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INHIBITION OF ANION TRANSPORT IN THE RED BLOOD CELL BY ANIONIC AMPHIPHILIC COMPOUNDS

II. CHEMICAL PROPERTIES OF THE FLUFENAMATE-BINDING SITE ON THE BAND 3 PROTEIN

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Flufenamate is a powerful inhibitor of anion exchange in red blood cells. It binds to the band 3 protein involved in the transport as discussed in the preceding paper (Cousin, J.-L. and Motais, R. (1982) Biochim. Biophys. Acta 687, 147–155). The present study is concerned with the chemical properties of the inhibitory binding site. Structure-activity studies were performed with two sets of compounds derivated from anthranilate (considered as the basic structure of flufenamate). The molar concentrations required to produce 50% inhibition (I_{50}) varied over more than a 10^4 range. The inhibitory activity was quantitatively correlated with the hydrophobic character of the molecules and the electron-withdrawing capacity of the substituents. Comparison between the inhibitory potency of flufenamate analogs made a definition of the contribution of each part of the molecule in the binding to the receptor possible. The results suggest that anionic inhibitors bind to a site which presents a positively charged groups at the water-protein interface whereas the hydrophobic part of the molecule is inserted into an hydrophobic and electron-donor region of the protein. The specificity of amphiphilic compounds towards anion transport is discussed.

Introduction

In the preceding paper [1] we showed that flufenamate is a powerful inhibitor of anion transport across the erythrocyte membrane; we consider that it binds to the membrane-associated domain of the band 3 protein which has been involved in the transport [2–4]. The present work deals with an attempt to obtain information about chemical properties of the inhibitor recognition site. A structure-activity study was carried out with two sets of derivatives and other related compounds.

Materials and Methods

Preparation of cells

Freshly withdrawn or recently outdated human blood (collected into citrate dextrose solution) was obtained from the blood bank and stored at 4°C. Bovine blood was collected into heparin by exsanguination and immediately transported to the laboratory and stored at 4°C. Before use the red cells were separated by centrifugation and plasma and buffy coat removed by aspiration; they were then washed three times in appropriate buffer and used as described below.

Abbreviation: SITS, 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic acid.

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^{36}*Chloride effluxes*

The chloride self-exchange fluxes in intact cells were measured at pH 7.4 0°C. Labelling of cells with ^{36}Cl, isolation of labelled cells, determination of radioactivity in cell-free medium, and automatic technic of flux measurement have been described previously [5]. However, in the present work the hematocrit was 0.5%. The composition of the solution used was: 150 mM NaCl, 10 mM KCl, 20 mM Tris, 10 mM(+)-D-glucose. In experiments with inhibitors, the cells were not incubated with them prior to the flux measurement: the drugs were only present in the experimental media at suitable concentration. The solutions used in experiments with ox red cells did not contain(+)-D-glucose.

Determination of lipophilic character of drugs

The hydrophobic properties of drugs were determined by thin-layer partition chromatography as previously described [6]. Silica gel Silanised (60 HF 254 Merck) was the stationary phase, and mixtures of water and acetic acid were the mobile phase. For a given compound, different R_F values were measured with different proportions of solvent and water in the mobile phase and the R_M value calculated from the following equation [7]:

$$R_M = \log\left(\frac{1}{R_F} - 1\right)$$

A particularly useful property of the R_M value, as contrasted with the R_F value is that it is a simple function of the relative volumes of the solvent phases.

The R_M values were plotted vs. proportion of water in the mobile phase, the intercept of the straight line with the ordinate axis (determined by regression analysis) gave the R_M value for pure water (R_{M,H_2O}). R_{M,H_2O} is a hydrophobic parameter equivalent to the logarithm of a partition coefficient.

Chemicals

Compounds II, III, VIII, IX–XII, XIV–XVIII, XX, XXI, were purchased from Aldrich Chemical Co.; I, IV, XIII from Fluka; V, VI, VII from Parke Davis; XXII from Sigma Chemical Co. The methyl ester of flufenamic acid was prepared by the small scale method of Schlenk and Gellerman [8]: 5.6 mg flufenamic acid was dissolved in acetone. Diazomethane was bubbled in the solution until complete reaction. The product of the reaction gave a simple peak in gas chromatography and a single spot in thin-layer partition chromatography performed as described above.

