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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Dengue virus replication inhibition by dibenzothiepin derivatives

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Abstract

The presented research uses both a target-based drug design strategy focused on dengue virus (DENV) helicase, and the repurposing of a known scaffold, the dibenzo[b,e]thiepine moiety, extensively used in antidepressants drugs. A series of dihydrodibenzo[b,e]thiepin derivatives were synthesized and tested at 10 µg/mL in HEK293 cells infected with DENV2. The replication inhibitory effect was average and depends on the chemical structure. The best antiviral effect was recorded for compounds, (*E*)-(2-methyl-6,11-dihydrodibenzo[b,e]thiepin-11-ylidene)amino butanoate (TM3) and (*E*)-(2-methyl-6,11-dihydrodibenzo[b,e]thiepin-11-ylidene)amino 3-fluorobenzoate (TM24); the concentrations resulting in a 90% (1 log) inhibiton of viral titers (IC_{90}) being calculated at 10 µM for TM3 and 0.25 µM for TM24. A molecular docking study has been conducted in order to predict the binding affinity of the tested compounds to DENV2 NS3 helicase and also on dopamine D4 receptor and to establish an in silico–in vitro correlation. The results obtained indicate that the antiviral mechanisms are complex and differ significantly depending on the structure. The majority of compounds appear to inhibit only the viral helicase, some of them both helicase and D4 receptors, and in the case of one compound the mechanism is elusive. We also observed that a 2-methyl substitution and S-oxidation on the dibenzo[b,e]thiepin scaffold significantly improves the inhibition of the viral replication.

Keywords Dengue fever · NS3 helicase inhibitor · D4 dopamine receptor antagonists · Drug repurposing · Dibenzothiepin · Molecular docking

Introduction

Dengue fever (DF) is a viral mosquito-borne disease caused by the infection of humans by one of the four dengue virus serotypes (DENV1, DENV2, DENV3, and DENV4), members of the *Flaviviridae* family, genus *flavivirus* (Bäck and Lundkvist 2013). DENV infection is associated with a variety of clinical outcomes. In most instances, these range from asymptomatic to DF, characterized by high fever, headache, myalgia, arthralgia, weakness, and cutaneous rash (Carabali et al. 2015). Most patients recover without complications around ten days after the onset of illness. Severe, life-threatening forms of the disease, dengue hemorrhagic fever, or shock syndrome, occur in approximately 5% of cases and are characterized by high fever, hemorrhagic manifestations, and thrombocytopenia (Rodriguez-Roche and Gould 2013). Dengue is ubiquitous throughout the tropics, being present in more than 100 countries, with ~390 million infections per year, of which 96 million manifest clinically (Bhatt et al. 2013). There are currently no licensed effective antiviral drugs to treat dengue infection and treatment is only supportive. Recently, a tetravalent vaccine was developed and approved in several endemic countries, but its efficacy is limited, especially for DENV1 and 2 (Low et al. 2017).

Even though most clinical studies are focused on prophylactic vaccines, the development of small-molecule inhibitors for dengue is increasing based on recent structural information and validation of several targets, both structural and nonstructural proteins of DENV (Lim et al. 2013). Most antiviral research is focused on targeting one or more of the seven nonstructural (NS) proteins, especially NS3, NS4B, and NS5 (El Sahili and Lescar 2017). NS3 contains a serine protease domain and a RNA helicase domain, NS5 has methyl-transferase and RNA-dependent

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RNA polymerase activities, while NS4B engages in a complex network of protein–protein interactions essential for viral replication and evasion of host immune responses (de Almeida et al. 2013; Xie et al. 2015).

The bipartite NS3 protease-NS3 helicase is crucial for the replication of dengue and other flaviviruses and is a very attractive target for antiviral development. Viral proteases are confirmed antiviral targets, with several inhibitors widely used clinically to treat human immunodeficiency virus or hepatitis C virus infections. However, protease inhibitors are associated with problematic metabolic adverse events caused by peripheral insulin resistance, including hyperlipidemia, lipodystrophy, and impaired glucose tolerance (Flint et al. 2009; Antonelli and Turriziani 2018). Considering this problem, the helicase active site of NS3 emerged as a target for developing DENV replication inhibitors (García et al. 2017). Despite these efforts, only a few inhibitors of NS3 helicase have been reported, including the antihelminthic drugs ivermectin and suramin, the benzoxazole derivative ST-610, and the primuline derivative ML283 (Sweeney et al. 2015). The lead ST-610 was well tolerated in strain 129 mice and AG129 mice after intraperitoneal administration with no visible adverse effects. ST-610 treatment reduced virus yield by five-fold compared to the control group (Byrd et al. 2013). Further, this drug design strategy is impaired by the structural similarity of the flavivirus NS3 helicase and human DDX3 proteins (Fang et al. 2016).

Drug repurposing has emerged as an alternative approach for rapid identification of effective therapeutics to treat challenging infectious diseases, like DENV (Zheng et al. 2017). Several well-known drugs from various therapeutic classes have been identified as potential candidates for treatment of DENV. Chloroquine, prednisolone, balapiravir, modipafant, lovastatin, mycophenolic acid, norclozapine, prochlorperazine, and ketotifen are some of the most important examples of this drug development strategy (Simanjuntak et al. 2015; Klug et al. 2016; Low et al. 2017).

