Structural Biology
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Lecture - 59
What makes a small molecule an ideal drug: Developing in silico ADMETox Model

Hi everyone, welcome again to the course on structural biology. We are at our last module. Today, after understanding the general view of drug discovery, rational drug discovery, traditional drug discovery, pharmacophore model, QSAR, de novo designing, and docking-based virtual screening. Today, we will focus on a beautiful question that I have already discussed a lot of times. When would a small molecule be considered a drug?

So, today's discussion would be majorly focused on the drugability of a molecule.

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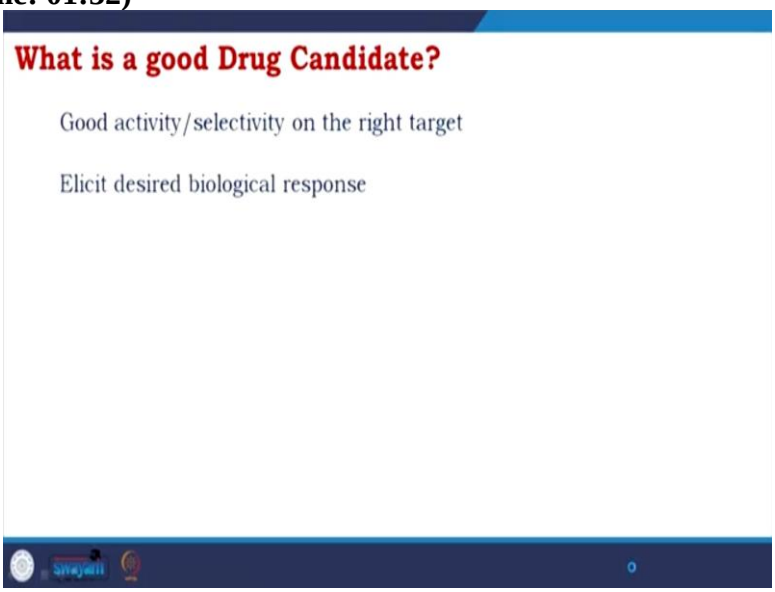

So, let us investigate what is a good drug candidate or the criteria that make a small molecule a good drug candidate? Our last few classes know that there is an obvious requirement of good activity and selectivity on the right target. A small molecule has to bind to the target, and then after binding, it would give some biological response. But a definition of a good candidate should not be restricted here.

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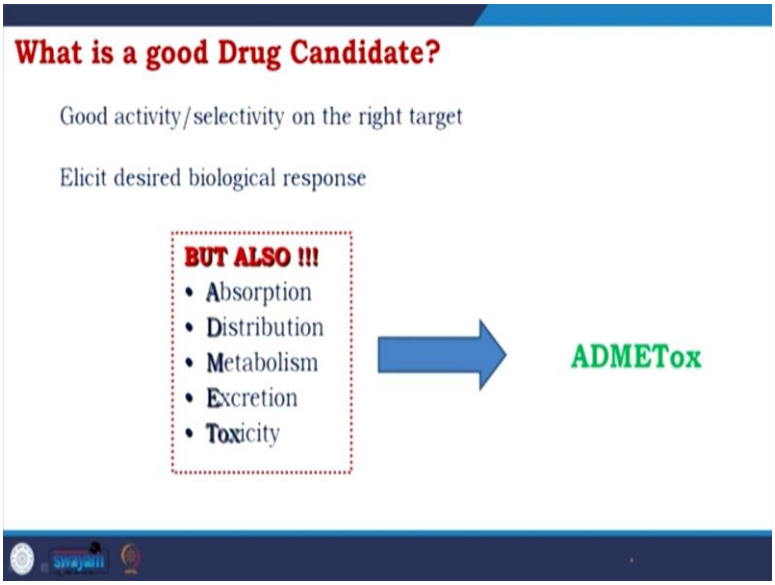

It should also have good absorption, good distribution, good metabolism, good excretion, and toxicity, known as ADMETox. ADMETox plays a critical role in drug designing because, as I have explained earlier, if a compound has all the quality to bind and show biological response, it still should not be considered a good drug until we know how this molecule is actually going and behaving in biology.

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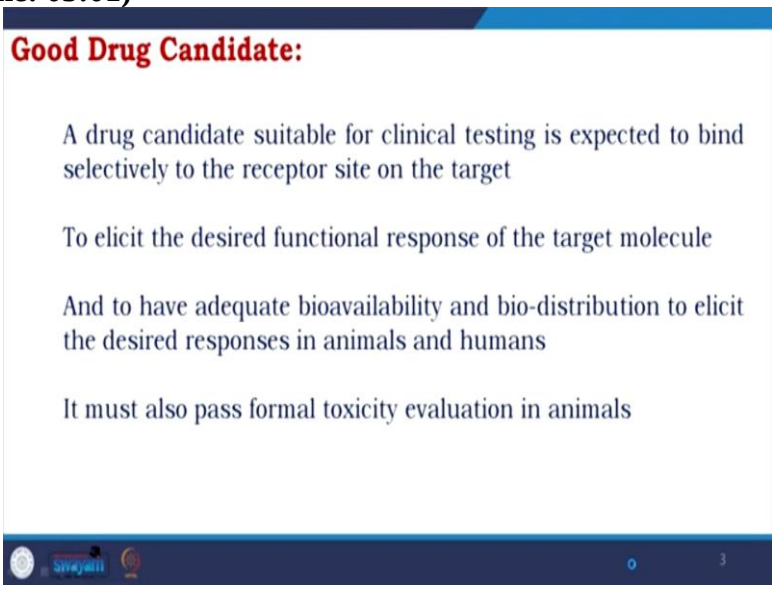

So, an ideal definition of a drug candidate, a drug candidate suitable for clinical testing, is expected to bind selectively to the receptor site on the target, as we have talked about, to elicit the desired functional response of the target molecule. In addition to having adequate bioavailability and biodistribution to elicit the desired response in animals and humans. So, you have adequate bioavailability should be available in the biological system. It should go

and dissolve, and it should distribute well. It must also pass formal toxicity evaluation in animals.

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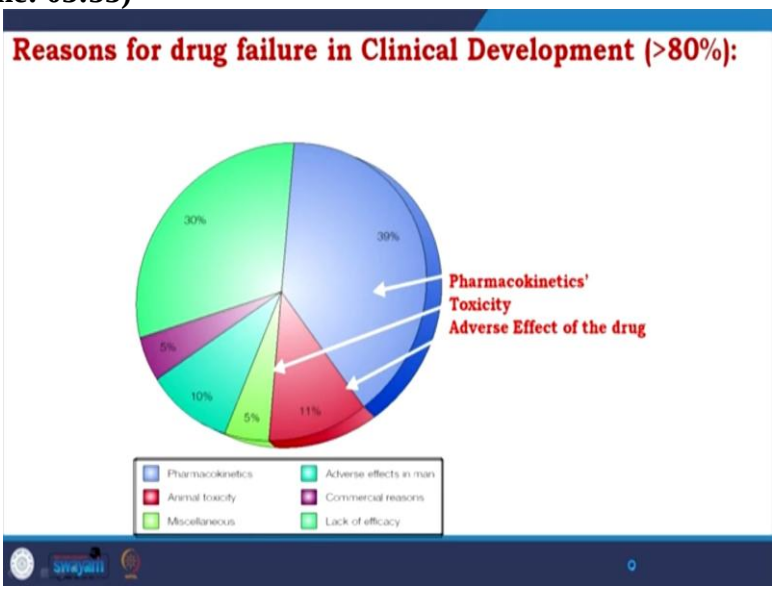

As you see, these are the major reasons for drug failure in clinical development. So, you see that, so many things pharmacokinetics, toxicity, adverse effect of drugs, they are all responsible.

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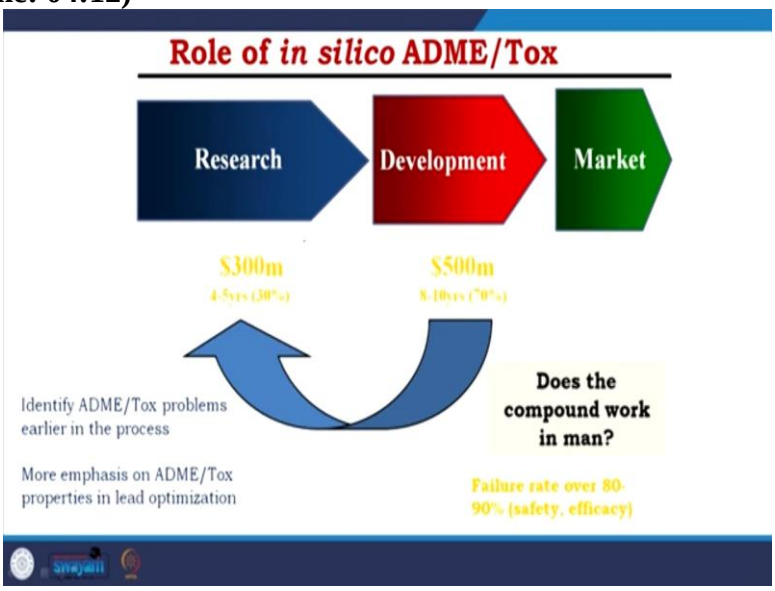

Your research to find out the target, at an average US dollar 300 million is spent on the research and 4 to 5 years it takes. Then you go for development, dollar 500 million again in 8 to 10 years. So, whatever you do here would not make any sense if it goes to the development.

So, the big question is, does the compound work in humans because you have to put it on the human system, and the failure rate is over 80 to 90% because you cannot kill or severely affect a person by giving a wrong drug. And that is where we have new thoughts as I continuously talk about; the major principle of improving drug discovery is replacing processes using computation replace experiment use computer.

So, the possibility we are exploring here is to identify ADMETox problems earlier in the process, emphasizing ADMETox properties in lead optimization. So, if we do something at the research level, it would help us save this money.

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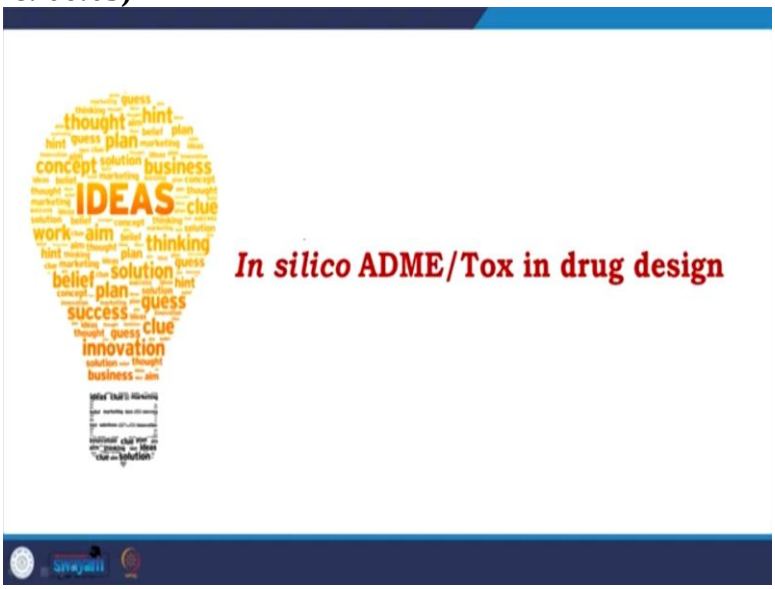

So, overall, looking at the definition of a good drug, looking at the critical role of ADMETox in the system and the thought that we need to reduce the time as well as the cost, the idea comes, is it possible that we will develop in silico ADMETox and introduce it in that drug designing. What is in silico ADMETox? In silico, ADMETox is to understand the process of ADMETox and make models understand parameters that are critical for that and develop in silico experimentation compared to what we do in biology.

What are the challenges? So, it was happening at the atomistic level up to the drug designing. So, it was possible to replace the process with a computer because there are logic, rules, and atom to atom connections. Whereas ADMETox depends on the biological effect completely and then it isn't easy actually to replace it, the ADMETox experiment with the computer or, in one word, develop in silico ADMETox.

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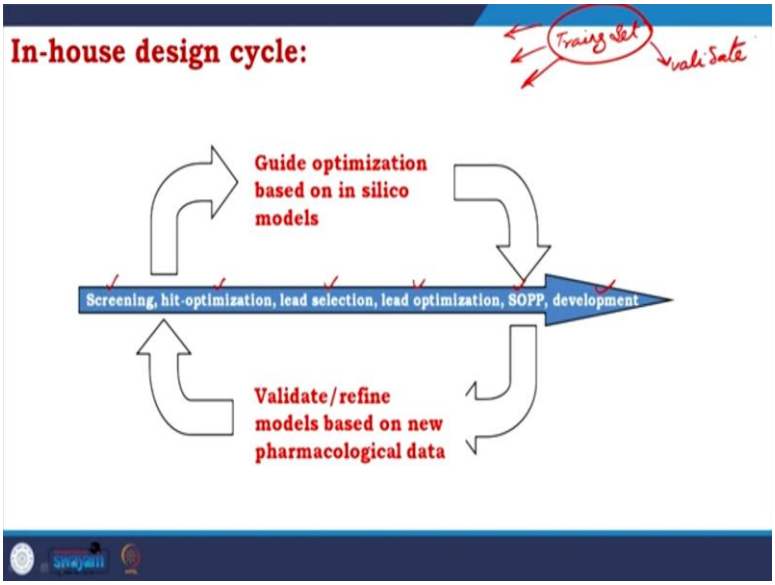

To start with, first, the focus is on in house design cycle. If you look at it as we all know, it starts from screening. It goes to hit optimization, lead selection, lead optimization, SOPP, and development. So, those are the process initially, screening then hit optimization, then lead selection, then lead optimization, SOPP, and then development. In this process, if we make a cycle where we guide optimization based on the in silico model and then validate a refined model based on new pharmacological data.

