VIRTUAL SCREEN OF SUBSTANCES WITH POTENTIAL ANTIVIRAL ACTIVITY AGAINST THREE FLAVIVIRUSES:

dengue virus, yellow fever virus and Zika virus

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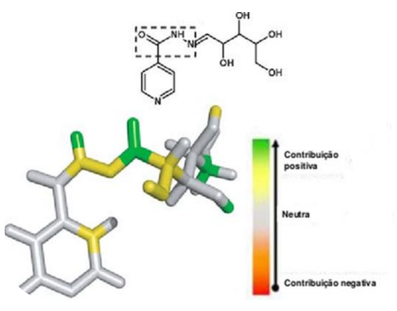
Introduction

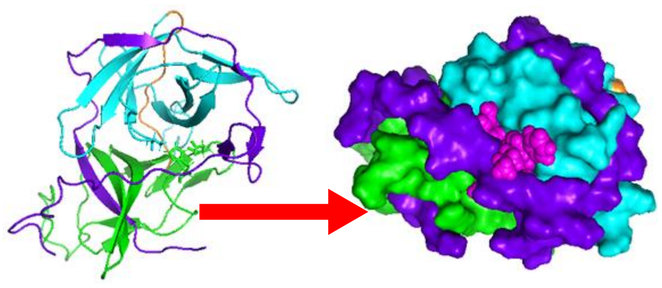
Approximately three billion people live in regions at risk of infections by flaviviruses. *Dengue virus* (DENV), *Zika virus* (ZIKV) and *Yellow fever virus* (YFV) presents outbreaks and severe complications. Currently, there are no antivirals available to treat these diseases. We screened and evaluated the potential antiviral activity of small molecules against these viruses, targeting the viral protease NS2B-NS3 ($NS3_{PRO}$). We used a combination of HQSAR models and structural molecular modelling, based on structures of peptidomimetic DENV-3 $NS3_{PRO}$ inhibitors and molecular docking studies to screen for new compounds (CPD). Binding sites of DENV-3 and ZIKV $NS3_{PRO}$ were assessed to build a pharmacophoric model for virtual screening (VS). Hits were selected after molecular dynamics (MD) simulations, with predictions of toxicity and biological activity. Biological activities were evaluated by the MTT assay. Antiviral activity was evaluated by plaque reduction, pre-treatment and virucide activity assays. Enzymatic inhibition assays against ZIKV $NS3_{PRO}$ were carried out.

