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# Topological estimation of proton–ligand formation constants of potential antitumour agents: Salicylhydroxamic acids

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**Abstract.** Proton–ligand formation constants of salicylhydroxamic acids (SHA) and their nuclear substituted derivatives have been estimated topologically using the normalized Wiener index, referred to as mean square Wiener index (Wms). Regression analysis of the data indicates that Wms can be used successfully for estimating and monitoring proton-ligand formation constants.

**Keywords.** Salicylhydroxamic acids; antitumour agents; topological indices; normalized Wiener index; mean square Wiener index.

## 1. Introduction

Selective inhibition by salicylhydroxamic acid (SHA) of deoxyribonucleic acid (DNA) synthesis in *Ehrlich ascites* tumour cells has been reported[1]. Characteristics of the inhibition were similar in some respects to those of hydroxyureas and of oxamylhydroxyamic acids[2,3]. Effects on the synthesis of DNA and of protein were nominal and were considered to be of secondary nature as a consequence of the lower rate of DNA formation. The inhibition of SHA was further evident immediately upon adding the compound to the cells, that is, no preincubation was necessary to evoke this effect. The rate of DNA synthesis resembled the control rate upon removal of the inhibitor by washing the cells, indicating no firm binding to the cells and no irreversible alteration of the cell by the compounds.

Gale and Hyenes[4] have shown that the structural features of compounds related to SHA influence the course of nucleic acid synthesis in a tumour-cell test system.

The physico-chemical and pharmacological potential of salicylhydroxamic acid and its nuclear substituted derivatives is determined by their corresponding protonation constants. These protonation constants in turn can be correlated with some of the topological indices.

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Prompted by the aforementioned observations [5], salicylhydroxamic acid and its nuclear substituted derivatives were synthesized and their protonation constants were assessed.

Application of topology and graph theory has indicated that the structure of an organic molecule acting as a drug can be represented by a number like the numerical presentation of their physico-chemical and pharmacological properties [5,6]. The molecular topological approach in quantitative structure-activity relationship (QSAR) essentially involves translation of molecular structures into characteristic numerical descriptors known as topological indices for predicting pharmacological activities. The advantage of topological indices is that they may be used directly in QSAR.

In view of the above, the work herein described was initiated for estimating the proton–ligand formation constants of salicylhydroxamic acids from the normalized Wiener index named as mean square Wiener indices (Wms). Our earlier study [4] indicated that the normalized Wiener index gives better results than the Wiener index itself. Different types of normalized Wiener indices can be obtained by imposing different types of normalization conditions. The definition of the normalized Wiener index used in the present study and its potential in modelling the proton–ligand formation constants of SHA derivatives is described below.

## 2. Methodology used

As stated above, it is known that certain invariants of molecular graphs, usually referred to as topological indices, can be used for establishing QSAR of interest in pharmacology. A number of successful QSAR studies were made [4] based on Wms by means of which we can determine the ways in which the structural features of SHA and SHA derivatives influence the course of nucleic acid synthesis in a tumour cell test system.

According to graph theory and topology [7,8], molecular structure of the organic molecules is transformed into a molecular graph wherein atoms constituting the molecules are termed as vertices and bonds joining the atoms are termed as edges. The vertices (atoms) are depicted by a dot (.) or a small circle (O), while the edges (bonds) are represented by lines (–). In doing so, all the carbon–hydrogen bonds are suppressed. Such molecular graphs are called hydrogen-suppressed molecular graphs or simply molecular graphs.

The molecular structure and molecular graph of the parent salicylhydroxamic acid (SHA) are as shown in figure 1.

It is worth recording that in the molecular graph the distance between the two vertices (atoms) is equal to the number of edges (bonds) in the shortest unit path connecting the respective vertices (atoms). These distances[9,10] are defined as the elements of a real $N \times N$ matrix $D(G)$, which is known as the distance matrix, where $N$ is the number of vertices (atoms) in molecular graph $G$. The elements of the distance matrix, $D_{ij}(G)$, are integers, for $i, j$ neighbours and the matrix is given by the expression:

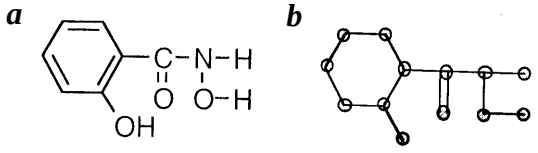


**Figure 1.** Molecular structure (**a**) and molecular graph (**b**) of SHA.

$$D_{ij}(G) = \begin{cases} 1, \text{ for } i \neq j, \\ 0, \text{ for } i = j. \end{cases} \quad (1)$$

The distance matrix is symmetric in relation to the principal diagonal and thus can be divided into two triangular off-diagonal submatrices. The total number of elements [11] in a triangular off-diagonal submatrice is equal to $\frac{1}{2} N (N - 1)$.

