DFT based computational methodology of IC_{50}

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Abstract

A new method derived from the relative toxicity equation termed as RICM, for the computation of IC_{50}, is reported here. It is tested for both organic and organometallic compounds as HIV-1 capsid A inhibitors and cancer drugs. Computed results match very well with the experiment. This new method is very easily applicable for the organic molecules as well as organometallic compounds. Most importantly, this method does not require any computation facility provided we know the dipole moments of the unknown compound and reference compound. Applicability and accuracy of this method showed very good agreement with the experiment. Since RICM needs only the dipole moment of a compound for the computation of IC_{50}, it may be used as a search criterion for the High Throughput Screening (HTS) used at the first step of the in-silico drug designing. This would ease the algorithm for HTS and increase the success rate.

keywords : Relative IC_{50} method, SVM, MLR, QCM.

1. Introduction

The complexity and high consumption of time and cost for the discovery of a new drug is reduced significantly by the use of computer-aided drug designing technique. There are different techniques used in in-silico drug designing which includes homology modeling [1], molecular dynamics simulation [2, 3, 4], molecular docking [5, 6, 7], high-throughput screening [8, 9], comparative molecular field analysis [10], 3D pharmacophore search [11],

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quantitative structure activity relationship (QSAR) [12, 13] etc. In-silico drug designing is implemented in all stages of drug research starting from pre-clinical stage to late-stages at the clinical trial. There are several properties like IC_{50}, $LogP$, cell uptake, toxicity, solubility, metabolic probability, etc., are measured before in vivo test and clinical trial of the new compound. Thus, development of methods for computation of these properties is extremely important in drug discovery. QSAR based methods like MLR [19], SVM [20], etc. are widely used for this purpose. These methods need large amount of experimental data for evaluation of IC_{50}. Molecular docking is reasonably accurate but requires high computation facilities. Recently, Bag and Ghorai [14, 15, 16] developed quantum-based method to compute IC_{50}, $LogP$ and toxicity which is straight forward and need low computational facility to implement. This method is very useful for the in-silico drug testing of a compound because in this method no large data set is required but, this method needs two parameters which depend on the enzyme-substrate binding free energy (Θ) and the enzyme-inhibitor binding nature (B) to compute IC_{50}. The computation of Θ is computationally very expensive. The computation of B is also not trivial. It depends on several external factors like temperature, cell type, etc. B is different for different types of inhibitors. Thus, application of this method to calculate IC_{50} in property based drug searching from a data set is not possible. To overcome these limitations, alternative method to compute IC_{50} is required. Here, we developed an alternative method which needs known value of IC_{50} of only one compound to compute IC_{50} of any unknown compound.

2. Relative IC_{50} method

The relative toxicity (γ) of a compound is defined as [14]

$$\gamma = \frac{\frac{\omega_{comp}}{\omega_{ref}}}{e^{(\mu_{comp}-\mu_{ref})}} \tag{1}$$

where μ_{comp} and μ_{ref} are the electric dipole moment of the compound under investigation and reference respectively. ω_{comp} and ω_{ref} [21, 22, 23] are the reactivity descriptors of the compound under test and reference respectively.

From the deffination of γ [14], we get

$$\gamma = \frac{(CC_{50})_{comp}}{(CC_{50})_{ref}} \tag{2}$$

where, $(CC_{50})_{comp}$ and $(CC_{50})_{ref}$ are the measure of the cyto-toxicity of compound and reference. Since, CC_{50} is strongly correlated with IC_{50} [25], we may replace CC_{50} by IC_{50} in equation

$$\gamma = \frac{(IC_{50})_{comp}}{(IC_{50})_{ref}} \tag{3}$$

Hence,

$$(IC_{50})_{comp} = (IC_{50})_{ref} \frac{\omega_{comp}}{\omega_{ref}} e^{(\mu_{ref} - \mu_{comp})} \tag{4}$$

When substrate is used as reference, we get absolute IC_{50} (AICM) of the compound. When, any other compound is used as reference, we get a relative IC_{50} (RICM). The computation of relative IC_{50} from Equation

$$(IC_{50})_{comp} = (IC_{50})_{ref} \times e^{(\mu_{ref} - \mu_{comp})} \tag{5}$$

