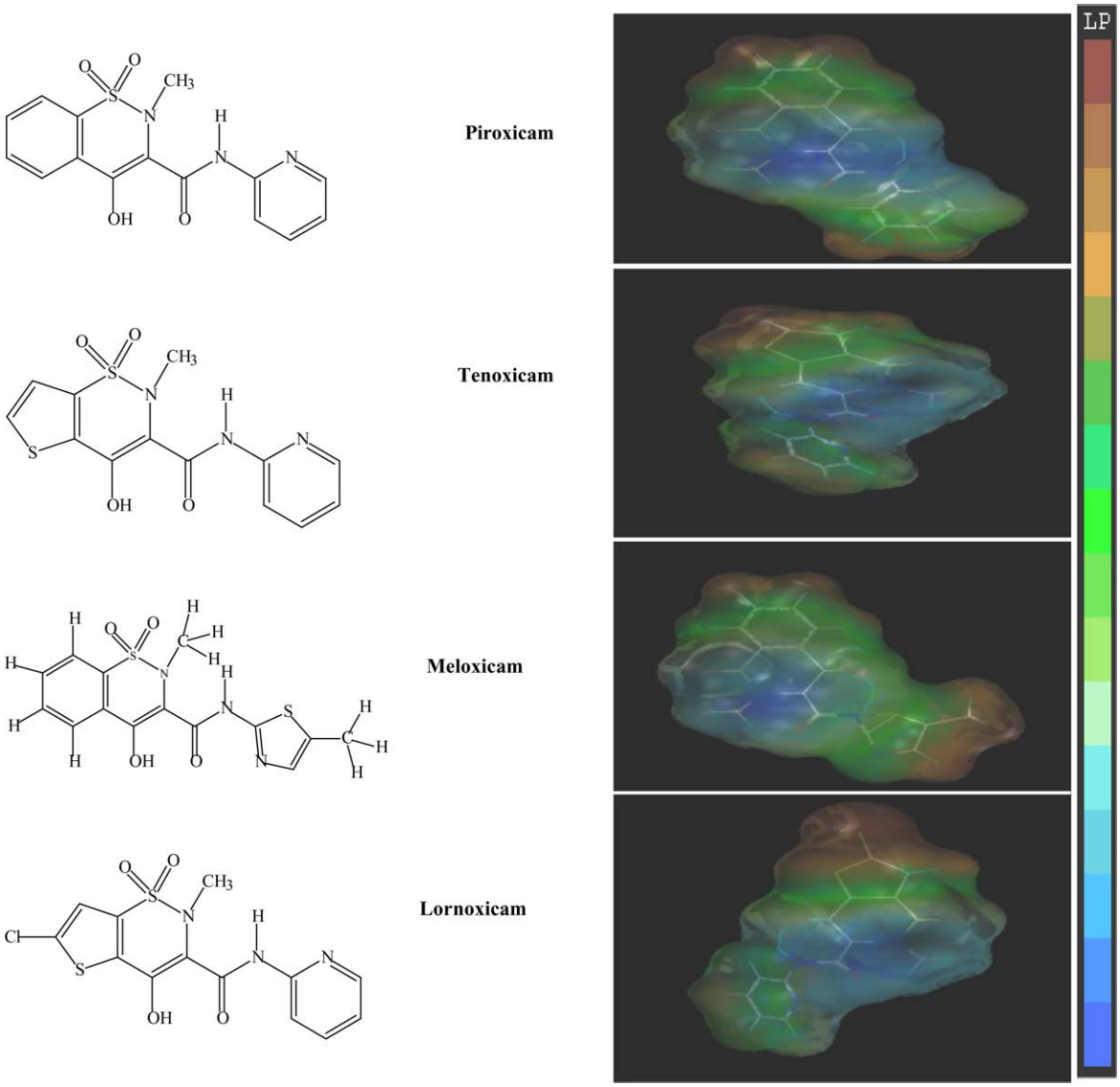

Fig. 1. Chemical structures of NSAIDs used in the study. On the right side are shown the lipophilic profiles of NSAIDs. Top (brown color) shows the most lipophilic segment while bottom (blue color) shows the most hydrophilic one.

It has been suggested that the variation in both efficacy and tolerability of NSAIDs, are partly due to their differences in their physicochemical properties (e.g. ionization constants (pK_a), solubility, partition coefficients), which determine their distribution in the body. Most NSAIDs are weak acids with pK_a values ranging from 3 to 5 (Tonnessen et al., 1989). In addition, lipid–aqueous partition coefficients ($\log P$ values) of NSAIDs influence their resorption from the upper GI tract and may affect their local gastromucosal tolerability. The aqueous solubility of acidic NSAIDs is pH dependent. Decreasing pH leads to an increase in the ratio of non-ionized to ionized drug, combined with an increase in solubility. For this reason membrane preparations were formed when the drug was in a neutral and acidic (pH 2.5) environments in order to explore any

differential effect of anionic or non-anionic forms of the molecules in membrane bilayers.

2. Experimental procedure

2.1. Materials

Dipalmitoyl-glycero-sn-3-phosphorylcholine (DPPC) was obtained from Avanti Polar Lipids Inc., AL, USA. The anti-inflammatory drugs were kindly donated by pharmaceutical companies PFIZER, "HELP EPE", NYCOMED DENMARK A/S and Boehringer Ingelheim, Greece, AE. Spectroscopy grade $CHCl_3$ (+99%) was purchased from Sigma–Aldrich (O.M.) Ltd. Athens, Greece. Stainless steel capsules were obtained from Perkin-Elmer, MA, USA.

