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Quantitative structure–activity relationship (QSAR) analysis of cationic complexes of heart perfusion imaging agents and subsequent proposition of two different uptake mechanisms

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Abstract

Some physicochemical and quantum-chemical discriptors of two series of cationic complexes of heart perfusion imaging agents ($[^{99m}Tc^{I}(NO)Cl(PL)_2]^+$ and $[^{99m}Tc\text{-Mine}]^+$ series) have been obtained using a semi-empirical quantum mechanics method — ZINDO/1. Quantitative structure–activity relationships (QSARs) between these descriptors and heart uptake were investigated by multiple linear regression analysis method, as the result of which, several equations were obtained. Fairly complete new heart uptake mechanisms, redox mechanism for $[^{99m}Tc^{I}(NO)Cl(PL)_2]^+$ series and passively transport mechanism for $[^{99m}Tc\text{-Mine}]^+$ series are proposed based on the results shown in the equations.

Introduction

Technetium-99m (Tc-99m)-labelled agents continue to play an important role in nuclear medicine and developments of new agents for the superior imaging characteristics of Tc-99m (^{99m}Tc, $T_{1/2} = 6.02$ h, $E_\gamma = 141$ keV). Therefore, an extensive effort has been expended to design a Tc-99m radiopharmaceutical that is taken up and retained by the myocardium (Deutsch et al 1981a, b, 1988; Dean et al 1986; Gerundini et al 1986; Gerundini & Maffioli 1989). The outcome of this research has been seen with the FDA approval of Cardiolite (Jones et al 1984; Iskandrian et al 1989; Kahn et al 1989; Kiat et al 1989; Wackers et al 1989), Cardiotec (Kelly et al 1989; Narra et al 1989; Seldin et al 1989; Leppo & Meerdink 1990; Stewart et al 1990; Leppo et al 1991; Taillefer 1992; Higley et al 1993) and Myoview (Kelly et al 1991; Diwakar & Wackers 1993; Tamaki et al 1994) and with the completion of clinical trials on TechnescanQ$_{12}$ (Rossetti et al 1992, 1994; Gerson et al 1994). However, the mechanism by which the heart takes up these agents is not clear, even now. It is generally accepted that Tl^+ acts physiologically as a K^+ analogue (Johnson & Abernathy 1983) and it is transported by the (Na^+, K^+)ATPase. At the same time, previous work has demonstrated that [^{99m}Tc]-DMPE is not transported by the (Na^+, K^+)ATPase of either human erythrocytes or neonatal rat myocytes (Gallagher & Lebowitz 1981). The (Na^+, K^+)ATPase is also not involved in the uptake of the Tc-99m isonitrile complexes.

Quantitative structure–activity relationships (QSARs) are mathematical models that statistically relate the biological activity of a compound to its physicochemical properties. Several recent studies have shown their importance to the prediction of biological activity (Lipinski et al 2001) and mechanism research (Agrawal & Khadikar 2002). However, although a great deal of research has been done concerning heart perfusion imaging agents, their heart uptake mechanism is still not clear. Thus, many difficulties are met while designing new radiopharmaceuticals. It is important to elucidate new mechanisms based on theoretical methods to make much more progress in this area. Therefore, two series of complexes $[^{99m}Tc^{I}(NO)Cl(PL)_2]^+$ and $[^{99m}Tc\text{-Mine}]^+$ complexes were calculated by the ZINDO/1 (based on a modified version INDO/1, Intermediates Neglect of Differential Overlap) method. The molecular structures of the two series are given in Figures 1 and 2. New heart uptake mechanisms are proposed based on the results obtained by multiple linear regression analysis.

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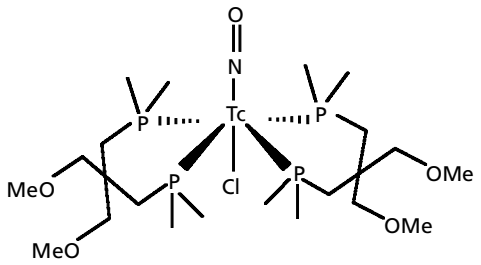


Figure 1 Structure of $[^{99m}Tc^{I}(NO)Cl(PL37)_2]^+$.

