Chemical and Biological Thermodynamics: Principles to Applications

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Lecture – 48
Isothermal Titration Calorimetry (ITC) in Drug Design

After discussing the principle of isothermal titration calorimetry and differential scanning calorimetry we have now started discussing the applications of both these calorimetric techniques in academics and in industry. Today we will talk about the use of isothermal titration calorimetry in rational drug design we will discuss how the changes in enthalpy entropy and free energy can be connected with the efforts to improve the existing molecules or new molecules with a focus towards novel drug design. There are lot of synthetic chemists molecular biologists and many other scientists working towards synthesizing designing and coming up with new drug molecules every molecule which has to act as a drug molecule has a specific function and sometimes the function can be it has to go and inhibit some process.

For example even if it is inhibiting the activity of an enzyme then inhibition of that particular activity will require a specific binding of that molecule or of that inhibitor with the enzyme and even if it is to be connected with the drug delivery again the focus comes on the particular affinity of that molecule towards that protein or towards that enzyme. That means, one of the efforts has been to improve the affinity either in the existing drug molecule or to come up with new molecules which should have improved affinity higher affinity of the order of the desired target.

We will discuss how isothermal titration calorimetry has given some guidelines over a period of years in this direction let us remember that the guidelines towards something do not come overnight one has to work with a variety of molecules suppose even if the base of a molecule is same. And then different substituents are added the effect of different substituents on the binding affinity can be checked thus by doing experiments with a variety of drug molecules structure property activity relations can be drawn and some guidelines can be drawn for rational drug design and target oriented synthesis.

Now, as I mentioned that there are large number of libraries of compounds available there are new molecular entities being added every year every month to this huge library

of compounds, but each compounds synthesized with an idea of making it as a drug does not generally end up in being a drug what are the required properties in a drug it should have a good oral bioavailability it should have good solubility it should have good membrane permeability amongst others.

Now for a drug to be orally bio available the molecule has to pass some stringent requirements and what are those requirements let us take a look at those requirements.

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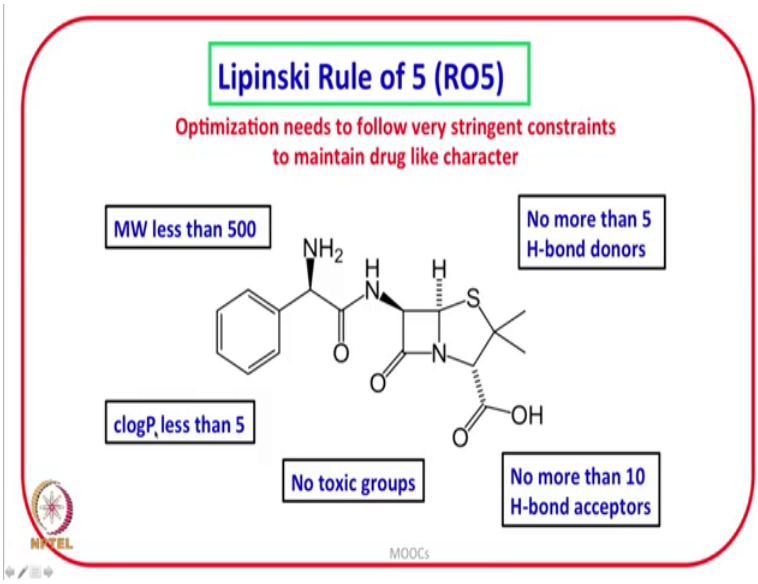

And let us go to the slide those requirements have to be met first of all in the form of Lipinski rule of 5 which is also sometimes called RO 5 what is that rule as I commented here optimization; optimization means even if you are working on an existing molecule you want to optimize the molecule. So, that it becomes an effective drug and that is what is commented over here that optimization needs to follow very stringent constrains to maintain drug like character and what are those suppose we want to optimize a molecule or come up with a new molecule which should be accepted as a drug like character. And as I said for a molecule to be for a molecule to have good bioavailability the stringent constraints are let us take a look at molecular weight should be less than 500 there should be no more than 5 hydrogen bond donors there should be no more than 10 hydrogen bond accepters and there should not be any toxic group and finally, seal of P should be less than 5.

