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A Quantitative Structure–Activity Relationship Study on a Series of Na^+, K^+-ATPase Inhibitors

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A quantitative structure–activity relationship (QSAR) study has been made on a new series of digitalis-like Na^+,K^+-ATPase inhibitors in which the guanylhydrazone group has been replaced by an aminoalkyloxime group. The correlations obtained have shown that the oxime moiety, primary amine group, overall size, and polarizability of the new type of substituents are higly beneficial to the Na^+,K^+-ATPase inhibition potency of the compounds and that their effect can be quantitatively assessed. The study also showed that the inotropic activity of the compounds is very well correlated with their Na^+,K^+-ATPase inhibition potency.

Keywords: Quantitative structure–activity relationship (QSAR); Na^+, K^+-ATPase inhibitors; Digitalis-like compounds; Cardiotonics

INTRODUCTION

Among the cardiotonics (agents against congestive heart failure), the most important group is the digitalis cardiac glycosides. Extensive studies on these glycosides established the clinical applicability of two of the compounds: digoxin (**1**) and digitoxin (**2**). Although these compounds are unquestionable in their utility for the treatment of congestive heart failure, they suffer from a low therapeutic index due to cardiac proarrhythmogenic activity. In a recent trial, a neutral effect was observed by digoxin on the mortality of patients with heart failure.[2] Therefore, attention has been focussed on investigating safer cardiotonic agents through the inhibition of Na^+,K^+-ATPase, the mechanism by which glycosides elicit their effect. Consequently, a series of 17β-guanylhydrazone derivatives of the digitoxigenin skeleton (**3**) were reported with the observation that the presence of a basic (guanidine) group at a 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 was essential for the Na^+, K^+-ATPase inhibition activity of the compounds.[3,4] The importance of a basic center and of a dipole was confirmed in a subsequent study by De Munari *et al.*[5] on a non-digitalis series. However, in order to further explore the requirements for strong Na^+,K^+-ATPase inhibition, Cerri *et al.*,[6] studied a new series of digitalis-like derivatives (**4**) in which the guanylhydrazone group was replaced by an aminoalkyloxime group. For these compounds, the general features that were observed qualitatively to be important were:[6]

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-unsaturated lactone in digitalis derivatives.
3. A correct distance of the amine group from the C-17 of the steroidal skeleton—a spacer of 6 atoms being the most suitable one.
4. Within the basic group, a primary amine was always more active than a tertiary amine.

All the above features were supported by a molecular modeling study.[6] What is still wanting is a study of the nature of the drugs–receptor interaction, recognition of the active sites in the receptor, and investigation of the role of physicochemical parameters and their contribution in

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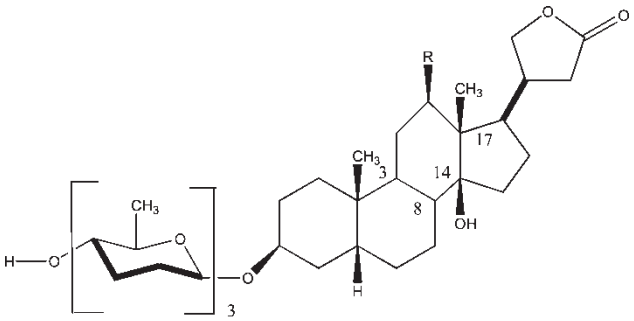
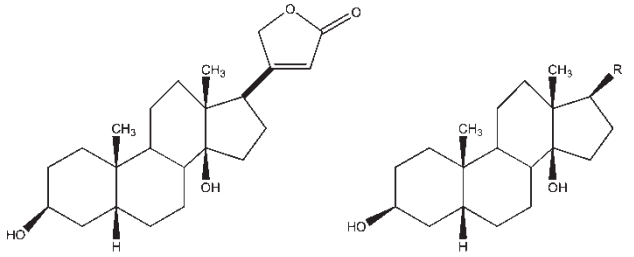


1, R = OH; **2**, R = H

the quantitative terms to the overall activity of the compounds. This all can be accomplished by a quantitative structure–activity relationship study on the compounds The object of this article is therefore to present with a critical discussion the results of such a study made on the series reported by Cerri *et al.*[6]



3 **4**

MATERIALS AND METHOD

The series of the compounds reported by Cerri *et al.*[6] is listed in Table I along with the physicochemical and structural parameters that were found to be useful in our QSAR study. Among these parameters, $^1\chi_R^v$ is Kier's first-order valence molecular connectivity index of the R-substituent, log P is the calculated hydrophobicity parameter, MR is the calculated molar refractivity index, MV is the calculated molar volume and pol is the calculated polarizability of the molecule. The log P was calculated using www.logP.com software and MR, MV, and pol were calculated using ACD/ChemSketch Freeware software. The $^1\chi^v$ was calculated as suggested by Kier and Hall[7] and as described below.

