A Novel Thermodynamic method For Quantifying Ligand-Receptor Interactions: The IC (1/3) Standard Vs IC (50)

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Abstract

A method based on the Concentration Derivative Product (CDP) is proposed to analyze the thermodynamics of Drug-Receptor interactions. CDP is defined as the product of the fractional inhibition f and it's concentration derivative. Assuming Michaelis-Menten kinetics, it is shown that for a number of drug-receptor systems (HIV protease, ACE, HMG-CoA, Opioid receptor inhibitors, etc.) f^{max} = 0.333, irrespective of the nature of bonding interactions and K_i. The CDP^{max} = [4 / (27 K_i)], and gives a measure of the

drug potency directly. CDP also gives thermodynamic information about the

ratio of the gradients of the inhibitor chemical potential in the free and bound

states.

Introduction

Inhibiting a vital enzyme involved in the replication cycle of a pathogen or a biochemical pathway is a common strategy employed in many drug discovery programs .[1] Thus inhibitors of HIV protease, Angiotensin Converting Enzyme (ACE) illustrate this strategy.[1] Detailed knowledge of the receptor helps in the a-priori design of the inhibitors. [2-15] . Design based on the three dimensional structure of the target protein has gained some prominence.[5-9] The 'Transition State mimetic concept ' has resulted in a number of successful AIDS drugs [10], viz. Saquinavir [14], Ritonavir [12], Indinavir [11], Nelfinavir and Amprenavir [15] etc.

Most drugs bind reversibly to the enzyme (receptor) and can be modeled by reversible Michaelis-Menten (MM) kinetics. [4] This accounts for the daily dosing and the biological half-life of 10-12 hours. The basic quantity of interest is the disassociation constant K_i and the binding free energy. This quantity is used as a marker for drug-potency, and all enzyme assay studies aim at estimation of this quantity. Apart from direct screening for drug-efficacy, a-priori theoretical estimates through molecular modeling can weed out unpromising candidates. Molecular mechanics and free energy

perturbation calculations can also give a - priori estimates of the K_{-i} to identify lead compounds [15 -18] For a number of drugs in current use, the K_{-i} values fall in the nM range. The free energies of binding estimated from the K_i fall in a rather narrow range of values [eg. HMG -CoA inhibitors [19,20]] , as will be shown later. The spectrum of K_i values also indicate inhibitor selectivity to the various Opioid receptors ,viz.. the μ ,δ,κ .[21, 22] . Thus it is possible to selectively block either of the above mentioned receptor subtypes, so as to get the desired pharmacological response.

It is possible to go beyond the K_i and free energy estimation to understand the intricacies of the thermodynamics of receptor–ligand interactions. In partcular, the binding free energy does not tell anything about the gradients of the chemical potentials of the ligand in the free and bound (to the receptor) states. All that we need to obtain this information is the binding assay data of the fractional inhibition of the receptor at various levels of inhibition, as a function of the equilibrium free ligand concentration.

In this work, we propose a thermodynamic method based on the analysis of the fractional inhibition f Vs concentration (of the free ligand)

data. The Concentration Derivative Product (CDP) will be defined as the product of f and it's concentration derivative. The relationship of CDP with the ratio of the ligand chemical potential in free and bound states will be shown. Finally the universality of the results (i.e. $f^{max} = 1/3$) will be shown for five different receptor-ligand systems. A new concentration standard [IC (1/3) = IC (50) / 2] will be defined at which the CDP attains it's maximum. It will also be further demonstrated that the influence of the experimental error in estimating the various thermodynamic quantities of interest will be minimal, when we carry out the measurements in the vicinity of IC (1/3). Thus it will be shown, that with less number of molecules, we get maximum thermodynamic information (such as the chemical potential gradient ratios), in addition to the usual binding free energy.

Formulation

In what follows, we assume the validity of reversible Michaelis-Menten kinetics. This is valid for a large number of ligands in the drug scenario where the drug molecules bind reversibly to the receptor . This is also fulfills the pharmaco dynamics requirement of

a physiological drug half life of 8-12 hours.