Results

Structure-activity relationship of anthranilate derivatives

The quantitative correlation between molecular modification and change in the inhibitory activity for a serie of congeners was based on the method developed by Hansch and coworkers [9–11]. Two sets of compounds derived from anthranilate (considered as the basic structure of flufenamate) were studied: N-substituted derivatives and compounds with substituents on the ring (Fig. 1).

The inhibitory activities were expressed as $1/I_{50}$, I_{50} being the molar concentration producing 50% inhibition of chloride self-exchange at Donnan equilibrium. As an approximation three types of properties may modulate bioactivity in a series of congeners: hydrophobic properties, electronic properties and steric effects of substituents.

(1) Hydrophobic properties are usually quantified by π, the Hansch constant, which is a measure of the free-energy of transfer of a substituent from an aqueous phase to a liphophilic phase. The constant π can be defined as $\pi = \log P_x - \log P_H$, where P_H is the octanol-water partition coefficient of the parent compound and P_x the octanol-water partition coefficient of the derivative. Log P can be obtained for numerous compounds from the literature or calculated according to the rules formulated by Hansch and coworkers from the substituent constants [11]. Unfortunately, for some of the derivatives the values of the constants are not available in the literature. Therefore we de-

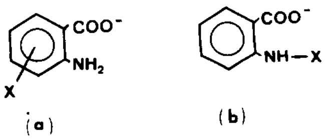


Fig. 1. (a) Anthranilate derivatives with substituents on the phenyl ring. (b) N-substituted anthranilate derivatives.

TABLE I

STRUCTURE-ACTIVITY RELATIONSHIPS OF N-SUBSTITUTED ANTHRANILATE DERIVATIVES

$\log 1/I_{50}$ represents the inhibitory activity of the compounds (I_{50} is the molar concentration reducing chloride self-exchange in the red cell by 50%). $R_{\mathrm{M,H_2O}}$ and $\log P$ measure the lipophilic character of the molecule.

No.	N-substituent	$\log \frac{1}{I_{50}}$ Man	$\log \frac{1}{I_{50}}$ Ox	$R_{\mathrm{M,H_2O}}$	$\log P$ (Refs. 16, 17)
I	$-H$	1.89	2.36	0.33	1.21
II	$-CH_3$	3.19	3.40	0.62	2.05
III	$-COCH_3$	2.82	3.25	0.90	1.88
IV	$-C_6H_5$ fenamic acid	4.74	5.01	2.27	4.36
V	$-C_6H_3(CH_3)_2$ mefenamic	5.57	5.83	2.64	5.37
VI	$-C_6H_2(Cl)_2(CH_3)$ meclofenamic acid	6.12	6.12	2.75	
VII	$-C_6H_4(CF_3)$ flufenamic acid	6.16	6.49	3.00	5.62
VIII	$-C_6H_4(NO_2)$	4.19	4.96	2.06	
IX	$-CH_2-C_6H_5$	5.00		2.15	

