Enzyme Science and Technology
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Module - IX
Enzyme Inhibitor Designing
Lecture - 40
Inhibitor Designing (Part-II: Modern Approach)

Hello everyone, this is Dr Vishal Trivedi from Department of Biosciences and Bio engineering IIT Guwahati. And what we were discussing? We were discussing about the different properties of the enzyme in the course Enzyme science and technology. And in this context, in the current module, we are discussing about the different approaches or different way to design the inhibitor for the enzyme.

In this context, if you recall in the previous lecture, we have discussed about how you can be able to identify the active site within the enzyme structures and how you can be able to use the biochemical approaches, bioinformatics approaches or the structural approaches to identify the active site.

And subsequent to that, we have also discussed about the traditional approach, how you can be able to use the traditional approach, what are different between the traditional as well as the computational approaches. And in today's lecture, we are going to discuss about the different types of computational approaches, what you can actually be able to use to design the inhibitor against the enzyme.

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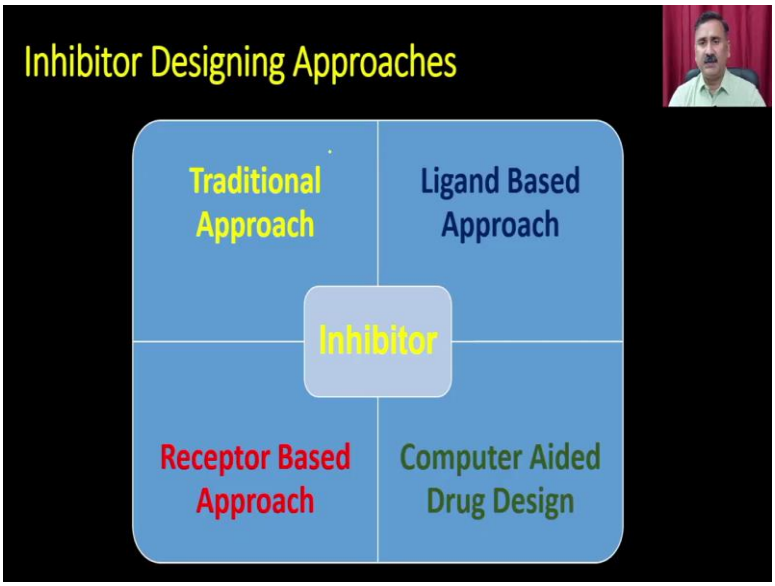

So, first, we will discuss about the Ligand Based Approach. So, before discussing about the this approaches, let us see what are different options available for the different types of inhibitor designing.

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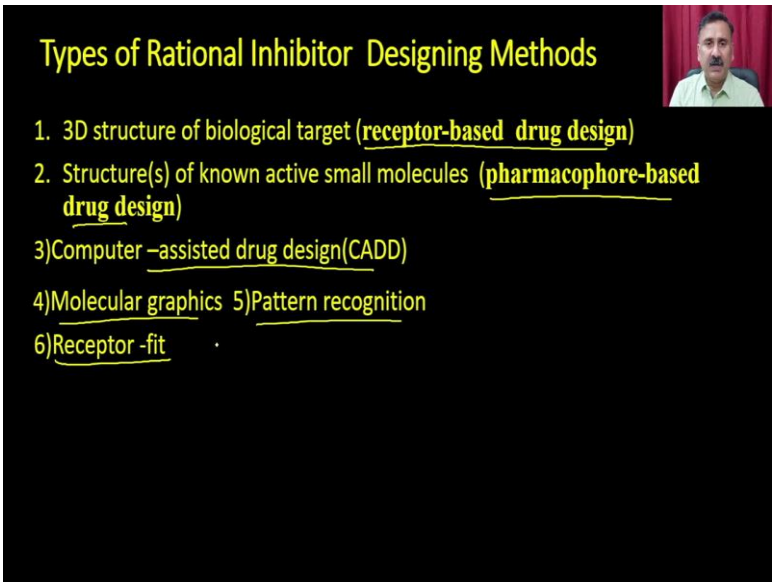

So, what you have is you have the receptor based approaches, you have the pharmacophore based approaches, you have the computer based approach, you have the molecular graphics, you have a pattern recognition and the receptor fit. So, many of these approaches, one can use depending upon the resources and as well as the expertise.

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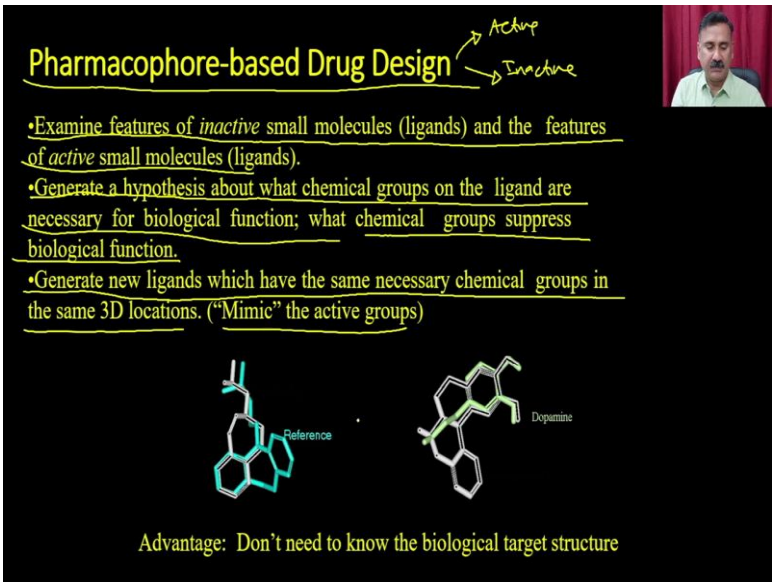

So, let us start first with the pharmacophore based approach. So, pharmacophore based inhibitor design. So, in this particular case, what you have to do is you have to take the two different types of molecule, one which is active and one which is inactive ok. So, what you have to do is you have to examine the features of the inactive molecules and as well as the features of the active molecule.

So, for example, in this case, right you have a reference molecule and you have the actual molecules. Then you generate a hypothesis about what chemical groups on the ligands are necessary for the biological function, what chemical groups suppresses the biological function and so on. And then you generate the new ligand, which have the same necessary groups in the same 3D locations or you mimic the active groups.

So, you see these are the, this is the reference group and this is the target ligands, what you want to design and that is how you can be able to do, you have to keep the active groups intact and you actually have to change the other groups and that is how you can be able to design the new molecules.