So, in between, we understand the role of machine learning, and we know that with a lot of experiments going on and a lot of experimental data already available, it is not difficult to make a training set, get the initial screening, and then validate it with a new cycle.

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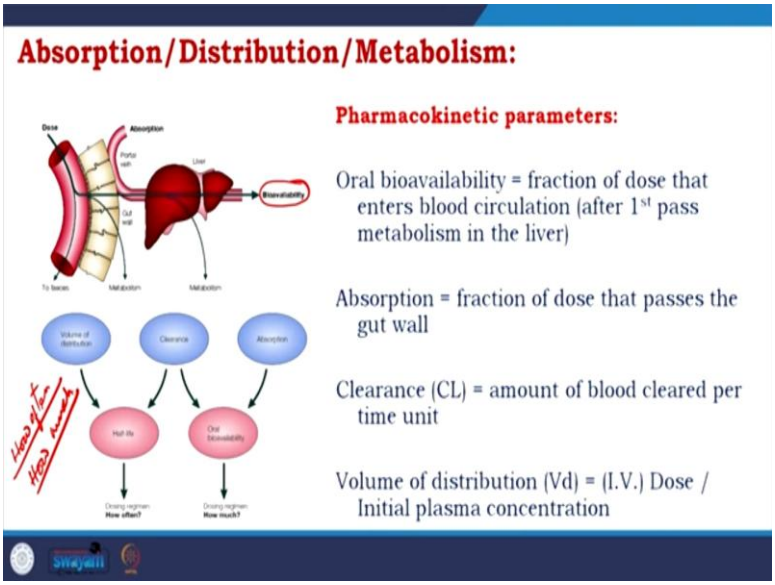

So, coming to absorption, distribution, and metabolism, you see that these systems go and directly come out in the excretion. Then, it comes into the system goes through metabolism, then it comes to the gut wall through the portal vein, come to the liver, again, including the metabolism, absorption happens. We get the drug available to the biological system. You have to consider the volume of distribution, its half-life, and the designed regimen. How often do you need to provide the drug?

Then you look at clearance, you look at absorption, oral bioavailability, and again, you design the dosing regimen. How often should the drugs be given? How much drug could be given in one go? These are two very critical parameters you have to understand. So, these are called pharmacokinetic parameters. You have to consider oral bioavailability, the fraction of dose that enters blood circulation after first-pass metabolism in the liver.

Absorption is the fraction of the dose that passes the gut wall. Clearance is the amount of blood cleared per time unit. Volume of distribution Vd equals IV and intravenous doses by initial plasma concentration.

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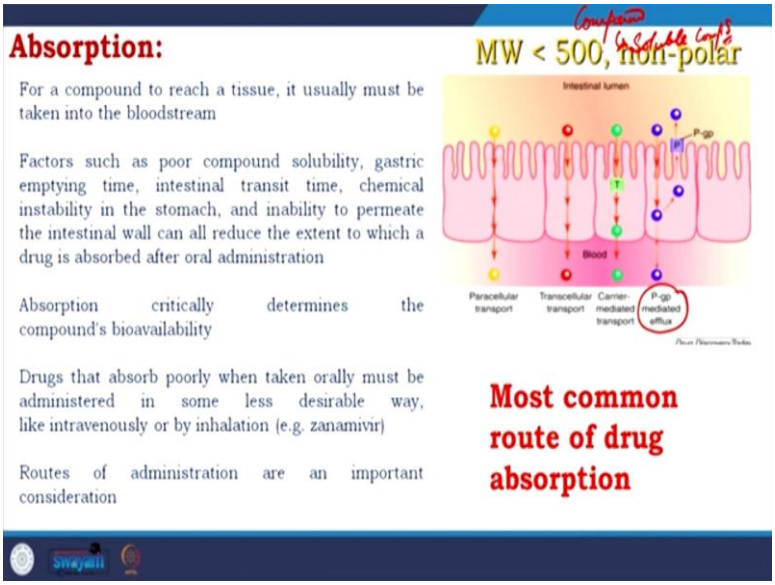

So, coming to absorption for a compound to reach a tissue, it usually must be taken into the bloodstream. Factors such as poor compounds solubility, gastric emptying time, intestinal transit time, chemical instability in the stomach, and inability to permeate the intestinal wall can reduce the extent to which a drug is absorbed after oral administration. So, you put the drug by oral administration, how it goes to the liver, how it becomes bio available, depends on the compound's solubility.

So, now when you say compound, it is a good soluble compound that has good quality to be a drug. Gastric emptying time, how much time does it take? Intestinal transit time, how much time it goes in the flow? Chemical instability in the stomach because in the stomach environment the compound stable or not that inability to permeate the intestinal wall, is it able to permeate, is it able to go through the intestinal wall, all of them reduce the extent to which a drug is absorbed after oral administration.

Absorption critically determines the compound's bioavailability. Drugs that absorb poorly when taken orally must be administered in some less desirable way, like intravenously or by inhalation, for example, zanamivir. Routes of administration are a very critical and very important consideration. So, if you look at the intestinal lumen to the blood, it comes through paracellular transport, transcellular transport, carrier-mediated transport, and Pgp, which is glycoprotein mediated efflux.

And these are the most common route of absorption and the criteria. Here are the criteria, the molecule being non-polar would be good. A molecule with less than 500 Dalton is good.

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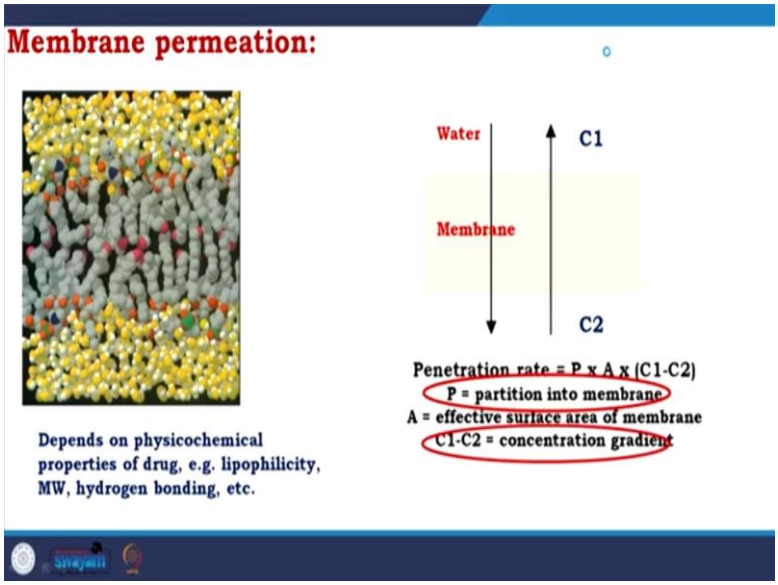

I talked about membrane permeation. If you look at the membrane, we talked about earlier. We talked about biological macromolecules lipids. You see that it is hydrophilic on both sides and hydrophobic inside. So, it comes from water, an aqueous environment to the hydrophobic membrane environment. Very important factors are P's penetration rate into A into C1 - C2, where P is the partition into the membrane, A is the effective surface area of the membrane, and C1 - C2 is the concentration gradient.

Penetration rate = P x A x (C1 – C2)

The partition into the membrane, which is the most critical factor here in the permeation, depends on the drug's physicochemical properties, such as lipophilicity molecular weight, hydrogen bonding, and all those factors. It also depends on the concentration gradient, which keeps developing in the system once you start dosing a drug, hydrogen bond donors, and acceptors.

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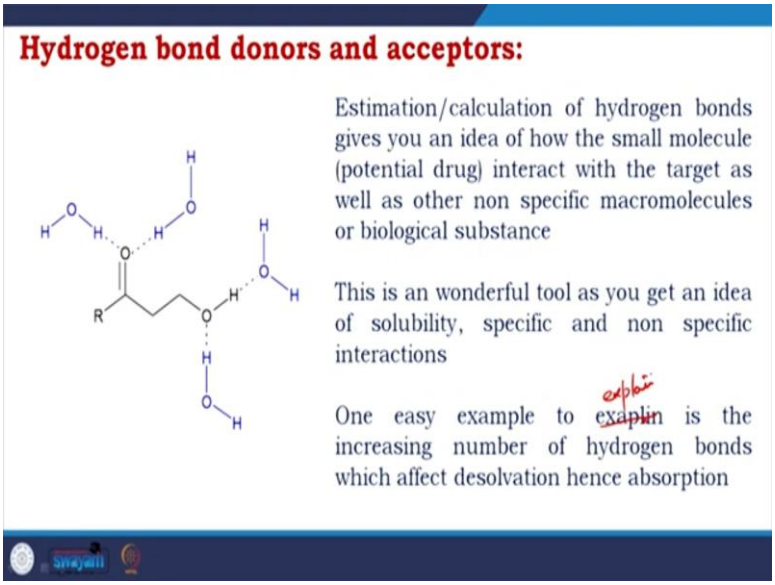

The estimation calculation of hydrogen bonds gives you an idea of how the small molecule, which is the potential drug, interacts with the target and other nonspecific macromolecules or biological substances. So, if you understand the initiative's goal, developing these in silico ADMETox is to make some rules that help you get some guidelines to make numbers so that you can correlate the number with the biological activities.

This will help you generate these numbers for different molecules and take a decision as much as this decision would be biologically relevant. Better could be your model. Because hydrogen bond is always a quantitative thing, you know that target. You could measure it. So, it is always a very helpful and critical parameter. This is a wonderful tool. By tool, I mean the estimation of hydrogen bond donor, hydrogen bond acceptor considering the small molecule interacting with the biological system.

This is a wonderful tool for getting an idea of solubility-specific and nonspecific interactions. One easy example is the increasing number of hydrogen bonds that affect desolvation. So, if you increase the number of hydrogen bonds, then what will happen? There would be a kind of incorporation of irreversible nature because more hydrogen bonds will make more tight binding, so opening once the complex form the opening would be difficult. Hence, absorption would be difficult.

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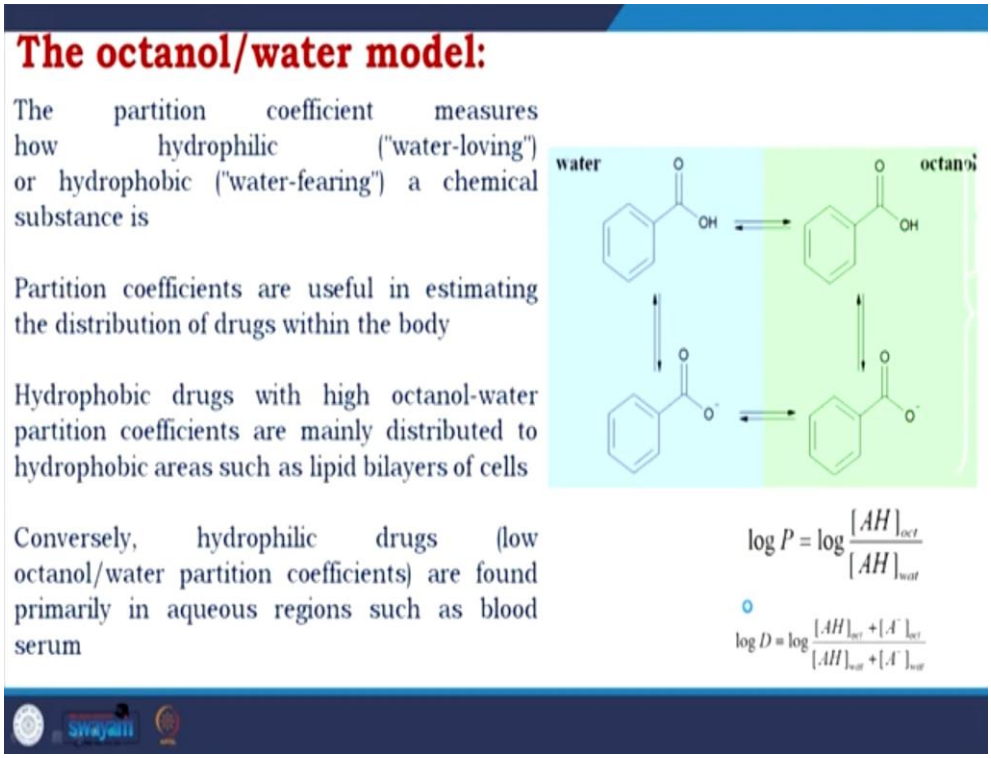

Coming to the partition coefficient, the partition coefficient is again a very important factor. Before going into biology, the context in which we are talking about if you consider partition coefficient according to the rule of basic science, a partition coefficient which is considered as P is related to distribution coefficient which is termed as D is like, partition coefficient and distribution coefficients are the mostly same thing or related thing or similar type of thing is the ratio of the concentration of a compound in a mixture of two immiscible solvents at equilibrium.