The research presented in this article uses both a target-based drug design strategy focused on DENV helicase and the repurposing of a known promiscuous scaffold, the dibenzothiepin moiety. The study is based on the structural similarities of prochlorperazine, ketotifen, norclozapine, ST-610, and dibenzo[b,e]thiepin derivatives previously designed as antidepressants. The antiviral activity of the isomer dibenzo[b,f]thiepin scaffold was demonstrated by the identification of 7-fluoro-11-(4-methylpiperazin-1-yl)-10,11-dihydrodibenzo[b,f]thiepin-2-ol, referred as SKI-417616 (Smith et al. 2014), a very close derivative of norclozapine (Fig. 1). Notably, the antiviral activity of SKI-417616 is primarily mediated through its inhibition of the D4 dopamine receptor pathway and downstream phosphorylation of EGFR-related kinase (ERK) (Smith et al. 2014). We therefore focused our in silico molecular docking analysis on both the DENV helicase and the D4 dopamine receptor.

Experimental methods

Synthesis of the compounds

A set of 20 dibenzo[b,e]thiepin derivatives were selected based on their molecular diversity and were prepared in a multistep synthesis as previously described using as starting materials 6,11-dihydrodibenzo[b,e]thiepin-11-ones **1**, converted to the corresponding 11-hydroximino-6,11-dihydrodibenzo[b,e]thiepin (OXH) and 11-hydroximino-2-methyl-6,11-dihydrodibenzo[b,e]thiepin (OXM) by treating with hydroxylamine hydrochloride. The dibenzothiepin

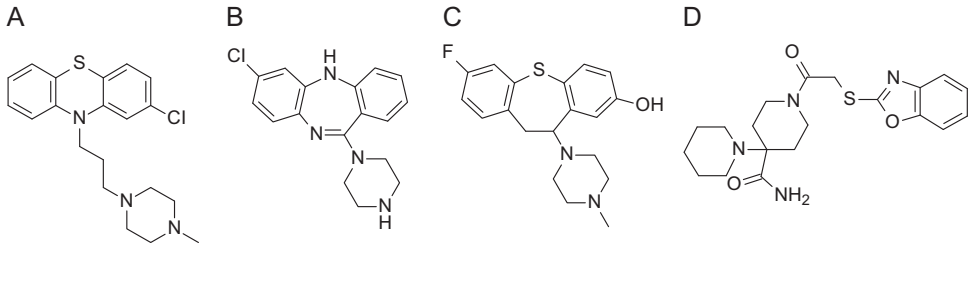

Fig. 1 The chemical structures of dengue replication inhibitors prochlorperazine **a**, norclozapine **b**, SKI-417616 **c**, and ST-610 **d**

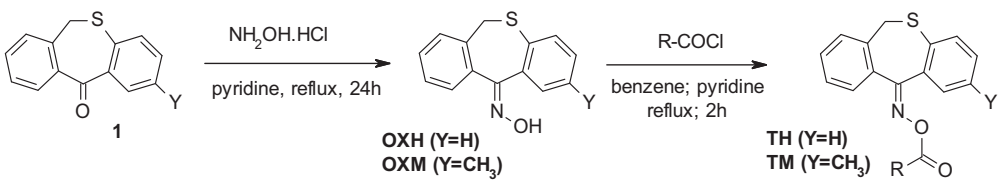

Fig. 2 Synthesis of dibenzothiepin derivatives of the TH and TM series

derivatives of the TH and TM series were synthesized by acylation of the oximes with various acid chlorides (Fig. 2).

The dibenzothiepin-5,5-dioxides code as SH and SM were prepared using two alternative synthesis pathways. One assumes the oxidation with hydrogen peroxide of the corresponding TH and TM compounds (Fig. 3). The other requires the following steps: the aforementioned ketones **1** were converted to the corresponding 5,5-dioxides **2** by oxidation with hydrogen peroxide and subsequently to the corresponding oximes **3**. The compounds from SH and SM series were prepared by acylation of the oximes with various acid chlorides (Fig. 4).

All reagents and solvents were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). The nuclear magnetic resonance (NMR) spectra were recorded on a Gemini 300BB instrument (Varian, USA), using DMSO-d6 as solvent; infrared spectra were recorded on a JASCO FT/IR-4200 spectrometer (JASCO, Japan); elemental analysis (using Perkin Elmer Series II 2400 CHNS/O Analyser, USA) and TLC were performed to assess the compounds' purity.

Structure activity relationships

A series of structural descriptors were calculated using DataWarrior application (http://www.openmolecules.org): molecular weight (MW), octanol/water partition coefficient (clogP), hydrogen bond donor (HD) and acceptor (HA) counts, number of rotatable bonds (RB), total surface area (TSA), polar surface area (PSA), shape index (ShI), molecular complexity (Cplx), non-hydrogen atoms (nH), non-carbon/hydrogen atoms (nCH), and molecular flexibility (Flx). DataWarrior application was used for several structure activity relationships (SAR) analyses. All the statistical analyses were performed with SPSS software (version 18.0 for Windows; SPSS Inc., Chicago, IL).