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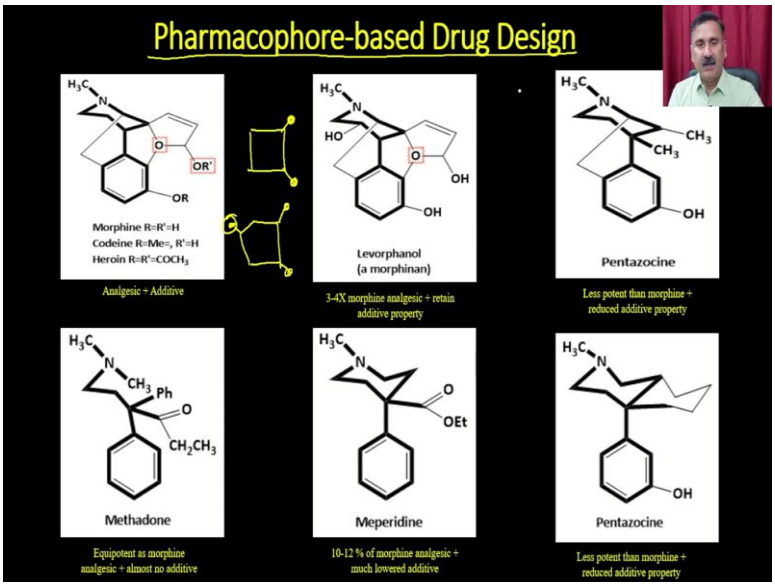

The same way people have also developed that is some of these kind of molecules. So, we have taken the examples from the natural sources, we have taken the examples from the biology and that is how you can be able to do the selection of the different types of molecules. So, you can actually be have the you know based molecule, for example, you will say that ok, these are the functional groups which are important, right.

So, you will keep these intact and instead of taking this, you can actually be able to do like designing like this, ok. So, you can actually be able to design the molecule like this, ok. So, in that case, you have designed you have preferred the new molecules and you can be able to use that for some more applications.

So, these I will actually ensure that it will go and hit the active site whereas, these groups you can actually be able to put as per the your additional requirements and that is how you can be able to use the pharmacophore based approach to design the molecules.

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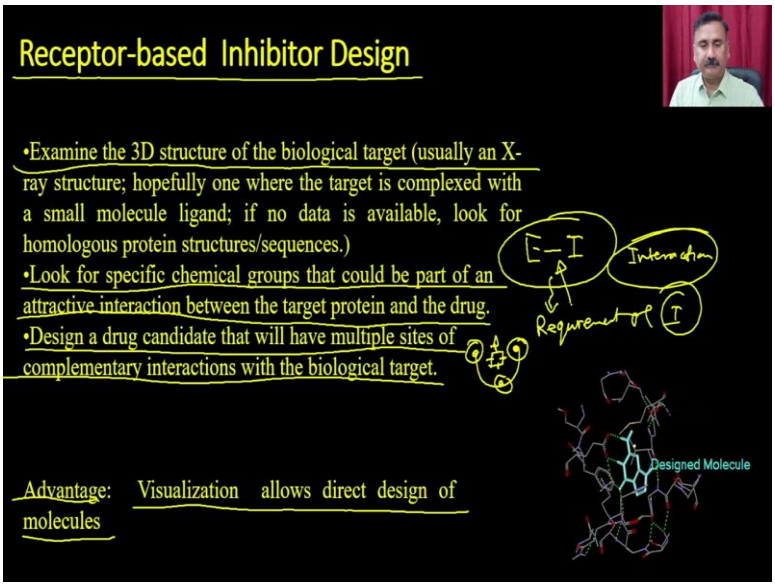

Now, the second approach is the receptor based inhibitor design. So, in the receptor based inhibitor design, you are actually going to examine the 3D structure of the biological target which means, you are going to examine the enzyme structures or if you are lucky and you are actually going to have the enzyme which is already complexed with the inhibitor, then your task is actually going to be facilitate even faster, right.

So, if you have such kind of complex available, then you are actually going to see what are the requirements of the inhibitor, ok. And what are the features, what are present in the enzyme to facilitate that and looking at that looking for the specific chemical group that could be a part of an attractive interaction between the target protein and the inhibitor, then you can actually be able to keep those interactions intact.

So, taking the interaction into account, you will keep the interaction intact, but you will change the molecules. For example, you have a active site like this, right. So, you can actually be able to have the inhibitor and you imagine that you have a group here you have a group here, you have a group here, right. So, now you cannot change the enzyme because these groups are intact, right. What you can do is you can actually be able to change the groups onto this, right. And you can actually be put additional groups based on the your requirements.

So, you can design a drug molecule that will have the multiple site of the complementary interaction with the biological targets. And what is the advantage of this group is that

you can actually be able to see that how the design molecule is fitting into the active site. And this is the one of the molecule, right.

So, what you can do is you can take the design molecule, you can put it into the active site and you can see whether it is mapping all the interactions what are crucial for the enzyme to interact with the inhibitor or not. And taking that into account, you can be able to ensure your or you may be more confident that this inhibitor is actually going to block the activity of this particular enzyme.

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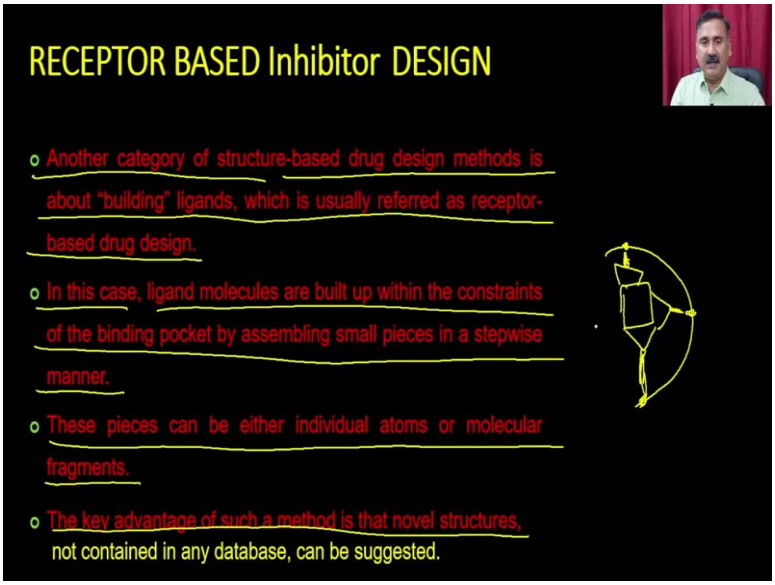

So, in the receptor based inhibitor design, you can actually have the you know structure based drug design method is about the building ligand which is usually referred as the receptor based drug design. So, in this case, the ligand molecules are built up within the constraint of the binding pocket by assembling the small pieces in a stepwise manner. These pieces can be either individual atom or the molecular fragments.

The key advantage of such a method is that novel structure, not contained in any database can be suggested. So, what it says is that suppose this is the active site and you know that these are the some of the groups what are present, right. So, what you can do is you can start very crude, you can start with a very crude molecule and then you can actually be start building the molecule based on that, right.

So, that you can be able to ensure the interaction of these groups with this particular new design molecule. And that is why you can actually have the iterative molecules, iterative the designing of the molecules.

