Review in Quantitative Structure Activity Relationships on Lipoxygenase Inhibitors

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Abstract: This paper reviews and evaluates all the published QSAR treatments of LOX inhibitors. This reveals that in almost all cases, the clog *P* parameter plays an important part in the QSAR relationships. In some cases the steric factors (B_1, B_5 and L) as well as the overall molar refractivity (CMR) or the substitutents molar refractivity (MR) are important. Electronic effects except for the Hammet's constant σ, are comparatively unimportant. The study shows that log *P* as calculated from the Clog *P* program is suitable for this form of QSAR study. Log *Po* of 2.77-3.76 was found to be ideal, for the biological response.

Keywords: QSAR, LOX inhibitors, lipophilicity, steric factors, electronic effects.

1. INTRODUCTION

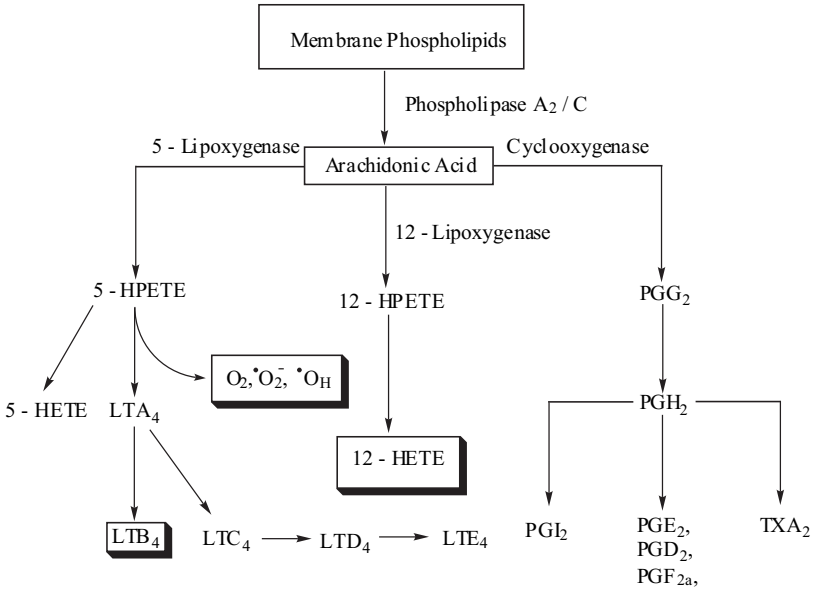
1.1. Eicosanoids

a. Arachidonic Acid Metabolism

The biosynthetic cascade of arachidonic acid has been the object of intense research. The major enzymatic routes of arachidonic acid metabolism in mammalian cells are the cyclooxygenase (COX) and the lipoxygenase (LOX) pathway in order to form the eicosanoids (Scheme I). The former catalyzes the production of prostaglandins (PGs), prostacyclins and thromboxanes (TX's). On the other hand, the LOX pathway results in formation of hydroxyperoxy-eicosatetraenoic acids (HETEs) and particularly leucotrienes, potent mediators of hypersensitivity and inflammatory reactions [1].

b. Lipoxygenases

Lipoxygenases are a number of cytosolic enzymes that catalyse the oxygenation of polyenic fatty acids to corresponding lipid hydroperoxides. The enzymes require a fatty acid substrate with two *cis*- double bonds separated by a methylene group. Arachidonic acid (AA), which contains several double bonds in its configuration, is metabolised to a number of products with the hydroperoxy group in different positions. For arachidonate these metabolites are called hydroperoxyeicosatetranoic acids (HPETEs). The HPETEs are unstable intermediates and are further metabolised by a variety of enzymes. All HPETEs may be converted to their corresponding hydroxy fatty acid (HETE) either by a peroxidase or nonenzymatically. Lipoxygenases



Scheme I. Arachidonic acid metabolism.

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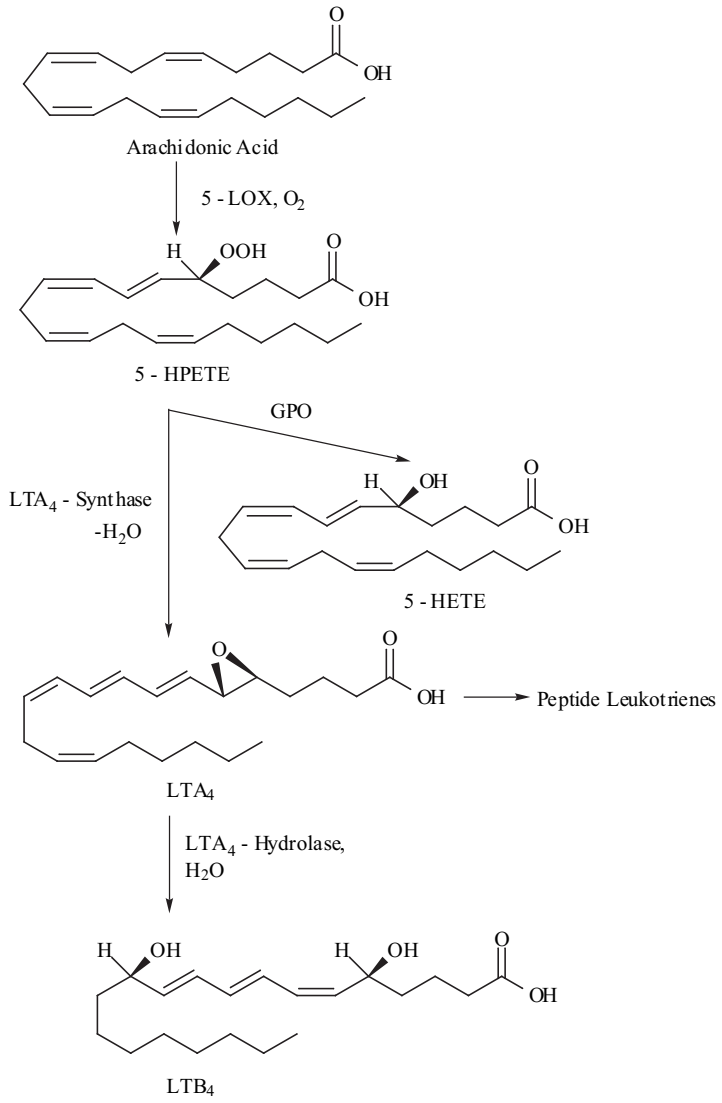


Scheme II. Metabolism of arachidonic acid by 5-lipoxygenase.

differ in their specificity of placing the hydroperoxy group (5-LOX, 12-LOX, 15-LOX), and tissues differ in the lipoxygenase(s) that they contain [2].

5-Lipoxygenase

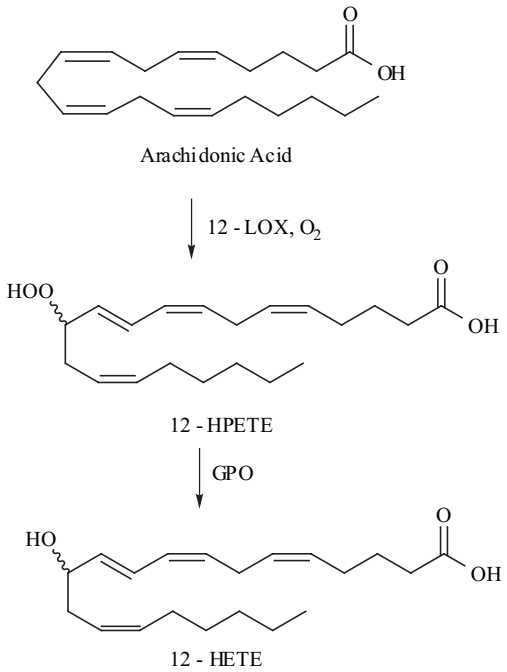
The name of 5-LOX of the enzyme is derived from the positional specificity of the oxygenation process (Scheme II). The position of the carbon bearing the hydroperoxy group is identified relative to the carboxylic group carbon of AA, which is designated as 1. Insertion of molecular oxygen at C_5 of the arachidonic acid gives 5-HPETE, which is rapidly transformed to an unstable 5,6-epoxide, leukotriene A_4 (LTA_4)- (5*S*)-5*E*, 6*E*-oxido-7*E*, 11*Z*-eicositetranoic acid [3]. Leukotriene A_4 is a precursor of the peptide leukotrienes (LTC_4, LTD_4, LTE_4) [4], formerly named of «slow reacting substances of anaphylaxis», which are potent mediators of hypersensitivity reactions. They can be implicated as mediators of certain respiratory, cardiovascular, renal, gastrointestinal and certain nervous system disorders. Thus, LTA_4 may undergo two diverse biochemical reactions. It produces LTB_4-(5*S,* 12*R*)-dihydroxy-6*Z*, 8*E,* 10*E,* 14*Z*-tetraenoic acid and may be conjugated with glutathione by glutathione transferase to produce LTC_4. Removal of glutamic acid by a γ-glutamyl transpeptidase gives LTD_4 [5]. Further removal of a glycine residue by a dipeptidase results in the formation of LTE_4.