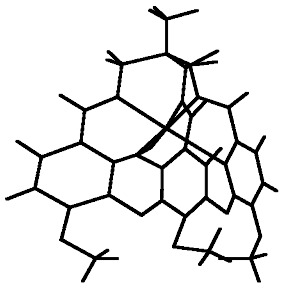


Figure 2 Structure of $[^{99m}Tc\text{-}Mine5]^+$.

Materials and Methods

Theoretical methods

A total of 26 molecules (Figures 3 and 4), whose names were consistent with those in original references, was employed in all the calculations (Chiu et al 1990; Marmion et al 1996). To determine the spin multiplicity of $[^{99m}Tc\text{-}Mine]^+$ complexes accurately, $[^{99m}TcL5]^+$ was optimized by ab-initio MO using the program Gamess (Chiu et al 1990). Comparing the energy calculated using spin multiplicity 2 and 4 (4, −311.5091 a.u.; 2, −311.4606 a.u.), 4 was adopted in subsequent calculations. The initial structures of the $[^{99m}Tc^{I}(NO)Cl(PL)_2]^+$ compounds were produced by Model Build Module, while $[^{99m}Tc\text{-}Mine]^+$ compounds were built on the basis of the structure of $[^{99m}TcL5]^+$, which has been created using X-ray data (Marmion et al 1996). The lowest energy conformations of each molecule were obtained by molecular dynamics using the simulated annealing method. These conformations were further refined by molecular mechanics. The structures were then optimized by semi-empirical quantum mechanics using the ZINDO/1 method. All these calculations were run on a PC using software Hyperchem suite for windows (release 6.0). The biological activity data were collected from the work of Chiu et al

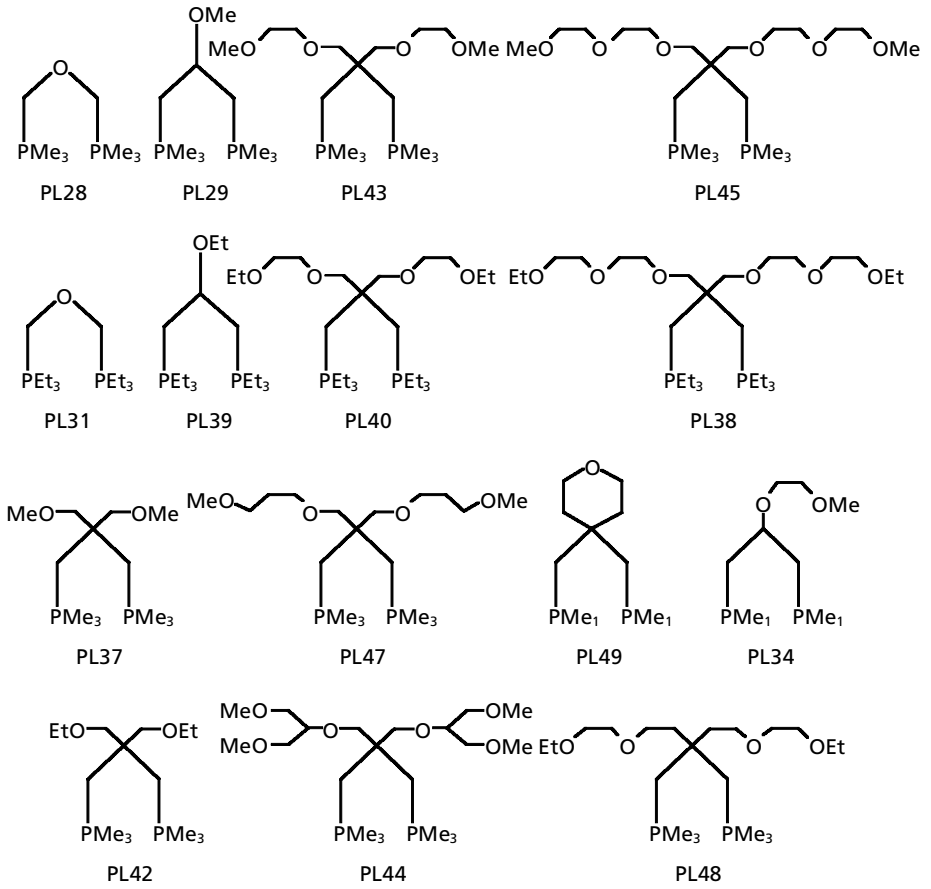
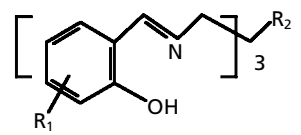
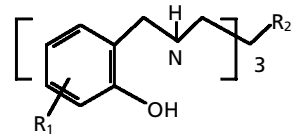


