DISCOVERY OF MULTITARGET ANTIVIRALS ACTING ON DENGUE VIRUS NS5-NS3 INTERACTION AND THE HOST Src/Fyn KINASES[1]

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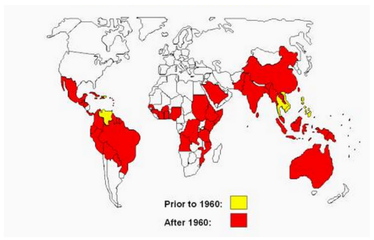
Background

Dengue Fever is the most widespread arthropod-borne disease in the world and accounts for more than 50-100 million patients per year, 99% of all reported cases of viral hemorrhagic fever and around 20.000 deaths. The causative agent is the Dengue Virus (DENV), transmitted through the bite of mosquitoes. WHO has classified Dengue as a major international public health concern. No drugs or vaccines are currently available for the treatment of this disease[2,3]

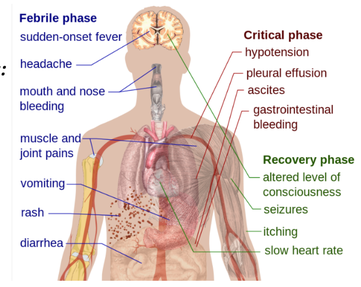
The vector:

Aedes aegypti

Worldwide diffusion:

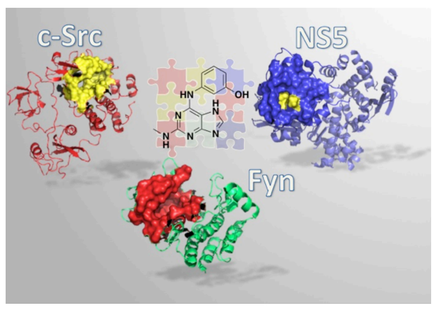


Symptoms:



Aim of the Work

Our aim is the development of small-molecule DENV inhibitors **TARGETING** both a crucial **VIRAL PROTEIN**-protein interaction (NS5-NS3) and an essential **HOST** cell **FACTOR** (Src kinases), to minimize the possibilities of drug-resistance.



c-Src INHIBITORS LIBRARY **3K Cpds**

↓ *VS on the allosteric site of DENV NS5*

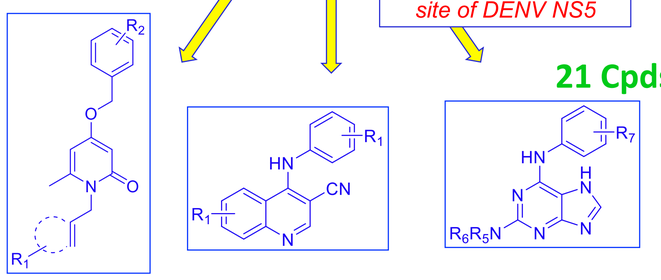
3 SCAFFOLDS selected

Virtual Library generation **10K Cpds**

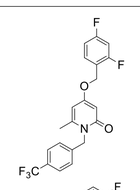
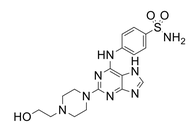
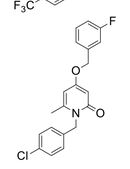
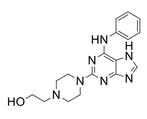
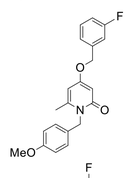
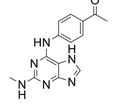
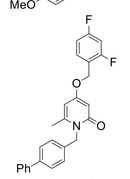
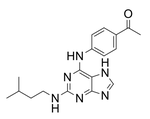
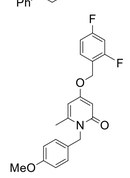
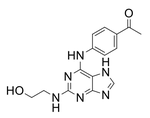
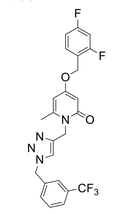
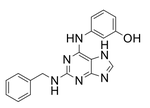
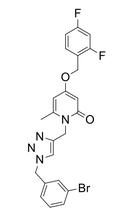
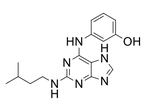
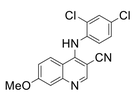
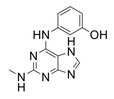
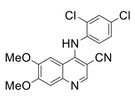
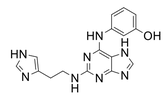
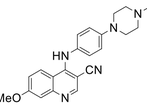
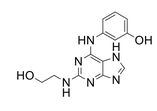
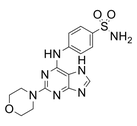
VS on the allosteric site of DENV NS5