12-Lipoxygenase

The 12-LOX pathway (Scheme III) is the main metabolic pathway of arachidonic acid in the skin, 12-HETE being the major product in most species [6]. The initial product of 12-LOX pathway is 12-HPETE, which is reduced to 12-HETE by a glutathione dependent peroxidase [7]. It has been observed the existence of two distinct 12-LOX enzymes: one in platelets and the other on the skin. That's why potent inhibitors of the *epidermal* 12-LOX did not inhibit *platelet* derived 12-LOX [8, 9].

15-Lipoxygenase

Mammalian 15-lipoxygenases are capable of oxygenating plasma lipoproteins [10, 11] and the ester lipids of the biological membranes [12, 13] without preceding action of a lipid-cleaving enzyme. The 15-LOX is receiving increased attention [14] as 15-LOX metabolites contribute to differentiation of reticulocites, the precursors of mature red blood cells [15]. In epithelial cells and eosinophils of the



Scheme III. Metabolism of arachidonic acid by 12-LOX.

airway, 15-LOX produces metabolites that may cause release of mucus, chemotaxis of inflammatory cells and contraction of bronchi [16]. The oxidation of low-density lipoproteins contributes to the development of aethero-sclerosis [17]. Inhibitors of 15-LOX can be broadly classified into two main categories: first competitive lipid substrate inhibitors and second, redox type inhibitors, which act by chelation or reduction of the Fe(III) of the active enzyme or by reaction with the fatty acid radical intermediate producing during the catalytic step.

Lipoxygenase Inhibitors

Lipoxygenases contain a «non-heme» iron per molecule in the active site as high-spin Fe(II) in the native state, and high-spin Fe(III) in the activated state [18-20]. It is supposed that iron changes its valence state during the catalytic reaction. The enzyme inhibitors can be broadly classified in the following categories:

a. Antioxidants and free radical scavengers, since lipoxygenation occurs *via* a carbon centered radical. These compounds inhibit the formation of this radical or trap it once formed. Moreover many LOX inhibitors also inhibit lipid peroxidation acting by scavenging chain-propagating peroxyl free radicals eg. circilliol, caffeic acid, NDGA, phenidone, BW755C.

b. Iron Chelators, they inhibit the enzyme because they are excellent ligands for the iron of the active site of the enzyme eg. N-hydroxyarachidonic acid amide, bufexamac.

c. Substrate and products analogues, they have analogue structure with the substrate eg. 5, 8 ,11,14-eicositetranoic acid (ETYA), 5,6-methano-LTA_4.

d. Structurally unrelated compounds eg. methoxythiazoles, indazolinones (2-(1-naphthylethyl)-indazolinone), ICI 216800.

1.2. Introduction on Qsar

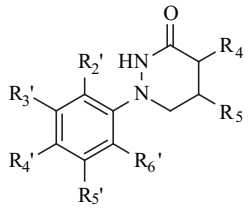
QSAR is a useful means for maximising the potency of a new lead compound. In the lead optimisation phase of the synthetic project, various QSAR procedures with the aid of computer technology have been proposed. Among them, the classical Hansch approach has been widely used leading to several successful examples. In the QSAR approaches, the prescription to optimise the lead structure is inferred from mathematical equations correlating variations in the congeneric molecules. The QSAR procedures are based on physical organic concepts and involve calculational operations.

Attempts have been made to perform QSAR studies in all categories of LOX inhibitors. However, a small number of QSARs are available on LOX inhibitors, the existing QSAR studies provide a great deal of information of the mechanism of drug enzyme interactions. A group wise description of them is presented here.

2. EARLIER WORK ON *IN VITRO* QSAR

a) LOX Inhibitors with Redox Potential Properties

i) Quantitative structure activity relationships (QSAR) of *phenyltetrahydropyridazin-3-[2H]-one analogues* (structure 1) indicated that enzymes inhibitory potency is increased by lipophilic substitutents at the 3' - and 4' -positions and substitutents with positive *F* value at the 4' -position also increase the potency, while substitutents at the 3' -position with a positive *R* value, decrease it. On the other hand, the potency decreases as the size of 2' -substituents increases. Thioketone analogues are about five times more potent than the corresponding carbonyl analogues [21].



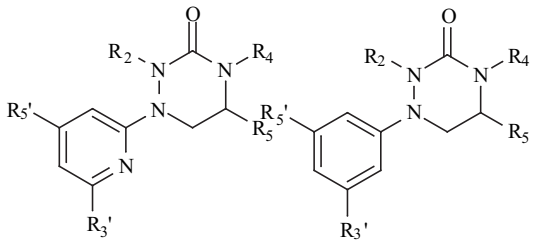
Phenyltetrahydropyridazin-3-[2H]-one analogues (structure 1)

$$\log 1/IC_{50} = -1.20\ (0.18)\ B2_{2'} + 0.42\ (0.11)\ \pi_{3'} - 0.67\ (0.32)\ R_{3'} + 0.51\ (0.09)\ \pi_{4'} - 4.76\ (0.59)\ B2_{4'} + 1.06\ (0.14)\ B2_{4'}^2 + 0.74\ (0.35)\ F_{4'} + 0.63\ (0.17)\ MR_4 - 0.09\ (0.05)\ MR_4^2 + 9.94\ (0.57) \quad \textbf{(1)}$$

n = 65, s = 0,313, r = 0.921, F = 33.94, p = 0.0001, $S_{(cv)}$ = 0.391, minimum $B2_{4'}$ = 2.25, optimum MR_4 = 3.47

ii) For *1-phenyl-[2H]-tetrahydrotriazine-3-one analogues* (structure 2) as inhibitors of 5-LOX, potency is increased by lipophililc substituents at the 3' -, 5' - and 5-positions and also with 3' - and 5' -

substituents that withdraw electrons and decreased with 3' -substituents on the pyridyl ring that donate electrons [22].

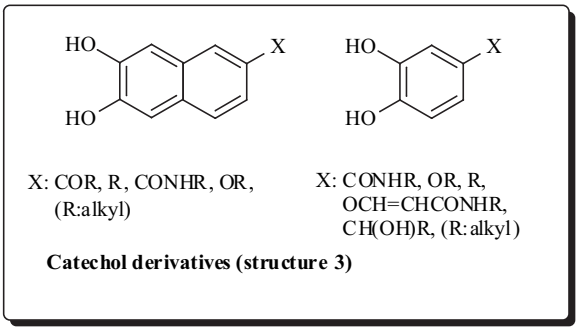


1-phenyl-[2H]-tetrahydrotriazine-3-one analogues (structure 2)

$\log 1/IC_{50} = 0.58\ (0.08)\ \pi_{5'} + 0.44\ (0.06)\ \pi_5 + 0.90\ (0.16)\ \pi_{3'} + 1.02\ (0.17)\ MR_4 + 0.24\ (0.06)\ MR_4{}^2 - 0.49\ (0.15)\ MR_2 - 0.33\ (0.11)\ I_{4OR} + 0.49\ (0.17)\ \Sigma\sigma_m - 0.89\ (0.46)\ \Sigma\sigma_p + 4.45\ (0.08)$ **(2)**

n = 56, RMSE = 0.247, r = 0.864, opt MR_4 = 2.13, $RMSE_{cv}$ = 0.291, F = 15.0, p = 0.0001

iii) For various *catechol derivatives* (structure 3), inhibitors of 5-LOX, variations in the inhibitory activity is explained in bilinear hydrophobic parameter (log P) terms and steric (molecular thickness) and electronic (proton nuclear magnetic resonance [^{1}H-NMR], chemical shift of the proton adjacent to the catechol group) parameter terms [23]. The hydrophobicity of the inhibitor molecule is important and the optimum value of log P is about 4.3-4.6 beyond which inhibition did not increase further.

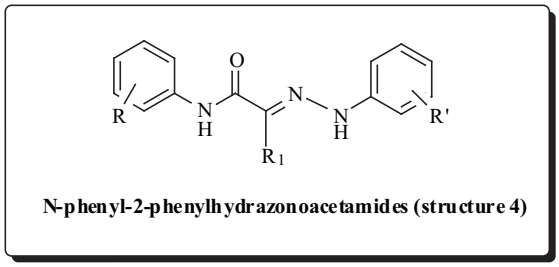


Catechol derivatives (structure 3)

$pI_{50} = 0.47 \log P - 0.68 \log (\beta 10^{\log P} + 1) - 0.82\ D_H - 0.29\ \Delta H_{C5} + 5.91$ **(3)**

n = 51, R= 0.86, S = 0.31, F = 24.92, log β = - 4.30, log P_o = 4.65

iv) Finally for *N-phenyl-2-phenylhydrazonoacetamides* (structure 4) as inhibitors of 15-LOX, a QSAR evaluation was carried out with different statistical methods: a) Free-Wilson, multiple linear regression (MLR) and partial least squares (PLS) b) a combined Free Wilson-Hansch approach. The resulting equations indicate that besides an electron donating group at the central amidrazone moiety, electronic effects at the arylhydrazone substituent play an important role for the biological activity [24].