Molecular docking

A molecular docking study has been conducted in order to predict the binding affinity of the tested compounds to DENV2 NS3 helicase (PDB code: 2BMF) and dopamine D4 receptor (PDB code: 5WIU). Crystal structures of the proteins have been retrieved from RCSB Protein Data Bank (https://www.rcsb.org/pdb/home/home.do), followed by the removal of solvent molecules and the addition of polar hydrogen atoms. AutoDock Tools 1.5.6 graphical user interface (Scripps Research Institute, San Diego, CA, USA) was used to compute Kollman and Gasteiger charges (Morris et al. 2009). The grid box was selected in order to include the known active sites and grid parameters were calculated using AutoGrid 4.0. Ligands were docked using AutoDock Vina 1.1.2 (Scripps Research Institute, San Diego, CA, USA) (Trott and Olson 2010) and protein–ligand interactions have been visualized with Discovery Studio® Visualizer (Accelrys Software Inc., San Diego, CA, USA). The molecular docking experiment was carried out on the synthetized dibenzothiepin derivatives used in their E conformation and known NS3 helicase inhibitors (ST-610) and dopamine D4 receptor antagonists (norclozapine, nemonapride, and SKI-417616) (Durcan et al. 1995; Smith et al. 2014; Wang et al. 2017).

Inhibition of DENV2 replication

Compounds were dissolved in 100% DMSO at 10 mg/mL. HEK293 cells were treated with 10 µg/mL final concentration of individual compounds and infected with DENV2 (NGC) at a multiplicity of 0.5 pfu/cell. At 72 h pi, culture

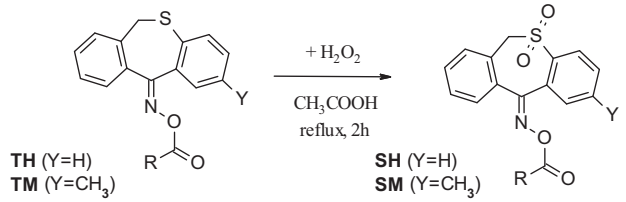

Fig. 3 Synthesis of dibenzothiepin-5,5-dioxides code as SH and SM (pathway 1)

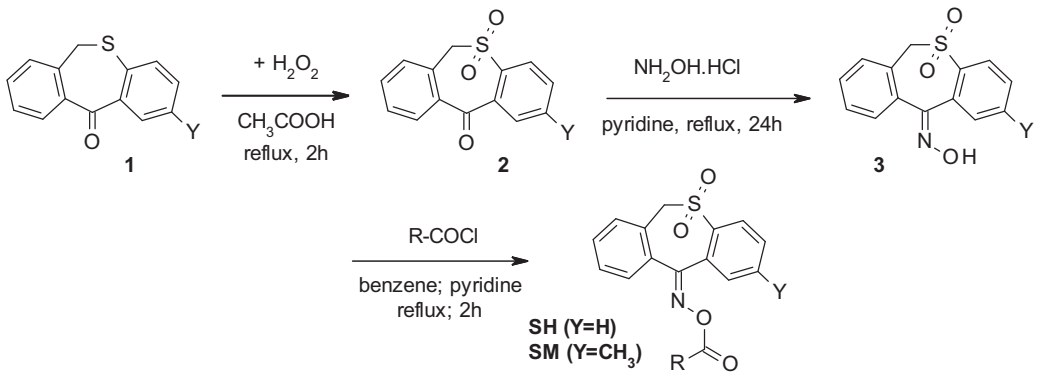

Fig. 4 Synthesis of dibenzothiepin-5,5-dioxides code as SH and SM (pathway 2)

Table 1 Compound structure and % inhibition (I%) on HEK293 cells infected with DENV2 after treatment with 10 µg/mL of individual compound

Code	I%	Series	Y	R	Reference
TH3	0	S	H	C_3H_7	Stecoza and Missir (1999)
TH6	17	S	H	2-furyl	Stecoza and Missir (1998)
TH25	0	S	H	4F-C_6H_4	Stecoza et al. (2008)
TH29	33	S	H	4Cl-C_6H_4	Ilie et al. (2009)
TH43	0	S	H	4C_3H_7-C_6H_4	Stecoza et al. (2013a)
TM3	92	S	CH_3	C_3H_7	Stecoza and Drăghici (2005)
TM5	35	S	CH_3	2-thienyl	Stecoza et al. (2013b)
TM8	62	S	CH_3	C_6H_5	Stecoza and Drăghici (2005)
TM9	20	S	CH_3	2CH_3-C_6H_4	Stecoza and Missir (2000)
TM24	89	S	CH_3	3F-C_6H_4	Stecoza and Missir (2001)
TM26	41	S	CH_3	4CF_3-C_6H_4	Stecoza et al. (2013b)
TM29	44	S	CH_3	4Cl-C_6H_4	Stecoza and Missir (2001)
SH5	73	SO_2	H	2-thienyl	Stecoza et al. (2009)
SH29	51	SO_2	H	4Cl-C_6H_4	Ilie et al. (2009)
SH35	69	SO_2	H	4Br-C_6H_4	Stecoza and Missir (2001)
SM6	72	SO_2	CH_3	2-furyl	Stecoza et al. (2013b)
SM7	75	SO_2	CH_3	1-methyl-pyrazol-4-yl	Stecoza et al. (2013b)
SM16	55	SO_2	CH_3	3CH_3O-C_6H_4	Stecoza and Missir (2001)
SM26	82	SO_2	CH_3	4CF_3-C_6H_4	Stecoza et al. (2013b)
OXH	0	S	H	–	Stecoza and Missir (1998)