The Michaelis-Menten rate law for the fractional loading or activity is [4]:

$$f = V / V_m = c / (K_M + c) \qquad (1)$$

Where V_m, K_M, c denote the maximum activity, the Michaelis constant and the concentration of the inhibitor respectively. $K_M = (k_{-1} + k_r) / k_1$, where k_1, k_{-1}, k_r are the forward and backward kinetic constants for binding and reaction respectively. [4] For our analysis involving inhibitors $k_r = 0.$, and $K_M = K_i$, the disassociation constant of the inhibitor.

We now define CDP by drawing an analogy with the concept of the Pressure Derivative Product of surface coverage in adsorption [23, 24].

$$CDP = f \, df / dc = f (1-f)^2 / K_i \qquad (2)$$

Eq.1 and Eq.2 have to be modified if enzyme inhibition studies are carried out in the presence of the substrate. [25]

The MM kinetics ,viz. Eq.1 , calls for a monotonic increase in f as c is increased. On the other hand, the slope df /dc progressively decreases with the concentration. Therefore CDP, which is the product an increasing and decreasing quantity, displays a maximum. If   CDP is plotted Vs f, the

location of f^{max} , CDP^{max} gives very valuable information on the thermodynamics of ligand binding.

From an experimental point of view, all one has to do is to plot the $f^2/2$ as a function of c. The slope of this curve will directly give CDP.

To locate the maxium of CDP ,we differentiate it with respect to c and set it equal to zero,

$$d/dc (f\, df/dc) = 0 \qquad (3)$$

and for MM kinetics, we get :

$$f^{max} = 1/3 = 0.33333 \quad (4.a)$$

$$CDP^{max} = (4/27) / K_i \quad (4.b)$$

The above equations clearly point out that the f^{max} is independent of the nature of receptor-ligand interactions and the binding constant (free energy). **On the other hand, the CDP ^{max} mirrors increasing affinity.**In the following, we consider the application of this method to a number of diverse drug scenarios.

Application to Drugs : Results

Firstly we apply the methodology to a number of HIV protease inhibitors. All these are currently in wide spread clinical use.These are

isosteres of either the hydroxy-ethyl amine or hydroxy-ethylene '**transition state mimicks'**. The values of the nihibition constants are taken from the medicinal chemistry literature, and these represent realistic systems.[1,10-15] It will be shown that the method is equally applicable to the proteases of the usual and wild type HIV strains. It will also be shown that the CDP attains it's maximum at f =1/3 , in both the cases. Only the values of CDP ^{max} show the difference in activity patterns of the drug . It will be shown that the CDP ^{max} gives a measure of drug –resistance (mainly caused by mutation in the amino acid sequence of the mutant strain HIV protease), in comparison to the wild HIV protease strain.

In Figure.1A, Figure.1B, the CDP is plotted Vs c and f respectively for five HIV- protease (wild type strain) inhibitors, which are successfully used in clinical practice .[1, 10-15] For the most potent ones, K_i are smaller; the CDP ^{max} are proportionately larger(see Eq.4.b). The f ^{max} is invariant and occurs at f = 1/3 = 0.3333.

Figure.2A and Figure.2B show the corresponding behavior for the mutant strain of HIV- protease [10-15], which shows drug resistance, as reflected

in the large values of K_i and smaller values of CDP ^{max} (see Eq.4.b).

Nevertheless, in every case $f^{max} = 0.3333$, as is true for the wild strain.

We now look at the case of the same inhibitor (β -Naltrexamine), but

three subtypes of Opioid receptors. viz. the μ,δ,κ which have their natural

substrates [21,22] . The selectivity of binding to various receptors mentioned

above is reflected in their K_i values(see also Table.2). Figure.3A and

Figure.3B give the CDP plots Vs c and f respectively. The invariance of the f

$^{max} = 1/3 = 0.3333$ is readily seen for these three receptors, irrespective of

their binding selectivity [21].