$^1\chi^v$ is calculated using the equation:

$$^1\chi^v = \Sigma\left(\delta_i^v \delta_j^v\right)^{-1/2} \tag{1}$$

where δ_i^v and δ_j^v are the vertex connectivity indices of atoms i and j, respectively, and the summation extends to all bonded pairs of nonhydrogenic atoms in the group or molecule. A unified definition of δ^v was given as[8]

$$\delta_i^v = \left(Z_i^v - h_i\right)/\left(Z_i - Z_i^v - 1\right) \tag{2}$$

where Z_i^v is the number of valence electrons of atom i, h_i is the number of hydrogen atoms attached to it, and Z_i is its atomic number.

The molecular connectivity index has been established to be a very important structural parameter, which signifies the degree of branching and the connectivity in the molecule or substituent. It also signifies the shape, size, length, and breath of the molecule. Since $^1\chi^v$ takes into account the valence electrons of atoms and the number of hydrogen atoms attached to them, it also measures the degree of saturation of the molecule or group. The presence of highly electronegative atoms (having a high value of Z^v) in the molecule or group will give a lower value of $^1\chi^v$. The calculation of $^1\chi_R^v$ has been made taking into account the carbon atom of the ring also to which the substituents are attached

In Table I, there are four dummy parameters I_1, I_2, I_3 and I_4. I_1 has been used with a value of unity for the substituents that have (E,E) isomerism (compounds 12–22, 30, 31). I_2 has been used with a value of unity for the substituents where the iminic double bond has been reduced to the corresponding hydroxyl function (24–29). I_3 has been used with a value of unity for such substituents that end with a primary amine group (NH_2) (6–8, 11, 16, 20–22, 26–28, 30–32), and I_4 has been used with a value of unity for the substituents that end with tertiary amine group ($N(CH_3)_2$) (3–5, 9, 10, 15, 19, 23, 25).

Table II gives the two activity parameters of the compounds, where IC_{50} is the concentration of the compound to inhibit 50% of Na^+,K^+-ATPase activity, and EC_{50} is the concentration producing 50% of the maximal increase in the force of contraction.

RESULTS AND DISCUSSION

A multiple regression analysis correlated the Na^+,K^+-ATPase inhibitory activity of the compounds as

$$\begin{aligned}\log(1/IC_{50}) = {} & 1.281(\pm0.983)^1\chi_R^v - 0.316(\pm0.187)(^1\chi_R^v)^2 \\ & + 0.509(\pm0.467)I_1 - 0.618(\pm0.523)I_2 \\ & + 2.077(\pm0.525)I_3 + 1.939(\pm0.791)I_4 \\ & + 3.573(\pm1.074)\end{aligned} \tag{3}$$

$$n = 32,\ r = 0.923,\ r_{cv}^2 = 0.74,\ s = 0.46,$$

$$F_{6,25} = 24.01(3.63),\ (^1\chi_R^v)_{opt} = 2.03$$

In this equation, n is the number of data points, r is the correlation coefficient, s is the standard

TABLE I A series of Na^+, K^+- ATPase inhibitors (**4**) and their physicochemical and structural parameters

