Structural Biology
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Lecture - 60
Structure-Based Drug Discovery: Case study and Conclusion

Hi everyone, welcome to this course in structural biology. We are going through the module of structure-based drug discovery. And today is the last class of the course.

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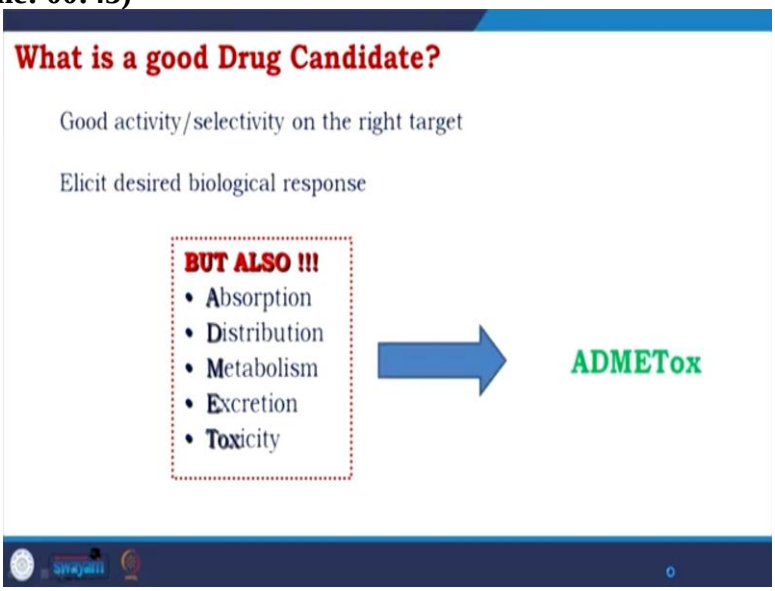

So, in the previous class, we talked about a good drug candidate, its qualities, good activity, selectivity on the right target, and eliciting a desired biological response.

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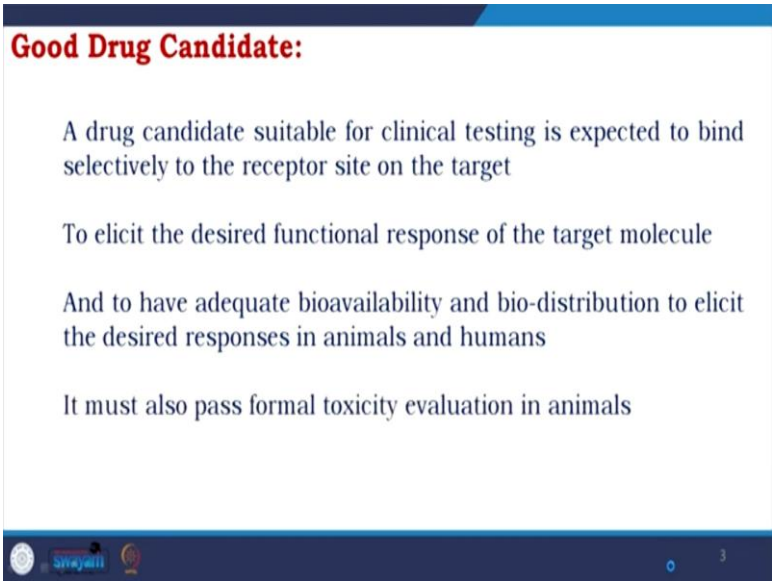

But at the same time, it should have ADMETox. So, a good drug candidate is a drug candidate suitable for clinical testing and is expected to bind selectively to the receptor and elicit the desired functional response. In addition to having adequate bioavailability and bio distribution to elicit the desired responses in animals and humans, it must also pass formal toxicity evaluation in animals.

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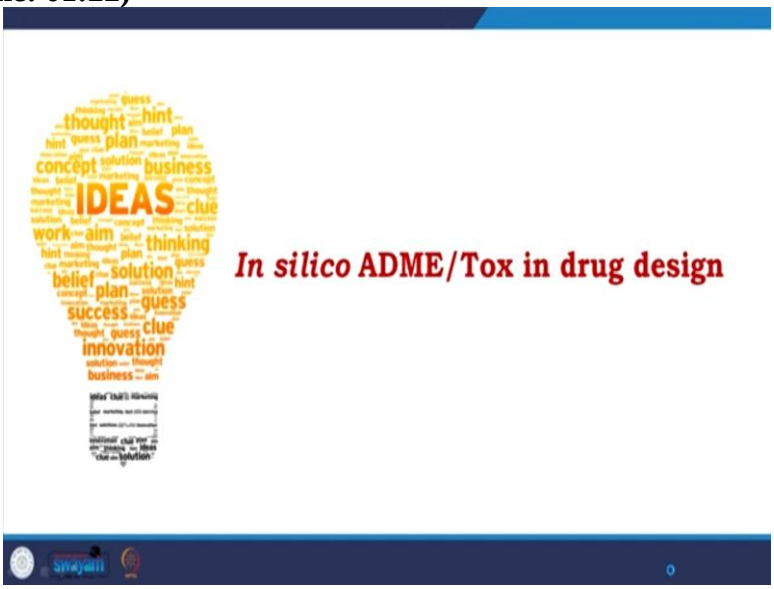

And a good amount of current research effort is wrestling on developing in silico ADMETox in drug designing. As I told you, the entire drug design cycle is now working through difficult drug targets. The problem is more difficult targets, more and more taken care which gets more and more longer, so more money, more time, and that makes the value of the drug a lot higher.

So, to solve the problem, one of the best ways is to replace the experimental procedures with computation, with computation means, with computational algorithms, with computation in the prediction of the structure, with computation in training, pharmacophore modeling, QSAR, docking, and all of them. But one of the major aspects is after doing all of them, and when you think that you have already gotten a successful drug, it could be that the molecule is not druggable. So, druggability has become an important issue that could be solved by replacing an in silico ADMETox process.

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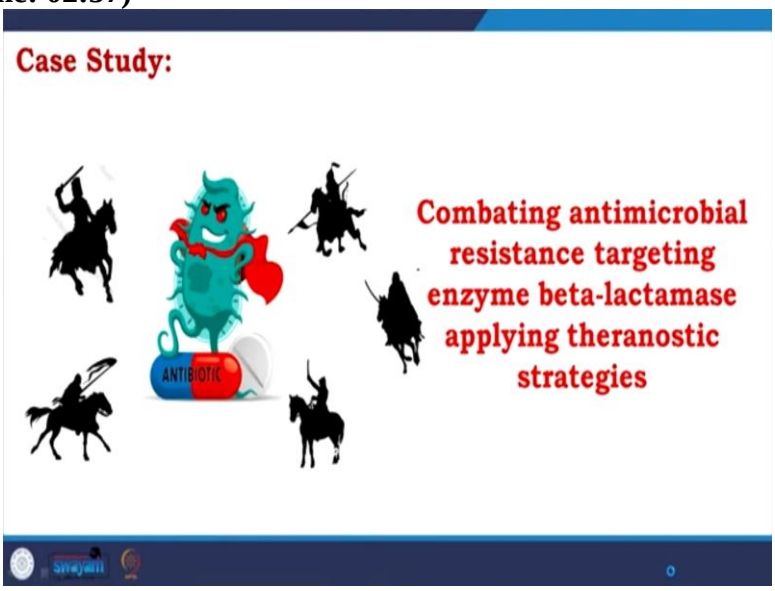

And after discussing in silico ADMETox, I have introduced the case study for combating antimicrobial resistance, targeting enzyme beta-lactamase applying theranostic strategies.

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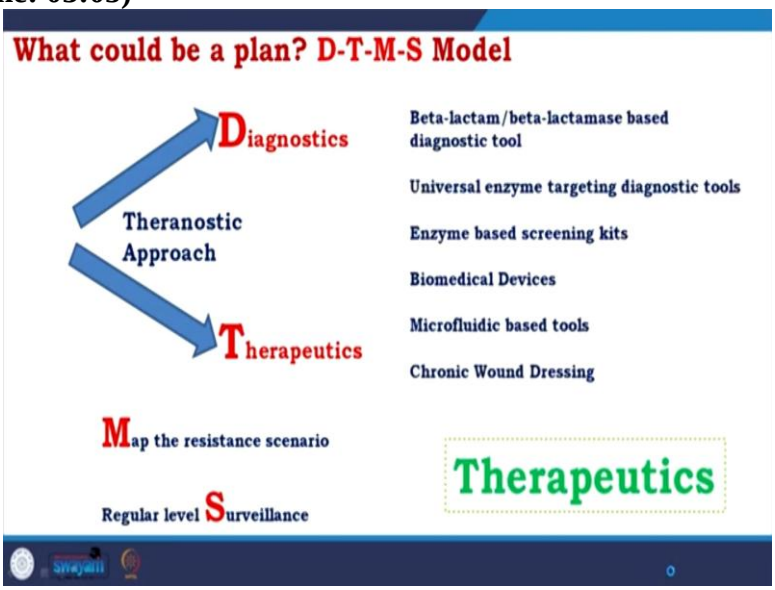

I talked about the D T M S model, and the D T M S model talked about diagnostics, the D is coming from there, the therapeutics the T is coming from there, mapping, the M is coming

from there, and surveillance the S is coming from there. So, many things you could do, but what we focus on here is therapeutics.