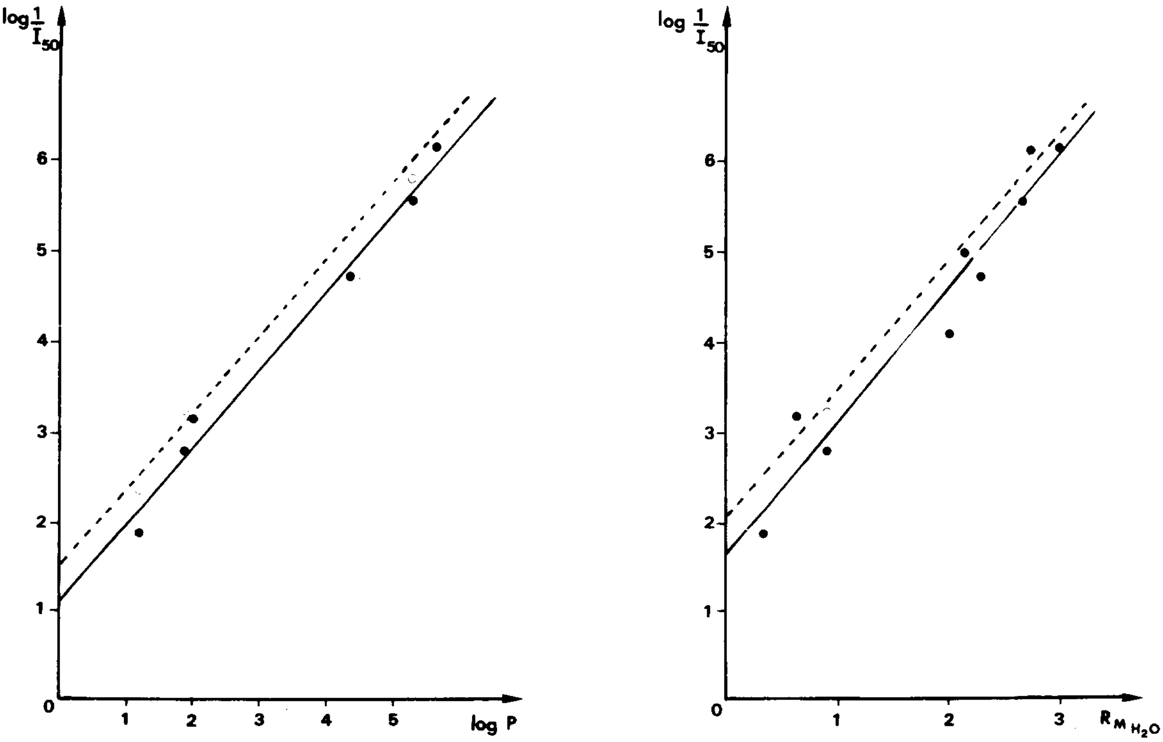


Fig. 2. Relation between inhibition of chloride permeability and lipophilic character of N-substituted anthranilate quantified by $\log P$ or $R_{\mathrm{M,H_2O}}$. Least squares regression line is superimposed on the data. (○) bovine red blood cells. (●) human red blood cells.

TABLE II

STRUCTURE-ACTIVITY RELATIONSHIPS OF ANTHRANILATE DERIVATIVES WITH SUBSTITUENTS ON THE PHENYL RING

$\log 1/I_{50}$ represents the inhibitory activity of the compounds (I_{50} is the molar concentration producing a 50% inhibition of chloride transport in human red blood cells). R_{M,H_2O} is a measure of the lipophilic character of the molecule and σ is the Hammett substituent constant.

No.	Substituent	$\log \frac{1}{I_{50}}$	$R_{M_{H2O}}$	σ (Ref. 17)
I	H	1.89	0.33	0.00
X	4-Cl	3.14	1.17	0.23
XI	5-Cl	3.44	1.06	0.37
XII	5-I	3.66	1.36	0.35
XIII	3,5-$(Cl)_2$	4.18	1.50	0.74
XIV	4,5-$(Br)_2$	4.60	1.71	0.62
XV	3-CH_3	2.32	0.71	−0.07
XVI	4-CH_3	2.25	0.76	−0.17
XVII	5-CH_3	2.54	0.53	−0.07

termined experimentally the hydrophobic properties for all the compounds by thin-layer partition chromatography and we obtained for each of them a R_{M,H_2O} value which is a hydrophobic parameter equivalent to $\log P$.

Because R_{M,H_2O} is determined with acetic acid-water mixtures as mobile phase it refers to the hydrophobicity of the acidic form.

(2) The electronic properties of a substituent were quantified by σ the Hammet constant defined by the equation:

$$\log k_x/k_o = \rho\sigma$$

where k_x is the rate or equilibrium constant for a member x of a congeneric set of compounds undergoing a reaction, k_o is the same constant for the unsubstituted compound, ρ is a constant characteristic of the reaction. σ values for simple substituent were obtained from the table published by McDaniel and Brown [12]. Those of multiple substituents were computed following the Hansch additivity rule [11].

(3) For the series so far studied we have found that the steric parameter can be neglected [6,13,14] and the results below indicate that this is also the case for anthranilate derivatives.

Concerning first the N-substituted anthranilate derivatives, the values of $\log 1/I_{50}$ (measured in human and bovine erythrocytes), R_{M,H_2O} and $\log P$ are given in Table I. From these data we have derived Equations 2–5 by the method of the least squares.