A New Concentration Standard : IC(1/3)

A quantity of interest in drug research is IC (50), viz., the

concentration needed to inhibit 50 % of the receptor sites. The advantage of

using the IC(50) standard is that $f = 0.5$ at IC(50) and IC(50) = K_i. Thus

IC(50) directly leads to the estimation of binding constants and free energy,

if we consider single component inhibition data, viz.in the absence of the

natural substrate. The IC(50) concentration standard is universally accepted

as a valid yard stick for drug-potency.

We now define a new concentration IC (1/3) at which one third of the receptors are blocked by the inhibitor, viz. f = 1/3 and CDP attains it's maximum. From Eq.1 and Eq.4a, we have:

$$IC \, (\, 1/3 \,) = I \, C \, (50) \, / \, 2 \; = \; K_i \, / \, 2 \qquad (\, 5 \,)$$

Thermodynamic Analysis of CDP^{max} and IC(1/3)

In this section, we try to analyze the thermodynamic significance of IC(1/3). As mentioned earlier, this is the concentration at which CDP attains the maximum. To understand thermodynamic significance of f^{max} and CDP^{max}, we express the CDP as a ratio of the gradients of the inhibitor chemical potential in the free and bound states. For equilibrium to exist, the chemical potentials of the inhibitor have to be equal in both the free and bound states, i.e., $\mu_c = \mu_s$. Here the suffixes c,s represent the free and bound (to the receptor) state chemical potentials.

Using this thermodynamic criterion, it is straight forward to show that:

$$(\partial f / \partial c)_T \; = \; (\, \partial\mu_c / \partial c)_T / \, (\partial\mu_s / \partial f)_T \quad (\, 6.)$$

$$f (\partial f / \partial c)_T = (\, \partial\mu_c / \partial c)_T / \, (\partial\mu_s / \partial [f^2/2])_T \quad (\, 7)$$

. From Eq.7 it is obvious that the CDP equals the ratio of gradients of two chemical potentials. The numerator is the gradient of free inhibitor chemical potential with respect to c. The denominator is the gradient of the bound state chemical potential with respect to a molecular pair –probability p_{11}. This pair probability p_{11} is a Mean Field Pair probability given by $p_{11} = f^2/2$. We recall that to physically interpret this pair probability, we visualize the ligand molecules as if they are arranged on adjacent points (nearest neighbor points) of a lattice of receptors. It is to be noted that each receptor point has either single occupancy (bound receptor)or none (vacant receptor site).This is analogous to the Ising model of ferromagnetism. It may be noted that the lattice model has a fair degree of success in explaining protein structure and dynamics .[3]

We further assume that the free state ligand chemical potential has a logarithmic function of c ,viz. $\mu = \mu^0 + RT \ln c$, and the CDP becomes

$$CDP = (RT/c) /(\partial \mu_s / \partial p_{11})_T \qquad (8)$$

The gradient $(\partial \mu_s / \partial p_{11})_T$ is the denominator of Eq.8. Using Eq.4.a and 4.b , we get this quantity as equal to 13.5 RT at CDP ^{max}. If we have a

detailed molecular picture based on molecular mechanics or dynamics, then we could compare the estimated gradient of the chemical potential with the above predicted value (which is universal for all systems obeying reversible MM kinetics) . Thus it (the CDP method) will form a wonderful complement to the otherwise computationally intensive molecular mechanics or dynamics.

It is to be noted that this gradient $(\partial\mu_s / \partial p_{11})_T$ never attains an extremum at $f^{max} = 1/3$. Only the ratio of two gradients, i.e. CDP displays a maximum. Besides this quantity has a universal value depending on the physiological temperature T (T =310^0 K in the present study) and is independent of the K_i, binding energy and the detailed model of binding . Thus the detailed nature of hydrogen bond (acceptor or donor) , dispersion interactions, hydrophobic interactions ,etc. involved in ligand binding does not affect this. This is in full agreement with the thermodynamic nature of the analysis .