Compound	R.	$^1\chi^v$	log P	MR	MV	pol	I_1	I_2	I_3	I_4
1	(E) CH═N─OH	0.792	3.63	91.35	251.3	36.21	–	–	–	–
2	(E) CH═N─OCH_3	1.182	4.21	96.32	275.7	38.18	–	–	–	–
3	(E) CH═N─$O(CH_2)_2N(CH_3)_2$	2.773	5.05	113.91	333.8	45.16	–	–	–	1
4	(E) CH═N─$O(CH_2)_3N(CH_3)_2$	3.273	4.43	118.52	349.9	46.98	–	–	–	1
5	(E) CH═N─$O(CH_2)_4N(CH_3)_2$	3.773	5.60	123.13	365.9	48.81	–	–	–	1
6	(E) CH═N─$O(CH_2)_2NH_2$	1.971	4.77	103.03	289.4	40.84	–	–	1	–
7	(E) CH═N─$O(CH_2)_3NH_2$	2.471	3.89	107.63	305.5	42.67	–	–	1	–
8	(E) CH═N─$O(CH_2)_4NH_2$	2.971	5.47	112.24	321.5	44.49	–	–	1	–
9	(E) CH_2CH═N─$O(CH_2)_2N(CH_3)_2$	3.182	4.43	118.52	349.9	46.98	–	–	–	1
10	(E,Z) $(CH_2)_2$CH═N─$O(CH_2)_2N(CH_3)_2$	3.682	5.49	123.13	365.9	48.81	–	–	–	1
11	(E,Z) $(CH_2)_2$CH═N─$O(CH_2)_2NH_2$	2.879	5.45	112.24	321.5	44.49	–	–	1	–
12	(E,E) CH═CHCH═N─OCH_3	1.849	4.61	105.53	307.9	41.83	1	–	–	–
13	(E,E) CH═CHCH═N─$O(CH_2)_2CH_3$	2.937	5.55	114.75	340.0	45.49	1	–	–	–
14	(E,E) CH═CHCH═N─$O(CH_2)_2OH$	2.546	4.24	111.18	322.0	44.07	1	–	–	–
15	(E,E) CH═CHCH═N─$O(CH_2)_2$ $N(CH_3)_2$	3.440	5.00	123.13	365.9	48.81	1	–	–	1
16	(E,E) CH═CHCH═N─$O(CH_2)_2NH_2$	2.638	4.88	112.24	321.5	44.49	1	–	1	–
17	(E,E)CH═C(CH_3)CH═N─OCH_3	2.260	5.21	109.96	323.0	43.59	1	–	–	–
18	(E,E)CH═C(CH_3)CH═N─$O(CH_2)_2OH$	2.956	4.76	115.61	337.2	45.83	1	–	–	–
19	(E,E)CH═C(CH_3)CH═N─$O(CH_2)_2N(CH_3)_2$	3.851	5.66	127.56	381.1	50.56	1	–	–	1
20	(E,E)CH═C(CH_3)CH═N─$O(CH_2)_2NH_2$	3.048	5.65	116.67	336.7	46.25	1	–	1	–
21	(E,E)CH═C(CH_3)CH═N─$O(CH_2)_3NH_2$	3.548	6.07	121.28	352.8	48.07	1	–	1	–
22	(E,E)CH═C(CH_3)CH═N─$O(CH_2)_4NH_2$	4.048	6.52	125.89	368.8	49.90	1	–	1	–
23	(E,E,E)$(CH═CH)_2$CH═N─$O(CH_2)_2N(CH_3)_2$	4.107	5.22	127.56	381.1	50.56	–	–	–	1
24	CH_2NHOCH_3	1.299	3.50	99.52	315.6	39.45	–	1	–	–
25	$CH_2NHO(CH_2)_2N(CH_3)_2$	2.890	3.71	117.14	372.3	46.43	–	1	–	1
26	$CH_2NHO(CH_2)_2NH_2$	2.088	3.25	107.69	335.8	42.69	–	1	1	–
27	$CH_2NHO(CH_2)_3NH_2$	2.996	3.41	112.32	352.3	44.52	–	1	1	–
28	$(CH_2)_3NHO(CH_2)_2NH_2$	3.088	4.37	116.95	368.8	46.36	–	1	1	–
29	(E) CH═$CHCH_2NHOCH_3$	2.041	4.44	110.57	330.4	43.83	–	1	–	–
30	(E,E) $(CH═CH)_2(CH_2)_2NH_2$	2.650	4.28	118.09	345.5	46.81	1	–	1	–
31	(E,E) $(CH═CH)_2(CH_2)_3NH_2$	3.150	5.71	122.72	362.0	48.65	1	–	1	–
32	$(CH_2)_7NH_2$	3.742	6.08	129.88	386.6	47.92	–	–	1	–
33	CHO	0.569	2.54	91.46	269.4	36.25	–	–	–	–
34	$(CH_2)_2$CHO	1.477	3.07	99.00	314.2	39.25	–	–	–	–
35	(E) CH═CHCHO	1.236	4.03	100.78	296.0	39.95	–	–	–	–
36	(E) CH═C(CH_3)CHO	1.764	3.01	105.26	312.3	41.73	–	–	–	–