Erythrocytes	n	r	s	F
Human				
$\log 1/I_{50} = 1.49 R_{M,H_2O} + 1.65$	9	0.972	0.38	120
$\log 1/I_{50} = 0.86 \log P + 1.11$	6	0.991	0.26	213
Bovine				
$\log 1/I_{50} = 1.42 R_{M,H_2O} + 2.09$	8	0.988	0.25	253
$\log 1/I_{50} = 0.84 \log P + 1.53$	6	0.992	0.23	249

(2-5)

In Eqns. 2–5 n is the number of points used in the regression, r is the correlation coefficient, s is the standard deviation and F is the decision statistic of the F test of significance. This value after tabular interpretation indicates the significance of the correlation found [34].

The regressions are highly significant and account for 95–98% of the variance in our data indicating that the hydrophobic character of a molecule is a very good predictor of its inhibitory activity. This is observed both in human (Eqns. 2 and 3) and bovine (Eqns. 4 and 5) red blood cells and whatever the index of hydrophobicity ($\log P$ or R_{M,H_2O}) used. This relation is illustrated in Fig. 2. The slopes are not significantly different for human and bovine red cells. The anion transport is therefore susceptible to the hydrophobic nature of the inhibitors to the same extent in both species. The incercepts are significantly different; thus bovine red blood cells are approximately 3-times more sensitive to any one of the inhibitors than human red blood cells.

σ substituent constants are not available in the literature so that their contribution in the inhibitory activity, if any, cannot be quantified.

Concerning anthranilate derivatives with substituents on the ring, Table II lists the values of $\log 1/I_{50}$ (measured in human red cells); R_{M,H_2O} and σ. The simple and multiple regression analysis gave the Equations 6–8. In Eqn. 8 the values in brackets below the coefficients are the t values (of Student's distribution) which are a measure of the contribution of the corresponding parameters to the correlation found.

	n	r	s	F
$\log 1/I_{50} = 1.93 R_{M,H_2O} + 1.15$	9	0.963	0.27	89
$\log 1/I_{50} = 2.71\sigma + 2.51$	9	0.940	0.34	52
$\log 1/I_{50} = \underset{(3.68)}{1.22} R_{M,H_2O} + \underset{(2.46)}{1.16\sigma} + 1.62$	9	0.982	0.20	80

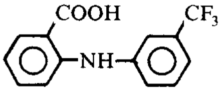
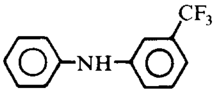
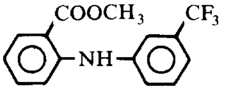
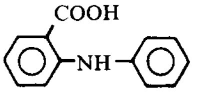
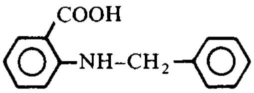
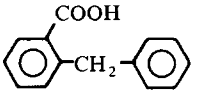
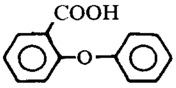
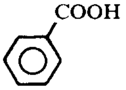
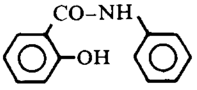
(6–8)

In terms of r and s, the lipophilic character of the molecule is a better predictor of activity than the electronic character but taking into account both characters we obtained the Eqn. 8 which is a significant improvement on Eqn. 6 ($P < 0.05$ as indicated by t and F tests). The positive coefficient of σ means that groups which attract electrons increase activity. The coefficient of determination of

TABLE III

INHIBITORY ACTIVITIES OF FLUFENAMATE RELATED COMPOUNDS

The inhibitory activities are expressed by $\log 1/I_{50}$ (I_{50} represents the molar concentration reducing the chloride self-exchange across the human erythrocyte membrane by 50%). The lipophilic character of the molecules is expressed by R_{M,H_2O}. Compound XIX was not soluble enough to determine exactly the I_{50} value.

