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Inhibition of zika virus infection by fused tricyclic derivatives of 1,2,4,5-tetrahydroimidazo[1,5-a]quinolin-3(3aH)-one

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Abstract

Zika virus (ZIKV) infection represents a significant threat to the global health system, and the search for efficient antivirals to ZIKV remains necessary and urgent. In this study, we extended the exploration of our previously discovered scaffold of 1*H*-pyrrolo[1,2-*c*]imidazol-1-one and revealed that two *trans* isomers of compounds **2** and **7** and one mixture with major *trans* isomer of compound **3** as novel tetrahydroquinoline-fused imidazolone derivatives are active against ZIKV infection but they are not virucidal. Western Blot and ELISA analyses of ZIKV NS5 and NS1 further demonstrate that compounds of (±)-**2**, (±)-**3** and (±)-**7** act as effective agents against ZIKV infection. We show that the N10's basicity is not the basic requirement for these compounds' antiviral activity in the current work. Importantly, tuning of some pharmacophores including substituents at arene can generate promising candidates for anti-ZIKV agents.

Graphical Abstract

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Appendix A. Supplementary data

Supplementary data-1 of chemistry and biology information in Word file and Supplementary data-2 of virucidal test protocol in Excel file to this article can be found online at <https://doi.org>

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Keywords

arboviruses; Zika virus; antiviral; 1*H*-pyrrolo[1,2-*c*]imidazol-1-one; tetrahydroquinoline-fused imidazolone derivatives

The major arboviruses, primarily spreading through arthropod vectors (mainly ticks and mosquitoes), are flaviviruses such as Zika virus (ZIKV), dengue virus (DENV), West Nile virus (WNV) and Japanese encephalitis virus (JEV), and alphaviruses such as chikungunya virus (CHIKV). These viruses have a monopartite, linear, positive sense single-stranded RNA genome, which encodes a single polyprotein with multiple transmembrane domains that is cleaved, by both host and viral proteases, into structural and non-structural proteins (NS). ZIKV was first isolated from a febrile sentinel rhesus monkey in Uganda in 1947¹ and the first confirmed human infection was reported in Uganda in 1962–63.² Due to clinical similarity, geographic overlap and serological cross-reactivity with closely related viruses, ZIKV infection was not only originally understood to be a mild self-resolving infection but also frequently misdiagnosed with dengue and chikungunya infection.^{3–6} However, it is now recognized that ZIKV can cause significant neurological defects in both neonates as pediatric microcephaly^{7,8} and adults as Guillain–Barré syndrome,⁹ which are different characteristics from other flaviviruses. Since the last major epidemic in the Americas in 2016, great efforts have been conducted in research of prevention and therapy development, but currently there is still no approved vaccine or specific antiviral against ZIKV.^{10–13} Clinical treatment of infected patients consists of only supportive care, and the best way to prevent ZIKV infection is to avoid mosquito bites. On the other hand, increased air travel means that it is more likely that a virus could be imported from one country into other countries in the tropical and subtropical regions,¹⁴ which is capable of establishing imported and local transmission.^{15–17} Thus, the endemic viruses are of significant concern and mosquito transmitted diseases remain an ongoing global threat. The search for efficient antivirals to ZIKV is of great medical significance.

In our previous studies of antiviral drug discovery, we demonstrated that certain derivatives of 1*H*-pyrrolo[1,2-*c*]imidazol-1-one, the fused bicyclic of pyrrolidine and 4-imidazolidinone, are potent NS2B-NS3 protease inhibitors of type II dengue virus (DENV-II). Among the compounds, compound **1** was discovered to be active against wild type DENV-II infection with EC₅₀ values of 39.4 μM.¹⁸ In order to improve the efficacy and to discover efficient antivirals targeting ZIKV, we extended our exploration of structure-activity relationship (SAR) by functionalization of 1*H*-pyrrolo[1,2-*c*]imidazol-1-one derivatives by designing, synthesizing and screening a series of tetrahydroquinoline-fused imidazolone compounds against ZIKV infection.