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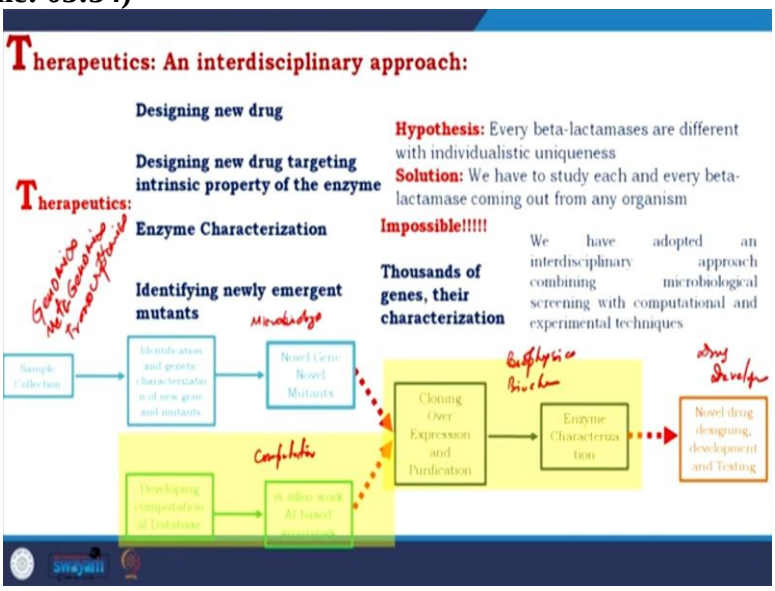

And I talked about the challenge in therapeutics in previous days when you were a drug designer of the 1930s, 40s, 50s, 60s, 70s. You have to design a new drug means, you have to design a new drug, but now when you are a drug designer of current days, you have to design a new drug targeting the intrinsic property of the enzyme. I explained what intrinsic property is and to get into the intrinsic property.

What is needed is enzyme characterization, identifying newly emergent mutants in case of drug resistance, and to do that, the hypothesis was every beta-lactamase is different with individualistic uniqueness's. So, what is the solution? We have to study every beta-lactamase coming out from any organism, their variants which is impossible. Because there are thousands of genes, even a thousand is an understatement. We need cloning over-expression characterization of all of them.

Now, adopting an interdisciplinary approach combining microbiological screening with computational and experimental techniques.

The microbiology part contains the sample collection, identification, and genetic characterization of new genes and mutants, novel gene, and novel mutants. You also include, we have talked about genes, but genomics, meta genomics, transcriptomics, and related NGS analysis are also included. On the other hand, again, there are a lot of things in computation.

But in broad develop a computational database, in silico work, then develop training set, then do machine learning-based predictions, machine learning-based group developments, classifications, and then go to cloning, overexpression purification, enzyme characterization,

and drug designing. So, we need many complicated steps and strategies because drug resistance is a big problem.

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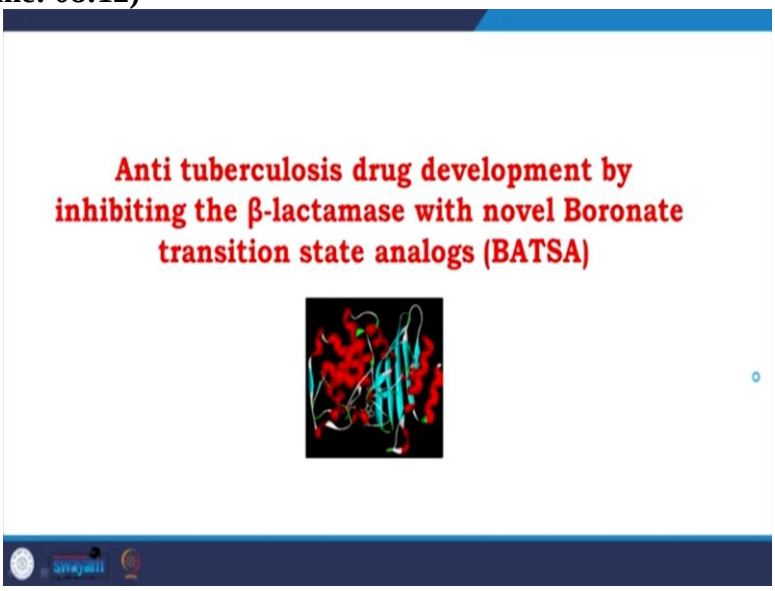

But I take the one simple, relatively simpler case study to develop anti-tuberculosis drug development by inhibiting the beta-lactamase with novel boronate transition state analogs protocol, BATSA, boronate transition state analogs. So, BATSA is a boric acid-based transition state analog.

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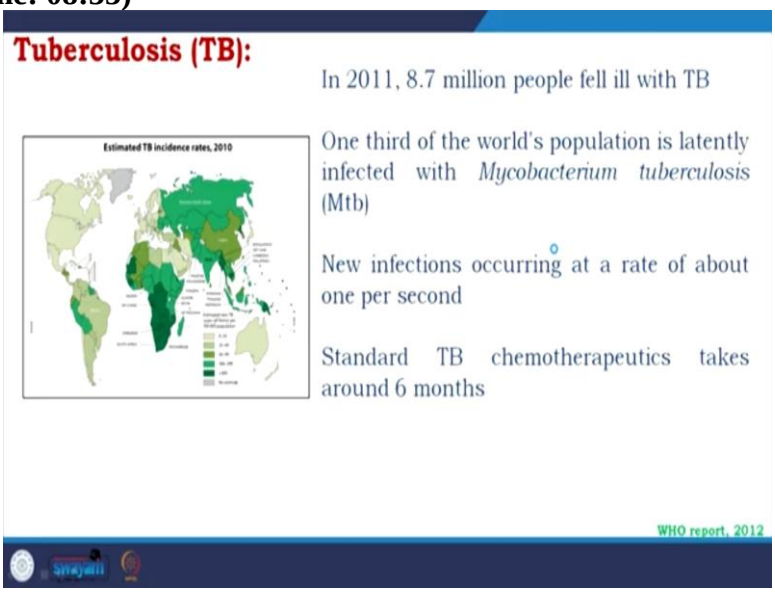
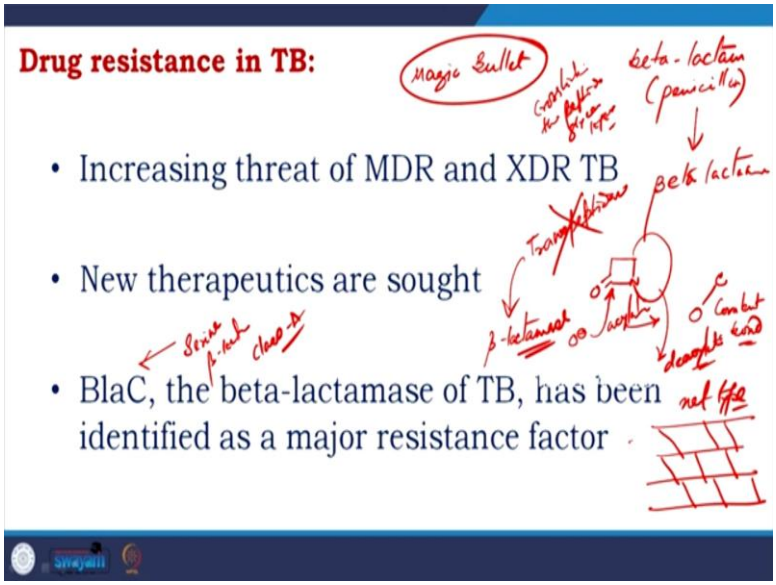


So, there is a lot to talk about TB, but I just talk about significant points in 2011, 8.7 million people fell in with TB. One-third of the world's population is latently infected with mycobacterium tuberculosis, new infections occurring at a rate of about one per second to the whole world. Standard TB chemotherapeutics takes around six months.



Drug resistance is another problem in tuberculosis, increasing the threat of MDR and XDR. MDR is multiple drug resistance. XDR is extensive drug resistance, so, whereas on one side, it is difficult to combat tuberculosis, on the other hand, it is becoming even more complicated than what we are looking at with the emergence of multiple drug resistance and extensively drug-resistant tuberculosis.

So, it is very important to get new therapeutics to combat this deadly pathogen. BlaC, the serine-based beta-lactamase of tuberculosis, has been identified as a major resistance factor.

If you remember, beta-lactam has a typical four-membered moiety with other groups. The enzyme transpeptidase attacks here and forms an OC. So, if you see, there is carbon, so this carbon involves a covalent bond. This is a rare case where an enzyme develops a covalent bond with the substrate. But because of that, the enzyme transpeptidase became inactivated, and transpeptidase was involved in cross-linking the peptidoglycan layer if you remember.

What is the effect of cross-linking of peptidoglycan layer? By cross-linking, they would form the net type environment, all of which are together. Now, they are much more powerful. The enzyme which took part in the cross-linking is transpeptidase. So, by inactivating the enzyme transpeptidase, beta-lactam, which is penicillin, makes the bacteria unable to develop the cross-linking of the outer cell layer.

As a result, the bacterial outer layer becomes weak, and when it goes through different environments with high pressure, with water going into it, bursts and bacteria die. Now, bacteria come up with an enzyme which is called beta-lactamase. There is 2 type of beta-lactamase. One is serine beta-lactamase, and Metallo beta-lactamase. Blac is the serine beta-lactamase belonging to class A, present in mycobacterium tuberculosis and identified as a major resistance factor because it does not allow any beta-lactams to work.