supernatants were collected and virus quantified by focus-forming assay (Smith et al. 2014). % inhibition (I%) is calculated as focus-forming units/mL for treated samples divided by control (DMSO)-treated samples. Compounds that showed inhibitory activity were re-examined using three-fold serial dilutions of compound to determine inhibitory concentrations of 90% (1 log) of virus production (IC_{90})

Results and discussion

Synthesis of the compounds

The structures and purity of the target compounds were confirmed by common analytical techniques, NMR, infrared, and elemental analysis, and were consistent with the literature data (Table 1). NMR spectral data of C-6 atom and the ester carbonyl showed the presence of only one of the stereoisomers in all synthesized compounds, except for compounds TH43 and TM24, which were obtained in ratio *Z*/*E* 1/4.5 and 1/3.6, respectively.

Inhibition of DENV2 replication

HEK293 cells were treated with 10 µg/mL of new dibenzo[b,e]thiepin derivatives and infected with DENV2 at a multiplicity of 0.5 pfu/cell. One percent was calculated by comparing with the negative control and are presented in Table 1. Most compounds presented an average replication inhibitory effect. Two compounds, TM3 and TM24, resulted in a ≥1 log reduction in virus titer. HEK293 cells were treated with three-fold serial dilutions of TM3 and TM24 and viral titers were quantified by focus-forming assay at 72 h pi. Concentrations resulting in a 90% (1 log) inhibition of viral titers (IC_{90}) were calculated at 10 µM for TM3, and 0.25 µM for TM24 (Fig. 5).

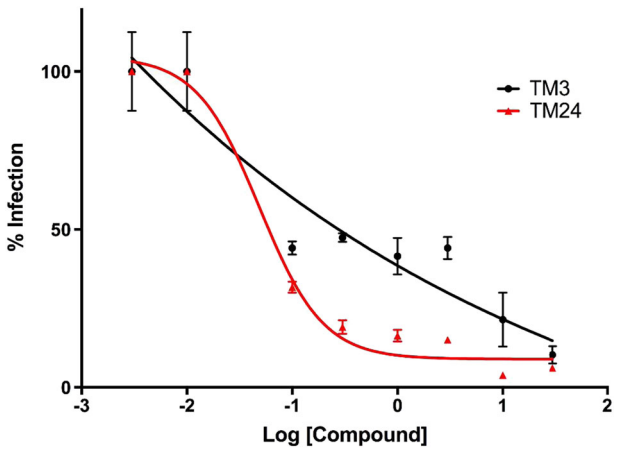

Fig. 5 Dose response of DENV2 to compounds TM3 and TM24. HEK293 cells were treated with three-fold serial dilutions of compounds. Culture supernatants were collected at 72 h pi and viral titers quantified by focus-forming assay. Concentrations resulting in a 90% (1 log) inhibition of viral titers (IC_{90}) are 10 µM for TM3 and 0.25 µM for TM24