N-phenyl-2-phenylhydrazonoacetamides (structure 4)

$\log 1/IC_{50} = 0.44\ (0.15)\ m\text{-}CF_3 - 0.60\ (0.08)\ NH_2 - 2.94\ (0.14)\ S\text{-}cHex - 1.41\ (0.14)\ S\text{-}Benz - 0.18\ (0.10)\ o\text{-}F' - 0.49\ (0.12)\ o\text{-}Cl' - 0.30\ (0.12)\ m\text{-}Cl' - 0.29\ (0.10)\ p\text{-}Cl' + 7.88\ (0.05)$ **(4)**

n = 27, r = 0.987, r^2 = 0.973, s = 0.149, q^2 = 0.955, F = 69.1

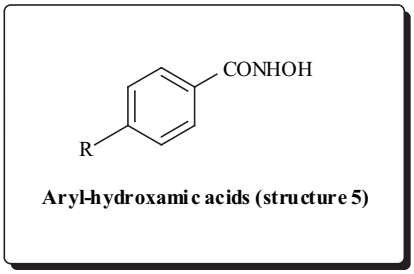
The best model generated, in a combined Free-Wilson-Hansch analysis with PLS and with the highest predictive power, is eq. 5

$\log 1/IC_{50} = 0.12\ \sigma - 0.05\ F + 2.12\ R + 0.07\ \pi - 0.01\ o\text{-}MR - 0.02\ p\text{-}MR - 0.77\ NH_2 - 3.15\ S\text{-}cHex - 1.60\ S\text{-}Benz + 0.49\ \sigma' - 0.89\ F' + 0.29\ R' - 0.29\ \pi'$ **(5)**

n = 26, r = 0.990, r^2 = 0.980, s = 0.138, q^2 = 0.932, F = 72.8, onc = 10

b) Iron Chelators

i) Evaluation of the quantitative structure activity relationships for *hydroxamic acids* (structure 5) revealed that the primary physicochemical feature influencing the *in vitro* 5-LOX inhibitory potencies of these compounds, is the hydrophobicity π while the σ constant of Hammet seems also to be important [25].

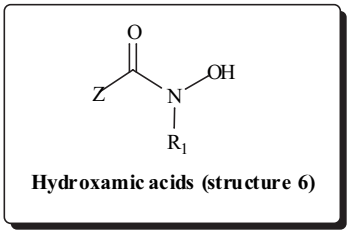


Aryl-hydroxamic acids (structure 5)

$\log 1/IC_{50} = 0.43\ (\pm 0.16)\ \sigma + 0.49\ (\pm 0.07)\ \pi - 1.80\ (\pm 0.07)$ **(6)**

n = 10, s = 0.49, r = 0.95

For hydroxamic acids with (structure 6) the primary physicochemical feature influencing the 5-lipoxygenase inhibitory potencies is also the hydrophobicity of the molecule as π' values and some indicator variables [26]:



Hydroxamic acids (structure 6)

$\log 1/IC_{50} = 0.55\ (0.04)\ \pi' - 1.05\ (0.08)\ I_{NH} - 0.64\ (0.08)\ I_1 + 4.25\ (0.18)$ **(7)**

n = 111, r = 0.916, s = 0.379, $F_{3,107} = 185.9$, $p<0.001$

The IC_{50} is a measure of the *in vitro* 5-LOX inhibitory potency in adherent rat basophilic leukemia cells. Parameter I_{NH} is an indicator variable having the value of 1 when the substitutent on the hydroxamate nitrogen R_1 is hydrogen, from the negative sign of I_{NH}, it is assumed that the presence of an alkyl group on the hydroxamate nitrogen is favorable for the inhibitory activity. I_1 is included to indicate whether the hydroxamic acid functionality is electronically insulated from the aromatic ring system. I_1 is assigned a value of 0 when the hydroxamate is directly attached to the aryl ring or attached through an unsaturated spacer unit and indicates that the presence of a saturated spacer units between the hydroxamate and the aryl ring system results in reduction in 5-LOX inhibitory activity.

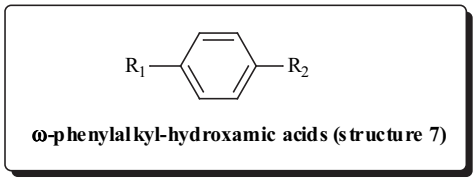
ii) From another study [27] concerning simple phenols the importance of hydrophobicity is apparent. The ki values of soybean LOX are used:

$\log 1/ki = 0.83 \log P + 0.39$ **(8)**

n = 12, $r^2 = 0.990$, s = 0.087

iii) A QSAR study has been made by Gupta [28] on some 5-LOX inhibitors belonging to

a) ω-phenylalkyl-hydroxamic acids (structure 7) [29]



ω-phenylalkyl-hydroxamic acids (structure 7)

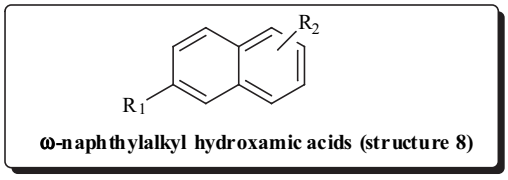
$pIC_{50} = 2.351\ (\pm 0.295)\ V_W(R_1) + 2.033\ (\pm 0.930)\ V_W(R_2) + 2.779$ **(9)**

n = 21, r = 0.970, s = 0.305, $F_{2,18} = 141.35$

$pIC_{50} = 1.005\ (\pm 0.182) \log P + 0.214\ (\pm 0.110)\ (\log P)^2 + 5.350$ **(9a)**

n = 21, r = 0.951, s = 0.387, $F_{2,18} = 84.14$

b) ω-naphthylalkyl hydroxamic acids (structure 8) [30]

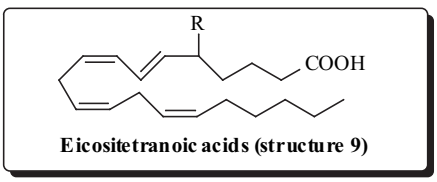


ω-naphthylalkyl hydroxamic acids (structure 8)

$pIC_{50} = 1.738\ (\pm 0.864)\ V_W(R_2) + 0.921\ (\pm 0.976)\ D + 0.079\ (\pm 0.679)\ V_W(R_1) + 3.564$ **(10)**

n = 19, r = 0.844, s = 0.557, $F_{3,15} = 12.40$

c) *eicositetranoic acids* (structure 9) [31]

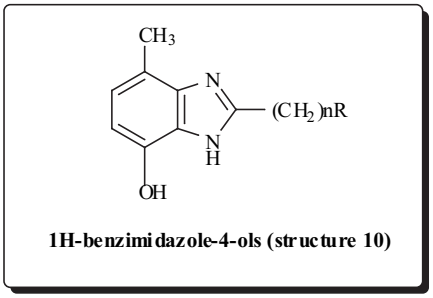


Eicositetranoic acids (structure 9)

$pIC_{50} = 1.215\ (\pm 0.429)\ D_1 + 0.621\ (\pm 0.423)\ D_2 - 0.148\ (\pm 0.079) \log P + 5.326$ **(11)**

n = 22, r = 0.896, s = 0.306, $F_{3,18} = 24.45$

and d) *1H-benzimidazole-4-ols* (structure 10) [32]



1H-benzimidazole-4-ols (structure 10)

$pIC_{50} = 1.260\ (\pm 0.896) \log P - 0.154\ (\pm 0.142)\ (\log P)^2 - 0.638\ (\pm 0.330)\ V_W + 3.952$ **(12)**

n = 20, r = 0.838, s = 0.162, $F_{3,16} = 11.22$

From the above equations (9, 9a, 10, 11, 12) it is shown that the hydrophobic character of the molecules and the size of their substitutents selectively govern their inhibitory activity. Since the enzyme active site possesses a non-heme ferric ion, a hydrophobic domain and a carboxylic acid binding site, the functional group of inhibitors must interact with the ferric ion, the substitutent on one side of it would be involved in hydrophobic interaction and that on the other side in van de Waals interaction with the enzyme, leading to an enhancement in the inhibitory activity of the inhibitors.

3. QSAR METHODOLOGY-RESULTS AND DISCUSSION

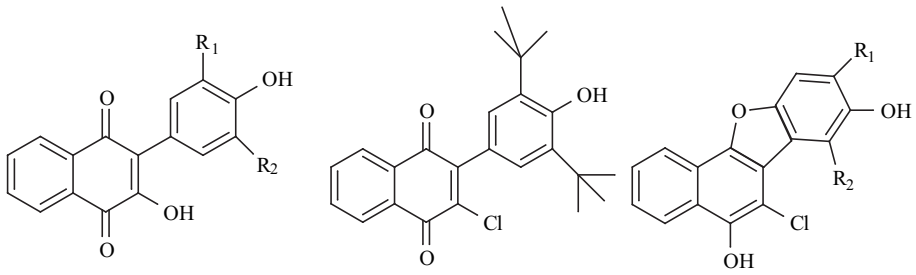
3.1. QSAR Methodology

The analysis was done by using the C-QSAR suite program (Biobyte) [33] through Internet. Clog *P* is the calculated octanol / water partition coefficient of the molecule and π is that of the substitutent. Clog *P* applies to the neutral form of partially ionized compounds B_1, B_5, and L are Verloop's sterimol parameters for the substitutents and σ is the Hammet electronic effect. CMR is the calculated molar refractivity for the whole molecule and MR is that for the substitutent. These values have been scaled by 0.1.

