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# A QSAR study on a series of simplified digitalis-like compounds acting on $Na^+,K^+$-ATPase

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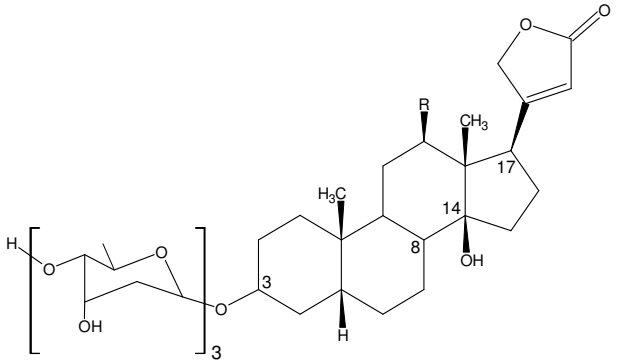
Among the cardiotonics (agents against congestive heart failure), the most important group is of the digitalis cardiac glycosides, but since these compounds suffer from a low therapeutic index, attention has been paid to investigating safer cardiotonic agents through the inhibition of $Na^+,K^+$−ATPase, the mechanism by which the digitalis cardiac glycosides elicit their action. Recently, a series of perhydroindenes were studied for their $Na^+,K^+$−ATPase inhibition activity. We report here a QSAR study on them to investigate the physicochemical and structural properties of the molecules that govern their activity in order to rationalize the structural modification to have more potent drugs. A multiple regression analysis reveals a significant correlation between the $Na^+,K^+$−ATPase inhibition activity of the compounds and Kier's first order valence molecular connectivity index of their $R_5$-substituents and some indicator parameters, suggesting that the $R_5$-substituents of the compounds containing atoms with low valence and high saturation and the $R_1$-substituents having =N−O− moiety will be conducive to the activity.

**Keywords:** Quantitative structure-activity relationship, $Na^+,K^+$−ATPase inhibitors, Perhydroindenes, Cardiotonic agents

Among the cardiotonics (agents against congestive heart failure), the most important group is of digitalis cardiac glycosides, although these glycosides have low therapeutic index due to cardiac pro-arrhythmogenic activity[1]. These glycosides elicit their effect through the inhibition of the enzyme $Na^+,K^+$−ATPase. Extensive studies on these glycosides have established the clinical applicability of two compounds: digoxin (**1**) and digitoxin (**2**). However, since these compounds suffer from a low therapeutic index, attention has been paid to investigating safer cardiotonic agents through the inhibition of $Na^+,K^+$−ATPase[2-8].

Cardiac glycosides are composed of three major components: a steroid ring system, a five- or six-membered lactone moiety, and 1 to 4 sugar residues. The sugars modify the water and lipid solubility of the glycoside molecules and affect their potency and duration of action. These cardiac glycosides are also referred to by the term *digitalis genin* or simply by *digitalis*. Efforts have been made to simply the digitalis skeleton in order to find simple compounds to act as cardiotonics. An encouraging effort has been made to synthesize the compounds with a hydrindane skeleton (**3**), which preserve the most distinctive part of the digitalis skeleton, *i.e*., C- and D-rings with a *cis* junction[9,10]. Cerri *et al.*[5] have also synthesized some 17β-aminoalkyloxime derivatives of the digitalis skeleton (**4**), which exhibit high inhibitory activity on $Na^+,K^+$−ATPase and high inotropic potency on guinea pig atrium; some of these compounds have been found to be more potent even the digoxin (**1**).




**1.** R=OH (digoxin); **2.** R=H (digitoxin)

The observation that replacement of butenolide ring with aminoalkyloxime chain could be advantageous has prompted Cerri *et al.*[7] to try such replacement on hydrindane skeleton (**3**) and synthesize a series of 1-(O-aminoalkyloximes) of perhydroindene derivatives as shown in Table 1.

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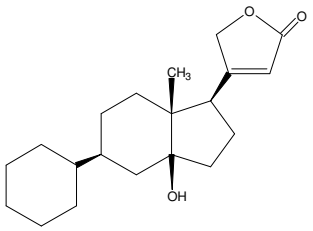
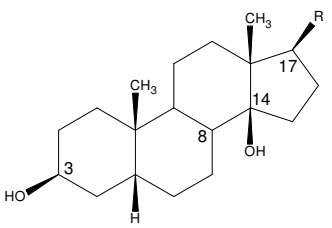



**3**




**4**

We have attempted here a quantitative structure-activity relationship (QSAR) study on this series of compounds in order to investigate the important features and the physicochemical properties of the compounds that govern their activity and thus to throw the light on their mechanism of interaction with the receptors.

