Review

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Quantitative Structure−Activity Relationship Studies on Na^+,K^+-ATPase Inhibitors

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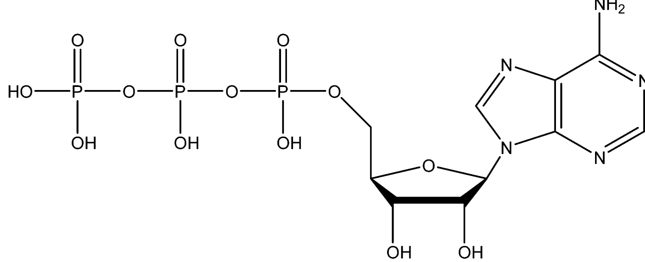
1. INTRODUCTION

The sodium, potassium adenosine triphosphatase (Na^+,K^+-ATPase), also known as sodium−potassium pump, is an enzyme (EC 3.6.1.3) found in the plasma membrane of all eukaryotic cells. In its enzyme entry EC 3.6.1.3, EC 3 refers to its main family (hydrolases), EC 3.6 to the subfamily (enzymes acting on acid anhydrides), EC 3.6.1 to the subsubfamily (enzymes in phosphorus-containing anhydrides), and EC 3.6.1.3 to its individual number. This enzyme, however, belongs to the class of ATPases that catalyze the exothermic dephosphorylation of adenosine-5′-triphophate (ATP, **1a**) to adenosine-5′-diphosphate (ADP, **1b**). This dephosphorylation reaction releases energy, which the enzyme (in most cases) harnesses to drive other chemical reactions that would otherwise not occur. The compound ATP is a multifunctional nucleoside triphosphate used in cells as a coenzyme. It is often called the "molecular unit of currency" of intracellular energy transfer.[1] It was first discovered in 1929 by Lohman[2] but was first synthesized in the laboratory by Todd[3] in 1948. However, it was proposed to be the main energy transfer molecule in the cell by Lipmann in 1941.[4] The process of dephosphorylation of ATP to release energy is common in all known forms of life.

Some of the ATPases are integral membrane proteins (anchored within biological membranes) and are thus called transmembrane ATPases. The Na^+,K^+-ATPase belongs to this class of ATPases. It is a heterodimeric transmembrane protein that transports sodium and potassium ions across cell membranes using energy from the hydrolysis of ATP. The energy released in the hydrolysis of ATP is used to drive the active transport in which the K^+ ions move into the cell and Na^+ ions out of the cell against their electrochemical gradients.[5] Thus, Na^+,K^+-ATPase helps the cells maintain their resting potential. In the resting state, cells contain a relatively high concentration of K^+ ions and low concentration of Na^+ ions.

The regulation of Na^+ and K^+ levels by Na^+,K^+-ATPase contributes to many essential cellular processes, such as maintenance of the membrane potential for muscle contraction and nerve propagation, regulation of cell volume, and transport of other ions, amino acids, neurotransmitters, and glucose.[5−8] This key enzyme also determines indirectly the intracellular Ca^{2+} concentration. In myocardial cells, the inhibition of Na^+,K^+-ATPase leads to an increase in intracellular accumulation of Na^+ ions and depletion of K^+ ions, resulting in an enhancement in the amount of Ca^{2+} ions and consequently an increase in the force of contraction (positive inotropic effect).



1a, adenosine-5′-triphosphate (ATP)

1b, adenosine-5′-diphosphate (ADP)

The increase in the force of myocardial contraction leads to increased cardiac output, decreased heart size, venous pressure, and blood volume, diuresis and relief of edema in patients with heart failure. The inhibition of Na^+,K^+-ATPase has been found of great value to be exploited to develop, particularly, potent inotropic agents for the treatment of congestive heart failure (CHF).

2. STRUCTURE OF NA^+,K^+-ATPASE

The enzyme Na^+,K^+-ATPase has been shown to consist of two or possibly three subunits of polypeptides designated as α, β,

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and γ, of which α is thought to contain the main locus of the receptor for glycosides.[5,9−13] In this structure, eight major hydrophobic sequences were identified that were suggested to represent the eight transmembrane (TM) domains of the unit.[14] A model (Figure 1) showing the above features was

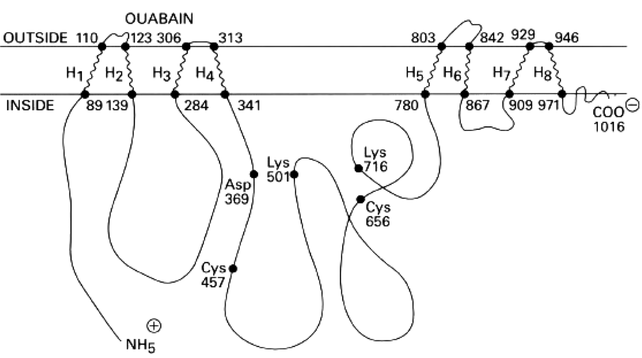


Figure 1. Wallick et al.'s model of α-subunit of Na^+,K^+-ATPase. H_1−H_8 represent the transmembrane (TM) hydrophobic sequences of the subunit. Reprinted with permission from ref 5. Copyright 1990 Elsevier.

published by Wallick et al.[15] The hydrophobic sequences are labeled H_1 to H_8, and the extracellular domains are specified by H regions on either side, for example, the first extracellular domain is designated as H_1−H_2. This model of Wallick et al. was based on the work of Shull et al.[16] The first two extracellular domains of the α-subunit, that is, H_1−H_2 and H_3−H_4, are supposed to be the putative binding sites since they contain several free carboxylic groups, which can provide several binding possibilities for ligands with different degrees of ionization.

3. NA$^+$,K$^+$-ATPASE INHIBITORS

All Na^+,K^+-ATPase inhibitors can be basically put into two groups, glycoside inhibitors and nonglycoside inhibitors, which are described as follows.

3.1. Glycoside Inhibitors

The glycoside inhibitors of Na^+,K^+-ATPase are better known as cardiac glycosides. As exemplified by **2a** and **2b**, they are the combination of an aglycone or genin and one to four sugars. The aglycone is chemically similar to bile acids and to steroids, such as adrenocortical and sex hormones, and constitutes the pharmacologically active portion of the glycosides. The sugars modify the water and lipid solubility of the glycoside molecules and thus affect their potency and duration of action.

Cardiac glycosides are also called *digitalis genin* or simply *digitalis*, because they are obtained from dried leaves of foxglove, *Digitalis purpurea* or *Digitalis lanata*. The best example of a compound obtained from the former is digitoxin (**2a**) and the one obtained from the latter is digoxin (**2b**). These are the two compounds whose clinical applicability has been established. The cardiac glycosides, however, can also be obtained from the seeds of *Strophanthus gratus*, for example, ouabain (**3**).

The cardiac glycosides can be put into two major classes: cardenolides and bufadienolides. The normal perhydrocyclopentanonephenanthrene nucleus is characterstic of the genin portion of all the members of both classes. They mainly differ in their lactone rings at the 17-position: a five-membered ring in cadenolides and a six-membered ring in bufadienolides.

2a(digitoxin), R=H; **2b**(digoxin), R=OH

3, ouabain

4,5

4 (digitoxigenin), R=

5 (bufalin), R=

A prototype of cardenolides is digitoxigenin (**4**), and one of bufadienolides is bufalin (**5**).

3.2. Nonglycoside Inhibitors

In this class, we put those compounds that are seco-D steroids, that is, the compounds in which the D ring of the steroid skeleton is broken, for example, cassaine (**6**),[17,18] or the compounds that have modified or totally different digitalis skeletons such as (**7**)[19] and (**8**).[20]

4. QSAR RESULTS AND DISCUSSION

Since Na^+,K^+-ATPase inhibitors have been found to constitute the most important class of cardiotonics, great attention has been paid to their structure−activity relationship (SAR) studies in order to design the most effective cardiotonics. In this respect, quantitative structure−activity relationship (QSAR) studies have been of great importance because they provide deeper insight into the mechanism of drug−receptor interaction and rationalize structural modifications to find potent drugs. It tries to explain the observed variations in biological activities of a group of congeners in terms of molecular variations caused by a change in the substituents. Among the various approaches initially developed for QSAR

6, cassaine

7

8, coumestans

studies,[21] the most historical has been the parametric method developed by Hansch,[22] which correlates the biological activity of molecules with their physicochemical, electronic, and steric properties. This parametric method by Hansch has been called Hansch Analysis. However, this is also called the *extrathermodynamic method* or *linear free energy-related approach* because all the molecular descriptors used in this method are linear free energy related terms (i.e., derived from rate or equilibrium constants). Also the biological activity terms, such as binding or inhibition constants, absorption, etc., are also linear free energy related terms.

However, the Hansch approach has not been limited to the use of only linear free energy related parameters but has been extended to use several mathematical descriptors, that is, topological indices, such as the Wienerr index,[23] the Hosoya index,[24] Randic's molecular connectivity index,[25] and many more defined by various authors[26] and initially used in quantitative structure−property relationship (QSPR) studies.

Apart from the Hansch approach, there have been a few more approaches, for example, Free−Wilson[27] or Fujita−Ban approaches,[28] discriminated analysis,[29] and pattern recognition technique,[30] that have been successfully applied to QSAR studies. Additionally, there have been some manual stepwise methods, such as the Topliss operation scheme,[31] Craig plots,[32] the Fibonacci search method,[33] and the sequential simplex strategy;[34] but these approaches have been of limited use.

All the above-mentioned QSAR approaches are related to 2D structures of the molecules. With the advent of the computer technologies came the era of 3D-QSAR studies, which led to the development of methods such as the distance geometry approach,[35] comparative molecular field analysis (COMFA),[36] comparative molecular similarity indices analysis (CoMSIA),[37] the hypothetical active site lattice (HASL) technique,[38] *de novo* ligand design,[39] and docking.[21d,40]

Using many of the methods described above, ample QSAR studies have been made on digitalis and digitalis-like compounds. A critical and comprehensive review of such QSAR studies is thought to be of great value in the design and development of potent cardiotonics, leading to this review.

Various qualitative SARs have presented some pictorial models of the actions of glycosides,[5,41−44] of which the most important is the presence of the steroid ring system, the 17β-lactone, and a 3β-sugar moiety. According to Repke,[45] the lactone ring, containing a carbonyl group conjugated with a double bond, is the most important functional group of the glycoside for interaction with the receptor. The carbonyl group was assumed to form a hydrogen bond with the receptor,[46] whose energy was calculated to be about 20 kJ/mol,[47] roughly equivalent to the binding energy of an average hydrogen bond. In naturally occurring cardiac glycosides, the lactone ring is always in β-orientation with respect to the steroid nucleus. Inversion of this ring to an α-configuration does not, for steric reasons, permit the steroid ring to approach the enzyme surface. This results in instability of the initial complex; the hydrogen bond breaks, and the compound becomes inactive.[48,49]

In fact, much importance has been given to the presence of the carbonyl group or its equivalent (e.g., CN) in the lactone ring.[50] Fullerton et al.[50] have insisted that the carbonyl group or its isostere is the sole binding entity and that the rest of the chain performs a purely passive role of positioning the carbonyl group. The hydrogen bonding through this group was considered to be the most likely binding force, and hence its position is important. In a more rigorous study on a group of compounds, Fullerton et al.[51] obtained an excellent correlation between the biological activity and the position of the carbonyl group or its isostere CN. For a set of a cardenolides, as shown in Figure 2, when structurally similar parts of the compounds with energetically favored conformations of the lactone ring were superimposed upon the digitoxigenin (**4**) and the distance (D) between the carbonyl oxygen of each compound and that of the digitoxigenin was calculated, the Na^+,K^+-ATPase inhibition activity was found to be significantly correlated with this D as[51]

$$\log(1/IC_{50}) = 6.47 - 0.457D$$
$$n = 9, r = 0.997 \qquad (1)$$

In eq 1, IC_{50} refers to the molar concentration of the compound leading to the 50% inhibition of the enzyme and n and r are the statistical parameters referring to the number of data points used in the correlation and the correlation coefficient, respectively. From this correlation, Fullerton et al.[51] suggested that for each 2.2 Å that the carbonyl oxygen of an analogue is displaced relative to its position in digitoxigenin, the activity drops by 1 order of magnitude.