We sought to functionalize 1*H*-pyrrolo[1,2-*c*]imidazol-1-one scaffold structure by enlargement of the ring size to a 6-membered ring and also by introduction of fused phenyl group based on the importance of quinoline moiety against infectious diseases.^{19–22} It is also predicted that phenyl reduces the electron density of the N10 to lessen the propensity to be protonation, resulting in more stability of the acetal. As the low electron density of the N10 makes the loss of the N10's basicity, we can investigate the necessity of the basicity of the

nitrogen in the scaffold for the antiviral activity. Meanwhile, analogs of phenyl hydrazine were investigated and substituent changes were made.

The synthetic procedure and ^1H NMR data of C1-H and C3a-H are illustrated in Scheme 1. Unlike hydrogenation of heterocycle ring of quinaldic acid catalyzed by expensive PtO_2 ,^{23,24} convenient reduction of methyl quinoline-2-carboxylate by NaBH_4 in acetic acid gave $(\pm)$ -1,2,3,4-tetrahydroquinoline $((\pm)$ -**8**) as racemic mixture. Then, protection of amine group by Cbz chloride afforded *N*-Cbz protected intermediate of $(\pm)$ -1-benzyl 2-methyl 3,4-dihydroquinoline-1,2(*2H*)-dicarboxylate $((\pm)$ -**9**). After hydrolysis of the ester $((\pm)$ -**9**) into the acid $((\pm)$ -**10**), phenyl hydrazine was introduced to form acyl hydrazine $((\pm)$ -**11**). To avoid migration of benzyl group under acid-catalysis (data not shown here),²⁵ deprotection of Cbz group from $(\pm)$ -**11** was conducted by 10% Pd/C to yield the corresponding key intermediate $(\pm)$ -**12**. Finally, diverse fused tricyclic ring derivatives of $(\pm)$ -1-(phenyl)-2-(phenylamino)-1,2,4,5-tetrahydroimidazo[1,5-*a*]-quinolin-3(3*aH*)-one **2**, **3**, **4** and **7** were synthesized by the catalysis of triethyl amine (TEA) according to our previously reported method.¹⁸ Furthermore, desilylation of $(\pm)$ -**2** and $(\pm)$ -**4** afforded corresponding $(\pm)$ -**5** and $(\pm)$ -**6**, respectively. Similar to the reported results¹⁸ of 1*H*-pyrrolo[1,2-*c*]imidazol-1-one derivatives, the electron-donating substitution at 3' and 4' of C1-Ar makes *cis* isomers of $(\pm)$ -**2**, and $(\pm)$ -**4** to $(\pm)$ -**6** inseparable from its *trans* isomer. As none of pure *trans* and *cis* isomers of $(\pm)$ -**3** were isolated by silica gel chromatography, compound $(\pm)$ -**3** used for anti-ZIKV assay in this study is the mixture of 5:1 *trans* and *cis* isomers (Fig. S1). Meanwhile, different from the separable chloro counterpart of 1*H*-pyrrolo[1,2-*c*]imidazol-1-one derivative,¹⁸ no pure *cis* isomer of $(\pm)$ -**7** (Fig. S2) was afforded but pure *trans* isomer of $(\pm)$ -**7** was obtained.