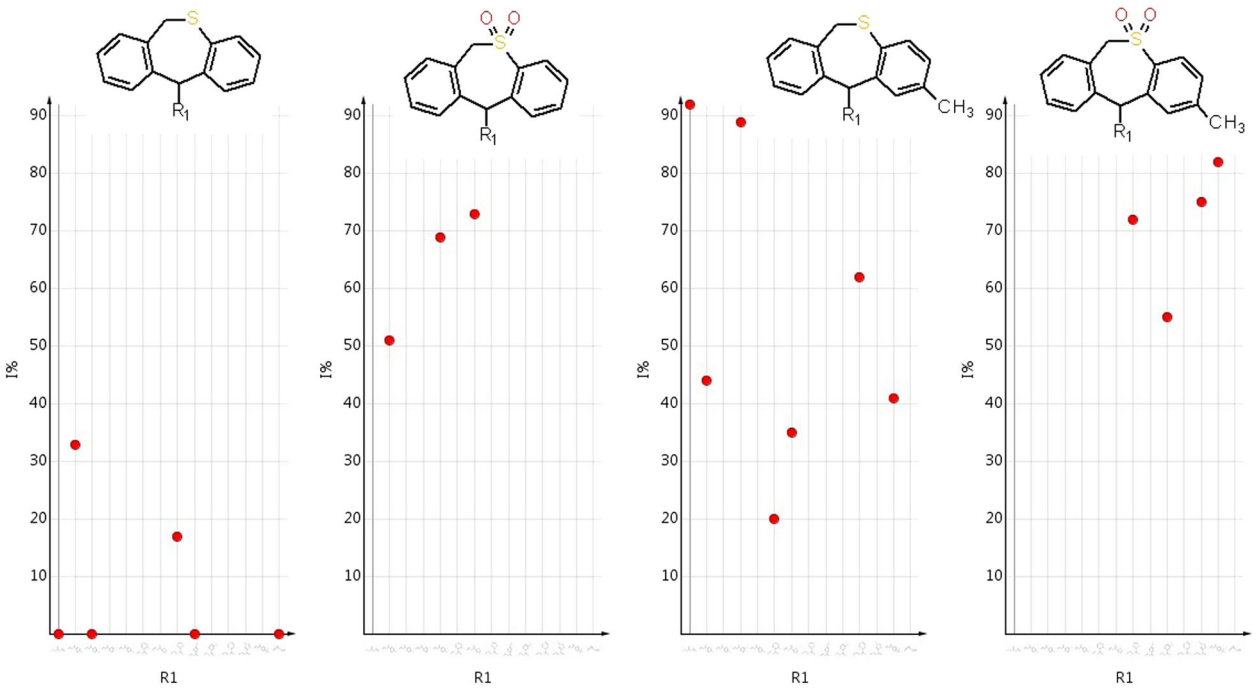

Fig. 6 Structure activity (I%) relationships using the most central ring method

Structure activity relationships

The analysis of I% values using the most central ring system for the new tested compounds revealed smaller effects for the unsubstituted dibenzothiepins and significant better inhibition for the 2-methyl-dibenzothiepins. The transformation in sulfones also increases the replication inhibition (Fig. 6).

The results of the central ring system SAR were confirmed using an independent samples *t*-test analysis of I% values, using as grouping variable the presence of the 2-methyl substitution, or of the sulfone group, respectively. The group of 2-methyl-6,11-dihydro-dibenzo[b,e]thiepin derivatives produced an average inhibition of 60.64% ($n = 11$) compared with 27.00% for the unsubstituted group ($n = 9$, $p < 0.05$) indicating the usefulness of this substitution (Y=CH_3). The tested 6,11-dihydro-dibenzo[b,e]thiepin-5,5-dioxide derivatives have an average I% of 68.14 ($n = 7$), significantly higher ($p < 0.05$) than 33.31 for the 6,11-dihydro-dibenzo[b,e]thiepin group ($n = 13$).

For all the compounds a series of significant descriptors were computed (Table 2) and the correlation with I% were tested using the Pearson coefficient (P). Statistical correlation was obtained for Cplx ($P = 0.645$), nCH ($P = 0.570$), HA ($P = 0.524$), MW ($P = 0.473$), PSA ($P = 0.454$), ShI ($P = -0.619$). This can be translated that a smaller shape index and a higher complexity favor the inhibition of viral replication. The dibenzothiepin scaffold could be improved by adding more heteroatoms to increase the molecular weight, the number of hydrogen bonds acceptors, and polar surface area.

Protein–ligand interactions and binding affinities

A molecular docking study was performed in order to predict the molecular mechanism of action for the tested compounds and to establish an in silico–in vitro correlation. The hypothesis of the viral inhibition of these compounds based on the structural similarity with ST-610, NS3 helicase inhibitor, and SKI-417616, D4 dopamine receptor antagonist, was tested using a docking study on both targets. The relative free binding energies (ΔG) of the ligands, estimated inhibitory constants (K_i), and residues involved in hydrogen bond formation are shown in Table 3. The inhibitory constant was calculated using the following formula:

$$K_i = \frac{\Delta G}{e K \times T}(M),$$

where R (gas constant) $= 1.9858775 \times 10^{-3}$ kcal K^{-1} and $T = 298.15$ K

Relative binding energies varied between −6.7 (OXH, TH3) and −9.5 kcal/mol (SM26) for DENV NS3 helicase and between −6.9 (TH3) and −10.7 kcal/mol (SM16) for dopamine D4 receptor. A linear regression analysis of I% values over the binding energies predicted on NS3 helicase resulted in a small correlation ($R = 0.404$) and pointed out the existence of several outliers. When the compounds TM3, TM24, SH5, TH25, and TH43 were removed from

Table 2 Structural descriptors for the tested dibenzothiepins

CODE	MW	cLogP	HA	HD	TSA	PSA	nCH	RB	ShI	Flx	Cplx
TH3	311.40	4.854	3	0	241.27	63.96	3	4	0.500	0.248	0.842
TH6	335.38	4.577	4	0	250.94	77.10	5	3	0.458	0.191	0.854
TH25	363.41	5.490	3	0	267.6	63.96	5	3	0.500	0.212	0.853
TH29	379.87	5.995	3	0	276.67	63.96	5	3	0.500	0.212	0.836
TH43	387.50	6.603	3	0	301.03	63.96	4	5	0.536	0.262	0.858
TM3	325.43	5.198	3	0	253.53	63.96	4	4	0.478	0.245	0.858
TM5	365.48	5.599	3	0	269.45	92.20	5	3	0.440	0.198	0.869
TM8	359.49	5.733	3	0	273.51	63.96	4	3	0.462	0.205	0.865
TM9	373.48	6.077	3	0	285.77	63.96	4	3	0.444	0.199	0.874
TM24	377.44	5.833	3	0	279.86	63.96	5	3	0.444	0.210	0.872
TM26	427.45	6.581	3	0	302.97	63.96	7	4	0.467	0.2640	0.873
TM29	393.89	6.339	3	0	288.93	63.96	5	3	0.481	0.210	0.853
SH5	383.45	4.422	5	0	268.28	109.42	7	3	0.423	0.207	0.905
SH29	411.86	5.161	5	0	287.76	81.18	7	3	0.464	0.218	0.882
SH35	456.32	5.280	5	0	290.97	81.18	7	3	0.464	0.218	0.882
SM6	381.41	4.088	6	0	274.29	94.32	7	3	0.407	0.198	0.918
SM7	395.44	2.961	7	0	284.82	99.00	8	3	0.429	0.228	0.922
SM16	421.47	4.829	6	0	306.86	90.41	7	4	0.433	0.235	0.924
SM26	459.44	5.747	5	0	314.06	81.18	9	4	0.437	0.267	0.921
OXH	241.31	3.685	2	1	180.09	57.89	3	0	0.471	0.186	0.814

