Monatshefte für Chemie 123, 487–491 (1992)

Monatshefte für Chemie
Chemical Monthly

QSAR of copper(II) complexes with cytotoxic properties

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Summary. A QSAR based on a multiple regression analysis for 15 copper(II) semi- and thiosemicarbazone complexes with cytotoxic properties is presented. "In vitro" cytotoxicity was selected as the dependent variable and Van der Waals volumes (*Vm*), octanol- water partition coefficients (log *P*), specific rate constants of the copper(II) complexes towards superoxide radicals (k_s) and variation in C=N vibration bands (ΔCN) in IR spectra of the complexes with respect to the free ligands were selected as the independent variables. The "stepwise regression" procedure and the "all possible regressions" were practiced in the analysis of the data. The orthogonality analysis proved noncollinearity among the variables. According to the obtained equation the two best copper(II) complexes were submitted to a broad "in vivo" screening study and resulted to be active against La, P-388 and L-1210 leukemias.

Keywords. QSAR; Thiosemicarbazones; Copper(II) complexes; Cytotoxicity; Antitumoral activity.

QSAR von Kupfer(II)-Komplexen mit cytotoxischen Eigenschaften

Zusammenfassung. Es wird eine quantitative Struktur-Aktivitäts-Beziehung basierend auf einer multiplen Regressionsanalyse für 18 Kupfer(II)- Semi- und Thiosemicarbazon-Komplexe präsentiert. Die „in vitro"-Cytotoxizität wurde als die abhängige Variable und Van-der-Waals-Volumina (*Vm*), Octanol-Wasser-Verteilungskoeffizienten (log *P*), spezifische Geschwindigkeitskonstanten der Kupfer(II)-Komplexe gegenüber Superoxid-Radikalen (k_s) und die Variationen der C=N-Vibrationsbanden (ΔCN) in den IR-Spektren der Komplexe gegenüber den freien Liganden als unabhängige Variablen eingeführt. Es wurde „stufenweise Regression" und die „alle möglichen Regressionen"-Prozedur in der Analyse der Daten verwendet. Die Orthogonalitätsanalyse zeigte Nichtkolinearität der Variablen an. Entsprechend den erhaltenen Gleichungen wurden die beiden besten Kupfer(II)-Komplexe einem breiten „in vivo"-Screening unterworfen. Sie waren gegenüber La, P-388 und L-1210 Leukemie aktiv.

Introduction

The biological properties of copper(II) complexes have received great attention in the past years. Antiinflammatory, antiulcer, anticonvulsant, radioprotective and anticancer activities have been studied with promising results in some cases [1].

These properties have been interpreted on the basis of the redox properties of copper(II) and, particularly, of its action towards superoxide radicals within the cells.

Up to date no multivariable quantitative structure-activity relationship (QSAR) for copper(II) compounds with cytotoxic properties has been reported. Correlations are presented by Coats et al. [2] for copper(II) KTSC complexes, although with relatively low correlation constants.

In the present paper a multiple regression analysis for 15 copper(II) complexes of semi- and thiosemicarbazones is presented.

The logarithm of the inverse of the "in vitro" cytotoxic activity ($\log 1/EC_{50}$) was selected as dependent variable in spite of its limitations as a measure of "in vivo" activity. Nevertheless, "in vitro" activity represents a fast and very unexpensive experimental parameter.

In vitro cytotoxic activity was studied as a function of the variables:

– Van der Waals molar volumes (*Vm*) [3] of the ligand substituents as a measure of steric effects.

– Logarithm of Octanol-water partition coefficients ($\log P$) as a lipophilic parameter [4]

– Specific rate constants (k_s) of the action of the copper(II) complexes towards superoxide radicals [5].

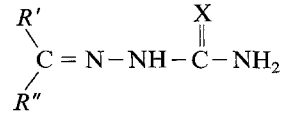
– Variation in C=N vibration bands (ΔCN) in IR spectra of the complexes with respect to the free ligands as a measure of the π-acceptor character of the bond with copper(II) ion through the nitrogen atom in semi- and thiosemicarbazones [6].