Figure 3 Structures of the ligands of $[^{99m}Tc^{I}(NO)Cl(PL)_2]^+$ complexes.



sal_3TAME derivatives

L.1 : R_1 = H, R_2 = CH_3 L.2 : R_1 = 5-CH_3, R_2 = CH_3
L.4 : R_1 = 5-F, R_2 = CH_3 L.5 : R_1 = 4-OCH_3, R_2 = CH_3
L.6 : R_1 = H, R_2 = CH_2OCH_3 L.7 : R_1 = H, R_2 = $CH_2O(CH_2)_2CH_3$
L.9 : R_1 = H, R_2 = $CH_2OCH_2(C_6H_5)$
L.14 : R_1 = 4-$OCH_2COOCH_2CH_3$, R_2 = CH_3
L.15 : R_1 = 4-$OCH_2COOC(CH_3)_3$, R_2 = CH_2OCH_3
L.16 : R_1 = 5-COOH, R_2 = CH_3



Reduced sal_3TAME derivatives

L.10 : R_1 = 5-CH_3, R_2 = CH_3
L.11 : R_1 = 4-OCH_3, R_2 = CH_3
L.12 : R_1 = H, R_2 = $CH_2O(CH_2)_2CH_3$
L.13 : R_1 = H, R_2 = $CH_2OCH_2(C_6H_5)$

Figure 4 Structures of the ligands of $[^{99m}Tc\text{-Mine}]^+$ complexes.

(1990) and Marmion et al (1996), which was represented as percent injected dose per organ (%ID/organ) ranging from 0.21∼2.26 (%ID/organ) and 0.2∼2.0% (ID/organ), respectively.

A total of twelve descriptors were obtained, of which four were geometric descriptors, and eight were electronic or quantum chemical descriptors. Geometric descriptors included molecular mass, molecular volume and two types of molecular surface area (S_1 (solvent accessible area — using a grid method) and S_2 (van der Waals surface area — a faster more approximate method). For electronic descriptors, dipole moment (μ), energy of the highest occupied molecular orbital (E_{homo}) and the lowest unoccupied molecular orbital (E_{lumo}) and five kinds of charges, including the maximum positive atomic charge (Q^{+max}), the maximum negative atomic charge (Q^{-max}), the sum of all positive atomic charges (ΣQ^+), the sum of all negative atomic charges (ΣQ^-), the sum of oxygen charges in the ether linkage (only for $[^{99m}Tc^{I}(NO)Cl(PL)_2]^+$ series) ($\Sigma Q^-(O)$), were determined. Calculation of geometric descriptors were carried out on the software Hyperchem (6.0) using the Quantitative Structure Activity Relationships (QSAR) Properties Module. Net charges of all atoms, E_{homo} and E_{lumo} were calculated by the ZINDO/1 method.

Statistical analysis

The QSAR study that we used is of the form of general equation:

$$\text{Log SP} = K_1\pi^2 + K_2\pi + K_3\sigma + K_4 e_s + K_5 \qquad (1)$$

Here, the dependent variable, SP, is a property of a series of compounds in a given system. Specifically, in this work, SP would be the heart uptake of the compounds. π is a hydrophobic parameter, σ is an electrostatic parameter, and E_s is a steric parameter. The equation coefficients, K_1, K_2, K_3, K_4 and K_5, are obtained by multiple linear regression. The data set was analysed using a standard program (GFA BASIC 4.38) on a PC computer. Step-wise regression analysis was used to determine the most significant descriptors. The regression coefficients were obtained by least-squares regression analysis. For each regression, the following descriptive information was provided: the number of data points used to derive the regression equation (n), the correlation coefficient (r), the standard error of the estimate (s), the value of F-test (F) and the cross-validated correlation coefficient ($r_{cr.val}$) derived from the predictive residual sum of squares (leave-one-out method). The leave-one-out procedure was used to determine the predictive ability of the mathematical model proposed. In this procedure, one compound was removed from the data set, the correlation equation was established from the remaining compounds and used to predict the discard compound. The process is repeated in turn for each compound in the data set (Kaixian et al 2000). All the coefficients were given with 95% intervals.