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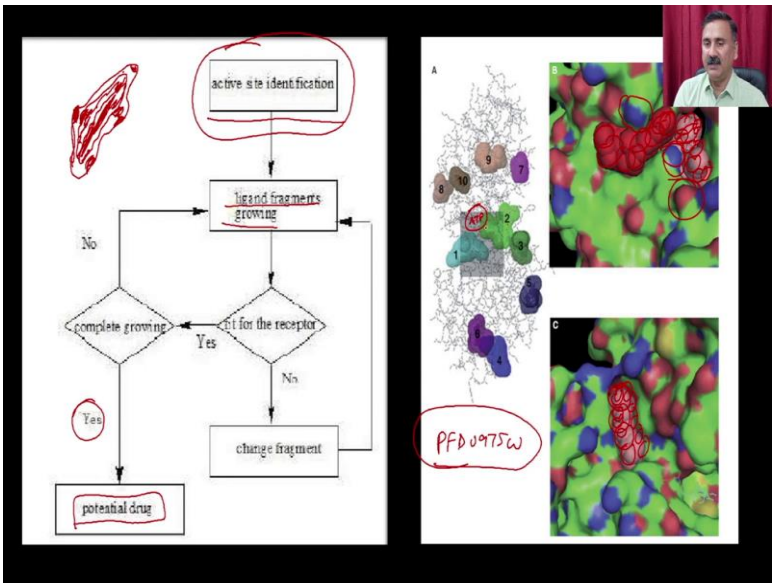

One of the example is that where we have done this, ok. So, in this what you are going to do is you are first going to identify the active site and so, first you will identify the active site, ok So, for example, you have this is the active site what you have designed, ok. So, this is the active site what you found.

And you found that there are some groups, some functional groups what are present in this. So, these functional groups are very crucial for the receptor to bind, right. For example, these are the groups. Now, what you are going to do is you are going to prepare a ligand fragments, ok. So, you are going to put a ligand fragment like this, ok.

Now, you are going to test whether this particular ligand fragment is fitting into this or not, right. If not then you are actually going to design another ligand, right. So, you are going to say ensure first that the t-dimensionally the ligand is fitting into this active site or not, right. So, you will fit like this.

Now, if it is fits into the structure receptor, then you are actually been you know in the right path if it is not then you are actually going to change the fragments, ok. And we grow that, right. So, now once the growth is over and you could have been able to get

nice fitting then what you can do is you can just put the functional groups onto this particular growing chain in such a way that it is actually going to ensure the molecular interactions.

And if that happens then you are actually going to get the new molecule or the new inhibitors. This is exactly what we have also done in our laboratory. And if you want to do this and you are actually going to first identify the binding pocket. So, what we did is we this is another this is same example of PFD 957w right. So, what we did is we have identified the binding pockets. So, you will see we have identified the 10 binding pockets.

And I am going to show you one example where this is the binding pocket. So, binding pocket 1 and 2, which is actually present within the ATP binding pocket. We have chosen and then we have started designing the inhibitors. So, what we did is we started with a very crude molecule, right. You see this, right. And then we started filling the balls, ok. So, you can actually be able to fill the balls so, that you can actually be able to know what are the 3D confirmation required, ok. Now, you see this, right.

So, if I start filling the 3 dimensional structures or 3 dimensional balls into this particular structure, I will be able to know what are the 3D confirmation required to bind this. And then you will see this is a one group what is present here. You have upon you know the one group here and so on. So, taking these into groups into the account and you see this is a group also here present.

So, you know first I will get the three dimensional confirmation and then I will put all these functional groups and that is how I am going to design the new inhibitor which will fit into this particular active site involving these two pockets. Now, let us take an another case study where we are actually going to show you how you can be able to design the inhibitors.

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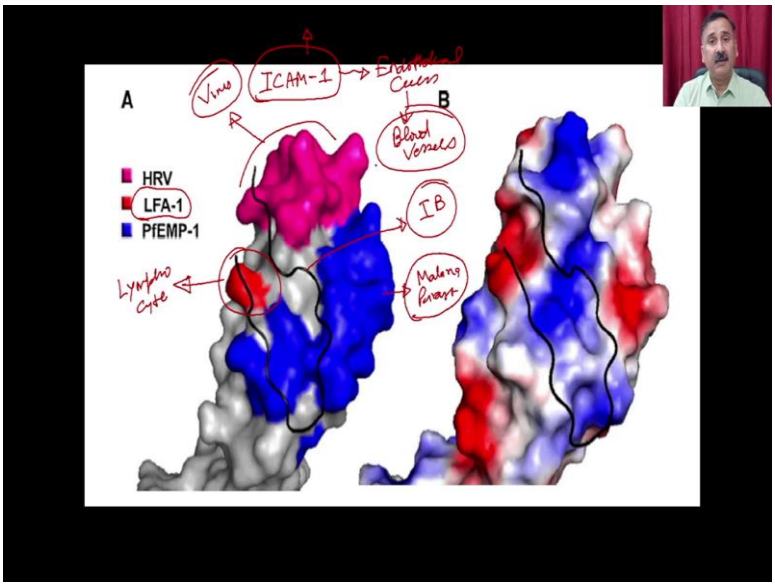

So, this is an example of the molecule which is called as the ICAM-1, ok. So, ICAM-1 is a molecule which is responsible for the binding of the different types of ligands and it is present on to the. Many of the proteins, but it also present on the endothelial cells and many of you if you do not know about endothelial cells endothelial cells are the cells which are actually making our blood vessels, ok.

So, our blood vessels are made up of the endothelial cells. Now, you see the ICAM-1, ICAM1- has three region. One is this region which is for the virus. Then you have this region, see this small portion, this is the region which from which it is actually binding the lymphocytes because this is the lymphocyte binding factor, ok And then this blue region what you see is actually for the malaria parasite. Here I am showing the same in terms of the positive and negative charges.

So, this is the topology of the different types of charges, right. So, and what you see this black colored thread is actually a peptide which is called as the inhibitory peptide and this peptide is known to disrupt the interaction of the ICAM-1 with the LFA-1, ok So, what we want is we want to modify this in such a way that it is actually going to go and bind here in such a way that it is actually going to disrupt the interaction of the malaria parasite with the ICAM-1 molecule.

Because when the malaria parasite interact with the ICAM-1 it actually interact it introduces the cytoadherence and that is how it is responsible for the development of cerebral malaria.

