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Research paper

Aminopurine and aminoquinazoline scaffolds for development of potential dengue virus inhibitors☆

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ABSTRACT

Previous efforts led to dicarboxamide derivatives like **1.3**, comprising either an imidazole, pyrazine or fenyl ring as the central scaffold, with many congeners displaying strong inhibitory effects against dengue virus (DENV) in cell-based assays. Following up on some literature reports, the rationale was borne out to preserve the pending groups, now attached to either a 2,6-diaminopurine or 2,4-diaminoquinazoline scaffold. Synthetic efforts turned out less straightforward than expected, but yielded some new derivatives with low micromolar *anti*-DENV activity, albeit not devoid of cellular toxicity. The purine **14** proved the most potent compound for this series with an EC50 of 1.9 μM and a selectivity index of 58, while the quinazoline **18a** displayed an EC50 of 2.6 μM with SI of only 2.

1. Introduction

The dengue virus (DENV), member of the Flaviviridae, is a very common viral infection transmitted by mosquito bites [2]. Dengue incidence and prevalence are rising considerably in endemic areas of the tropical and subtropical regions. It is estimated that approximately 390 million infections occur each year [2] and cases continue to rise worldwide [3]. Indeed, global warming and increasing international contacts and travelling make up the perfect breeding ground for an exponential increase in Dengue as well as other previously exotic virus outbreaks carried by arthropods. The most recent newcomer is the Zika virus with outbreaks in Central and South America reaching pandemic levels especially in Brazil. This virus is likewise member of the Flaviviridae and related to dengue, yellow fever and West Nile viruses [4]. Like dengue it spreads through mosquito bites, and is expected to spread further over both American continents. The symptoms are usually mild, but infection of pregnant women leads to severe birth defects and poor pregnancy outcomes [5].

Although DENV is much more prevalent and wide-spread, currently there still is neither any vaccine nor any antiviral therapy available for DENV [6,7]. Indeed, dengue vaccine development is not straightforward, as an immunogenic response is needed against all four serotypes of DENV. When a “vaccinated” person becomes infected with a serotype against which this patient is not (or insufficiently) protected, an aggravated form of the disease will develop, due to an incompletely understood mechanism, antibody-dependent enhancement (ADE) with increased viral loads [8]. However, a tetravalent dengue vaccine under development by Sanofi-Pasteur recently was registered in Brazil [9]. This should avoid the ADE complication, but meanwhile in addition, a fifth serotype was reported [10] further complicating vaccine development.

Regarding antiviral therapy, the intensive efforts of many research groups led to a large variety of possible targets and a host of compounds have been reported to be able inhibiting DENV growth in a laboratory setting. Thus recently, a series of 2-aroyl-3-arylquinoline was reported to strongly inhibit DENV2 RNA expression without significant cell cytotoxicity [11]. However, many more reports are appearing each year and an excellent review of Klein et al. tries to cover all aspects on the medicinal chemistry of dengue virus [12], while another review of Soliman et al. specifically discusses the potential of targeting non-structural proteins

☆ This is part 5 in a series on dengue virus inhibitors, part 4 being reference [1].

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and especially proteases for combating neglected diseases as caused by arboviruses with in particular Dengue virus [13]. In addition it was recently determined that host molecular chaperones like Hsp70 are required for viral entry, RNA replication and virion production and allosteric inhibitors of Hsp70 potently inhibit DENV replication [14]. We likewise recently reported on tritylated nucleosides [15–17] as well as imidazole and pyrazine dicarboxamides [1,18] as potential inhibitors of both Dengue and Yellow fever virus, but have not been able to uncover the mechanism of action of the latter although we suspect the RNA polymerase. Bisaryl amide compounds likewise have been reported as weak inhibitors of influenza virus, supposedly interacting with APOBEC3G, an RNA editing enzyme [19]. In addition, DENV NS5 RdRp inhibitors binding in its palm subdomain were uncovered recently using an X-ray based fragment screening methodology, resulting in low micromolar EC50 values in cell-based assays [20]. Finally and alternatively, the vector of dengue fever, *Aedes aegypti*, could be targeted in an effort to control the spread of dengue virus [21,22].