As it can be observed, the first two parameters are related to transport factors. The last two parameters have electronic characteristics related to redox processes, although k_s is a kinetic factor.

Experimental Part

The synthesis of the ligands and complexes is described in [7].

Van der Waals molar volumes were calculated for the substituents (*R'*, *R''*) in the quasiplanar molecules:



according to Bondi's parameters [3]; X=O for semicarbazones and X=S for thiosemicarbazones. The corresponding copper(II) complexes were not involved in the calculation of *Vm*, the reported values must be considered as approximated.

For the determination of the octanol-water partition coefficients, $1 \cdot 10^{-4} - 1 \cdot 10^{-5}$ mol dm^{-3} octanol solutions of the complexes were prepared and 20 cm^3 of each were mixed with 200 cm^3 of water for an hour and finally centrifuged.

The concentration of the complex in octanol phase was determined spectrophotometrically at the corresponding wavelengths given in Table 1. Both octanol and water were previously saturated with each other.

The specific rate constants were determined spectrophotometrically as described in [7] at 37°C, assuming a first order reaction to the complex. Xanthine – Xanthineoxidase was used as a source of superoxide radicals.

Table 1. Values of the determined parameters for copper(II) complexes (the units are: EC_{50}: mol dm^{-3}; k_s: s^{-1}; *Vm*: $cm^3\,mol^{-1}$; ΔCN: cm^{-1})

Complexes	$\log 1/EC_{50}$	k_s (10^3)	$\log P$	*Vm*	ΔCN + δNH_2
Cu(*FuSC*)$_2Cl_2 \cdot 3\,H_2O$	3.69	2.21	0.58	88.06	13
Cu(*FuSC*-H)$_2$	4.01	5.58	0.46	88.06	13
Cu(*FuTSC*)$Cl_2 \cdot 4\,H_2O$	4.80	2.36	1.58	50.79	26
Cu(*SaSC*)(*Py*)$Cl \cdot H_2O$	4.21	4.62	1.85	110.00	16
Cu(*SaTSC*)(*Py*)$Cl \cdot H_2O$	5.60	2.48	3.50	117.04	53
Cu(*AlSC*-H)$Cl \cdot H_2O$	3.86	2.65	1.43	25.16	36
Cu(*AlTSC*-H)$Cl \cdot H_2O$	4.52	2.34	0.93	31.92	21
Cu(*PSC*)$_2Cl_2$	3.63	1.13	1.18	41.45	13
Cu(*PTSC*-H)(*PTSC*)Cl	5.64	3.33	0.99	96.42	67
Cu_2(*ATSC*-H)$_2$(*ATSC*)$Cl_2 \cdot H_2O$	4.48	5.14	5.40	111.47	16
Cu(*ATSC*-H)$Cl \cdot 2\,H_2O$	3.93	5.46	5.43	74.31	10
Cu(*ABTSC*)$Cl_2 \cdot H_2O$	4.16	13.50	1.84	71.18	20
Cu(*MFTSC*)$Cl_2 \cdot 4\,H_2O$	3.89	2.30	1.73	61.01	33
Cu(*BTSC*-H)$Cl \cdot 2\,H_2O$	3.65	1.12	3.06	64.09	27
Cu(*BTSC*)(*BTSC*-H)$Cl \cdot 2\,H_2O$	3.93	1.69	3.70	128.18	27

FuTSC: furfural thiosemicarbazone; *FuSC*: furfural semicarbazone; *SaTSC*: salicylaldehyde thiosemicarbazone; *SaSC*: salicylaldehyde semicarbazone; *AlTSC*: acetaldehyde thiosemicarbazone; *AlSC*: acetaldehyde semicarbazone; *PTSC*: pyruvic acid thiosemicarbazone; *PSC*: pyruvic acid semicarbazone; *ATSC*: acetophenone thiosemicarbazone; *ABTSC*: *o*-amino benzaldehyde thiosemicarbazone; *MFTSC*: methyl furyl cetone thiosemicarbazone; *BTSC*: benzaldehyde thiosemicarbazone

All the above described experimental measurements were repeated three times. Medium values are given in Table 1.