The mean-square Wiener index (Wms) is defined as the mean of the square of the elements $D_{ij}(G)$ of the off-diagonal submatrix:

$$\text{Wms} = \frac{1}{N(N-1)} \sum_{ij} D_{ij}^2 . \quad (2)$$

Experimental protonation constants of SHA and its derivatives are determined by a potentiometric method proposed earlier [12,13].

It should be mentioned that SHA has the formula given in figure 1 and is expected to dissociate in two steps:

$$H_2A \rightleftharpoons H^+ + HA^-, \quad (3)$$

$$HA^- \rightleftharpoons H^+ + A^{2-}. \quad (4)$$

In the dianion $A^{2-}$, both the hydroxamic and the phenolic OH groups lose a proton. In the monoanion form the proton is dissociated from the hydroxamic function (NHOH). There is equilibrium between the two forms, each of the monoanionic species being stabilized by very strong intramolecular hydrogen bonds.

Hence, the proton–ligand formation equilibrium can be expressed as shown below and the logarithmic representation of their formations is given by their respective equilibrium constants (i.e. $\log pk_1^{\text{H}}$ and $\log pk_2^{\text{H}}$ respectively.

$$H^+ + A^{2-} \rightleftharpoons HA^-, \quad (5)$$

$$HA^- + H^+ \rightleftharpoons H_2A. \quad (6)$$

## 3. Results and discussion

SHA and its derivatives, their abbreviations and their protonation constants are recorded in table 1. Values of Wms as calculated from (2) for SHA-derivatives are also summarized in table 1. Regression parameters for the correlations of Wms with the proton-ligand formation constants are given in table 2. Table 3 records the experimental and estimated proton–ligand formation constants together with the difference between their experimental and estimated values.

In table 1 are also listed the logarithms of the so-called [12,13] practical protonation constants $pK_1^{\text{H}}$ and $pK_2^{\text{H}}$ of the respective salicylhydroxamic acids. The protonation constants for SHA-derivatives pertain to the equilibria below:

$$pK_1^{\text{H}} = \frac{[HA^-]}{[A^{2-}][H^+]}, \quad (7)$$

**Table 1.** SHA derivatives, their abbreviations, mean square Wiener index and experimental protonation constants ($\log pK_1^H$ and $\log pK_2^H$).

| Compound | Abbrev. | Mean square Wiener index | Protonation constants | |
|---|---|---|---|---|
| | | | $\log pK_1^H$ | $\log pK_2^H$ |
| Salicylhydroxamic acid | SHA | 55·16 | 9·28 | 6·18 |
| 3-Amino-salicylhydroxamic acid | 3-ASHA | 83·38 | 7·68 | 4·96 |
| 5-Amino-salicylhydroxamic acid | 5-ASHA | 87·54 | 7·68 | 4·96 |
| 3,5-Diamino-salicylhydroxamic acid | 3,5-DASHA | 114·20 | 7·68 | 4·96 |
| 3-Bromo-salicylhydroxamic acid | 3-BSHA | 62·02 | 7·29 | 4·18 |
| 5-Bromo-salicylhydroxamic acid | 5-BSHA | 63·72 | 7·29 | 4·18 |
| 3,5-Dibromo-salicylhydroxamic acid | 3,5-DBSHA | 70·05 | 6·90 | 3·40 |
| 3-Chloro-salicylhydroxamic acid | 3-CSHA | 62·02 | 7·31 | 4·22 |
| 5-Chloro-salicylhydroxamic acid | 5-CSHA | 63·72 | 7·31 | 4·22 |
| 3,5-Dichloro-salicylhydroxamic acid | 3,5-DCSHA | 70·05 | 6·94 | 3·48 |
| 3-Fluoro -salicylhydroxamic acid | 3-FSHA | 62·02 | 7·34 | 4·28 |
| 5-Fluoro-salicylhydroxamic acid | 5-FSHA | 63·72 | 7·34 | 4·28 |
| 3,5-Difluoro-salicylhydroxamic acid | 3,5-DFSHA | 70·05 | 7·00 | 3·60 |
| 3-Iodo-salicylhydroxamic acid | 3-ISHA | 62·02 | 7·33 | 4·26 |
| 5-Iodo-salicylhydroxamic acid | 5-ISHA | 63·72 | 7·33 | 4·26 |
| 3,5-Diiodo-salicylhydroxamic acid | 3,5-ISHA | 70·05 | 6·98 | 3·56 |
| 3-Methyl-salicylhydroxamic acid | 3-MSHA | 62·02 | 7·74 | 5·08 |
| 5-Methyl -salicylhydroxamic acid | 5-MSHA | 63·72 | 7·74 | 5·08 |
| 3,5-Dimethyl-salicylhydroxamic acid | 3,5-DMSHA | 70·05 | 7·80 | 5·20 |
| 3-Methoxy-salicylhydroxamic acid | 3-MeSHA | 73·22 | 7·57 | 4·74 |
| 5-Methoxy-salicylhydroxamic acid | 5-MeSHA | 78·75 | 7·57 | 4·74 |
| 3,5-Dimethoxy-salicylhydroxamic acid | 3,5-DMeSHA | 95·32 | 7·46 | 4·52 |
| 3-Nitro-salicylhydroxamic acid | 3-NSHA | 83·38 | 6·94 | 3·48 |
| 5-Nitro-salicylhydroxamic acid | 5-NSHA | 87·54 | 6·94 | 3·48 |
| 3,5-Dinitro-salicylhydroxamic acid | 3,5-DNSHA | 114·20 | 6·20 | 2·00 |