1.1. Rationale: building on previous leads

Among our previously synthesized heterocyclic compounds, analogue **1.2** exhibited the most potent *anti*-DENV activity with an EC_{50} = 0.5 μM and a selectivity index (SI) of above 235 while the initial lead (**1.1**) having an imidazole dicarboxamide central scaffold displayed an EC_{50} = 2.5 μM. In addition, evaluation of the congeners with a pyrazine central scaffold lead to compound **1.3** being the most potent congener for this series with again an EC_{50} = 0.5 μM and SI of over 235. In addition, the latter compound upon removal of the methyl moiety of the benzene ring at the right, displayed strong YFV inhibition with EC_{50} = 0.4 μM (Fig. 1). Unfortunately, we have been unable to generate resistant viruses and no target so far could be pinpointed.

Searching for structurally analogous compounds, it has been reported in literature that a series of anthranilic acid derivatives are potent inhibitors of the hepatitis C NS5B polymerase. One of the compounds (**1.4**, Fig. 2) of this series displayed an IC_{50} of 17 nM and very low cellular toxicity affording selectivity indexes over 7000 using the MTS cell proliferation assay [23]. The Novartis corporate compound archive also led to the identification of *N*-sulfonylanthranilic acid **1.5** which inhibited DENV RdRp with an IC_{50} of 0.7 μM [24]. In addition, scientists at NITD identified compound **1.6** as one of their inhibitors which displayed an average EC_{50} of 119 nM against dengue virus serotype 2 in a human cell line [25]. In another series of compounds, 2,4-diaminoquinazoline derivative **1.7** was observed to display both the highest antiviral potency (EC_{50} = 2.8 nM, SI > 1000) and an excellent pharmacokinetic profile against DENV [26]. Finally, a series of substituted quinazoline-2,4-diamines (**1.8**) has been shown to display interesting anti-Leishmania activities with favorable physicochemical properties [27].

Combination of the structural information in these reports with our previous lead structures for DENV inhibition within the imidazole (**2.1**) and the phthalic acid series (**2.2**), inspired us to envisage compound series like **3.1** or **3.2** for their potential antiviral activity, as shown below (Fig. 3).

2. Results and discussion

2.1. Synthetic procedures

The 2,6-dichloropurine and 2,4-dichloroquinazoline scaffolds are the logic precursors for synthesis of the compound series **3.1** and **3.2**. Both share a 2,4-dichloropyrimidine ring allowing selective reaction to introduce different aniline substituents. Coupling of two different anilines can be done selectively by optimization of the temperature around 60 °C and 90 °C, respectively. Both steps proceed via a straightforward S_NAr mechanism on the 2,6-dichloropyrimidine ring. Hereto, either a preformed heterocycle substituted aniline could be used, or a concluding Suzuki reaction on a brominated aniline can afford the target compounds **3.1** and **3.2**.

Corroborating on these plans, somewhat unexpectedly 2,6-dichloropurine **4** displayed no nucleophilic substitution at C6 using 2-bromo-toluidine (**5**) under different reaction conditions [28] (either in Et_3N, *n*-BuOH at 100 °C for 12 h, or in Et_3N, amyl alcohol, 100–110 °C for 16 h or in presence of DIPEA in acetonitrile, 80–90 °C for 18 h) (Scheme 1). Likewise, nucleophilic substitution at the C4 position of 2,4-dichloroquinazoline [29] (**7**) to obtain **8** proved not possible using **5** in either amyl alcohol or acetonitrile at elevated temperatures. This lack of reactivity could be attributed to the use of the sterically hindered electron-poor aniline [30] **5**. According to the literature [31], however, the pKa value of a trifluoroacetyl (TFA) protected aniline approaches the pKa for phenol (9.9 and 10.0 respectively). Activation of the sterically hindered electron-poor aniline nitrogen by a TFA group therefore will lower the pKa of the anilide **9** increasing its nucleophilicity under basic conditions. The corresponding **9** was easily obtained in 90% yield by treatment of **5** with triflic anhydride and Et_3N in DCM at rt for 3 h. In parallel, reaction of 2,6-dimethylquinolin-4-ol (**10**) with hydrazine afforded the envisaged pyrazole containing aniline **11** as described previously [32].