Results and Discussion

$[^{99m}Tc^{I}(NO)Cl(PL)_2]^+$ complexes

See Table 1 for descriptors used in equations, heart uptake (HU) and its log value.

$$\log(\text{HU}) = 7.925\ (\pm 3.168) + 1.809\ (\pm 0.332)\ E_{lumo} - 0.00041\ (\pm 0.000140)\ S_2 - 29.572\ (\pm 11.886)\ Q^{+max} \qquad (2)$$

$$n = 15,\ s = 0.141,\ r = 0.899,\ F = 15.509,\ r_{cr.val} = 0.807$$

In equation 2, log(HU) correlated well with a linear combination of E_{lumo}, Q^{+max} and S_2. Figure 5 shows the relationship between actual and predicted log(HU). Since lumo usually is the electron acceptor during the redox reaction, a lower E_{lumo} would make for electron filling in. At the same time, because an atom with higher Q^{+max} has more opportunities to be attacked by negatively charged groups, the energy barrier of reduction is easier to overcome. Thus a cationic complex with lower E_{lumo} and higher Q^{+max} would more easily undergo reduction from cation to its neutral form. So a monovalent

Table 1 Descriptors used in equations, heart uptake (HU; ID%/g) and its logarithm value.

Complex	HU	log(HU)	E_{lumo} (eV)	S_2	ΣQ^+ (a.u.)	ΣQ^- (a.u.)	ΣQ^- (O) (a.u.)	Q^{+max} (a.u.)
$[^{99m}Tc^{I}(NO)Cl(PL28)_2]^+$	0.21	−0.678	−0.269	458.0	3.501	−2.511	−0.478	0.269
$[^{99m}Tc^{I}(NO)Cl(PL29)_2]^+$	0.68	−0.167	−0.063	483.0	4.578	−2.955	−0.557	0.262
$[^{99m}Tc^{I}(NO)Cl(PL31)_2]^+$	1.40	0.146	0.017	630.8	4.298	−3.371	−1.065	0.276
$[^{99m}Tc^{I}(NO)Cl(PL34)_2]^+$	0.48	−0.319	0.158	458.3	3.735	−2.735	−0.489	0.264
$[^{99m}Tc^{I}(NO)Cl(PL37)_2]^+$	2.26	0.354	−0.033	676.4	5.077	−3.389	−1.106	0.263
$[^{99m}Tc^{I}(NO)Cl(PL38)_2]^+$	1.12	0.049	0.124	594.5	4.289	−3.290	−1.019	0.266
$[^{99m}Tc^{I}(NO)Cl(PL39)_2]^+$	1.26	0.100	0.169	1378.9	7.245	−6.245	−3.309	0.263
$[^{99m}Tc^{I}(NO)Cl(PL40)_2]^+$	1.30	0.114	0.020	482.2	4.344	−3.344	−0.606	0.262
$[^{99m}Tc^{I}(NO)Cl(PL42)_2]^+$	1.22	0.086	0.180	990.8	6.101	−5.155	−2.213	0.263
$[^{99m}Tc^{I}(NO)Cl(PL43)_2]^+$	0.83	−0.081	0.222	600.5	5.059	−4.059	−1.115	0.264
$[^{99m}Tc^{I}(NO)Cl(PL44)_2]^+$	1.43	0.155	0.128	989.5	5.380	−4.380	−2.118	0.263
$[^{99m}Tc^{I}(NO)Cl(PL45)_2]^+$	0.46	−0.337	0.289	1126.2	6.503	−5.503	−3.223	0.264
$[^{99m}Tc^{I}(NO)Cl(PL47)_2]^+$	1.52	0.182	0.139	1377.5	6.427	−5.476	−3.214	0.264
$[^{99m}Tc^{I}(NO)Cl(PL48)_2]^+$	0.66	−0.180	0.222	1003.8	5.841	−4.618	−2.183	0.263
$[^{99m}Tc^{I}(NO)Cl(PL49)_2]^+$	0.32	−0.495	0.112	1159.9	5.904	−4.905	−2.147	0.264