So, these 5 constraints 1 2 3 4 5 that is why it is called RO5 Lipinski rule of 5. So, in addition to the molecular weight and the hydrogen bonding abilities in the form of hydrogen bond donor or hydrogen bond acceptor toxicity also becomes a very stringent requirement toxicity comes later, but before that C log P should be less than one now this log P; P is partition coefficient C log P or log P actually tells about the hydrophobicity of a molecule because P is partitioning coefficient between octanol and water octanol will octanol has one hydroxyl group and there are 8 carbons.

That means, it is a largely hydrophobic molecule and when we have a mixture of octanol and water and you add a hydrophobic moitute to this it will partition between octanol and water. So, if the molecule is largely hydrophobic it will partition more into octanol and if the molecule is largely hydrophilic it will partition more into water. So, therefore, if there is more partitioning into octanol; that means, the molecule is more hydrophobic and the requirement is let us take a look at this slide that log P or C log P is should be less than 5; that means, there is a maximum limit set for the hydrophobicity of the molecule and the effort has to be that P or log P should be less than 5.

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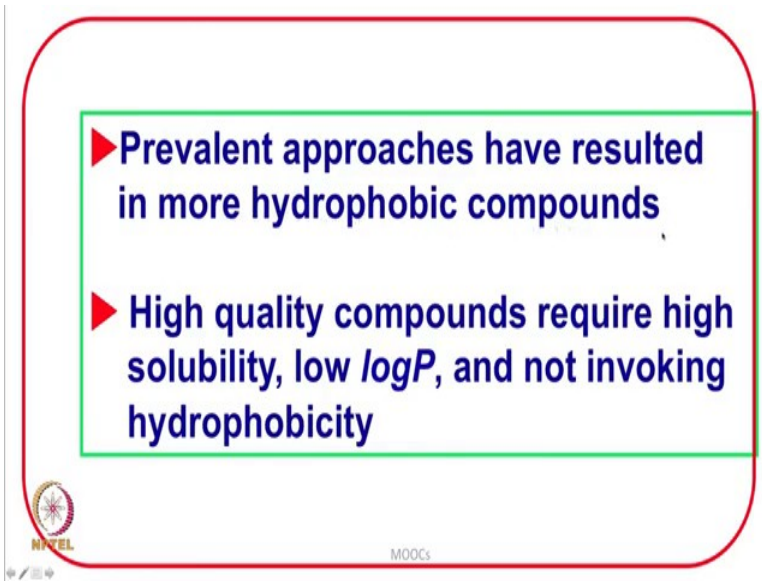

Now, let us take a look at this slide prevalent approaches have resulted into more hydrophobic compounds once again commenting upon the synthesis of new molecular entities with an objective of rational drug design or novel drug design we generally come across the information from the literature that most of the approaches in this direction

result into more hydrophobic compounds, but then the question is are these hydrophobic compounds or the compounds in which the hydrophobic component is more is it good or is it bad for a molecule to act like a drug and I just mentioned C log P should be less than 5. So, their it refers to that the hydrophobicity should not exceed a certain value and I will talk a little more about it that what the literature has suggested whether the hydrophobic content should be more or polarity should be more.

Now, let us took take a look at the next comment high quality compounds require high solubility low log P and not invoking hydrophobicity now this information is based upon the literature information if we say C log P should be less than 5; that means, we are we are putting the high quality compounds in that category and that is why this comment is made that [ha/high] high quality compounds require good solubility. So, that C log P is below 5 and that can be obtained only if we do not invoke higher content of hydrophobicity in the molecule.