In order to analyze the relative configuration of these compounds, the single crystal of the major isomer of compound $(\pm)$ -**7** was cropped and its crystal structure (CCDC 1915004) of the relative *trans* configuration of C1-H and C3a-H was determined as shown in Fig. 2. Since the electron-donating substitution and weak electron-withdrawing group (EWG) of chloro group at C1-Ar generates less quantity of minor *cis* isomer and two isomers are quite difficult to isolate by silica gel chromatography, pure *trans* isomer and the mixture of *trans* and *cis* isomers were used for the structure analysis (Supplementary data-1, Fig. S2). Compared with ^1H NMR data (Scheme 1) of pure $(\pm)$ -**7** *trans* isomer with the mixture of $(\pm)$ -**7** *trans* and *cis* isomers, the chemical shifts (δ) of C1-H and C3a-H of $(\pm)$ -**7** *trans* isomer are recognized 5.81 and 4.54 ppm while those of $(\pm)$ -**7** *cis* isomer are 6.12 and 4.25 ppm (Supplementary data-1, Fig. S2). Based on the current and previous facts that the chemical shift of C1-H of *cis* isomer is higher than that of *trans* isomer but the chemical shift of C3a-H of *cis* isomer is lower than that of *trans* isomer, it is concluded from ^1H NMR data of the mixture of *trans* and *cis* isomers of $(\pm)$ -**3** that the chemical shifts (δ) of C1-H and C3a-H of $(\pm)$ -**3** *trans* isomer is 5.62 and 4.44 ppm while those of $(\pm)$ -**3** *cis* isomer is 5.92 and 4.18 ppm (Supplementary data-1, Fig. S1). The chemical shift (δ) difference of C3-H between two isomers is around 0.6 ppm in the reported results of 1*H*-pyrrolo[1,2-*c*]imidazol-1-one derivatives; meanwhile, the chemical shift (δ) difference of C1-H between two isomers is about 0.3 ppm in this study. Even though the chemical shift (δ) difference of C7a-H between two isomers is not obvious in the reported results of 1*H*-pyrrolo[1,2-*c*]imidazol-1-one

derivatives, the chemical shift (δ) difference of C3 α -H between two isomers is significantly 0.2 to 0.3 ppm for compounds ($\pm$)-**3** and ($\pm$)-**7** in this study. ^1H NMR data of C1-H and C3 α -H of compounds of ($\pm$)-**2** to ($\pm$)-**7** are in Scheme 1 and the stereochemistry of the major isomers of ($\pm$)-**2** to ($\pm$)-**6** was designated as *trans* configuration as ($\pm$)-**7** *trans* isomer even though no pure *cis* isomer was got for the structure analysis in this study. The weak electron-withdrawing group (EWG) property of chloro at *para*-position makes higher chemical shifts of C1-H and C3 α -H of ($\pm$)-**7** compared with ($\pm$)-**2** to ($\pm$)-**6** bearing two electron-donating groups (EDGs) of hydroxyl, methoxy and *t*-butyldimethylsilyloxy (OTBS) at 3'- and 4'-positions.