In all equations, n represents the number of data points, r the correlation coefficient, s is the standard deviation of the regression equation, q^2 defines the cross-validated r^2 [34] while log *Po* represents the optimum hydrophobicity and *F* the *F*-statistics-significance level. The bilinear model of Kubinyi [35] was used in eqs. 26, 28, β is the ratio of the volumes of the organic phase and the aqueous phase.

3.2. Results and Discussion

Many thousands of compounds have been screened as LOX inhibitors in industrial laboratories and a large number of active compounds with novel structures are undergoing clinical trials. This evaluation provides data sets suitable for Quantitative Structure-Activity Analysis. The laboratory tests utilized in identifying lipoxygenase inhibitors are:



2-(3-Halogen-4-hydroxyphenyl)-1,4-Naphthoquinone derivatives (structure 11)

human granulocytes, rat basophilic leukemia cells (RBL-1) and human whole blood assay (HWBL). All the activity data has been collected from the literature [37-48]. The activity is expressed in molar concentration. IC_{50} is the 50% inhibitory concentration.

For drugs acting as LOX inhibitors [21-26] hydrophobicity is an important property, it is also a significant factor in the susceptibility of drugs to attack by the P-450 enzymes [36].

A QSAR study was made for the following list of compounds:

I. 2-(3-Halogen-4-hydroxyphenyl)-1,4-naphthoquinone derivatives [37-39]

II. 10-Aminomethylene-1,8-dihydroxy-9(10)H-anthraquinones [38, 40]

III. (3-Pyridylmethyl)benzoquinone derivatives [38, 41, 42]

IV. Symmetrical bis(heteroarylmethoxyphenyl)alkylcarboxylic acids [43, 44]

V. (E)-3-(1,4 - benzoquinolyl)-2-[(3-pyridyl) – alkyl]-2-propenoic acid derivatives [42, 45]

VI. 2,3-dihydro-5-benzofuranols [42, 46]

VII. N-hydroxyurea derivatives [42, 47, 48]

VIII. Nonsteroidal Anti-Inflammatory Drugs as Scaffolds for the Design 5-Lipoxygenase Inhibitors [49]

IX. (6,7-Diaryldihydropyrrolizin-5-yl) acetic acids [39, 50]

X. (Methoxyalkyl)thiazoles [51]

XI. 2 – substituted - 1 – naphthols [52]

XII. 9, 10-Dihydro-1,8-dihydroxy-9-oxo-2-anthracenecarboxylic and hydroxamic acids [53]

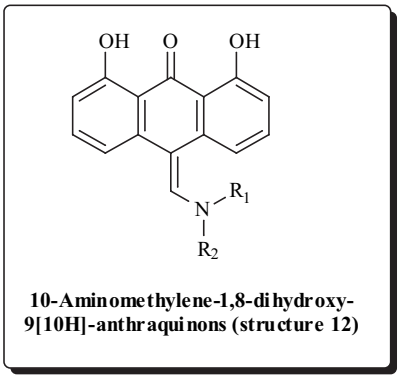
I. The biochemical basis for the mode of action of *2-(3-Halogen-4-hydroxyphenyl)-1,4-Naphthoquinone Derivatives* [37-39] (structure 11) is uncertain, but there is growing evidence that it is related to their redox activity leading to the production of free radicals and active oxygen species. Naphthoquinone derivatives posses redox properties and can be expected to inhibit the LOX reaction by inducing free radical species. For this group of naphthoquinone derivatives equation (13) was obtained:

$$\log 1/IC_{50} = 0.058\ (\pm 0.033)\ \text{clog}\ P + 5.023\ (\pm 0.162) \quad \textbf{(13)}$$

$n = 5$, $r = 0.956$, $r^2 = 0.913$, $q^2 = 0.825$, $s = 0.036$, $F_{1,3} = 30.75$, $\alpha = 0.5$

Equation 13 gave a good correlation between observed and calculated IC_{50} values. IC_{50} is the biological activity of the compounds for the inhibition of the production of LTB_4 in human granulocytes. The parameter clog *P* represents the theoretically overall calculated lipophilicity of the molecule and governs the variations in activity, so an increase of the lipophilicity, increases the inhibitory activity of the compounds.

II. The equation derived for *10-Aminomethylene-1,8-dihydroxy-9[10H]-anthraquinones* [38, 40] (structure 12) shows that the inhibitory activity of the compounds depends on their lipophilicity (experimental measured lipophilicity) [38]. The negative sign indicates that the inhibitory activity increases as the lipophilicity decreases.



10-Aminomethylene-1,8-dihydroxy-9[10H]-anthraquinons (structure 12)

$$\log 1/IC_{50} = -0.995\ (0.924)\ \log P_E + 9.717\ (4.231) \quad \textbf{(14)}$$

$n = 7$, $r = 0.778$, $r^2 = 0.605$, $q^2 = -0.307$, $s = 0.493$, $F_{1,5} = 7.66$, $\alpha = 0.05$

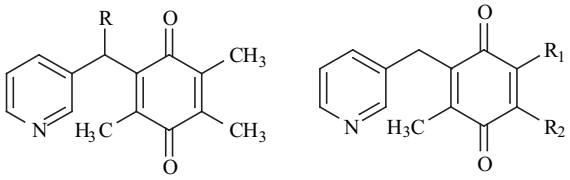
Although no parameterization for substituents R_1, R_2 has been done, all derivatives are well predicted. The phenyl ring with its substituents does not appear to reach an hydrophobic surface. No role for an electronic effect was found. This indicates the equation is giving a skewed picture. Attempts to derive a better equation using steric parameters did not succeed. The number of data points is small.

The experimental values of lipophilicity were correlated to log P_E the theoretically calculated clog P values. Equation 15 is derived:

clog P = 1.043 (0.614) log P_E + 0.167 (2.075) **(15)**

n = 9, r = 0.835, r^2 = 0.698, q^2 = 0.441, s = 0.489, $F_{1,7}$ = 1.796

III. *(3-Pyridylmethyl)benzoquinone derivatives* [38, 41, 42] (structures 13, 14) inhibit Thromboxane A_2 synthase and leukotriene biosynthesis enzymes. For the inhibition of 5-LOX the experiments were made in human whole blood assay. In the following relationship (structure 13):



(structure 13) **(structure 14)**

(3-Pyridylmethyl)benzoquinone derivatives

log $1/IC_{50}$ = 0.490 (0.234) CMR - 1.914 (0.421) I_{COOH} -1.641 (0.535) I_{Ph} + 2.174 (2.077) **(16)**

n = 19, r = 0.957, r^2 = 0.916, q^2 = 0.858, s = 0.359, $F_{3,15}$ = 6.949, α = 0.01

CMR is the overall calculated molar refractivity. Since MR is primarily a measure of the bulk of the substituent, the positive coefficient with this term indicates that molecules are contacting polar space in the enzyme, not hydrophobic space. A positive coefficient might suggest an interaction depending on the polarizability of the substituents although there is a little evidence for the importance of such an effect. On the other hand I_{COOH} and I_{Ph} (indicator variables having a value of 1 when R has a carboxylic or phenyl group) have negative signs, which means that the presence of these groups decreases the inhibition of LOX.

However, if the experimental measured lipophilicity log P_E is used in place of CMR equation 17 is obtained:

log $1/IC_{50}$ = 0.498 (0.149) log P_E - 1.439 (0.562) I_{Ph} + 4.461 (0.533) **(17)**

n = 19, r = 0.921, r^2 = 0.849, q^2 = 0.810, s = 0.467, $F_{2,16}$ = 33.118, α = 0.01

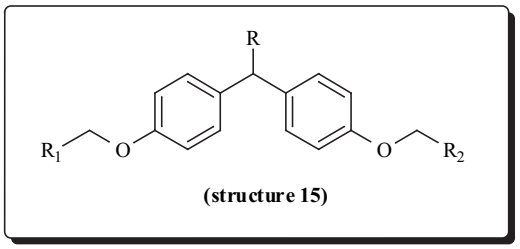
For compounds (structure 14) equation 18 is derived:

log $1/IC_{50}$ = 0.586 (0.240) log P_E + 5.473 (0.396) **(18)**

n = 6, r = 0.959, r^2 = 0.920, q^2 = 0.859, s = 0.324, $F_{1,4}$ = 45.843, α = 0.01

Equation (18) shows that there is a linear relationship between the biological activity log$1/IC_{50}$ and log P_E (experimental lipophilicity). Equation 18 is very simple. Since log P is by far the most important parameter, it would seem that the simple linear relationship is associated with the positive diffusion of the compounds to the active site.

IV. A parabolic equation is obtained for *symmetrical bis (heteroarylmethoxyphenyl) alkylcarboxylic acids* [43, 44] (structure 15). The laboratory test used for the observed IC_{50} values, is the human neutrophil leukotriene biosynthesis assay.