cationic Tc-99m complex possessing these characteristics might suffer in-vivo reduction, which reduces the amount of complex in cationic form. However, monovalent cationic species are well-known heart-affinity substances, which implies that this in-vivo reduction leads to poor heart uptake. So these two descriptors may be interpreted in terms of reduction, which highlights the importance of the stability of the complex with respect to redox chemistry in determining the efficacy of heart uptake of Tc-99m cations as myocardial agents. The S_2 mainly indicated that the complex with a small molecular surface area exhibited appropriate heart uptake, which may be understood by considering the biological permeation.

$$\log(HU) = 9.5195(\pm 3.434) + 1.876(\pm 0.364)E_{lumo} - 0.132(\pm 0.048)\Sigma Q^+ - 34.296(\pm 12.693)Q^{+max} \quad (3)$$

$$n = 15,\ s = 0.147,\ r = 0.890,\ F = 14.034,\ r_{cr.val} = 0.80$$

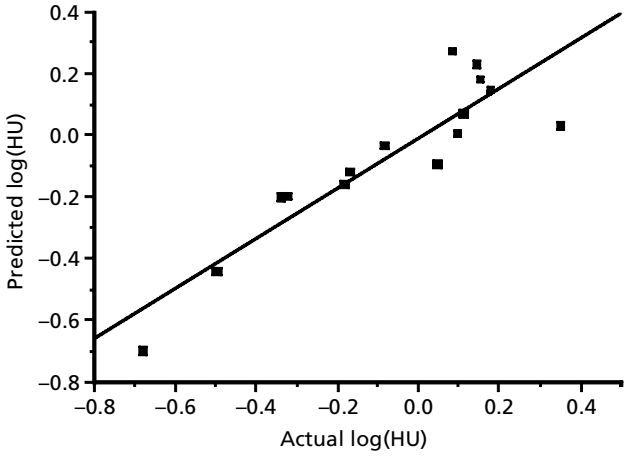


Figure 5 Relationship between actual and predicted (via equation 2) log(HU) for $[^{99m}Tc^{I}(NO)Cl(PL)_2]^+$ complexes.

In equation 3, the complex with higher ΣQ^+ would pull negatively charged groups that would attack the atom with Q^{+max}. This pull would lead to reduction of the complexes and impair heart uptake.

$$\log(HU) = 8.125(\pm 3.434) + 1.938(\pm 0.398)E_{lumo} + 0.122(\pm 0.048)\Sigma Q^- - 29.749(\pm 12.831)Q^{+max} \quad (4)$$

$$n = 15,\ s = 0.152,\ r = 0.888,\ F = 12.858,\ r_{cr.val} = 0.781$$

$$\log(HU) = 8.248(\pm 3.357) + 1.874(\pm 0.369)E_{lumo} + 0.131(\pm 0.049)\Sigma Q^-(O) - 31.294(\pm 12.606)Q^{+max} \quad (5)$$

$$n = 15,\ s = 0.149,\ r = 0.882,\ F = 13.663,\ r_{cr.val} = 0.764$$

That the activity correlated with ΣQ^- in equation 4 was easily understood taking into account the good correlation of ΣQ^- with ΣQ^+ (r = 0.98). Good statistical quality of equation 5 could be explained by the fact that ΣQ^- (O) was evidently correlated with ΣQ^- (r = 0.951). Comparing these two equations showed that ΣQ^- (O) is a better descriptor than ΣQ^- (equation 4, r = 0.888; equation 5, r = 0.882). As is reported (Johnson & Abernathy 1983), complexes formed from ligands with ether linkages have significantly higher heart uptake than complexes formed from corresponding ligands without ether linkages. The presence of O atom provided additive hydrophilic effects which reduced the protein bonding of Tc-99m complexes, while this bonding would make the complex clear rapidly with blood flow instead of entering the myocardium (Johnson & Abernathy 1983; Kelly et al 1991). Therefore, the ether linkages are not there only to help ΣQ^- (O) push negatively charged groups, but also to increase the hydrophilicity to improve heart uptake. So ΣQ^- (O) was more appropriate than ΣQ^-.