For the compounds of ($\pm$)-**2** to ($\pm$)-**7**, we investigated their inhibitory activity against ZIKV infection in a viral titer assay (in Vero cells for 48 h)²⁶ (Supplementary data-1). We measured ZIKV production in compound-treated human glioblastoma cells (SNB-19 line) (incubation for 24 h) infected by PRVABC59 strain at multiplicity of infection (MOI) of 1 as illustrated in Supplementary data. Niclosamide, one of the previously identified active compounds against ZIKV infection,^{26,27} was used as a positive control in each antiviral assay. We conducted initial antiviral screening using one concentration in triple experiments and then advanced the active compounds from initial screening to generate EC₅₀ values with at least 7 gradient concentrations. The cytotoxicity (Table 1) of each compound in this study was tested in SNB-19 cells (24 h treatment) and Vero cells (48 h treatment) for the duration corresponding to the incubation time in anti-ZIKV screening. Among the compounds reported in this study, three compounds of ($\pm$)-**2**, ($\pm$)-**3** and ($\pm$)-**7** were active against ZIKV infection as shown in Table 1. In particular, compound ($\pm$)-**2** exhibits low micromolar EC₅₀ value (1.56 μM) with SIs of higher than 64.1 in SNB-19 cell line and 17.3 in Vero cell line. Compound ($\pm$)-**6** with two hydroxyl groups at 3'- and 4'-positions and compound ($\pm$)-**5** with 3'-methoxy group and 4'-hydroxyl group are inactive; in contrast, compound **3** with 3'-hydroxyl group and 4'-methoxy group is active against ZIKV infection with 7.40 μM of EC₅₀ value and SI values of 13.5 and 8.0 in SNB-19 and Vero cell lines, respectively. As *trans* isomer of ($\pm$)-**3** is the major isomer, the inhibitory activity of ($\pm$)-**3** may be attributed to its *trans* isomer; however, the precise EC₅₀ value of *trans* isomer of ($\pm$)-**3** against ZIKV can only be determined after the pure *trans* isomer of ($\pm$)-**3** is obtained. Silylation of 4'-hydroxyl group of compound ($\pm$)-**5** makes compound ($\pm$)-**2** exhibit very potent anti-ZIKV activity, which is possibly attributed to the introduction of TBS group consistent with a previously reported observation.²⁸ Whereas, bis-silylation of 3',4'-hydroxyl groups of compound ($\pm$)-**6** is not able to enhance the anti-ZIKV activity of compound ($\pm$)-**4**. Interestingly, compounds of ($\pm$)-**4**, and ($\pm$)-**5** are almost not cytotoxic to SNB-19 and Vero cell lines. 4'-Chloride compound ($\pm$)-**7** possesses good anti-ZIKV activity, with an EC₅₀ of 3.40 μM and SIs are 15.5 in SNB-19 cell line and 12.9 in Vero cell line. Some images from the titer plates used to calculate the EC₅₀ values of the compounds are shown in Fig. S3. With these anti-ZIKV results, we further conducted the virucidal tests (Supplementary data-2)²⁹⁻³¹ of these active compounds of ($\pm$)-**2**, ($\pm$)-**3** and ($\pm$)-**7** at 4 concentrations around their EC₅₀ values, and virucidal test results showed that they are not virucidal, indicating that anti-ZIKV activity of this series of compounds is not due to virucidal (neutralising) effect. As a whole, these results revealed that this structural scaffold has a valuable pharmacological feature and it is possible that the reduced N10's basicity is not the key for the antiviral activity, and

importantly, tuning of some pharmacophore/s including substituents can produce effective antiviral drug candidates against ZIKV infection.

Anti-ZIKV activity of compounds ($\pm$)-**2**, ($\pm$)-**3** and ($\pm$)-**7** were confirmed with additional assays including Western Blot (Fig. 3) and ELISA (enzyme-linked immunosorbent assay) (Fig. 4). As shown in Fig. 3, Western Blot experiments indicate that expressions of both ZIKV NS5 and NS1 were effectively reduced by treatment of 5 μ M of ($\pm$)-**2**, ($\pm$)-**3** and ($\pm$)-**7** as 5 μ M of the positive control Niclosamide compared to vehicle control (ZIKV DMSO). Meanwhile, ELISA experiments (Fig. 4) demonstrate that amount of ZIKV NS1 in the supernatant of infected cell culture was also decreased by 10 μ M of Niclosamide, ($\pm$)-**2**, ($\pm$)-**3**, and ($\pm$)-**7** relative to vehicle control (ZIKV DMSO). It should be noted that ZIKV NS1 expressions detected by ELISA were more significantly inhibited by addition of ($\pm$)-**2** at 24 hrs than at 16 hrs after cells were infected. As a whole, compounds of ($\pm$)-**2**, ($\pm$)-**3** and ($\pm$)-**7** act as effective agents against ZIKV infection.

In summary, we designed and synthesized several tetrahydroquinoline-fused imidazolone derivatives based on our previously discovered lead against DENV¹⁸ and discovered that *trans* isomers of compounds ($\pm$)-**2**, ($\pm$)-**3** (5/1 *trans/cis*) and ($\pm$)-**7**, as major isomers, exhibit good antiviral activity against ZIKV infection. Western Blot experiments for ZIKV NS1 and NS5 and ELISA experiments for ZIKV NS1 also demonstrated that compounds of ($\pm$)-**2**, ($\pm$)-**3** and ($\pm$)-**7** possess anti-ZIKV activity. However, the anti-ZIKV activity was not related to virucidal effect. It seems that the N10's basicity is not the basic requirement for the current compounds' antiviral activity in this work but the conclusion needs further SAR study. Our results show that optimal substitution at arene and its modification of C1-phenyl will benefit the antiviral activity. This study validates our concept of developing novel tetrahydroquinoline-fused imidazolone-based therapeutics as antiviral (specifically, anti-ZIKV) drugs. We are planning to test these compounds' activity against DENV and other arboviruses in the future.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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References