However, reaction of 2,6-dichloropurine (**4**) with **9** failed likewise to afford the desired substitution, and hence **4** was first protected on the imidazole nitrogen with 4-methoxybenzyl chloride (PMBCl) (Scheme 2). This resulted in a 2:1 mixture of the 9- and 7-PMB regioisomers [33] **12a** and **12b** respectively, which were conveniently separated by column chromatography.

Nucleophilic aromatic substitution at the C6-position of **12a** with the acylated aniline **9** was accomplished using K_2CO_3 in 1,2-dimethoxyethane (DME) at reflux for 24 h to obtain **13** in 48% yield. Various other reaction conditions were less successful, with decreased yields using NaH or Cs_2CO_3 in DME, while no reaction took place with K_2CO_3 in an aprotic solvent like DMF, even at elevated temperature. Deprotection of the PMB group using TFA in DCM at rt for 5 h afforded **6** in 51% yield. In contrast with **5**, the

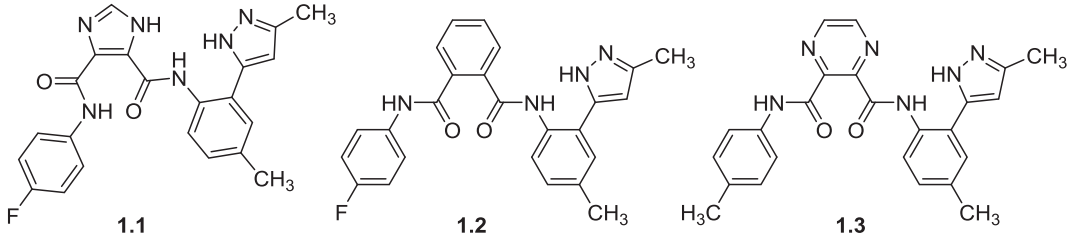


Fig. 1. Structures of the most active *anti*-DENV compounds from our previous work.

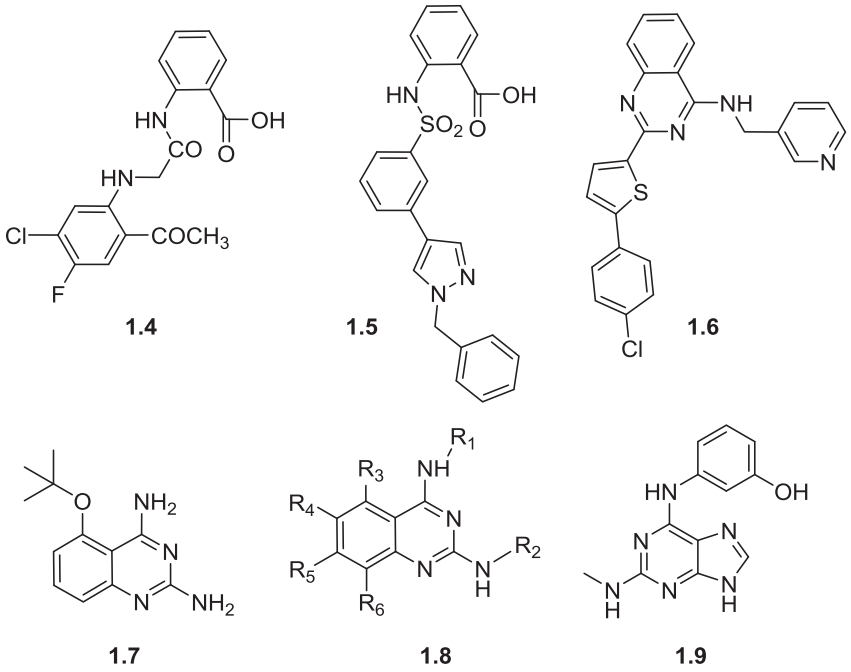


Fig. 2. Compounds with biological activity and sharing some structural aspects with our series or displaying antimicrobial activity.