$[^{99m}$Tc-Mine$]^+$ complexes

See Table 2 for descriptors used in equations, heart uptake and its log value.

$$\log(\mathrm{HU}) = -6.467(\pm 1.263) + 19.766(\pm 3.725)Q^{+\max} - 0.011(\pm 0.003)\mu \quad (6)$$

$$n = 11,\ s = 0.012,\ r = 0.897,\ F = 16.528,\ r_{cr.val} = 0.842$$

In equation 6, there was a negative correlation between log(HU) and μ, and a positive correlation between log(HU) and Q^{+max}. Figure 6 shows the relationship between actual and predicted log(HU); μ reflects the polarity of compounds. Since a major component of the myocyte membrane is of low polarity (Sands et al 1986), appropriate low polarity of Tc-99m complexes could account for the uptake by the cell membrane. On the contrary, substantially increasing the polarity of a Tc-99m complex reduced the heart uptake (Sands et al 1986). The results presented here suggest that (Na^+,K^+) ATPase was not involved in the uptake of these complexes. Instead, these complexes were passively transported because of their low polarity. So lower μ is good for increasing heart uptake. At the same time, complexes with high Q^{+max} had good heart uptake, which should be understandable in terms of electrostatic attraction. There are two layers of membranes surrounding the mitochondria of myocytes — the outer one is relatively permeable and resembles the cell membrane and the inner one is impermeable and resembles the bacterial cell membrane. There are proteins in the inner membrane that have negative charge by which the monocationic complex of Tc-99m would be trapped, which indicates increasing heart uptake. Therefore, Tc-99m complex with a higher Q^{+max} would bind more tightly to the mitochondria.

$[^{99m}TcL16]^{2-}$ is an outliner in the regression which is not used in the equation. It should be noted that all complexes are monovalent cationic species that are well-known heart-affinity substances, except $[^{99m}TcL16]^{2-}$. The fully deprotonated form of this ligand generates a complex of −2 overall charge at physiological pH as the three carboxylates offset the inherent +1 charge of the complex. The complex with negative charge would not tend to be trapped by negatively charged proteins in mitochondria. Therefore, $[Tc^{99m}L16]^+$ was expected to have only background values for heart uptake, so it should not be used in equation 1. However, $[Tc^{99m}L14]^+$ is an outliner too. Guinea-pig distribution assays typically showed 1∼2% of the injected dose in the heart tissue at 5 min post-injection, with a heart-to-liver ratio ≤1. Only the complexes $[Tc^{99m}L14]^+$ and $[^{99m}TcL16]^{2-}$ were significantly below the mean for myocardial uptake. $[Tc^{99m}L14]^+$ has an apparent +1 charge upon injection. Nevertheless, it showed a distribution similar to $[Tc^{99m}L16]^{2-}$, indicating that metabolism of the simple ethyl group, which could lead to an anionic COO^- group, was probably rapid in guinea-pigs. However, that $[Tc^{99m}L15]^+$ is not an outliner indicated the greater resistance of its *t*-butyl ester group to in-vivo ester hydrolysis during the time course of the experiment. $[Tc^{99m}L12]^+$ is also an outliner in the equation. It is reaction of the ligand L12 with technetium that yields complex $[^{99m}TcL7]^+$ containing the analogous imine ligand L7 (Marmion et al 1996).

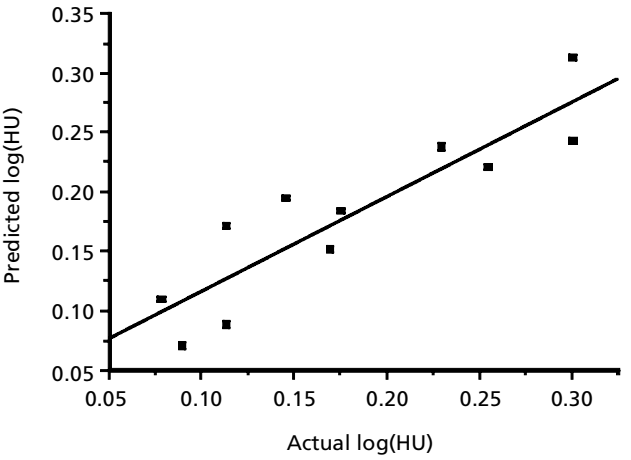


Figure 6 Relationship between actual and predicted (via equation 6) log(HU) for the $[^{99m}$Tc-Mine$]^+$ complexes.