1. Dick GW, Kitchen SF, Haddow AJ. Zika virus. I. Isolations and serological specificity. *Trans R Soc Trop Med Hyg.* 1952;46:509–520. [PubMed: 12995440]
2. Wikan N, Smith DR. Zika virus: a history of newly emerging arbovirus. *Lancet Infect Dis* 2016;16:e119–26. [PubMed: 27282424]
3. Baud D, Gubler DJ, Schaub B, et al. An update on Zika virus infection. *Lancet.* 2015;390:2099–109.
4. Campos GS, Bandeira AC, Sardi SI. Zika virus outbreak, Bahia, Brazil. *Emerg Infect Dis.* 2015;21:1885–1886. [PubMed: 26401719]
5. Cao-Lormeau VM, Musso D. Emerging arboviruses in the Pacific. *Lancet.* 2014;384:1571–1572. [PubMed: 25443481]

6. Cardoso CW, Paploski IA, Kikuti M, et al. Outbreak of exanthematous illness associated with Zika, chikungunya, and dengue viruses, Salvador, Brazil. *Emerg Infect Dis.* 2015;21:2274–2276. [PubMed: 26584464]
7. Mlakar J, Korva M, Tul N, et al. Zika virus associated with microcephaly. *N Engl J Med.* 2016;374:951–958. [PubMed: 26862926]
8. Calvet G, Aguiar RS, Melo ASO, et al. Detection and sequencing of Zika virus from amniotic fluid of fetuses with microcephaly in Brazil: a case study. *Lancet Infect Dis.* 2016;16:653–660. [PubMed: 26897108]
9. Brasil P, Sequeira PC, Freitas AD, et al. Guillain-Barré syndrome associated with Zika virus infection. *Lancet.* 2016;387:1482. [PubMed: 27115821]
10. Kublin JL, Whitney JB. Zika virus research models. *Virus Res.* 2018;254:15–20. [PubMed: 28811170]
11. Richner JM, Diamond MS. Zika virus vaccines: immune response, current status, and future challenges. *Curr Opin Immunol.* 2018;53:130–136. [PubMed: 29753210]
12. Saiz JC, Martín-Acebes MA. The race to find antivirals for zika virus. *Antimicrob Agents Chemother.* 2017;61 pii:e00411–17. [PubMed: 28348160]
13. Wahid B, Ali A, Rafique S, Idrees M. Current status of therapeutic and vaccine approaches against Zika virus. *Eur J Internal Med.* 2017;44:12–18. [PubMed: 28797534]
14. Guzman MG, Halstead SB, Artsob H, et al. 2010. Dengue: a continuing global threat. *Nat Rev Microbiol.* 2010;8:S7–16. [PubMed: 21079655]
15. Barrows NJ, Campos RK, Liao KC, et al. Biochemistry and Molecular Biology of Flaviviruses. *Chem Rev.* 2018;118:4448–4482 and references cited therein. [PubMed: 29652486]
16. Castanha PMS, Nascimento EJM, Braga C, et al. Dengue Virus-Specific Antibodies Enhance Brazilian Zika Virus Infection. *J Infect Dis.* 2017;215:781–785. [PubMed: 28039355]
17. Bardina SV, Bunduc P, Tripathi S, et al. Enhancement of Zika Virus Pathogenesis by Preexisting Antiflavivirus Immunity. *Science.* 2017;356:175–180. [PubMed: 28360135]
18. Weng Z, Shao X, Graf D, et al. Identification of fused bicyclic derivatives of pyrrolidine and imidazolidinone as dengue virus-2 NS2B-NS3 protease inhibitors. *Eur J Med Chem.* 2017;125:751–759. [PubMed: 27721158]