Table 3 Predicted ligand affinities of the new compounds on DENV2 NS3 helicase (PDB: 2BMF) and dopamine D4 receptor (PDB: 5WIU)

Code	NS3 helicase			Dopamine D4 receptor		
	ΔG (kcal/mol)	K_i (μM)	H-bonding residues	ΔG (kcal/mol)	K_i (μM)	H-bonding residues
TH3	−6.7	0.6897	ASP290	−8.1	1.1447	SER94
TH6	−7.5	0.2114	ASP290	−8.9	0.2964	SER94
TH25	−8.3	0.1076	GLY320, HIS487	−10	0.0462	SER94
TH29	−8.8	0.3510	–	−9.8	0.0648	–
TH43	−7.6	2.6634	–	−9.1	0.2114	THR434
TM3	−6.8	10.2857	–	−6.9	8.6873	–
TM5	−7.6	2.6634	VAL544	−8.5	0.5825	ASP115, THR434
TM8	−8.6	0.4920	ARG387, LYS388	−7.6	2.6634	–
TM9	−7.6	2.6634	–	−10.6	0.0168	LEU187
TM24	−8.4	0.6897	–	−10.2	0.0330	LEU187
TM26	−8.8	0.0909	LYS388, ASP603	−8.7	0.4155	–
TM29	−7.9	1.6047	–	−9.6	0.0909	–
SH5	−7.3	4.4207	–	−9.6	0.0909	ASP115
SH29	−8.4	0.6897	LYS388	−10	0.0462	HIS414, THR434
SH35	−8.4	0.6897	LYS388	−8.7	0.4155	GLN417
SM6	−8.3	0.8166	LYS388	−9.2	0.1786	LEU187
SM7	−8.5	0.5825	ARG599	−8.8	0.3510	LEU187
SM16	−8.8	0.2114	–	−10.7	0.0142	ASP115, SER196, HIS414
SM26	−9.5	0.1076	LYS388, ASP603	−10.5	0.0199	ASP115
OXH	−6.7	1.6047	-	−7.9	1.6047	CYS185
ST−610	−8.2	0.9668	LEU542	−10.5	0.0199	CYS185
SKI-417616	−7.1	6.1971	LYS388	−8.9	0.2964	ARG186
Norclozapine	–	–	–	−9.3	0.1508	ASP115
Nemonapride	–	–	–	−9.5	0.1076	ASP115

the set, the correlation of I% values with the docking energies for NS3 helicase was statistically significant ($R = 0.832$, $n = 15$). This result suggest the inhibition of the viral helicase as the mechanism for the majority of the compounds, and the existence of other mechanisms for compounds TM3, SH5, and TM24 (Fig. 7). In the case of D4 dopamine receptor, the docking results were not correlated with I% values ($R = 0.025$). One major

limitation of the study is the use of I% values for the SAR analyses.

In the case of TM3, SH5, and TM24, I% is higher than predicted by the helicase inhibition and is probably due to other mechanism. The docking on D4 receptor indicate that TM24 and SH5 have a dual mechanism, antagonism on D4 dopamine receptor, and a helicase inhibition. However, in the case of the third outlier, compound TM3, a very low affinity is predicted for the D4 receptor; the high viral inhibition being produced by neither mechanisms and will be the subject of future research. On the other hand, compounds TH25 and TH43 have a lower inhibitory effect than predicted by the docking on helicase, and also on D4 receptor. The causes of this mismatch can be multiple, the most probable being the low solubility or a high cellular metabolism.