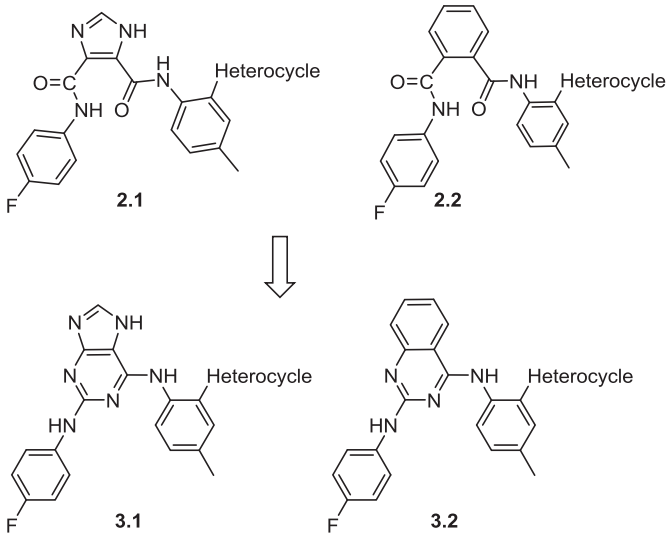


Fig. 3. New envisaged structures for DENV inhibition based on aminopurine or aminoquinazoline scaffolds.

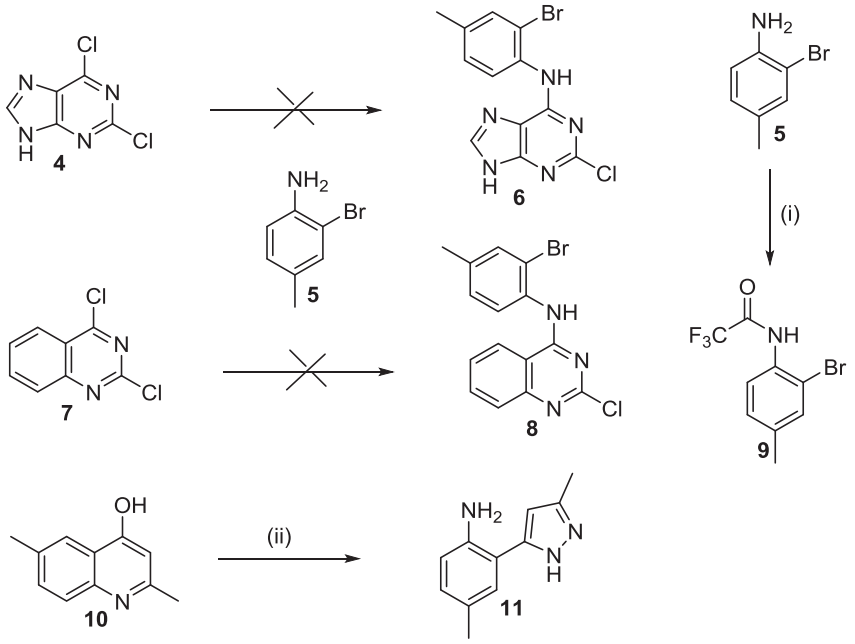
hindered aniline **11** displayed adequate nucleophilicity and 37% of **14** was obtained on refluxing **12a** and **11** in DME for 24 h, followed by deprotection to afford **15** in moderate yield. Direct substitution of **4** with **11** to provide **15** proved feasible as well on small scale in low yield (50 mg batch), albeit the reaction proved not reproducible (Scheme 2).