19. Delvecchio R, Higa LM, Pezzuto P, et al. Chloroquine, an Endocytosis Blocking Agent, Inhibits Zika Virus Infection in Different Cell Models. *Viruses.* 2016;8:E322. [PubMed: 27916837]
20. Kumar A, Liang B, Aarthy M, et al. Hydroxychloroquine Inhibits Zika Virus NS2B-NS3 Protease. *ACS Omega.* 2018;3:18132. [PubMed: 30613818]
21. Li F, Li XM, Sheng D, et al. Discovery and preliminary SAR of 14-aryloxy-andrographolide derivatives as antibacterial agents with immunosuppressant activity. *RSC Adv* 2018;8:9440–9456.
22. Han Y, Pham HT, Xu H, Quan Y, Mesplède T. Antimalarial drugs and their metabolites are potent Zika virus inhibitors. *J Med Virol.* 2019;doi: 10.1002/jmv.25440.
23. Nagata R, Tanno N, Kodo T, et al. Tricyclic quinoxalinediones: 5,6-dihydro-1H-pyrrolo-[1,2,3-de] quinoxaline-2,3-diones and 6,7-dihydro-1H,5H-pyrido [1,2,3-de] quinoxaline-2,3-diones as potent antagonists for the glycine binding site of the NMDA receptor. *J Med Chem.* 1994;37:3956–3968. [PubMed: 7966156]
24. Brindisi M, Butini S, Franceschini S, et al. Targeting dopamine D3 and serotonin 5-HT1A and 5-HT2A receptors for developing effective antipsychotics: synthesis, biological characterization, and behavioral studies. *J Med Chem.* 2014;57:9578–9597. [PubMed: 25343529]
25. Liu Z, Xu J, Ling B, et al. Synthesis of Benzyl Phenol from Benzyl Aryl Ether by Polyphosphoric Acid-Catalyzed Benzyl Rearrangement. *Chin J Org Chem.* 2019;39:2515–2524.
26. Lee EM, Titus SA, Xu M, Tang H, Zhang W High-Throughput Zika Viral Titer Assay for Rapid Screening of Antiviral Drugs. *Assay Drug Dev Techn.* 2019;17:128–139.
27. Xu M, Lee EM, Wen Z, et al. Identification of small-molecule inhibitors of Zika virus infection and induced neural cell death via a drug repurposing screen. *Nat Med.* 2016;22:1101–1107. [PubMed: 27571349]

28. Vernekar SKV, Qiu L, Zhang J, et al. 5'-Silylated 3'-1,2,3-triazolyl Thymidine Analogues as Inhibitors of West Nile Virus and Dengue Virus. *J Med Chem.* 2015;58:4016–4628. [PubMed: 25909386]
29. Malik FZA, Allaudin ZN, Loh HS, et al. Antiviral and virucidal activities of *Duabanga grandiflora* leaf extract against Pseudorabies virus in vitro. *BMC Complement Altern Med.* 2016;16:139. [PubMed: 27216794]
30. Chen M, Aoki-Utsubo C, Kameoka M, et al. Broad-spectrum antiviral agents: secreted phospholipase A2 targets viral envelope lipid bilayers derived from the endoplasmic reticulum membrane. *Sci Rep.* 2017;7:15931. [PubMed: 29162867]
31. Faral-Tello P, Mirazo S, Dutra C, et al. Cytotoxic, Virucidal, and Antiviral Activity of South American Plant and Algae Extracts. *The Sci World J.* 2012;2012:174837.

Highlights

Extended exploration of 1*H*-pyrrolo[1,2-*c*]imidazol-1-one scaffold were conducted.

Two tetrahydroquinoline-fused imidazolone derivatives are active against ZIKV.