Table 2 Descriptors used in equations, heart uptake (HU) and its logarithm value.

Complex	HU	log(HU)	Q^{+max} (a.u.)	μ (Debyes)
$[^{99m}TcL1]^+$	1.3	0.114	0.340	7.771
$[^{99m}Tc\ L2]^+$	1.5	0.170	0.339	7.138
$[^{99m}Tc\ L4]^+$	1.3	0.114	0.338	11.839
$[^{99m}Tc\ L5]^+$	2.0	0.301	0.347	7.956
$[^{99m}Tc\ L6]^+$	1.4	0.146	0.341	7.693
$[^{99m}Tc\ L7]^+$	1.8	0.255	0.342	6.540
$[^{99m}Tc\ L9]^+$	1.7	0.230	0.342	5.164
$[^{99m}Tc\ L10]^+$	1.2	0.090	0.338	13.217
$[^{99m}Tc\ L11]^+$	1.2	0.079	0.341	15.078
$[^{99m}Tc\ L12]^+$	1.7	0.230	0.353	12.178
$[^{99m}Tc\ L13]^+$	2.0	0.301	0.345	10.186
$[^{99m}Tc\ L14]^+$	0.2	−0.699	0.345	22.746
$[^{99m}Tc\ L15]^+$	1.5	0.176	0.349	23.441
$[^{99m}Tc\ L16]^+$	0.2	−0.699	0.347	10.243

For the $[^{99m}Tc^I(NO)Cl(PL)_2]^+$ series, log(HU) correlated well with a linear combination of E_{lumo}, Q^{+max} and S_2, while for the $[^{99m}$Tc-Mine$]^+$ series, there was a negative correlation between log(HU) and μ, and a positive correlation between log(HU) and Q^{+max}. By comparison, higher Q^{+max} was unfavourable for heart uptake of $[^{99m}Tc^I(NO)Cl(PL)_2]^+$ series but good for $[^{99m}$Tc-Mine$]^+$ series, while E_{lumo} did not correlate with heart uptake in the latter series. As their molecular structures were considered, their uptake mechanisms were supposed to be different. The structure of $[^{99m}Tc^I(NO)Cl(PL)_2]^+$ series was like a cone. The core of Tc-N=O is on the top of the cone, in which the nitrogen atom possessing the Q^{+max} would easily be attacked by negatively charged groups because of its open manner. Taking the low E_{lumo} into account, the uptake of complexes of this series would be greatly interfered with by in-vivo reduction. Its uptake mechanism was a redox mechanism.

For [^{99m}Tc-Mine]$^{+}$ series, the atom Tc, which possesses Q^{+max}, was surrounded by ligands with bulk volumes. Therefore, groups with negative charge could not reach it, and it would not easily undergo reduction. Furthermore, a complex with higher Q^{+max} would bind more tightly to the mitochondria in terms of electrostatic attraction. So the uptake of this series was decided by how many molecules were trapped in the mitochondria. Its uptake mechanism was by passive transport.

Conclusion

Two series of cationic complexes of heart perfusion imaging agents were calculated and analysed. For [^{99m}Tc^{I}(NO)Cl(PL)$_2$]$^{+}$ series, log(HU) correlated well with a linear combination of E_{lumo}, Q^{+max} and S_2, while for [^{99m}Tc-Mine]$^{+}$ series, log(HU) correlated with μ and Q^{+max}. Two kinds of new heart uptake mechanism were proposed: a redox mechanism ([^{99m}Tc^{I}(NO)Cl(PL)$_2$]$^{+}$ series) and passive transport ([^{99m}Tc-Mine]$^{+}$ series). Although it seemed that these two series have different mechanisms, it could be deduced that the two mechanisms may both occur during the heart uptake process, yet one of them may be the main mechanism for certain complexes.

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