Thomas et al.,[43,52] however, suggested that the lactone ring could be replaced by an open-chain α,β-unsaturated moiety (Figure 3a) and that the lactone or its open-chain analogues could be bonded to the receptor by a "two-point" attachment, involving a hydrogen bond and an ion−dipole interaction, in which the fractional positive charge on C20 might be involved (Figure 3b). But this "two-point" attachment concept was supposed to be very empirical.

But from studies on some series of 17β-guanylhydrazone derivatives of the digitoxigenin skeleton, such as those listed in Tables 1 and 2, where the lactone ring is replaced by a guanylhydrazone substituent-bearing chain or shifted from the original position by a spacer, Cerri et al.[53,55] reported that the presence of a basic (guanidine) group at the correct distance, a 1,2-polarized iminic double bond, or a 1,4-polarized conjugate system, which could mimic that of α,β-unsaturated lactone of digitoxigenin was essential for the Na^+,K^+-ATPase inhibition. The importance of a basic center and of a dipole in Na^+,K^+-ATPase inhibition was confirmed in a subsequent study by De Munnari et al.[18]

1-5

1. R^1 = , R^2 = H 2. R^1 = , R^2 = OH 3. R^1 = , R^2 = H

4. R^1 = , R^2 = H 5. R^1 = , R^2 = H

6,7

6. R^1 = , R^2 = H 7. R^1 = , R^2 = H

8 9

Figure 2. Cardenolides studied by Fullerton et al.[51]

For such a series of compounds as studied by Cerri et al.[53] (Table 1), Gupta and Paleti[56] found the ATPase inhibiton activity to be significantly correlated with van der Waals volume (V_w) of the substituents as shown by eq 2. This finding not only indicated that such a replacement of the lactone ring will uphold the Na^+,K^+-ATPase inhibition activity of the compounds but also suggested that the activity would be controlled by the shape and size of the substituents. Equation 2 indicates that the activity will not increase with the increase in the size of the substituent until its V_w, the van der Waals volume, reaches an optimum value ($V_{w,o}$) of 1.11 × 10^2 Å^3. In the derivation of this equation, however, compound **5** was not included because

$$\log(1/IC_{50}) = 11.269 - 10.901(\pm 8.416)V_w + 4.899(\pm 4.267){V_w}^2$$

$$n = 9,\ r = 0.921,\ s = 0.14,\ F_{2,6} = 16.86\ (10.92),\ V_{w,o} = 1.11 \quad (2)$$

Figure 3. (a) An open-chain α,β-unsaturated moiety (A = a heteroatom) that can replace the lactone ring at the 17-position of digitalis; R is usually an oxygen- or nitrogen-containing group. (b) Interaction of the open chain with the digitalis receptor by the "two-point" attachment model of Thomas et al.[43,52] involving a hydrogen bond and an ion−dipole interaction. The chain is shown as lying within a cleft on the enzyme surface. The charge distribution on atoms is due to the resonance phenomenon as shown in panel a. Reprinted with permission from ref 43. Copyright 1974 John Wiley and Sons.

it behaved as an outlier. Its observed activity (6.80) is much higher than that predicted by eq 2 (5.63). In the whole series, compound **5** has the highest activity. Cerri et al.[53] have suggested that in the case of this compound, the association of a basic guanidine group and a polarized system, which closely resembles the α,β-unsaturated lactone system present in digitoxigenin, permits a very strong interaction with the receptor, resulting in a very high inhibition of the enzyme. This suggestion and the correlation obtained is not far off from the observation of Thomas et al. as modeled in Figure 3. In eq 2, F is another statistical parameter, called Fisher ratio. It refers to the ratio of the variances between the observed and calculated activities. The subscripts in it stand for the degrees of freedom, where the first figure refers to number of independent variables k (descriptors) and the second one refers to the value of $(n - k - 1)$, n being the number of data points used. A higher value of F compared with the one given in parentheses, which is the standard statistical value of it at 99% level, indicates the level of significance of the correlation.

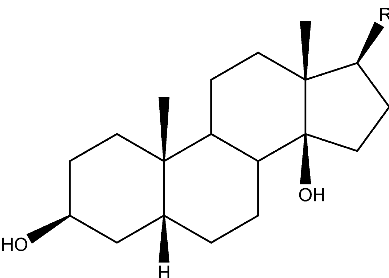
For another series of digitoxigenin derivatives bearing guanylhydrazone substituents at 17β-position (Table 2), Quadri et al.[55] studied the effect of the basicity of hydrazone substituents on binding to the enzyme. They tried to correlate the Na^+,K^+-ATPase binding affinity of the compounds with their pK_a values. A significant correlation was obtained between the two (eq 3), suggesting that a great portion of the variance in the activity (about 75%, $r^2 = 0.749$) could be accounted for by the changes in the basicity of the compounds. The positive coefficient associated with pK_a indicated that the higher the pK_a value (the greater the tendency to protonate), the higher the binding affinity. An equivalent result, using the proton affinity calculated by molecular orbital method (AM1), was also obtained by these authors (eq 4).

$$\log(1/IC_{50}) = 4.195 + 0.238(\pm 0.042)pK_a$$
$$n = 13,\ r = 0.865,\ s = 0.47,\ F_{1,11} = 32.81\ (9.65) \quad (3)$$

$$\log(1/IC_{50}) = -21.488 + 0.121(\pm 0.029)PA$$
$$n = 13,\ r = 0.784,\ s = 0.59,\ F_{1,11} = 17.57\ (9.65) \quad (4)$$

Both eqs 3 and 4 thus suggested that an ion-pair interaction could take place between a carboxylate residue of the enzyme

Table 1. Digitalis-like Compounds and Their Biological Activities[53]



compd	R	$V_w{}^a$ (10^2 Å^3)	log(1/IC$_{50}$), obsd	log(1/IC$_{50}$), calcd, eq 2
1	(*E*)-CH=N−N=C(NH$_2$)	0.737	5.80	5.89
2	(*E*)-CH=N−N−NH(2-imidazolinyl)	1.073	5.20	5.21
3	(*E*)-CH=N−NH [2-(1,4,5,6-tetrahydropyrimidinyl)]	1.158	5.30	5.22
4	CH$_2$−NH−N=C(NH$_2$)$_2$	0.775	5.70	5.76
5	(*E*,*E*)-CH=CH−CH=N−N=C (NH$_2$)	1.003	6.80	5.26
6	(*E*,*Z*)-CH=CH−CH=N−NH(2-imidazolinyl)	1.261	5.20	5.31
7	(*E*,*E*)-CH=CH−CH=N−NH(2-imidazolinyl)	1.261	5.60	5.31
8	(*E*,*E*,*E*)-(CH=CH$_2$)$_2$−CH=N−N=C(NH$_2$)$_2$	1.269	5.10	5.32
9	(*E*)-CH=CH(2,5-dihydro-5-oxo-3-furyl)	0.969	5.40	5.31
10	2,5-dihydro-5-oxo-3-furyl(digitoxigenin)	0.663	6.30	6.18

[a]Calculated according to ref 54.

Table 2. Hydrazone Derivatives of Digitoxigenin and Their Na^+,K^+-ATPase Inhibition Activity and Physicochemical Properties[55]

compd	R	pK_a	V_w (Å^3)	MR	PA (kcal/mol)	obsd	cacld eq 7	cacld eq 8
1		9.35	82.8	27.588	226.23	7.0	7.1	6.7
2		1.78	78.8	27.189	217.46	5.1	5.1	5.5
3		6.34	116.5	40.139	221.99	5.7	5.5	5.2
4		3.44	112.2	38.561	217.16	4.6	4.9	4.6
5		8.42	104.8	35.168	227.47	6.7	6.4	6.3
6		10.36	120.2	39.923	232.98	6.6	6.6	6.8
7		8.05	116.0	40.463	229.24	6.0	6.0	6.2
8		5.76	107.3	36.856	224.58	5.7	5.6	5.8
9		8.62	148.0	53.921	229.89	5.2	5.3	5.3
10		9.49	98.2	32.096	227.87	6.7	.8	6.6

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Table 2. continued

compd	R	pK_a	V_w (Å^3)	MR	PA (kcal/mol)	obsd	cacld eq 7	cacld eq 8
11	CH=N–NH–C(=S)NH$_2$	0.86	90.4	35.180	219.93	4.7	4.4	5.2
12	CH=N–N=C(NHCH$_3$)(NO$_2$)	2.37	123.9	39.742	215.67	4.5	4.5	4.3
13	CH=N–N=(4-oxoimidazolidin-2-ylidene)	3.58	101.9	35.397	216.48	4.7	5.1	4.7

and the protonated 17β-hydrazone group and that is what Thomas had hypothesized.

However, the most statistically significant models were found by Quadri et al.[55] when an additional parameter, V_w (van der Waals volume) or MR (molar refractivity index), was further included (eqs 5−8). The negative coefficient of V_w or MR, however, in these models suggested that an increase in the molecular size of the compound will have an adverse effect, which may be due to some steric effect.

$$\log(1/IC_{50}) = 6.422 + 0.275(\pm 0.021)pK_a - 0.023(\pm 0.004)V_w$$

$$n = 13,\ r = 0.973,\ s = 0.23,\ F_{2,10} = 89.81\ (7.56) \quad (5)$$

$$\log(1/IC_{50}) = -24.171 + 0.144(\pm 0.022)PA - 0.023(\pm 0.007)V_w$$

$$n = 13,\ r = 0.903,\ s = 0.43,\ F_{2,10} = 21.97\ (7.56) \quad (6)$$

$$\log(1/IC_{50}) = 6.310 + 0.263(\pm 0.020)pK_a - 0.061(\pm 0.010)MR$$

$$n = 13,\ r = 0.974,\ s = 0.23,\ F_{2,10} = 91.86\ (7.56) \quad (7)$$

$$\log(1/IC_{50}) = -24.475 + 0.147(\pm 0.017)PA - 0.074(\pm 0.015)MR$$

$$n = 13,\ r = 0.944,\ s = 0.33,\ F_{2,10} = 40.87\ (7.56) \quad (8)$$

However, in order to further explore the requirements for strong Na^+,K^+-ATPase inhibition, Cerri et al.[57] studied a new series of digitoxigenin derivatives (Table 3a) in which the guanylhydrazone group was replaced by an aminoalkyloxime group. For these compounds, the general features that were observed qualitatively to be more important were as follows:[57]

1. The presence of an amino function in the 17β-substituent.
2. The contemporaneous presence of an α,β-unsaturated oxime group, mimicking the electronic situation of the natural 17β-unsatutrated lactone in digitalis derivatives.
3. A correct distance of the amino group from C17 of the steroidal skeleton, a spacer of six atoms being the most suitable one.
4. Within the basic group, a primary amine was always more active than a tertiary amine.