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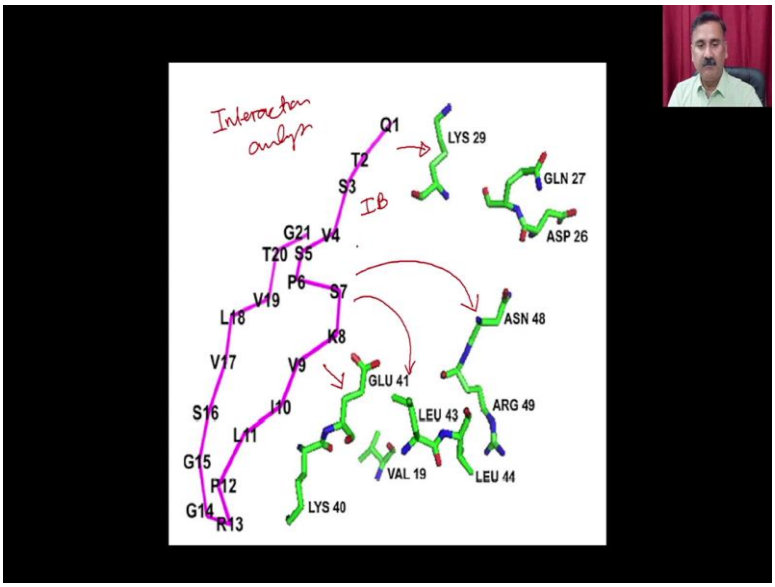

First thing what we did is we did the interaction analysis. So, this is the IB peptide and what we have identified, we have identified the crucial residues which are responsible for the interactions. And considering these interactions what we did is we started modifying the fragments, ok. So, remember that when we were talking about the receptor based inhibitor design we started designing the fragments, ok.

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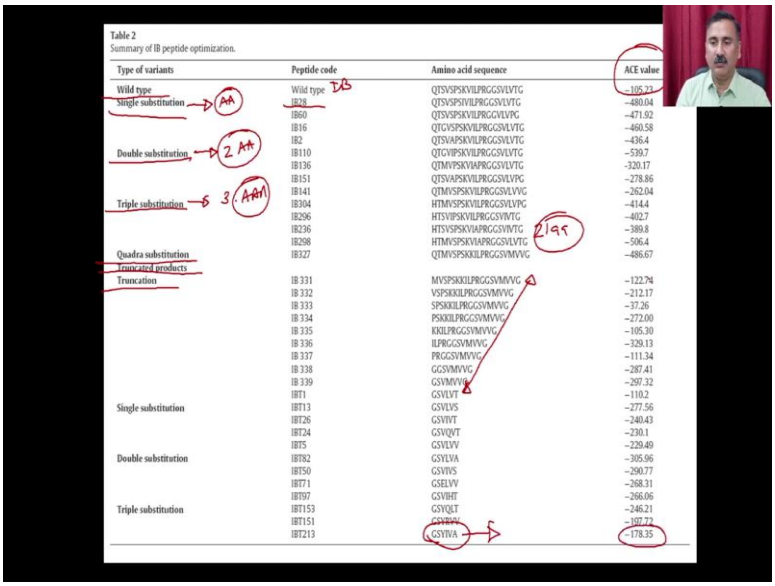

Table 2
Summary of IB peptide optimization.

Type of variants	Peptide code	Amino acid sequence	ACE value
Wild type	Wild type	QTSVSPSKVILPRGGSVLVTG	−105.23
Single substitution	IB28	QTSVSPSIVILPRGGSVLVTG	−480.04
	IB60	QTSVSPSKVILPRGGVLVPG	−471.92
	IB16	QTGVSPSKVILPRGGSVLVTG	−460.58
Double substitution	IB2	QTSVAPSKVILPRGGSVLVTG	−436.4
	IB110	QTGVIPSKVILPRGGSVLVTG	−539.7
	IB136	QTMVPSKVIAPRGGSVLVTG	-320.17
	IB151	QTSVAPSKVILPRGGSVLVPG	−278.86
Triple substitution	IB141	QTMVSPSKVILPRGGSVLVVG	−262.04
	IB304	HTMVSPSKVILPRGGSVLVPG	−414.4
	IB296	HTSVIPSKVILPRGGSVIVTG	−402.7
	IB236	HTSVSPSKVIAPRGGSVIVTG	−389.8
	IB298	HTMVSPSKVIAPRGGSVLVTG	−506.4
Quadra substitution	IB327	QTMVSPSKKILPRGGSVMVVG	−486.67
Truncated products			
Truncation	IB 331	MVSPSKKILPRGGSVMVVG	−122.74
	IB 332	VSPSKKILPRGGSVMVVG	−212.17
	IB 333	SPSKKILPRGGSVMVVG	−37.26
	IB 334	PSKKILPRGGSVMVVG	−272.00
	IB 335	KKILPRGGSVMVVG	−105.30
	IB 336	ILPRGGSVMVVG	−329.13
	IB 337	PRGGSVMVVG	−111.34
	IB 338	GGSVMVVG	−287.41
	IB 339	GSVMVVG	−297.32
	IBT1	GSVLVT	−110.2
Single substitution	IBT13	GSVLVS	−277.56
	IBT26	GSVIVT	−240.43
	IBT24	GSVQVT	−230.1
	IBT5	GSVLVV	−229.49
Double substitution	IBT82	GSYLVA	−305.96
	IBT50	GSVIVS	−290.77
	IBT71	GSELVV	−268.31
	IBT97	GSVIHT	−266.06
Triple substitution	IBT153	GSVQLT	−246.21
	IBT151	GSYEVT	−192.72
	IBT213	GSYIVA	−178.35

So, what we did is we did the wild types of molecule which is the IB peptide, right. And then we started modifying the fragments. So, we first started making the mutations into the main chain, right. So, we did the single substitutions, double substitutions, triple sub single substitution means, we have changed the amino acid, one amino acid at a time

Double substitution means, we changed the 2 amino acid at a time. And triple substitution means, we have changed 3 amino acid in the sequence at a same time, ok. And what we found is that its binding into the cavity is changing when we are doing this. And then we also did the quadra and truncated and all that. After that since this is a 21 amino acid long peptide we started producing the truncations, ok

And we started putting the truncation. So, that we will going to have the smaller peptide and that smaller peptide is also going to have the same efficiency as this particular large peptide. Because you know that the large peptide does not get into the target site and it will actually going to have the more non-specific interaction. And then we also did the optimization of the smaller peptide and ultimately, we got the best peptide which is the this peptide, ok. And you will see this is also binding into the active site.