And in most cases, when we come to biology, we consider an aqueous or polar solvent with a non-polar hydrophobic solvent. So, in pharmaceutical sciences, both phases usually are solvents. Most commonly, one solvent is water, while the second one is hydrophobic such as octanol. So, here, the molecule is partitioned between a polar solvent, water, and a hydrophobic solvent, octanol.

Why are we doing that? If you remember, here, we have water and a membrane. The membrane is hydrophobic to mimic the situation we take octanol. Now, if you see the partition coefficient P,

the log P = log $[AH]_{octanol}$ / $[AH]_{water}$

[AH] is the concentration of the molecule in octanol by the concentration of the molecule in water. So, the log partition coefficient is the log of the concentration of hydrophobic by the concentration of water the aqua solvent.

Coming next, the partition coefficient measures how hydrophilic water-loving or hydrophobic water-fearing a chemical substance is. Partition coefficients are useful in estimating the distribution of drugs within the body. Hydrophobic drugs with high octanol-water partition coefficients are mainly distributed to hydrophobic areas such as the lipid bilayer of the cell. Conversely, the low octanol water partition coefficients of the hydrophilic drugs are found primarily in aqueous regions such as blood serum.

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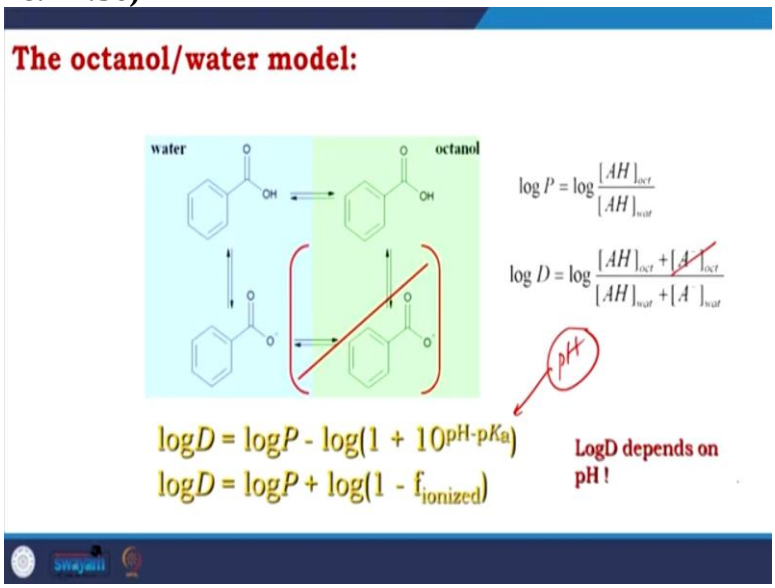

Very interestingly, you could see that as I told the log P equal to the log concentration of the molecule in octanol by the concentration of the molecule water,

$\log D = \log P - \log (1+10^{pH-} pKa)$

log D is the log of the concentration of the molecule plus the concentration of the ionized part of the molecule in octanol divided by the concentration of the non-ionized molecule plus the concentration of the ionized molecule. But, if you think, because octanol is hydrophobic, no ionization will occur.

$\log D = \log P - \log (1+10^{pH-} pKa)$

$\log D = \log P + \log (1 - f_{ionized})$

So, the value of log D, log D is the distribution coefficient depending on the pH. And now, you understand that there is dependence in pH, so you have to look at the system and determine the pH.

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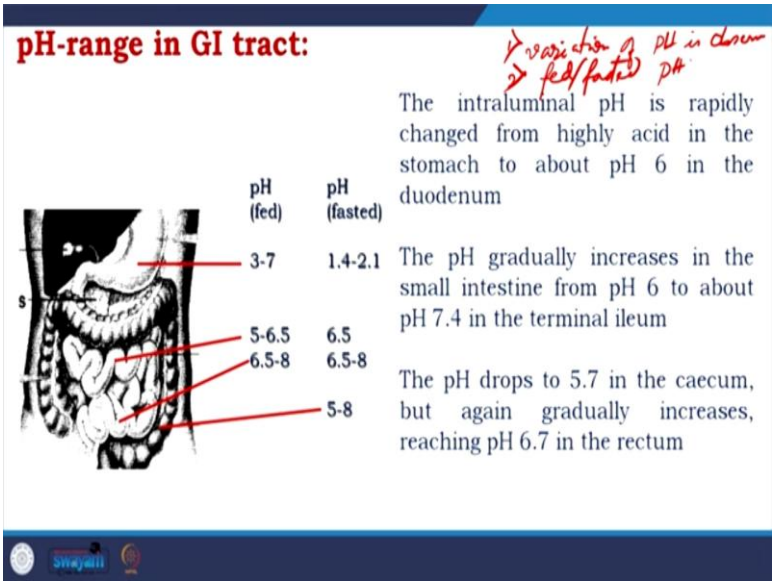

So, it is now important to see the pH range in the GI tract. The intraluminal pH is rapidly changed from highly acidic in the stomach to about 6 in the duodenum. So, if you see, there are two things, one there is a variation, so, variation of pH is absorbed in the system.

So, the intraluminal pH is rapidly changed from highly acidic in the stomach to about pH 6 in the duodenum. The pH gradually increases in the small intestine from pH 6 to about pH 7.4 in the terminal ileum.

The pH drops to 5.7 in the caecum but gradually increases, reaching pH 6.7 in the rectum. We know now about log P and log D, we know P is partition coefficient, and D is distribution coefficient. Still, today, I will introduce a new number that changes the spectrum of ADMETox, especially in silico ADMETox. So, instead of the log P value, we will talk about the ClogP value.

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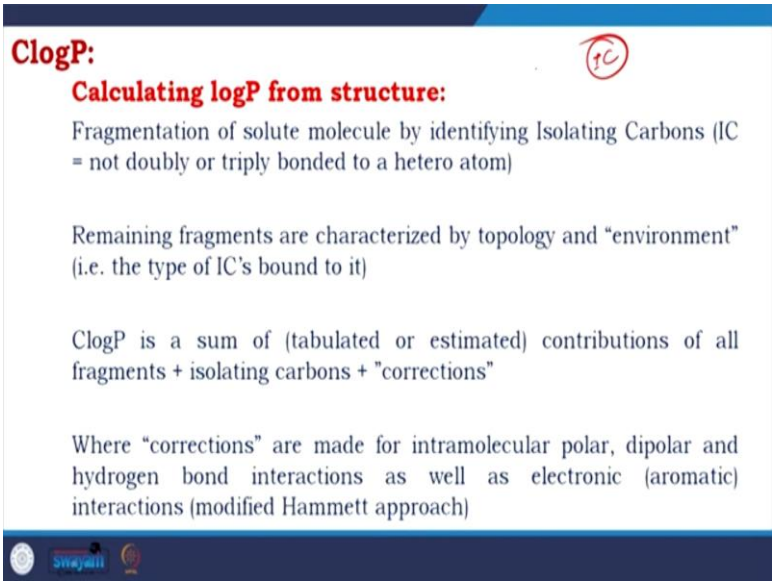

Which is calculated log P, so how do we calculate log p from the structure? When I say structure, we need the structure of the small molecule. So, fragmentation of solute molecule by identifying isolating carbons which are not doubly or triply bonded to a hetero atom, so our first job is to identify the ICs the isolated carbons which are not doubly or triply bonded to a heteroatom. The remaining fragments are characterized by topology and environment.

ClogP is a sum-up contribution of all the fragments, isolating carbons, and corrections. Corrections are based on effect corrections made for intra molecular polar, dipolar and hydrogen bond interaction and electronic interactions brought from the modified Hammett approach. So, we are calculating that log P which we call ClogP. ClogP considered the isolated carbon than the other remaining fragments and corrections made because of polar, bipolar, intermolecular hydrogen bonds and electronic effects.

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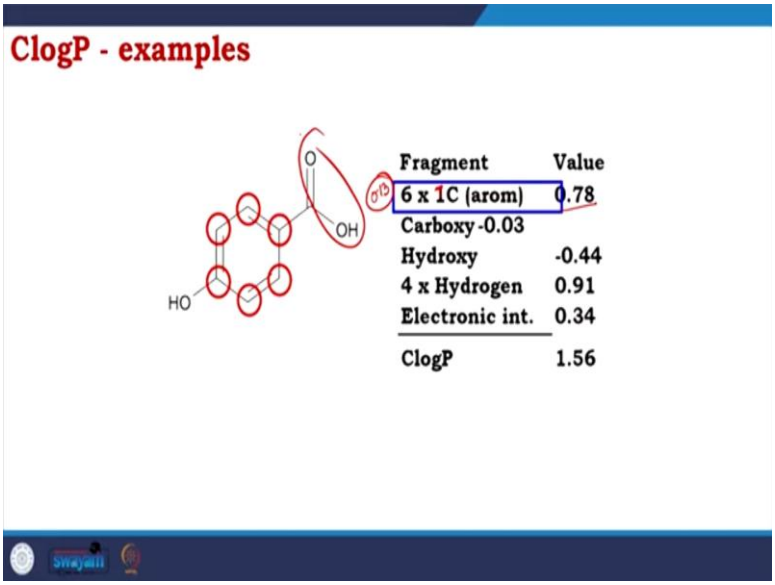

So, let us see how ClogP calculation works? So, first, you have six isolated carbons. So, you have six isolated carbon, which means each has a value of 0.13. You get 0.78 because of having a carboxy group, and you get minus 0.03. So, first, you have these 6 carbons, which gives you 0.13 x 6 = 0.78.

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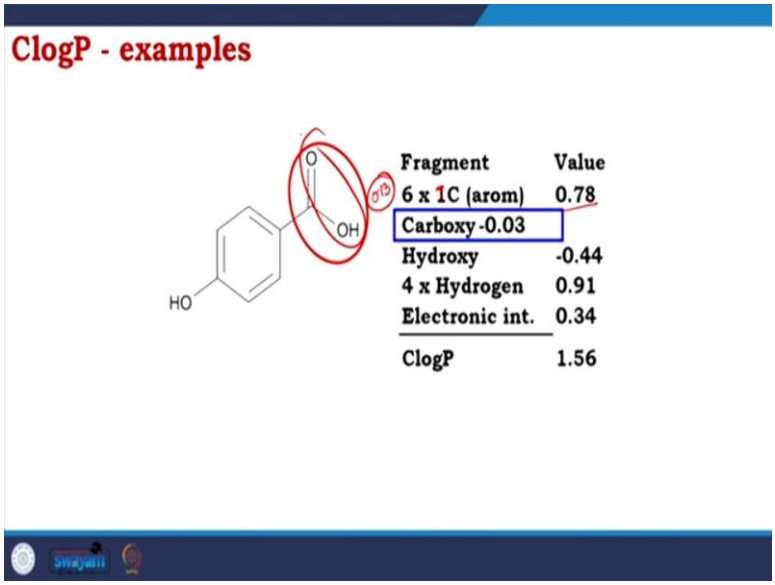

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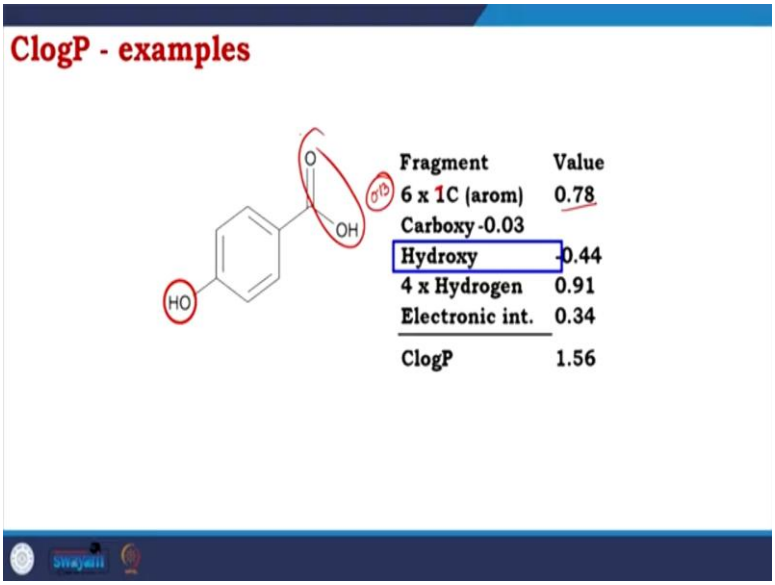

Then you go to the carboxy, which gives you a correction of 0.03. Then you come to hydroxy, which gives you minus 0.44.