Most of the above features were supported by a molecular study of Cerri et al.[57] In order to investigate the physicochemical properties of 17β-substituents in these compounds, Gupta et al.[58] conducted a QSAR study on them. A multiple regression analysis correlated the Na^+,K^+-ATPase inhibitory activity of these compounds with Kier's first-order valence molecular connectivity index ($^1\chi^v$) of 17β-substituents as

$$\log(1/IC_{50}) = 1.281(\pm 0.983)\,{}^1\chi^v_R - 0.316(\pm 0.187) \times ({}^1\chi^v_R)^2 + 0.509(\pm 0.467)I_1 - 0.618(\pm 0.523)I_2 + 2.077(\pm 0.525)I_3 + 1.939(\pm 0.791)I_4 + 3.573(\pm 1.074)$$

$$n = 32,\ r = 0.923,\ r_{cv}^2 = 0.74,\ s = 0.46,\ F_{6,25} = 24.01\ (3.63),\ ({}^1\chi^v_R)_{opt} = 2.03 \quad (9)$$

In this equation, I_1−I_4 are some indicator variables that were used to specify the role of certain important characteristics of the substituents. I_1 was used with a value of unity for the substituents that have (*E,E*) isomerism (compds **12**−**22**, **30**, and **31**), I_2 was used with a value of unity for the substituents where the iminic double bond has been reduced to the corresponding hydroxyl function (compds **24**−**29**), I_3 was used with a value of unity for such substituents that end with a primary amino group (NH_2) (compounds **6**−**8**, **11**, **16**, **20**−**22**, **26**−**28**, and **30**−**32**), and I_4 was used with a value of unity for the substituents that end with tertiary amine group ($N(CH_3)_2$) (compds **3**−**5**, **9**, **10**, **15**, **19**, **23**, and **25**).

The correlation expressed by eq 9 is fairly significant and its r_{cv}^2 (the square of cross-validated correlation coefficient obtained from jackknife leave-one-out procedure) indicates its good predictive ability. The parabolic dependence of activity on $^1\chi^v_R$ suggested that the shape and size of the substituent would control the activity but with an optimum value of 2.03 for $^1\chi^v_R$. Although $^1\chi^v_R$ accounts for all shape, size, length, and breadth,[25] here it can be length only because the substituents are mostly linear chains.

Cerri et al.[57] have pointed out that the most suitable substituents can be those that have a chain length of six atoms preceding the amine group. Such an ideal substituent in Table 3a is possessed by compound **7**, which has its $^1\chi^v_R$ around that

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Table 3. Series of 17*β*-*O*-Aminoalkoximes of 5*β*-Androstane-3*β*,14*β*-diol[57] with Some Structural Parameters and Their Observed and Calculated Biological Activities

(a) 17*β*-O-Aminoalkoximes of 5*β*-Androstane-3*β*,14*β*-diol and Structural Parameters

compd	R	$^1\chi^v$	MR	pol	I_1	I_2	I_3	I_4
1	(*E*)-CH=N–OH	0.792	91.35	36.21	0	0	0	0
2	(*E*)-CH=N–OCH_3	1.182	96.32	38.18	0	0	0	0
3	(*E*)-CH=N–$O(CH_2)_2N(CH_3)_2$	2.773	113.91	45.16	0	0	0	1
4	(*E*)-CH=N–$O(CH_2)_3N(CH_3)_2$	3.273	118.52	46.98	0	0	0	1
5	(*E*)-CH=N–$O(CH_2)_4N(CH_3)_2$	3.773	123.13	48.81	0	0	0	1
6	(*E*)-CH=N–O $(CH_2)_2NH_2$	1.971	103.03	40.84	0	0	1	0
7	(*E*)-CH=N–$O(CH_2)_3NH_2$	2.471	107.63	42.67	0	0	1	0
8	(*E*)-CH=N–$O(CH_2)_4NH_2$	2.971	112.24	44.49	0	0	1	0
9	(*E*)-CH_2CH=N–$O(CH_2)_2N(CH_3)_2$	3.182	118.52	46.98	0	0	0	1
10	(*E*,*Z*)-$(CH_2)_2CH$=N–$O(CH_2)_2N(CH_3)_2$	3.682	123.13	48.81	0	0	0	1
11	(*E*,*Z*)-$(CH_2)_2CH$=N–$O(CH_2)_2NH_2$	2.879	112.24	44.49	0	0	1	0
12	(*E*,*E*)-CH=CHCH=N–OCH_3	1.849	105.53	41.83	1	0	0	0
13	(*E*,*E*)-CH=CHCH=N–$O(CH_2)_2CH_3$	2.937	114.75	45.49	1	0	0	0
14	(*E*,*E*)-CH=CHCH=N–$O(CH_2)_2OH$	2.546	111.18	44.07	1	0	0	0
15	(*E*,*E*)-CH=CHCH=N–$O(CH_2)_2N(CH_3)_2$	3.440	123.13	48.81	1	0	0	1
16	(*E*,*E*)-CH=CHCH=N–$O(CH_2)_2NH_2$	2.638	112.24	44.49	1	0	1	0
17	(*E*,*E*)-CH=$C(CH_3)CH$=N–OCH_3	2.260	109.96	43.59	1	0	0	0
18	(*E*,*E*)-CH=$C(CH_3)CH$=N–$O(CH_2)_2OH$	2.956	115.61	45.83	1	0	0	0
19	(*E*,*E*)-CH=$C(CH_3)CH$=N–$O(CH_2)_2N(CH_3)_2$	3.851	127.56	50.56	1	0	0	1
20	(*E*,*E*)-CH=$C(CH_3)CH$=N–$O(CH_2)_2NH_2$	3.048	116.67	46.25	1	0	1	0
21	(*E*,*E*)-CH=$C(CH_3)CH$=N–$O(CH_2)_3NH_2$	3.548	121.28	48.07	1	0	1	0
22	(*E*,*E*)-CH=$C(CH_3)CH$=N–$O(CH_2)_4NH_2$	4.048	125.89	49.90	1	0	1	0
23	(*E*,*E*,*E*)-$(CH{=}CH)_2CH$=N–$O(CH_2)_2N(CH_3)_2$	4.107	127.56	50.56	0	0	0	1
24	CH_2NHOCH_3	1.299	99.52	39.45	0	1	0	0
25	$CH_2NHO(CH_2)_2N(CH_3)_2$	2.890	117.14	46.43	0	1	0	1
26	$CH_2NHO(CH_2)_2NH_2$	2.088	107.69	42.69	0	1	1	0
27	$CH_2NHO(CH_2)_3NH_2$	2.996	112.32	44.52	0	1	1	0
28	$(CH_2)_3NHO(CH_2)_2NH_2$	3.088	116.95	46.36	0	1	1	0
29	(*E*)-CH=$CHCH_2NHOCH_3$	2.041	110.57	43.83	0	1	0	0
30	(*E*,*E*)-$(CH{=}CH)_2(CH_2)_2NH_2$	2.650	118.09	46.81	1	0	1	0
31	(*E*,*E*)-$(CH{=}CH)_2(CH_2)_3NH_2$	3.150	122.72	48.65	1	0	1	0
32	$(CH_2)_7NH_2$	3.742	129.86	47.92	0	0	1	0
33	CHO	0.569	91.46	36.25	0	0	0	0
34	$(CH_2)_2CHO$	1.477	99.00	39.25	0	0	0	0
35	(*E*)-CH=CHCHO	1.236	100.78	39.95	0	0	0	0
36	(*E*)-CH=$C(CH_3)CHO$	1.764	105.26	41.73	0	0	0	0

(b) Observed and Calculated Biological Activities

compd	log (1/IC_{50})		log (1/EC_{50})	
	obsd[57]	calcd, eq 9	obsd[57]	calcd, eq 11
1	4.00	4.39		
2	4.49	4.65		
3	6.00	6.63		
4	6.60	6.32	5.89	5.89
5	5.70	5.85	5.33	5.77
6	6.80	6.95	6.21	6.60
7	7.70	6.89	6.74	7.00
8	6.30	6.67	5.54	5.55
9	6.20	6.39	5.74	5.89

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Table 3. continued

	(b) Observed and Calculated Biological Activities			
	log (1/IC$_{50}$)		log (1/EC$_{50}$)	
compd	obsd[57]	calcd, eq 9	obsd[57]	calcd, eq 11
10	7.00[a]	5.95	6.19	5.77
11	7.10	6.72	5.96	5.55
12	5.40	5.37		
13	5.00	5.12		
14	5.20	5.30	4.41	4.83
15	7.52[a]	6.69	7.24	7.26
16	7.70	7.34	7.30	7.04
17	5.00	5.36		
18	5.10	5.11	4.64	4.91
19	7.22	6.27	7.15[b]	6.01
20	7.52	7.13	7.15	7.12
21	6.70	6.73	6.77[b]	5.67
22	5.90	6.17	5.40	5.54
23	5.20	5.45	4.70	4.51
24	4.10	4.09		
25	4.30[a]	5.96		
26	5.80	6.33	5.10[b]	6.50
27	6.40	6.03	5.41	5.31
28	6.00	5.97	4.92	5.47
29	4.40	4.25		
30	7.10	7.33	6.42	6.59
31	6.49	7.06	7.46	6.74
32	5.90	6.02		
33	4.60	4.20	4.96	4.94
34	4.40	4.78		
35	6.60[a]	4.67	5.40	4.74
36	5.80	4.85		

[a]Not used in derivation of eq 9. [b]Not used in derivation of eq 11

of $({}^1\chi^v_R)_{opt}$. Compound **7** fortunately has the highest activity in the series, but there are several other compounds, e.g., **10**, **11**, **15**, **16**, **19**, **20**, and **30**, that also have high activity but their ${}^1\chi^v_R$ values are much greater than $({}^1\chi^v_R)_{opt}$, suggesting that their activity should be much lower than that of compound **7**. Here comes the role of other structural features of the compounds. The positive coefficients of the indicator variables I_1, I_3, and I_4 in eq 9 suggested that a substituent with (E,E) isomerism or one ending with primary amine or tertiary amine will have an additional beneficial effect. The negative coefficient of I_2, however, indicated that the reduction of iminic bond to hydroxyl amines would be detrimental to the activity. This shows the importance of the polarized iminic bond.

Polarizability of the compounds has been found to be important in the inhibition of Na^+,K^+-ATPase. For a set of cardenolides (Table 4), Repke et al.[59] calculated the relative dipole−dipole attraction forces, which gave information about the relative statistical probabilities of the receptor occupancy, called W_{rel}. As the simplest congener, digitoxigenin, was arbitrarily chosen as reference compound with a value of 1 given to its W_{rel}. Then, the Na^+,K^+-ATPase inhibition activity of compounds was shown to be significantly correlated with W_{rel} as

$$\log(1/IC_{50}) = 2.54W_{rel} - 2.93$$
$$n = 8,\ r = 0.95 \quad (10)$$

Table 4. A Series of Cardenolides and Their Na^+,K^+-ATPase Inhibition Activity and Relative Statistical Probability of Receptor Occupancy[59]

no.	compd	structural difference (referred to **1**)	W_{rel}	log(1/IC$_{50}$) obsd	log(1/IC$_{50}$) cacld, eq 10
1	digitoxigenin		1.00	−0.175	−0.39
2	gitoxigenin	16β-OH	0.69	−1.000	−1.18
3	16α-gitoxigenin	16α-OH	0.97	−1.300	−0.47
4	digoxigenin	12β-OH	0.77	−0.680	−0.97
5	gitoxigenin-16-acetate	16β-$OCOCH_3$	1.20	0.301	0.12
6	gitoxigenin-16-nitrate	16β-ONO_2	1.45	0.825	0.75
7	dihydrodigitoxigenin	sat. lactone	0.63	−1.500	−1.33
8	$\Delta^{8(14)}$-anhydrodigitoxigenin	$\Delta^{8(14)}$	0.05	−2.770	−2.80

For the compounds of Table 3a, Gupta et al.[58] had found, as exhibited by eq 11, that the polarizability of the compounds could also be an important governing factor for the inotropic activity ($\log(1/EC_{50})$), which has a significant linear relationship with the Na^+,K^+-ATPase inhibition activity ($\log(1/IC_{50})$) (eq 12). In eqs 11 and 12, EC_{50} refers to the concentration of the compound producing 50% of the maximal increase in force of contraction studied in electrically driven guinea pig left atrium. In eq 12, the intercept is almost zero and the slope is

nearly 1; hence IC_{50} can be taken as a good measure of inotropic activity.

