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Design, synthesis, optimization and antiviral activity of a class of hybrid dengue virus E protein inhibitors

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ABSTRACT

The β-OG pocket is a cavity in the flavivirus envelope (E) protein that was identified by *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 6986 as a promising site for the design of antiviral agents that interfere with virus entry into the host cell. The availability of the X-ray crystal structure of the dengue virus (DENV) E protein provided an opportunity for in silico drug design efforts to identify candidate inhibitors. The present study was set up to explore whether it is possible to generate a novel class of molecules that are hybrids between two hit compounds that have been reported previously by *ACS. Chem. Biol.* **2008**, *3*, 765 following an in silico screening effort against the DENV E protein. First, a library of twenty hybrid molecules were designed and synthesized to explore the feasibility of this strategy. Antiviral evaluation in a virus-cell-based assay for DENV proved this approach to be successful, after which another twenty-four molecules were produced to further explore and optimize the potency of this novel class of hybrid inhibitors. In the end, a molecule was obtained with an EC_{50} against dengue virus serotype 2 in the low micromolar range (**23**, 1.32 ± 0.41 μM).

Dengue fever is a mosquito-transmitted viral disease that in the past decades has become endemic in most, if not all, tropical and sub-tropical regions around the world. Yearly, an estimated 50 to 200 million infections are caused by dengue virus (DENV),[1] with a fatal outcome in over 2.5% of the cases.[2] Globalization and climate change are drivers that continue to increase the impact of this infectious disease on human health.[3] The pharmaceutical industry is heavily investing in the development of an efficient vaccine, which until now still has failed to fulfill its promise.[4] Several antiviral drugs for the treatment or prevention of DENV infections are in the (pre)clinical stage, but it will take several more years before the first one will reach the clinic. In this latter context, any stage in the viral replication cycle may represent a possible target for the development of such small-molecule inhibitors.[5–7]

In 2003, Modis and colleagues reported the crystal structure of the DENV envelope (E) protein (1OKE).[8] In this structure, a density was observed that could be attributed to an *n*-octyl-β-D-glucoside (β-OG) molecule, a detergent that is commonly used for the production of protein crystals. The cavity in the E protein in which this molecule fits, also designated the β-OG pocket, quickly drew the attention for structure-based drug screening and design efforts, especially because it was shown that fusion of the virus envelope with host cell membranes requires a pronounced conformational change of the E-protein as well as this cavity.[9,10] In this context, it was put forward that freezing the E protein in its pre-fusion state by filling the β-OG pocket with a small molecule would prevent virus fusion and thus would offer a novel and promising strategy for the development of a drug for the treatment or prevention of DENV infections.

In 2008, Zhou and colleagues performed a large-scale in silico screen from which the 23 top-ranking compounds were selected for evaluation in a yellow fever virus (YFV) assay as alternative for DENV.[11] Several compounds showed inhibitory activity, by which they validated this novel drug discovery approach. The purpose of the present study is to explore whether a novel hybrid chemical scaffold with more potent antiviral activity against DENV can be developed based on compounds M02 and D03 that have been reported in the aforementioned study (Fig. 1). Even though these compounds were shown to only have inhibitory activity in the higher micromolar range in the YFV assay (M02: IC_{50} = 51 ± 7 μM and D03: IC_{50} = 31 ± 0.3 μM, respectively),

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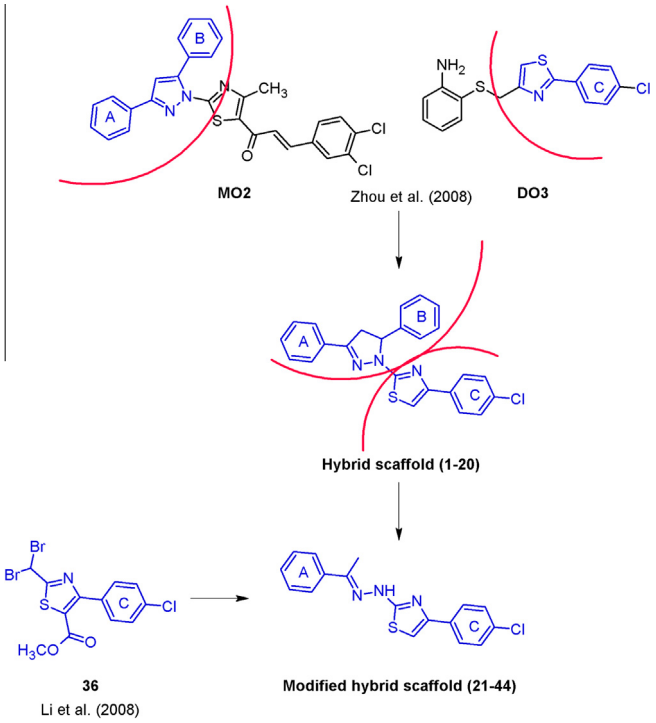


Figure 1. Design of library molecules (**1**–**44**).