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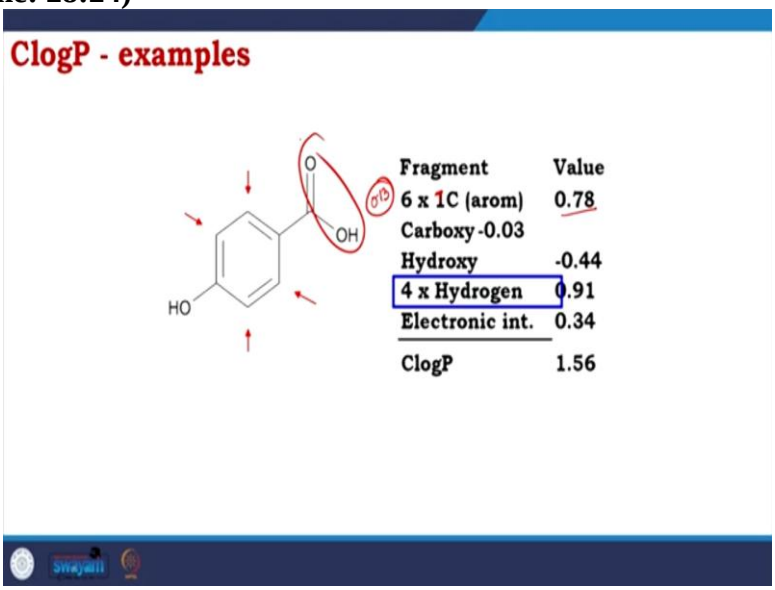

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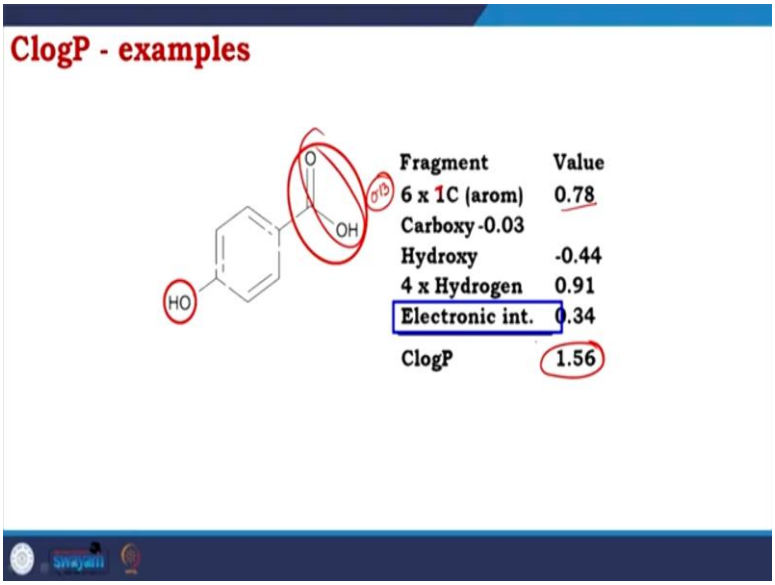

Then you have four hydrogen's, which gives you 0.91. And then, you get the electronic interaction of the carboxyl and hydroxyl group, which gives you the value of 0.34. Overall, the ClogP is 0.78 + 0.91 + 0.34 - 0.44 - 0.03, is 1.56. So, this is the calculated log P. I am sure that now you are very excited to know the actual experimental value. I would not make it late and show you the value of 1.58.

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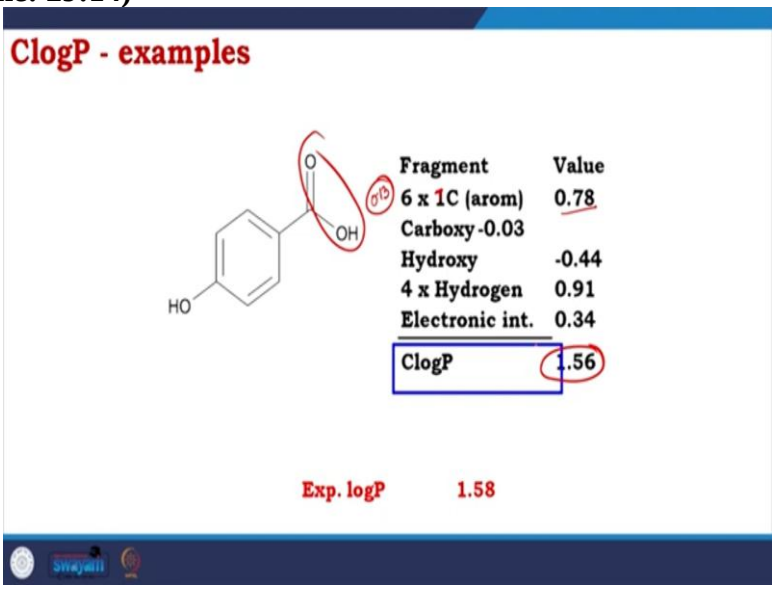

So, now you can understand how ClogP is making the revolution because the ClogP value comes 1.56, whereas the experimental value is 1.58.

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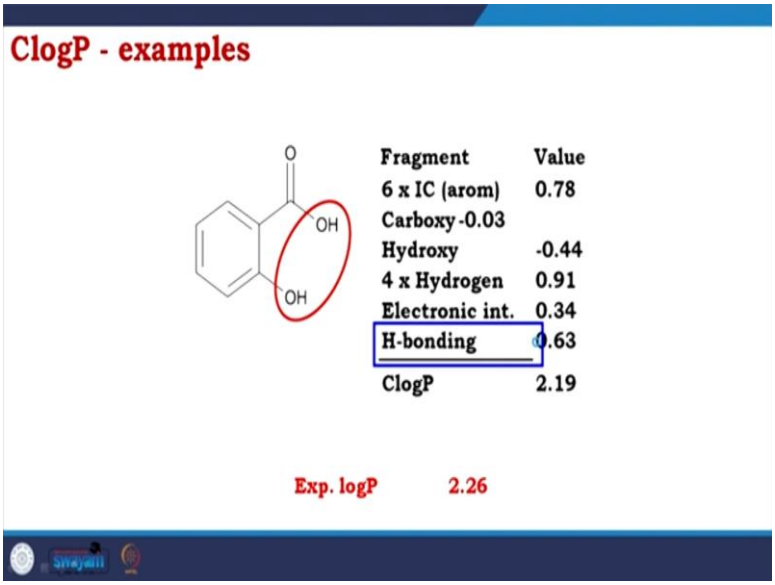

Let us take another example where the hydroxyl group changes to the ortho position. So, here you also have an extra thing: the hydrogen bonding between the carboxyl and hydroxyl groups. Because of that, 0.63 would be added, making the value 2.19, whereas the experimental log p-value is 2.26, again, very close. And if you are thinking that I am picking up the best examples and presenting to you, yes, I am, but then I am taking you to a more revolutionary work again.

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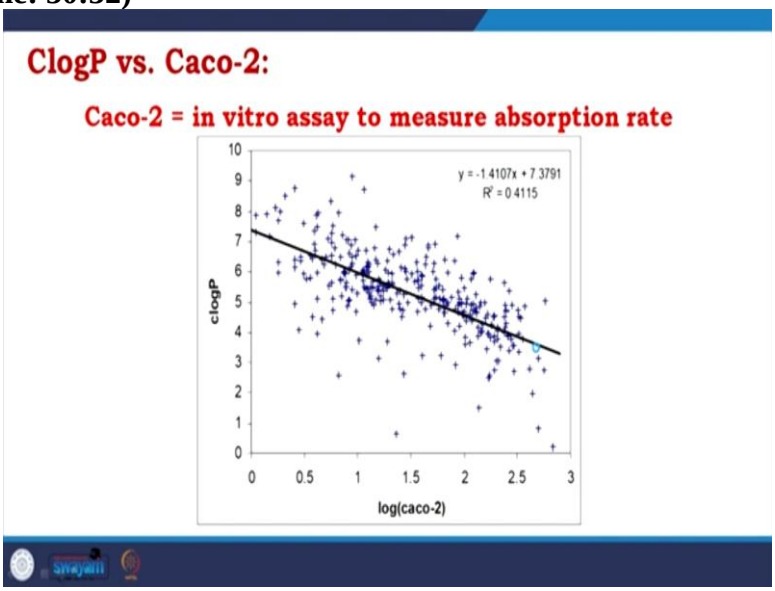

Which is a plotting between the ClogP versus Caco-2. Caco-2 is the value coming from in vitro assay to measure the absorption rate. So, this is an experiment, Caco-2 values are coming through the experiment. And when we plot the ClogP and log Caco-2, you see a linear regression. You see a trend, which is very critical. To get a trend between the

theoretical calculations with the experimental value means, now, you could replace the experiment with a computer calculation.

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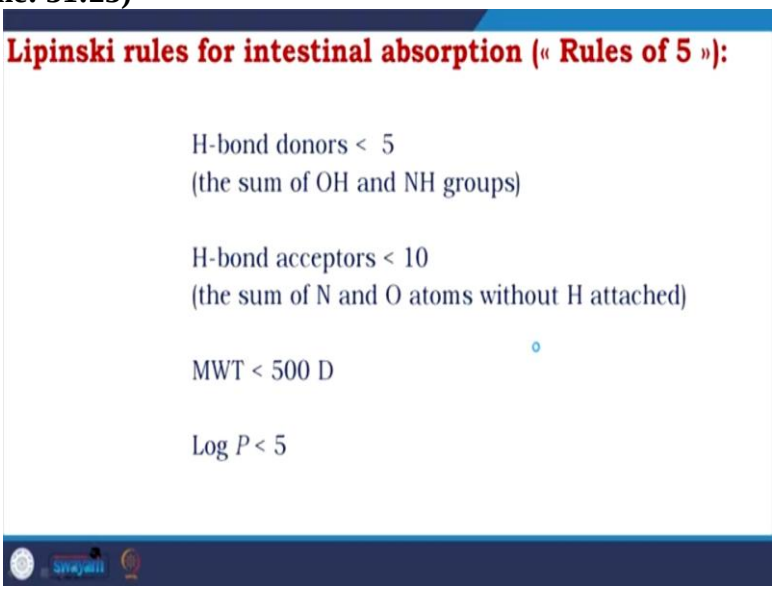

And all these factors are provided with a rule called Lipinski rules for intestinal absorption or Lipinski rule of 5. According to that rule, the hydrogen bond donors should be less than five, which is the sum of hydroxyl and NH groups. Hydrogen bond acceptors should be less than ten, the sum of N and O atoms without hydrogen attached. The molecular weight would be less than 500 Dalton, and the log P would be less than 5.

Because all the values are multiplied by 5, it is called the Lipinski rule of 5. Lipinski rules for intestinal absorption or Lipinski rules of 5 gives you the first theoretical model to apply on a small molecule even before you decide to do the screening. So, even before the screening, it is possible to check the druggability of the molecule using the Lipinski rule.

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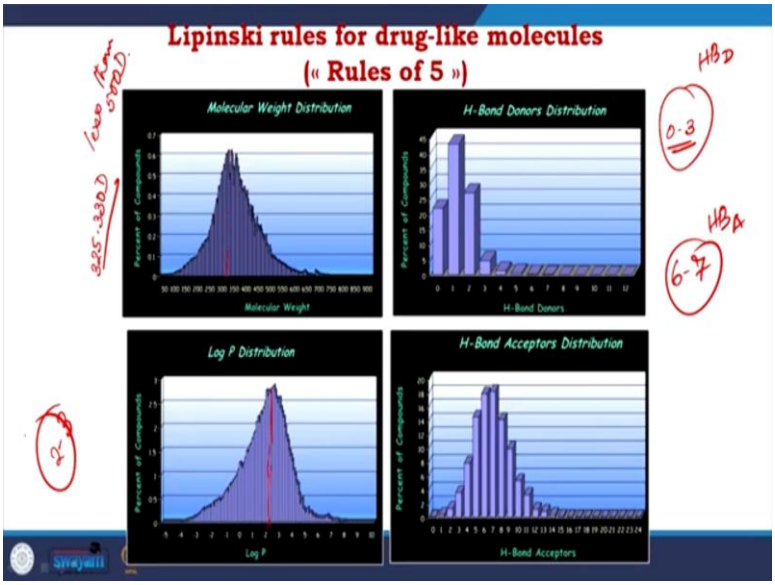

How does the Lipinski rule work? If you see the Lipinski rule for drug molecules, you will see the distribution for molecular weight, and we say it should be less than 500 Dalton. If you plot it here, the peak comes around 325 to 330 Dalton. For the hydrogen bond donor, the peak is between 0 to 3. Hydrogen bond acceptor the peak is kind of 6 to 7. The log P distribution comes around 2 to 3. So, this shows that when you make a big sample, you still get the values that they are obeying the Lipinski rule.