2.2. Methods

2.2.1. Sample preparation

Appropriate amounts of the phospholipid with or without NSAID were dissolved in spectroscopic grade chloroform. The solvent was then evaporated by passing stream of O_2-free nitrogen over the solution at 50 °C and the residue was placed under vacuum (0.1 mmHg) for 12 h. For measurements this dry residue was dispersed in appropriate amounts of bi-stilled water by vortexing.

2.2.2. DSC

After dispersion in water (50%, w/w), portions of the samples (ca. 5 mg) were sealed in stainless steel capsules and stored at freezer. Thermograms were obtained on a Perkin-Elmer DSC 7 calorimeter. All samples were heated using a scanning rate of 2.5 °C/min at different periods until identical thermograms were obtained. The duration of equilibration time above the phase transition of the sample and the use of slower scanning rate did not affect the quality of the thermal profile. The temperature scale of the calorimeter was calibrated using indium (T_m = 156.6 °C) as standard sample and DPPC bilayers (T_m = 41.2 °C).

2.2.3. Raman spectroscopy

The spectra were obtained at 4 cm^{-1} resolution from 3500 to 400 cm^{-1} with interval 2 cm^{-1} using a Perkin-Elmer NIR FT-spectrometer (Spectrum GX II) equipped with CCD detector. The measurements were performed at a temperature range of 25–43°. The laser power and spot (a Nd:YAG at 1064 nm) were controlled to be constant at 400 mW during the experiments. One thousand five hundred scans were accumulated and back scattering light was collected.

2.2.4. Molecular modeling

SYBYL 6.8 version from Tripos was used in order to explore the stereoelectronic properties of NSAIDs under study in absence or presence of DPPC bilayers. More specifically, the $NSAID_S$ were incorporated into DPPC bilayers built by (Tieleman et al., 1997). In order to localize the preferred topography of the energy minimized molecules into membrane bilayers NSAIDs were incorporated in different locations and having different orientations in terms of the membrane normal axis. The generated model bilayer with incorporated drug was optimized using the AMBERall atom forcefield as implemented in SYBYL 6.8. Molecular dynamics at 37 °C were carried out in order to relax the system, which was afterwards minimized and the energy was calculated for each minimized structure. The lipophilic profiles of the molecules were also calculated. By default the color mapping of the lipophilic surfaces is relative, not based on absolute values. The color scale runs from the most lipophilic to the most hydrophilic parts of the molecule. Lipophilicity force field profile is a powerful tool when one seeks to compare the lipophilicity molecular potential of different molecules having similar pharmacological profiles (Croizet et al., 1990). Superimposition between NSAIDs using the torsional flexible method was achieved in order to reveal their stereoelectronic similarities and differences and provide a reasonable explanation for the DSC scans. In this method, the structures are fitted together by suitably modifying each structure's rotable torsional angles. The one structure is the working and undergoes torsional angle modifications and the others are the targets which remain fixed during the superimposition process. The equivalent atoms used for the matching during the superimposition process were the common ones in all structures, i.e. the hetero-phenyl ring, the sulfoxide group, the hydroxyl group and the peptide bond. The resulted structures were energy minimized and then superimposed using rigid body method in which only translations and rotations of the energy minimized

molecules are considered. All the theoretical calculations were performed using subroutines of SYBYL 6.8 running in HP workstation.

3. Results

3.1. Differential scanning calorimetry

Differential scanning calorimetry (DSC) is a fast and relatively inexpensive technique that allows the study of the thermotropic properties of the membranes in the absence and presence of bioactive molecules. Therefore, it is used in our laboratory as a diagnostic technique to investigate differential effects that may be caused by the incorporation of additives under study. When such differential effects are observed, then techniques that offer complementary and more detailed information on the thermal and dynamic properties of membranes, with or without the presence of additives are applied.

Fig. 2 shows DSC scans of DPPC/NSAIDs bilayers formed in aqueous environment (pH 7.0). Fully hydrated DPPC bilayers show a characteristic thermogram consisting of a broad low enthalpy transition at 35.3 °C and a sharp enthalpy main transition at 41.2 °C. The DPPC bilayers exist in the gel phase (L'_{β}) for temperatures lower than 33 °C, and in the liquid crystalline phase for temperatures higher than 42 °C (L'_{α}). In between 33 and 42 °C the phospholipid bilayers exist in $P_{\beta'}$ or ripple phase (Mouritsen and Jorgensen, 1994; Janiak et al., 1976; Mason, 1998). The obtained DSC scan of fully hydrated DPPC multibilayers shows a pretransition centered at 35 °C and a peak maximum at 41.2 °C. The main phase transition is accompanied by several structural changes in the lipid molecules as well as systematic alterations in the bilayer geometry, but the most prominent feature is the *trans-gauche* isomerization-taking place in the acyl chain conformation. The average number of *gauche* conformers indicates the effective fluidity, which depends not only on the temperature, but also on perturbations due to the presence of a drug molecule intercalating between the lipids (Janiak et al., 1976).