(structure 15)

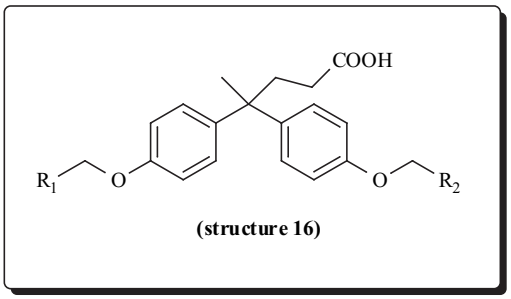
log $1/IC_{50}$ = 1.445 clog P – 0.112 clog P^2 + 0.136 MR + 2.408 **(19)**

n = 33, r = 0.787, r^2 = 0.620, q^2 = 0.502, s = 0.211, $F_{3,29}$ = 23.598, α = 0.01

optimum clog P : 6.462, from 4.557 to 7.033

Log P is the most important parameter followed by MR. The fact that clog P has been used to model hydrophobicity implies that all the parts where substitutents have been entered, hydrophobic contact have been made. Parabolic dependence on clog P provides an optimum hydrophobicity 6.462 which is high. The calculated log P values for this set are high enough (from 5.853 to 9.841). It is likely that R_1-R_2 substitutents do contact a hydrophobic space. Unfortunately the compounds included in this set contain rather little variation in R_1 and R_2.

For Bis(2-quinolylmethoxyphenyl)alkylcarboxylic acids derivatives (structure 16), equation 20, is obtained:



(structure 16)

log $1/IC_{50}$ = 0.217 clog P + 5.890 **(20)**

n = 4, r = 0.953, r^2 = 0.908, q^2 = 0.466, s = 0.063, $F_{1,2}$ = 19.75, α = 0.05

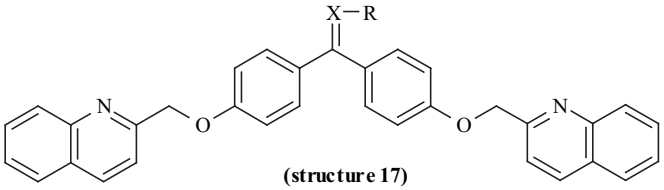
The lipophilicity describes better the inhibitory activity.

For the compounds (structure 17) with an heterocyclic substitution on the diphenolic acid template, the molar refractivity of the substitutent R_2 (MR-R_2) is the most significant.

log $1/IC_{50}$ = -5.777 MR-R_2 + 31.91 **(21)**

n = 5, r = 0.959, r^2 = 0.920, q^2 = - 6.987, s = 0.179, $F_{1,3}$ = 34.468, α = 0.01

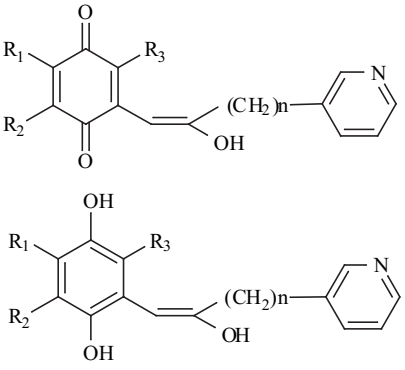
The negative MR-R_2 brings out a small steric effect of substitutents in the R_2 position. The negative steric term implies that the critical effects are on (in) an active site on an macromolecule which is not hydrophobic.



(structure 17)

Symmetrical bis(heteroarylmethoxyphenyl)alkylcarboxylic acids

V. For a group of compounds *(E)-3-(1,4 - benzoquinolyl)-2-[(3-pyridyl) – alkyl]-2- propenoic acid derivatives* [43, 45] (structure 18) that inhibit both 5-Lipoxygenase and Thromboxane A_2 Synthase, the following equation is derived:



(E)-3-(1,4 - benzoquinolyl)-2-[(3-pyridyl) - alkyl]-2- propenoic acid derivatives (structure 18)

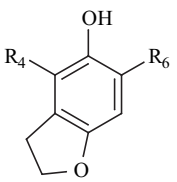
$\log 1/IC_{50} = 0.640\ (0.257)\ \text{clog}\ P - 1.174\ (0.510)\ I_{OH} - 0.402\ (0.137)\ L_{-R1} - 0.602$

$(0.305)\ MR\text{-}R_2 - 2.840\ (0.504)$ **(22)**

$n = 18,\ r = 0.895,\ r^2 = 0.801,\ q^2 = 0.648,\ s = 0.272,\ F_{4,\ 13} = 13.080,\ \alpha = 0.01$

There is a small difference between r^2 and q^2 while s is good. I_{OH} (indicator variable having a value of 1 when the OH group is present) has negative sign, which means that the presence of these groups decreases the inhibitory activity. L_{-R1} refers on the length of the substitutent R_1, an increase of that decreases the inhibition of the enzyme. $MR\text{-}R_2$ (molecular refractivity of the substitutent R_2) implies steric effects (negative sign), while an increase of the lipophilicity as clog *P* increases the inhibitory activity.

VI. For *2,3-dihydro-5-benzofuranols* [42, 46] (structure 19) the IC_{50} values refers to PMN' s cells. The 6-isomers are more potent as LOX inhibitors than the 4-isomers.



(structure 19)

2,3-dihydro-5-benzofuranols

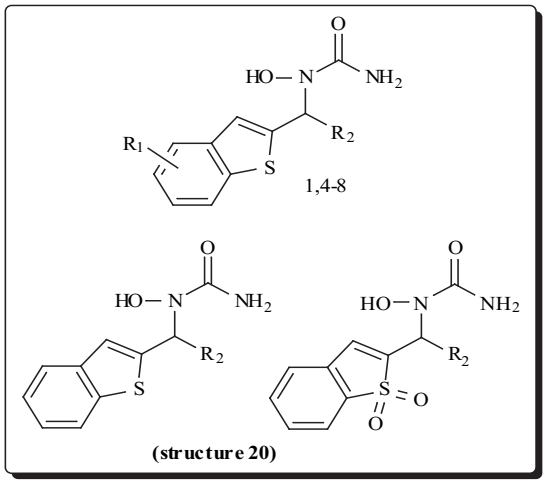
$\log 1/IC_{50} = 0.658\ (0.093)\ \text{clog}\ P + 0.779\ (0.245)\ I_{\text{-O-R6}} + 0.382\ (0.204)\ I_{\text{- C-R6}} + 4.038\ (0.334)$ **(23)**

$n = 47,\ r = 0.924,\ r^2 = 0.853,\ q^2 = 0.825,\ s = 0.302,\ F_{4,\ 43} = 83.274,\ \alpha = 0.01$

The lipophilicity as clog *P* plays important role while indicator variables for the presence of an oxygen atom or a carbon at the R_6 substitutent, instead of a sulfur expressed by $I_{\text{-O-R6}}$ and $I_{\text{-C-R6}}$ increases the inhibitory potency. Perhaps the dimensions of the substitutents, as Verloop parameters could possibly better describe the inhibition but because of the absence of values for the majority of the substitutents we couldn't use them for the derivation of the equation.

VII. N-hydroxyurea analogues [42, 47, 48] have been found to act by chelation with the iron of the active site of the enzyme. These derivatives have been studied as subgroups.

For the first subgroup (structure 20):



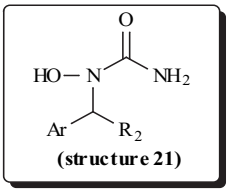
(structure 20)

$\log 1/IC_{50} = 0.647\ (0.169)\ \text{clog}\ P + 4.419\ (0.463)$ **(24)**

$n = 7,\ r = 0.975,\ r^2 = 0.951,\ q^2 = 0.930,\ s = 0.148,\ F_{1,\ 5} = 96.972,\ \alpha = 0.01$

The most important parameter is clog *P*.

For the following analogues (structure 21) a bilinear equation has been derived. Two compounds have been omitted.



(structure 21)

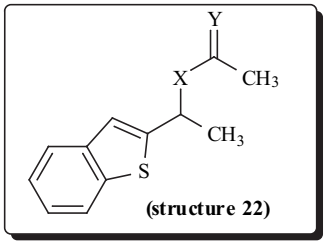
$\log 1/IC_{50}$ = 0.935 (0.333) clog *P* - 1.015 (0.612) log (β.10clog *P* + 1) + 3.993 (0.592) **(25)**

n = 18, r = 0.899, r^2 = 0.808, q^2 = 0.638, s = 0.301, $F_{3, 14}$ = 19.665, α = 0.01

optimum clog *P* : 3.689, log β: -2.620

The inhibitory activity first linearly increases with increase in hydrophobicity up to a clog *P* 3.689. Also the slope of the right hand portion of the linear model (-0.020) is different from zero.

For the analogues with structure 22 clog *P* gives a good correlation.

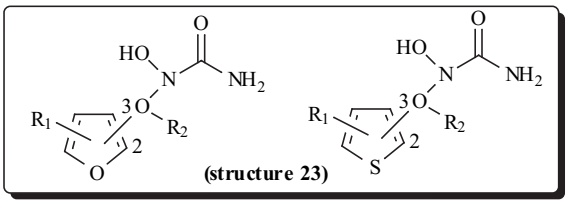


(structure 22)

$\log 1/IC_{50}$ = 0.403 (0.130) clog *P* + 5.185 (0.345) **(26)**

n =19, r = 0.846, r^2 = 0.715, q^2 = 0.668, s = 0.257, $F_{1, 17}$ = 42.643, α = 0.01

For the furyl and thiazolyl-hydroxamic acids (structure 23) hydrophobicity clog *P* in a bilinear model describes well the inhibitory activity.