$$\log(1/EC_{50}) = 133.10(\pm 74.306)\text{pol} - 52.874(\pm 29.437)\text{MR} + 1.489(\pm 0.544)I_1 + 2.343(\pm 0.649)I_3 + 3.238(\pm 1.003)I_4 - 14.840(\pm 4.057)$$

$$n = 21,\ r = 0.926,\ r_{cv}^2 = 0.72,\ s = 0.40,\ F_{5,15} = 18.07\ (4.56) \quad (11)$$

$$\log(1/EC_{50}) = 0.918(\pm 0.193)\log(1/IC_{50}) - 0.052(\pm 1.252)$$

$$n = 23,\ r = 0.907,\ s = 0.39,\ F_{1,21} = 97.48\ (8.02) \quad (12)$$

Equations 5−8 and 11 have also expressed exclusively that the bulk of the molecules could be an important factor to control the activity by producing steric hindrance in drug−receptor interaction. However, in both series of compounds (Tables 2 and 3a) for which eqs 5−8 or 11 were obtained, the change in the bulk of compounds is accounted for by the change in the shape and size of 17β-substituents. In a series of grayanotoxins (Table 5), Shirai et al.[60] showed that the bulkly substituents at their 14β-position may also create a steric problem. These authors synthesized a series of 14β-acylated grayanotoxins (Table 5) and measured their positive inotropic potency (in terms of EC_{50}, the molar concentration leading to 50% of the maximum inotropic effect) in isolated guinea pig papillary muscle, which they found to have significant parabolic correlation with van der Waals volume (V_w) of 14β-substituents as

$$\log(1/EC_{50}) = 3.751(\pm 1.592)V_w - 3.371(\pm 1.296) \times V_w^2 + 5.653$$

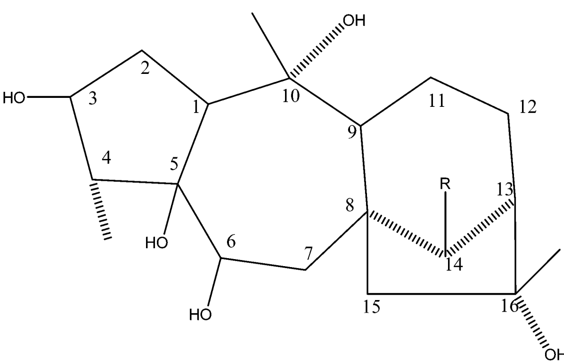
$$n = 12,\ r = 0.894,\ s = 0.25,\ F_{2,9} = 17.84\ (8.02) \quad (13)$$

This correlation gives an optimum value of $V_w = 0.556 \times 10^2$ Å^3, suggesting that the 14β-substituents could produce the steric effect only after they cross this limit of V_w. Up to this limit, however, the substituent would favor the activity, which may be due to its involvement in dispersion interaction with the receptor, which is in tune with dipole−dipole interaction as discussed in other cases. Shirai et al., however, have also pointed out that an overall hydrophobic−lipophilic balance, too, of the molecule could be important for the inotropic effect of acylated grayanotoxins studied by them.[60] They had found that acylation of the equivalent 14β-hydroxyl group in grayanatoxins had increased their inotropic potency.

In the derivation of eq 13, compound **12** was not included, because it had exhibited aberrant behavior. Equation 13 predicted very high activity of this compound compared with its observed activity. Its low observed activity may be due to the typical steric effect of the *t*-butyl group.

Hydroxyl groups at positions other than 3 and 14 of the steroid ring generally decrease the activity.[61,62] This means that the portion of the receptor that binds the steroid has hydrophobic characteristics or else that the additional hydroxyl

Table 5. Grayanotoxins and Their Inotropic Activity[60]



compd	R	V_w (10^2 Å^3)	log(1/EC_{50}) obsd	log(1/EC_{50}) cacld, eq 13
1	H	0.013	5.61	5.70
2	OH	0.081	6.15	5.93
3	$OCOCH_3$	0.423	6.31	6.64
4	$OCOC_2H_5$	0.577	6.67	6.70
5	OCO-n-C_3H_7	0.731	6.81	6.59
6	OCO-n-C_4H_9	0.885	6.41	6.33
7	OCO-n-C_5H_{11}	1.039	5.75	5.91
8	OCO-n-C_6H_{13}	1.193	5.32	5.33
9	$OCOCH_2CH_2Cl$	0.172	6.96	6.61
10	$OCOC_6H_{11}$	1.039	6.17	5.91
11	$OCOC_6H_5$	0.963	5.79	6.14
12[a]	OCO-t-C_4H_9	0.785	5.78	6.52
13	$OCOCH(OH)CH_3$	0.595	6.54	6.69

[a]Not included in the derivation of eq 13.

groups interfere with the tight fit of the molecule in the receptor cleft.

The classical SAR studies have indicated the importance of 3β- and 14β-hydroxyl groups, but subsequent studies showed that activity was not abolished if these groups were removed. The 3β-hydroxyl group does not contribute to binding but provides an essential point of attachment for the sugar residues. Although in grayanatoxins the acylation of the 14β-hydroxyl group has been found by Shirai et al.[60] to increase the inotropic activity, the removal of it in glycosides does not abolish the activity; rather its presence significantly increases the activity, and it probably has a direct binding role. According to Naaido et al.,[63] this group may be involved in hydrogen bonding with the receptor.

The mechanism by which the sugar component of cardiac glycosides confers its effect is not well-known. It was only proposed that the sugars protect the 3β-hydroxyl group from epimerization or conjugation;[64] either of these biotransformations leads to a relatively inactive compound. In systematic studies on the role of sugars in the action of glycosides, Yoda et al.[65−68] found that glycosides with 6-deoxy sugars (i.e., those with 5′-methyl groups) were the most potent of all glycosides and concluded that this group was a key factor in the interaction of the glycoside moiety with the receptor. These authors proposed that binding of cardiac glycoside occurs in two steps: first binding of the steroid and then a slower interaction of the sugar residues.[67,68]

In a recent study, Stanton et al.[69] reported that the nature of substituent at the 17β-position is an important determinant of the activity. In most active glycosides, it is a monounsaturated five-membered furanone. In certain naturally occurring compounds such as bufalin (**5**), this ring is replaced by a six-memberd pyranone with two double bonds, which is accompanied by a noticeable increase in enzyme inhibitory potency. This has been verified by the high potency of **9**. A reduction in ring size to an epoxide as present in **10** is detrimental to the activity. This was also indicated in an earlier study by Paula et al.,[70] but in **10**, the modifications of the cyclopentanoperhydrophenanthrene moiety may also be responsible for compound's inactivity.

Stanton et al.[69] also performed a partial least-squares (PLS) regression analysis on a set of cardiac glycosides. The PLS regression analysis, also known as *projection to latent structures* technique, is an alternative approach to the linear multiple regression analysis (Hansch approach) fitted by least-squares, if the number of independent variables (descriptors) is relatively high compared with the number of data points (number of compounds in a given series) and several of the independent variables are mutually correlated.[70] In such a situation, the PLS analysis generates latent variables from the linear combinations of the descriptors, which are then used to correlate the dependent variables. These latent variables are orthogonal to each other. The weighting coefficient of a descriptor in a latent variable shows its contribution to the dependent variable.

From PLS analysis, Santon et al.[69] found two most important descriptors of the inhibitory activity of the compounds: first the ninth-order chain molecular connectivity index ($^9\chi$) and second the sum of the hydrogen atom-type electrotopological state index for potential internal hydrogen bonds separated by three edges (*SHBint3*).[71] The primary instance of a nine-membered chain (**a**−**i**) occurs in the C and D rings of the steroidal nucleus and an additional occurrence of such a nine-membered chain (**j**−**r**) can also be observed in some compounds such as **11**.

9

10

Both primary and additional nine-membered chains are shown by bold faces in **11**. The calculation of $^9\chi$ takes into account all nine bonds of nine-membered rings.

The feature of *SHBint3* is illustrated in structure **12**, where there are five instances of potential internal hydrogen bonds, occurring between O and OH in each case, separated by three edges as indicated by *a-b-c-h*, *h-c-d-i*, *i-d-e-j*, *j-e-f-g*, and *g-b-c-h*.

Another important feature for the activity of cardiac glycosides indicated by Stanton et al.[69] as well as Paula et al.[72] is the *cis*−*trans* stereochemistry of bonds C5−C10 and C13−C14, connecting rings A and B and C and D, respectively. A *cis* fusion of the rings at either or both the bonds can be more favorable to the activity than the transfusion. An example of this could be cited of digitoxigenin (**13**) and uzarigenin (**14**), where the former with *cis* fusion at both the bonds has 25 times higher activity than the latter with *trans* fusion at C5−C10 and the *cis* fusion only at C13−C14. However, it has been pointed out that this is not an absolutely essential condition.

Paula et al.,[72] in fact, have pointed out much importance of the saturation of the lactone ring. The saturation of this ring causes an increase in relative biological activity of almost 2 orders of magnitude. As reported previously by Farr et al.,[73] this seemingly minor structural modification abolishes the planarity of the lactone ring system, which might result in a repositioning of the compound in the binding pocket of the enzyme.

Comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA) were also conducted on glycoside binding with Na^+,K^+-ATPase by some authors. The CoMFA calculates the steric and electrostatic interaction energies for a molecule binding with the receptor, and CoMSIA is simply a modified version of

11

12

13, digitoxigenin

14, uzarigenin

CoMFA that calculates not only the steric and electrostatic interaction energies but also the hydrophobic and hydrogen-bond energies. In both the methods, the different interaction energies calculated for a series of molecules at different grid points are correlated with the biological activity using PLS method and are represented as three-dimensional contour maps in which contours of various colors represent locations on the molecular structure where low or high steric, electrostatic, hydrophobic, hydrogen-bond donor, or hydrogen-bond acceptor interactions would take place. A detailed discussion of these methods can be found in refs 21d, 36, and 37. In a comparative molecular similarity indices analysis (CoMSIA) of cardiac glycoside binding with Na^+,K^+-ATPase, Paula et al.[72] had derived a model (Figure 4) that revealed a green area about the α-sugar (Figure 4A) indicating that the presence of steric bulk here would increase ligand binding affinity. The contours in Figure 4C indicated electrostatic interactions that are dominated by a large blue area between the lactone ring and the steroid C16 atom in which an increase in positive charge would favor ligand binding. Additionally, a small red area at the lactone ring in the proximity of the carbonyl group indicated that the presence of negative charge on the ligand would improve binding, which may be due to the attractive electrostatic interactions between the carbonyl oxygen and the positively charged counterparts in the enzyme, such as protonated nitrogen atoms on lysine, arginine, proline, and histidine side chains.

CoMSIA also revealed hydrophobic contours as shown in Figure 5, where Figure 5A indicates that the presence of polar groups in positions C1 and C19 (yellow regions) at the steroidal ring would be detrimental to high-affinity binding. This is verified by relatively low affinity of certain compounds, such as ouabagenin (**15a**), strophanthidine (**15b**), and strophanthidol (**15c**), but compounds like ouabain (**15d**) and cyamarin (**15e**), which also have polar groups at these regions, still have high affinity. The expected loss in activity in them seems to be compensated by the presence of sugar rings in them. This emphasizes the importance of the presence of at least one sugar moiety in cardiac glycosides for high binding affinity. The gray contour about the α-sugar in Figure 5A describes a region where polar groups on the ligand may improve the binding.