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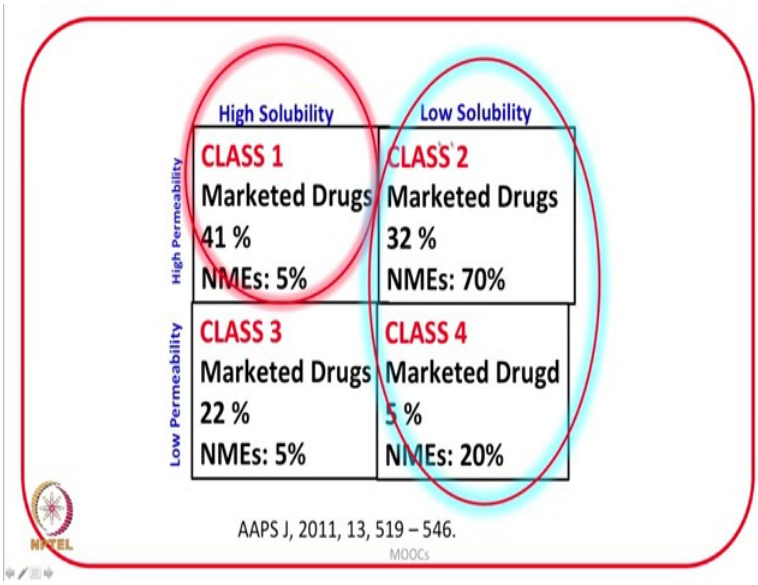

Analysis of lot of data available from literature over a number of years has suggested some guidelines or has given some important information let us take a look at this slide let me divide the drugs available into the market into 4 categories class one where the membrane permeability is high and solubility is also high this is the most preferred class class 2 where the high permeability and low solubility is observed class 3 low

permeability and high solubility and class 4 which is the least preferred that is low permeability and low solubility this is not preferred.

Now, the data suggests that out of forty one percent marketed drugs the new molecular entities only 5 percent of the new molecular entities fall into the category of high permeability and high solubility this is a point worth noticing that only 5 percent of the new molecular entities the newly synthesized drugs only 5 percent fall into high permeability and high solubility category whereas, 90 percent 70 percent or plus 20 percent 90 percent of the new molecular entities fall into the category of low permeability, low solubility or high permeability low solubility and solubility you see is the major concern over here there is still a fraction 5 percent where you have permeability low. But solubility is sufficient, but the major problem which is faced by the industry is this ninety percent of the new molecular entities will either have high permeability, but low solubility or low permeability and low solubility and that is the problem being faced by pharmaceutical industries that is why I made that comment over there that the high quality compounds means the solubility efforts increasing the solubility those efforts should be strengthened.

Now let us take a look at some more trends.

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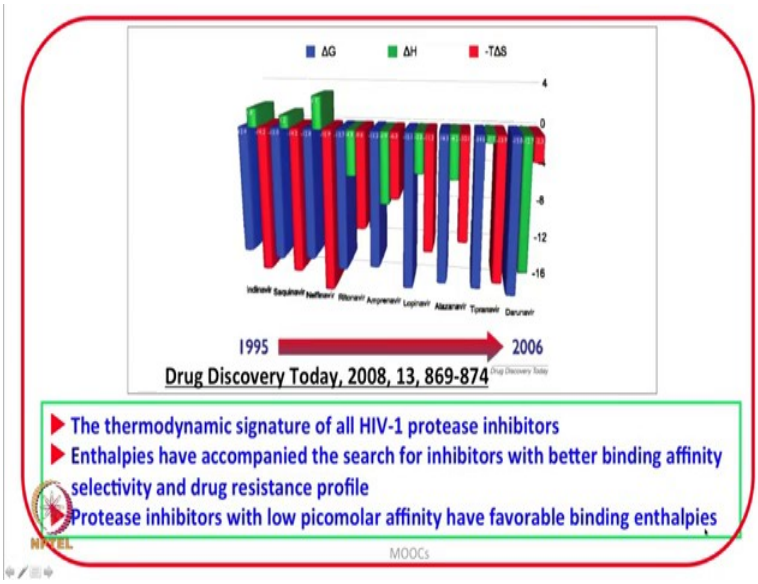

Now, this let us take a look at this data very carefully this data corresponds to the affinity binding of the HIV protease inhibitors.

And the associated changes in standard free energy standard enthalpy and standard entropy over a period of about 10 to 11 years this data it gives some interesting information the green bars upwards represent enthalpy change upwards we consider positive and downwards. Let us consider negative delta G naught is represented by the blue bars and minus T delta S is represented by the red bars and the trend is for end in a way to drownaway in 2006; drownaway was the quite used FDA approved HIV protease inhibitor.