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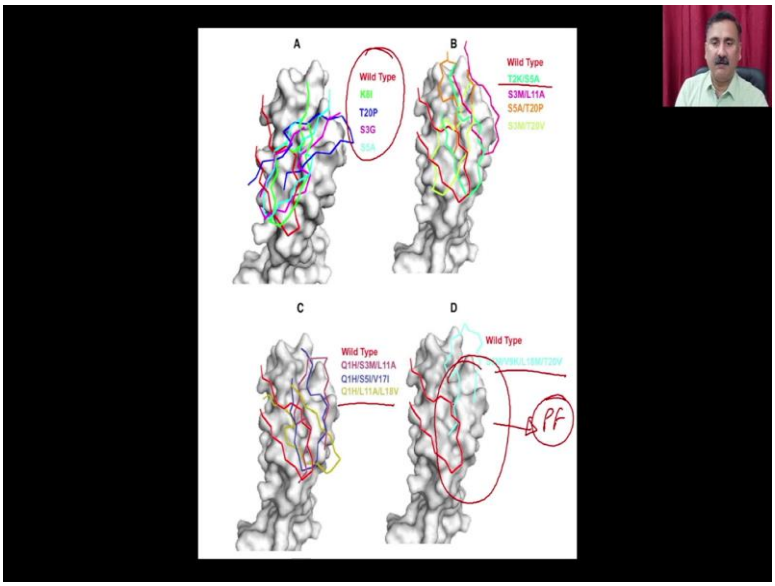

Now, if you see how it is binding. So, when we are doing the substitution onto the peptide it is actually you know rotating and binding and changing its conformation, binding site and all that. And so, when we did the you know double substitutions, triple substitutions and ultimately, we did the quadra substitutions.

And that is how it has actually got changed completely and it is actually started mapping this particular site and that is how it is actually going to disrupt the interaction with the plasmodium falciparum. So, since taking these into the account we have designed the new molecule.

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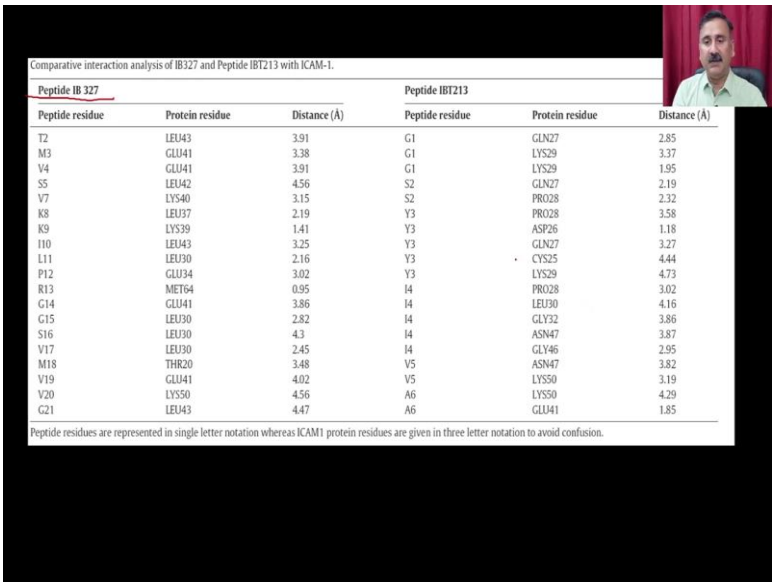

Comparative interaction analysis of IB327 and Peptide IBT213 with ICAM-1.

Peptide IB 327			Peptide IBT213		
Peptide residue	Protein residue	Distance (Å)	Peptide residue	Protein residue	Distance (Å)
T2	LEU43	3.91	G1	GLN27	2.85
M3	GLU41	3.38	G1	LYS29	3.37
V4	GLU41	3.91	G1	LYS29	1.95
S5	LEU42	4.56	S2	GLN27	2.19
V7	LYS40	3.15	S2	PRO28	2.32
K8	LEU37	2.19	Y3	PRO28	3.58
K9	LYS39	1.41	Y3	ASP26	1.18
I10	LEU43	3.25	Y3	GLN27	3.27
L11	LEU30	2.16	Y3	CYS25	4.44
P12	GLU34	3.02	Y3	LYS29	4.73
R13	MET64	0.95	I4	PRO28	3.02
G14	GLU41	3.86	I4	LEU30	4.16
G15	LEU30	2.82	I4	GLY32	3.86
S16	LEU30	4.3	I4	ASN47	3.87
V17	LEU30	2.45	I4	GLY46	2.95
M18	THR20	3.48	V5	ASN47	3.82
V19	GLU41	4.02	V5	LYS50	3.19
V20	LYS50	4.56	A6	LYS50	4.29
G21	LEU43	4.47	A6	GLU41	1.85

Peptide residues are represented in single letter notation whereas ICAM1 protein residues are given in three letter notation to avoid confusion.

And what we have found is that the this new molecule which is the IB 327 is as efficient as the IB peptide. This is the IB peptide, this is the smaller peptide, this is the larger peptide and both are actually having the very very strong interaction with the all the residues what we have identified that were used for the interactions. So, this is the receptor based studies what you can actually be able to perform to design the new inhibitors.

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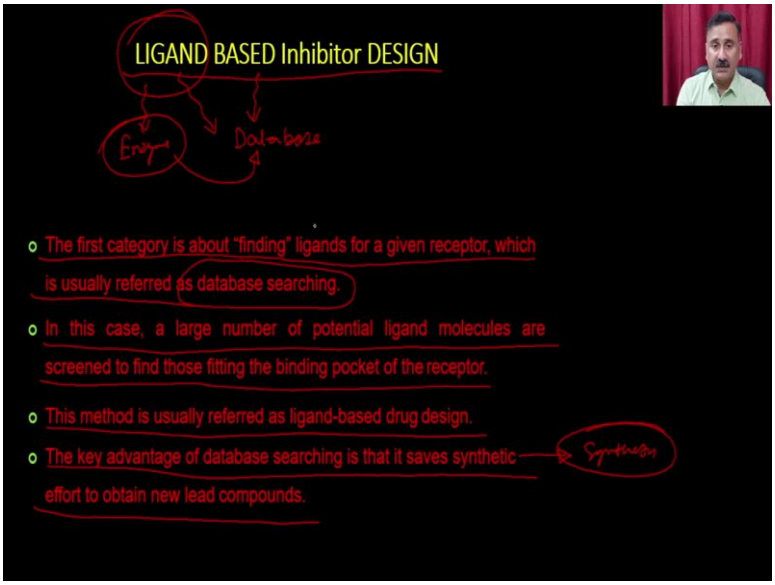

Then let us move on to the nibs molecule and the next molecule is the ligand based inhibitor design. So, the first category is about finding ligand for a given receptor which is usually referred as the database searching. So, ligand based inhibitor; that means, you are actually going to search the database taking this ligand into account. Ok. You know that this ligand actually binds to that particular enzyme or it is actually an inhibitor for this particular ligand.

So, now you use this and you identify the another molecule into the database. So, in this case a large number of potential ligand molecules are screened to find those fitting the binding pocket of the receptor. This method is usually referred as the ligand bit inhibitor design.

The key advantage of the database searching is that it saves the synthetic efforts to obtain the new lead molecule which means, it actually does not it reduces the efforts to synthesize these molecules and then you test them into the enzyme assay and all that. Because if this ligand is good and if you search into the database and could found the alternate molecule fitting exhibiting the similar kind of properties this means that also is also going to be an inhibitor.