(structure 23)

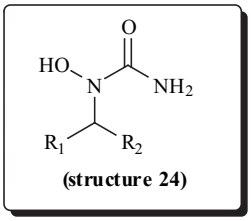
$\log 1/IC_{50}$ = 0.820 (0.166) clog *P* – 1.002 (0.713) log (β.10clog *P* +1) + 4.129 (0.272) **(27)**

n= 31, r = 0.937, r^2 = 0.878, q^2 = 0.857, s = 0.268, $F_{1, 27}$ = 64.694, α = 0.01

optimum clog *P* : 3.763 (0.776), log β: - 2.691 (1.749)

There is a resemblance between equations 25 and 27. The fact that clog *P* has been used to model lipophilicity implies that all the parts where substituents have been entered, hydrophobic contacts have been made.

For hydroxamic acids with structure 24 the molar refractivity of the R_2 substitutent plays the most important role

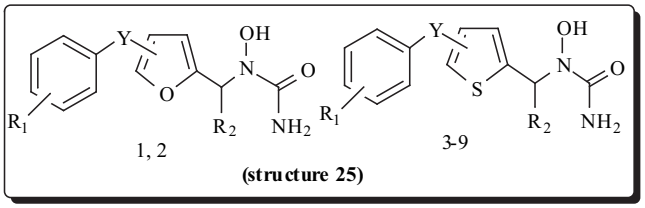


(structure 24)

$\log 1/IC_{50}$ = 0.372 (0.375) MR-R_2 – 4.282 (0.763) **(28)**

n = 5, r = 0.876, r^2 = 0.768, q^2 = 0.233, s = 0.138, $F_{1, 3}$ = 10.052, α = 0.1

Equation 29 describes the inhibitory activity in HWBL (human whole blood assay). I_5 is an indicator variable which takes the value of 1 for the presence of a substitutent is present at the 5 position of the furyl or the thienyl ring (structure 25).

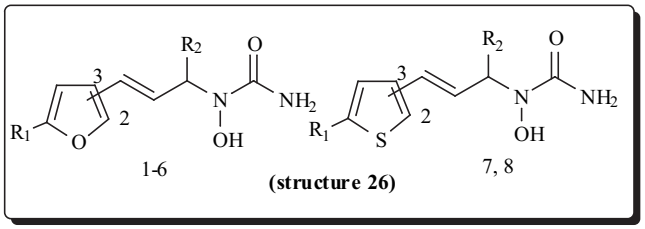


(structure 25)

$\log 1/IC_{50}$ = 0.248 (0.283) clog *P* + 0.489 (0.388) I_5 + 6.175 (0.522) **(29)**

n = 8, r = 0.905, r^2 = 0.819, q^2 = 0.646, s = 0.183, $F_{1, 5}$ = 11.332, α = 0.01

Equation 30 is developed for the inhibitory activities in RBL-1 cells. It seems that a C-group in the 5-position is the best inrelation to the *in vitro* inhibitory activity compared to the other substituents (structure 26).



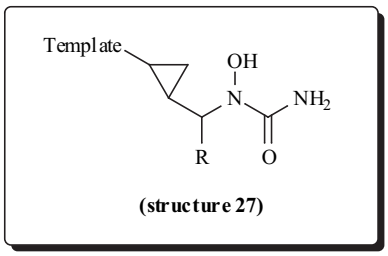
(structure 26)

$\log 1/IC_{50}$ = 0.738 (0.249) clog *P* + 4.587 (0.394) **(30)**

n = 9, r = 0.936, r^2 = 0.876, q^2 = 0.749, s = 0.189, $F_{1, 7}$ = 49.223, α = 0.01

In the following equations the lipophilicity seems to play the most important role.

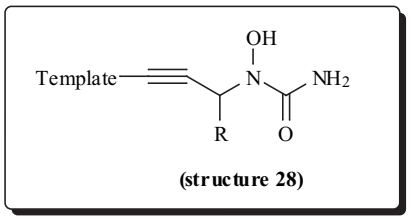
$\log 1/IC_{50}$ = 0.593 (0.130) clog *P* + 4.607 (0.275) **(31)**



(structure 27)

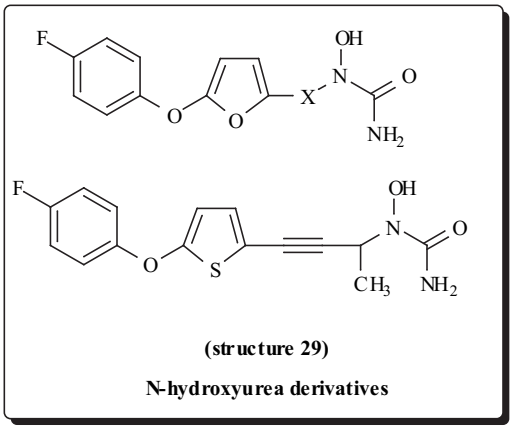
n = 5, r = 0.993, r^2 = 0.986, q^2 = 0.342, s = 0.102, $F_{1, 3}$ = 211.451, α = 0.01

$\log 1/IC_{50}$ = 0.475 (0.399) clog *P* + 4.980 (1.127) **(32)**



(structure 28)

n = 6, r = 0.856, r^2 = 0.733, q^2 = 0.542, s = 0.464, $F_{1, 4}$ = 10.966, α = 0.5



(structure 29)

N-hydroxyurea derivatives

For the fluoro-derivatives of N-hydroxyurea (structure 29) the parabolic depedence on clog *P* provides an optimum hydrophobicity 2.744.

$\log 1/IC_{50}$ = - 7.055 (3.789) clog *P* + 1.272 (0.717) clog P^2 + 16.607 (4.942) **(33)**

n = 9, r = 0.922, r^2 = 0.849, q^2 = 0.505, s = 0.085, $F_{2,6}$ = 17.023, α = 0.01

optimum clog *P* : 2.744, from 2.685 to 2.988

VIII. *Nonsteroidal Anti-Inflammatory Drugs as Scaffolds for the Design 5-Lipoxygenase Inhibitors* [49] (structure 30) are inhibitors of COX and they have been modified in order to inhibit LOX. Their IC_{50} values for the inhibitory activity are measured *in vitro* by the human whole blood assay. The following equation is derived:

$\log 1/IC_{50}$ (HWBL) = -0.533 (0.129) clog *P* + 1.342 (0.204) $I_{1\text{-}OH}$ + 6.135 (0.328) **(34)**

n = 6, r = 0.997, r^2 = 0.993, q^2 = 0.977, s = 0.059, $F_{2,3}$ = 228.3, α = 0.01

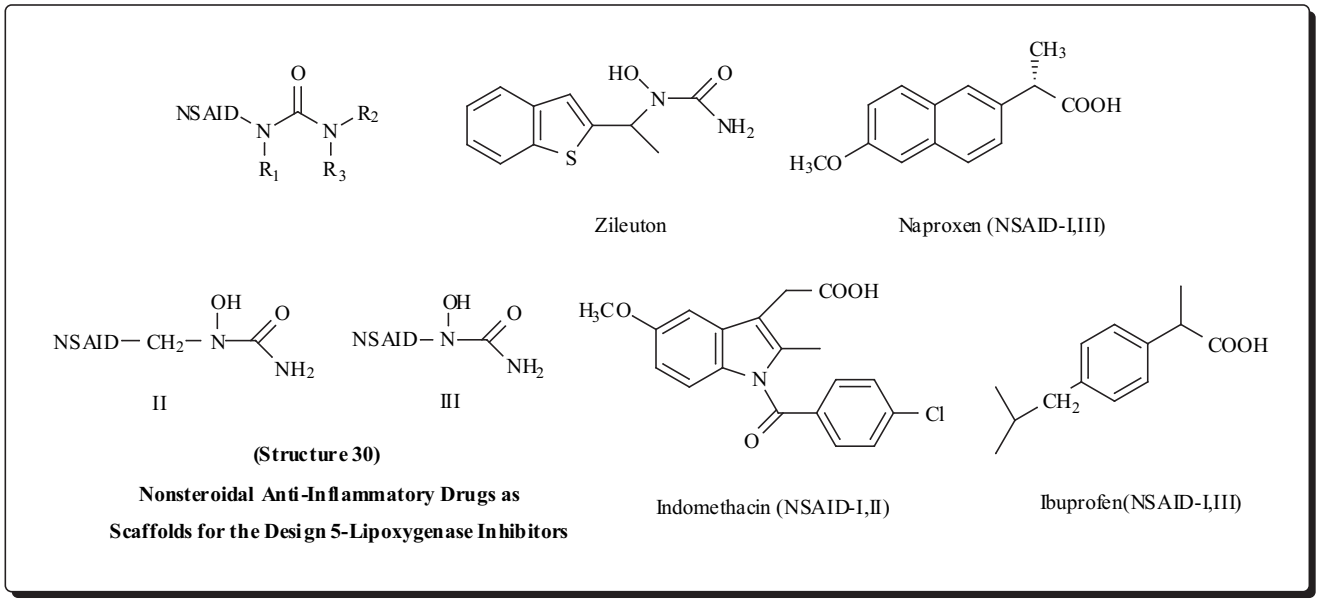
No collinearity problems were found among the parameters used in equation. The correlation is not exceedingly sharp but it is significant in statistics terms. Hydrophilicity (negative sign at clog *P*) seems to be important. $I_{1\text{-}OH}$ indicator for the examples where an OH is present seems to be important. The presence of the OH-group at the at the R_1 substitutent increases the biological activity.