At low concentration of 99:1 phospholipid:drug molar ratio (designated as $x_{drug} = 0.01$) all NSAIDs affect

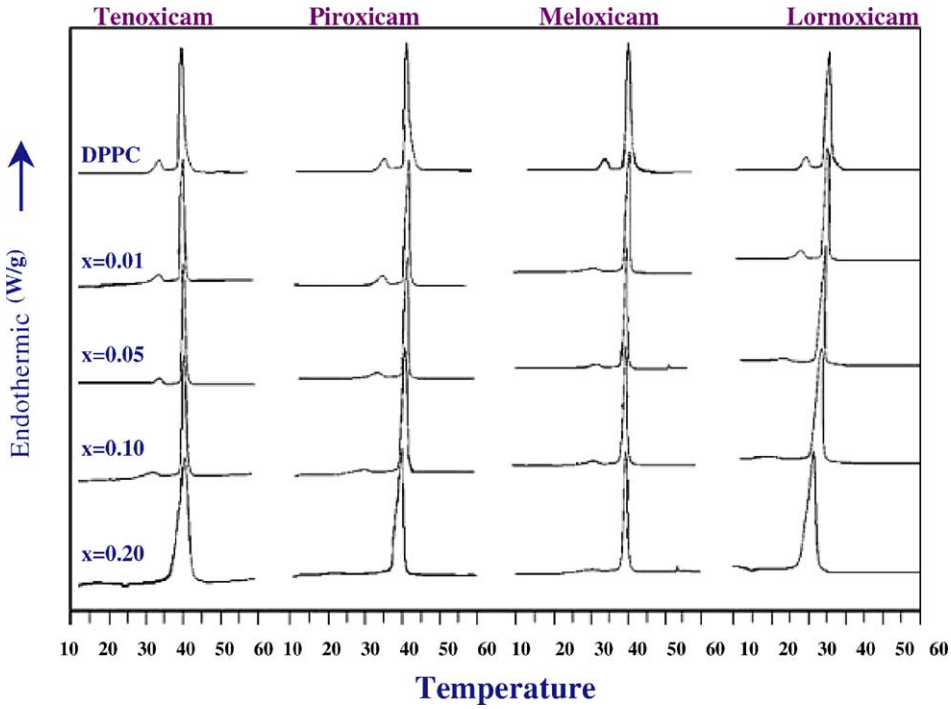

Fig. 2. DSC scans of DPPC bilayers containing either one of the four NSAIDs meloxicam, lornoxicam, piroxicam or tenoxicam at pH 7.

Table 1
Values of phase pretransition and transition temperatures (T_m), half-width temperature and enthalpy changes ($\triangle H$) of DPPC with or without NSAIDs with the use of water

pH	Sample description	$T_{m_{1/2}}$ (°C)	T_m (pretran)	T_m (°C)	$\triangle H$	$\triangle H$ (J/g)	$T_{m_{1/2}}$ (°C) (pretran)
7.0	DPPC alone	0.50	36.64	42.67	5.37	42.51	0.40
	DPPC + tenoxicam ($x = 0.01$)	0.40	34.98	41.76	4.69	43.32	0.40
	DPPC + tenoxicam ($x = 0.05$)	0.40	34.54	41.60	4.70	43.79	0.60
	DPPC + tenoxicam ($x = 0.10$)	0.50	35.34	41.95	4.34	42.22	0.70
	DPPC + tenoxicam ($x = 0.20$)	1.15	–	38.82	–	41.99	–
	DPPC + piroxicam ($x = 0.01$)	0.50	35.06	41.72	5.00	42.55	0.44
	DPPC + piroxicam ($x = 0.05$)	0.60	33.78	41.18	4.67	42.75	0.60
	DPPC + piroxicam ($x = 0.10$)	0.85	39.90	40.29	3.93	42.00	0.76
	DPPC + piroxicam ($x = 0.20$)	0.90	–	41.10	–	41.84	–
	DPPC + meloxicam ($x = 0.01$)	0.40	32.13	44.21	3.15	43.40	0.60
	DPPC + meloxicam ($x = 0.05$)	0.40	33.69	41.28	2.88	43.00	0.64
	DPPC + meloxicam ($x = 0.10$)	0.45	32.60	40.85	2.84	41.51	0.66
	DPPC + meloxicam ($x = 0.20$)	0.50	33.05	42.66	1.57	38.94	0.90
	DPPC + lornoxicam ($x = 0.01$)	0.50	35.59	42.18	5.38	41.95	0.44
	DPPC + lornoxicam ($x = 0.05$)	0.50	31.06	41.21	3.19	41.93	0.60
	DPPC + lornoxicam (x = 0.10)	0.80	29.30	41.25	1.49	41.87	1.00
	DPPC + lornoxicam ($x = 0.20$)	1.00	–	38.92	–	41.62	–
2.5	DPPC alone	0.65	36.47	42.20	4.73	43.58	0.38
	DPPC + tenoxicam ($x = 0.01$)	0.5	35.76	42.04	4.93	43.23	0.40
	DPPC + tenoxicam ($x = 0.05$)	0.5	35.34	41.82	4.94	43.41	0.54
	DPPC + tenoxicam ($x = 0.10$)	0.7	33.16	41.24	3.66	42.90	0.80
	DPPC + tenoxicam ($x = 0.20$)	1.2	–	38.29	–	36.85	–
	DPPC + piroxicam ($x = 0.01$)	0.55	36.44	42.87	4.75	41.69	0.44
	DPPC + piroxicam ($x = 0.05$)	0.6	33.93	41.70	3.57	43.05	0.58
	DPPC + piroxicam ($x = 0.10$)	0.7	28.92	40.85	3.90	42.90	0.90
	DPPC + piroxicam ($x = 0.20$)	0.9	–	39.85	–	42.36	–
	DPPC + meloxicam ($x = 0.01$)	0.65	39.21	44.63	3.41	43.94	0.70
	DPPC + meloxicam ($x = 0.05$)	0.4	34.38	42.03	1.76	42.39	0.80
	DPPC + meloxicam ($x = 0.10$)	0.5	35.05	42.13	2.52	36.86	0.84
	DPPC + meloxicam ($x = 0.20$)	0.6	–	41.28	1.57	36.20	0.90
	DPPC + lornoxicam ($x = 0.01$)	0.65	35.59	42.84	5.15	41.48	0.38
	DPPC + lornoxicam ($x = 0.05$)	0.65	31.08	42.04	2.44	42.92	0.60
	DPPC + lornoxicam ($x = 0.10$)	0.55	29.30	40.90	1.46	42.13	0.90
	DPPC + lornoxicam ($x = 0.20$)	1.2	–	39.34	–	40.64	–

the pre-transition breadth by broadening it and the main phase transition by lowering the phase transition temperature. At $x_{drug} = 0.05$ (95:5 phospholipid:drug ratio) a further broadening of the pre-transition is observed (see Table 1).