No.	Compound	formula	$\log \frac{1}{I_{50}}$	R_{M,H_2O}
VII	Flufenamic acid		6.16	3.00
XVIII	3-(Trifluoromethyl)-diphenylamine		5.36	3.04
XIX	Flufenamic acid methyl ester		<4.50	3.72
IV	Fenamic acid		4.74	2.27
IX	*N*-benzyl-anthranilic acid		5.00	2.15
XX	*o*-Toluyl-benzoic acid		3.10	2.10
XXI	*o*-Phenoxy-benzoic acid		2.30	1.75
XXII	Benzoic acid		1.85	0.88
XXIII	Salicylanilide		4.32	2.13

Eqn. 8 indicate that 96% of the variance is 'explained' by the regression equation. In other words, inhibitory activity of this set of compounds is well described by a combination of both hydrophobic and electronic parameters and it does not appear necessary to take into account any other parameter such as a steric effect. Furthermore hydrophobicity can be obtained by substitution either on the ring or on the amino group, indicating that no specific steric arrangement is needed.

Since the basic structure is anthranilate, the contribution of carboxyl and amino groups to the inhibitory activity cannot be infered from the above structure-activity relationship. We analysed the role of these groups by comparing the inhibitory potency of analogs of flufenamate.

Table III shows that removal of the carboxyl group from the flufenamate molecule (compound VII and XVIII) decreases the inhibitory activity by a factor 6 without affecting R_{M,H_2O} *. Esterification of the carboxyl group by diazomethane leads to XIX and increases the hydrophobicity but nevertheless decreases the inhibitory activity. These findings indicate that the carboxyl group is important for inhibitory activity and suggest that the dissociated form is more active than the neutral form. This confirms our previous results obtained with niflumate, a very close analog of flufenamate [15].

Comparison of the inhibitory activities of a number of analogs of *N*-phenylanthranilate (fenamate IV) in which the amino bridging group is replaced by other single-atom or multi-atom bridges, indicates that the amino bridging group is more effective than the -O- or CH_2 group (compounds XXI and XX, respectively). Compound XXI has a lower R_{M,H_2O} value than the three others and therefore is expected to have lower activity. However the difference in R_{M,H_2O} values is not sufficient to explain the difference in $\log 1/I_{50}$ values according to Eqn. 2. It must be concluded that an -NH-group is a structural requirement for inhibitory activity.

If one assumes that I_{50} represents the dissociation constant of the site-inhibitor complex, the variation of the molar free-energy ΔG associated with the complex formation can be calculated. Comparison of the ΔG values of related compounds give the contribution ΔG_p of each part of the molecule to the overall ΔG for the flufenamate (-7.8 kcal·mol^{-1}). The results are given in Table IV. It can be seen that hydrophobic regions are essential but that the hydrophylic group and particularly the -NH- group play a role. The larger contribution of -CF_3 compared with phenyl ring must be attributed to its hydrophobic character and its strong electron-withdrawing capacity.

Discussion

Flufenamate [1] and niflumate [15] are among the most powerful reversible inhibitors of anion transport in red blood cells. We have obtained strong evidence suggesting that they bind to band 3 protein which is involved in this transport. The arguments can be summarized as follow:

(a) Flufenamate and niflumate bind to high affinity binding sites and low affinity binding sites on the erythrocyte membrane.

(b) The number of sites in the first class corresponds to the number of band 3 copies per cell.

TABLE IV

CONTRIBUTION OF EACH GROUP OF THE FLUFENAMATE MOLECULE TO THE VARIATION OF THE MOLAR FREE-ENERGY ΔG AS ASSOCIATED TO THE DRUG RECEPTOR-COMPLEX FORMATION AT 0°C

ΔG_p represents the partial contribution of each part of the molecule. It is calculated from the difference in ΔG of compounds taken from Table III. ΔG is calculated from I_{50} according to Eqn. 9.

Compound	Group	$-\Delta G_p$ kcal·mol^{-1}
VII-XVIII	—COOH	1.0
XXII-(—COOH)	—C_6H_5	1.3
VII-IV	—CF_3	1.8
IX-XX	—NH—	2.4
IX-IV	—CH_2—	0.3

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* The observation that R_{M,H_2O} values are similar can be explained as follows: R_{M,H_2O} is determined with acetic acid-water as the mobile phase in thin-layer partition chromatography and therefore is related to the hydrophobicity of the unionized form of the carboxyl form.

(c) The dissociation constant for this class is equal to the I_{50} for anion transport.

(d) These sites disappear when chloride transport is inhibited by covalently bound 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic acid (SITS) a specific inhibitor of anion transport.

(e) Conversely niflumate inhibits the binding reaction of SITS with sites related to anion transport.