As for purines, nucleophilic aromatic substitution of the quinazoline ring is directed preferably to the C4-position displaying higher reactivity. Hence, the quinazoline **7** reacted swiftly with **9** or **11** using K_2CO_3 in DME at reflux conditions to afford **8** and **16** in 88% and 61%, respectively (Scheme 3). Further nucleophilic substitution at C2 of the quinazoline **16** in presence of conc. HCl under reflux in acetone [29] proved unsuccessful due to degradation of the starting material. Also Buchwald coupling conditions ($Pd_2(dba)_3$ [34], BINAP, Cs_2CO_3, Toluene, reflux for 7 h) lead to degradation. Microwave conditions [35] (toluidine, EtOH, 30 min at 150 °C) furnished a low yield reaction (20%), but optimal results finally were obtained upon heating in IPA at 100 °C in a sealed tube [36] affording 66% and 46% of **18a** and **18b**, respectively.

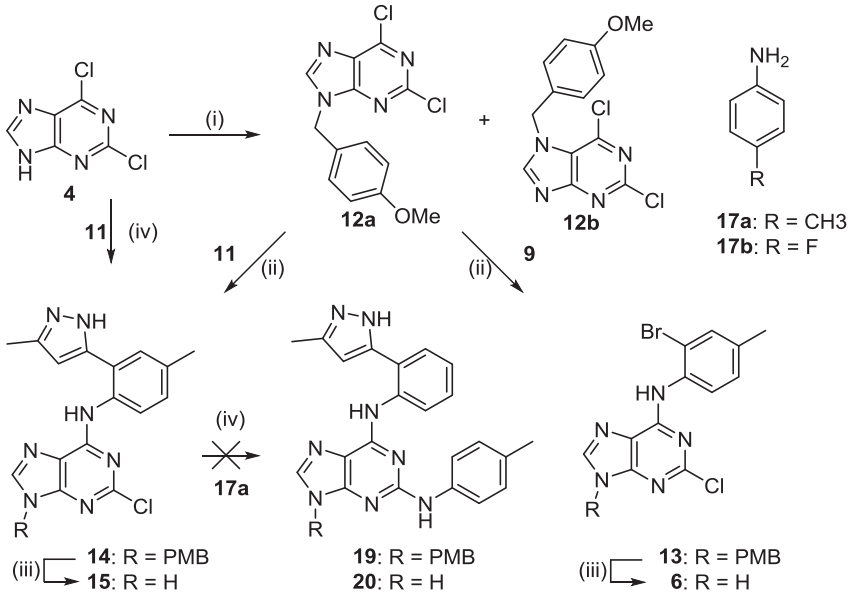
Introduction of amines via substitution of chlorine at the C2-position of purines on many occasions in the past has shown to be problematic, especially with an electron donating nitrogen already present at C6. Ciszewski et al. carefully analyzed the problem and found slight acidic catalysis using TMSCl to be optimal [37]. However, upon treatment of **15** with low molecular % amounts of TMSCl in refluxing n-butanol for 12 h, the starting material had disappeared and a brown solid was filtered, but the desired product could not be detected. Preserving the PMB group at N9 as in **14**, substitution at C2-position likewise proved unsuccessful under various Buchwald-Hartwig [38] conditions with toluidine (**17a**) (either ($Pd(OAc)_2$, BINAP, Cs_2CO_3, toluene, reflux; $Pd_2(dba)_3$, BINAP, Cs_2CO_3, dioxane, MW at 150 °C for 15 min; or $Pd(OAc)_2$, BINAP, KOtBu, toluene, reflux) (Scheme 2). Possibly, complexation of the catalyst by interaction with the pyrazole moiety causes poor reactivity of **14** towards palladium catalyzed cross-coupling reactions. Unfortunately, heating in IPA which was successful for the quinolone congeners, likewise did not afford the desired compound. The work on 2,6,9-trisubstituted purines of Houzé et al. [38] with prior functionalization of the C2 position starting from a C2-amine like 2-amino-6-chloropurine could have solved our issue but was not attempted further.