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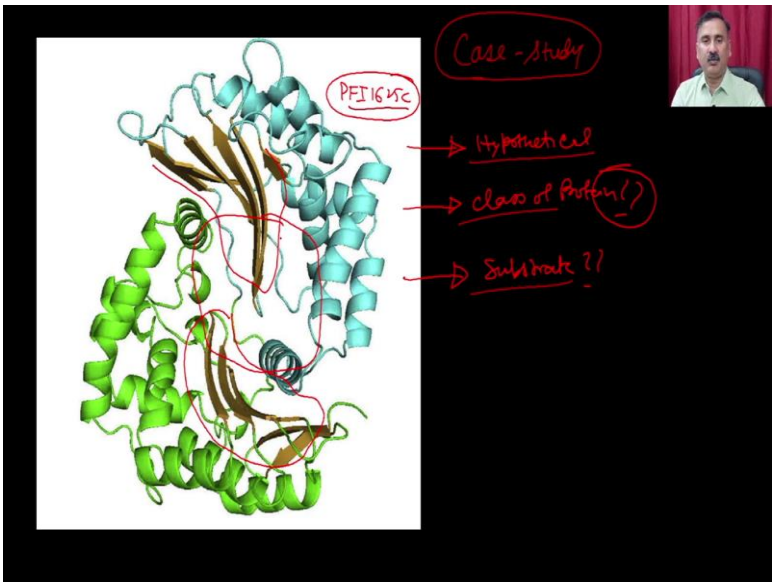

Now, let us see another molecule or another story or case study how you can be able to use this to design the use the ligand based approach ok. So, this is another protein, but we were working in our laboratory which is called as the PFI 1625C and this is a

hypothetical protein and it actually does not know anything about this class of enzyme and all that. So, what we did is we did the homology modeling and we prepared a homology model.

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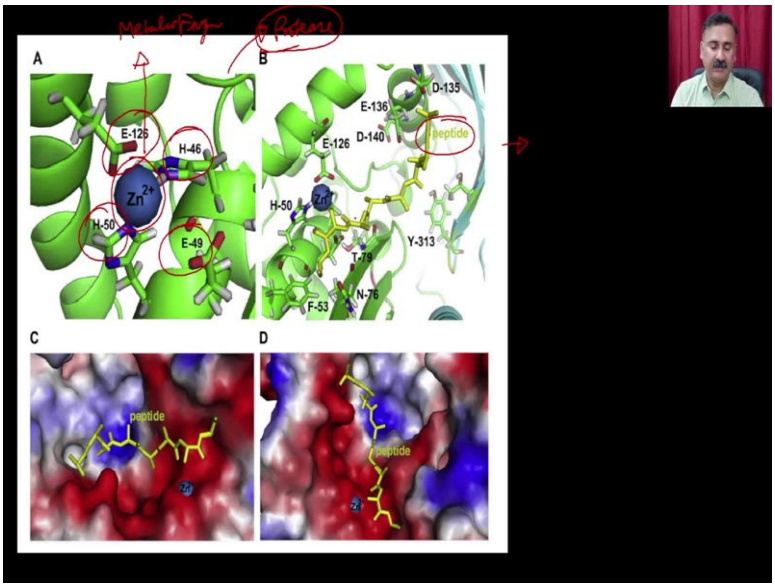

And based on this homology model what we found is that it also has a protein bound zinc which means one information is clear that it is a metalloprotein ok. So, it has a metalloenzyme right because it has a metal. Then it also has the some of these E residues and considering these E residues and the three dimensional structure of the active site we would be found is that it is actually a protease.

And when we know that the protease then we also identify the peptide, which actually fits into this particular active site and how this peptide can be cleaved. So, considering these peptide sequence which was you know starting from the very crude sequence we have identified the refined sequence which will fit into this.

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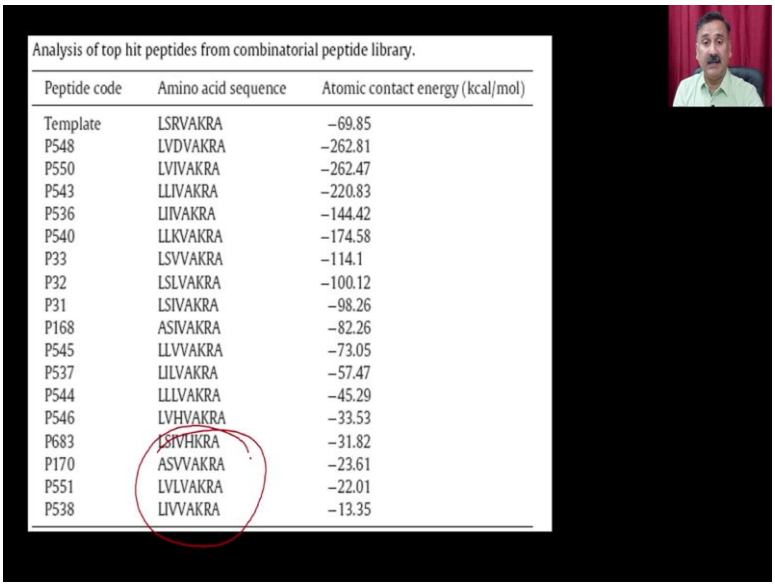

Analysis of top hit peptides from combinatorial peptide library.

Peptide code	Amino acid sequence	Atomic contact energy (kcal/mol)
Template	LSRVAKRA	−69.85
P548	LVDVAKRA	−262.81
P550	LVIVAKRA	−262.47
P543	LLIVAKRA	−220.83
P536	LIIVAKRA	−144.42
P540	LLKVAKRA	−174.58
P33	LSVVAKRA	−114.1
P32	LSLVAKRA	−100.12
P31	LSIVAKRA	−98.26
P168	ASIVAKRA	−82.26
P545	LLVVAKRA	−73.05
P537	LILVAKRA	−57.47
P544	LLLVAKRA	−45.29
P546	LVHVAKRA	−33.53
P683	LSIVHKRA	−31.82
P170	ASVVAKRA	−23.61
P551	LVLVAKRA	−22.01
P538	LIVVAKRA	−13.35

So, we have done the similar kind of approach we have done the substitutions and all that and what we found is that these are the amino acid sequences which fit into the active site very nicely.