For the high throughput screening (HTS), we may use the absolute IC_{50} of the inhibitor which may be calculated as follows

$$(IC_{50})_{inhibitor} = (IC_{50})_{substrate} \frac{\omega_{inhibitor}}{\omega_{substrate}} e^{(\mu_{substrate} - \mu_{inhibitor})} \tag{6}$$

here $(IC_{50})_{substrate}$ is the activity of the substrate. The activity of the substrate for a particular process is unique, but, very difficult to compute; though we can measure it experimentally. For the computation of the electric dipole moments and reactivity descriptors (ω) of the test compound and the substrate one could use any advanced quantum computation method like density functional theory (DFT) [26, 27], the equation of motion coupled cluster method (EOMCC) [28, 29], the Fock-space multireference coupled cluster method (FSMRCC) [30, 31, 32] transition probability approximated CI (TPA-CI) [33], etc.

3. Computational details

To test validity of the proposed method, we choose small organic molecules of different classes. The calculation of IC_{50} involves ω. Thus, we perform geometry optimization of the compounds to calculate ω and dipole moments.

All geometry optimizations are done using Gaussian 09 package.[42] We optimize structures of all compounds mentioned here without any symmetry

constraints and confirmed the minimum energy structures by the harmonic vibrational frequency calculation with all positive mode of vibrational frequency. The convergence thresholds we set to 0.000015 Hartree/Bohr for the forces, 0.00006 Å for the displacement and 10^6 Hartree for the energy change. Calculations of organic molecules are performed with the density functional theory (DFT) [26, 27] with unrestricted Becke's three parameter hybrid exchange functional [43] combined with Lee−Yang−Parr non-local correlation function [44], abbreviated as B3LYP using 6-311G basis set. For osmium carbonyl nitrosyl clusters and cluster derivatives DFT is used with unrestricted Becke's three parameter hybrid exchange functional [43] combined with exchange component of Perdew and Wang's 1991 functional [45, 46, 47, 48] and LanL2DZ basis set [49, 50] along with the corresponding Los Alamos relativistic effective core potentials [51] which is implemented in Gaussian 09 package.

4. Results and discussion

The IC_{50} of 15 small organic compounds are computed as HIV-1 Capsid inhibitor [52] and compared with the experiments [19]. To test the efficiency of this method, we compared our results with other theoretical methods which are commonly used in the industries and academics for the in-silico drug test.

4.1. Validity and accuracy

To test the validity and accuracy of the proposed methods we computed IC_{50} using AICM and RICM methods and compared with the experiments.

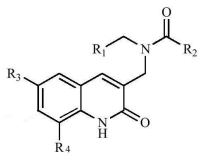
4.1.1. AICM method

Absolute IC_{50} of 15 small organic compounds are computed using Equation

4.1.2. RICM method

We consider same 15 compounds to compute IC_{50} using RICM. We randomly chose two compounds from these compounds as reference compound. Compound-7 and compound-8 are chosen as reference compounds. We have taken two different compounds as reference to test whether calculated IC_{50} depends on the choice of the reference or not. Computed results are presented in Table

Table 1: Absolute IC_{50} of 15 HIV-1 capsid inhibitors using AICM (Eqn.



Compound	R_1	R_2	R_3	R_4	IC_{50} (μM) (AICM)	IC_{50} (μM) (expt.)
Comp-1			CH_3	H	24.4	16.7
Comp-2			CH_3	H	17.8	25.6
Comp-3			CH_3	H	25.8	23.6
Comp-4			CH_3	H	13.3	8.20
Comp-5			CH_3	H	13.4	24.8
Comp-6			CH_3	H	12.3	24.1
Comp-7			CH_3	H	23.6	23.6
Comp-8			CH_3	H	34.3	38.9
Comp-9			C_2H_5	H	21.8	13.9
Comp-10			OCH_3	H	7.6	7.87
Comp-11			CH_3	H	10.5	11.7
Comp-12			CH_3	H	14.0	12.9
Comp-13			CH_3	H	6.5	5.00
Comp-14			CH_3	H	11.3	8.6
Comp-15			H	CH_3	3.7	7.9