As it can be observed in Fig. 3 there is no linearity between T_m and increasing drug concentration. Such linearity is however evident with $T_{m_{1/2}}$ both for the pre- and main transition temperatures (Fig. 4). Both $T_{m_{1/2}}$ increase as drug concentration increases.

At $x_{drug} = 0.10$ (90:10 phospholipid:drug ratio), tenoxicam affects only the pre transition by broadening it while piroxicam as well as lornoxicam cause broadening both to the pre- and main transition temperatures. Meloxicam causes broadening of the pre-transition but it does cause small narrowing of the main phase transition. At $x_{drug} = 0.20$ (80:20 phospholipid:drug ratio) tenoxicam, piroxicam and lornoxicam cause broadening of the main phase transition of DPPC bilayers and significant lowering of the main phase transition temperature. This is not observed with meloxicam which at this high concentration causes lowering of the phase transition temperature by only 1.5 °C but no broadening of the phase transition. Tenoxicam and lornoxicam cause abolishment of the pre-transition while piroxicam and meloxicam a significant broadening. The

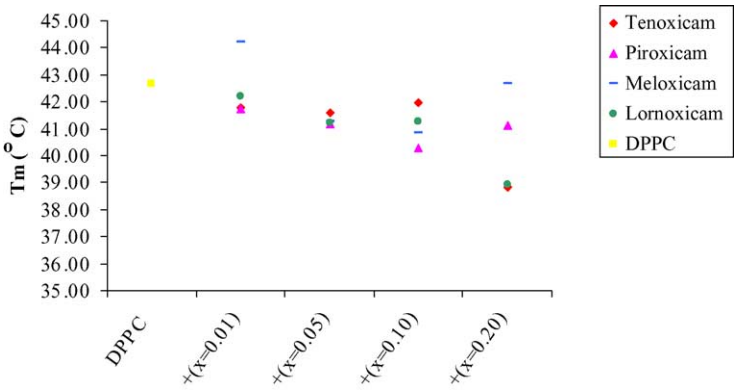

Fig. 3. T_m vs. DPPC bilayers with different drug additions at pH 7.0. The drug represents any of the four NSAIDs meloxicam, lornoxicam, piroxicam or tenoxicam.

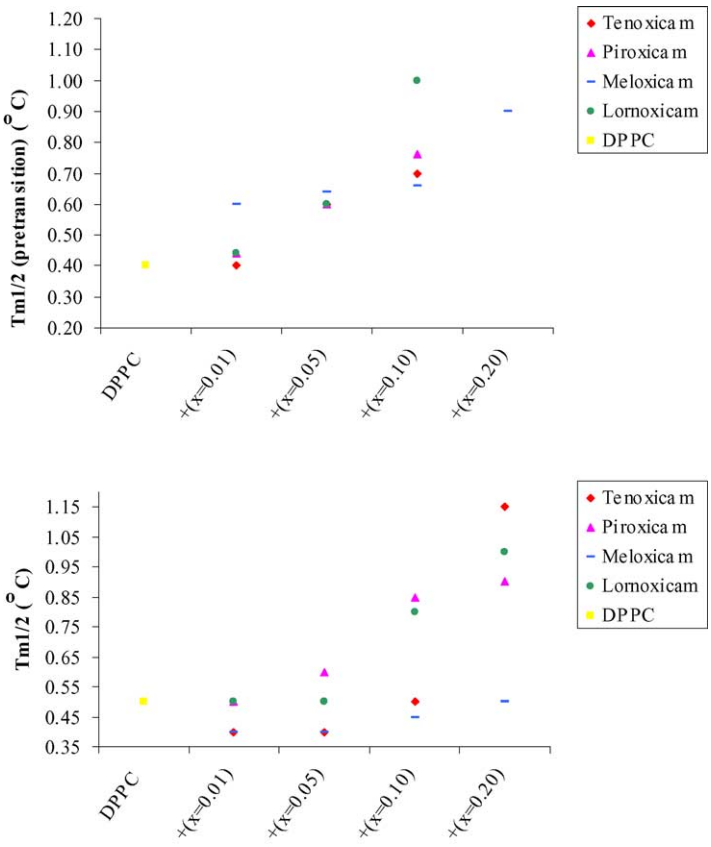

Fig. 4. $T_{m_{1/2}}$ vs. DPPC bilayers with different drug additions at pH 7.0. The drug represents any of the four NSAIDs meloxicam, lornoxicam, piroxicam or tenoxicam.