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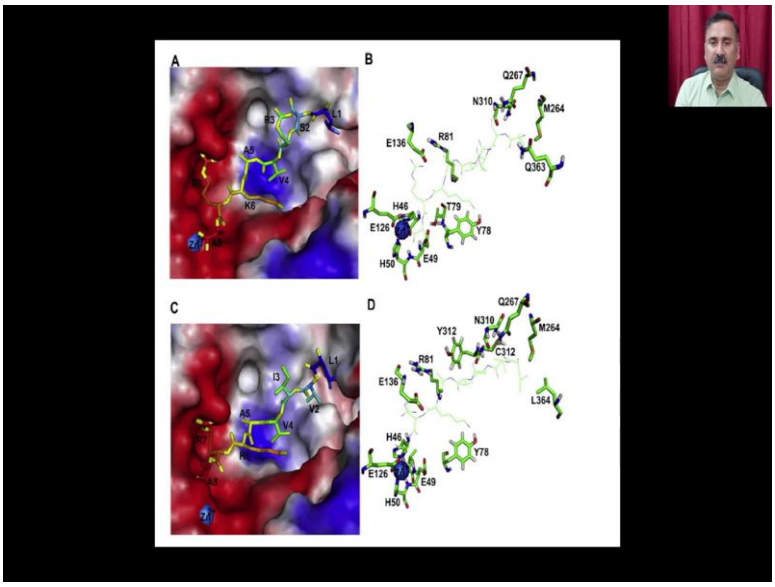

And these are the sequence these are the peptide final peptide which fit into the active site and can be actually be able to cleaved.

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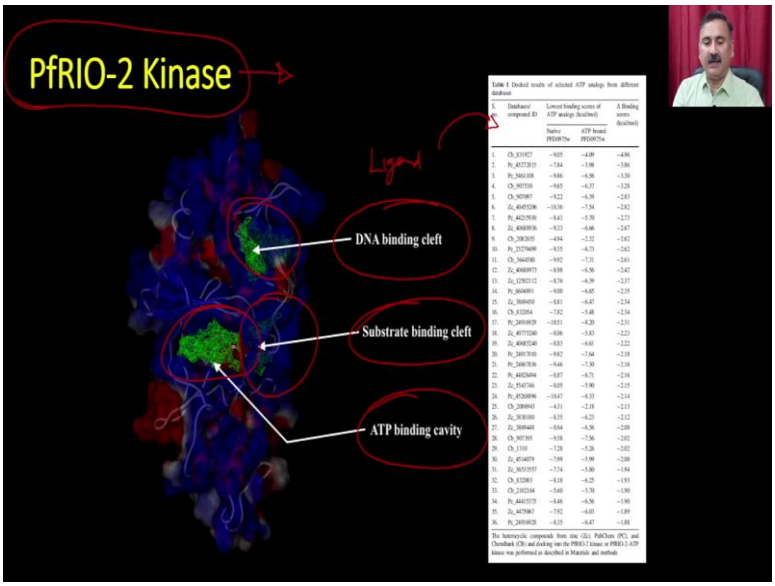

Apart from that there was another enzyme, which is called as the PFRIO-2 Kinase and that also can be used for the ligand based approach. So, like PFRIO-2 kinase has a DNA binding cleft it also has a substrate binding cleft and it also has the ATP binding cleft right. So, this is a substrate binding cleft and this is the ATP binding cleft. Taking these into the account what we have done is we have taken the ligand.

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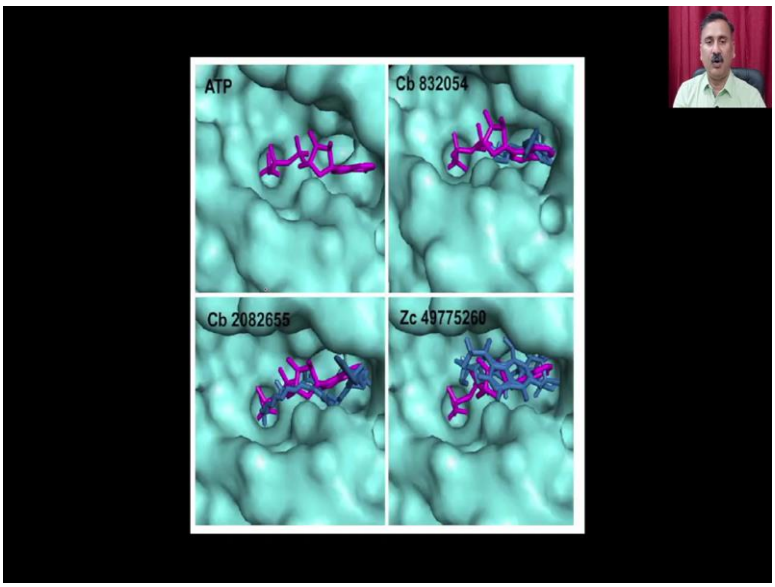

And we have searched different types of molecule into the database. So, these are the different molecule what represent the database. We docked all these molecule to ensure

that they are binding into the ATP binding pocket and then we have selected some of these molecules like the this molecule, this molecule and this molecule and then you see that they are very nicely fitting into the active site. This means, they are the potential molecules right.

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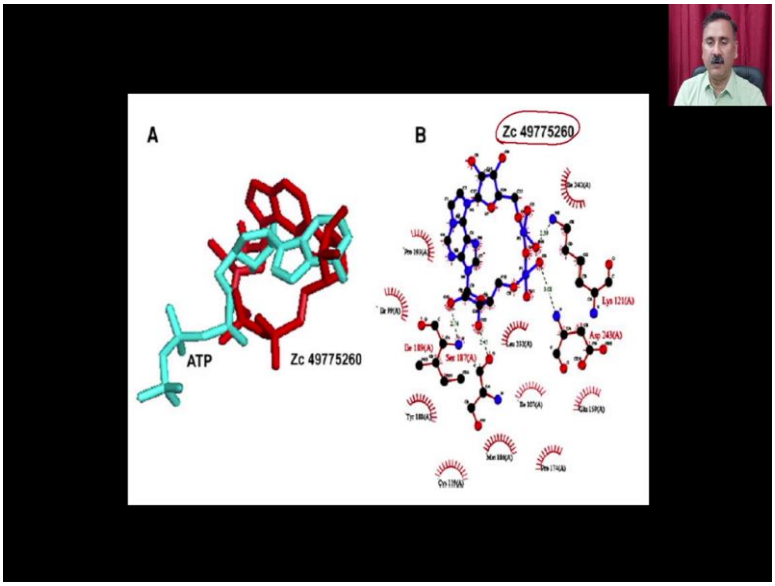

And then this is the best molecule what we have identified right from the database and we have studied how what will be the drug like properties of this enzyme.

