Fundação Oswaldo Cruz – Fiocruz – Rio de Janeiro, Brazil

Instituto Nacional de Infectologia Evandro Chagas – INI

Chloroquine analogs, synthesized by thermal and ultrasonic means, are endowed with anti-Zika virus activity

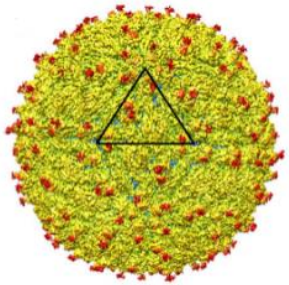
Giselle Barbosa-Lima, PhD

giselle.barbosa@ini.fiocruz.br

Washington DC

February 2017

Introduction



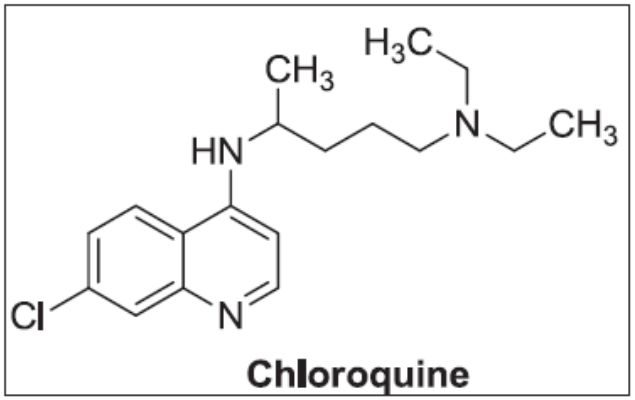
Sirohi et al. (2016) *Science*

Zika virus

- Zika virus (ZIKV) was recently associated with a dramatic increase in neurological disorders;

- Effective antiviral drugs able to inhibit ZIKV replication are necessary;

- The quinoline-containing antimarial drug, chloroquine, showed anti-ZIKV potency around 10 μM – at least 10-times higher than that observed for compounds with antiviral activity;



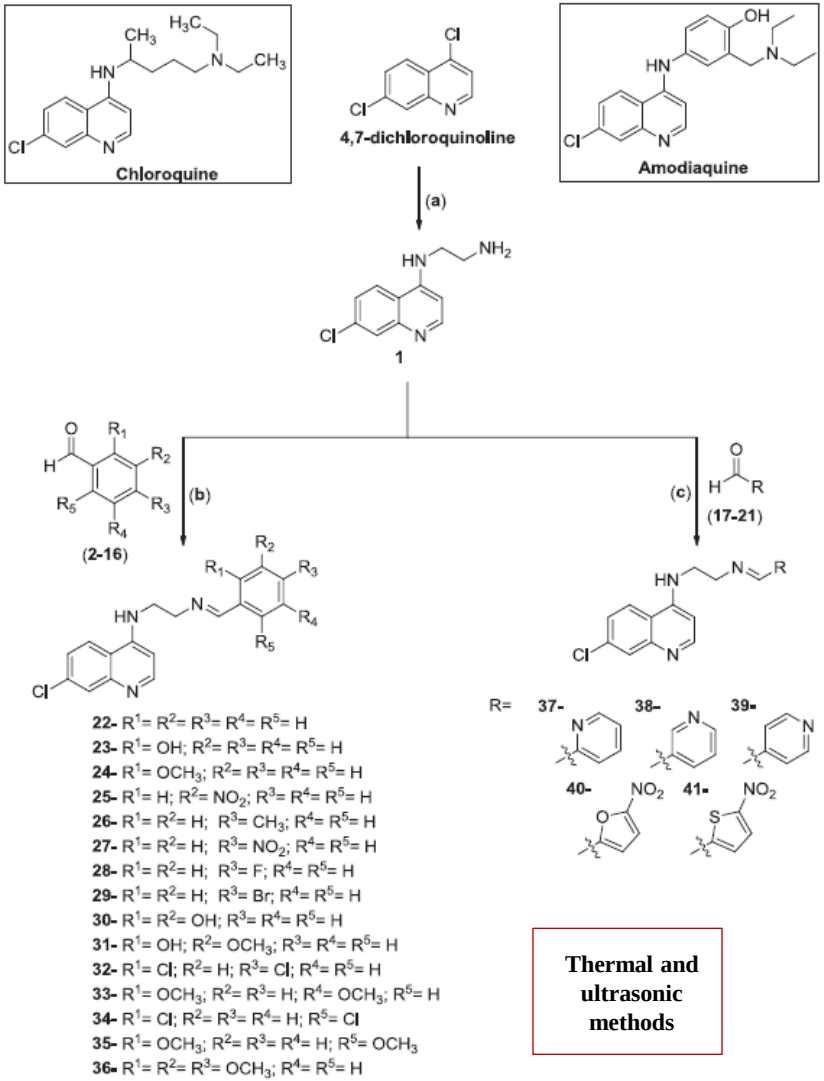
Chloroquine

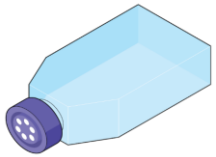
- While this information indicates that the chloroquine chemical structure is promising against ZIKV, medicinal chemistry driven approaches could lead to derivatives with improved potencies in anti-ZIKV therapies.