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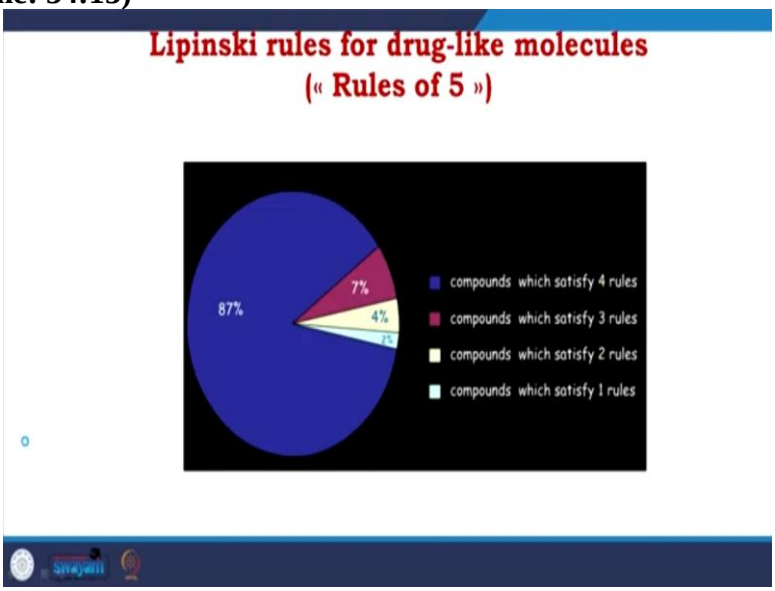

And then a bigger statistic even makes it more convenient, 87% of good drug molecule satisfy the 4 of the four rules of Lipinski, 7% compound satisfy three rules, 4% compound satisfy two rules, 2% compound satisfy 1 rule.

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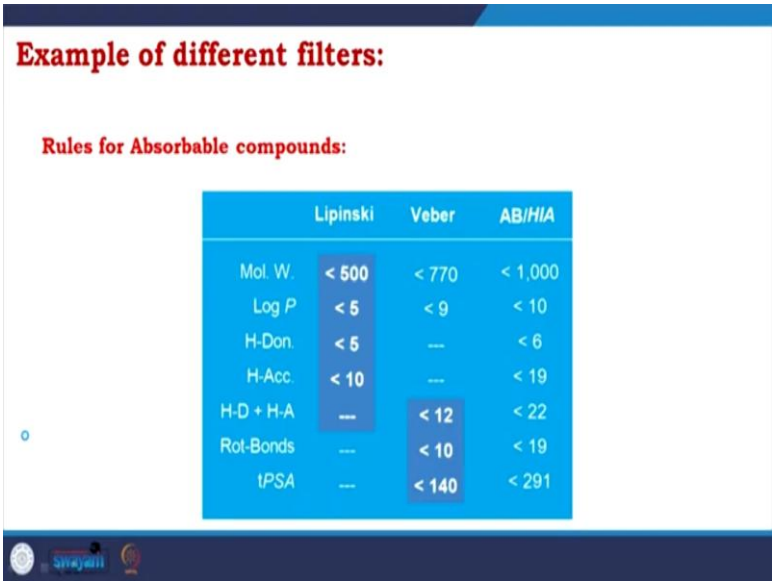

Besides Lipinski, there are other examples of different filters. So, here we compare Lipinski's rule of 5 with Veber and ABIHIA. So, we know that Lipinski's molecular weight should be less than 500 Dalton, log P should be less than 5, hydrogen donor to be less than 5, and hydrogen acceptor is less than 10. In Veber, they say that the molecular weight should be less than 770, log P should be less than 9, they did not give a separate measure of donor and acceptor the thing that the whole thing should come together. So, they make hydrogen bond donor and acceptor, and they make number it should be less than 12, rotational bonds in a lot of case you will see that the bond rotation takes a major role in the flexibility of the molecule and more rotational bond means, more confirmation and difficult to predict. So, they introduce rotational bond, which should be less than 10, tPSA, which is the surface area that distributes, less than 140.

In ABIHIA, the molecular weight is less than 1000, log P is less than 10, the hydrogen donor is less than 6, hydrogen acceptor is less than 19, hydrogen bond donor and acceptor should be less than 22, the rotational bond would be less than 19, and tPSA is less than 291.

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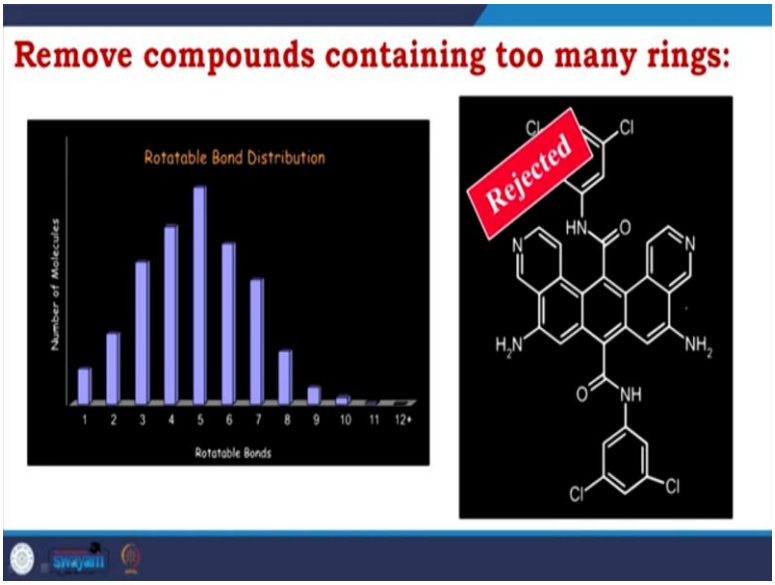

Now, you would see some exceptions or say some factors included in the filter to get a better result. I talked about the rotatable bond distribution, so when you have compounds containing too many rings, they are often rejected, reason, there are reasons about their size as well as when they have an aromatic bulk distribution that gives them a flatness, and because of the flatness, they could bind to any macromolecular active site, or they could be making a nonspecific binding and all other things.

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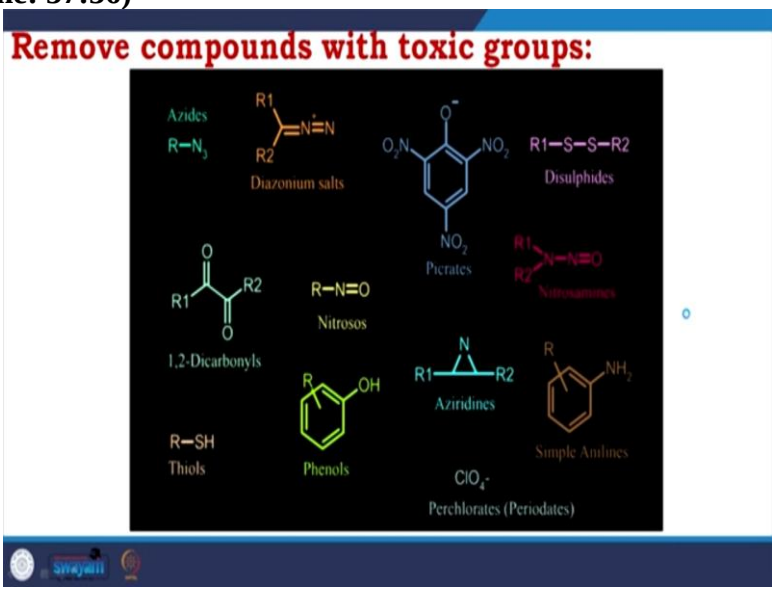

We also remove compounds that have a toxic group like Azide, Diazonium salts, Picrates, Disulfides, 1, 2 Dicarbonyls, Thiols, Phenols, Nitrosos, Aziridines, Periodates, Simple Anilines, they all are Nitrosamines when we see the possibility of a chemical compound to go inside the biological system and so, the toxic effect we try as much as possible to reduce or to remove those compounds or functional groups.

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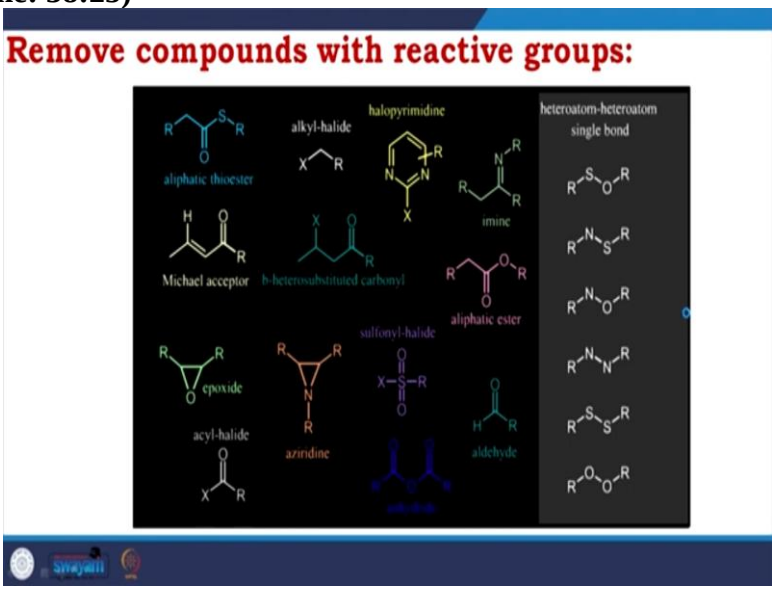

We also try to remove compounds with reactive groups like aliphatic thioesters, alkyl halide, halopyrimidine, imine, Michael acceptor, aliphatic esters, epoxides, acyl halide, aziridine all those sulphonyl halides, which are hetero atom single bonds, all of these we do not normally allow to be incorporated in a druggable molecule.

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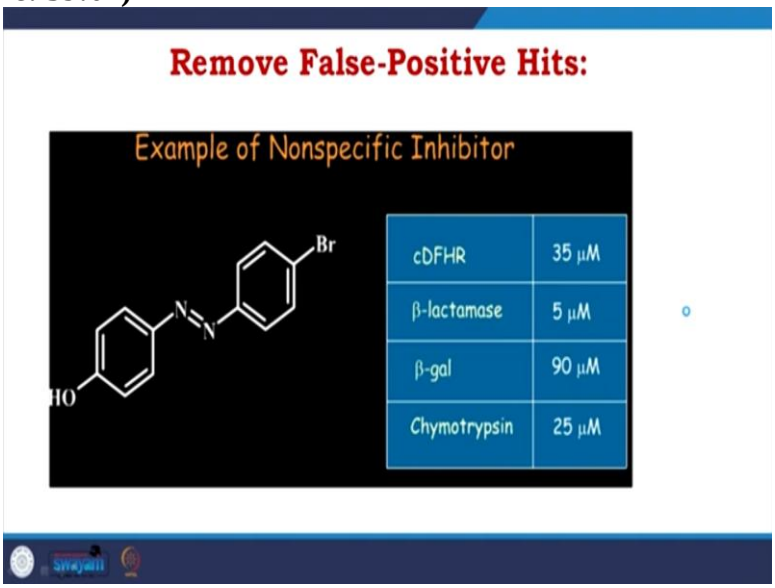

Also, we remove inhibitors, or we do not include inhibitors which give a good number which means nonspecific binding to many inhibitors, like this inhibitor, it binds to DFHR, Beta-lactamase, Beta-galactosidase, and Chymotrypsin, so it binds to different proteins, different active sites, so we consider these as a nonspecific inhibitor.

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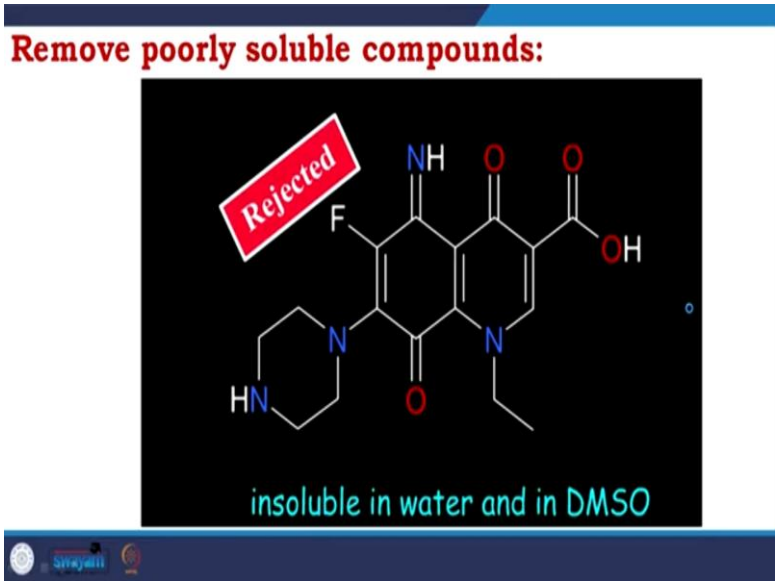

Also, as we have already explained about solubility, we cannot understand that we remove poorly soluble compounds. And if we see molecules that are insoluble in water, we try in DMSO because DMSO is polar aprotic, and then if it is not soluble in DMSO.