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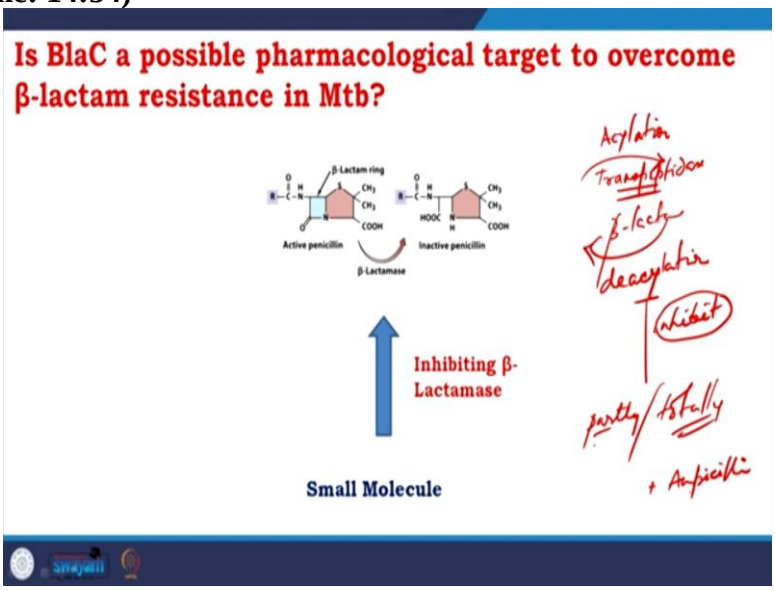

Is Blac a possible pharmacological target to overcome beta-lactam resistance in mycobacterium tuberculosis? So, as I told you, we think there are two steps: acylation and deacylation. Acylation is taken by transpeptidase, whereas beta-lactamase goes through step acylation and deacylation. Through deacylation, it makes the drug ineffective.

The concept is somehow if we inhibit the deacylation step. So, first, the small molecule goes and inactivates the beta-lactamase, and it would be added with, let us say, ampicillin, penicillin, and all this. So, it would be a combination of the inhibitor molecule and the classic beta-lactams. So, we are looking for small molecules which could go and at least partially stop the deacylation step.

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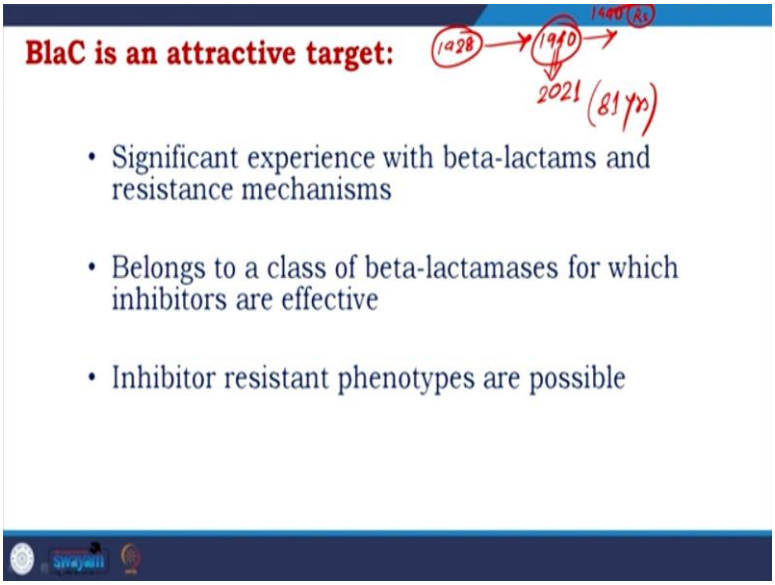

Significant experience with beta-lactams and resistance mechanism, so, as I told you in 1928, penicillin was invented, in 1940 that drug came in the market and it says that around 1940 resistance gene was also there. So, think about from 1940 to 2021, which is 81 years, this gene is mutated around the world, making variants. This gene product is responsible for inactivating a group of drugs that the doctors prescribe. More than 75% of antibiotic is beta-lactams.

So, there are significant research efforts to understand. So, we have previous data. As we have discussed, we are continuously going through innovative computational algorithms and new computational power, so this is one of the good targets. It belongs to a class of beta-lactamase for which inhibitors are effective, inhibitor-resistant phenotypes are possible. Once you get the drug activated on the wild-type beta-lactamase, BlaC.

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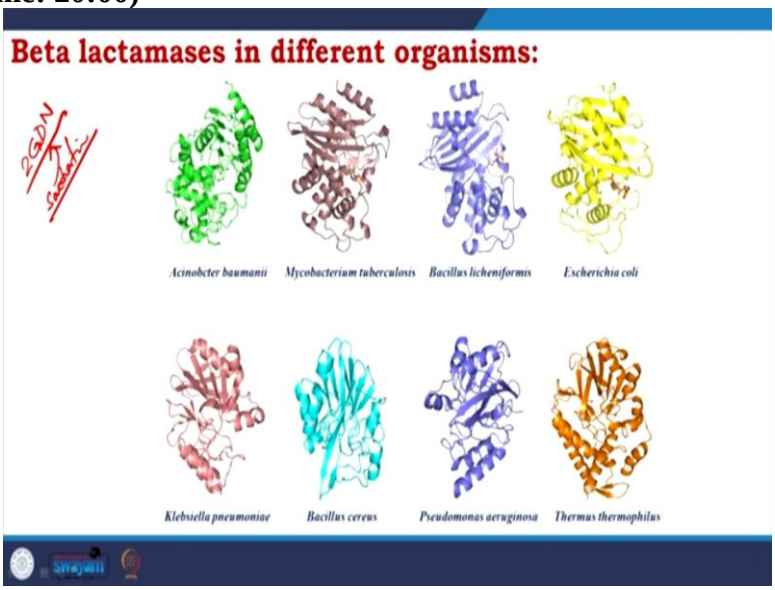

As I told you, beta-lactamase is vividly studied, so you will see that you will get the structure for different class A beta-lactamase like here. I have given some examples, beta-lactamase from Acinetobacter baumanii, Mycobacterium tuberculosis itself have their structure.

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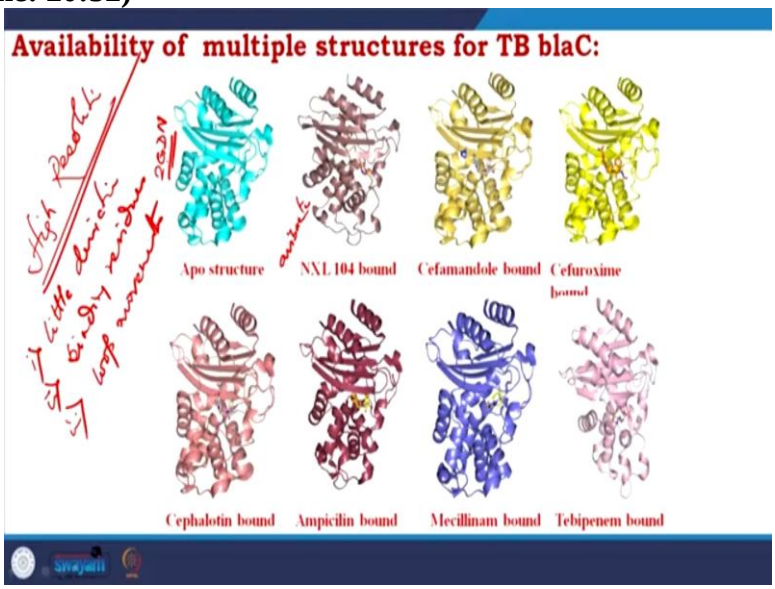

Also, if you take a close look at the tuberculosis beta-lactamase structure, as I told you, the Apo structure, the 2GDN one, and then structures we got NXL104 which is, avibactam bound, Cefamandole bound, Cefuroxime bound, Cephalotin bound, Ampicillin bound, Mecillinam bound, and Tebipenem bound. So, different complex structures will exist, and they are all high-resolution structures. So, they are of the same proteins, but if you compare them, you see little deviations, you will see binding residues, you see loop movements. All of those pieces of information are extremely critical towards designing the drugs.

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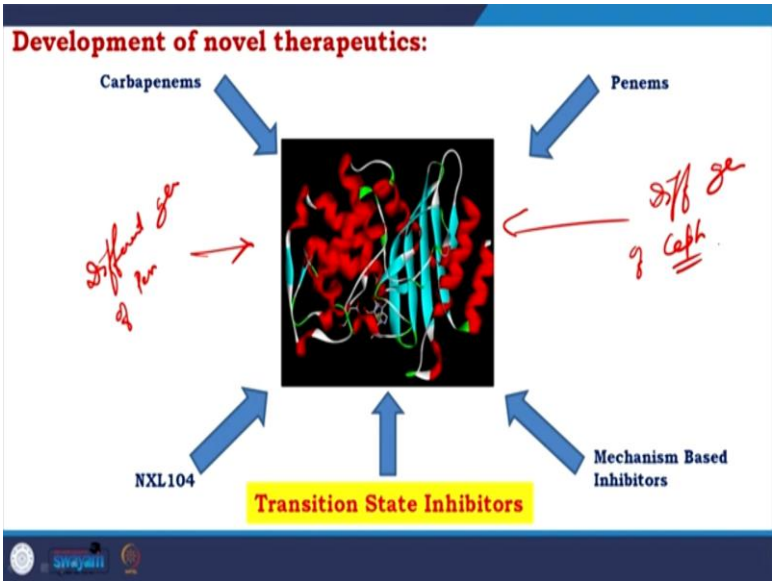

As I told you, there are lots of drugs, and new drugs developed, drugs tried like Carbapenems, Avibactam, Penems, mechanism based inhibitors definitely different generation of penicillins, different generation Cephalosporins. So, all those drugs are characterized, giving us more reflection about the enzyme, BlaC, from tuberculosis. And with that knowledge, this case study focuses on transition-state inhibitors. What is a transition state inhibitor? I have explained it already.