The H-bond donor contour map (Figure 6A) obtained by Paula et al.[72] provided, for the first time, evidence for favorable interactions between a H-bond donating hydroxyl group located in the proximity of substituents at C4′ on the α-sugar and corresponding acceptor groups on the enzyme. Figure 6C shows additional favorable hydrogen bonding between the ring oxygen of the α-sugar acting as H-bond acceptor and an active site of the enzyme acting as H-bond donor. The purple area in the proximity of C1 and C19 in Figure 6A indicates unfavorable interactions between the enzyme and the hydroxyl or carbonyl groups of ouabagenin, strophanthidine, or strophanthidol (**15a–c**) in these positions. Likewise,

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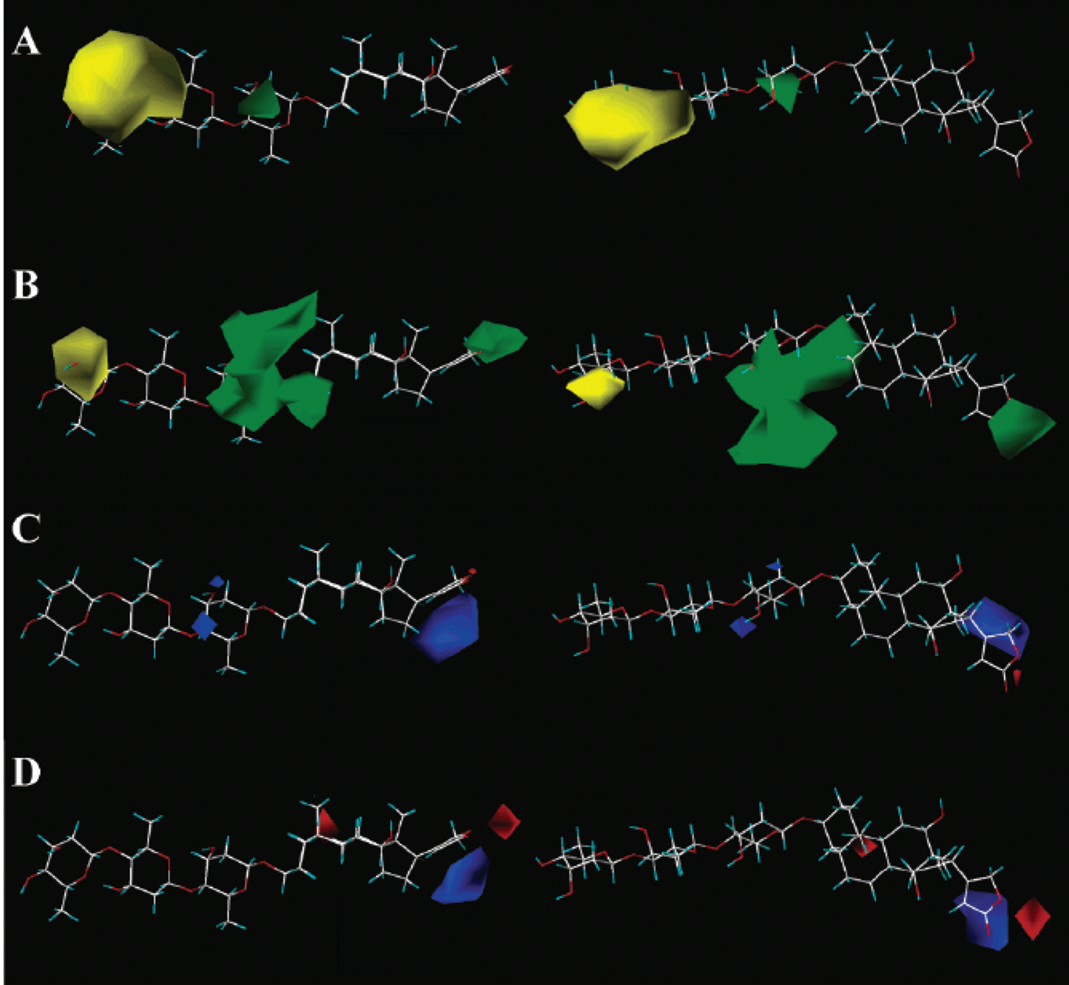

Figure 4. Steric and electrostatic CoMSIA contour maps of cardiac glycosides for ligand binding (A, C) and enzyme inhibition (B, D). The structure of digoxin is displayed for reference. In contours A and B, the green areas represent the regions where the addition of steric bulk can increase the activity, and the yellow areas indicate the regions where addition of steric bulk can decrease the activity. In contours C and D, the blue areas represent the regions where the addition of positive charge can increase the activity, and red areas indicate the regions where the addition of positive charge can decrease the activity. Reprinted with permission from ref 72. Copyright 2005 American Chemical Society.

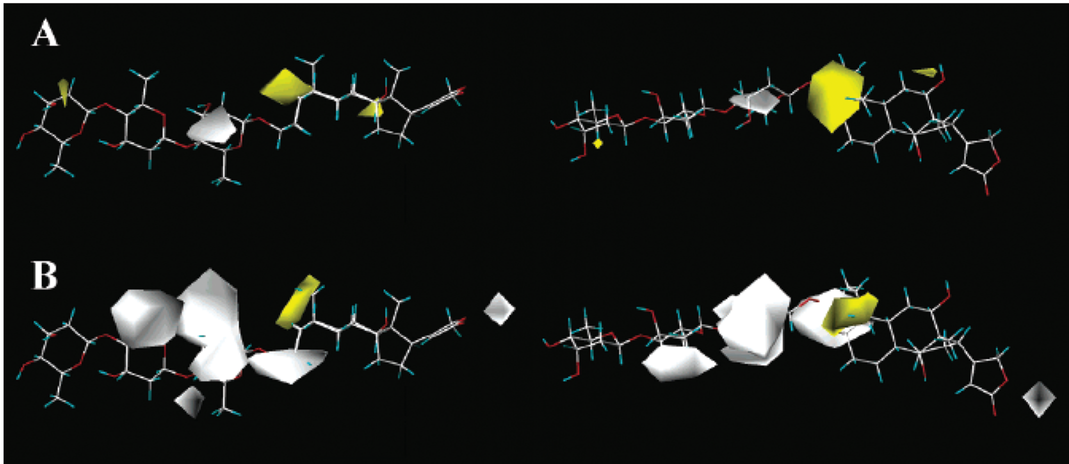

Figure 5. The hydrophobic CoMSIA contour maps of cardiac glycosides for ligand binding (A) and enzyme inhibition (B). The structure of digoxin is displayed for reference. In the contours, the yellow areas indicate the regions where the increase in hydrophobic character can increase the activity of the compound and the gray areas the regions where the increase in hydrophobic character may lead to decrease in the activity of the compound. Reprinted with permission from ref 72. Copyright 2005 American Chemical Society.

15

15a (ouabagenin): R_1 = H, R_2 = R_3 = R_4 = OH
15b (strophanthidine): R_1 = R_2 = R_4 = H, R_3 = (=O)
15c (strophanthidol): R_1 = R_2 = R_4 = H, R_3 = OH
15d (ouabain): R_1 = rhamnose, R_2 = R_3 = R_4 = OH
15e (cymarin): R_1 = cymarose, R_2 = R_4 = H, R_3 = (=O)

the orange contour in Figure 6A about the ring oxygen of the γ-sugar indicates the successive reduction in binding affinity upon addition of more sugar rings to the α-sugar. This again confirms the importance of the presence of only one sugar ring at the 3-position.

Paula et al.[72] observed that substitution of the five-membered lactone ring of cardenolides with the six-membered lactone of bufadienolides caused binding affinity to decline but inhibitory potency to increase. In a previous study, workers of the same group, Farr et al.,[73] reported that the most potent inhibitors were some bufadienolides with their six-membered unsaturated lactone rings rather than cardenolides with five-membered lactone rings at 17β-position. However, for most compounds a correlation was found between the binding affinity and the inhibitory potency.[72,73]

In the previous study, Farr et al.[73] had made a comparative molecular field analysis (CoMFA) on the same series of cardiac glycosides for their Na^+,K^+-ATPase inhibition, whose results were almost consistent with those of CoMSIA performed by Paula et al.[72] However, the CoMFA contour map of digoxin inhibition of Na^+,K^+-ATPase allowed Farr et al.[73] to make some preliminary predictions for the binding site in the enzyme for digitalis. Currently, there are two prominent models for the

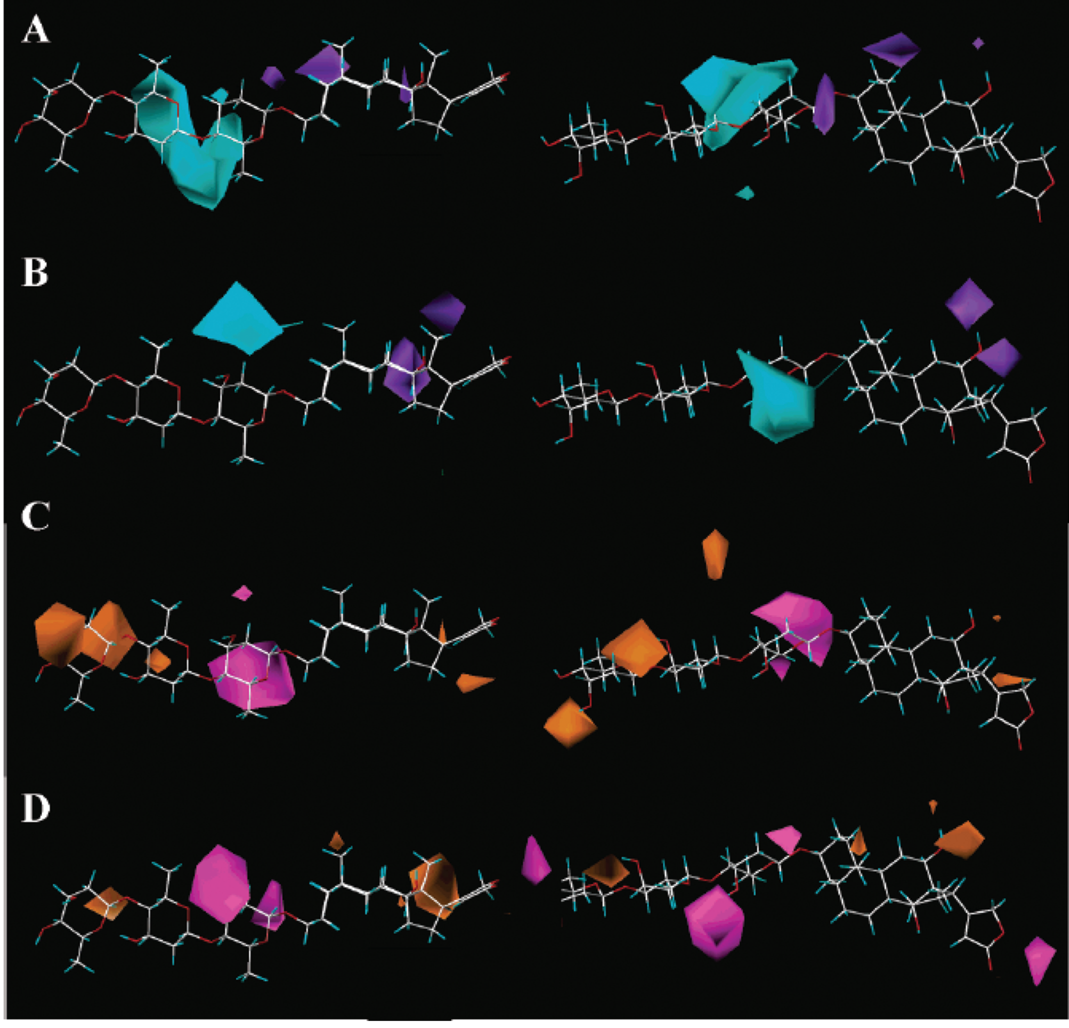

Figure 6. The H-bond donor and H-bond acceptor CoMSIA contour maps of cardiac glycosides for ligand binding (A, C) and enzyme inhibition (B, D). The structure of digoxin is displayed as a reference. In contours A and B, the cyan areas represent the regions where the presence of an H-bond donor group can increase the activity, and purple areas represent the regions where the presence of H-bond donor group can decrease the activity. In contours C and D, the pink areas represent the regions where the presence of an H-bond acceptor can increase the activity, and the orange areas represent the regions where the presence of H-bond acceptor can decrease the activity. Reprinted with permission from ref 72. Copyright 2005 American Chemical Society.

same: the Repke model[6] and the Lingrel model.[7] The Repke model puts the bound ligand within the membrane at the interface of the two α subunits formed by adjacent H_1 and H_2 transmembrane (TM) segments (Figure 1) and the Lingrel model puts the same across the extracellular exposed portion of the α subunit. Farr et al. claimed that their contour plots are consistent to both the models, because these plots showed that the steric and electrostatic fields, surrounding the ligands and being important to the enzyme inhibition, covered approximately 20 Å (from lactone ring to α sugar), which is almost the same distance that was suggested by Repke et al.[6] to be the depth (19 Å) of the digitalis binding cleft; and for the compatibility with the Lingrel model they argued as follows: The structure of Na^+,K^+-ATPase is not known, but the structure of a closely related calcium pump of the sarcoplasmic reticulum has been determined[74] and found to possess 10 TM α-helical segments grouped together in a TM domain to have a diameter of approximately 40 Å. This size could easily include a cardiotonic binding site with a diameter of 20 Å on the extracellular surface, fulfilling the condition of the Lingrel model.