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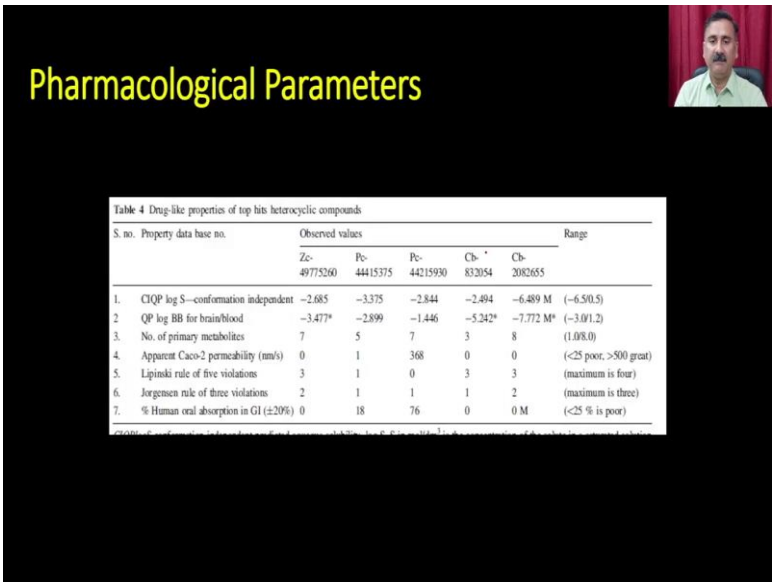

Table 4 Drug-like properties of top hits heterocyclic compounds

S. no.	Property data base no.	Observed values					Range
		Zc-49775260	Pc-44415375	Pc-44215930	Cb-832054	Cb-2082655	
1.	CIQP log S—conformation independent	−2.685	−3.375	−2.844	−2.494	−6.489 M	(−6.5/0.5)
2	QP log BB for brain/blood	−3.477*	−2.899	−1.446	−5.242*	−7.772 M*	(−3.0/1.2)
3.	No. of primary metabolites	7	5	7	3	8	(1.0/8.0)
4.	Apparent Caco-2 permeability (nm/s)	0	1	368	0	0	(<25 poor, >500 great)
5.	Lipinski rule of five violations	3	1	0	3	3	(maximum is four)
6.	Jorgensen rule of three violations	2	1	1	1	2	(maximum is three)
7.	% Human oral absorption in GI (±20%)	0	18	76	0	0 M	(<25 % is poor)

So, we have found that [FL] this molecule is following the Lipinski rules and all the other kind of parameters. And then we what we did is we have actually be able to use that molecule to identify the similar kinds of drugs what are present into the database.

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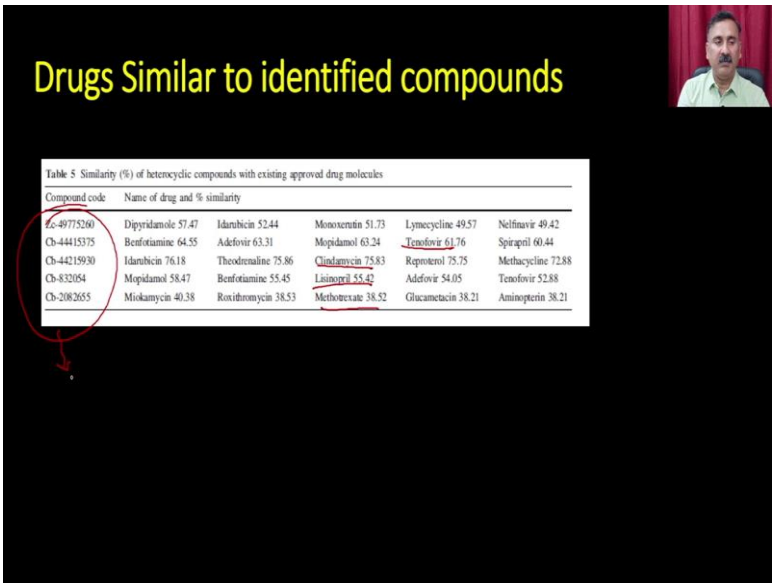

So, these are the drugs what are present ok and these could be a alternative to these compounds for testing their inhibitory activities.

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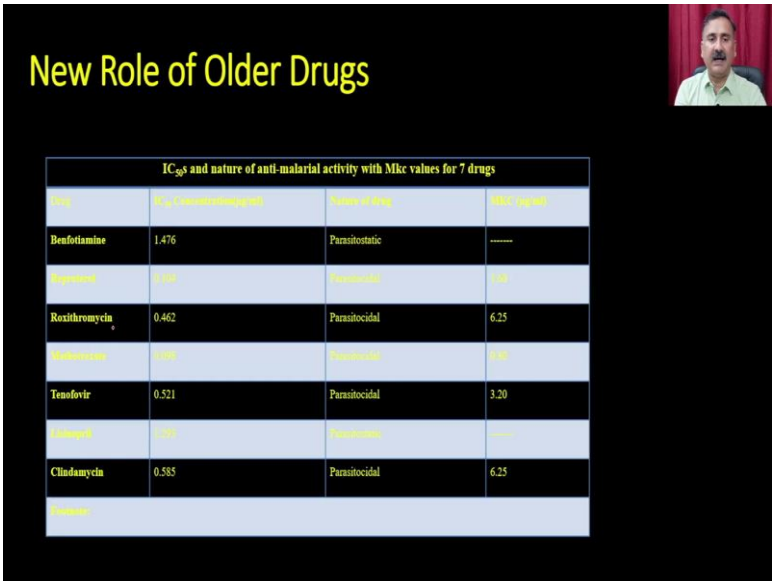

And that is how we have assigned the new role of the older drugs which means we have said that these are the drugs which are actually going to work instead of those molecules

and they can be used in the clinics. So, this is all about the some of the more inhibitor designing approach. So, what we have discussed?

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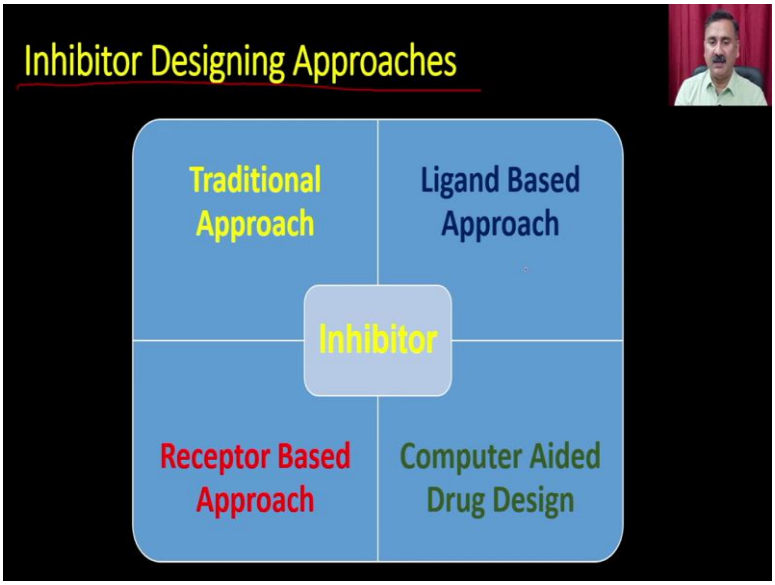

We have discussed the ligand based approach and we also discussed about the receptor based approach. In our subsequent lecture we are going to discuss about the computer aided inhibitor design and we also going to show you a demo about how you can be able to use the different types of tools to design the inhibitors. So, with this I would like to conclude my lecture here.

Thank you.