deviation, F is the F-ratio between the variances of calculated and observed activities, and the data within the parenthesis with ± sign are the 95% confidence intervals. The figure within the parenthesis following the F-value is the standard F-value of 99% level. This equation represents a very significant correlation between the inhibition potency of the compounds and $^1\chi^v_R$ and the indicator varaibles. The parabolic correlation between activity and $^1\chi^v_R$ suggests that initially there would be an increase in the activity with $^1\chi^v_R$ but this will reach a maximum when $^1\chi^v_R$ acquires an optimum value, $(^1\chi^v_R)_{opt}$ equal to 2.03. After this value of $^1\chi^v_R$, the activity will start decreasing. This puts a limit to the beneficial role of the length of the R-substituent (although χ accounts for all shape, size, length, and breadth, here it can be length only since all the substituents are mostly linear chains).

Cerri *et al.*[6] have pointed out that the most suitable substituents can be those that have a chain length of 6 atoms preceding the amine group. Such an ideal substituent in Table I is possessed by compound 7 which has its $^1\chi^v_R$ value around that 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, 30, which also have high activity but their $^1\chi^v_R$ is much greater than $(^1\chi^v_R)_{opt}$, suggesting that their activity should be much lower than that of compound 7. Here the role comes in of other structural features of the compounds that are described by our indicator parameters I_1, I_2, I_3, and I_4. We have characterized and quantified the effect of 4 structural features by these 4 parameters. As already defined, $I_1 = 1$ has been used for the substituents that have (E,E) isomerism, $I_2 = 1$ stands for the substituents where the iminic double bond has been reduced to the corresponding hydroxyl function, $I_3 = 1$ stands for the substituents that end with a primary amine group, and $I_4 = 1$ stands for the substituents that end with a tertiary amine group, $N(CH_3)_2$. Now with the positive coefficient of I_1, Equation (3) suggests that an (E,E) isomer will have a better effect than any other isomer. Similarly, with the positive coefficients of I_3 and I_4, the equation indicates that substituents having a primary or tertiary amine will be much better than those having any other group. However, a slightly higher coefficient of I_3 than that of I_4 suggests that the primary amine will have an edge over the tertiary amine. While the primary amine

TABLE II The observed and calculated biological activities of compounds of Table I

	$\log(1/IC_{50})$		$\log(1/EC_{50})$	
Compound	Obsd[a]	Calcd. Eq (3)	Obsd.[a]	Calcd. Eq (5)
1	4.00	4.31	–	–
2	4.49	4.81	–	–
3	6.00	5.84	–	–
4	6.60	5.84	5.89	5.89
5	5.70	5.68	5.33	5.77
6	6.80	6.68	6.21	6.60
7	7.70	6.93	6.74	7.00
8	6.30	7.02	5.54	5.55
9	6.20	5.85	5.74	5.89
10	7.00[b]	5.95	6.19	5.77
11	7.10	7.02	5.96	5.55
12	5.40	5.30	–	–
13	5.00	5.73	–	–
14	5.20	5.66	4.41	4.83
15	7.52[b]	6.69	7.24	7.26
16	7.70	6.85	7.30	7.04
17	5.00	5.55	–	–
18	5.10	5.73	4.64	4.91
19	7.22	5.51	7.15[c]	6.01
20	7.52	6.90	7.15	7.12
21	6.70	6.81	6.77[c]	5.67
22	5.90	6.56	5.40	5.54
23	5.20	5.48	4.70	4.51
24	4.10	4.02	–	–
25	4.30[b]	5.96	–	–
26	5.80	5.84	5.10[c]	6.50
27	6.40	6.10	5.41	5.31
28	6.00	6.10	4.92	5.47
29	4.40	4.64	–	–
30	7.10	6.85	6.42	6.59
31	6.49	6.89	7.46	6.74
32	5.90	6.86	–	–
33	4.60	3.98	4.96	4.94
34	4.40	5.12	–	–
35	6.60[b]	4.67	5.40	4.74
36	5.80	5.37	–	–

[a] Taken from Ref 6. [b] Not used in the derivation of Eq 3. [c] Not used in the derivation of Eq 5.

may give around 120 times better activity, the tertiary amine may give only around 90 times better activity than any other functional group. However, it is obvious that both groups are highly conducive to the inhibition potency of the compounds.