21 Cpds

Starting from known c-Src inhibitors, a virtual screening (VS) on the allosteric pocket of DENV NS5 let us to identify molecules that could inhibit the NS3-NS5 protein-protein interaction.[4] Three cheap-to-produce scaffolds were selected and used as input to generate a virtual library of 10K analogues. A second VS allowed to select the best ranking compounds for chemical synthesis and biological studies.



Compounds and Cellular Assays

Cpds	Structure	EC_{50} (uM)	CC_{50} (uM)	Cpds	Structure	EC_{50} (uM)	CC_{50} (uM)
4a		<4.8	<4.8	**14b**		NA	437
4b		<6.4	<6.4	**14c**		NA	474
4c		<6.4	<6.4	**14d**		42 28 (>4)[a]	>177
4d		NA	10	**14e**		59 (1)	85
4e		<4.6	<4.6	**14f**		72 (2)	160
7a		NA	40	**14g**		69 (>2)	>150
7b		NA	36	**14h**		20 7.7 (8)	151 19
11a		NA	19	**14i**		5.3 6.6 (32)	168 30
11b		<5.7	<5.7	**14l**		10 6.0 (>15)	>149
11c		NA	7.9	**14m**		7.4 0.7 (>24)	>175
14a		NA	373	Ribavirine		42 4 (>10)	409

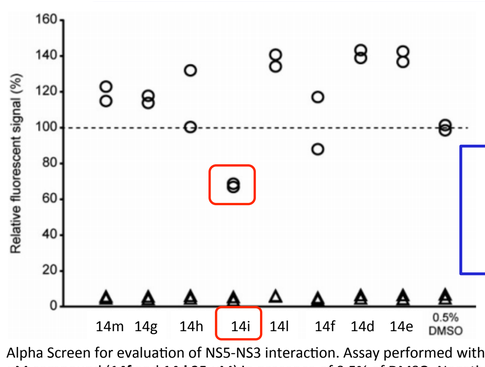
()[a]: Selectivity Index, must be >10 for an antiviral compound

Kinase Inhibition Assay

Cpd	c-Src (ID $_{50}$ uM)[a]	Fyn (ID $_{50}$ uM)[a]
4a	40.0	NA[b]
4c	NA	NA
7a	NA	NA
11a	24.4 (0.2)[c]	2.0
11b	0.62 (0.03)[c]	0.22
11c	NA	NA
14b	7.4 (0.26)[d]	4.8
14c	4.2 (3.14)[d]	4.2
14a	0.9 (0.02)[d]	0.5
14g	3.7	4.1
14h	1.7	1.7
14i	4.9	3.6
14l	1.1	0.7
14m	2.6	2.1

[a]mean of at least two experiments; [b]NA= inhibition at 100 µM; [c]see ref 5;[d]see ref 6

NS5-NS3 Interaction Assay

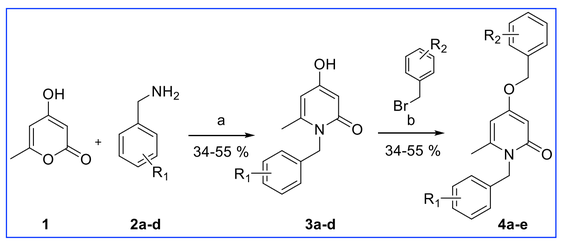


14i inihibits NS3-NS5 interaction

Alpha Screen for evaluation of NS5-NS3 interaction. Assay performed with 25 nM protein and 50 µM compound (**14f** and **14d** 25 µM) in presence of 0.5% of DMSO. Negative control with HF-DHFR and GB-NS5 in the presence of respective compounds (triangles). Results are percentage or luminescent signal obtained by positive control (0.5 % DMSO, 100 % dashed line)