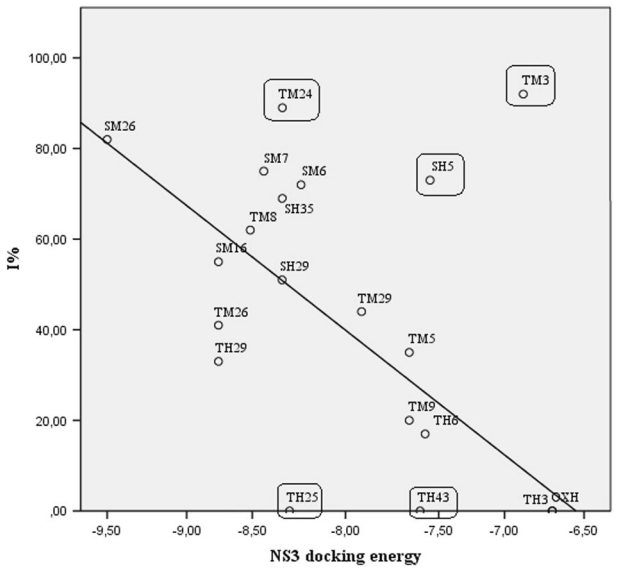

Fig. 7 Linear correlation of I% values for the new dibenzothiepin derivatives and the calculated binding energy on DENV2 NS3 helicase (PDB code: 2BMF). Outliers are boxed

Looking at docking results on both targets, the compounds SH5, SM6, and SM7 appear to have also a dual mechanism, I% values being a sum of helicase inhibition and D4 antagonism. Chemically, they share a common penta-atomic heterocyclic acyl scaffold.

The docking results are confirmed in the case of the control compounds, ST-610, as helicase inhibitor, and SKI-417616, norclozapine, and nemonapride as D4 antagonists. In both methods, OXH and TH3 have low affinity for the target proteins, in accord with the low antiviral effect.

The results of the docking on helicase protein indicate SM26 as the lead for development of dibenzothiepins as dual helicase/D4 inhibitors. In silico modeling suggests that SM26 forms three hydrogen bonds with the NS3 helicase and attaches to the nucleic acid binding site by interacting with several residues that were previously shown to bind the viral RNA strand (Xu et al. 2005): two hydrogen bonds with LYS388, pi-cation interaction with ARG387, alkyl interaction with LEU443, pi-alkyl interaction with ARG599 and van der Waals interaction with ILE365 (Fig. 8). Moreover,

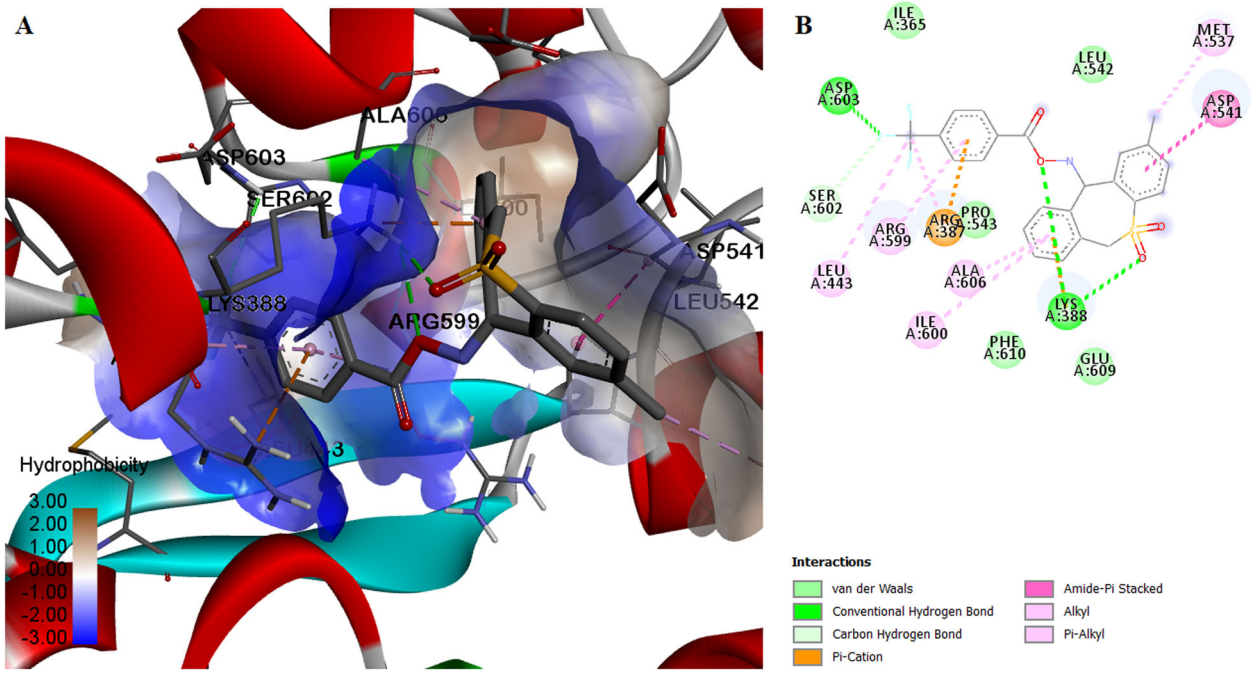

Fig. 8 **a** 3D conformation of helicase-SM26 complex. **b** 2D diagram of helicase–SM26 complex interactions