(f) Proteolytic dissections which do not affect Cl^- transport are without effect on binding and conversely those which inhibit Cl^- transport decrease the number of binding sites in the same ratio.

By a comparative study of flufenamate analogs (determination of free-energy change associated with the binding, correlation between inhibitory activity and physico-chemical properties of compounds), we attempt to gain insight into the chemical characteristics of the binding site.

The results obtained by the determination of ΔG_p (Table IV) indicate that hydrophobic regions of the flufenamate molecule play a leading part but hydrophilic -COO$^-$ and -NH- groups also contribute to the binding. This is confirmed by the observation that the inhibitory activity of chloride transport is mainly accounted for by the hydrophobic character of the molecules (as measured by R_{M,H_2O} as well as $\log P$) (see Tables I and II).

$\log P$ refers to the transfer of a compound from water to octanol, whereas R_{M,H_2O} results from partition chromatography using silanized silica gel as a stationary phase. Thus R_{M,H_2O} refers to an adsorption process in which only one face of the molecule is concerned in hydrophobic adsorption [36,37]. For a series of compounds the slope of $\log 1/I_{50}$ versus hydrophobic parameters is a measure of the extent to which the binding of the inhibitor to the site ressembles transfer from water to octanol or adsorption to hydrophobic gel. The values observed (1.49 for R_{M,H_2O} and 0.86 for $\log P$) indicate that the binding to the site is not a complete transfer from water to hydrophobic phase and more than an adsorption process. Thus not all the water molecules have to be stripped off at the time of binding. We therefore suggest that binding site is a surface rather than a pocket. That is also supported by the fact that steric effect is negligible.

In the above correlations, the inhibitory activities are related to hydrophobicity of the neutral form of the molecules since R_{M,H_2O} was determined with acetic acid-water mixtures as a mobile phase (see Materials and Methods) suggesting that the unionized form could be the active one. On the contrary, in recent work, we showed that for niflumate the anionic form is the active one and we gave above evidence indicating that it is the same for flufenamate. At first sight these two facts appear inconsistent. However they can be reconciled assuming that, upon binding, the negative charge of carboxylate anion is neutralized by a cationic locus on the protein; the complexed anion behaves then as a neutral molecule with respect to 'hydrophobic interactions'.

Eqn. 8 shows that the electron withdrawing ability of the substituents contributes significantly to the regression. Thus the more lipophilic and the more electron-attracting the substituents are, the greater the activity they confer to the parent compound. This suggests the presence of electron-donnor groups in the binding site and the participation of charge transfer in the stability of the receptor-inhibitor complex.

The -NH- bridging group appears to be necessary. Thanks to this group, the anthranilate moiety can readily form intramolecular hydrogen bonds of the type (-N-H ---O) in a six membered ring in such a way that the affinity of hydrophilic groups for the aqueous phase would be reduced. This is shown by the positive difference in the hydrophobicity (as measured by $\log P$) of anthranilate and its *para* isomer (Table V, Ref. 17). An intramolecular hydrogen bond is also suggested by

TABLE V

PHYSICO-CHEMICAL PROPERTIES OF AMINOBENZOIC ACID ISOMERS

Hydrophobic character is expressed as the logarithm of the partition coefficient ($\log P$) and pK_a values of amino benzoic acid in water.

Compound	$\log P$	pK_1	pK_2
o-NH_2	1.21	2.09	4.80
m-NH_2		3.08	4.80
p-NH_2	0.68	2.50	4.87

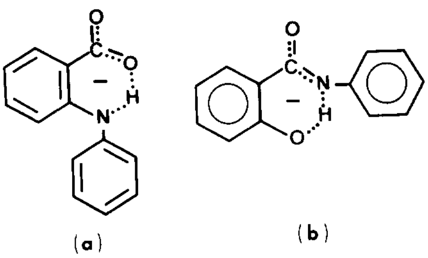

Fig. 3. Planar structure of the fenamate (a) and salicylanilide (b) anions stabilized by electron delocalization and hydrogen bonding.

the lower pK_a value of anthranilate compared with *meta* and *para* isomers (Table V; Ref. 18). The intramolecular hydrogen bond makes fenamate (with hydrophilic -NH- bridging group) more lipophilic than *o*-phenoxybenzoate (with hydrophilic -O- bridge) and approximately as lipophilic as *o*-toluylbenzoate (with hydrophobic -CH_2- bridging group). The two last compounds cannot form an intramolecular hydrogen bond. However the large differences observed in the inhibitory activities of compounds with an -NH-bridge (IV and IX) and the others with -CH_2- (XX) or -O- bridges (XXII) cannot be explained by a difference in the hydrophobicity of the molecules according to Eqn. 2.