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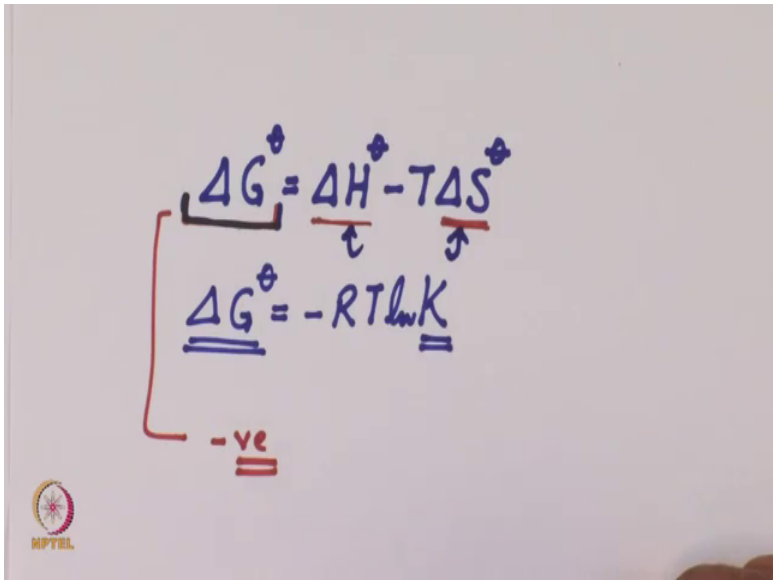

Now, let us take a look at this equation delta G naught is equal to delta H naught minus T delta S naught as I mentioned that one of the efforts in industry is to improve the affinity and improving the affinity means that what should be done suppose if we want to improve the affinity by an order of 5. Now we know that delta G naught is equal to minus r T log K and if I want to improve the affinity by 10 raise to the power 5; that means, by an order of 5 we can easily calculate that how much it will contribute to the value of delta G naught this equation will permit that.

However delta G naught has to be negative this has to be negative and the contributions to delta G naught come from delta H naught and delta S naught represented in this as minus T delta S naught reaction or the binding can be exothermic can be endothermic it can generate entropy it can reduce entropy the example that I what would like to discuss with you gives some general trends observed over at least a period of 10 to 11 years. Let

us take a look at the slide 1995 look at the binding the association was accompanied by positive change in enthalpy endothermicity and over the years if you see the green bars which were upwards are turning to downwards green bars. That means, over a period of about 10 to 11 years the new molecules showed affinity with more exothermicity see it changes from endothermicity towards exothermicity and this trend suggests that over this period the new molecules have shown a trend changing from endosermicity to more exothermicity.

Now, this figure which represents the thermodynamic signature of all hiv one protease inhibitors suggests that enthalpies have accompanied the search for inhibitors with better binding affinity selectivity and drug resistance profile in pharmaceutical industry we are not only concerned about the binding affinity, but the selectivity and the drug resistance profile also now binding affinity let us go back to this discussion binding affinity affinity is talked in terms of delta G naught and affinity can be engineered. Now I am using the word very carefully engineered the affinity can be engineered in a molecule by either working on delta H naught or delta S naught.

If we want to have a more negative value of delta G naught delta H naught if it is more negative that will support and if delta S naught also becomes more positive that will also support; that means, the binding affinity can be engineered either by making the interaction more exothermic or a combination of exothermicity and a lot of entropy generation and let us discuss a bit more about that the next comment protease inhibitors with low picomolar affinity have favorable binding enthalpies and that is what I was commenting upon that picomolar affinity you remember in one of the previous lectures. I said that physical chemists would like to talk in terms of association and biochemists would like to talk in terms of dissociation picomolar means we are talking here in terms of dissociation and one can convert this dissociation constant in those association constant and talks in terms of the binding affinity or binding constant.