## Materials and Methods

The perhydroindene derivatives and their $Na^+,K^+$–ATPase inhibition activities were reported by Cerri et al.[7] Among the various physicochemical and topological descriptors that were tried to derive the best QSAR model, Kier's first-order valence molecular connectivity index $^1\chi^v$ was found to be the most important parameter. Additionally, some indicator parameters were also found to govern the activity. These indicator parameters have been defined later. The $^1\chi^v$ was, however, calculated as follows[11]. According to Kier and Hall[11], it is defined as:

$$^1\chi^v = (\Sigma\ \delta_i^v\ \delta_j^v)^{-1/2} \qquad \ldots (1)$$

where $\delta_i^v$ and $\delta_j^v$ are the vertex connectivity indices of atom i and j, respectively, and the summation extends to all bonded pairs of non-hydrogenic atoms in the group or molecule. For the second and third rows of atoms, a unified definition of $\delta_i^v$, as expressed by Eq. (2)[12], is used. In this equation, $Z_i^v$ is the number of valence electrons of the atom i, $h_i$ is the number of hydrogen atoms attached to it, and $Z_i$ is its atomic number.

$$\delta_i^v = (Z_i^v - h_i) / (Z_i - Z_i^v - 1) \qquad \ldots (2)$$

## Results and Discussion

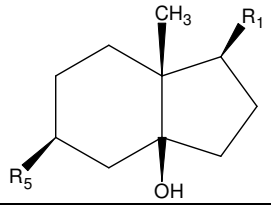
The whole data set contains 45 compounds. This set is divided into two subsets, the training and test sets. In the test set, the compounds are arbitrarily selected keeping in mind the wide structural diversity and span in the activity data. The compounds of test set are given with bold in Table 2. The remaining compounds are taken for the training set. A multiple regression analysis is performed on the training set and the best correlation that we could find is:

$$\log(1/IC_{50}) = 0.772\ (\pm 0.177)\ ^1\chi^v_{R5} - 1.060\ (\pm 0.488)\ I_1 - 0.895\ (\pm 0.402)\ I_2 - 0.275\ (\pm 0.253)\ I_3 + 3.579\ (\pm 0.504)$$
$$n = 33,\ r = 0.900,\ r^2_{cv} = 0.69,\ s = 0.32,\ F_{4,28} = 29.94(4.07) \qquad \ldots (3)$$

where $^1\chi^v_{R5}$ refers to the molecular connectivity of only $R_5$-substituent and $I_1$, $I_2$, and $I_3$ are three indicator variables. $I_1$ is used with a value of 1 for the first 5 compounds (Table 1), where the $R_5$-substituents are attached with the nucleus through a =CH– or a $–CH_2–$ bridge. Similarly, $I_2$ is used with a value of 1 for such $R_1$-substituents which do not contain =N–O– moiety. Such $R_1$-substituents are present in compounds 8, 9, 29 and 38 (Table 1). The last indicator variable $I_3$ is used with a value of unity for such compounds (compounds 23-38 in Table 1) that have $R_5$-substituent as $\beta$-$C_6H_{11}$.

We tried many other physiochemical parameters, but the best correlation that we could find is as shown by Eq. (3). In this equation, $IC_{50}$ refers to the minimum concentration of the compound leading to the 50% inhibition of $Na^+,K^+$–ATPase. Among the statistical parameters, *n* is the number of data points, *r* is the correlation coefficient, $r^2_{cv}$ is the square of cross-validated correlation coefficient obtained from leave-one-out (LOO) jackknife procedure, *s* is the standard deviation, *F* is the F-ratio between the variances of calculated and observed activities and the data within the parentheses with ± sign are 95% confidence intervals. The figure within the parenthesis following the F-value is the standard F-value at 99% level. The values of these statistical parameters in Eq. (3) show that the correlation obtained is quite significant. This correlation suggests that the $Na^+,K^+$–ATPase inhibition activity of this series of compounds is basically controlled by the molecular connectivity of $R_5$-substitutent. This molecular connectivity takes into account the nature (valence and unsaturation) of the atoms, their connectivity and size of the molecule. The presence of large number of atoms with low valence and high saturation leads to high value of $^1\chi^v$. Thus, a $R_5$-substitutent containing such atoms may have high inhibition activity. Such group may be more hydrophobic and thus may be involved in hydrophobic interaction with the receptor.