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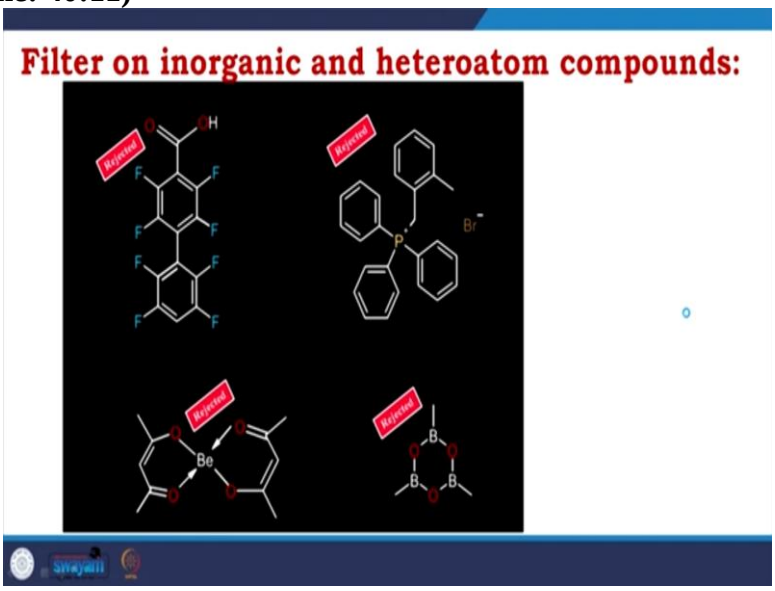

We generally remove them. We tried to remove or reject inorganic and heteroatom compounds because we already studied the known biological compounds or known biologically allowed atomic-containing compounds. Whereas we have bromine, fluorine, beryllium, and boron, all these new elements are hetero atoms inorganic in nature. We generally do not involve them. Still, it is said that, nowadays, a lot of those moieties are successfully included.

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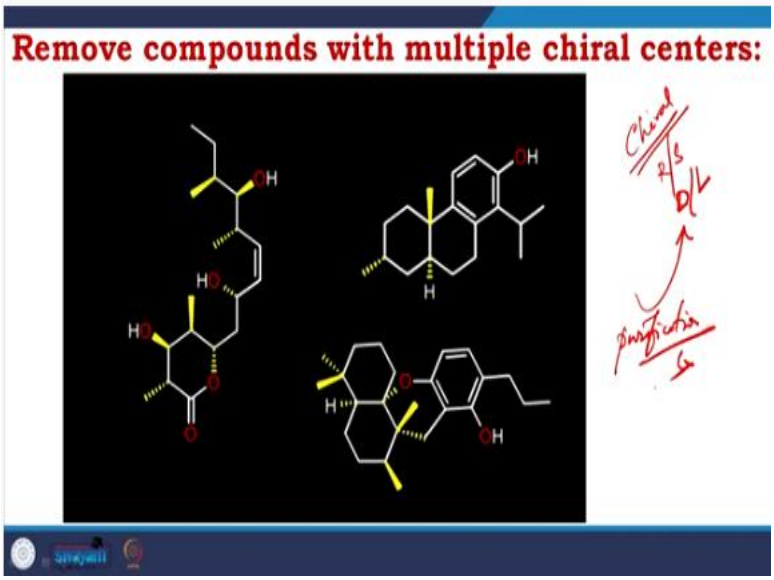

Compounds with multiple chiral centers are always a very big problem. Because as you know, when it is a chiral molecule, it has R S or D L, so one reason is purification. In each round, you need a lot of purification, and even after careful purification, you still have both stereoisomers.

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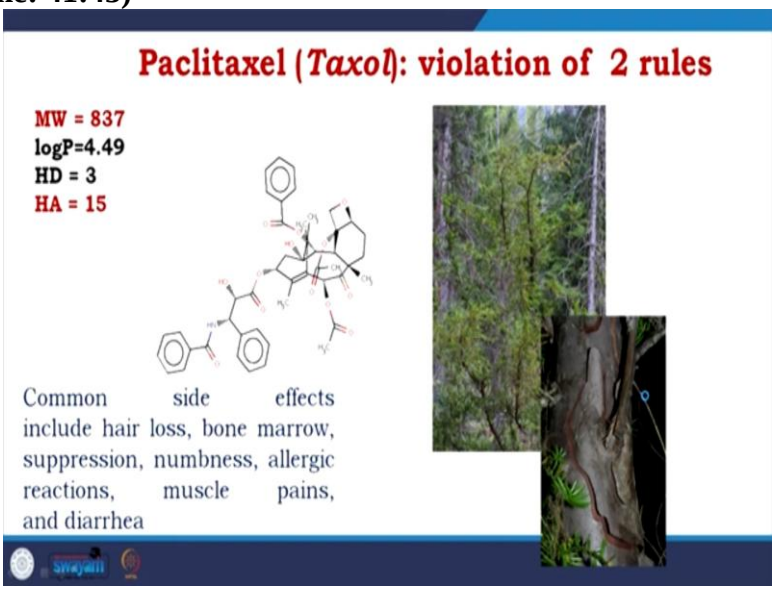

But with all of these, all you do correctly, still you could have come up with, there are situations where the drug is working, but it is not obeying the rules. A very good example is Paclitaxel Taxol, the anticancer drug; you see the compound isolated from the bark of the tree. It maintains two rules, one the molecular weight, which is 837. You see, this is quite a big compound and hydrogen acceptor equal to 15.

But even after that, it is considered a good drug, but these drugs are common side effects, including hair loss, bone marrow suppression, numbness, allergic reaction, muscle pains, and diarrhea. So that again tells you, so, why you use this? Because it is used as an anticancer and

is a very effective anticancer drug. But that does not mean it is side-effect-free, which again proves the importance of the established in silico model.

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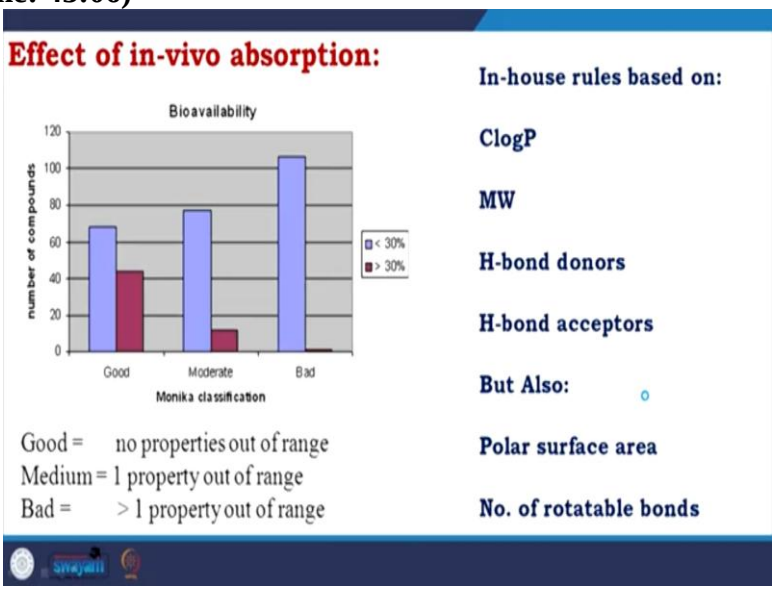

So, we have seen that in vitro effect now. When you have the compounds, you put them in bioavailability; good is no properties out of range, the medium is one property out of range, and bad is greater than one property out of range where the rules are based on ClogP. So, now, the log P considered in all the rules changed to ClogP. So, we had log P, and now we are considering ClogP, molecular weight, hydrogen bond donor-acceptor. But now, we also include the number of rotatable bonds and the polar surface area we have seen in other filters.

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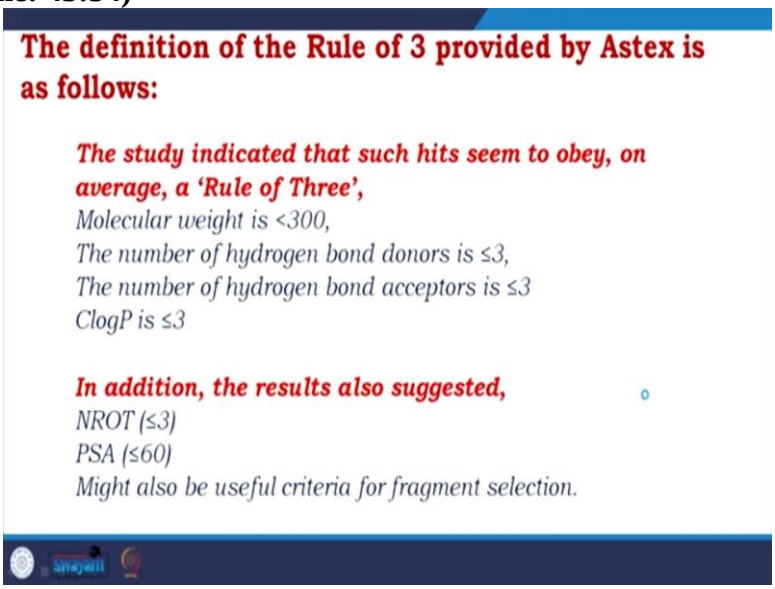

So, the definition of the rule of 3, instead of the rule of 5 of Lipinski, we call it to rule of 3 provided by Astex. The study indicated that such hits seem to obey a rule of 3. Molecular weight should be less than 300 instead of 500. The number of hydrogen bond donors is less

than equal to 3, the number of hydrogen bond acceptors is less than equal to 3, ClogP is less than equal to 3.

But in addition, the results suggested the number of the rotatable bond would be less than equal to 3, and PSA is less than equal to 60. Including them, which is called the rule of 3, is now a beautiful criterion to check the drug ability of the molecule.

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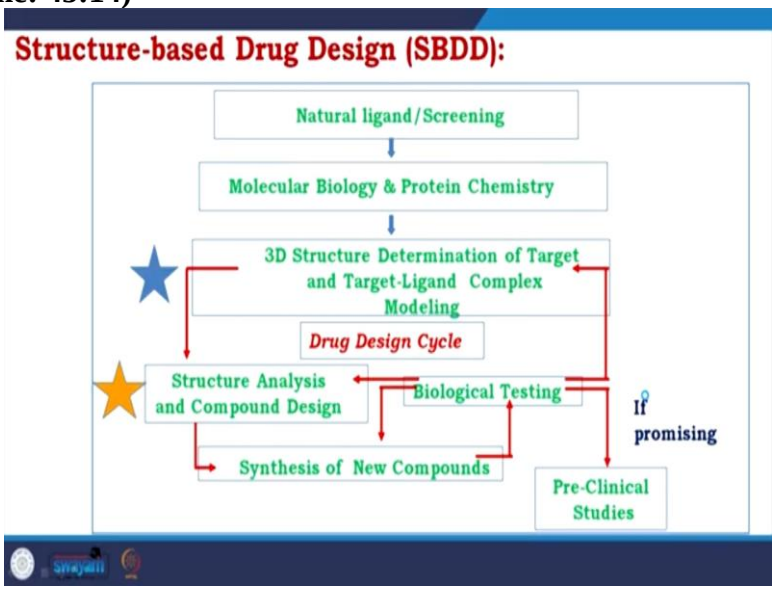

So, as we take a look at structure-based drug discovery, we have the natural ligand, we get them directly, or we get them through screening, we go for molecular biology evaluation, protein biochemistry then we get the 3D structure determination of target and target ligand complex, if not, we go for modeling. Then we go for structural analysis and compound designing, biological testing, synthesis of new compounds, and if promising, we go for preclinical studies.

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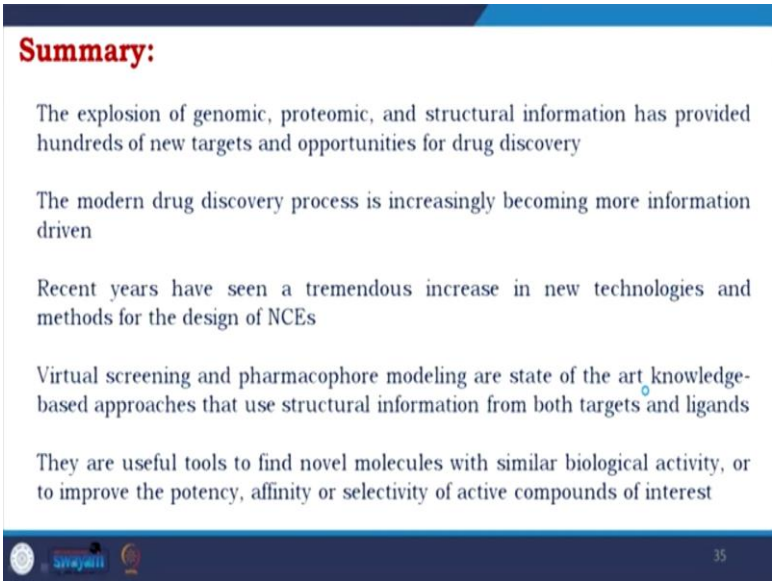

So, coming to the summary, the explosion of genomic, proteomic, and structural information has provided hundreds of new targets and opportunities for drug discovery. The modern drug discovery process is increasingly becoming more information-driven. Recent years have seen a tremendous increase in new technologies and methods for designing new chemical entities. Virtual screening and pharmacophore modeling, which we talked about, are state-of-the-art knowledge-based approaches that use structural information from both targets, the receptor, the biological macromolecules, as well as the ligand. They are useful tools for finding novel molecules with similar biological activity or improving the potency, affinity, or selectivity of active compounds of interest.