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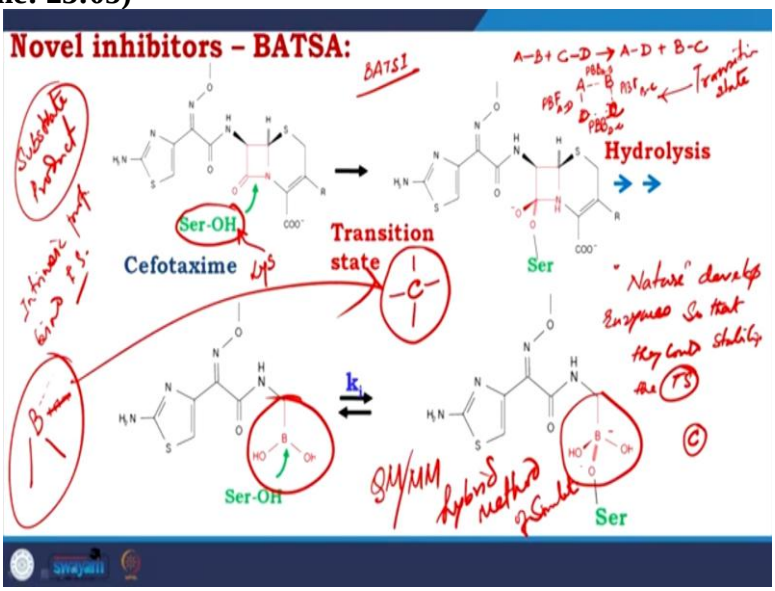

You have A bond B reacting to C bond D will make A bond D and B bond C. This is partial bond-breaking A B, partial bond-breaking DC, partial bond-forming A D, partial bond-forming BC.

This state where all the bonds are forming and breaking is called the transitions state. Nature has developed an enzyme to stabilize the transition state of a small molecule substrate. Mother Nature develop or design enzymes in such a way that they can stabilize the transition state. Because, by stabilizing the transition state, they could lower the activation barrier needed to perform a reaction. And that is the main reason a biocatalyst, an enzyme, could perform a reaction at normal physiological temperature and pressure.

This is the most critical part. The transition state needs to be stabilized and whatever is needed to perform the reaction is to break the bond and form the bond. So, what pushes it is the enzyme by properly allocating its amino acids to help the optimized position in terms of geometry, in terms of energy for a transformation. So, binding to transition state is the intrinsic property of the enzyme.

I am saying that when we get the pattern using a machine learning algorithm, we now design it. Here I am talking about the inhibited designing: BATSAs, Boronic Acid Based Transition State Analogs, also called BATSIs, Boronic Acid Based Transition State Inhibitors. So, look at the core concept now. As you now understand, our goal is to get the transition state.

Because getting transition state means, you get the connection of intrinsic binding to the enzyme, and you make those analogs, they will bind to the enzyme much more tightly, you understand? So, we looked at the reaction pattern of Cefotaxime, and this is the enzyme where the hydroxyl group of the serine, the lysine, takes the proton, attacks, and forms a bond like that.

Now, if you take boron, boron would mimic that transition state by introducing the four bonds because one bond is, boron has three bonds, but here one bond would be forming and one bond would be breaking, four bonds which is mimicking the carbon. So, by introducing a boronic acid, we mimic that transition state.

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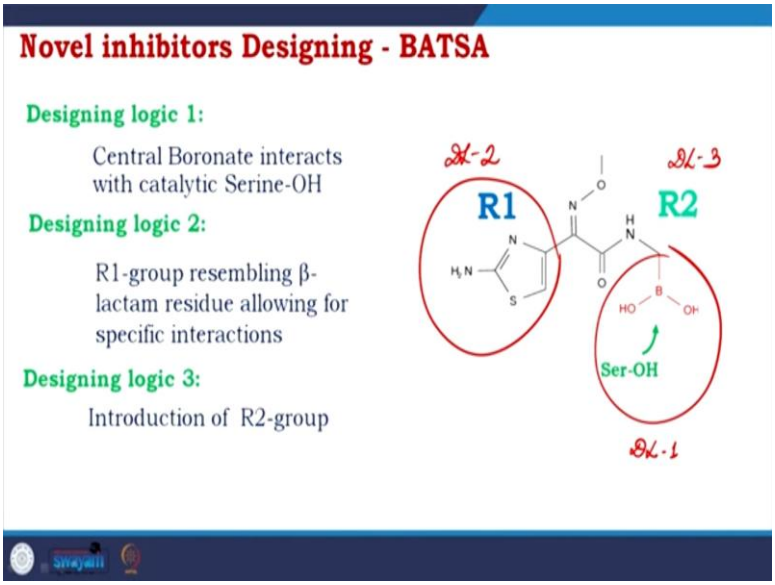

So, how we have designed the BATSAs, we have three designing logic behind the development of that compound. First, we introduce the central boronate, which interacts with the catalytic serine hydroxyl. So, this interaction is designing logic 1, and designing logic 2 introduces the R1 group. So, we take the best beta-lactam moieties interacting with the beta-lactamase. So, the designing logic 2 is, R1 group resembling beta-lactam residue allowing for specific interaction. We already know some beta-lactam drugs that have excellent binding, so we take their moiety. In designing logic three, we are very adventurous. So, we put any possible group, hydroxyl, amino, methyl, chloride like halides, phenyl group, everything. Introducing the central boronates, the R1 group, which are already tested, marketed, approved beta-lactams, the best moiety and designing logic three is introduced here.

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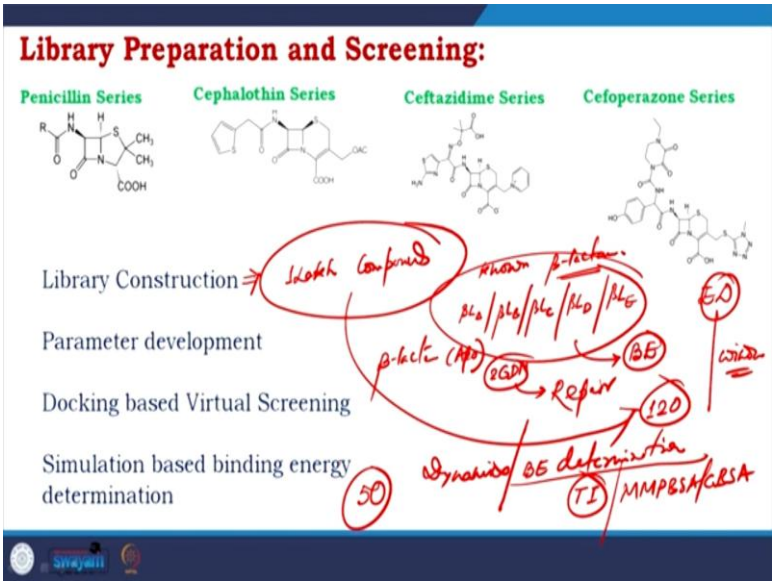

As I told you, we take penicillin, cephalothin, ceftazidime, and cefoperazone, so these are the moieties we have taken. By taking this moiety, we make the changes we talked about, which helps us, so we make the library. It contains around 1 lakh compounds, then we develop the parameters of all of them and perform docking-based virtual screening.

We had 1 lakh compounds, and we are down to around 120. For these 120, we do dynamics, dynamics followed by binding energy determination. We use two methods. One is thermodynamic integration, and another is MMPBSA GBSA and then cut them off to 50.

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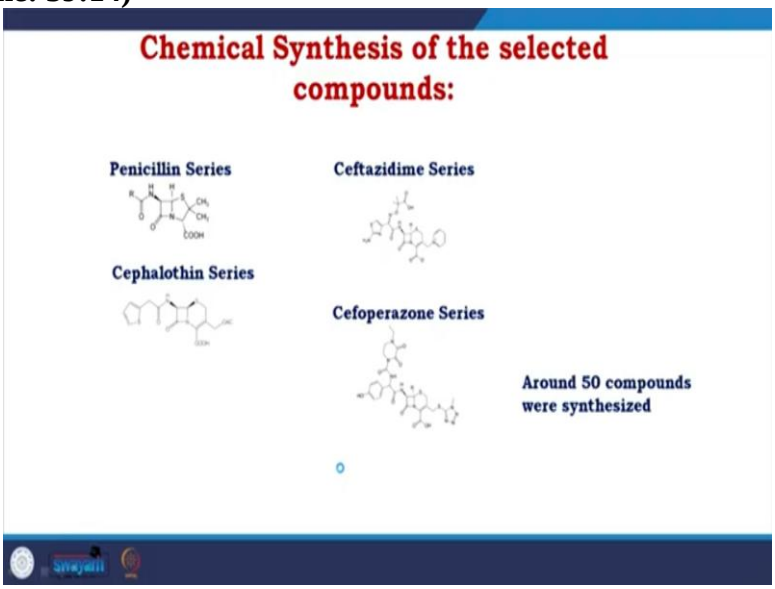

All the 50 compounds from all the series, penicillin series, cephalothin series, ceftazidime series, and cefoperazone series, are all synthesized. Now, what we have, we have 50 compounds in hand, the real compounds. From our imagination of designing logic, we do the

library construction. We do the docking-based virtual screening, simulation, binding and determination, and cutoff to 50. Then we synthesize them, so now we have it in hand.

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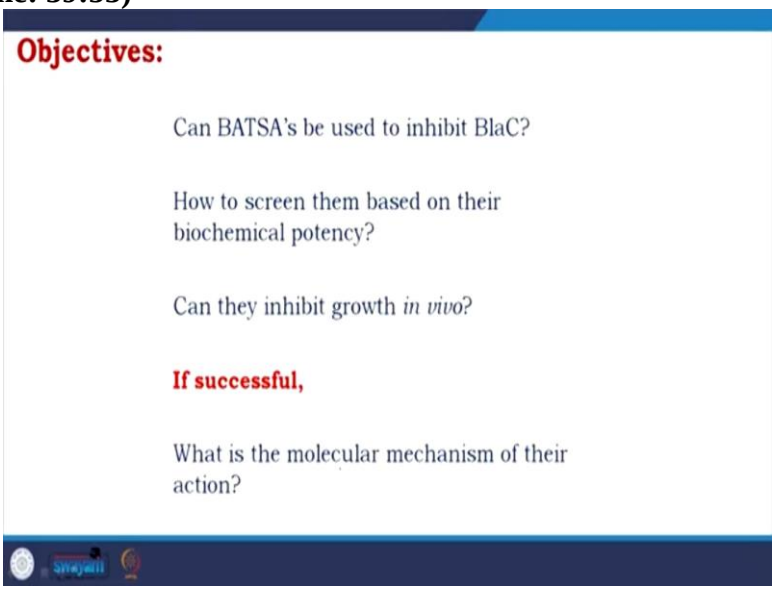

So, now we start the project with the following objectives, can BATSAs inhibit BlaC? So, BlaC is our target, so our question is, can the designed compounds we started from around 1 lakh coming down to 50 be used to inhibit BlaC. How to screen them based on their biochemical potency? We need to develop a biochemical assay because we can get the biochemical potency of a molecule.