In comparison to the Repke model, however, Farr et al. claimed that their results were far more consistent with the Lingrel model. According to Repke et al.,[6] a series of five hydrophobic residues along the surface of a side of each of the H_1 and H_2 TM segments interacts with the lactone and steroid rings forming the binding site. This should generate steric interactions along the length of the aglycone on both its α and β sides. Instead, Farr et al. observed the steric interactions to be restricted to the α-sugar and lactone and the electrostatic interactions to occur along the length of entire ligand molecule. This observation resembled more the Lingrel model than the Repke model. This point has been further stressed by Ball et al.[75]

Through a docking study, the Paula group tried to give a more detailed picture of digitalis binding with Na^+,K^+-ATPase.[76] By docking a series of cardiac glycosides onto the modeled extracellular surface of the α subunit, these authors were able to pinpoint a consensus drug-binding site that accommodated the lactone ring moiety oriented toward the H_1-H_2 loop and the sugar moieties directed toward the H_9-H_{10} loop, with two to three easily identifiable likely hydrogen-bond interactions between the drug and receptor. The steroidal moiety was found to occupy the space between H_2-H_4 and H_5-H_6. Thus, Keenan et al.[76] claimed that this model of binding was consistent with the Lingrel model.[6]

Gobbini et al.[77] recently performed a study on a series of istaroxime (**16**) derivatives. They first explored the chemical space around the 6-position of the steroidal moiety by changing the functional groups at that position and maintaining a basic oxime chain at the 3-position. All of the compounds were tested for their Na^+,K^+-ATPase inhibitory potencies. Many of the compounds tested *in vivo* were found to be safer than digoxin, the classic digitalis compound currently used as inotropic agent to treat congestive heart failure. These authors then performed CoMFA and CoMSIA studies on these istaroxime derivatives. From CoMFA steric and electrostatic contour plots (Figure 7), Gobbini et al. found that the area surrounding the heterocyclic rings of the oximic chain and the space under the substituent in the 6α-position (green polyhedra) could be favored steric areas to increase the inhibitory potency. This observation was supported by the fact

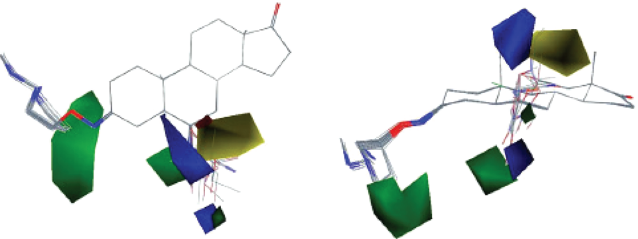

Figure 7. CoMFA steric and electrostatic cotour maps. The green polyhedra reperesent sterically favored areas, and the yellow ones represent the sterically disfavored areas. The blue polyhedra represent the positively charged favored areas, and the red ones represent the negatively charged favored areas. In the maps, all the training and test set molecules are shown for reference. Reprinted with permission from ref 77. Copyright 2010 Elsevier.

that most of the compounds with heterocyclic rings in the oxime chain had higher inhibitory potency than the corresponding compounds with a linear oxime chain and that the compounds with 6α substitution also had relatively better activity. A yellow contour toward the 6β face, however, indicated that a bulky substituent at this position would not favor the activity.

Regarding electrostatic interactions, the contours with blue polyhedra exhibited the areas where positively charged substituents may favor the Na^+,K^+-ATPase inhibitory activity. These regions are above position 6 and on the axis of the 6α-position. A small red contour above the 6β-position close to the disfavored steric region (yellow) indicated the area where a negatively charged substituent may increase the activity.

16, istaroxime

The CoMSIA's steric and electrostatic contours were found to be comparable to the corresponding CoMFA contours, but they additionally provided H-bond donor, H-bond acceptor, and hydrophobic contours (Figure 8). Contours in Figure 8a indicated the 6α-region where the presence of an H-bond acceptor may favor the activity (magenta contour) and two small regions in the 6β-face and on the axis of the 4α-position where the H-bond acceptor groups may be detrimental to the activity (red contours). Notwithstanding, a smaller region close to the 4α-position accounts for the very high potency of compounds probably because of a 6-oxo substituent. The contour maps of H-bond donor field (Figure 8b) indicated four regions (cyan polyhedra) where the presence of an H-bond donor may increase the activity: two regions close to the oxime chains, one small region near the 4-position, and a small region near the 6α-position. The contour maps of hydrophobic fields (Figure 8c) indicated the hydrophobic area (in violet) to be

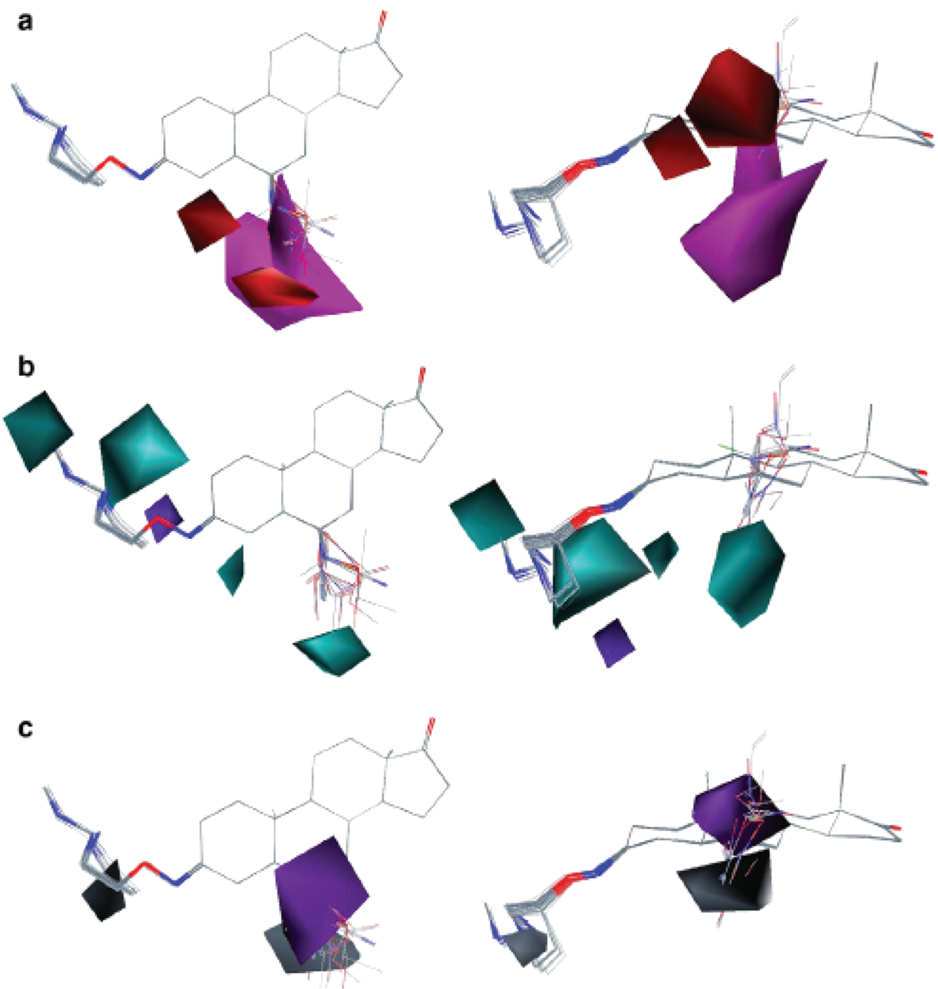

Figure 8. CoMSIA H-bond donor, H-bond acceptor, and hydrophobic contour plots for androstane derivatives. In contour a, the magenta polyhedra represent the favored H-bond acceptor areas, and the red ones represent the disfavored H-bond acceptor areas. In contour b, the cyan polyhedral represent the favored H-bond donor areas, and the purple ones represent the disfavored H-bond donor areas. In contour c, the violet polyhedra represent the favored hydrophobic areas, and the gray ones represent the disfavored hydrophobic areas. In all of the contours, all the training and test molecules are displayed for reference. Reprinted with permission from ref 77. Copyright 2010 Elsevier.

present on the axis of a double-bonded substituent at the 6-position.

A coumestan derivative, 2-methoxy-3,8,9-trihydroxy coumestan (PCAL36, **17**), was also studied for its Na^+,K^+-ATPase inhibition by Pôças et al.[20] PCAL36 is a synthetic isomer of wedelolactone, a naturally occurring coumestan isolated from *Eclipta prostrate.* Coumestans are a class of isoflavonoids. Wedelolactone and some synthetic derivatives of coumestan were earlier studied for Na^+,K^+-ATPase inhibition by da Silva et al.[78] Pôças et al.'s study on the molecular mechanism of Na^+,K^+-ATPase inhibition by PCAL36 indicated that this compound inhibits the enzyme by oxidizing its sulfhydryl groups, which are essential for its catalytic activity. It has been reported by many authors[79−82] that Na^+,K^+-ATPase is inhibited by the oxidation of its free sulfydryl groups.