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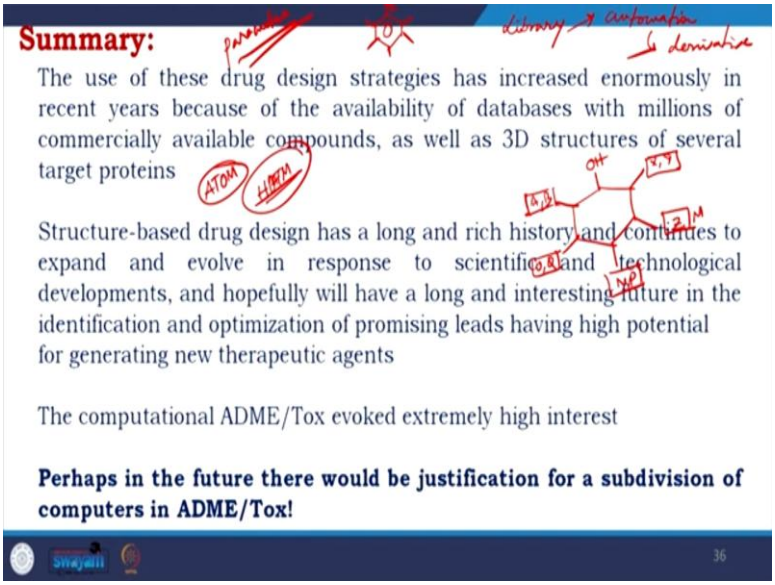

The use of these drug designing strategies has increased enormously in recent years because of the availability of databases with millions of commercially available compounds and 3D structures of several target proteins. So, first, we understand more about library. We understand more about automation.

Automation to build up derivatives from a lead compound, we identify a lead compound. As I talked about this, the lead compound phenol identifies the positions and makes the available groups according to the possible interactions. And then, we include all the possible opportunities which could, we think, contribute to the positive interaction of the small molecule with the biological target.

And then, we allow the program where the automation is coming. It is always difficult to make more compounds included in the library if you design and write. But now, we could deal with millions with the increasing power of the computation, so, once you get the lead, you would not be restricted to the making of the derivatives by just drawing it. You will need a computer to automate the information.

Then, we allow the development of permutation and combination of all those options coming into the molecule, making millions of them and keeping them in the library. So that first development of the library then, we do the parametrization, if you remember when I was talking about the PDB file which you have looked in the structure, I told that in the PDB file there are two parts, one is an atom, and another is a heteroatom.

While the atom corresponds to the known molecules like if you have components, the amino acids, the nucleosides, the lipids, the common carbohydrates, phosphates and all of them, water, they all the bond length, bond distance, dihedral, and all those parameters are already

there in the library. So, the molecules for which the things are known are termed atoms. So, they are already there in the library.

On the contrary, the new molecules are coming up, but it is impossible to make all of them parameterization. So that who are included in the heteroatom, HET atom, is another concern, and we have to calculate for every new molecule. Now, again, with computation's innovation, we develop programs that could automate this. Structure-based drug design has a long and rich history and continues to expand and evolve in response to scientific and technological developments.

And hopefully, we will have a long and interesting future in identifying and optimizing promising leads with high potential for generating new therapeutic agents. But as we have discussed today, it should not be limited to only identifying the molecule through high throughput screening, getting the small molecule to the lead, lead to derivative, lead optimization, and drug.

You should also know when this molecule has the potential to be a drug or not, and in that respective interaction of computational ADMETox evoked extremely high interest. Now, we know whether the molecule we are picking up they are going to the end cycle or not. If we could estimate that, if it could be a drug, if it could interact and make biological response, it would not be considered a poison which I have discussed in the last class.

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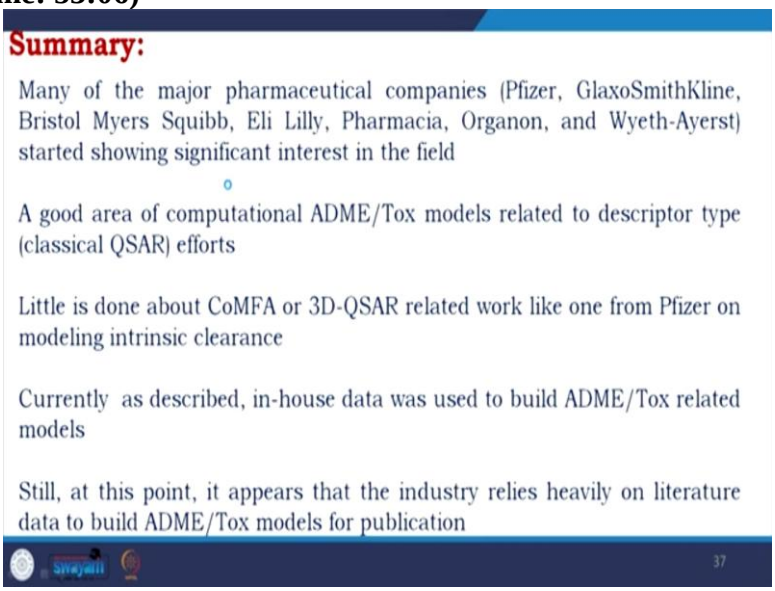

Many major pharmaceutical companies like Pfizer, GlaxoSmithKline, Bristol Myers Squibb, Eli Lilly, Pharmacia, Organon, and Wyeth Ayerst started showing significant interest in the field. A good area of computational ADMETox model related to descriptor type, classic QSAR, efforts. But little is done about CoMFA or 3D QSAR related work.

We have seen from Pfizer, which is modeling a compound's intrinsic clearance. As described, in-house data was used to build ADMETox related models, which I mentioned earlier. Still, at this point, it appears that the industry relies heavily on literature data to build the ADMETox model for publication because the field is in its young stage.

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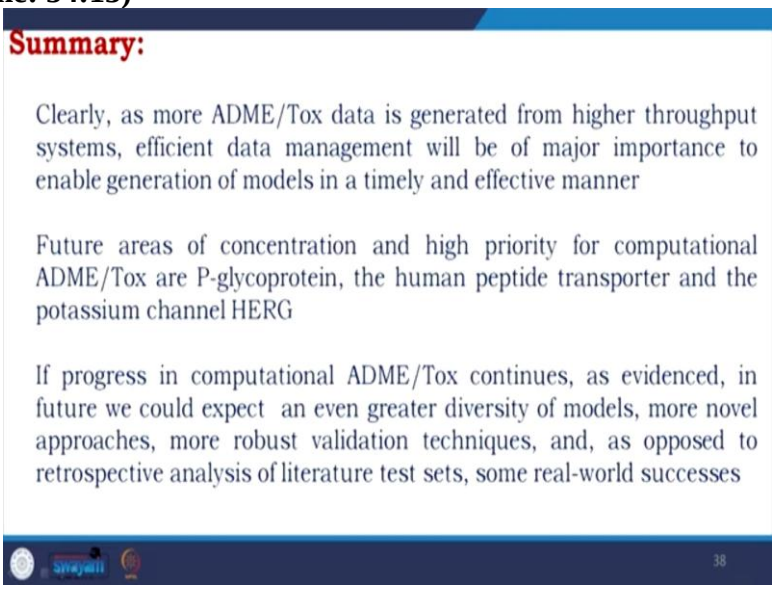

As more ADMETox data is generated from higher throughput systems, efficient data management will be of major importance to generate models in a timely and effective manner. Future areas of concentration and high priority for computational ADMETox are P glycoprotein, the human peptide transporter, and the potassium channel, HERG, but many more are explored.

Suppose progress in computational ADMETox continues as evidence in the future. In that case, you could expect an even greater diversity of models, more novel approaches, more robust validation techniques, and as opposed to a retrospective analysis of literature test sets, some real-world success in the field of in silico ADMETox.

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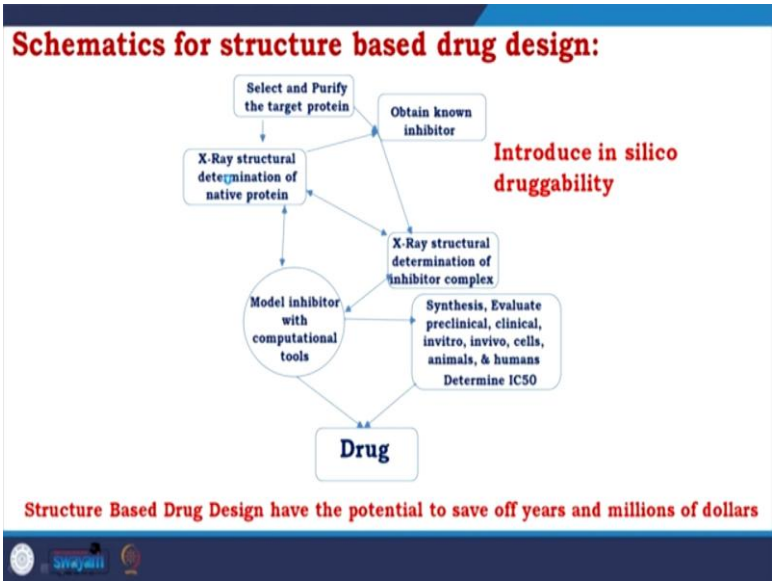

So, in continuing in the schematics for structure-based drug designing where we see first, we select and purify the target protein, obtain the known inhibitor, and go for extra structural determination of native protein. Then, we come to extra structure determination of inhibitor complex, model inhibitor with computational tools, synthesis, evaluate, preclinical, clinical, in vitro, in vivo, cell, animal, and human determine the IC50 come to a drug.

But now, we know that from today's discussion, we have to introduce in silico druggability that could help us save a lot of money that would help us select some compound or reject some compound at the initial level of the study, the initial level of the project which will save millions of dollars. So, structure-based drug design has the potential to save off years and millions of dollars. That is what we are, understanding through the discussions of the last two days.

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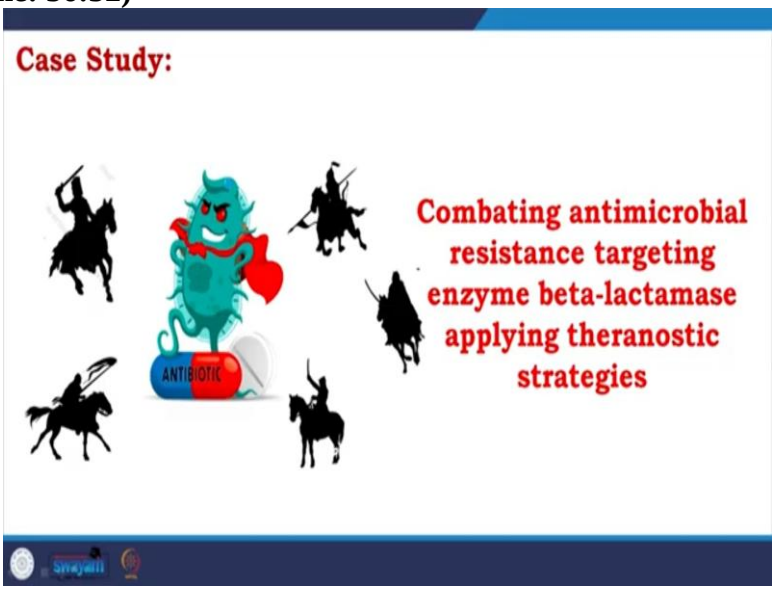

After that, I would come to the case study, in which I discussed beta-lactamase, the basis of that. I talked about a very serious thing, which is antimicrobial resistance. So, this case study is about combating antimicrobial resistance targeting enzyme beta-lactamase applying theranostics strategies. What is meant by theranostic? It isn't easy only to apply therapeutic strategy, though you are doing therapeutics. So, a combination of therapeutic and diagnostics is needed, and together, it is called theranostic.

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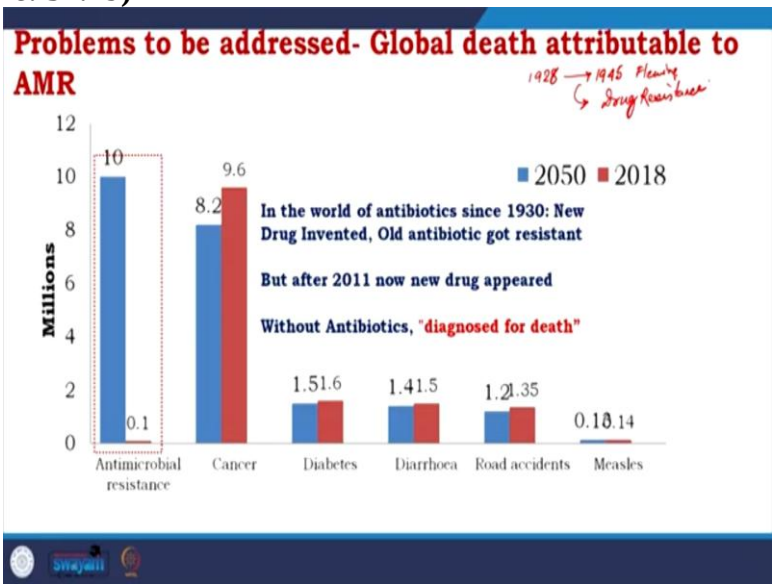

How important is the problem of drug resistance? So, if you see, here, you could see several options and their numbers in millions, the brown color columns give you the presentation at 2018. At the same time, the blue color column gives you the idea of 2050. We have chosen all the factors causing a significant number of deaths. If you compare measles, there is no significant increase from 18 to 2050. If you consider road accidents, it is decreasing, diarrhea; again, you could say it is similar or decreasing.