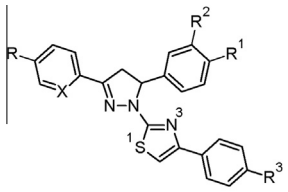
because YFV and DENV have a ~43.8% sequence identity at the amino acid level of the E protein (and even higher for the amino acids that line the β-OG pocket),[12] it was envisaged that compounds with similar or even a more potent inhibitory effect against DENV could be obtained. A similar approach was reported by Ref. 13 which yielded methyl-4-(dibromomethyl)-2-(4-chlorophenyl)
thiazole-5-carboxylate, a compound that later was developed into a metabolically more stable analogue by Ref. 14. Few more reports on the identification of fusion inhibitors through High-Throughput Virtual Screening (HTVS) are available.[12,15–17]

So far, all inhibitors of DENV infection that target the β-OG pocket have not been further developed. However, novel compound series undoubtedly will contribute to a better understanding of this pocket as target for small-molecule inhibitors.

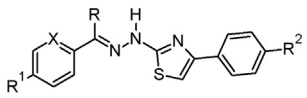
A first series of twenty compounds (**1**–**20**, Table 1) was designed as hybrid molecules by grafting half of the structure of compound M02 onto half of the structure of compound D03 (see Ref. 11) (Fig. 1). The resulting pyrazoline derivatives (**1**–**20**) were synthesized through the reactions outlined in Scheme 1A.[18] The synthesis starts with the preparation of chalcones from appropriate benzaldehydes and acetophenones in the presence of sodium hydroxide (60%) using methanol as solvent at room temperature for a period of 48 h. The resulting chalcones were converted into the respective pyrazolines by the reaction of thiosemicarbazide in the presence of sodium hydroxide under reflux using methanol as solvent for a period of 8–10 h. The final compounds (**1**–**20**) are obtained by the reaction of appropriate pyrazolines with either *p*-chloro phenacyl bromide or *p*-phenyl phenacyl bromide under reflux using ethanol as solvent for a period of 30–45 min.

None of the twenty molecules were able to inhibit the development of virus-induced cytopathic effects (CPE, virus-induced cell death) when compound and virus were added to the cells at the same time. However, when virus and compound were mixed and pre-incubated for 2 h at 37 °C, four compounds (**9**, **13**, **19**, **20**)

Table 1
Structural formulas of compounds **1**–**44**



1-20



21-44

Code	X	R	R^1	R^2	R^3	Code	X	R	R^1	R^2	R^3
1	CH	–H	–H	–H	–Cl	**23**	CH	$–CH_3$	–OH	–Cl	–
2	CH	–Cl	–H	–H	–Cl	**24**	CH	$–CH_3$	$–OCH_3$	–Cl	–
3	CH	–OH	–H	–H	–Cl	**25**	CH	$–CH_3$	$–NO_2$	–Cl	–
4	CH	$–OCH_3$	–H	–H	–Cl	**26**	N	$–CH_3$	–H	–Cl	–
5	CH	$–NO_2$	–H	–H	–Cl	**27**	CH	–H	–H	–Cl	–
6	N	–H	–H	–H	–Cl	**28**	CH	–H	–Cl	–Cl	–
7	CH	–Cl	–OH	$–OCH_3$	–Cl	**29**	CH	–H	–OH	–Cl	–
8	CH	–Cl	–OH	–H	–Cl	**30**	CH	–H	$–OCH_3$	–Cl	–
9	CH	$–OCH_3$	–OH	$–OCH_3$	–Cl	**31**	CH	–H	$–NO_2$	–Cl	–
10	CH	$–OCH_3$	–OH	–H	–Cl	**32**	N	–H	–H	–Cl	–
11	CH	–H	–H	–H	$–C_6H_5$	**33**	CH	$–CH_3$	–H	$–C_6H_5$	–
12	CH	–Cl	–H	–H	$–C_6H_5$	**34**	CH	$–CH_3$	–Cl	$–C_6H_5$	–
13	CH	–OH	–H	–H	$–C_6H_5$	**35**	CH	$–CH_3$	–OH	$–C_6H_5$	–
14	CH	$–OCH_3$	–H	–H	$–C_6H_5$	**36**	CH	$–CH_3$	$–OCH_3$	$–C_6H_5$	–
15	CH	$–NO_2$	–H	–H	$–C_6H_5$	**37**	CH	$–CH_3$	$–NO_2$	$–C_6H_5$	–
16	N	–H	–H	–H	$–C_6H_5$	**38**	N	$–CH_3$	–H	$–C_6H_5$	–
17	CH	–Cl	–OH	$–OCH_3$	$–C_6H_5$	**39**	CH	–H	–H	$–C_6H_5$	–
18	CH	–Cl	–OH	–H	$–C_6H_5$	**40**	CH	–H	–Cl	$–C_6H_5$	–
19	CH	$–OCH_3$	–OH	$–OCH_3$	$–C_6H_5$	**41**	CH	–H	–OH	$–C_6H_5$	–
20	CH	$–OCH_3$	–OH	–H	$–C_6H_5$	**42**	CH	–H	$–OCH_3$	$–C_6H_5$	–
21	CH	$–CH_3$	–H	–Cl	–	**43**	CH	–H	$–NO_2$	$–C_6H_5$	–
22	CH	$–CH_3$	–Cl	–Cl	–	**44**	N	–H	–H	$–C_6H_5$	–