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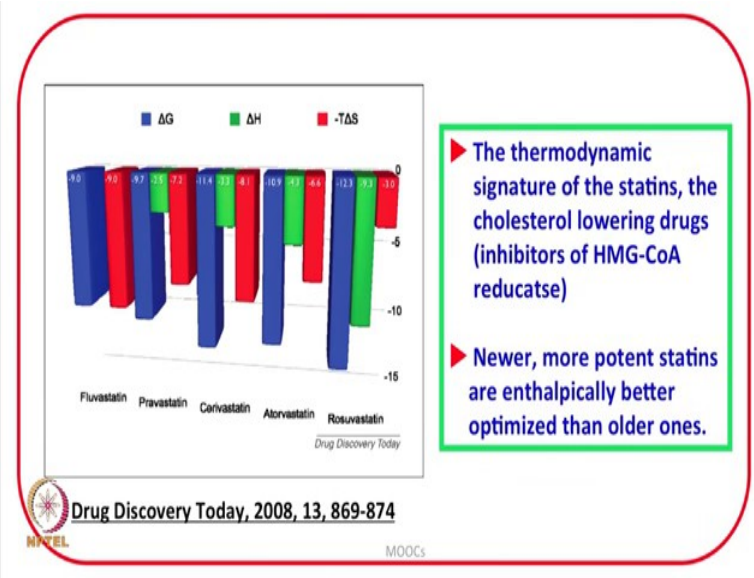

The trend is more clearly seen in this figure this figure is on the Statins; statins are the cholesterol lowering drugs and these act by inhibiting the hmg co a redactors and therefore, the affinity of the statins to the enzyme becomes very important and the efforts have been made to achieve the optimum affinity. Now here if we look at the trend once again the trend is towards more exothermic binding of the statins starting from fluvastatin to rosuvastatin we see that the green bars the lengths of the green bar is more though extent of exothermicity is more when we started from earlier and made progress towards the later years and of course, we should at the same time not forget the changes in the entropy.

But engineering binding affinity in a molecule in terms of enthalpy becomes much more easier as I we will discuss later on, but a general comment which I can make over here is that from the first in class this drug if I say fluvastatin as the first in class in this period to the best in class the best in class in this period I will call as rosuvastatin. So, from the first in class to the best in class the trend has been enthalpic that is over the number of years the new drug molecules were binding with the more exothermic character.

Now, let us take a look at the comment the thermodynamic signature of statins the cholesterol lowering drugs inhibitors of HMG-CoA reductase have shown a some general trend that is from a lower exothermicity to more exothermicity in binding and that is what is commented over here that newer more potent statins are enthalpically

better optimized than older ones this is enthalpically better optimized than this one that is what the comment reference to.

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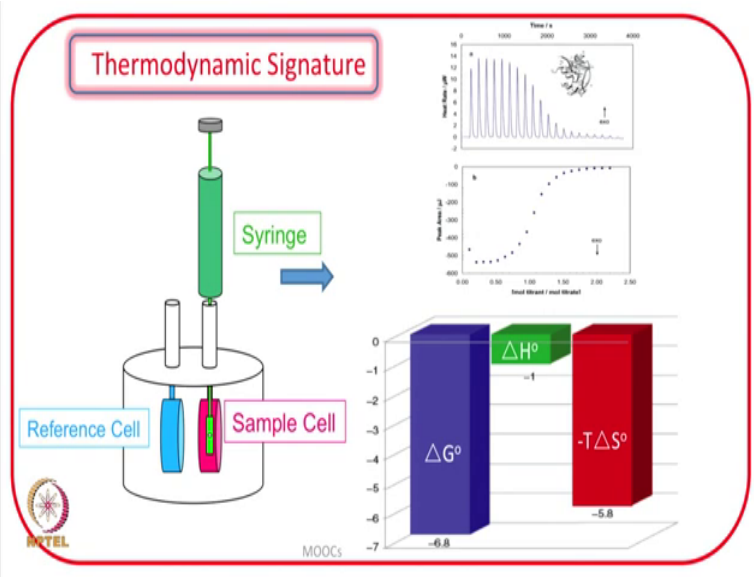

And as I mentioned that when we want to engineer binding affinity in a drug molecule we have to talk about the thermodynamic signature which is shown in this figure; that means, delta H naught and delta S naught are the 2 important contributions which will decide the sign and magnitude of delta G naught.