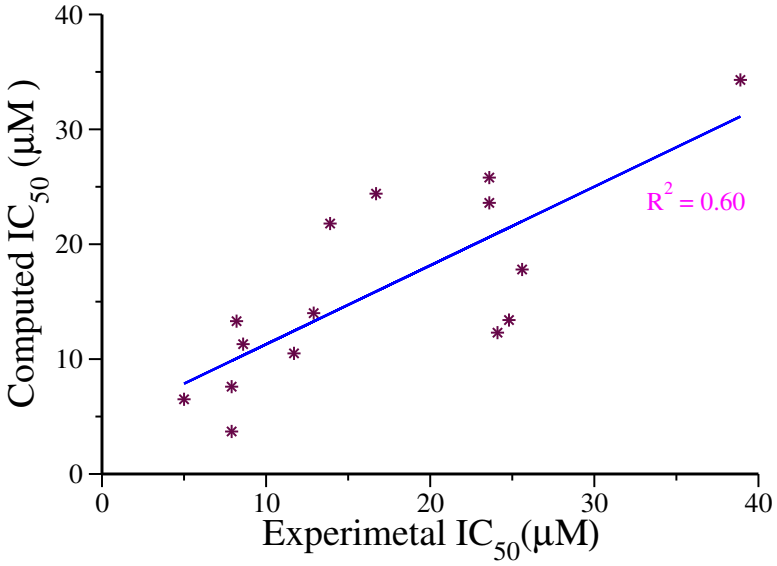


Figure 1: Correlation between AICM and experiment.

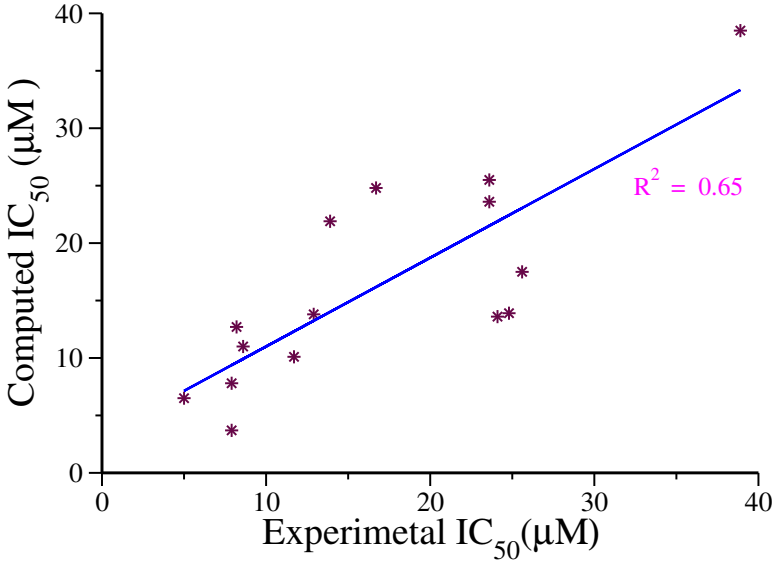


Figure 2: Correlation between RICM and experiment.

Table 2: Comparison of RICM with experiment.

Compound	IC_{50} (μM) expt. [19]	IC_{50} (μM) RICM (Ref-1*)	IC_{50} (μM) RICM (Ref-2*)
Comp1	16.7	24.8	23.8
Comp2	25.6	17.5	17.7
Comp3	23.6	25.5	25.8
Comp4	8.2	12.7	12.8
Comp5	24.8	13.9	14.0
Comp6	24.1	13.6	13.7
Comp7	23.6	23.6	23.8
Comp8	38.9	38.5	38.9
Comp9	13.9	21.9	22.1
Comp10	7.9	7.8	7.9
Comp11	11.7	10.1	10.3
Comp12	12.9	13.8	13.9
Comp13	5.0	6.5	6.6
Comp14	8.6	11.0	11.1
Comp15	7.9	3.7	3.7
Mean Deviation		-0.56	-0.47
Mean Abs. Deviation		4.1	4.1