2.2. Assessment of antiviral activity

Our previous work entailing imidazole-4,5-and pyrazine-2,3-dicarboxamides targeting dengue virus (see chemical structures **1.1**–**1.3**) resulted in several compounds endowed with submicromolar inhibitory acitivities and high selectivity indices. Combined with literature reports on some quinazoline derivatives displaying strong dengue inhibitory properties (structures **1.6**–**1.8**), we sought to combine some features of both series in either a



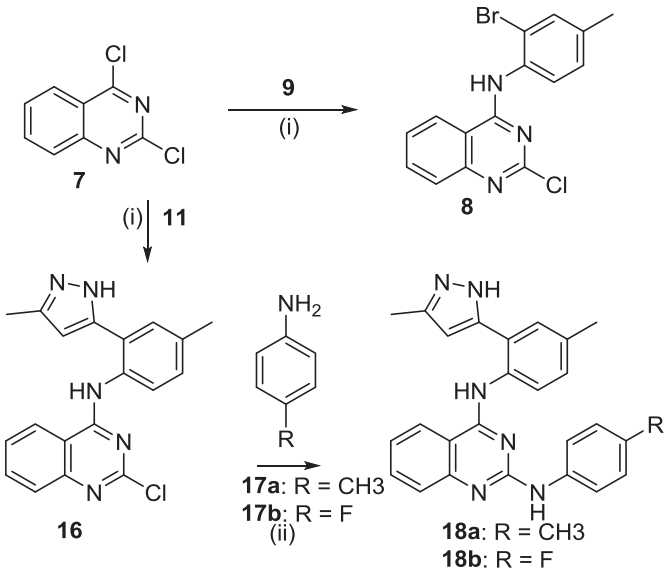
Scheme 1. i) triflic anhydride, Et_3N, DCM, rt, 3 h (90%); (ii) NH_2NH_2/NH_2NH_2. 2HCl, ethylene glycol, 200 °C, sand bath, 5 h (75%).



Scheme 2. i) PMBCl, K_2CO_3, DMF, rt., 24 h (**12a**: 55%; **12b**: 26%); ii) K_2CO_3, DME, reflux, 24 h (48% of **13** (along with 25% recovery of **12a)** and 37% of **14**; iii) TFA, DCM, rt, 5 h (51%); iv) K_2CO_3, DME, reflux, 24 h (35% of **15**).

quinazoline or purine functionalized scaffold (see Fig. 3). The resulting compounds were evaluated for their inhibitory properties against DENV (serotype 2) in Vero-B cells. Following completion of our work presented here, Vincetti et al. [39] disclosed a series of double substituted purine compounds with some of them displaying micromolar inhibitory effects, targeting the dengue virus NS5 polymerase at an allosteric pocket. Best results for this series were obtained for the 2,6-diaminopurine congener **1.9** with an EC_{50} of 5.3 μm and having strong resemblance to our target compounds. Hence, the results for compounds **1.3** and **1.9** are included here in Table 1 as reference compounds.

Only for the diaminoquinazoline series the double modified target compounds **18a** and **18b** comprising the 3-methyl-1*H*-pyrazol-5-yl-phenyl moiety were attained. To our satisfaction we noted the strong inhibitory effect on denge virus proliferation (2.6 μm and 6.7 μm, respectively, based on RNA viral load determination), as was hoped for based on our starting rationale. However, both compounds are about 10 times less active compared to the lead dicarboxamide **1.3** (EC_{50} 0.5 μm) [1] being at the same time much more toxic and devoid of selectivity. The mono-aminated quinazolines **8** and **16** proved only twice less inhibitory, but displayed lower toxicity, endowing these congeners with a selectivity index (SI) of around 10. In the purine series, the intermediate N9 or N7-methoxybenzyl protected derivatives **12a,b** already displayed inhibitory activity concomitant with toxicity in the lower micromolar range, while the mono-aminated purines **6**



Scheme 3. i) K_2CO_3, DME, reflux, 6–12 h (88% for **8** and 61% for **16**); ii) IPA, 100 °C, 4–5 h (66% for **18a** and 46% of **18b**).