Efforts were made to simplify the digitalis skeleton in order to find simple compounds to act as cardiotonics. Among such efforts, an encouraging effort was to synthesize the compounds with a hydrindane skeleton (**18**), which preserved the most distinctive part of the digitalis skeleton, that is, the C and D rings with a *cis* junction.[83,84] The

HO, O, O, H_3CO, OH, OH

17, PCAL36

observation that the replacement of the butenolide ring with aminoalkyloxime chains could be advantageous prompted Cerri et al.[19] to try such replacement on the hydrindane skeleton and synthesize a series of 1-(*O*-aminoalkyloximes) of perhydroindene derivatives as shown in Table 6a. Seelam et al.[85] conducted a QSAR study on them to explore the physiochemical properties and important features of the substituents that are important for the Na^+,K^+-ATPase inhibitory activity of the compounds. These authors found the dominant role of R_5-substituents rather

Table 6. A Series of Perhydroindene Derivatives with Their Observed Na^+,K^+-ATPase Inhibition Activity[19] and Their Physicochemical Parameters and Observed and Calculated Na^+,K^+-ATPase Inhibition Activities

1-40 41-43

(a) Perhydroindene Derivatives with Their Observed Na^+,K^+-ATPase Inhibition Activity

compd	R_1	R_5	$\log(1/IC_{50})$
1	(*E*)-$CH=NO(CH_2)_2N(CH_3)_2$	(*Z*) $=CHC_6H_5$	4.30
2	(*E*)-$CH=NO(CH_2)_2N(CH_3)_2$	(*Z*) $=CHC_6H_5$	5.10
3	(*E*)-$CH=NO(CH_2)_2N(CH_3)_2$	α-$CH_2C_6H_{11}$	4.00
4	(*E*)-$CH=NO(CH_2)_2N(CH_3)_2$	β-$CH_2C_6H_5$	5.49
5	(*E*)-$CH=NO(CH_2)_2N(CH_3)_2$	β-$CH_2C_6H_{11}$	5.40
6	(*E*)-$CH=NO(CH_2)_2N(CH_3)_2$	β-C_6H_5	5.49
7	(*E*)-$CH=NO(CH_2)_2NH_2$	β-C_6H_5	5.40
8	(*E*)-$CHNHO(CH_2)_2N(CH_3)_2$	β-C_6H_5	5.00
9	(*E*)-$CHNHO(CH_2)_2NH_2$	β-C_6H_5	4.70
10	(*E*)-$CH=NO(CH_2)_2N(CH_3)_2$	β-(3-$H_3CC_6H_4$)	5.30
11	(*E*)-$CH=NO(CH_2)_2NH_2$	β-(3-$H_3CC_6H_4$)	5.49
12	(*E*)-$CH=NO(CH_2)_2N(CH_3)_2$	β-(4-$H_3CC_6H_4$)	5.00
13	(*E*)-$CH=NO(CH_2)_2NH_2$	β-(4-$H_3CC_6H_4$)	5.20
14	(*E*)-$CH=NO(CH_2)_2N(CH_3)_2$	β-(3-HOC_6H_4)	5.00
15	(*E*)-$CH=NO(CH_2)_2N(CH_3)_2$	β-(4-HOC_6H_4)	5.60
16	(*E*)-$CH=NO(CH_2)_2N(CH_3)_2$	β-(3-$HOCH_2C_6H_4$)	5.30
17	(*E*)-$CH=NO(CH_2)_2NH_2$	β-(3-$HOCH_2C_6H_4$)	5.60
18	(*E*)-$CH=NO(CH_2)_2N(CH_3)_2$	β-(4-$HOCH_2C_6H_4$)	5.10
19	(*E*)-$CH=NO(CH_2)_2NH_2$	β-(4-$HOCH_2C_6H_4$)	5.60
20	(*E*)-$CH=NO(CH_2)_2N(CH_3)_2$	β-(4-$(H_3C)_2N(CH_2)_2OC_6H_4$)	6.00
21	(*E*)-$CH=NO(CH_2)_2N(CH_3)_2$	β-(3-C_5H_4N)	4.89
22	(*E*)-$CH=NO(CH_2)_2N(CH_3)_2$	β-(4-C_5H_4N)	5.60
23	(*E*)-$CH=NO(CH_2)_2N(CH_3)_2$	β-C_6H_{11}	5.80
24	(*E*)-$CH=NO(CH_2)_3N(CH_3)_2$	β-C_6H_{11}	5.49
25	(*E*)-$CH=NO(CH_2)_4N(CH_3)_2$	β-C_6H_{11}	5.00
26	(*E*)-$CH=NO(CH_2)_2NH_2$	β-C_6H_{11}	6.00
27	(*E*)-$CH=NO(CH_2)_3NH_2$	β-C_6H_{11}	6.10
28	(*E*)-$CH=NO(CH_2)_4NH_2$	β-C_6H_{11}	4.89
29	(*E*)-$CH=NN=C(NH_2)_2$	β-C_6H_{11}	4.20
30	(*E,Z*)-$CH_2CH=NO(CH_2)_2N(CH_3)_2$	β-C_6H_{11}	5.80
31	(*E,Z*)-$CH_2CH=NO(CH_2)_2NH_2$	β-C_6H_{11}	5.80
32	(*E,Z*)-$(CH_2)_2CH=NO(CH_2)_2N(CH_3)_2$	β-C_6H_{11}	5.30
33	(*E,Z*)-$(CH_2)_2CH=NO(CH_2)_2NH_2$	β-C_6H_{11}	5.49
34	(*E,E*)-$CH=CHCH=NO(CH_2)_2N(CH_3)_2$	β-C_6H_{11}	6.00
35	(*E,E*)-$CH=CHCH= NO(CH_2)_2NH_2$	β-C_6H_{11}	6.00
36	(*E,E*)-$CH=C(CH_3)CH=NO(CH_2)_2N(CH_3)_2$	β-C_6H_{11}	5.70
37	(*E,E*)-$CH=C(CH_3)CH=NO(CH_2)_2NH_2$	β-C_6H_{11}	5.89
38	(*E,E*)-$CH=CHCH=NN=C(NH_2)_2$	β-C_6H_{11}	4.80
39	(*E*)-$CH=NO(CH_2)_2N(CH_3)_2$	β-(*cis*-4-HOC_6H_{10})	5.80
40	(*E*)-$CH=NO(CH_2)_2N(CH_3)_2$	β-(*trans*-4-HOC_6H_{10})	6.00
41	(*E*)-$CH=NO(CH_2)_2N(CH_3)_2$		7.40
42	(*E,E*)-$CH=CHCH=NO(CH_2)_2N(CH_3)_2$		7.60
43	(*E,E*)-$CH=CHCH=NO(CH_2)_2NH_2$		7.80
digitoxigenin			7.20
uzarigenin			6.60

Table 6. continued

(b) Physicochemical Parameters and Observed and Calculated Na^+,K^+-ATPase Inhibition Activities

compd	$^1\chi^v_{R5}$	I_1	I_2	I_3	log(1/IC50) obsd[a]	log(1/IC50) calcd, eq 14	log(1/IC50) LOO[b]
1	2.44	1	0	0	4.30	4.40	4.52
2[d]	2.44	1	0	0	5.10	4.40	
3[d]	3.60	1	0	0	4.00	5.30	
4[c]	2.63	1	0	0	**5.49**	**4.55**	
5	3.60	1	0	0	5.40	5.30	5.19
6	2.16	0	0	0	5.49	5.25	5.23
7	2.16	0	0	0	5.40	5.25	5.23
8	2.16	0	1	0	5.00	4.35	3.91
9[c]	2.16	0	1	0	**4.70**	**4.35**	
10	2.56	0	0	0	5.30	5.56	5.57
11	2.56	0	0	0	5.49	5.56	5.56
12	2.56	0	0	0	5.00	5.56	5.59
13	2.56	0	0	0	5.20	5.56	5.58
14	2.28	0	0	0	5.00	5.34	5.37
15	2.28	0	0	0	5.60	5.34	5.32
16[c]	2.44	0	0	0	**5.30**	**5.46**	
17	2.44	0	0	0	5.60	5.46	5.45
18	2.44	0	0	0	5.10	5.46	5.49
19	2.44	0	0	0	5.60	5.46	5.45
20[d]	4.23	0	0	0	6.00	6.84	
21	2.00	0	0	0	4.89	5.12	5.15
22	2.00	0	0	0	5.60	5.12	5.07
23[c]	3.13	0	0	1	**5.80**	**5.72**	
24	3.13	0	0	1	5.49	5.72	5.74
25[d]	3.13	0	0	1	5.00	5.72	
26	3.13	0	0	1	6.00	5.72	5.69
27	3.13	0	0	1	6.10	5.72	5.68
28[d]	3.13	0	0	1	4.89	5.72	
29	3.13	0	1	1	4.20	4.83	5.16
30	3.13	0	0	1	5.80	5.72	5.71
31	3.13	0	0	1	5.80	5.72	5.71
32	3.13	0	0	1	5.30	5.72	5.76
33	3.13	0	0	1	5.49	5.72	5.74
34	3.13	0	0	1	6.00	5.72	5.69
35	3.13	0	0	1	6.00	5.72	5.69
36	3.13	0	0	1	5.70	5.72	5.74
37	3.13	0	0	1	5.89	5.72	5.70
38	3.13	0	1	1	4.80	4.83	4.84
39	3.06	0	0	0	5.80	5.94	5.95
40	3.06	0	0	0	6.00	5.94	5.94
41	4.96	0	0	0	7.40	7.41	7.41
42	4.96	0	0	0	7.60	7.41	7.26
43[c]	4.96	0	0	0	**7.80**	**7.41**	
44[c]	4.36	0	0	0	**7.20**	**6.94**	
45[c]	4.36	0	0	0	**6.60**	**6.94**	

[a]Taken from ref 19. [b]Calculated from leave-one-out procedure. [c]Test set compounds. [d]Not included in the derivation of eq 14.

than R_1-substituents and correlated the Na^+,K^+-ATPase inhibitory activity of the compounds with Kier's first-order valence molecular connectivity index $^1\chi^v$ of R_5-substituents and some indicator parameters. The whole data set contained 45 compounds. This set was divided into two subsets, the training set and the test set. In the test set, the compounds were arbitrarily selected keeping in mind the wide structural diversity and span in the activity data. The compounds of the test set are given in bold and with superscript c in Table 6b. The remaining compounds were taken for the training set. A multiple regression analysis was performed on the training set, and the best correlation that could be found was

$$\log(1/IC_{50}) = 0.772(\pm 0.177)^1\chi^v_{R5} - 1.060(\pm 0.488)I_1 - 0.895(\pm 0.402)I_2 - 0.275(\pm 0.253)I_3 + 3.579(\pm 0.504)$$

$$n = 33,\ r = 0.900,\ r_{cv}^2 = 0.69,\ s = 0.32,\ F_{4,28} = 29.94(4.07) \quad (14)$$

In this equation, the indicator parameter, I_1, has been used with a value of 1 for the first five compounds (Table 6a) where the R_5-substituents are attached with the nucleus through a =CH− or a $-CH_2-$ bridge. Similarly, I_2 has been used with a value of 1 for R_1-substituents that do not contain a =N−O− moiety. Such R_1-substituents are present in compounds **8**, **9**, **29**, and **38** (Table 6a). The last indicator variable, I_3, has been used with a value of unity for compounds that have R_5 = β-C_6H_{11}. Such compounds are **23**−**38** in Table 6a. These three indicator parameters have negative coefficients in the correlation, suggesting that some R_1- and R_5-substitutents of a particular nature will not be conducive. For R_5-substituents, while I_1 indicated that any substituent with an alkyl or alkene bridge will not be tolerated, I_3 indicated that a β-C_6H_{11} substituent will also not be tolerated. Probably such substituents might create steric problems. In R_1-substituents, I_2 suggested that substituents other than those containing the =N−O− moiety will be deleterious to the activity. This means that the =N−O− moiety may have some specific role because of its electronic nature. Maybe it participates in some dipole−dipole interaction with the receptor. As already discussed, the dipole−dipole interaction has been found to be important in digitalis interaction. However, the major dependence of activity on molecular connectivity of R_5-substituents suggested that shape and size of R_5-substituents will play a great role in the inhibition activity of the compounds, because the molecular connectivity index defines the molecular shape and size of the compounds.[25]

18

In deriving eqs 14, however, certain compounds as indicated in Table 6b with superscript d (compounds **2**, **3**, **20**, **25**, **28**) were not included because they had exhibited aberrant behavior. All these compounds, except **2**, had their observed activities much lower compared with their corresponding activity values calculated from eq 14. The reasons for such discrepancies were well explained by Seelam et al.[85] as follows. Compound **2** is the geometrical isomer of **1** and with its *Z*-configuration seems to have better activity than the one with *E*-configuration. Compound **3** is the only compound in which R_5-substituent has α-configuration. The same R_5-substituent attached with β-configuration appears to be more conducive (compound **4**). Compound **20** may have the lower activity because of its long R_5-substituent that might create the steric hindrance. The lower activity of compounds **25** and **28** could be attributed to their R_1-substituents containing the longest primary amine (with a spacer of seven atoms). As suggested by Cerri et al.,[57] an amine chain with a spacer of six atoms is the most suitable, and this is exemplified here by compound **27**. Thus these outliers indicated what kind of substituents might not be favorable to the inhibition potency of the compounds.