IX. For *(6,7-Diaryldihydropyrrolizin-5-yl) acetic acids* [39, 50] (structure 31) equation 35 is derived:

$\log 1/IC_{50}$ = 1.268 (0.272) $B_{1\text{-}4}$ + 0.126 (0.080) $B_{5\text{-}4}$ – 0.782(0.299) I_4 + 3.958 (0.335) **(35)**

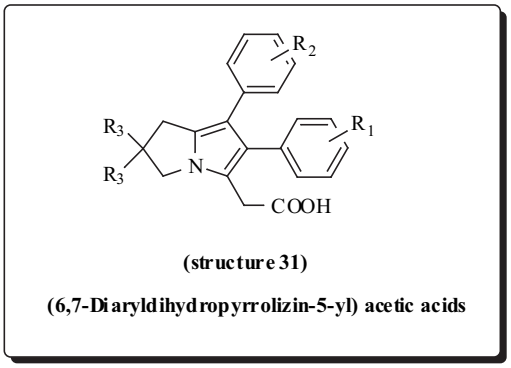
n = 15, r = 0.957, r^2 = 0.915, q^2 = 0.582, s = 0.147, $F_{3,11}$ = 10.733, α = 0.01

B_1 and B_5 are Verloop's parameters for the substitutents. B_1 is the measure of the minimum width, B_5 is an attempt to define overall volume. Both terms have a positive effect on the inhibition. I_4 is an indicator variable indicating the presence of p-substitutent at the 4-position of the phenyl ring.



(Structure 30)

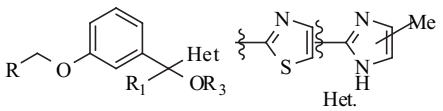
Nonsteroidal Anti-Inflammatory Drugs as Scaffolds for the Design 5-Lipoxygenase Inhibitors



(structure 31)

(6,7-Diaryldihydropyrrolizin-5-yl) acetic acids

X. *(Methoxyalkyl)thiazoles* [51] (structure 32) are LOX inhibitors. Their mechanism of inhibition is not well defined. They don't possess redox properties and they don't act as iron chelators for the iron of the active site of the enzyme. For this group of derivatives eq. 36 is derived:



(structure 32)

(Methoxyalkyl)thiazoles

$$\log 1/IC_{50} = 0.551\ (0.333)\ \text{clog}\ P + 2.998\ (1.648) \quad \textbf{(36)}$$

n = 6, r = 0.917, $r^2 = 0.840$, $q^2 = 0.634$, s = 0.370, $F_{1,4} = 21.051$, $\alpha = 0.05$

XI. *2 – substituted - 1 – naphthols* [52] (structure 33) are inhibitors of leukotrienes biosynthesis. Parabolic depedence on CMR, theoretically calculated molar refractivity provides an optimum CMR value 6.672:

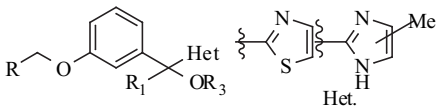
$$\log 1/IC_{50} = 5.296\ (2.739)\ \text{CMR} - 0.397\ (0.208)\ \text{CMR}^2 - 10.181\ (8.716) \quad \textbf{(37)}$$

n = 11, r = 0.847, $r^2 = 0.717$, $q^2 = 0.340$, s = 0.422, $F_{2,8} = 10.11$, $\alpha = 0.01$

optimum CMR: 6.672, from 6.366 to 7.074

The molar refractivity is primarily a measure of the bulk. Its positive sign indicates that the larger molar refractivity leads to, the better inhibitory activity. The main difficulty is the uncertainly as to whether interaction is occurring with polar and hydrophobic space.

For the compounds with (structure 34) equation 38 is developed:



(structure 32)

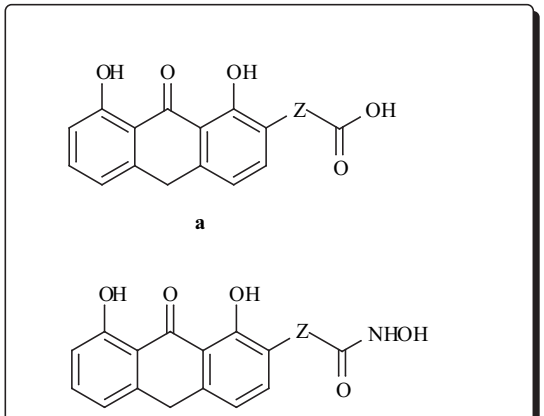
(Methoxyalkyl)thiazoles

$$\log 1/IC_{50} = 0.913\ (0.202)\ \text{clog}\ P + 1.521\ (0.283)\ I_H - 0.449\ (0.414)\ \Sigma\sigma - 1.066\ (0.288)\ \Sigma\pi + 1.566\ (1.043) \quad \textbf{(38)}$$

n = 33, r = 0.923, $r^2 = 0.852$, $q^2 = 0.787$, s = 0.336, $F_{4,28} = 40.252$, $\alpha = 0.01$

An increase of the lipophilicity clog *P,* increases the inhibitory potency. I_H is an indicator variable indicating the presence of an H at X ($I_H = 1$, when X = H). From the positive sign, it is assumed that the absence of a substitutent at X is favorable for the activity. The parameter $\Sigma\sigma$ refers to the sum of σ–Hammet electronic factor, and would seem to imply a negative role for electron attracting groups in all positions. Again we find a negative hydrophobic effect ($\Sigma\pi$).

XII. *9, 10-Dihydro-1,8-dihydroxy-9-oxo-2-anthraquinocarboxylic and hydroxamic acids* [53] (structures 35). Anthraquinones possess redox properties and are among the major topical remedies for the treatment of psoriasis. The most notable and prevalent are the staining and irritation of the non-affected skin. In these compounds the active methylene protons at C-10 were replaced by suitable substitutents which permit control over the release of active oxygen species. log P_E is the experimental measured lipophilicity.



a

b

(structures 35)

9,10-Dihydro-1,8-dihydroxy-9-oxo-2-anthracenecarboxylic and hydroxamic acids

$$\log 1/IC_{50} = -0.490\ (0.223)\ \log P_E + 0.643\ (0.117)\ \text{CMR} + 1.132\ (0.663) \quad \textbf{(39)}$$

n = 8, r = 0.991, $r^2 = 0.981$, $q^2 = 0.952$, s = 0.082, $F_{2,5} = 132.803$, $\alpha = 0.01$

CMR is the most important parameter, a measure of the bulk and the polarizability. The net take home message is that hydrophobicity is relatively unimportant (and negative) and there is an interrelationship (CMR vs log *P* 0.590). The number of compounds is low (n = 8), but the coefficient is high of the correlation r = 0.991.

More significant is eq. 40:

$$\log 1/IC_{50} = 0.396\ (0.143)\ \text{CMR} + 1.580\ (1.292) \quad \textbf{(40)}$$

n = 9, r = 0.927, $r^2 = 0.860$, $q^2 = 0.754$, s = 0.207, $F_{1,7} = 42.976$, $\alpha = 0.01$

4. CONCLUSIONS

From the reported QSAR studies for different series of LOX inhibitors, it is evident that there is a strong positive correlation between lipophilicity and activity. The biological results used in the study are not derived all from cell-free assays, so a lipophilic interaction at the binding position of the enzyme could be explained by the enrichment of the cell membrane.

Steric parameters B_1, B_5, L, MR, CMR seem to play important and significant part in the correlations. The existence of linear correlations between inhibition values and log *P* simply suggests that log *P* values were not great enough to establish the upper limit for the inhibition (values for optimum lipophilicity). The negative coefficient with clog *P* lacks hydrophobic terms. In the cases where the

relationship between log $1/IC_{50}$ and log *P* is well approximated by parabola or a bilinear model, then the role of the lipophilic character of LOX inhibitors can be at least roughly separated from their electronic and steric characteristics.

For some structural features, we had to use indicator variables as a device to account for the effect of a specific feature that cannot be accounted for by a more specific parameter.