Aim

To evaluate the ability of chloroquine analogs to inhibit ZIKV replication

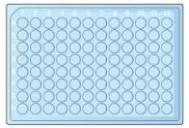
Experimental design





Vero cells

Treatment: 10 µM of compounds 1, 22-41 and chloroquine



ZIKV infection

ZIKV RNA quantitation RT-qPCR

Best hits (inhibition of ~≥75% of ZIKV replication)

- **Cytotoxicity assay (CC_{50})**
- **Antiviral assay (EC_{50})**

Scheme 1 Synthesis of N-(2-(arylmethylimino)ethyl)-7-chloroquinolin-4-amine derivative

Barbosa-Lima et al. (2017) *Eur J Med Chem*

Results

Chloroquine analogs inhibit ZIKV replication

Entry	ZIKV RNA inhibition at 10 μM (%)	Ultrasound Yied %/time (s)	Thermal Yied %/time (min)
1	43 ± 8.0	–	–
22	76 ± 1.8	52/120	58/60
23	57 ± 5.1	74/30	69/30
24	70 ± 2.7	69/180	76/60
25	59 ± 5.6	80/60	80/60
26	66 ± 8.1	76/60	74/30
27	82 ± 3.8	80/60	81/60
28	75 ± 3.2	64/120	59/180
29	52 ± 5.2	83/60	80/30
30	NE	88/30	65/30
31	74 ± 5.1	74/60	78/30
32	NE	74/45	71/30
33	51 ± 1.3	59/30	68/30
34	NE	77/30	73/30
35	75 ± 3.2	70/30	73/30
36	41 ± 3.1	60/30	74/30
37	70 ± 2.3	64/120	53/60
38	80 ± 6.7	40/120	47/30
39	74 ± 4.2	50/120	52/30
40	72 ± 5.3	49/30	60/30
41	78 ± 3.5	77/30	70/60
Chloroquine	47 ± 5.3	–	–

Table 1 Comparison of classical and ultrasonic methods for the preparation of the N-(2-(arylmethylimino)ethyl)-7-chloroquinolin-4-amine derivatives and the inhibition of ZIKV replication. NE – Non evaluated. These substances did not have their antiviral activity evaluated because they were cytotoxic, reducing the cell viability in more than 80 % at 10 μM.

Adapted from Barbosa-Lima et al. (2017) *Eur J Med Chem*

Results

Chloroquine analogs are more potent than chloroquine to inhibit ZIKV activity

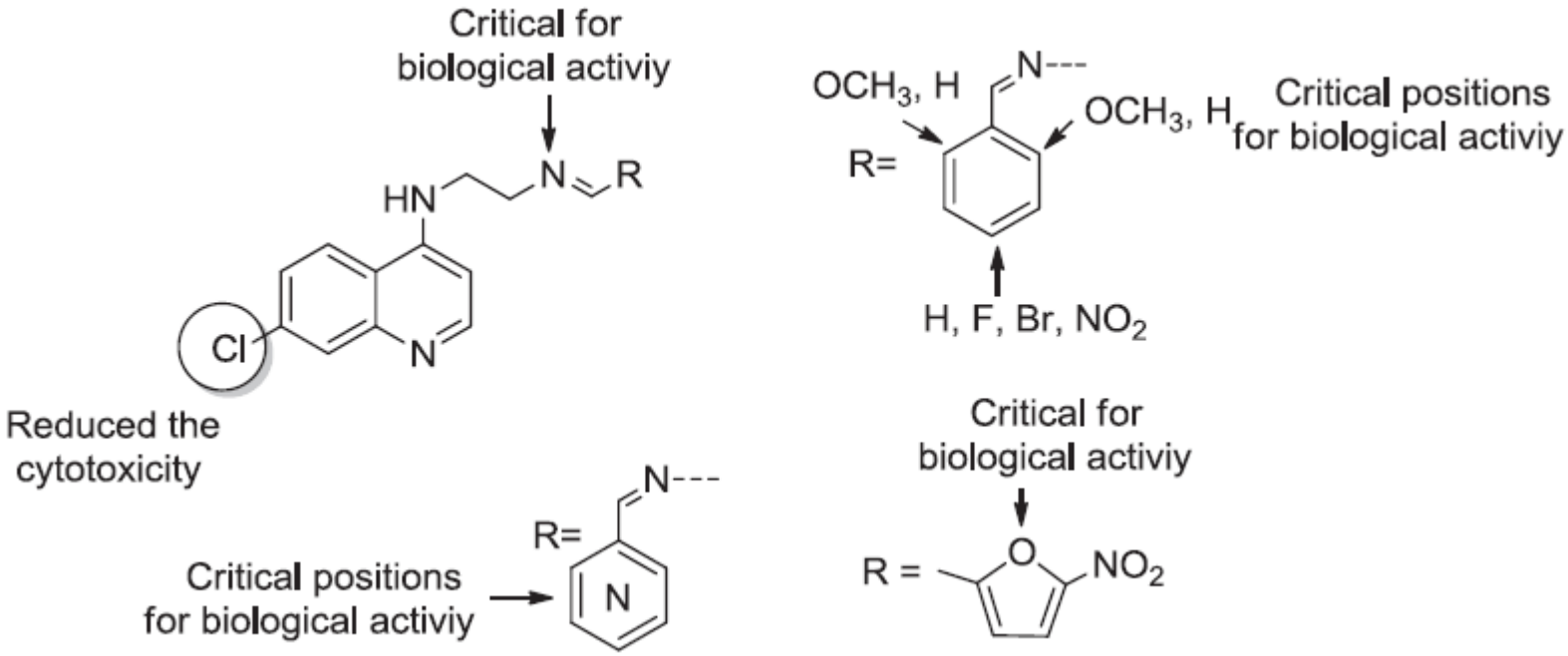
Compound	EC_{50}	CC_{50}	SI[a]	*c*Log*P*[b]
22	1.2 ± 0.05	489 ± 16	407	4.14
27	1.5 ± 0.03	512 ± 27	341	4.10
28	1.5 ± 0.08	534 ± 42	356	4.31
31	1.6 ± 0.02	487 ± 41	304	3.38
35	1.5 ± 0.08	458 ± 36	305	4.16
38	3.4 ± 0.08	578 ± 26	170	2.91
39	1.5 ± 0.09	571 ± 52	380	2.91
40	0.8 ± 0.07	412 ± 26	515	3.48
41	1.5 ± 0.03	478 ± 34	318	4.13
Chloroquine	12 ± 3.2	412 ± 24	34	5.00