"In vitro" cytotoxic activity was determined spectrophotometrically at the Institute of Oncology and Radiobiology of Cuba in KB cellular lines according to the protocol established by the N. C. I.. The cytotoxic activity is expressed as the medium effective dosis (ED_{50}) that corresponds to the quantity of substance (in μg/ml) necessary to kill 50% of the tumorigenic cells.

Results and Discussion

The data were analyzed using a system of statistical and standard programs. The "stepwise regression" and the "all possible regression" procedures were used in the analysis. Both methods indicated the same determining variables.

The variable ΔCN enters first and k_s enters second. If the *F* value is changed (i. e. *F* to enter and *F* to remove), significant variations are not observed.

On the other hand, the "all possible regression" procedure demonstrated that any other regression equation where ΔCN is not present, explains the unsatisfactory variability of data. Other variables do not influence the regression significantly.

Therefore, the obtained regression equation is:

$$\log 1/EC_{50} = 1.3 \cdot 10^{-4} k_s + 0.033\,\Delta CN + 3.04$$

with the multiple correlation coefficient of 0.803.

Table 2. Mean values and standard deviations of the variables

Variable	Mean	Standard deviation
k_s	2917.333	1552.429
Vm	77.295	31.929
ΔCN	26.067	16.002
log *P*	2.244	1.616
$\log 1/EC_{50}$	4.267	0.643

Table 3. Variance analysis

Source	*df*	*SS*	*MS*	*F*
Regression	2	3.728	1.864	10.876
Residual	12	2.057	0.171	
Total	14	5.785		

Table 4. Antitumoral activities in experimental leukemias

	ILS (%) La	(dosis in mg/kg)	
		P-388	L-1210
Cu(*SaTSC*)(*Py*)Cl·H_2O	36 (15)	24 (15)	30 (10)
Cu(PTSC-H)(PTSC)Cl	49 (4)	27 (4)	24 (4)

The residuals were examined graphically. This analysis showed that there was neither strong evidence of the non-homogeneity of the variance, nor abnormal distribution or anomaly in the data.

The orthogonality analysis proved non-collinearity among the variables. The mean values and standard deviations of the variables are given in Table 2. The variance analysis is presented in Table 3.

As it was briefly indicated above, the variation in the C=N vibration bands in the IR spectra of copper complexes with respect to the corresponding free ligands (ΔCN) is a measure of the displacement of the electronic density due to the metal-ligand bond. The coordination increases the wave number of this vibration mode which is interpreted as a reinforcement of the C=N bond. This increase of the electronic density between the atoms in the C=N group can be attributed to the π-acceptor character of this nitrogen atom as it was considered from quantum-mechanic calculations by Chumakov [6].

The determining role of the C=N group in the biological properties of thiosemicarbazone complexes was earlier proposed on the basis of quantum-mechanic calculations [8]. Therefore, the present results confirm the prediction in Ref. [8].

Since ΔCN is the determining factor in the studied correlations it is expected that the cytotoxic action of these copper complexes is via redox processes.

As can be observed from Table 1, the semicarbazone complexes are less cytotoxic and present lower values of ΔCN than the corresponding thiosemicarbazones, as has been pointed out in earlier investigations by Agrawal [9].

In order to fully confirm the validity of these results, the two copper complexes with highest ΔCN values and also highest $\log 1/EC_{50}$ calculated values were submitted to a broad "in vivo" screening study in La, P-388 and L-1210 leukemias.

The results are presented in Table 4. As it can be observed both complexes resulted to be active against these experimental leukemias in mice. In this way, the validity of the multiple regression is proved.

Acknowledgement

The authors thank Dr. N. P. Konovalova of the Physical Chemical Institute of Moscow for the antitumoral determinations.

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Received May 27, 1991. Revised September 30, 1991. Accepted October 8, 1991