Relationship Between Binding Free energy and CDP ^{max}

We now extend the analysis after making a comparison with the conventional thermodynamic analysis involving estimation of the free energy of ligand-receptor interaction. From the inhibition constant K_i [or IC(50)],

the free energy of binding is found using the logarithmic relationship $\Delta G^0 =$

-RT ln K_b = RT ln K_i , where K_b , K_i are the binding and disassociation

constants respectively. Tables 1 and 2 give the free energies of binding for

a nunber of receptor-ligand systems such as the HIV- Protease inhibitors. [10-15] Opioid receptor inhibitors [21, 22], Angiotensin Converting Enzyme (ACE)

inhibitors [26] , HMG -CoA inhibitors [19,20] etc. In every case the successful drug

candidates have IC(50) or K_i value of 0.05 to 2 nM. The free energy spread is

also rather narrow, varying from -10 to -14 kcal /mole(see Tables 1&2). The

CDP^{max} show a direct correlation with the binding free energy ,as it should. (

see Eq. 4.b). ***It is to be noted that this quantity CDP^{max} estimated at***

IC(1/3) gives a direct measure of binding free energy as well as the

chemical potential gradients, as mentioned above.

Table 3 summarizes the essential findings about all the systems

studied. The universality of the conclusions drawn from a purely

thermodynamic analysis can be easily seen. From the second column it is

obvious that the CDP^{max} and the free energy differ by a constant value of 1.1

kcal / mole at a fixed temperature. The gradient of the bound state ligand

chemical potential with respect to a pair probability is a constant given by

8.32 kcal /mole, at the physiological temperature of 310 ^{0} K. Since the HIV protease inhibitors we have studied are ' transition state mimetics' , we would have thought that the estimation of the chemical potential gradient would help in drawing firm conclusions about the activated complex involved in the hydrolysis of the amide bond. Unfortunately, at the level of theory pursued in our work, no firm conclusions about the activated complex can be drawn [27, 30].

The HIV protease is known to be a homodimeric beta strand protein , with sites S1,S2,S3, S1',S2',S3', the sites being symmetrically placed. In future, one could visualize the concept of 'tethered ligands' [31] where in a pair of small ligands will act as an effective inhibitor. It is thus hoped that small molecule inhibitors(which will have enhanced oral availability) can make a ' **dual ligand**'. In searching for lead compounds, thus the molecular pair probability p_{11} and the gradient of bound state chemical potential will become very useful quantities.

Minimizing The Interference Of Experimental Error

The error of estimating the CDP from experimental data is minimal at points near $f^{max} = 1/3$. By following the line of argument followed in a

previous study(successfully employed in the study of adsorption[23]), this fact can be rigorously proved. But we omit the details in the present study, and only present the numerical results. This is can also be seen from Figure.4, where the the CDP error variance Vs deviation from f^{max} are shown for a number of HIV protease inhibitors. The experimental error in f   is assumed to be Gaussian. The data presented are simulated results The plot is shown for HIV protease inhibitors, but the same holds for all the other systems.

Concluding Remarks

Some brief comments are in order regarding the significance of CDP peaking at intermediate loading of f = 1/3. If we follow simple qualitative arguments, this has mainly origin in the configurational entropy of binding . The role of configurational entropy in protein folding is well understood [3, 28- 30] . For many proteins, the difference   between entropy and enthalpic contributions leading to the native structure is about 14 kcal/mole [3] . For the drugs analyzed, the free energy change is of the same order (see Tables 1,2). When the 'native structure ' (receptor in active confirmation) is disrupted as a result of ligand (inhibitor) binding , we would expect the binding free energy released to be of the same order(viz. 14 kcal/mole).

How much of the protein (receptor) conformation is distorted due to inhibition can be understood through a study of low frequency hinge-vibrations of the receptor, before and after binding. This is because, the HIV protease has flap regions, which control the accesss of the ligand to the receptor. These flap vibrations contribute appreciably to the vibrational – entropy change following drug binding. We do not attempt to quantify this in the present study, but this can be an interesting topic of a future publication.

Lastly, the concentration standard IC(1/3) is just half of the often used IC(50).***Thus with less quantity of lead molecules, we get more information which complements that obtained from Molecular modeling.*** Besides experimental error has very little influence (see Figure.4),if we collect accurate inhibition data around f = 1/3 and IC(1/3) as stated before.

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Table 1.