Table 2 Potency, cytotoxicity and lipophilicity of the most active quinoline derivatives against ZIKV replication. [a]SI, selective index is determined by the ratio between CC_{50} and EC_{50} values. [b]Calculated using www.molinspiration.com

Barbosa-Lima et al. (2017) *Eur J Med Chem*

Results

Structure-activity relationship analysis indicated that different factors are critical for anti-ZIKV activity

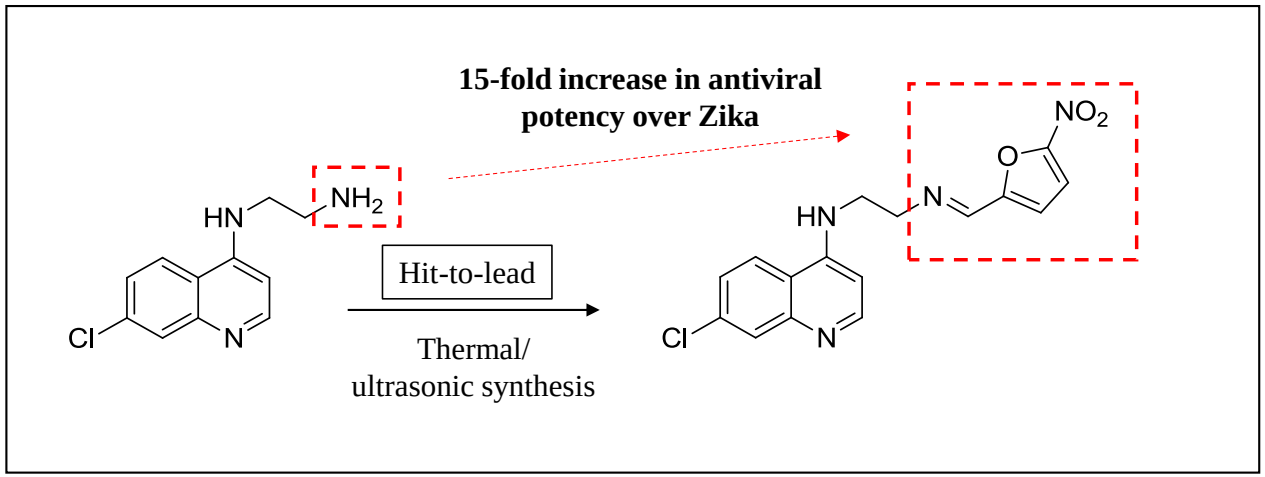


Scheme 2 Critical factors for the biological activity: number, the positions and the types of substituents.

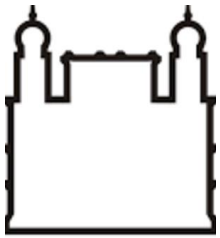
Barbosa-Lima et al. (2017) *Eur J Med Chem*

Conclusion

- We report the synthesis of a series of twenty chloroquine analogs obtained by thermal and ultrasonic means;
- Our study reveal the anti-ZIKV activity of these derivatives and also the critical factors for biological activity;
- Finally, our results represent an improvement of chloroquine chemical structure towards the development of future anti-ZIKV therapies.



Thank you!



Thiago Moreno
Patrícia Bozza
Caroline Freitas
Andressa Marttorelli

José Cerbino
Yasmine Rangel
Alexandre Vizzoni

Marcus Nora

Ligia Pinto