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6. REFERENCES

[1] Goodman-Gilman, Pharmacological Basics of Theurapeutics, William B. Campbell, Chapter *24*, p.600.
[2] Samuelsson. *Science,* **1983**, *220*, 568.
[3] Borgeat, P.; Sammuelsson, B. *Proc. Natl. Acad. Sci. USA*, **1979**, *76*, 3213.
[4] Murphy, R. C.; Hammarstrom, S.; Sammuelsson, B. *Proc. Natl. Acad. Sci. USA*, **1979**, *76*, 4275.
[5] Piper, P. J. *Physiol. Rev.*, **1984**, *64*, 744.
[6] Ruzicka, T.; Vitto, A.; Printz, M.P. *Biochim. Biophys. Acta,* **1983**, *751*, 359.
[7] Bryant, R.W.; Simon, T.C. *J. Biol. Chem.,* **1982**, *257*, 14937.
[8] Nakadate, T.; Yamamoto, S.; Aizu, E.; Kato, R. *J. Pharm. Pharmacol.,* **1985**, *37*, 71.
[9] Opas, E.; Argenbright, L.W.; Pacholok, S.D.; Weinstein, M.J.; Humes, J.L. *Clin. Res.,* **1987**, *35*, 795.
[10] Belkner, J.; Wiesner, R.; Rathmann, J.; Barnett, J.; Sigal, A.; Kuhn, H. *Eur. J. Biochem.,* **1993,** *213*, 251.
[11] Kuhn, H.; Belkner, J.; Suzuki, H.; Yammamoto, S. *J. Lipid. Res.,* **1994,** *35*, 1749.
[12] Kuhn, H,; Belkner, J.; Wiesner, R.; Brash, .R. *J. Biol. Chem.,* **1990,** *265,* 18351.
[13] Yammamoto, S. *Biochem. Biophys. Acta,* **1992,** *1128*, 117.
[14] Strasser, M.; Cooper, P.; Dewald, B.; Payne, T. *Helv. Chim. Acta,* **1992,** *1128*, 117.
[15] Sigal, E.; Craik, Ch. S.; Highland, E.; Grumberger, D.; Costello, L.L.; Dixon, R.A.F.; Nadel, J.A. *Biochem. Biophys. Res. Commun.,* **1988,** *157,* 457.
[16] Musser, J.H.; Kreft, A.F. *J. Med. Chem.,* **1992,**. *35*, 2501.
[17] Berliner, J.A.; Heinecke, J.W. *Free Rad. Biol. Med*., **1996,** *20*, 707.
[18] Hilbers, P.M.; Rossi, A.; Finazzi-Agro, A.; Veldink, G.A.; Vliegenthart, F.G. *J. Biochim. Biophys. Acta,* **1994**, *1211*, 239.
[19] Stallings, C.W.; Kroa, B.A.; Carroll, R.T.; Mertzer, A.L.; Funk, M.O. *J. Mol. Biol.,* **1990**, *211*, 685.
[20] Boyington, J.C.; Amzel, L.M. *J. Biol. Chem.,* **1990**, *165*, 12771.
[21] Kim, K.H.; Martin, Y.C.; Brooks, D.W.; Dyer, D.; Carter, G.W. *J. Pharm. Sci.,* **1994**, *83*, 433.
[22] Kim, K.H.; Martin, Y.C.; Brooks, D.W. *Quant. Struct. Act. Relat.,* **1996**, *15*, 491.
[23] Naito, Y.; Sugiura, M.; Yamaura, Y.; Fukaya, C.; Yokoyama, K.; Nakagawa, Y.; Ikeda, T.; Senda, M.; Fujita, T. *Chem. Pharm. Bull.,* **1991**, *39*, 1736.
[24] Fleischer, R.; Frohberg, P.; Buge, A.; Nuhn, P.; Wiese, M. *Quant. Struct.- Act Relat.,* **2000,** *19*, 162.
[25] Summers, J.B.; Gunn, B.P.; Martin, J.G.; Martin, M.B.; Mazdiyasni, H.; Stewart, A.O.; Young, P.R.; Bouska, J.B.; Goetze, A.M.; Dyer, R.D.; Brooks, D.W.; Carter, G.W. *J. Med. Chem.,* **1988**, *31*, 1960.
[26] Summers, J.B.; Kim, K.H.; Mazdiyasni, H.; Holms, J.H.; Ratajczyk, J.D.; Stewart, A.O.; Dyer, R.D.; Carter, G. W. *J. Med. Chem.,* **1990**, *33*, 992.
[27] Mitsuda, H.; Yasumoto, K.; Yamamoto , A. *Archiv. Biochem. Biophys.,* **1967,** *118*, 664.
[28] Gupta, S.P.; Gupta, G.K. *J. Enzyme Inhib.,* **1990**, *3*, 179.
[29] Summers, J.B.; Mazdiyasni, H.; Holms, J.H.; Ratajczyk, J.D.; Dyer, R.D.; Carter, G.W. *J. Med. Chem.,* **1987**, *30*, 574.
[30] Kerdesky, F.A.J.; Schmidt, S.P.; Holms, J.H.; Dyer, R.D.; Carter, G.W.; Brooks, D.W. *J. Med. Chem.,* **1987**, *30*, 1177.
[31] Kerdesky, F. A. J. ; Holms, J. H. ; Schmidt, S. P. ; Dyer, R. D. ; Carter, G. W. *Tetrahedron Lett.,* **1985**, *26*, 2143.
[32] Buckle, D.R. ; Foster, K.A. ; Taylor, J.F. ; Tedder, J.M. ; Thody, V. E. ; Webster, R.A.B.; Bermudez, J.; Markewell, R.E.; Smith, S.A. *J. Med. Chem.,* **1987**, *30*, 2216.
[33] Biobyte Corp., 201 West 4th Street, Suite 204, Claremont, CA 91711, USA
[34] Cramer, R.D.; Bunce, J.D.; Patterson, D. E. *Quant. Struct.-Act Relat.,* **1988**, *7*, 18.
[35] Kubinyi, H. *Arzneim. Forsch.,* **1978**, *28*, 598.
[36] Hansch, C.; Zhang L. *Drug Metab. Rev.,* **1993**, *25*, 1.
[37] Wurm, G.; Schwandt, S. *Pharmazie,* **1999**, *54*, 487.
[38] Hadjipavlou-Litina D.; Pontiki E. *«Internet Electron. J. Mol. Design,* **2002***, I*, 134.
[39] 1ο International and 10ο Panhellenic Pharmaceutical Congress **2001** p. 68-69, No 25 and No 26, Abstracts «*Farmakeutiki*», *14* (II), p. 60, No 25 and No 26.
[40] Muller, K.; Sellmer, A.; Prinz, H. *J. Med. Chem.,* **1997**, *32*, 895.
[41] Ohkawa, S.; Terao, S.; Terashita, Z.; Shibouta, Y.; Nishikawa, K. *J. Med. Chem.,* **1991**, *34*, 267.
[42] 3rd European Graduate Student Meeting on Fankufurt-Main, **2001** (Abstracts. *«Archiv der Pharmazie. Med. Chem.» 2001/2*, p. 18, No 46)
[43] Kolasa, T. ; Gunn, D. E.; Bhatia, P. ; Basha, A. ; Craig, A. R. ; Stewart, O. A. ; Bouska, B. J. ; Harris, R. R. ; Hulkower, I. K. ; Malo, E. P. ; Randy, L. B. ; Carter, W. G. ; Brooks, D. W. C. *J. Med. Chem.,* **2000**, *43*, 3322.
[44] 4th European Graduate Student Meeting on Fankufurt-Main, **2002**, Abstract Book p.55
[45] Hibi, S.; Okamoto, Y.; Tagami, K.; Numata, H.; Kobayashi, N.; Shinoda, M.; Kawahara, T.; Harada, K.; Miyamoto, K.; Yamatsu, I. *J. Med. Chem.,* **1996***, 39*, 3148.
[46] Hammond, M. L.; Kopka, I. E.; Zambias, R. A.; Caldwell, C. G.; Boger, J.; Baker, F.; Bach, T.; Luell, S.; Mac Intyre, D. E. *J. Med. Chem.,* **1989** *32* (5): 1006.
[47] Stewart, A. O.; Bhatia, P. A.; Martin, J. G.; Summers, J. B.; Rodriques, K. E.; Martin, M. B.; Holms, J.H.; Moore, J. L.; Craig, R. A.; Kolasa, T.; Ratajczyk, J. D.; Mazdiyasni, H.; Kedersky, F. A. J.; DeNinno, S. L.; Maki, R. G.; Bouska, J. B.; Young, P. R.; Lanni, C.; Bell, R. L.; Carter, G. W.; Brooks, C. D. W. *J. Med. Chem.,* **1997,** *40*, 1955.
[48] 10th Panhellenic Symposium of Pharmaceutical Chemistry, **2002**, p.45.
[49] Kolasa, T.; Brooks, D. W. C. ; Rodriques, E. K. ; Summers, B. J. ; Dellaria, F. J. ; Hulkover, I. K. ; Bouska, J. ; Bell, L. R.; Carter, W. G. *J. Med. Chem.,* **1997**, *40*, 819.
[50] Laufer, A. S. ; Augustin, J. ; Dannhardt, G. ; Kiefer Werner *J. Med. Chem.,* **1994**, Vol. *57*, No 12.

[51] Geoffrey, T.; Bird, C.; Bruneau, P. ; Crawley, C. G. ; Edwards, P. M. ; Foster, J. S. ; Girodeau, J. M. ; Kingston, F. J. ; McMillan M. R. *J. Med. Chem.,* **1991***, 34*, 2176.

[52] Batt, G. D. ; Maynard, D. G. ; Petraitis, J. J. ; Shaw, E. J. ; Galbraith, W. ; Harris, R.R. *J. Med. Chem.,* **1990**, *33*, 360.

[53] Muller, K. ; Prinz H. *J. Med. Chem.,* **1997**,*40*, 2780.

$RMSE_{CV}$: is the average residual standard error of estimation of the predicted potencyfrom the leave one-out validation analysis calculated from the corresponding PRESS (Predictive, Residual sum of squares) value.