We have checked the in silico ADMETox and understood that because we have used the major moiety as the already established beta-lactam moieties, all our compounds are non-toxic. And also, when we say that we introduce any group, we do not introduce groups that could have toxicity. So, we need biochemical potency. If some of the molecules we have now in hand, so, biochemical potency, can they also inhibit the growth in vivo, in real tuberculosis cells system?

And if we are successful, so, if BATSA could inhibit, it goes through biochemical and in vivo testing.

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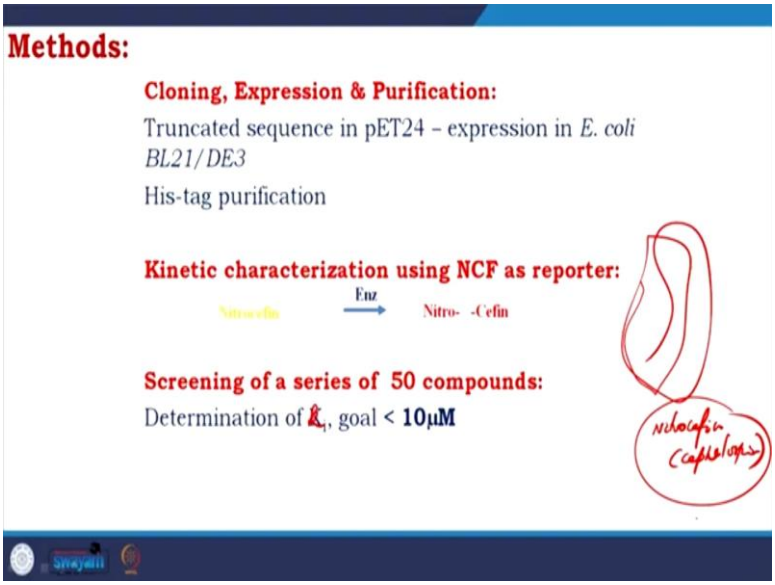

So, first, we do the cloning expression and purification of the BlaC enzyme. We make the truncated version 39 amino acid of the protein was truncated, deleted out, and clone it in the plasmid pET24 expression in E. coli BL21 DE3 expression cells. We use His tag for primary purification and size exclusion chromatography for secondary purification. We develop or take the help of kinetic characterization using nitrocefin as a reporter.

Nitrocefin is an amazing compound. It is pale yellow. In the presence of beta-lactamase, it changed the color to red. The major advantage here is the target here is the four-membered rings and the color development is related to the electronic transition followed by the enzyme attack. So, the enzyme that affects nitrocefin must be attacking the four-membered rings.

We want to go for screening of a series of 50 compounds that are synthesized, so we put criteria, the ki, the inhibitory constant of the inhibition.

So, when nitrocefin binds, the yellow color becomes bright red. Now, all those inhibitors would compete with nitrocefin. The inhibitor would be potent enough to bind, and less color will be generated. So, by calculating the difference in intensity of the red color present here, you could calculate the inhibitory constant.

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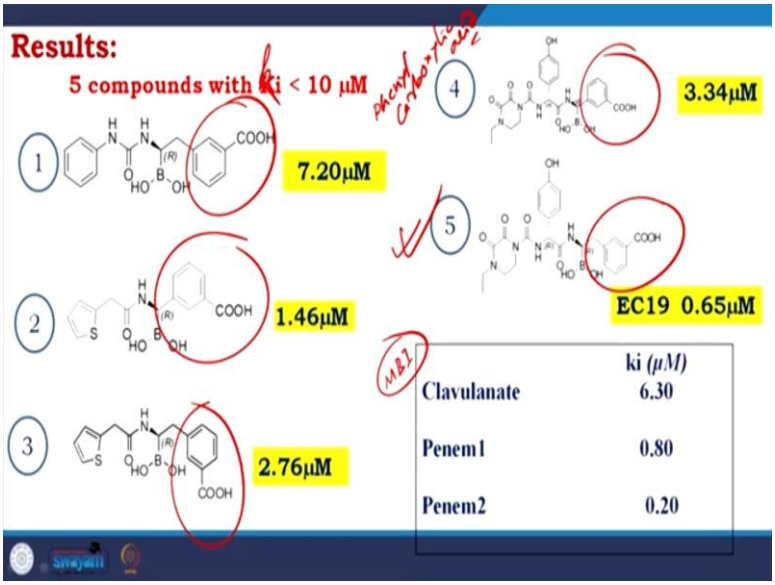

I will show you the result, five compounds we get which have the ki less than 10 μM. So, this is the first one which has 7.2 μM. This is the second one which is having 1.46 μM, the third one we get 2.76 μM, fourth one 3.34 μM and the fifth one which is 0.65 μM(this compound is EC19). I have given some established compounds, clavulanate is a very established mechanism-based inhibitor. It have the ki of 6.3 μM, Penem 1 and 2 again have 0.8 and 0.2 μM. So that shows that this is a quite good compound, at least in terms of the design followed by the biochemical assay. So, we decided to test this one, the fifth one for in vivo.

We have introduced amino and hydroxyl groups only benzene, halides, anything, and everything, but surprisingly, all the compounds we got are phenyl carboxylic acid. So, that gives us a thought that this is very important or made us excited to know about the role of this.

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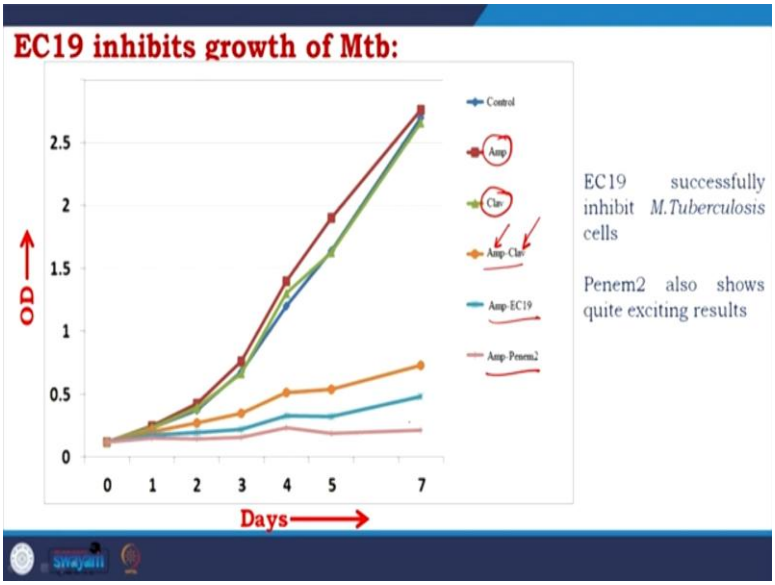

We go for in vivo testing, in the in vivo testing, what we did, we have the tuberculosis cells, and we use combinations, we use Ampicillin, Clavulanate, and we use Ampicillin-Clavulanate combination, Ampicillin-EC19 and Ampicillin-Penem2. If you see the combination of a beta-lactam along with the beta-lactamase inhibitor works here. So, we get good data for Ampicillin-Clavulanate complex, Ampicillin-EC19 complex and Ampicillin-Penem2 complex. So, EC19 successfully inhibits M. tuberculosis cells. Penem2 also suggests quite exciting results.

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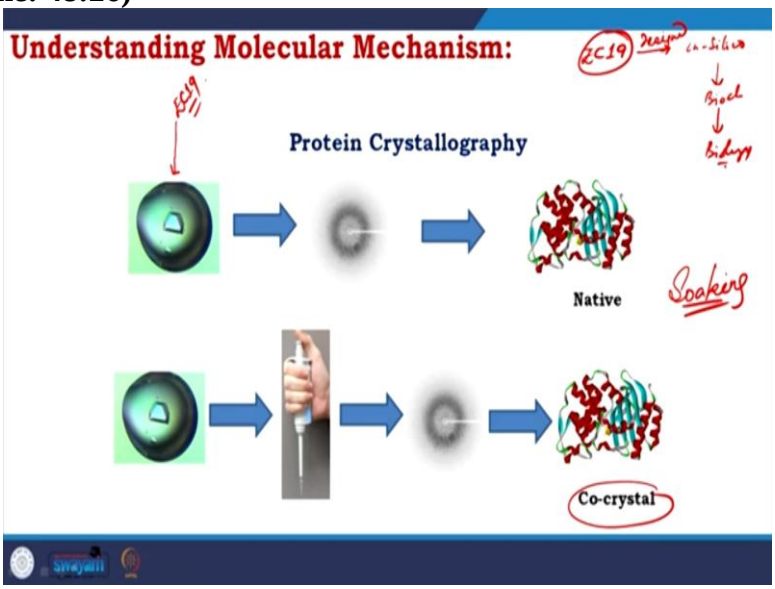

So, if you think about the EC19, EC19 was designed, comes in silico screening, goes biochemical, and then biology. So, now, we want to look at the molecular mechanism, and for that, as a crystallographer, I go for protein crystallography. For protein crystallography, there are two steps, to get the complexes. You get the protein crystal in the native form or

crystallize the protein with the substrate. Interestingly, with many tries, I was never able to cocrystallize the drug with the plus.