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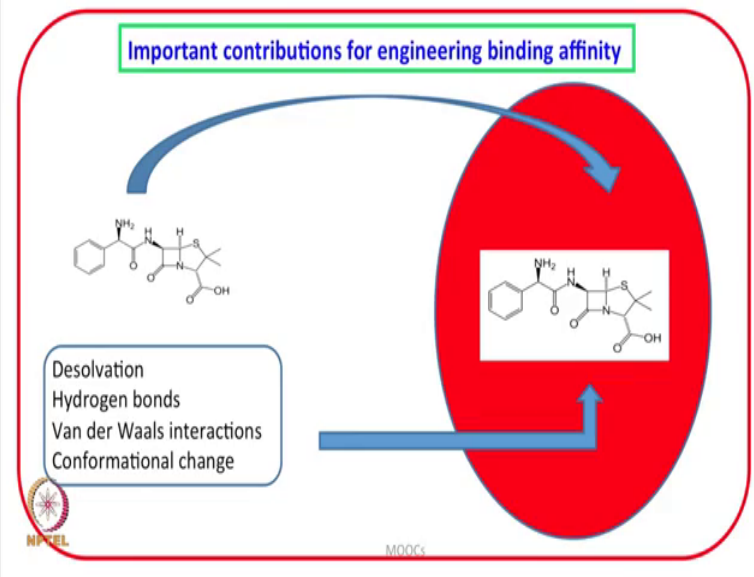

So, what are the important contributions for engineering binding affinity now let us say that this is the binding cavity I represent this orange this cavity as the binding cavity and let us say I am interested we are interested that this molecule should go and fit into this cavity and bind over there this is the process that we are interested in. So, what are the important contributions for this molecule to go and effectively bind over there let us remember that this molecule when it is free in aqueous solution it is surrounded by lot of water molecules. And therefore, before this molecule is removed from water and put into this binding cavity water has to be removed from the immediate vicinity of this molecule and that is what is called desolvation; this desolvation not only refers to the removal of water molecules from the vicinity of this drug molecule, but also the water molecules which may be occupying the binding cavity.

Now, when this molecule is placed into this binding cavity it will undergo some interactions with the constituents of this binding cavity and what are those interactions these can be hydrogen bonding interactions it can be hydrophobic association. Also there can be Van Der Waals changes interactions and there can be conformational change desolvation hydrogen bonds Van Der Waals interactions conformational change and if there are largely hydrophobic groups there can be hydrophobic interactions also.

Now, we need to go through it a little bit more carefully and closely one is that the water molecules have to be removed from immediate vicinity of this molecule as well as from the binding site and we talked about that once this molecule is placed over here. These interactions will set up first is the desolvation; desolvation is removal of water molecules and these interaction hydrogen bonding Van Der Waals interactions conformational change or some hydrophobic interactions may also setup between the various groups of the drug molecule and the constituents of the binding cavity and each point which is mentioned over here will contribute to delta G naught delta H naught and delta S naught.

So, therefore, when we engineer binding affinity in a drug molecule or we synthesize a new molecule with a good binding affinity we need to take care of several things one is the desolvation how much energy it will cost to de solve it, how much energy it will give back when it form hydrogen bond Van Der Waals interactions, what will be the contribution to delta G naught or delta H naught because of hydrophobic interactions and conformational change does it cost in terms of delta G naught or delta H naught or does it give a reward in terms of delta G naught or delta H naught various thermodynamic

quantities when measured give some guidelines in these directions in answering these questions.

So, what we have discussed in this lecture is that over a longer sufficiently longer duration of time the thermodynamic data gives sufficient guidelines towards the next step for example, what we observed that the HIV 1 protease inhibitors or we took the example of statins the data suggested that the drug molecules from the first in class to the best in class have shown affinity which is more enthalpically driven and similarly the contribution of enthalpy of or contribution of entropy which makes changes to delta G naught gives lot of guidelines and these issues we will discuss in further detail in the next lecture.

Thank you very much.