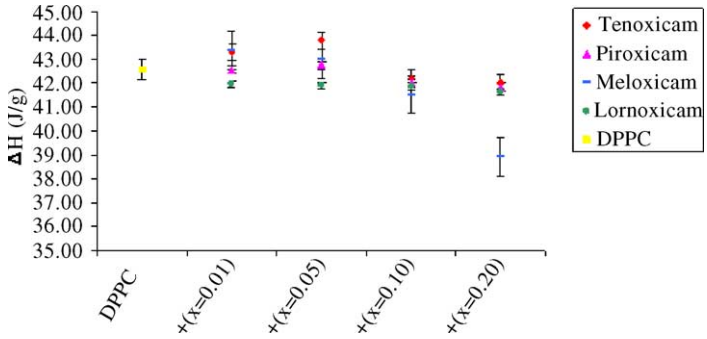

Fig. 5. Average $\triangle H$ (and S.D.) vs. DPPC bilayers with different drug additions at pH 7.0. The drug represents any of the four NSAIDs meloxicam, lornoxicam, piroxicam or tenoxicam.

effects of T_m and $T_{m_{1/2}}$ of the phase transition and pretransition for all preparations at different concentrations are shown in Figs. 3 and 4.

The drug molecules under study do not appear to affect significantly the enthalpy change ($\triangle H$) of the main phase transition of DPPC bilayers up to $x_{drug} = 0.10$. At $x_{drug} = 0.20$ only meloxicam causes lowering of the enthalpy change (see Fig. 5).

Fig. 6 shows DSC scans of the same NSAIDs on DPPC bilayers using identical concentrations and buffer (pH 2.5). The scans resemble those obtained at pH 7.0 (see also Table 1). This indicates that the acidity of the bilayers does not affect significantly the thermal changes caused by NSAIDs.

3.2. Raman spectroscopy

Since the methylene C–H stretching mode region provides the most intense features in Raman spectrum of a lipid sample, the 2800–3100 cm^{-1} interval has been commonly used to monitor changes in the lipid chain lateral packing properties in both gel and

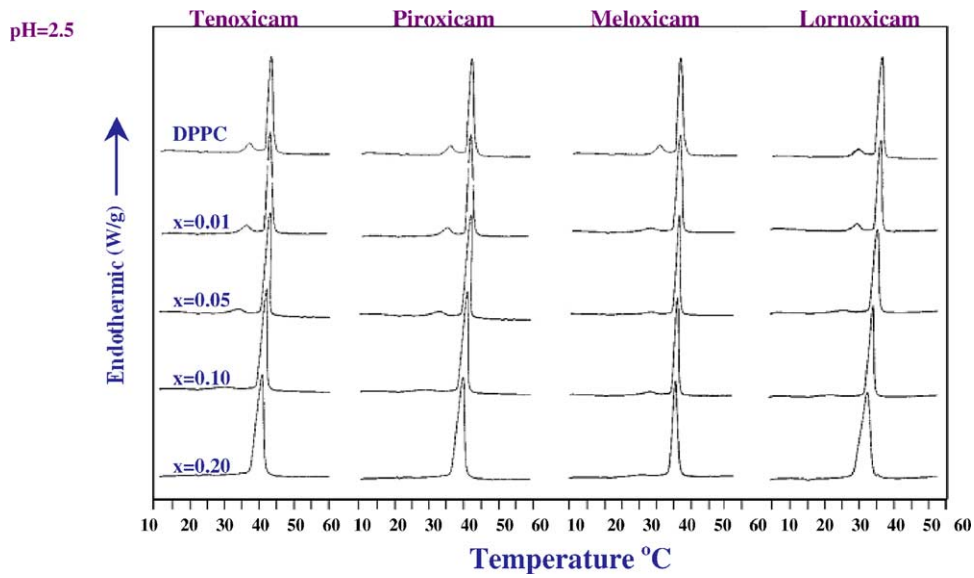

Fig. 6. DSC scans of DPPC bilayers containing either one of the four NSAIDs meloxicam, lornoxicam, piroxicam or tenoxicam at pH 2.5.

liquid crystalline uni- and multilamellar bilayer systems (Yellin and Levin, 1977; Zinder et al., 1980). At the spectrum of the gel state the methylene C–H symmetric and asymmetric stretching appear as the most intensed partially overlapped features at 2847 and 2883 cm^{-1}, respectively. The 2936 cm^{-1} peak represents, in part, a Fermi resonance component of the acyl chain terminal methyl C–H symmetric stretching mode. When dispersions are heated, intramolecular chain-disordering process exists and the intensity in the 2936 cm^{-1} region increases and broadens. In contrast, the 2883 cm^{-1} frequency decreases in intensity and shifts in frequency to 2892 cm^{-1} as the bilayer gets more disordered and enters the liquid crystalline state. In the liquid crystalline state the half width of the 2883 cm^{-1} line increases several fold. Since the empirical I_{2936}/I_{2883} peak height intensity ratio has been demonstrated to be a measure of the interchain order/disorder processes of the lipid acyl chains in bilayers, it was used to compare the members of the present series of phospholipids dispersions in absence and presence of drug molecules (O'Leary et al., 1984). Although the C–H stretching mode region consists of a congested, complex set of vibrational transitions, the peak height intensity ratios described above provide a sensitive probe of intramolecular (conformational) chain disorder concomitantly with intermolecular chain–chain packing disorder (Zinder et al., 1980). The C–C stretching mode region in the 1050–1150 cm^{-1} spectral interval directly reflects intramolecular *trans*/*gauche* conformational changes within the hydrocarbon chain region of the lipid matrix. The temperature profiles derived from the intensity ratios $I_{1090(gauche)}/I_{1130(trans)}$ peak height intensity ratios are also useful in comparing bilayer disorder–order characteristics between similar bilayers (O'Leary et al., 1984).