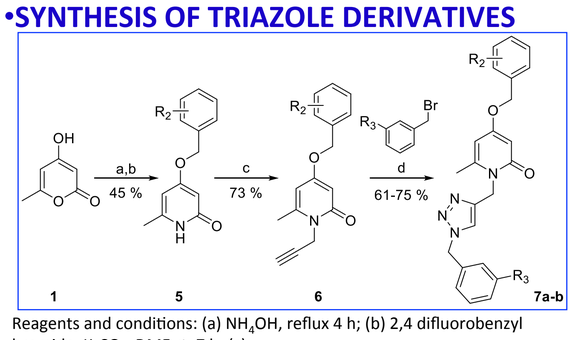
Chemistry

•SYNTHESIS OF 2-PYRIDONES DERIVATIVES



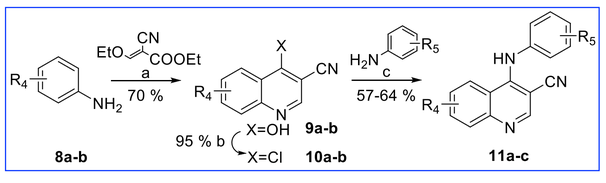
Reagents and conditions: (a) H_2O, reflux 2-7 h; (b) K_2CO_3,DMF, rt, 2 h

•SYNTHESIS OF TRIAZOLE DERIVATIVES



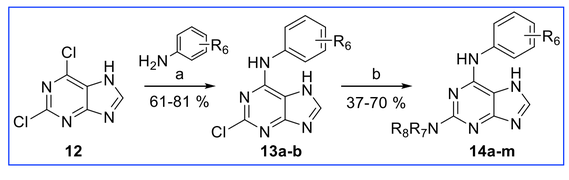
Reagents and conditions: (a) NH_4OH, reflux 4 h; (b) 2,4 difluorobenzyl bromide, K_2CO_3, DMF,rt, 7 h; (c)

•SYNTHESIS OF QUINOLINE DERIVATIVES



Reagents and conditions: (a) (i) neat, µW, 120 °C, 5 min; (ii) $(Ph)_2O$, µW, 230 °C, 7 min; (b) $POCl_3$, reflux, 2 h; (c) NaH, DMF, reflux, 2 h

•SYNTHESIS OF PURINE DERIVATIVES



Reagents and conditions: (a) *n*-BuOH, NEt_3,µW, 70°C, 10 min; (b) method A (for **14a-m**) NR_5R_6, *n*-BuOH, µW, 170 °C, 10 min then 150 °C, 10 min; method B (for **14l**) *n*-BuOH, NEt_3, µW, 130°C, 10 min

Conclusion

- A cheap **FIRST-IN-CLASS** inhibitor (**16i**) of Dengue virus replication has been identified.
- Compound **16i** proved to inhibit NS3-NS5 replication, c-Src/Fyn kinases and is 10 times more potent than ribavirin in cell-based assays.

References

1) Vincetti, P. et al. *J. Med. Chem.* **2015**, *58*(12), 4964-4975; 2) Stevens A.J. et al. *J. Med. Chem*. **2009**, *52(24)*, 7911-7926; 3) Lim S.P. et al. *Antivir. Res*. **2013**, *100*, 500-519; 4) Zou G. et al. *J. Bio.l Chem*. **2011**, *286(16)*, 14362-14372; 5) Boschelli, D. H. et al. *J. Med. Chem.* **2001**, *44*, 822-833; 6) Huang, H. et al. *Bioorg. Med. Chem.* **2010**, *18*, 4615-4624