**Table 2.** Data for linear regression between the logarithm of protonation constants and Wms.

| | $i$ | $a_i$ | $b_i$ | Standard deviation | Correlation coefficient |
|---|---|---|---|---|---|
| $\log pK_1^H$ | 1 | – 0·0901 | 13·000 | 0·0900 | – 0·9056 |
| | 2 | – 0·0025 | 7·6210 | 0·0870 | – 0·9062 |
| $\log pK_2^H$ | 1 | – 0·0403 | 6·6648 | 0·0910 | – 0·9296 |
| | 2 | – 0·0050 | 5·3241 | 0·2118 | – 0·8953 |

$$pK_2^H = \frac{[H_2A]}{[HA^-][H^+]}. \tag{8}$$

The $pK_1^H$ and $pK_2^H$ values were determined earlier [3], by means of previously described experimental techniques [12,13]. It is observed that when Wms is correlated with each of the

**Table 3.** Observed and estimated protonation constants for SHA derivatives.

| | $\log pK_1^H$ | | | $\log pK_2^H$ | | |
|---|---|---|---|---|---|---|
| Compound | Observed | Estimated | Residue | Observed | Estimated | Residue |
| SHA | 9·28 | Outlier | – | 6·18 | Outlier | – |
| 3-ASHA | 7·68 | 7·65 | 0·03 | 4·96 | 4·90 | 0·06 |
| 5-ASHA | 7·68 | 7·64 | 0·04 | 4·96 | 4·88 | 0·08 |
| 3,5-DASHA | 7·68 | 7·57 | 0·11 | 4·96 | 4·75 | 0·21 |
| 3-BSHA | 7·29 | 7·41 | 0·12 | 4·18 | 4·16 | 0·02 |
| 5-BSHA | 7·29 | 7·25 | 0·04 | 4·18 | 4·09 | 0·09 |
| 3,5-DBSHA | 6·90 | 6·68 | 0·22 | 3·40 | 3·84 | – 0·44 |
| 3-CSHA | 7·31 | 7·41 | 0·10 | 4·22 | 4·16 | 0·06 |
| 5-CSHA | 7·31 | 7·25 | 0·06 | 4·22 | 4·09 | 0·13 |
| 3,5-DCSHA | 6·94 | 6·69 | 0·25 | 3·48 | 3·84 | – 0·36 |
| 3-FSHA | 7·34 | 7·41 | – 0·41 | 4·28 | 4·16 | 0·12 |
| 5-FSHA | 7·34 | 7·25 | 0·09 | 4·28 | 4·09 | 0·19 |
| 3,5-DFSHA | 7·00 | 6·69 | 0·31 | 3·60 | 3·84 | – 0·24 |
| 3-ISHA | 7·33 | 7·41 | – 0·08 | 4·26 | 4·16 | 0·10 |
| 5-ISHA | 7·33 | 7·25 | 0·08 | 4·26 | 4·09 | 0·17 |
| 3,5-ISHA | 6·98 | 6·69 | 0·29 | 3·56 | 3·84 | – 0·28 |
| 3-MSHA | 7·74 | 7·70 | 0·04 | 5·08 | 5·01 | 0·07 |
| 5-MSHA | 7·74 | 7·70 | 0·04 | 5·08 | 5·00 | 0·08 |
| 3,5-DMSHA | 7·80 | 7·68 | 0·12 | 5·20 | 4·97 | 0·23 |
| 3-MeSHA | 7·57 | 7·67 | – 0·10 | 4·74 | 4·95 | – 0·21 |
| 5-MeSHA | 7·57 | 7·66 | – 0·09 | 4·74 | 4·93 | – 0·19 |
| 3,5-DMeSHA | 7·46 | 7.62 | – 0.16 | 4·52 | 4·84 | – 0·32 |
| 3-NSHA | 6·94 | 5.48 | 1.46 | 3·48 | 3·30 | 0·18 |
| 5-NSHA | 6·94 | 5.11 | 1.83 | 3·48 | 3·13 | 0·35 |
| 3,5-DNSHA | 6·20 | 2·71 | 3·49 | 2·00 | 2·06 | – 0·06 |

proton–ligand formation constants, the point belonging to SHA deviate considerably from the linear plots, whereas the other points are grouped into two families, I and II. In the first family are included all the halogen-substituted derivatives together with nitro-derivatives. The other family (II) contains amino-, methyl- and methoxy-derivatives of SHA. Statistical analysis has indicated that for both the families, good correlations are obtained.