So, what I did, I did soaking, what soaking means? I get the native crystal, and I start soaking that drug, EC19.

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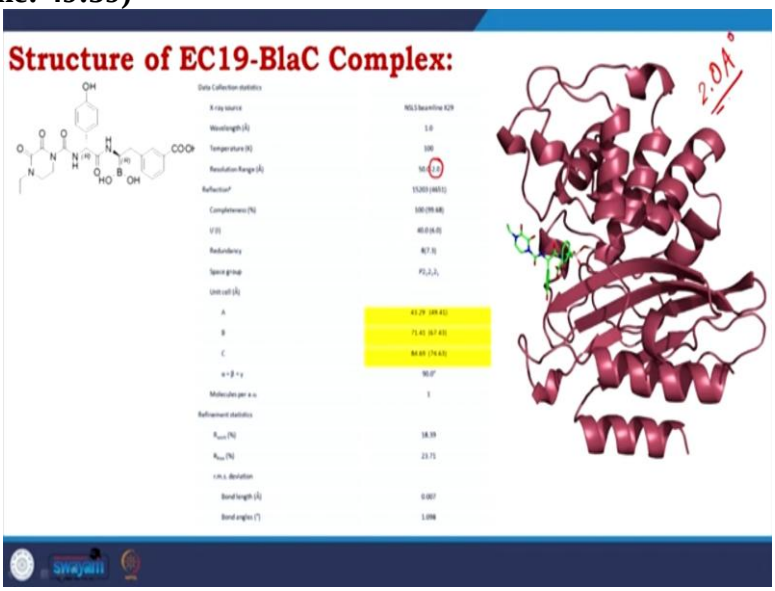

I managed to get the crystal, and I showed you a comparison of data collection statistics.

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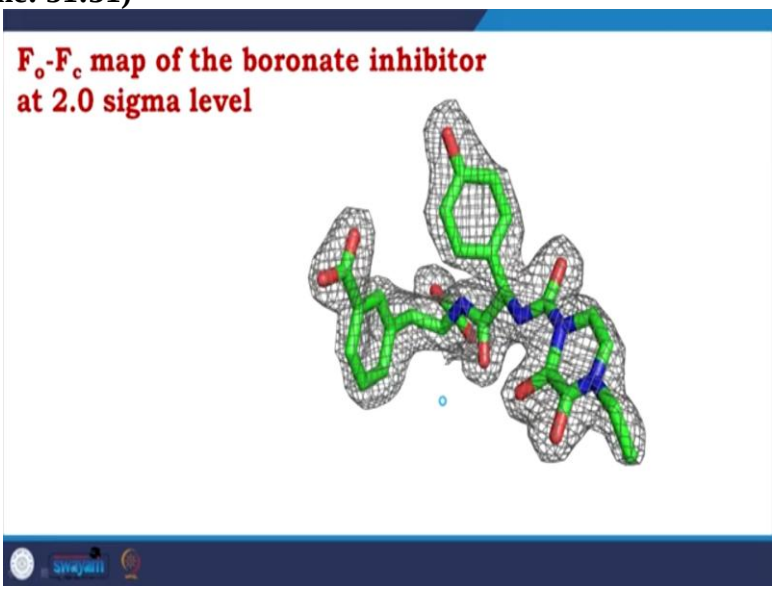

This is the F0 Fc map of the boronate inhibitor at the two sigma level, which clearly says the good quality of the structure we have solved.

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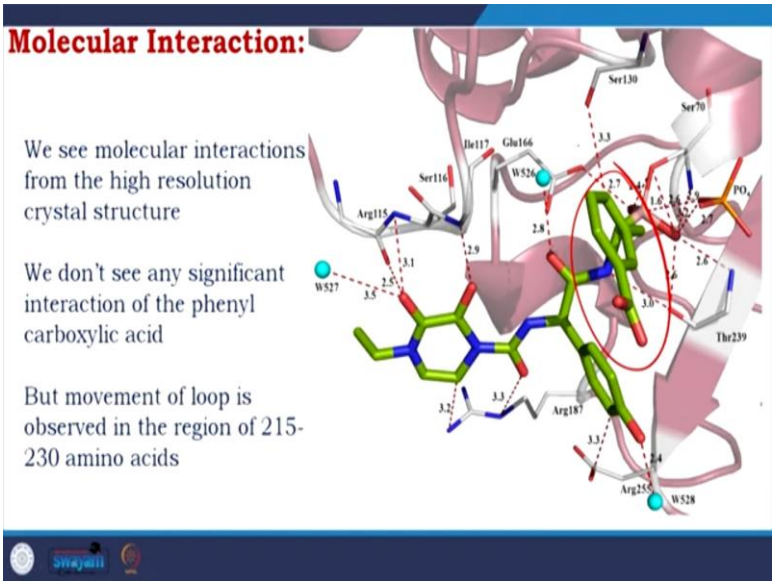

This is the molecule of interaction, and what we see here, we see molecule interactions from the high-resolution crystal structure which we found. So, what was the resolution? We have collected the crystal as 2 Angstrom resolution. But very interestingly, we do not see any significant interaction of the phenyl carboxylic acid. These are the phenyl carboxylic acids you are expecting if you look at. So, as I told you, we see a significant movement in 215 to 230 positions.

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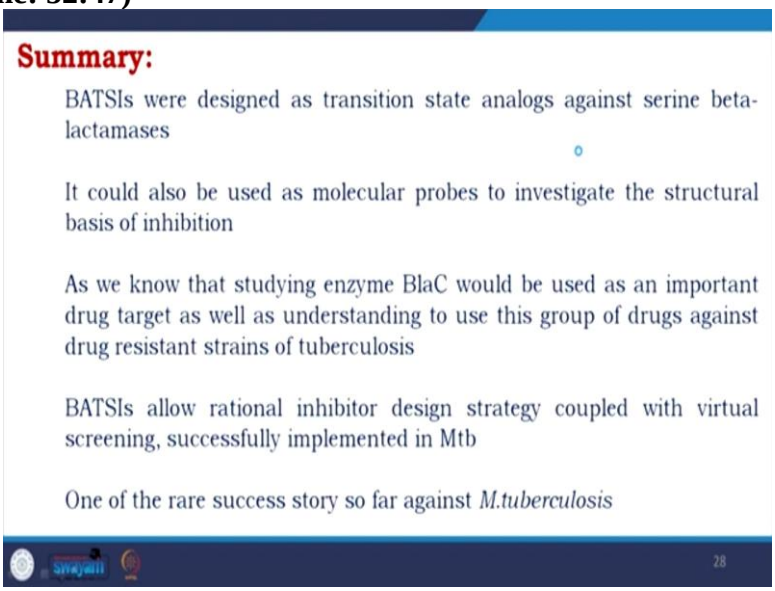

So, in summary, BATSIs were designed as transition state analogs against serine beta-lactamases. It could also be used as molecular probes to investigate the structural basis of inhibition. As we know, studying enzyme BlaC would be used as an important drug target and understanding to use this group of drugs against a drug-resistant strain of tuberculosis.

BATSIs allow rational inhibitor design strategy coupled with virtual screening successfully implemented in Mycobacterium tuberculosis.

This is one of the rare success stories against tuberculosis because drug designing is not a very good thing, a very successful thing against tuberculosis.

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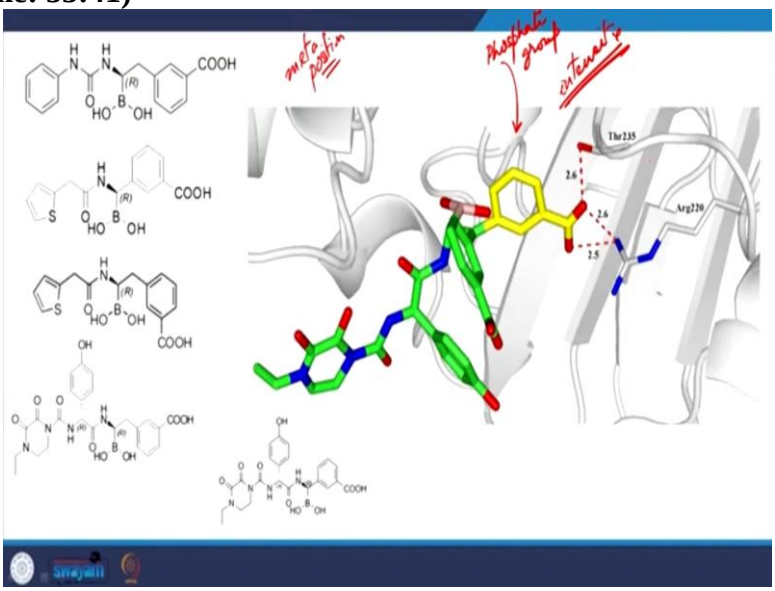

But as I told you, we look at them, and we see that, in all of them, there is a carboxylic group in the meta position of the phenyl ring. So, I was baffled, but when I compared, I see that there is a phosphate group common to any beta-lactamase structure of tuberculosis.

And then we see, as I told Still, the intensity is there, which is good enough for a phenyl carboxylic acid. So, we work on that and, to our surprise, find the alternative confirmation of this phenyl carboxylic acid, and we get two very important interactions.