Fig. 7 shows the Raman spectra of the four oxicams used in our studies. The characteristic of these spectra is that region 500–1500 cm^{-1} is loaded with high intensity peaks. Several low intensity peaks are also observed in the region of 3000 cm^{-1}.

Fig. 8 shows the Raman spectra of DPPC bilayers at the gel in the pretransition and liquid crystalline phases with and without the addition of NSAID. The peaks of DPPC bilayers are well characterized in previous publications (Omura and Muraishi, 1997). The DPPC bilayers containing NSAID are enriched with peaks attributed to drug as expected. This enrichment of the spectrum due to the presence of NSAID creates difficulties in the analysis. (a) Peaks attributed to the drug interfere with the peaks due to DPPC bilayers. This peak overlapping masks the effects of the drug and unables to study its possible location in membrane bilayers. (b) The calculation of *gauche*:*trans* ratio is limited to the ratio of I_{2936}/I_{2883} which presents the least interference.

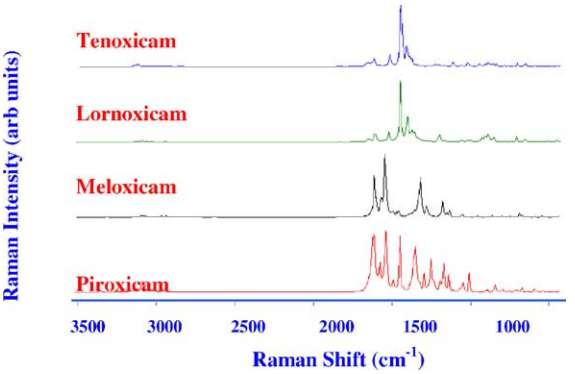

Fig. 7. Raman spectra from 3500 to 500 cm^{-1} for the four NSAIDs meloxicam, lornoxicam, piroxicam or tenoxicam.

The intensity attributed to the drug is subtracted during calculations. The profile of the DPPC bilayers which shows the I_{2936}/I_{2883} cm^{-1} ratio that reflects directly the *gauche*:*trans* ratio at the various preparations containing $x_{drug} = 0.20$ versus temperature is shown in Fig. 9. These preparations were chosen for comparison because at DSC experiments showed the maximum differences. As it can be observed from the I_{2936}/I_{2883} cm^{-1} ratio versus temperature meloxicam shows a different profile than the other three oxicams. At gel phase (25 °C) the DPPC bilayers containing meloxicam have similar *gauche*:*trans* ratio as DPPC bilayers alone. At pretransition temperature (36 °C) DPPC bilayers containing meloxicam show lower ratio than DPPC bilayers alone. This is in accordance with DSC data which show a broadening of the pre-transition temperature when meloxicam is present in DPPC bilayers. Above the phase transition temperature the preparations of DPPC bilayers alone or with the presence of meloxicam show similar *gauche*:*trans* ratios. However, the other three oxicams show consistently higher *gauche*:*trans* ratio.