You consider diabetes; again, it is like similar or decreasing, cancer decreasing quite significantly. Whereas all of them have similar number antimicrobial resistance, which you understand very well-staying home nowadays in the COVID era, but trust me, if you could get the heat of COVID so much, imagine there are the number of bacteria's, they are around us, they are all developing resistance.

And the result is here from 0.1 million to 10 million. That is a significant increase. So, it is one of the most critical global problems. So, in the world of antibiotics, since 1930, a new drug was invented, old antibiotics got resistant. What is meant by resistance? If you remember, I talked about the story. In 1928, penicillin was invented, and in 1945 Fleming

was awarded the Nobel prize. From 1928 to 1945, Fleming did not do much work on antibiotic resistance.

And why he did not do the work was understood from his noble achieving, noble awarding talk where he talks in volume to the world that, yes, innovation of antibiotic penicillin is giving you a magic bullet to fight against bacteria, but remember, if you overuse it, it will come back, the nature will fight back, nature help bacteria to fight back and when it fights back, it could create drug resistance.

So, what is drug resistance? When you have an antibiotic, you are applying it to the bacteria, and bacteria develop a mechanism so that the drug is not working is called antibiotic resistance. Now, why is this significant state of enhancement because the war is going, you keep inventing one antibiotic, bacteria come up with the resistance. But, the problem is once one bacteria get one resistance mechanism, it is now spreading in the whole world, the entire world. If one bacteria gets a resistance gene, it could pass it to another bacteria. There are several processes of horizontal gene transfer, like conjugation and transformation. And that is why the problem is, in the entire world today, one bacteria get the mechanism to one drug, and it would start spreading it to the entire world. So, as I told you, from 1930, drugs came, drugs became resistant, but I will show you data. After 2011 no new drug appeared. And without antibiotics, what will happen? Think about without antibiotics, no pregnancy, no operation, even a small wound could be very critical towards death. So, the condition without antibiotics is considered as diagnosed for death.

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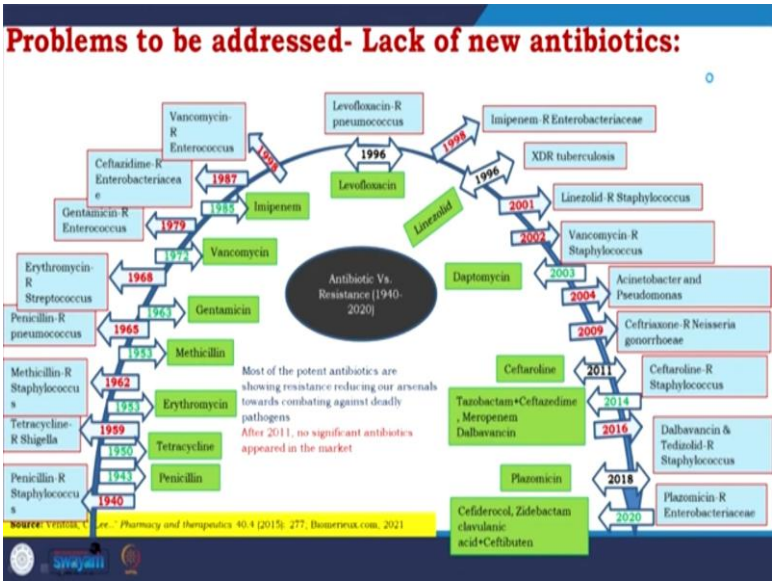

The green line talks about the appearance of drug whereas the red ones talk about resistance. This is the antibiotic versus resistance journey from 1940 to 2020. The significant things are that most of the potent antibiotics show resistance, reducing our arsenal towards combating deadly pathogen.

And as I told you, after 2011, no significant antibiotics appeared in the market. So, there was a continuous war going on, but gradually, currently, we are losing the war. We are not making new antibiotics, and the old antibiotics are resistant.

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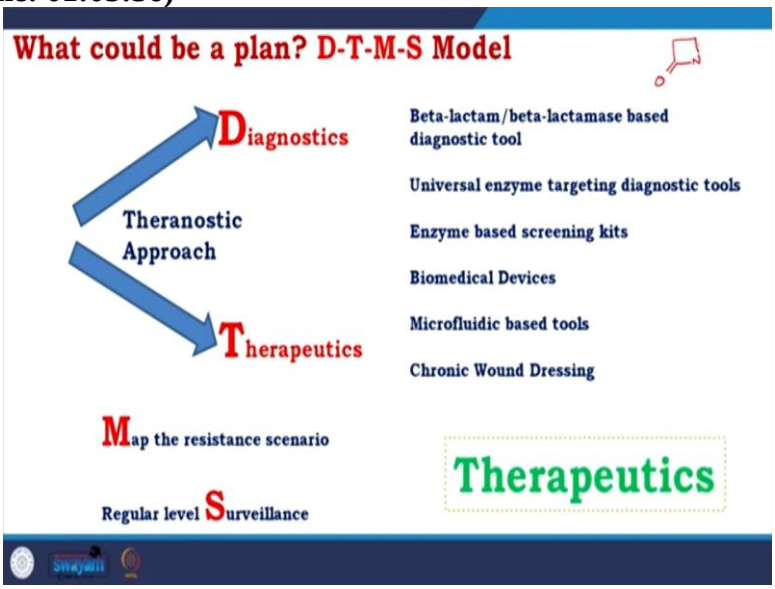

A probable solution is termed as D T M S model. What is the D T M S model? D is from diagnostics. T is from therapeutics, then you need to understand from where the resistances are developing. So, for every country, you need to map the resistance. Is it the hospital, is it

the animal farm, is it the canal where the wastewater, the dirty water is going, so, by getting every information is called map the resistance scenario.

So, the aim is from there and regular level surveillance, what is that? So, you measure, you make a map of India, now, you know that these are the animal from where the resistance is coming, what type of resistance is coming which gene is getting resistance. So, based on that, you have to control the usage of the drug. You have to take measures to control the emergence of the new resistance. So, this is called surveillance. So, D T M S is diagnostics, therapeutics, mapping, and surveillance.

This is considered an upcoming map, an upcoming model to fight against those deadly pathogens. This story is about beta-lactam. So, in diagnostics, beta-lactam, and beta lactamase-based diagnostic tools must be developed. If you look at the beta-lactam structure, you see a unique four-membered ring. Now, you know about all the biological macromolecules. If you see that, you will never see any cyclic molecule present in biology with a four-membered ring.

Because it is unique, you could utilize beta-lactam-based diagnostics, which would be specifically working for beta-lactamase. Once you have that, you could develop enzyme-based screening kits used as biomedical devices.

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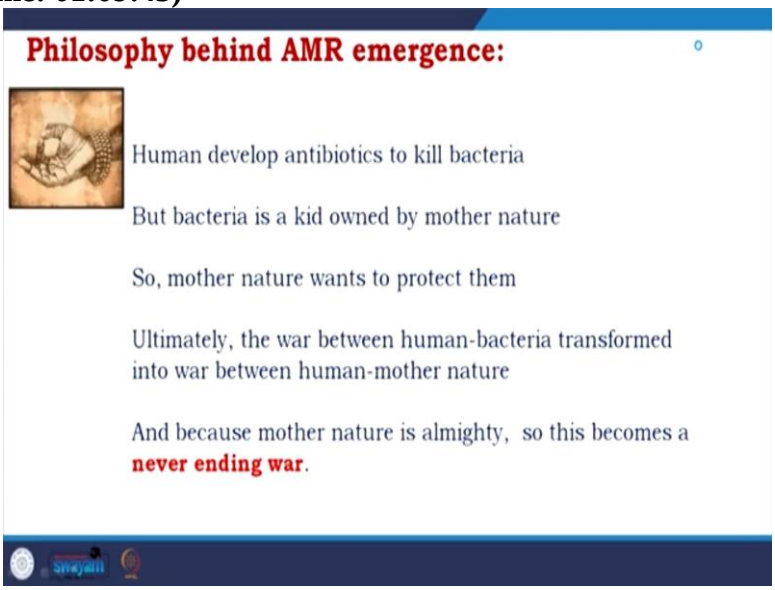

So, if you are thinking, why is this happening? I want to share the philosophy behind the emergence of antimicrobial resistance. Humans develop antibiotics to kill bacteria. But, you know, if you look at from the perspective of Mother Nature, human is her creation, but at the same time, bacteria, the microorganism, is also created by her. So, Mother Nature wants everyone to grow and be the most intellectual and powerful community.

If we develop some mechanisms to kill them, Mother Nature wants to protect them with her unthinkable creativity; it helps them come back. So, ultimately, what I was trying to talk about a war between bacteria and humans is a war between humans and Mother Nature. And because, we all know, we all realize how creative, how organized Mother Nature is taking care of the entire living world, this becomes a never-ending war, at least from their side.

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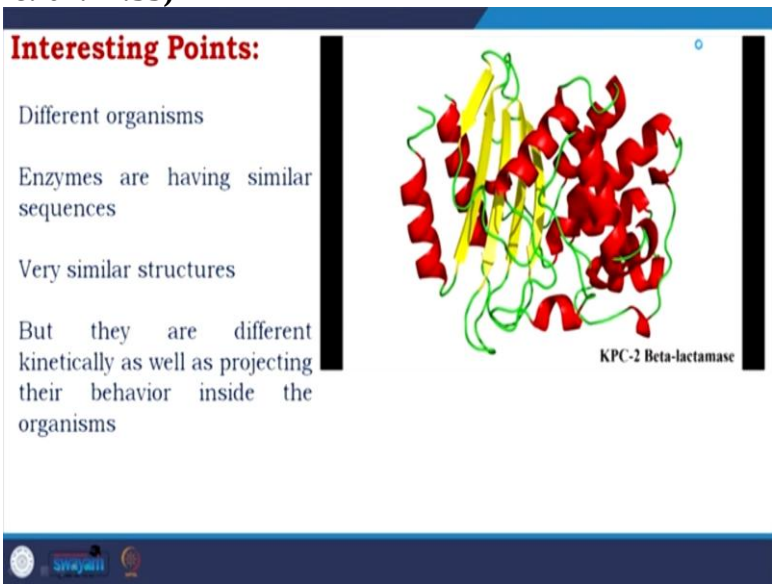

So, coming back to therapeutics, some interesting points, if you look at the movie here, in this movie, you see that a lot of different serine beta-lactamase Class A is presented. Interestingly, those are enzymes coming from different organisms, those enzymes have very similar sequences, and if you look at the movie, you realize that they have very similar structures. The 3D folds are almost identical. But they are different kinetically and project their behavior inside the organism.

So, one enzyme is different from another, but even one enzyme goes to E. coli and Klebsiella, they behave differently. And because of that, there is so much diversity here. And when you apply drugs, they come up with resistance; they come up with the power to diffuse the effect of the antibiotic.

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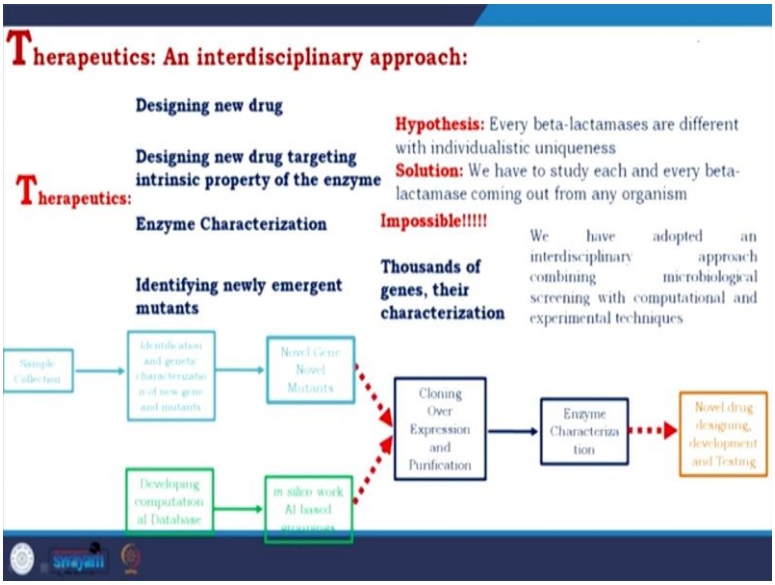

So, a very integrated interdisciplinary approach needs to be taken. You need to design new drugs with a heavy fact in mind that these drugs should hit the enzyme so that they should have the universal capability to inhibit each enzyme. Second, it would be very difficult for the organisms to develop resistance. So, as a drug designer of this new world, you have to design a new drug targeting the intrinsic property of the enzyme. If, by thorough interdisciplinary study, we could hit that intrinsic property and design drug through that, then it would be not easy. It is not impossible. Still, it would be difficult for the organisms to develop resistance.

So, we have to characterize the enzyme biochemically, biophysical, structurally, dynamically in every aspect in detail. And we also identify the newly emergent mutants. So, what are the common mutant comings? What are the mutational hotspots, many things you have to consider as a drug designer of current days?

So, you have to adopt an interdisciplinary approach combining microbiological screening with computational and experimental characterization to understand techniques. So, you have to divide the areas, understand what type of genes are involved, what variation, do sample collections from the hotspots where resistance happens, and get them by mapping. So, sample collection, identification, and genetic characterization of new gene and mutants and novel characterization or identification of the novel gene, novel mutants.