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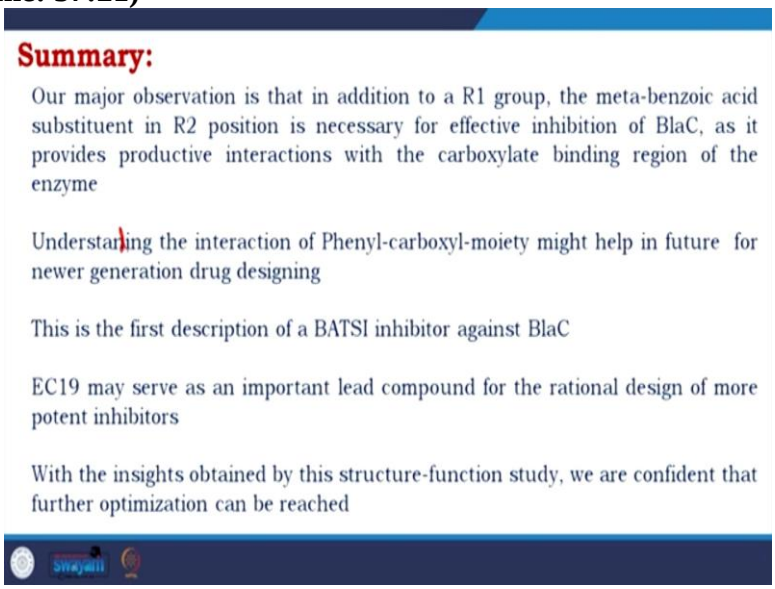

So, as I told our major observation, in addition to the R1 group, the beta benzoic acid substituent in R2 position is necessary for effective inhibition of BlaC, as it provides productive interactions with the carboxylate binding region of the enzyme. Understanding the interaction of phenyl carboxyl moiety might be helpful for new generation drug designing. And this is the first description of a BATSI or BATSA inhibitor against BlaC.

EC19 may serve as an important lead compound for the rational design of more potent inhibitors. With the insight obtained by this structure-function study, we are confident that further optimizations can be reached and though I am not going into the detail. In the next new synthesis cycle, we mimic the position, the alternative confirmer, and we introduce a lot of groups like carboxylates, sulfate, phosphate, all of them along with the other part modification that gives us new libraries which we are currently working.

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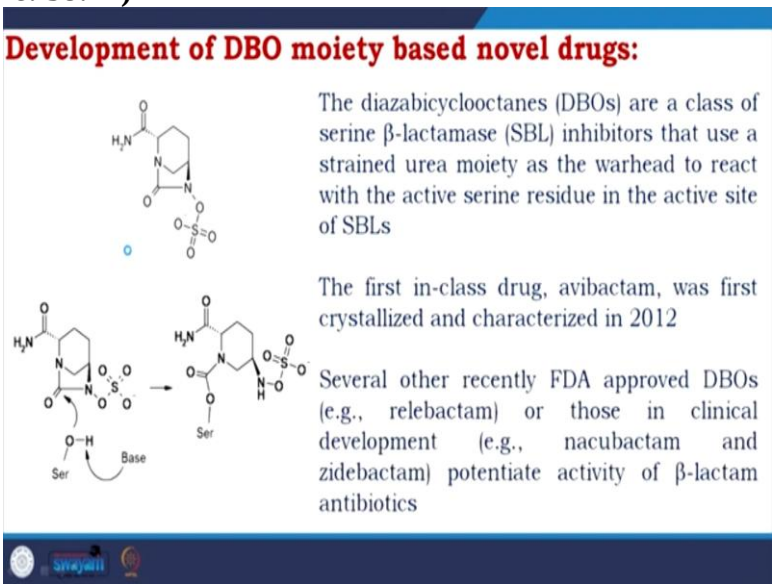

So, a brief description of others, we work with DBO moieties. So, DBOs, the diazabicyclooctanes, are class serine beta-lactamase inhibitors that use strained urea moiety, as you see here, as the warhead to react with the active serine residue in the active site of serine beta-lactamases. Interestingly, they are probably the very few non beta lactam working as beta lactamases inhibitors.

The first in the class drug, avibactam, was crystallized and characterized in 2012. Several other recently FDA-approved DBOs are there. For example, relebactam is FDA approved, and few are in the clinical development, nacubactam, zidebactam.

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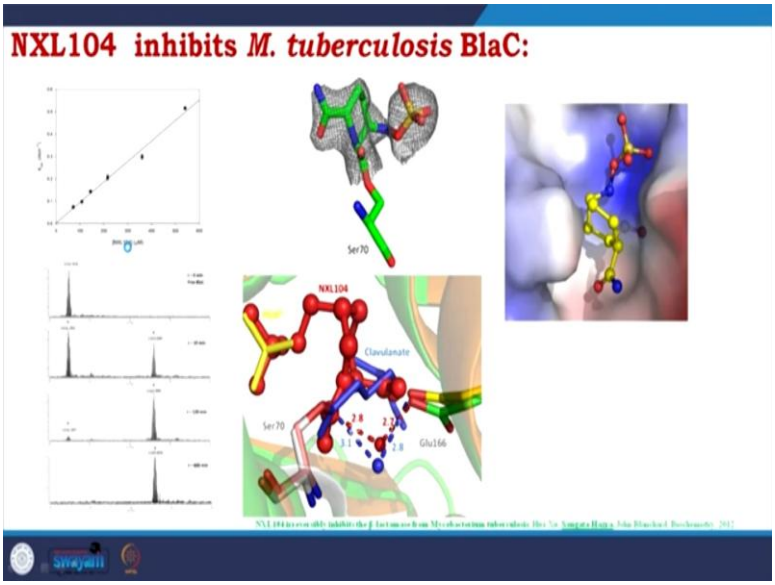

And we worked on them as I told. The avibactam is also called an NXL104. So, we did the kinetics. We did the mass spectrometry analysis of the time range of the reaction, we got the structure, and this is the comparison of the beta-lactam with NXL104 and Clavulanate.

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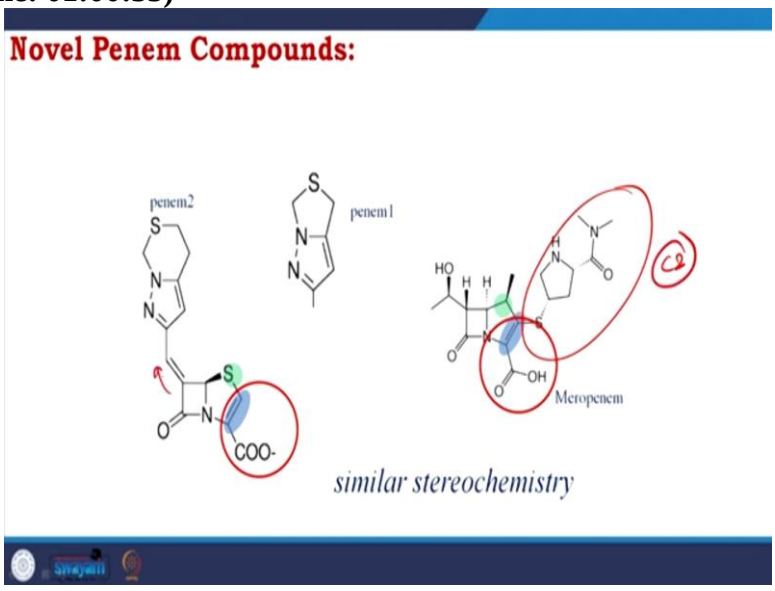

We also design a novel penem compound. If you see, this is the Meropenem which is carbapenem. The penems are different but, so, similar stereochemistry whereas, the substitution is, C2 substitution, here the substitution is in another side.

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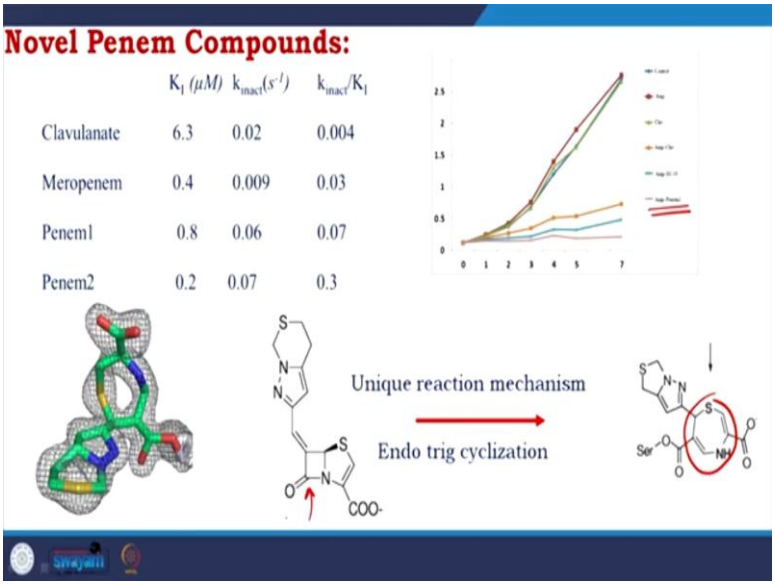

That is the difference. So, we designed those penem 1 and penem 2, if you see their values, the kinetic values, you see, we have determined the kinetic values, I have already shown the in vivo work, we have crystallized penem 2 and very interestingly, if you see, the drug now converted this, there is a change, and it converted to a seven-membered ring. It is a unique reaction mechanism, and we propose an Endo trig cyclization.

So, it is extended cyclization making the seven-membered rings. You will see the presence of a beautiful seven-membered ring here. So, we hypothesize that when a bacterial enzyme attacks here, there is a modification, rearrangement of the ring and a 7 member ring containing a new compound develops. Because of this, there is no report of resistance against this class of novel penems.