* Reference-1 is compound-7 and Reference-2 is compound-8

4.2. Test of the efficiency of AICM and RICM : comparison with other theoretical methods

We observe that the relative IC_{50} method is very easy to implement and produces good results. We compare our method with other existing methods. First we compare our method with two QSAR based methods, MLR [19] and SVM [20] and one quantum computation based method (QCM) [16]. Among these three methods MLR and SVM are widely used in academics and pharmaceutical industries. We also compare our method with molecular mechanic based docking method separately. We intentionally divide our study in two different sets because MLR and SVM method do not use any information about the enzyme or effect of the surroundings. These methods completely depend on the IC_{50} values of numbers of known compounds and values of their descriptors. While, QCM method uses the chemical properties of the compound under test. In QCM method, the effect of enzyme is included through a constant term Θ and the effect of other parameters like temperature, enzyme concentration, substrate concentration, etc., which influence the value of IC_{50} are implicitly counted through another constant B. But, a docking method explicitly used the structural and chemical properties of enzyme as well as the inhibitor. In that sense, docking based methods are considered as the best to compute IC_{50} or binding constant (generally termed as K_i). The search for an alternative method to docking is continued due to the high requirement of computation time and storage facilities of docking.

4.2.1. Comparison with MLR, SVM and QCM

AICM and RICM are compared with MLR, SVM and QCM. For the comparison, we perform the regression analysis of these methods with respect to the experiment and regression plots are presented in Figure

4.2.2. Comparison with the docking based method

Since docking based methods are considered as the best for the computation of binding constant and IC_{50}, we compared our method with docking based method. We used docking server [56] for our calculation. In this particular docking method [57] semi-empirical quantum-mechanical computation is used for the calculation of partial charge of both the ligand and the protein. Crystal structure of HIV-1 capsid protein (p24) (PDB ID -1e6j) [58] is used after proper cleaning. Computed results are used for the regression analysis. Regression results of AICM , RICM and docking method (marked as DOC in the figure) are plotted and presented in Figure

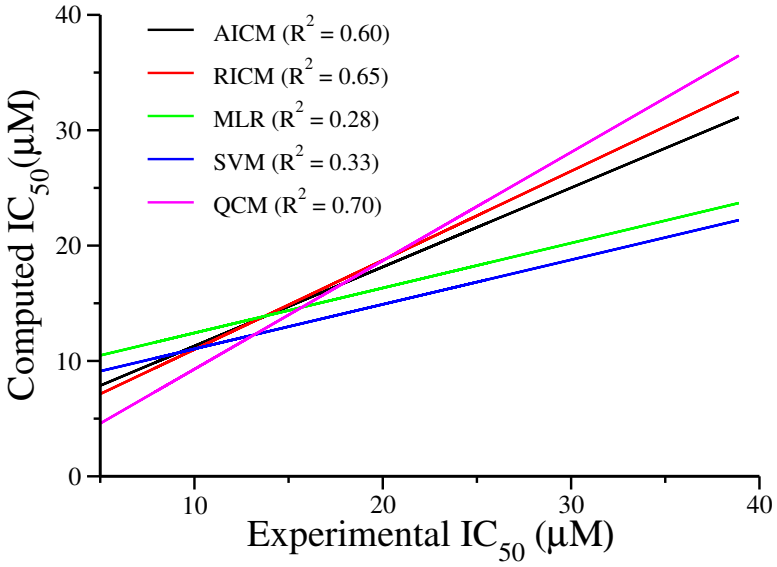


Figure 3: Comparison of AICM, RIAM, QCM, MLR and SVM with respect to their regression with respect to experiment.