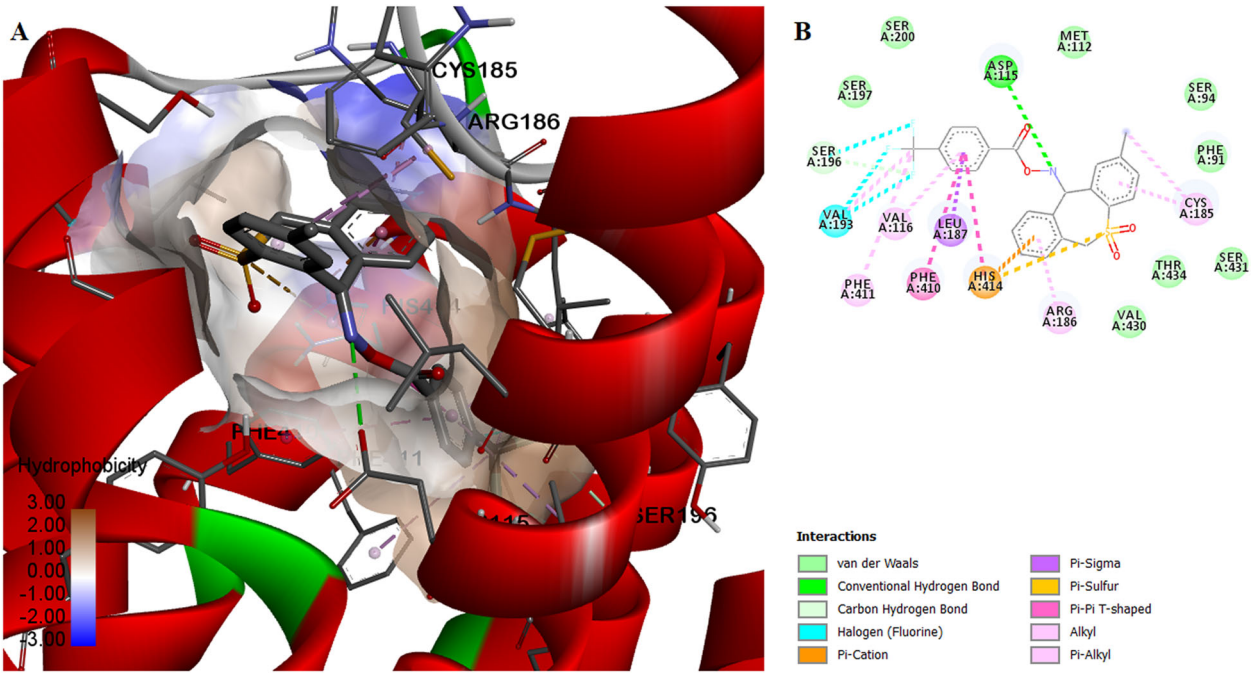

Fig. 9 **a** 3D conformation of D4-SM26 complex. **b** 2D diagram of D4-SM26 complex interactions

the sulfone and methyl moieties of the dibenzothiepin nucleus may play a major role in the antiviral activity of SM26, in agreement with the SAR results.

SM26 interacts with several dopamine D4 receptor residues that bind nemonapride in the crystal structure of the nemonapride–D4 receptor complex (Wang et al. 2017) and showed a five-fold lower inhibitory constant. Thus, SM26 forms pi-cation and pi-sulfur interactions with HIS414, carbon–hydrogen bond and halogen bond with SER196, pi-sigma interaction with LEU187 and van der Waals interactions with PHE91 (Fig. 9).

Judging by the docked poses of the ligand in the active sites, dibenzothiepin derivative SM26 might be able to inhibit NS3 helicase and dopamine D4 receptor in an orthosteric manner.

On the basis of both molecular docking results and SAR analysis, it can be stated that the presence of sulfone and methyl moieties on dibenzothiepin central ring contributes to a higher affinity for NS3 helicase and higher antiviral activity. In most cases, the sulfone group acted as a hydrogen bond acceptor, while the methyl group was involved in alkyl interactions with residues present in the helicase active site. The lack of sulfone moiety from both TM3 and TM24 suggests that the methyl radical is an essential pharmacophore responsible for a more efficient inhibition of viral replication. Thus, a strategy for designing new dibenzothiepins with NS3 helicase inhibitor activity may consist in replacing the methyl group with other lipophile moieties capable of accepting hydrogen bonds (e.g. –O–CH_3, –S–CH_3, –CF_3).

Conclusions

A series of dibenzo[b,e]thiepin derivatives were synthesized and tested at a concentration of 10 µg/mL in HEK293 infected with DENV2. Two compounds, (*E*)-(2-methyl-6,11-dihydrodibenzo[b,e]thiepin-11-ylidene) amino butanoate (TM3) and (*E*)-(2-methyl-6,11-dihydrodibenzo[b,e]thiepin-11-ylidene)amino 3-fluorobenzoate (TM24), resulted in a ≥1 log reduction in virus titer. Structural activity relationship analysis indicated that a 2-methyl substitution on the 6,11-dihydrodibenzo[b,e]thiepin scaffold significantly improves the inhibition of the viral replication.

The results indicated that despite the high degree of chemical similarity within the analyzed series of new compounds, the antiviral mechanisms are complex and differ significantly depending on the structure. The majority of compounds appear to inhibit only the viral helicase, some of them both helicase and D4 receptors, and in the case of TM3 the mechanism is elusive.

Considering the extensive use of the dibenzo[b,e]thiepin scaffold in antidepressants drugs, the proposed strategy of repurposing them as future therapies in dengue infection is promising and could be successful.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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