It seems that N10's basicity is not the key for antiviral activity in this work.

Tuning of some pharmacophores results in promising candidates for anti-Zika agents.

Fig. 1.
Evolution of the previous DENV-II inhibitor (**1**)¹⁸ to the designed ZIKV inhibitors (**2** to **7**).

Fig. 2.
The crystal structure of compound (±)-7 *trans* isomer (CCDC 1915004).

Fig. 3. Expression of viral NS proteins of ZIKV NS5 and NS1 detected by Western Blot analysis. SNB-19 cells were infected by ZIKV for 4 hrs using PRVABC59 strain (MOI = 1) and then treated with vehicle control (0.1% DMSO) of ZIKV DMSO or 5 μ M of Niclosamide, **2**, **3** and **7**, respectively, and after 24 hrs, proteins were extracted and then subjected to Western blot analysis to determine the expression level of ZIKV NS5 and NS1. GAPDH was used as a loading control. A compound not included in this report was loaded in the red arrow-indicated lane, therefore this band was not quantified. The intensity of protein bands were quantitated by ImageJ image analysis software. The fold change value was calculated normalizing NS5/NS1 bands to GAPDH then normalized to the DMSO control, as follows: (compound's NS5 or NS1/compound's GAPDH)/(DMSO control's NS5 or NS1/DMSO control's GAPDH). The error bars show mean $\pm$ SD (p value; *** $<$ 0.0005, **** $<$ 0.0001 for significance respective to ZIKV DMSO control, by one-way ANOVA).

Fig. 4. Quantification of ZIKV NS1 protein detected by ELISA analysis.

SNB-19 cells were infected by ZIKV for 16 hrs (A) or 24 hrs (B), using PRVABC59 strain (MOI = 1), and then treated with vehicle control (0.1% DMSO) of ZIKV DMSO or 10 μ M of Niclosamide, **2**, **3** and **7**, respectively, and after 24 hrs, cell supernatant was subjected to ELISA analysis of ZIKV NS1 to determine the concentration of secreted ZIKV NS1 to the medium of infected and treated cells. The error bars show mean $\pm$ SD, *p* values are indicated in the figure, by one-way ANOVA as compared to the ZIKV control group.

Scheme 1.

Reagents and conditions: (a) AcOH, NaBH₄, 5 °C, 6 h. (b) DCM, CbzCl, NaHCO₃, 30 °C, 9 h. (c) MeOH/H₂O (V/V 3/2), NaOH, 60 °C, 2 h. (d) DCM, phenylhydrazine, IBCF, TEA, 0 °C, 2h. (e) THF, H₂, Pd/C, rt, 6 h. (f) MeCN, N₂ atmosphere, aldehyde 13 ~ 16, TFA, 60 °C, 1.5 h. (g) THF, TBAF, 0 °C, 5 min.

Table 1.

The EC₅₀ (50% effective concentration), CC₅₀ (50% toxic concentration) and SI (selectivity index, CC₅₀/EC₅₀) of these compounds.

Cmpd	EC ₅₀ (μM)	To SNB-19 cell line ^a		To Vero cell line ^b	
		CC ₅₀ (μM)	SI	CC ₅₀ (μM)	SI
(±)- 2	1.56 ± 0.21	> 100	> 64.1	27.00 ± 0.61	17.3
(±)- 3 (<i>trans/cis</i> 5/1)	7.40 ± 0.37	> 100	> 13.5	59.30 ± 0.43	8.0
(±)- 4	NA ^c	> 100	/ ^d	> 100	/
(±)- 5	NA	> 100	/	> 100	/
(±)- 6	NA	94.60 ± 1.0	/	50.40 ± 0.16	/
(±)- 7	3.40 ± 0.38	52.60 ± 1.1	15.5	44.00 ± 1.18	12.9

^aIncubated for 24 h.

^bIncubated for 48 h.

^cNA: not active as EC₅₀ values are higher than 40 μM.

^d/: not available.