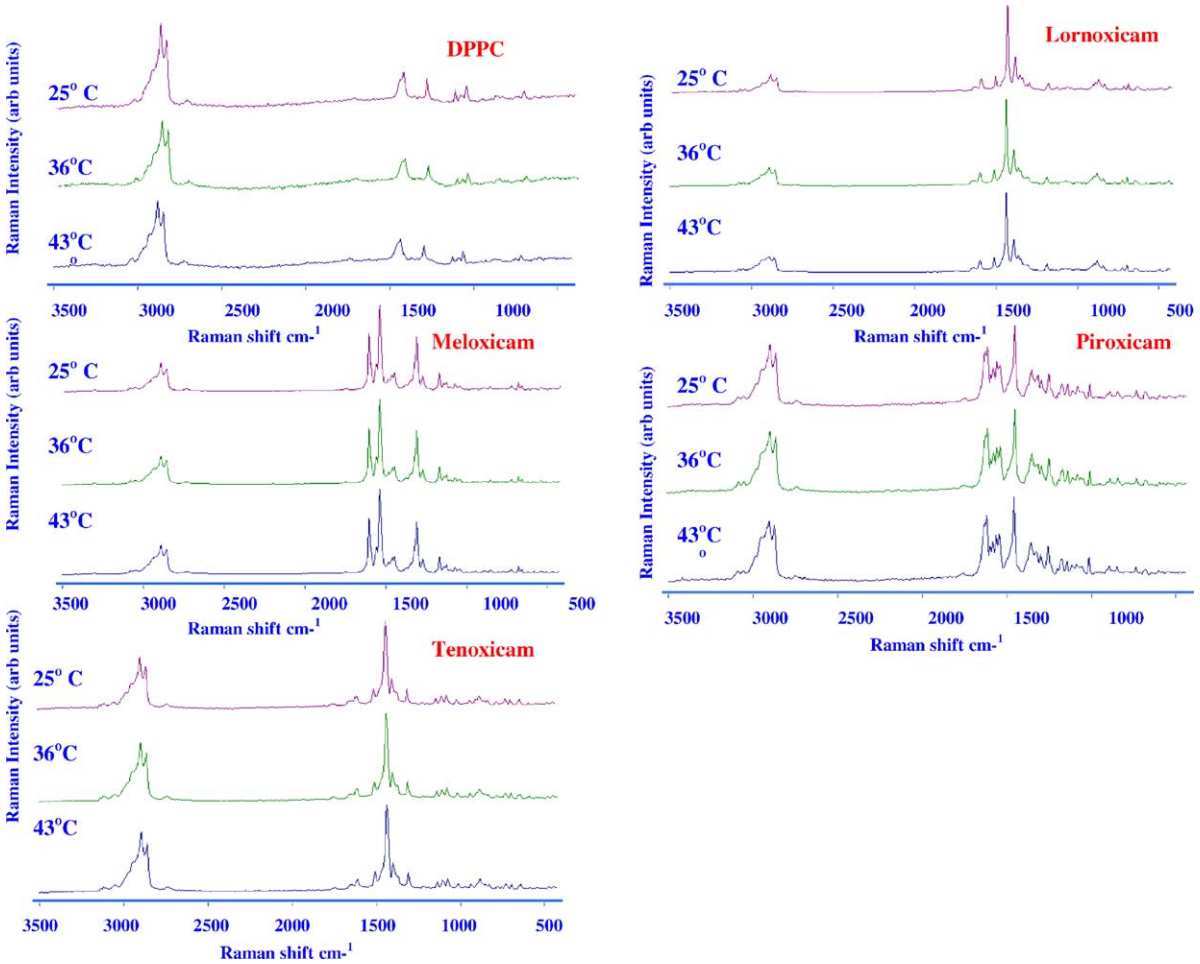

Fig. 8. Raman spectra from 3500 to 500 cm^{-1} for DPPC bilayers containing either one of the four NSAIDs meloxicam, lornoxicam, piroxicam or tenoxicam.

3.3. Molecular modeling

The most favored location of a representative energy minimized structure of lornoxicam in DPPC bilayers after applying dynamics experiment is shown in Fig. 10.

The hydrophilic part of lornoxicam orients towards the polar part of the membrane bilayers while its hydrophobic segment in the upper part of the lipophilic segment. The lipophilic profiles of the four NSAIDs reveal that only meloxicam has some topographical variation of the lipophilic component which may responsible for its differences on the thermal effects and *gauche*:*trans* ratio observed using DSC and Raman spectroscopy correspondingly (Fig. 1). Superimposition of the four oxicams shown in Fig. 11 reveals the perfect superimposition of the rigid segments and protruding of their side groups where no equivalent groups can be sought.

4. Discussion

We have applied a combination of DSC, Raman spectroscopy and molecular modeling to study the thermal effects and physicochemical properties of the NSAIDs in membrane bilayers. According to molecular modeling results the drugs are located in a

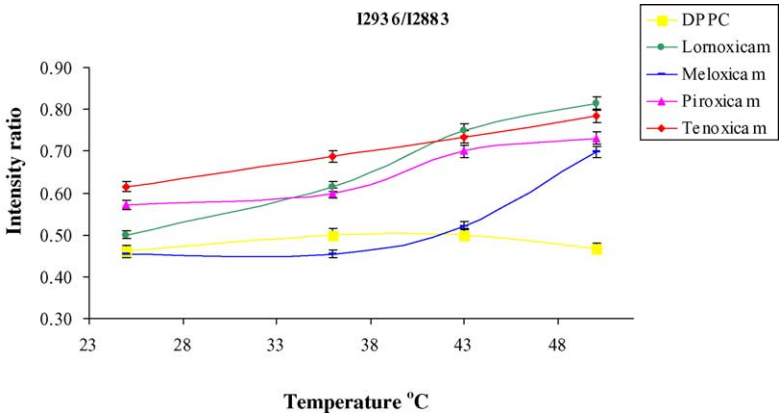

Fig. 9. I_{2936}/I_{2883} cm^{-1} ratio vs. temperature for DPPC bilayers (pH 7) containing either one of the four NSAIDs melxicam, lornoxicam, piroxicam or tenoxicam.

similar topography inside the membrane bilayers. Each molecule is located near the head-group of the DPPC molecules with the capability to form hydrogen bonding with water molecules or the head-group itself.

Literature data for the structure and physicochemical properties of meloxicam have been already reported. Meloxicam exists in the following ionization forms (Luger et al., 1996):

cation ↔ zwitterion ↔ enol ↔ anion

The other structurally similar molecules under study will be expected to exist in similar forms. The obtained DSC data show that the thermal effects of these molecules are similar at pH 2.5 and 7 where oxicams exist in anionic, enolic and zwitterions forms. These data are in agreement with molecular modeling which show that NSAIDs are localized in the vicinity of headgroup independently if they are in a dipole or anionic forms.