Inhibition constants, free energies of binding and CDP [max] for HIV protease

inhibitors , T =310 ^{0} K. Both wild and mutant proteases are studied. K_i

values are taken from ref [10].

Inhibitor	HIV Wild Type Protease			HIV A-44 Mutant Protease		
	K_i ,nM	ΔG^0 kcal/mole	CDP [max], nM^{-1}	K_i ,nM	ΔG^0 kcal/mole	CDP [max], nM^{-1}
Mk-944a ref [10]	0.049	-14.63	3.02	8.	-11.49	$1.85\ 10^{-2}$
Indinavir ref [11, 10]	0.24	-13.65	0.617	15.	-11.1	$9.88\ 10^{-3}$
Saquinavir ref [14, 10]	0.062	-14.48	2.39	15.	-11.1	$9.88\ 10^{-3}$
Ritonavir ref [12, 10]	0.062	-14.48	2.39	60.	-10.25	$2.47\ 10^{-3}$
Nelfinavir ref [13, 10]	0.14	-13.98	1.06	16.	-11.06	$9.26\ 10^{-3}$

Table 2.

Inhibition constants and free energies of binding , CDP ^{maxx} for three groups

of receptors at T=310 ^{0} K.

Inhibitor	Receptor	K_i, nM	ΔG^0, Kcal /mole	CDP ^{max}, nM $^{-1}$
β -Naltrexamine Ref [21]	Opioid , μ	0.45	-13.26	0.33
	δ	2.57	-12.19	$5.76 \ 10^{-2}$
	κ	0.7	-12.99	0.21
Captopril Ref [26]	ACE	1.7	-12.44	$8.71 \ 10^{-2}$
Enalaprilet Ref [26]	ACE	0.18	-13.83	0.823
Compactin Ref [26]	HMG - COA Reductase	1.4	-12.56	0.106
Mevinolin Ref [26]	HMG - COA Reductase	0.64	-13.05	0.231

Table 3

Universality in the thermodynamics of receptor -ligand interactions at CDP

^{max} for reversible Michaelis- Menten kinetics at T = 310 ^{0} K.

f ^{max}	$\Delta G^0 + RT \ln (CDP^{max})$	Gradient of the bound state chemical potential

		at CDP ^{max}
0.333	-1.18 kcal / mole	8.32 kcal /mole

LEGEND TO FIGURES

Figure.1A   Plot of the Concentration Derivative Product (CDP) Vs concentration of Inhibitor for five HIV protease (wild type) inhibitors displaying maxima, Saquinavir and Ritonavir have the same inhibition constants and plots. T= 310 ^{0}K

Figure.1B   CDP plots Vs fractional inhibition f. Note the invariance of f ^{max} =1/3.

Figure,2A Plot of CDP Vs the concentration of inhibitor for five HIV protease (mutant strain) inhibitors. Indinavir and Saquinavir have the same inhibition constants and plots. The drug resistance of the mutant strain is seen readily in the higher values of K $_I$ and lower values of CDP, CDP ^{max}

Figure. 2.B CDP plots Vs f . Note the location of f ^{max} = 1/3

Figure. 3.A plot of CDP Vs concentration of inhibitor for β - Naltrexamine / Opioid receptors μ , δ , κ

