THE USE OF COMPETITIVE LIGAND BINDING
RESULTS IN QSAR STUDIES

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SUMMARY. — *The use in QSAR studies of parameters measuring the ability of an unlabeled molecule to compete for the binding site with a radioligand is examined.*

It is shown that in order to employ them within a recently developed formal model it is previously necessary to carry out a linear transformation. This necessarily demands the adoption by investigators of a standard measuring procedure.

RIASSUNTO. — *Viene considerata la possibilità di applicare in relazioni quantitative struttura chimica/attività (QSAR) grandezze in grado di misurare la capacità di una molecola non marcata di competere con un legante marcato per il* sito *recettoriale.*

Viene dimostrato che per usare tali grandezze è necessaria una preliminare trasformazione lineare. Ciò richiede l'adozione di un procedimento standard per la loro misura.

Recently (1), it has been shown that, if we consider a weak interaction between a drug D, and a macromolecular receptor, the following formula accounts for the equilibrium constant, K:

$$\log(K_i) = A + B \log(M_{D_i}) + C \log(\sigma_{D_i}) + D \log(ABC)_{D_i} + E\Delta\varepsilon_i \qquad [1]$$

where A, B, C, D, E are constants, M, σ, and ABC are, respectively, the mass, the symmetry number and the product of the three moments of inertia about the three principal axes of rotation, "i" refers to the i-th drug molecule and $\Delta\varepsilon_i$ is the difference between the ground-state energy of the complex and the ground-state energies of the drug and the receptor. As the molecular structure of the receptor is normally not known, $\Delta\varepsilon_i$ is calculated in an approximate way (1).

We must note that eq. [1] expresses a physically-based functional relation between $\log(K_i)$ and the drug's molecular-electronic properties,

that is originated from statistical-mechanical and quantum-mechanical laws. Eq. [1] must not be confounded with a purely statistical correlation problem. Here, statistical analysis is employed, not to see if there is a good equation, but to find the best one.

This method has given excellent results when applied to indole derivatives (2), phenylalkylamines (3, 4), carbamate insecticides (5) and MAO inhibitors (6).

Here we must keep in mind that the fitting of eq. [1] will give the same results if we work with the true K_i's or with values obtained through linear transformations of them.

In recent years, investigations on the specificity of the receptor binding have often measured the ability of an unlabeled molecule to compete for the binding site with a radioligand. The value generally reported is the IC_{50}, i.e., the concentration of non-labeled ligand at which the binding of the radioligand is decreased by 50%. This technique has been employed extensively with narcotic analgesics (7), benzodiazepines (8) and adrenergic agents (9). As the IC_{50} is not a true equilibrium constant, it cannot be formally employed in eq. [1].

In this note, we analyze the conditions necessary to carry out QSAR analysis with the IC_{50} instead of K in eq. [1]).

Let us consider a receptor saturation experiment using a radiolabeled ligand for the case of a fixed concentration of labeled ligand and increasing concentrations of unlabeled ligand. In has been shown atht the IC_{50} may be expressed as (10):

$$IC_{50} = \frac{K_i}{K^+} \left[\frac{[K^+/(1-f^+)] + (1-0.5\,f^+)\,R_t}{[K^+/(1-f^+)] + 0.5\,R_t} \right] \{ [K^+/(1-f^+)] + 0.5[1+K'/K_i]\,R_t \} \qquad [2]$$

where K, f^+, R_t and K^+ are, respectively, the drug-receptor equilibrium constant, the fractional saturation of the receptors by the labeled ligand in the absence of unlabeled competitor, the total concentration of receptors and the radioligand-receptor equilibrium constant.

If we consider a group of IC_{50}'s obtained with a series of experiments that were carried out with the same total quantity of receptors, with the same labeled ligand and with the same final concentration of labeled ligand, it follows that R_t, f^+ and K^+ are constants for the whole group of molecules under study. In these conditions, and using eq. [2], K_i may be written as:

$$K_i = \frac{IC_{50}\,(1-f^+)}{1+(1-f^+)\,(1-0.5\,f^+)\,R_t/K^+} - \frac{0.5\,R_t\,(1-f^+)}{1+0.5\,(1-f^+)\,R_t/K^+} = a\,IC_{50} - b \qquad [3]$$

where a and b are constants.

The inspection of eq. [3] reveals that we cannot transform the IC_{50} into K because we do not know the constants a and b. This problem can be avoided by the analysis of a and b. First, we must note that, as always $K_i > 0.0$, it follows that:

$$a \ IC_{50} - b > 0.0 \qquad [4]$$

This condition will impose a restriction to the possible values of a and b chosen for a given set of IC_{50}'s.

The examination of receptor saturation experiments (7-9) shows that in general $R_t < IC_{50}$, $0.1 \ nM \leqslant IC_{50} \leqslant 1000 \ nM$. With these data, we way use for example $K^+ = 1.0$, $R_t = 0.1 \ nM$ and $f^+ = 0.7$. This gives $a = 0.294$ and $b = 0.0147$, that we propose to employ as transformation constants in future QSAR studies. Note that it is possible to propose other values for a and b from the analysis of a given experiment. It must remain clear that the couple of constants proposed are arbitrary and that they only serve as transformation constants. If, in a given experiment, the values of R_t, f^+ or K^+ are determined, their values can be used to obtain values for a and b that also will be useful.

Anyway, this or any other similar choice will permit us to use eq. [3] to obtain a set of parametrized K_i values and to apply the formal quantum-mechanical model developped in ref. (1).

On the other hand, if we arbitrarily make $R_t \rightarrow 0$ in eq. [2], we get:

$$K_i = IC_{50} \, (1 - f^+) = \frac{IC_{50}}{1 + (f^+/K^+)} = Q \ IC_{50} \qquad [5]$$

where f^+ is the free concentration of the labeled ligand and Q is constant for the whole group of molecules considered. Eq. [5] is not rigorous, but is normally employed to calculate K (see refs. 11 and 12 for the mathematical formalism). Note that if we employ eq. [5] we can replace directly $\log(K_i)$ by $\log(IC_{50})$, probably obtaining misleading results.

As an example of the lack of a standard method to measure the IC_{50}, we may cite the case of narcotic agonists and antagonists. In different receptor saturation experiments with rat brain, using the same experimental technique (13), it was employed 1.5 nM of $[^3H]$-naloxone (13, 14), 2.6 nM of $[^3H]$-naloxone (15), 1.0 nM of $[^3H]$-naloxone (16) and 1.0 nM of $[^3H]$-diprenorphine (17) as final concentration of labeled ligand. From the above analysis, it is clear that it is not possible to use together the IC_{50} values reported in refs. 13 to 17 for a QSAR analysis. These facts indicate that it is absolutely necessary that the workers in this field define a group of standard experiments for use in all the laboratories.

The analysis presented here should open new areas for the application of the formal model exposed in ref. (1) to research on narcotics, benzodiazepines, dopamine and adrenergic molecules etc.

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