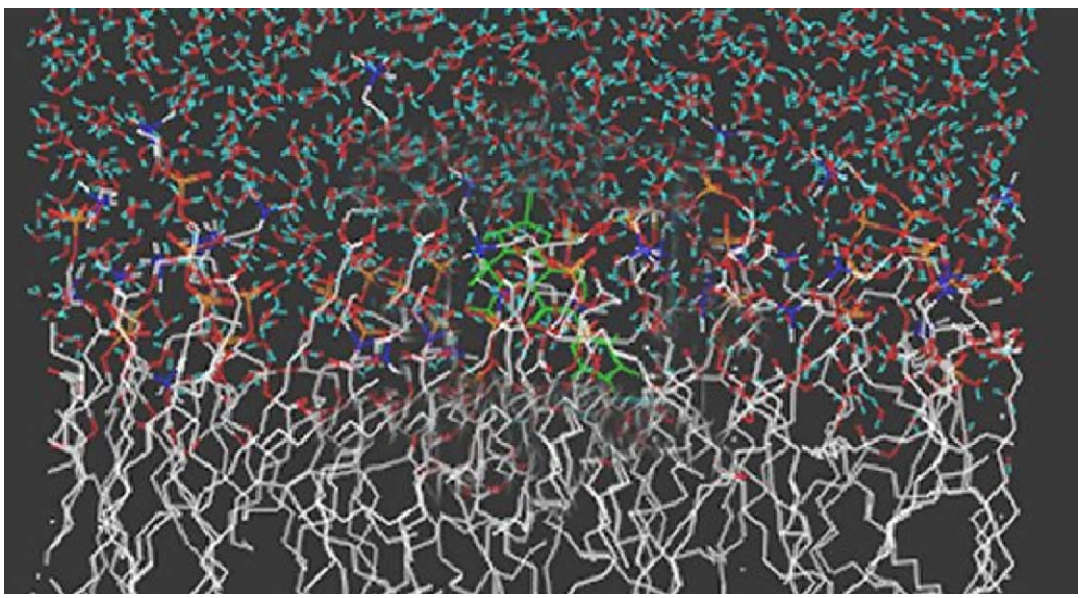

Fig. 10. Localization of a representative NSAID lornoxicam into DPPC bilayers. This bilayer contains 128 DPPC ligands and 3910 water molecules and was constructed by Tieleman et al. (1997).

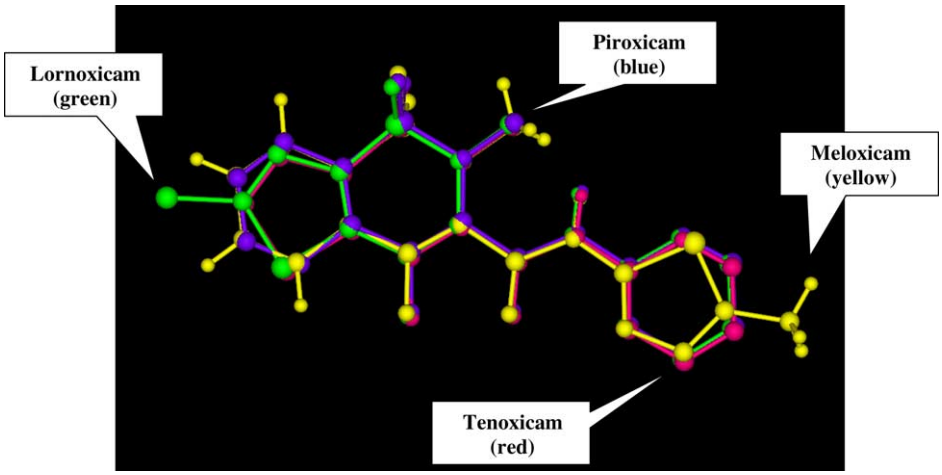

Fig. 11. Superimposition between the four oxicams meloxicam, lornoxicam, piroxicam and tenoxicam.

Their differences in their thermal changes can be explained from their different lipophilicity. Literature data (Luger et al., 1996) (see Table 2) show the lipophilicities for three of the NSAIDs under study. Calculation of their lipophilicities agree that the most lipophilic molecule is meloxicam (Table 1). The perturbing effect as defined by the degree of $T_{m_{1/2}}$ increase and T_m lowering of the main phase transition is inversely proportional to the lipophilicity of the molecules. This is expected from the molecular modeling results. Meloxicam shows the least perturbing effect because its lipophilic moiety is accommodated better in the hydrophobic core of the membrane bilayers. Tenoxicam has the lowest $\log P$ value and highest perturbing effects. Piroxicam has intermediate perturbing effects and intermediate lipophilicity. Lornoxicam has additional chlorine in the thiophene ring. It is therefore expected to be more lipophilic than tenoxicam (calculations confirm this). Its perturbing effects are then expected and indeed are found to be between tenoxicam and meloxicam. Direct comparison between piroxicam and lornoxicam cannot be made because the lack of experimental data for lornoxicam.

The effects of these molecules on the pre-transition are more complicated. Meloxicam is still having the least effect in the lowering and broadening of pre-transition temperature and increase of pre-transition breadth. The other three oxicams do not follow the same term described for the main-phase transition. Tenoxicam for example does appear to affect in less degree the pre-transition than piroxicam and lornoxicam.

Meloxicam is the only oxicam that causes lowering of $\triangle H$ when is present at high concentration in DPPC bilayers. A plausible explanation for this observation is the following. Meloxicam causes an increase of *trans*:*gauche* at pretransition temperatures and broadening of the pre-transition. This may result in conformational changes of acyl changes that favor the melting in less energy expense.

Huang and Shen, 1981 studied the thermal effects of indomethacin, flurbiprofen and diflunisal in membrane bilayers. Based on comparative DSC studies with molecules that their localization in membrane bilayers is known the authors concluded that the active anti-inflammatory structures insert deeply into the hydrocarbon region of the bilayer, whereas the inactive compounds probably bind mainly to the carbonyl region near the surface. Significant differences were observed in the thermal scans at pH 4.5 and 10.0 showing that

Table 2
Partition coefficients ($\log P$ values) of NSAIDs in *n*-octanol-buffer at different pH values

NSAID	$\log P$ value at pH						
	2	3	4	5	6	7.4	12.0
Meloxicam	2.43	2.68	2.34	1.91	1.01	0.07	−0.13
Piroxicam	1.43	1.67	1.68	1.57	1.03	−0.14	−0.70
Tenoxicam	0.77	0.84	0.82	0.67	0.12	−0.75	−1.03

the degree of ionization is important for drug perturbation. Oxicams, however, were found to span both the polar and hydrophobic segments and their differences were not attributed to their localisation but lipophilicity. These structural differences and their perturbation on membranes may related to their different pharmacological activity. NSAIDs flurbipofen, indomethacin and lulindac inhibit COX-1 and 2 while oxicams have some preferential COX-2 inhibition.

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