Figure.3.B CDP plots Vs f showing invariance of f ^{max} = 1/3

Figure.4 Plot of error variance of CDP Vs experimental error. Notice the striking minimum at f = 1/3. The experimental error is Distributed as a Gaussian with zero mean and standard deviation of 0.01,and simulated with random numbers.
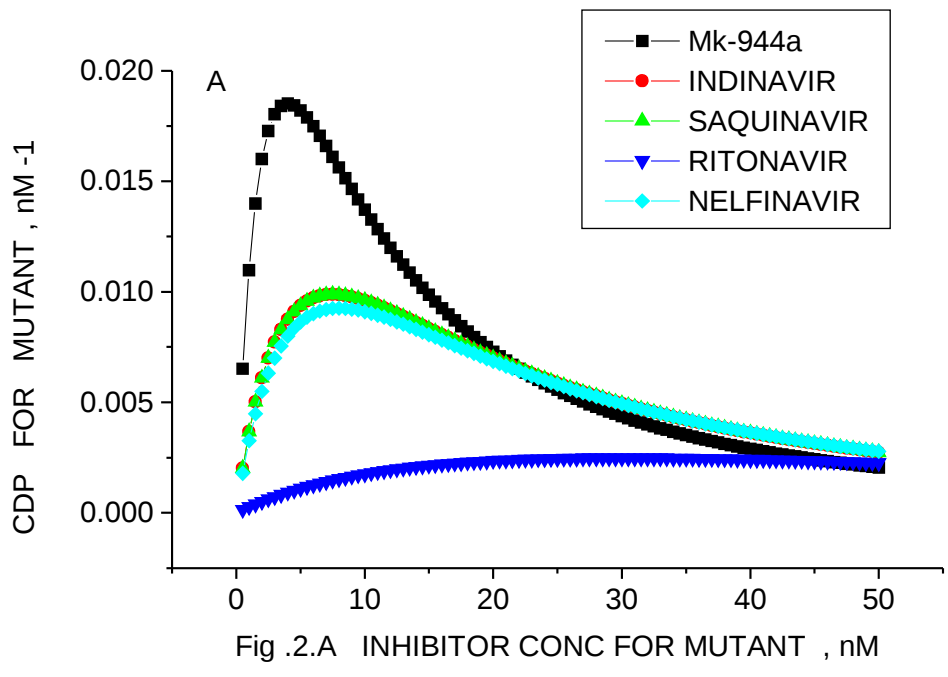
Fig.1. A   INHIBITOR   CONC, nM