Table 1—A series of perhydroindene derivatives and their observed $Na^+,K^+$-ATPase inhibition activity[7]




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

[a]**4;** R = (E) $CH=NO(CH_2)_2N(CH_3)_2$.; [b]**4;** R = (E,E) $CH=CHCH=NO(CH_2)_2N(CH_3)_2$.; [c]**4;** R = (E,E) $CH=CHCH=NO(CH_2)_2NH_2$

Table 2—The physicochemical parameters and observed and calculated $Na^+,K^+$-ATPase inhibition activities of compounds of Table 1

| Compd | $1\chi^v_{R5}$ | $I_1$ | $I_2$ | $I_3$ | log(1/$IC_{50}$) | | |
|---|---|---|---|---|---|---|---|
| | | | | | Obsd[a] | Calcd., Eq.(3) | LOO |
| 1 | 2.44 | 1 | 0 | 0 | 4.30 | 4.40 | 4.52 |
| 2[c] | 2.44 | 1 | 0 | 0 | 5.10 | 4.40 | — |
| 3[c] | 3.60 | 1 | 0 | 0 | 4.00 | 5.29 | — |
| **4**[b] | 2.63 | 1 | 0 | 0 | **5.49** | **4.55** | — |
| 5 | 3.60 | 1 | 0 | 0 | 5.40 | 5.29 | 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**[b] | 2.16 | 0 | 1 | 0 | **4.70** | **5.56** | — |
| 10 | 2.56 | 0 | 0 | 0 | 5.30 | 4.35 | 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**[b] | 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[c] | 4.23 | 0 | 0 | 0 | 6.00 | 6.85 | — |
| 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**[b] | 3.13 | 0 | 0 | 1 | **5.80** | **5.72** | — |
| 24 | 3.13 | 0 | 0 | 1 | 5.49 | 5.72 | 5.74 |
| 25[c] | 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[c] | 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 | 7.40 | 7.26 |
| **43**[b] | 4.96 | 0 | 0 | 0 | **7.80** | **7.41** | — |
| **44**[b] | 4.36 | 0 | 0 | 0 | **7.20** | **6.95** | — |
| **45**[b] | 4.36 | 0 | 0 | 0 | **6.60** | **6.96** | — |

[a]Taken from ref. (7). [b]Test set compounds. [c]Not included in the derivation of Eqs. (3) and (6)-(8)

The validity of the correlation is judged by the value of its $r^2_{cv}$ which is calculated as:

$$r^2_{cv} = 1 - [\Sigma_i( y_{i, obsd} - y_{i, cald})^2 / \Sigma_i( y_{i, obsd} - \bar{y}_{obsd})^2] \quad \ldots (4)$$

where $y_{i, obsd}$, and $y_{i, cald}$ are the observed and calculated activity values of compound i, respectively and $\bar{y}_{obsd}$ is the average of the observed activities of all compounds used in the correlation. The correlation

is supposed to be valid if $r^2_{cv} > 0.60$. From this point of view, the correlation expressed by Eq. (3) seems to be quite valid. However, the predictive ability of any correlation equation is measured by using it to predict the activity of the compounds in the test set and calculating the value of $r^2_{pred}$, which is defined as:

$$r^2_{pred} = 1 - [\Sigma_i( y_{i, obsd} - y_{i, pred})^2 / \Sigma_i( y_{i, obsd} - \bar{y}_{obsd})^2] \quad \ldots (5)$$

where $y_{i, pred}$ is the predicted activity of compound i. A value of $r^2_{pred} > 0.5$ signifies a good predictive ability of the correlation. For Eq (3), $r^2_{pred}$ value is as high as 0.85. The activity values predicted from this equation for test set compounds are given (in bold) in Table 2. A comparison shows that these predicted values are in very good agreement with the corresponding observed ones. In the training set also, the calculated values are found to be in excellent agreement with the observed ones. All these observations can be better visualized in the graphs drawn between the calculated/predicted and observed activities (Figs 1 and 2).

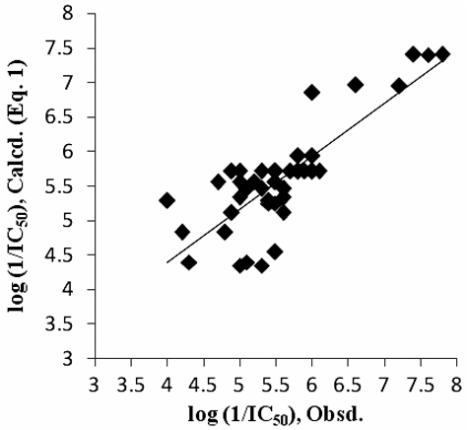



Fig. 1—A plot of calculated activity vs observed activity for training set compounds