The negative coefficient of I_2, however, indicates that reduction of oximes to hydroxylamines is detrimental to the activity, hence oximes should be preferred, otherwise there can be about 4 times decrease in 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, the Na^+,K^+-ATPase inhibition activity was shown to have an excellent correlation with the dipole moment ($r = 0.95$).[9] Thus, not the only iminic bond, but other double bonds also present in R-substituents may be important. The importance of some other electronic features in the inhibition of Na^+,K^+-ATPase inhibition has also been discussed in a recent review.[10]

Cerri *et al.* have also reported for some of the compounds studied their inotropic activity (Table II) on electrically driven guinea pig left atrium in terms of EC_{50}, the concentration of the compound producing 50% of the maximal increase in force of contraction. For only oximes (compounds 4–26 in Table II), these authors reported a good correlation existing between EC_{50} and IC_{50} ($n = 17$, $r = 0.918$) but found that the correlation had significantly dropped when all the compounds including the oximes, hydroxylamines, alkenes, alkanes, and aldehydes of Table II were included ($n = 25$, $r = 0.460$; Table II has only 24 compounds with EC_{50} values but Cerri *et al.* had taken one more compound where at C-17 the attachment of the substituent was through a double bond but we have not considered such compounds in our correlations as they belong to a different series and were few in number). Notwithstanding the findings of Cerri *et al.*, we found a significant correlation existed between EC_{50} and IC_{50} for all the compounds of Table II except one (compound 22) which behaved as an outlier (Equation (4)). In Equation (4), the intercept is almost zero and the slope is near 1, hence there should not be much difference between EC_{50} and IC_{50} values, and consequently the IC_{50} values themselves can be taken as a good estimate of inotropic activity.

$$\log(1/EC_{50}) = 0.918(\pm 0.193)\log(1/IC_{50}) - 0.052(\pm 1.252) \quad (4)$$

$$n = 23,\ r = 0.907,\ s = 0.39,\ F_{1,21} = 97.44(8.02)$$

The inotropic activity, however, has been found to be correlated with physicochemical and indicators parameters as

$$\log(1/EC_{50}) = 133.130(\pm 74.306)\text{pol} - 52.874(\pm 29.437)\text{MR} + 1.489(\pm 0.544)I_1 + 2.343(\pm 0.649)I_3 + 3.238(\pm 1.003)I_4 + 14.840(\pm 4.057) \quad (5)$$

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

This correlation does not exhibit any effect on inotropic activity of the molecular connectivity of the R-substituent nor does it show any negative effect of reduction of oximes to hydroxylamines (absence of I_2). However, as in the case of Na^+,K^+-ATPase inhibition activity, the beneficial roles of (E,E) isomers and the amine groups of the substituents are also exhibited in inotropic activity. It is, however, to be noted that while in the case of

Na^+,K^+-ATPase inhibition the primary amine dominates over the tertiary amine, it is just the reverse in the case of inotropic activity. This difference can be assumed to be due to the different conformations of the enzyme molecules with which IC_{50} and EC_{50} were measured. The IC_{50} was measured with purified Na^+,K^+-ATPase isolated from dog kidney and the EC_{50} was measured on the whole guinea pig atrium. Since EC_{50} has been found to be linearly related to IC_{50} (Equation (4)), the inotropic activity seems necessarily to be a result of the inhibition of Na^+,K^+-ATPase and particularly the same isoform of the enzyme (α1) that is contained in the dog kidney; the guinea pig atria contains α1 and α3. Hence should the enzyme may be same with which IC_{50} and EC_{50} could be measured, the difference in the roles of primary and tertiary amines in the two systems can be attributed to only the difference in their conformations. This conformational difference may also be the reason for the absence in the inotropic activity of the negative effect of the reduction of the oximes to corresponding hydroxyl amines and of any role of molecular connectivity of the R-substituents.

We further find from Equation (5) that the polarizability of the molecule can be a major controlling factor of inotropic activity, but a very bulky molecule may be detrimental to it due to some steric effect, as there appears a molar refractivity term with a negative coefficient. Since the molar refractivity term is related to molar volume, its negative effect may be attributed to a size effect through a steric role. This steric role of the molecule may also be attributed to the conformation of the enzyme molecule.

The log P and MV parameters that were also calculated assuming that they might also play some role were found to be of little importance in this study.

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