The beauty of eq 14 was that it could also accommodate very well the last two compounds (**41** and **42**) of Table 6a, which did not belong to perhydroindene series of this table. Further, its r_{cv}^2 value being greater than 0.6 exhibited its good prediction ability. As obvious from Table 6b, the activity values predicted from it for the compounds of both the training and test sets were in excellent agreement with their corresponding observed ones (Table 6b).

5. AN OVERVIEW

This review essentially presents the features of Na^+,K^+-ATPase inhibitors that are important for the inhibition of the enzyme Na^+,K^+-ATPase and highlights the forces involved in the enzyme−inhibitor interaction. The enzyme Na^+,K^+-ATPase consists of three subunits of polypeptides designated as α, β, and γ, of which α is thought to contain the main locus of the receptors for cardiac glycosides, the most widely studied class of Na^+,K^+-ATPase inhibitors. In this structure, eight major hydrophobic sequences (H_1−H_8) were identified, which were suggested to represent the eight transmembrane (TM) domains of the α subunit (Figure 1), where the first two domains H_1−H_2 and H_3−H_4 were supposed to be the putative binding sites since they contain several free carboxylic groups providing several binding possibilities for ligands with different degrees of ionization.

According to qualitative SARs, the most important features in glycosides for binding with Na^+,K^+-ATPase are the steroid ring system, the 17β-lactone, and a 3β-sugar moiety as in ouabain (**3**). According to Repke,[45] the lactone ring, containing a carbonyl group conjugated with a double bond, is the most important functional group of the glycoside for interaction with the receptor, because it forms the hydrogen bond with the receptor. In naturally occurring cardiac glycosides, the lactone ring is always in β-orientation with respect to the steroid ring. Inversion of this ring to α-configuration abolishes the activity of the compound, because it produces steric hindrance for the steroid ring to approach the enzyme surface and the formation of the hydrogen bond does not take place. The hydrogen bond through the carbonyl group or its equivalent (CN) in the lactone ring is considered to be the most likely binding force; hence its position and the orientation of the lactone ring are important. In fact, an excellent correlation was obtained between the biological activity and the position of the carbonyl group or its isostere CN relative to that in digitoxigenin (eq 1).[51] Notwithstanding, the "two-point" attachment concept of Thomas et al.,[43,52] in which the lactone ring was replaced by an open-chain α,β-unsaturated moiety (Figure 3), was supposed to be very empirical. In a recent study also, the nature of the substituent at the 17β-position has been found to be an important determinant of activity.[69] In most glycosides, it is a monosaturated five-membered furanone, and in certain naturally occurring compounds such as bufalin (**5**), it is a six-membered pyranone with two double bonds. However, while a six-membered ring may lead to an increase in the activity as for **9**,[69] a reduction in size to an epoxide as in **10** could be detrimental to the activity.[72]

The saturation of the lactone ring has been indicated to be another important factor to favor the activity,[72] because it might abolish the planarity of the lactone ring, leading to a better repositioning of the compound in the binding pocket of the enzyme.[73]

Cerri et al.,[53,55] however, reported that the lactone ring could be replaced by a guanylhydrazone substituent-bearing chain or

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shifted from the original position by a spacer, as represented by compounds in Tables 1 and 2, since the presence of a basic (guanidine) group at the correct distance, a 1,2-polarized iminic double bond, or a 1,4-polarized conjugate system, which could mimic that of the α,β-unsaturated lactone of digitoxigenin, might do as well as the lactone ring. The importance of a basic center and of a dipole in Na^+,K^+-ATPase inhibition was confirmed in a subsequent study by De Munnari et al.[18] The effect of the basicity of hydrazone substituents on binding to enzyme was shown by Quadri et al.[55] by correlating the binding affinity of the compounds of Table 2 with their pK_a values (eq 3) or their proton affinity (eq 4). However, these authors also showed that increase in size of such compounds may have an adverse effect (eqs 5−8). Some similar conclusions were drawn by Gupta et al.[58] when they correlated the inhibition activity of compounds of Table 3a with Kier's first-order valence molecular connectivity index and some indicator variables (eq 9). In such a series, the importance of the polarized iminic bond was discussed.

Polarizability of the compounds has been found to be important in the inhibition of Na^+,K^+-ATPase as exhibited by eq 10 obtained by Repke et al.[59] for a series of cardenolides (Table 4) and by eq 11 obtained by Gupta et al.[58] for the compounds of Table 3a.

As already discussed, the bulk of the molecules or substituents may create the steric problems. For a series of grayanotoxins (Table 5), eq 13 obtained by Shirai et al.[60] showed that the bulky substituents at the 14β-position of the compounds will create the steric problem.

In cardiac glycosides, the positions of hydroxyl groups have their own importance. At positions other than 3 and 14 of the steroid ring, the presence of OH groups leads to a decrease in the activity. Classical SAR studies have indicated the importance of 3β- and 14β-hydroxyl groups, but subsequent studies showed that the activity was not abolished if these groups were removed. In glycosides, however, the presence of an OH group at the 14β-position has been found to increase the activity, probably because of its direct binding role. According to Naaido et al.,[63] this group may be involved in hydrogen bonding with the receptor.

The mechanism by which the sugar component of cardiac glycosides confers its effect is not well-known. However, some authors proposed that the binding of cardiac glycosides occurs in two steps: first binding of the steroid and then a slower interaction of the sugar residues.[67,68] It is also proposed that sugars protect the 3β-hydroxyl group from epimerization or conjugation;[64] either of these biotransformation leads to a relatively inactive compound. However, through a CoMSIA study on glycosides, Paula et al.[72] emphasized the importance of the presence of at least one sugar moiety in cardiac glycosides because it can compensate the loss in activity that may occur due to the presence of polar groups, if any, at positions C1 and C19. But these authors also indicated the successive reduction in binding affinity upon addition of more sugar rings to α-sugar and thus stressed the importance of the presence only one sugar ring at the 3-position.

In place of 3α-sugar, Stanton et al.[69] found the importance of a nine-membered chain as indicated in **11**, like the one in C and D rings of the steroidal nucleus.

In connection to the steroidal nucleus, several important features have been pointed out from CoMFA, CoMSIA, and docking studies. One of them is the *cis*−*trans* stereochemistry of bonds C5−C10 and C13−C14, connecting rings A and B and C and D, respectively.[69,72] A *cis* fusion of the rings at either or both of the bonds can be more favorable to the activity than the *trans* fusion. Another important feature is the presence of polar groups in positions C1 and C19. The polar groups at these positions have been pointed out to be detrimental to high binding affinity (Figure 5A). In Figure 6A, the purple area in the proximity of C1 and C19 indicated unfavorable interactions between the enzyme and the hydroxyl or carbonyl groups of glycosides such as **15a−c**.

Another important point in the steroidal ring is the 6-position, which has been pointed out by CoMFA and CoMSIA studies on istaroxime (**16**) derivatives.[77] The space under the substituent in the 6α-position has been indicated to be one of the favored steric areas (Figure 7), and the regions above position 6 and on the axis of the 6α-position have been indicated to be the areas where the positively charged substituents may favor the Na^+,K^+-ATPase inhibitory activity (Figure 7). A small area above the 6β-position has been pointed out to be the region where a negatively charged substituent may increase the activity. Further, the presence of an H-bond acceptor at the 6α-region has been indicated to favor the activity, while the same at 6β-face was found to be unfavorable to the activity (Figure 8).

Regarding the binding of a whole cardiac glycoside molecule with the enzyme, the docking study of Keenan et al.[76] pointed out that the lactone ring is oriented toward the H_1−H_2 loop and the sugar moieties are directed toward the H_9−H_{10} loop, with two to three easily identifiable likely hydrogen-bond interactions with the receptor. The steroidal moieties were found to occupy the space between H_2−H_4 and H_5−H_6. Thus Keenan et al. claimed that this model of binding was consistent with the Lingrel model. However, many authors have reported that Na^+,K^+-ATPase is inhibited by the oxidation of its free sulfhydryl groups.[79−82]

In a study on compounds with a simplified digitalis skeleton such as **18**, Cerri et al.[19] found the dominant role of R_5-substituents rather than R_1-substituents (at the place of lactone ring). For the series of compounds (Table 6a), eq 14 obtained by Seelam et al.[85] expressed the role of molecular connectivity index of R_5-substituents, which usually defines the shape and size of the molecules.

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Biography

Satya P. Gupta is now a *Professor of Eminence* in the Department of Applied Sciences at Meerut Institute of Engineering and Technology

(MIET), Meerut. He obtained his M.Sc. and D.Phil. degrees from University of Allahabad, Allahabad, in 1967 and 1971, respectively. For his doctoral degree, he worked with Professor Bal Krishna in Quantum Chemistry, leading to the development of a new molecular orbital method, known as IOC-ω-technique (inclusion of overlap charges in ω technique). The method was found at that time very useful in dealing with the problems of π-electron systems. After his doctorate, Gupta spent a couple of years at Tata Institute of Fundamental Research (TIFR), Mumbai, working with Professor Girjesh Govil on the structure and functions of biomembranes. In 1973, he joined Birla Institute of Technology and Science (BITS), Pilani, from which he retired in July 2008 and joined MIET, Meerut. He has long been working on the theoretical aspects of drug design. He has made notable contributions in this area, for which in 1989 the Ranbaxy Research Foundation Award, a coveted national award, was bestowed upon him, and in 1985 he was made Fellow of the National Academy of Sciences, India (*F. N. A. Sc.*). He has been an advisory member of the Technical Resource Group on Research and Development on HIV/AIDS, constituted by Ministry of Health and Welfare, Government of India. He has acted as a Guest Editor of *Current Medicinal Chemistry* and *Combinatorial Chemistry & High Throughput Screening*, both published by Bentham Science Publishers BV, The Netherlands and U.S.A., and served as Editor-in-Chief of its four well-known journals, namely, *Cardiovascular and Hematological Agents in Medicinal Chemistry*, *Current Computer-Aided Drug Design*, *Current Enzyme Inhibition*, and *Current Bioinformatics.* Besides, He guest-edited two volumes of *Topics in Heterocyclic Chemistry* (Springer-Verlag Heidelberg Germany) and recently edited a book entitled "Ion Channels and Their Inhibitors" for Springer-Verlag Heidelberg, and a book entitled "Matrix Metalloproteinase Inhibitors: Specificity of Binding and Structure-Activity Relationships" for Springer Basle AG. Dr. Gupta has also been on the Editorial Board of *Indian Journal of Chemistry, Section B* (CSIR, New Delhi) and presently is on the editorial board of *Letters in Drug Design and Discovery*, *Online Journal of Organic Chemistry*, and *BenSci Journal*, all three from Bentham Science, and *Research and Reports in Medicinal Chemistry*, Dove Press, New Zealand. Additionally, he has authored a book entitled "Quantum Biology", New Age International Publishers, New Delhi, 1996. and a book entitled "QSAR and Molecular Modeling", 2011, Anamaya Publishers, New Delhi, and Springer, Dordrecht, the Netherlands. Dr. Gupta has contributed more than a half dozen reviews in *Chemical Reviews* in addition to many in *Progress in Drug Research*, *Current Medicinal Chemistry*, and some others, besides hundreds of excellent original publications in various internationally reputed journals.

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