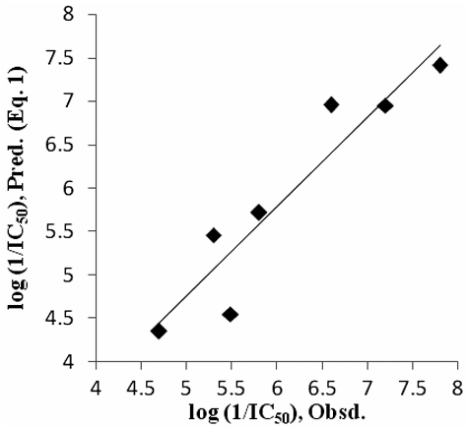



Fig. 2—A plot of predicted activity vs observed activity for test set compounds

All the three indicator parameters $I_1$, $I_2$, and $I_3$ have negative coefficients in the correlation, suggesting that some $R_1$- and $R_5$-substitutents of particular nature will not be conducive. For $R_5$-substituents, while $I_1$ indicates that any substituent with an alkyl or alkene bridge will not be tolerated, $I_5$ indicates that a $\beta$-$C_6H_{11}$ substituent will also not be tolerated. Probably such substituents might create steric problems. In $R_1$-substituents, $I_2$ suggests that substituents other than those containing =N–O– moiety will be deleterious to the activity. It indicates that =N–O– moiety may have some specific role because of its electronic nature. May be it participates in some dipole-dipole interaction with the receptor. All the three indicator parameters are statistically quite significant in the correlation. If they are removed one by one from the correlation, the significant reductions in the values of statistical parameters are successively observed (Eqs. 6-7).

$$\log (1/IC_{50}) = 0.711 (\pm 0.179)\,{}^1\chi^v_{R5} - 0.944 (\pm 0.507)\, I_1 - 0.976 (\pm 0.421) I_2 + 3.646 (\pm 0.532)$$
$$n = 33,\ r = 0.881,\ r^2_{cv} = 0.63,\ s = 0.34,$$
$$F_{3,29} = 33.66\ (4.54) \quad \ldots (6)$$

$$\log (1/IC_{50}) = 0.726 (\pm 0.234)\,{}^1\chi^v_{R5} - 0.852 (\pm 0.661)\, I_1 + 3.509 (\pm 0.693)$$
$$n = 33,\ r = 0.777,\ r^2_{cv} = 0.54,\ s = 0.44,$$
$$F_{2,30} = 22.84\ (5.39) \quad \ldots (7)$$

$$\log (1/IC_{50}) = 0.711(\pm 0.254)\,{}^1\chi^v_{R5} + 3.500 (\pm 0.755)$$
$$n = 33,\ r = 0.716,\ r^2_{cv} = 0.44,\ s = 0.48,$$
$$F_{1,31} = 32.55(7.53) \quad \ldots (8)$$

In deriving Eqs (3) and (6) to (8), however, certain compounds as indicated in Table 2 with superscript 'c' (compds 2,3,20,25,28 ) are not included as they exhibit aberrant behavior. All these compounds, except 2 have their observed activities much lower as compared to their corresponding activity values calculated from Eq. (3). The reasons for such discrepancies are not hard to find. Let us take one by one. 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 is with $\alpha$-configuration. The same $R_5$-substituent attached with $\beta$-configuration appears to be more conducive (Compd 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 may be attributed to

Table 3— Correlation matrix showing the mutual correlations among the variables used

| | $^1\chi^v_{R5}$ | $I_1$ | $I_2$ | $I_3$ |
|---|---|---|---|---|
| $^1\chi^v_{R5}$ | 1.000 | – 0.111 | 0.090 | – 0.314 |
| $I_1$ | | 1.000 | 0.035 | 0.218 |
| $I_2$ | | | 1.000 | – 0.183 |
| $I_3$ | | | | 1.000 |

their $R_1$-substituents containing the longest primary amine (with $–(CH_2)_4–$ moiety). The optimal length of the primary amine appears to be with $–(CH_2)_3–$ moiety, as exemplified by compound 27. Thus, these outliers indicate what kind of substituents may not be favorable to the inhibition potency of the compounds.

The specialty of Eq.(3) is that it has also very well accommodated the last two compounds (41 and 42) of Table 1, which do not belong to perhydroindene series of this table. As shown in Table 3, the variables used in deriving Eq.(3) have no significant mutual correlation.

## Conclusion

From this study we find that the $Na^+$,$K^+$–ATPase inhibition activity of the compounds is a significant function of molecular connectivity of $R_5$-substituents. The large $R_5$-substituents having atoms with low valence and high saturation may be quite conducive to the activity. In $R_1$-substituents, the =N–O– moiety is shown to be quite crucial. Its replacement by =N–N= will not be beneficial. Also, a very large $R_1$-substituent is not suggested to be conducive to the inhibition activity of the compounds.

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