Hence, the following expressions can be used,

$$\log pK_1^H = a_1 \mathrm{Wms} + b_1, \tag{9}$$

for amino-, methyl- and methoxy-derivatives of SHA and

$$\log pK_2^H = a_2 \mathrm{Wms} + b_2, \tag{10}$$

for fluoro-, chloro-, bromo-, iodo- and nitro-substituted SHA.

Calculated values of the coefficients $a_1$ and $b_1$, $i = 1, 2$ in (9) and (10), as well as the data showing the quality of respective correlations are given in table 2. Perusal of table 2

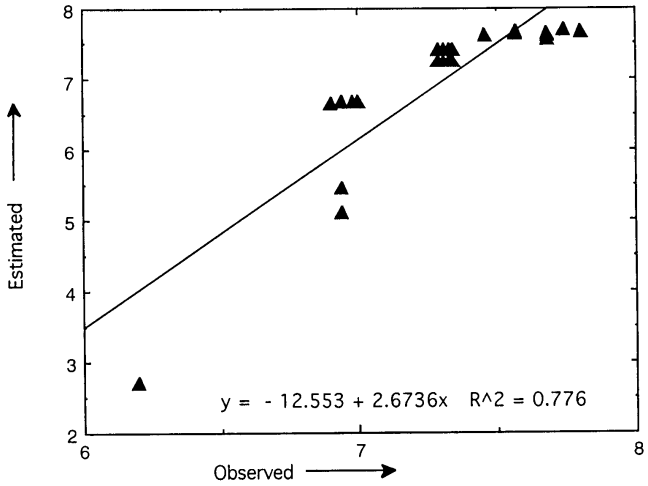


**Figure 2.** Correlation of observed and estimated log$pK_1^H$ of SHA derivatives used in the present study.

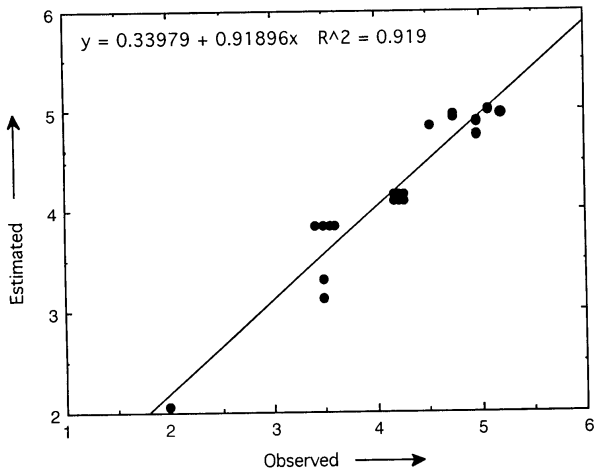


**Figure 3.** Correlation of observed and estimated log$pK_2^H$ of SHA derivatives used in the present study.

indicates that the proton–ligand formation constants (log$pK_1^H$ and log$pK_2^H$) for SHA-derivatives can be accurately estimated by means of Wms.

Recent work by us [14,15] indicates that the pharmacological properties of SHA-derivatives are a function of their protonation constants. In view of this and on the basis of the results obtained in the present study, it is possible to use Wms to infer the pharmacological activities of SHA-derivatives.

It is worth mentioning that the categorising of the correlations into two families (I and II) is due to $\pi$-electron-donating and -withdrawing effect of the respective substituents.

Electron-donating substituents (amino, methyl, methoxy) form the family II while electron-withdrawing substituents (nitro, halogens) belong to family I. It is worthy of mention that the amino- and methoxy-groups are typical $\pi$-donors, whereas the methyl-group increases the electron density in the aromatic nucleus due to hyperconjugation.

Final evidence in favour of our result is obtained by plotting estimated proton–ligand formation constants against their observed values. Such plots are shown in figures 2 and 3 respectively for $\log pK_1^H$ and $\log pK_2^H$. Results show that the proton–ligand formation constants of the nuclear substituted SHA-derivatives are sensitive to the number, position and shape of the substituents, as reflected by their Wms values.

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