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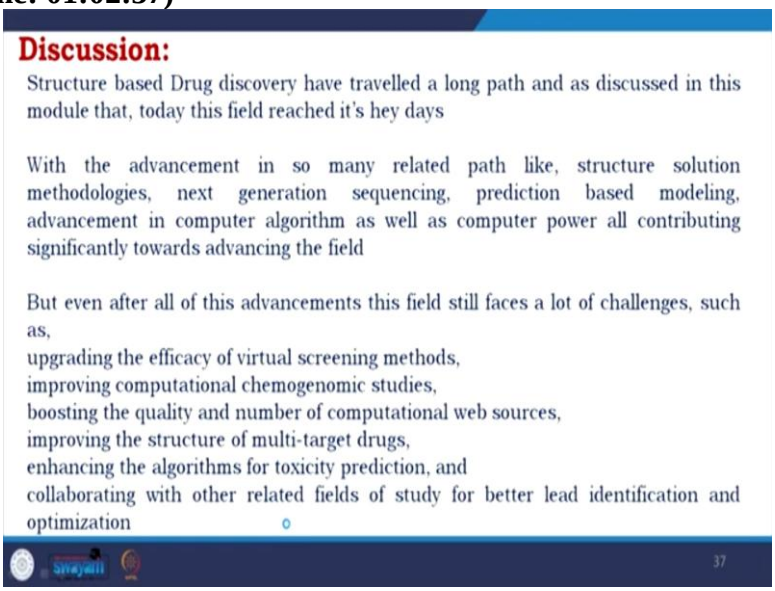

Structure-based drug discovery has traveled a long part, and as discussed in this module, today this field reached its hay days, it is on the top, a lot of work is going on. The advancement in so many related paths like structure solution methodologies, next-generation sequencing, prediction-based modeling, advancement in a computer algorithm, and computer power contribute significantly to advancing this field.

But even after all of this advancement, this field still faces a lot of challenges, such as upgrading the efficacy of virtual screening methods, as we have discussed, improving computational chemogenomic studies, boosting the quality and number of computational web sources, improving the structure of multi-target drugs, enhancing the algorithms for toxicity predicts the ADMETox, collaborating with other related fields of study for better lead identification and optimization.

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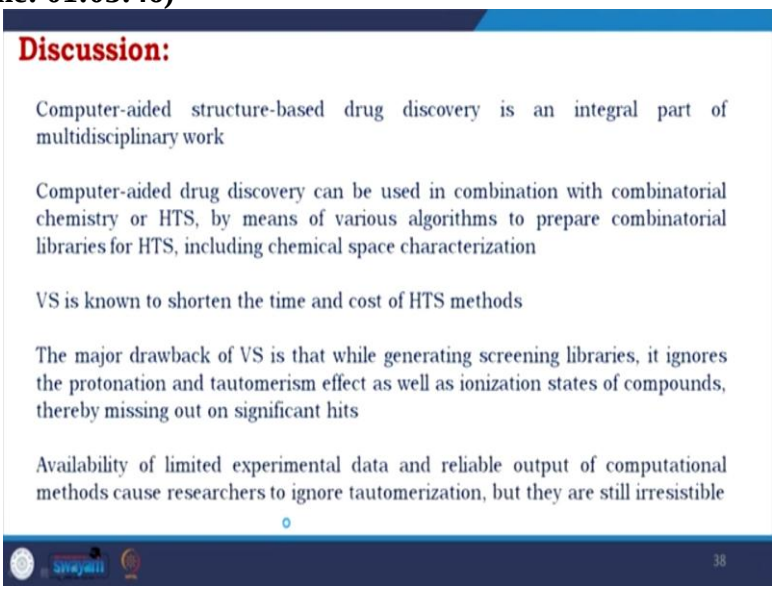

Computer-aided structure-based drug discovery is an integral part of multidisciplinary work. Computer-aided drug discovery can be combined with combinatorial chemistry or high throughput screening using various algorithms to prepare combinatorial libraries for high throughput screening, including chemical space characterization. Virtual screening is known to shorten the time and cost of high throughput screening methods.

The major drawback of virtual screening is that, while generating screening libraries, it ignores the protonation and tautomerism effect and ionization states of compounds, thereby missing out on significant hits. The availability of limited experimental data and reliable output of computational methods cause researchers to ignore tautomerization, but they are still irresistible. You have to work on them.

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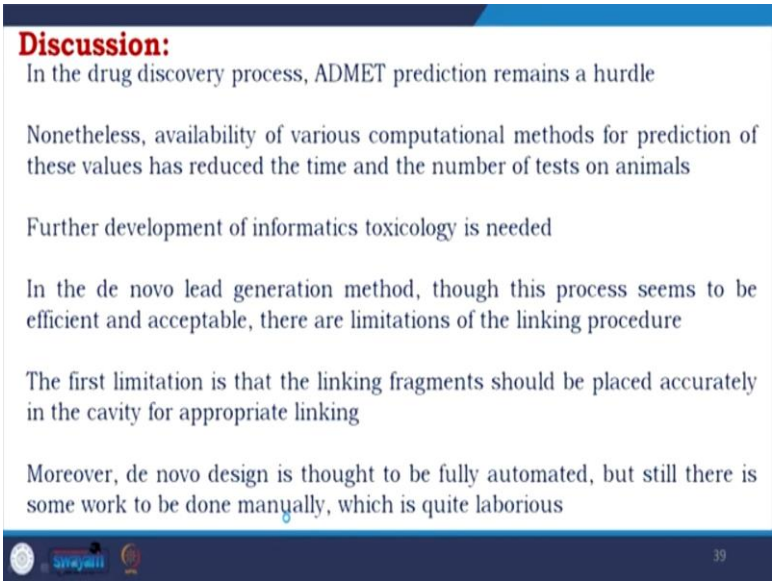

ADMET prediction remains a hurdle in the drug discovery process, as we have discussed in detail. Nonetheless, the availability of various computational methods to predict these values has reduced the time and number of tests on animals. Further development of informatics toxicology is needed. In the de novo lead generation method, though this process seems to be efficient and acceptable, there are limitations of the linking procedures.

The first limitation is that the linking fragments should be placed accurately in the cavity for appropriate linking. Moreover, de novo design is thought to be fully automated, but still, some work is to be done manually, which is quite laborious.

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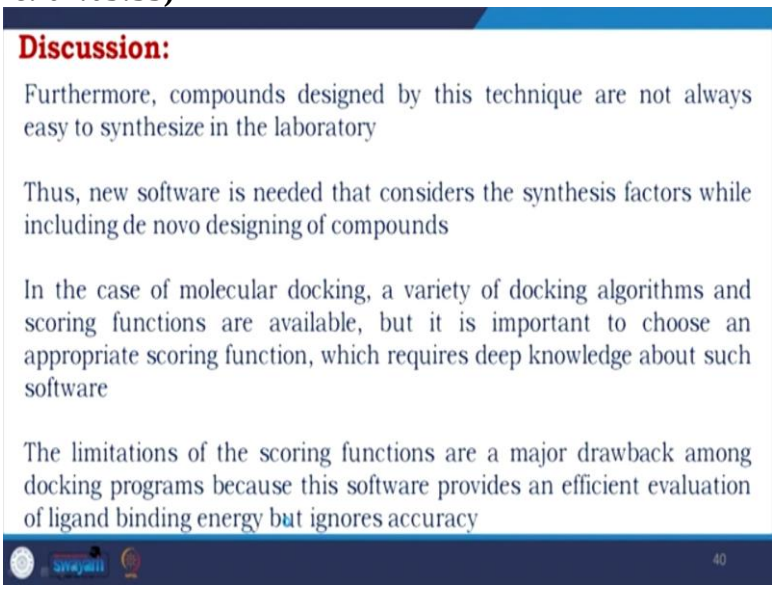

Furthermore, compounds designed by this technique are not always easy to synthesize in the laboratory. Thus, new software is needed that considers the synthesis factors while including de novo designing of compounds. You design it, but you have to think about the possibility of

synthesis. That is what is ignored still now. In the case of molecular docking, various docking algorithms and scoring functions are available.

But it is important to choose an appropriate scoring function, as I have discussed details that require a deep knowledge about such software. The limitations of the scoring functions are a major drawback among docking programs because this software provides an efficient evaluation of ligand binding energy but ignores the accuracy.

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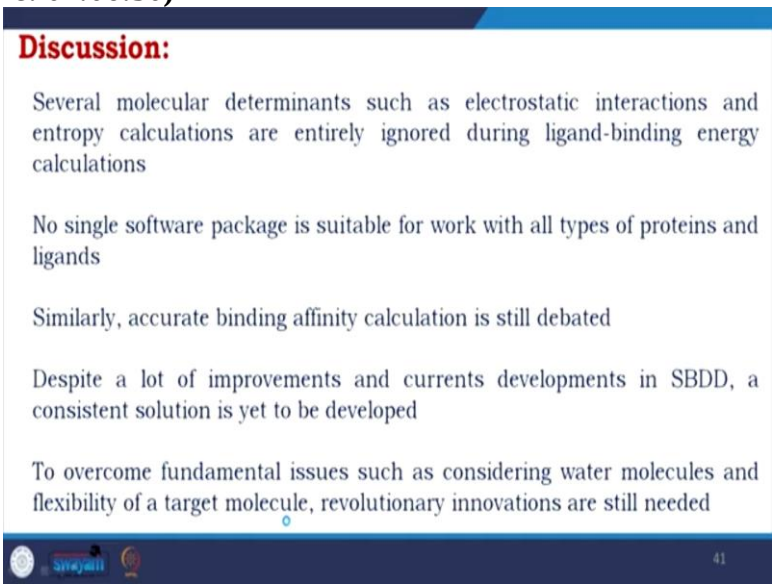

Many molecular determinants such as electrostatic interactions and entropy calculations are ignored during ligand binding energy calculation. No single software package is suitable for working with all types of proteins and ligands. Similarly, accurate binding affinity calculation is still debated. Despite many improvements and current development in structure-based drug discovery, a consistent solution is yet to be developed.