Methods

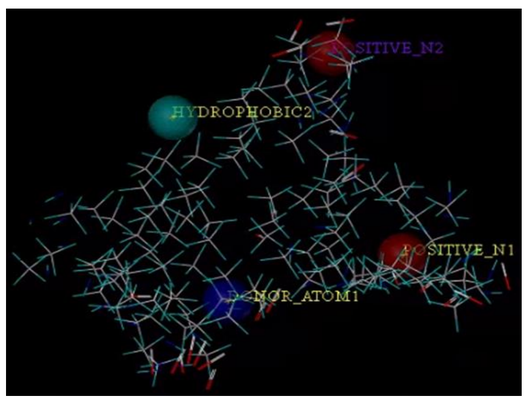
1. HQSAR



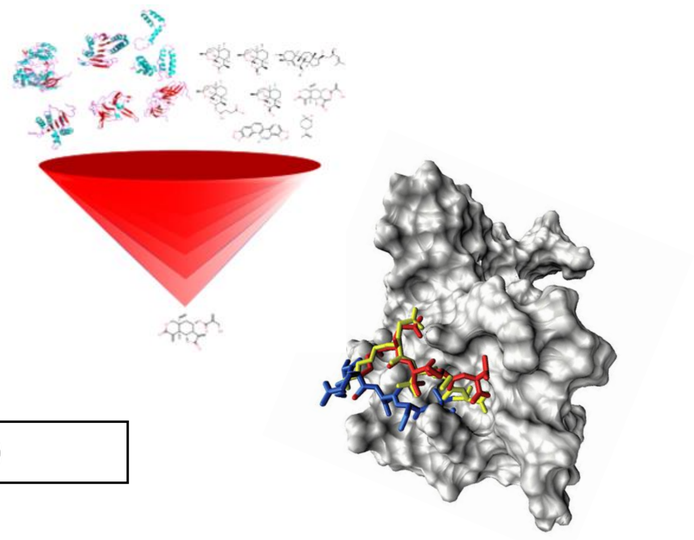
2. Structure mapping



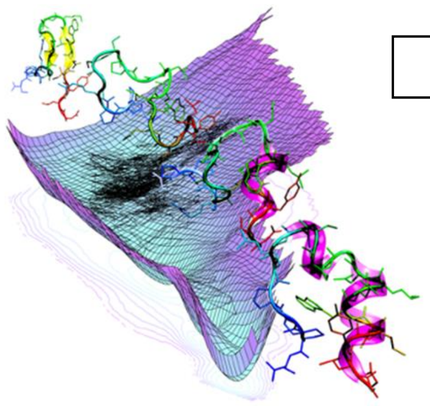
3. Pharmacophore



4. Virtual screen (VS)



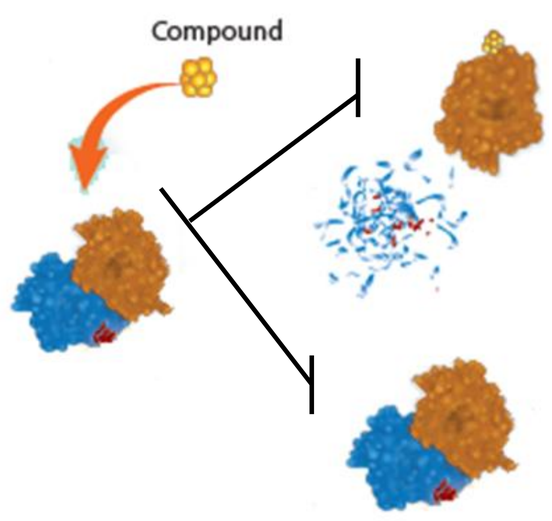
5. Molecular dynamics (MD)