An intramolecular hydrogen bond also 'stabilizes' the structure of the molecule in which an electron pair of nitrogen, as well as a negative charge of carboxyl are delocalized increasing the resonance in the molecule. From this we propose the planar structure shown in Fig. 3 for the fenamate anion. Neither the *o*-phonoxy- nor the *o*-toluylbenzoate can maintain this planar structure.

It is interesting to note that such a planar structure has been propsed [19] for salicylanilide anion (XXIII). This drug has the same hydrophobicity and inhibitory activity as its analog fenamate (IV) both in human and bovine red cells (Table III; Ref. 20). As it can be seen in Fig. 3 the spatial organization of the molecules is not exactly the same, confirming that no specific steric arrangement is needed. This structure in which electrons are delocalized could be involved in charge transfer and (or) in Van de Waals-London dispersion forces. These forces were also suggested, for niflumate binding, from thermodynamic considerations [15]. All the observations discussed above suggest that the binding site of flufenamate, as niflumate, exhibits a positively charged group(s) close to a hydrophobic surface with electron-donor groups.

It is well known that anion transport is reversibly inhibited by various chemically unrelated compounds [21–26] all of which have, however, an amphiphilic structure. The question arises: do the conclusions drawn about the nature and the properties of the flufenamate inhibitory site hold good for the anionic amphiphilic inhibitors? Two arguments support this idea:

The first is the observation that many of these agents such as furosemide, salicylate, dinitrophenol, ethacrynic acid etc reduce that rate of dinitrophenylation of the band 3 protein [27] suggesting that the site of action of these inhibitors could be also the band 3 protein.

The second evidence arises from structure-activity studies which have been made for several classes of anionic inhibitors including derivatives of benzoate, salicylate, phenoxyacetate (to which belongs ethacrynic acid), phenol, benzenesulfonate and stilbenedisulfonate [6,13,14,28]. As for anthranilate derivatives, for each set of compounds, the inhibitory activity is correlated with both hydrophobicity of the molecules and electron-attracting effect of the substitents. It is interesting to note in this context that furosemide which reduces the rate of dinitrophenylation of the band 3 protein is an anthranilate derivated and fits Eqn. 2 ($\log 1/I_{50} = 3.74$; $R_{M,H_2O} = 1.34$).

These facts strongly suggest a common mode of action. The observation that stilbenedisulfonate (SITS), when covalently bound, prevents the binding of niflumate and flufenamate and conversely that niflumate reduces the rate of irreversible binding of SITS to sites related to anion transport [1,15] also supports this view.

However, amphiphilic compounds such as phenol, benzoate, salicylate [25], furosemide [29] and niflumate [15] have been shown to be non-competitive or mixed inhibitors of anion transport whereas benzenesulfonate and stilbenedisulfonate derivatives, when reversible, compete with anions for the transport site [30,31].

These data suggest that all these inhibitors bind to the same cavity but at different regions which overlap.

Flufenamate and other anionic amphiphilic compounds are also known to exhibit various biochemical and physiological effects. Structure-activity studies showed that the features of the inhibition of anion transport in red blood cell bear tight similarities with those observed for uncoupling activity in mitochondria, binding to serum albumin [16,32] and inhibition of prostaglandin biosynthesis [33]. We showed similar correlation between uncoupling activity and inhibition of anion transport for phenol derivatives [14] *. These observations suggest that different pharmacological effects of these anionic drugs have a common denominator at the molecular level: interaction with a protein site bearing the characteristics discussed above.

These drugs also inhibit glucose transport in the human red blood cell but in this case the mode of action is different. Indeed we provide evidence suggesting that inhibition results from a perturbation of the lipid phase of the membrane [1,15,35].

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* In this context it must be pointed out that the inhibitory effect cannot be due to uncoupling effect of these drugs since mammalian red cells do not contain mitochondria and that anions are passively transported in these cells.