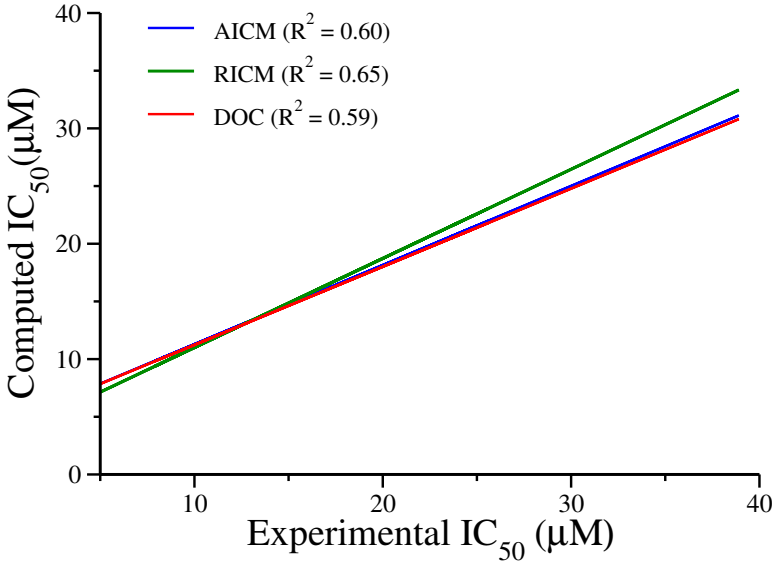


Figure 4: Comparison of AICM and RIAM with docking based method.

Table 3: Calculated and experimental IC_{50} of BMx compounds as HIV-1 capsid assembly inhibitors. Substrate is used as reference.

Compound	$IC_{50}(\mu M)$ experimental [53]	$IC_{50}(\mu M)$ Computed (RICM)
BM1	0.062	0.057
BM2	0.26	0.23
BM3	0.11	0.22

Table 4: IC_{50} of tri-ruthenium cluster derivatives for A2780cisR cell line

Compound	IC_{50} (computed) (μM)	IC_{50} (experimental) [54] (μM)
$Ru_3(CO)_{11}P$	0.127	0.135 ± 0.01
$Ru_3(CO)_{10}P_2$	0.401	0.570 ± 0.23
$Ru_3(CO)_9P_3$	284.6	> 300

P = glucose-modified bicyclophosphite ligand

4.3. Test of generality

So far, RICM and AICM are tested with the same set of compounds as HIV-1 capsid A inhibitors. It is also necessary to test the general applicability of these methods. Thus, we extend our study for a different class of compound and further as different class of drug with different types of compounds (organometallic compounds in particular).

4.3.1. Computation of IC_{50} of different class of HIV-1 capsid inhibitors using AICM

We test the applicability of AICM to calculate IC_{50} of HIV-1 capsid assembly inhibitors of a different class of chemical compounds. Due to the availability of less number of compounds of this class, only AICM is used for the computation of IC_{50}. These compounds are taken from the literature [53] which are termed as BMx. Results are presented in

4.3.2. Computation of IC_{50} of organometallic compounds as cancer drug using RICM

To find the generality of RICM method we compute IC_{50} for two sets of organometallic compounds as cancer drug. The cancer drugs which are considered here affect the G1 phase of cell division. Hence, for these drugs, computation of IC_{50} using AICM is not possible as there is no substrate. RICM

Table 5: IC_{50} of tri-osmium cluster and cluster derivatives for MDA-MB-231 cell line

Compound	IC_{50} (computed) (μM)	IC_{50} (experimental) [55] (μM)
$Os_3(CO)_{12}$	10.0	> 10.0
$Os_3(CO)_{10}(CH_3CN)_2$	4.4	6.2 ± 1.2
$Os_3(CO)_{10}(H)_2$	8.7	9.7 ± 1.8
$Os_3(CO)_{10}(H)(OH)$	4.6	8.5 ± 0.9

is tested for two different cell lines with different sets of organometallic compounds for which experimental IC_{50} values are known. First, tri-ruthenium cluster derivatives of glucose-modified bicyclophosphite ligand are tested for A2780cisR cell line. The experimental and computed values of IC_{50} are listed in Table-

5. Conclusion

So far there was no straight forward method to compute IC_{50} of a compound. QSAR based methods, like MLR, SVM, which are available for the computation of IC_{50} of a compound require huge data set for machine learning process for different class of drug. Accuracy of the computed result heavily depends on the span of the dataset used. Thus, it is difficult to use computed IC_{50} value as a search criterion. RICM, on the other hand, needs only the dipole moment values of reference compound and the compound under search. Thus, it is very easy to use computed IC_{50} as a search criterion. AICM could be used to compute IC_{50} of any compound when information about the substrate is available. Both AICM and RICM are found very accurate compare to QCM and docking method. They are better than MLR and SVM methods. It is expected that if we replace dipole moment by volume to dipole ratio or charge density, better agreement with experiment may be obtained. Formulation of new algorithm for drug search using AICM,RICM and QCM is possible. Such search analogy should speed up the search process as well as the chance of success to find out an effective drug.

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