Fig 1. B FRACTIONAL   LOADING
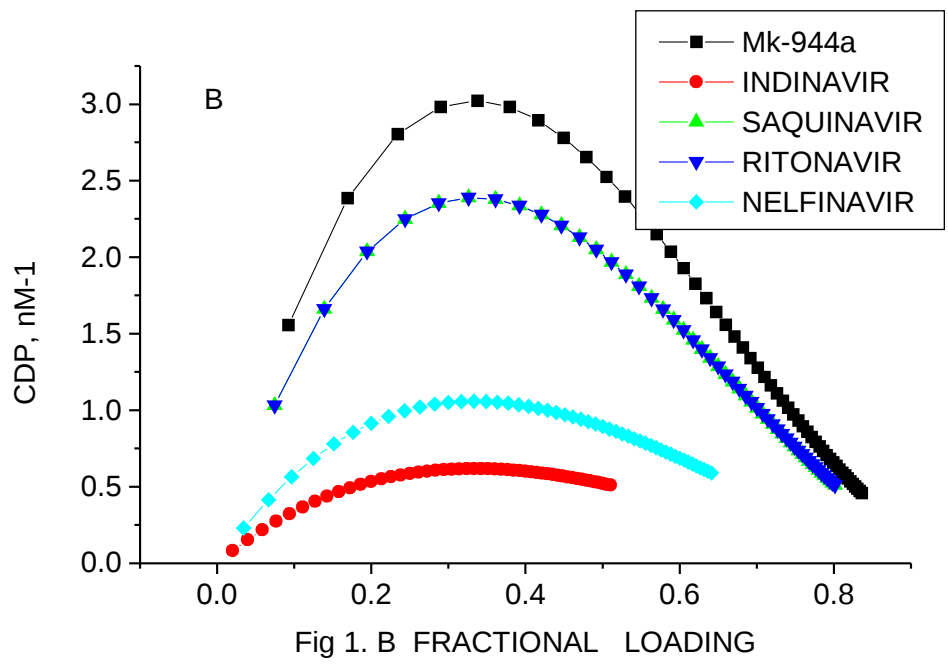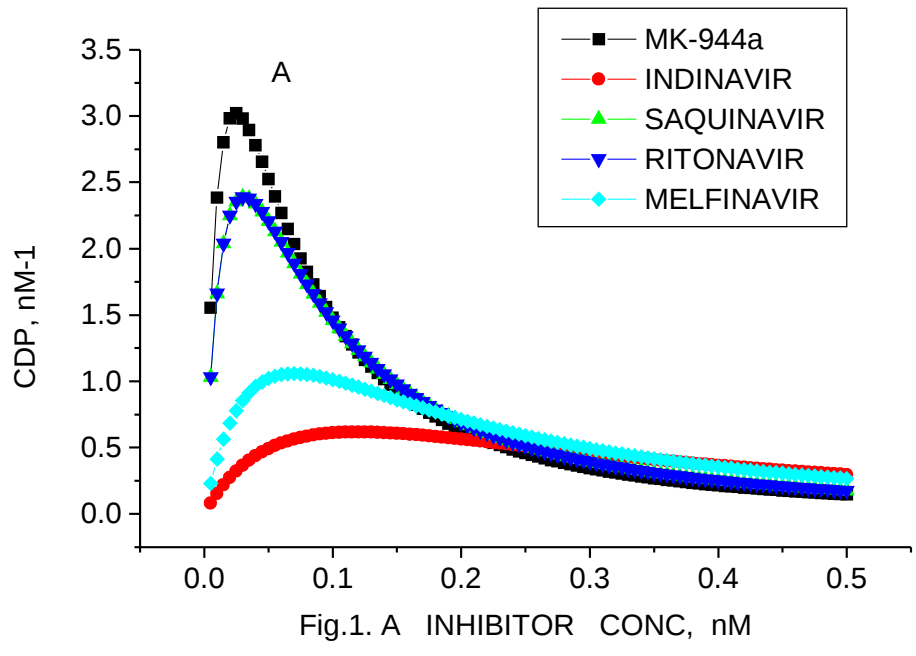
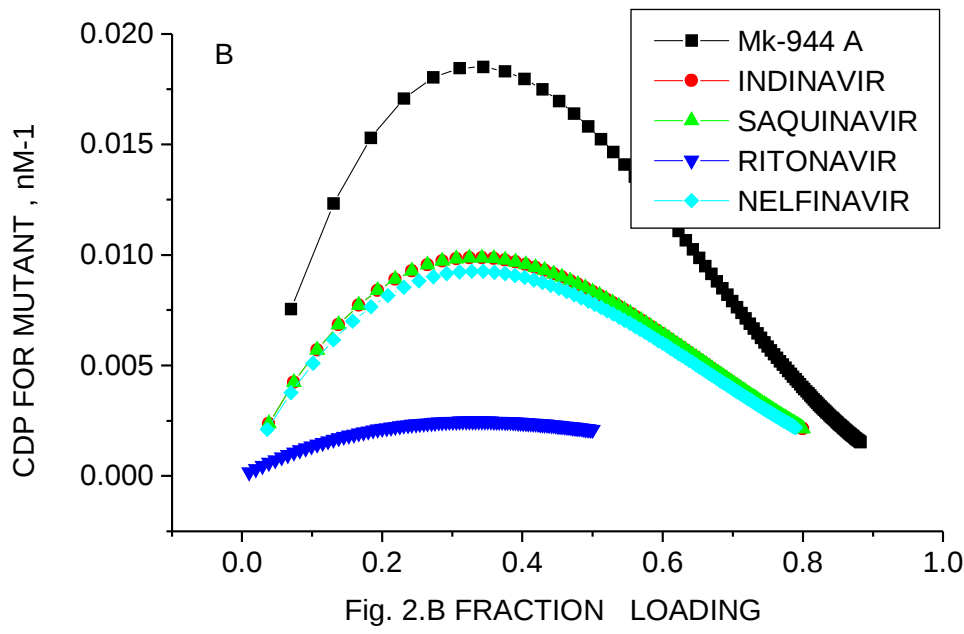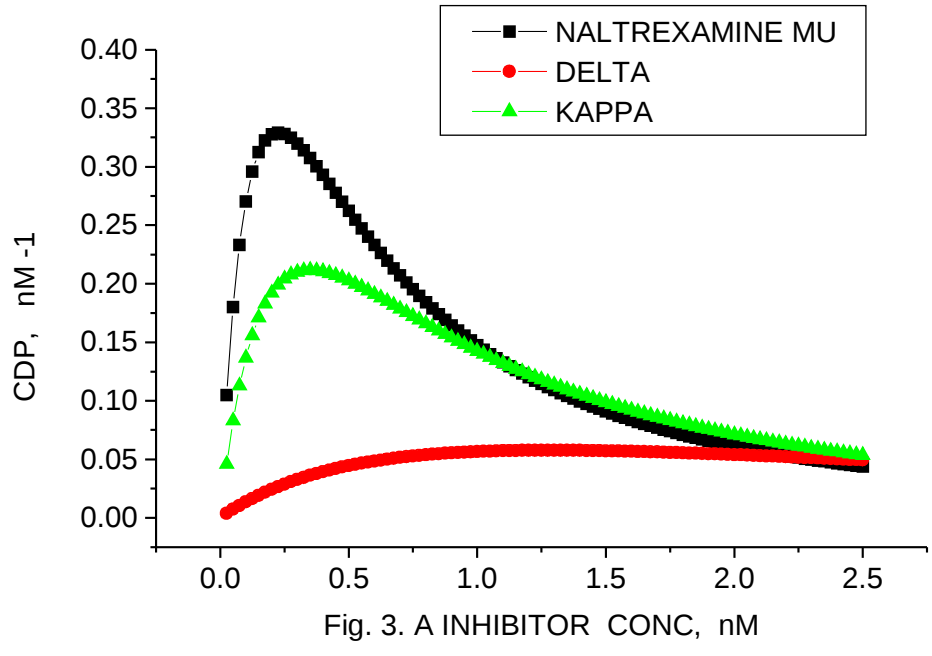