6. Biological predictions

7. Antiviral assays

8. Enzymatic inhibition



Results

Figure 1. HQSAR model: important fragments to activity

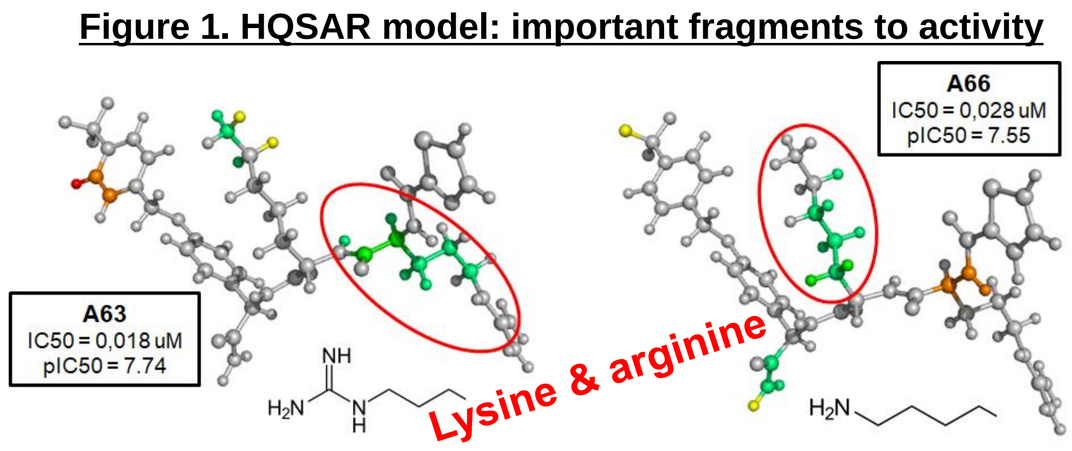


Figure 2. Structure mapping: sites and pockets of interaction

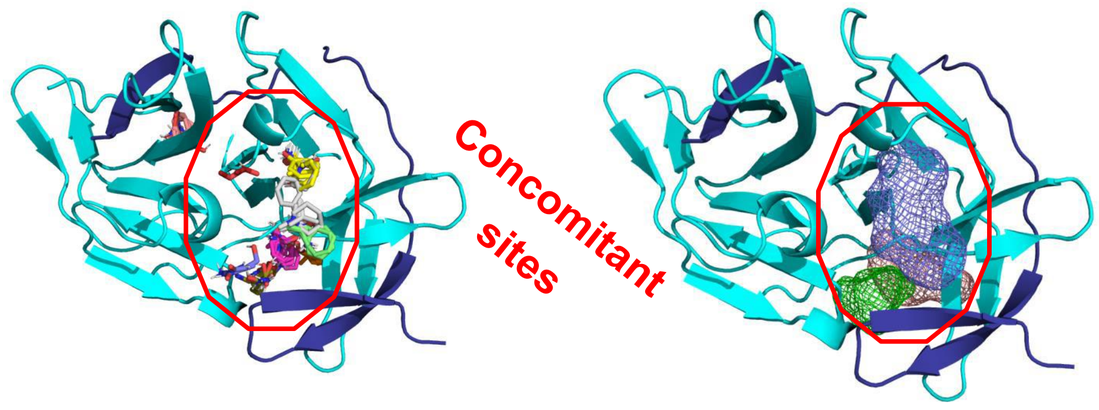


Figure 3. Molecular dynamics: CPD stability & target aminoacids

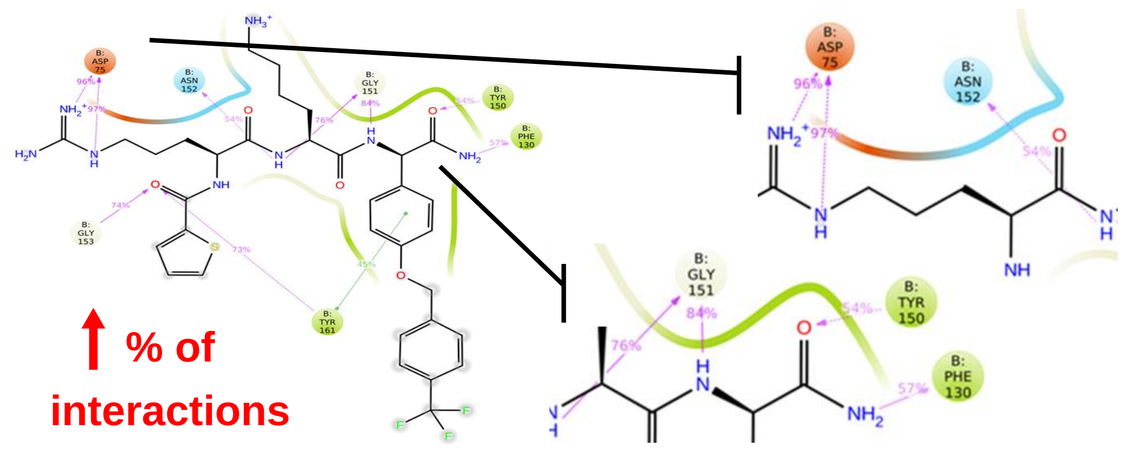


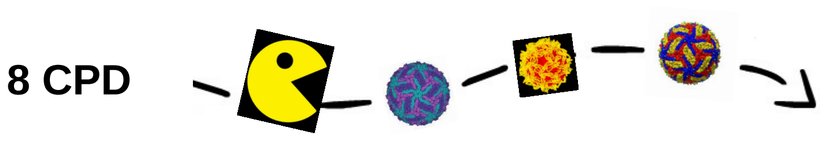
Table 1. Antiviral activity of CPD against ZIKV, YFV & DENV

Cells	Vero	ZIKV MOI 0.1		YFV MOI 0.1		BHK-21	DENV-2 MOI 0.1		DENV-3 MOI 0.1	
CPD	CC_{50} (µM)	EC_{50} (µM)	SI	EC_{50} (µM)	SI	CC_{50} (µM)	EC_{50} (µM)	SI	EC_{50} (µM)	SI
156	141.74 ± 4.6	NA	-	NA	-	106.7 ± 7.8	NA	-	37.5 ± 0.8	2.85
136	16.87 ± 1.49	12 ± 0.4	1.42	4.79 ± 0.2	3.52	9.5 ± 0.6	4.2 ± 0.14	2.26	NA	
128	47.59 ± 2.9	NA	-	24.2 ± 1.2	1.97	30 ± 4.1	9.5 ± 0.12	3.16		
140	21.38 ± 1.07	NA	-	6.4 ± 0.12	3.36	11.1 ± 0.3	NA			
158	17.3 ± 1.67	NA	-	4.6 ± 0.13	3.74	11.3 ± 0.1	NA			-
Ribavirin	>100	4.1 ± 0.3	>24.39	40.9 ± 5.3	>2.44	25.1 ± 0.2	NA		NA	-

NA: Not Active; Selectivity Index (SI) = CC_{50}/EC_{50}; CPD: Compounds; MOI: Multiplicity of infection = virus/cell

5/8 were active

Figure 4. Enzymatic inhibition assay



3/8 inhibited ZIKV NS3pro

IC_{50} from 28 to 69 µM

Conclusion

HQSAR showed the importance of lysine and arginine-like fragments and a VS of 7,600,000 CPD identified 8 potential inhibitors to $NS3_{PRO}$..

Five CPD (5/8) were active against ZIKV, YFV or DENV-2!

One CPD (136) was active against all three viruses and it reduced 1.0 to 1.5 log_{10} of all viruses titer in plaque reduction assays.

CPD 136 has a potential multiflavivirus activity!

ZIKV $NS3_{PRO}$ inhibition assays showed three (3/8) active compounds. CPD 136 is from a class of drugs such as CPD 140 and 158.

CPD 136 is a confirmed inhibitor and potential drug candidate!

SUPPORT: CAPES FAPEMIG DECIT Ministério da Saúde CNPq