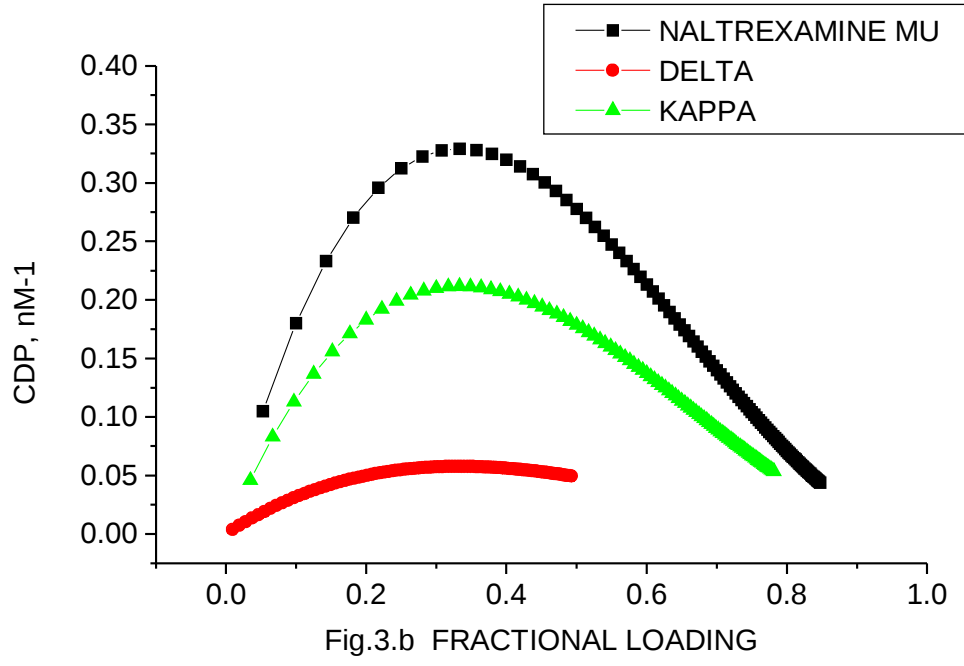Fig .2.A   INHIBITOR CONC FOR MUTANT , nM

Fig. 2.B FRACTION   LOADING

Fig. 3. A INHIBITOR CONC, nM

Fig.3.b FRACTIONAL LOADING

Fig.4   DEVIATION FROM THE f (max)