Table 1
Dengue inhibitory activity and cytotoxicity of the various obtained compounds.

compound	MW	MTS/PMS CC_{50} (μm)	RT-qPCR EC_{50} (μm)	SI
12a	308	32.4	17.6	2
12b	308	12.3	9.9	1
13	457	>109[a]	7.2	15
6	337	>148	33.5	4
14	459	>109[a]	1.9	58
15	339	>147	28.9	5
8	347	>144[a]	10.9	13
16	349	97	9.2	11
18a	420	5.8	2.6	2
18b	424	8.7	6.7	1
1.3	426	>117	0.5	>235
1.9	256	168	5.3	32

[a] Deviating CC_{50} on visual inspection of cell viability: 63 μm (**13**); 10 μm (**14**); 32 μm (**8**).

and **15** displayed only marginal activity around 30 μm with an SI of about 5. Finally, their precursors **13** and **14** still functionalized with the methoxybenzyl moiety, proved most active and selective of this series. The pyrazolyl containing purine **14** herein showed most promising with an EC_{50} of 1.9 μm and an SI of 109. Visual inspection for cell abnormalities (in contrast to inhibition of cell metabolism as determined in the MTS cell viability assay) however tempered our enthusiasm and afforded a reduced SI of only 5. In comparison, the diaminated purine **1.9** displayed inhibitory effects at 5 μm with an SI of 32 [39].

2.3. Molecular docking on dengue virus NS5 polymerase

Vincetti et al. described a novel series of dengue virus inhibitors targeting an allosteric pocket coined “cavity B” on DENV-NS5 [39] with their purine derivative **1.9** (16i in the original publication) interacting with crucial residues within this cavity. With our compounds displaying structural similarity to the reported compounds, a similar modeling study was carried out. Hereto, the envisaged compounds were docked in cavity B [40] of the dengue virus DENV-3 RNA-dependent RNA polymerase (DENV-3 RdRp NS5 pdb code 2j7u [41] being part of the NS3-NS5 protein interface. This cavity is conserved in DENV RdRps so our docking models based on the DENV-3 structure should be a good model for the experimental inhibition measurements obtained with DENV-2 [40].

The ligands were positioned in cavity B simulating induced fit docking by introducing flexibility in some protein residues defining cavity B (Leu326, Leu327, Lys329, Thr858, Trp859, Asn862, and Ile863). Due to high flexibility of the shallow cavity multiple binding modes for the different analogues are observed having a binding energy difference of less than 0.2 kcal/mol for different conformations. Fig. 4 shows a docking pose in cavity B for several of the new reported compounds as well as for the reference compound **1.9** (structure 16i in the respective reference) [39]. Selection of this conformation is based on the docking score (in the top 5 out of 50) and maximal hydrophobic contact with the residues Leu327, Lys329, Trp859 and Ile863, which were proven before to be important for NS3-NS5 interaction by mutational analysis. Remarkably, our docking result for this reference compound is different from the one reported by Vincetti, with 3 hydrogen bonds instead of one but absence of the reported cation-pi interaction with Lys329 which was not observed in our study. However, the inhibitor shows hydrophobic contact with Lys329, Trp859 and Ile863, 3 out of 4 residues important for viral replication (no contact with Leu327, using DENV3 numbering). This deviation is not surprising, as often different docking positions are found when using different docking programs [42] Using a similar procedure allowed to dock the other compounds, all binding cavity B and displaying different hydrogen bonds and hydrophobic contacts. All hydrophobic contacts with the key amino acids are given in the caption for Fig. 4. The nice hydrophobic fit for **14** (panel D) is noteworthy. Compounds **18a** and **18b** (panels E and F) likewise display good interaction. The docking of compound **14** is different when compared to orientation of compounds **15** and **18** despite the fact they have a similar scaffold. This likewise is not remarkable, as small changes in substituents may result in a completely different binding mode [43]. Nevertheless, all these docking experiments may help to explain the experimental findings. From the obtained results it seems plausible that binding may interfere with NS3-NS5 protein interaction and concomitant initiation of RNA synthesis [39] but only a qualitative interpretation can be provided.

3. Conclusion

Based on our previous findings for dicarboxamide derivatives like **1.3** endowed with strong inhibitory effects on dengue virus, and on the biological activities of 2,4-diaminoquinazoline structures as reported in literature, efforts were undertaken to combine both features based on either a central quinazoline or purine heterocyclic scaffold.

Accurate activity data were obtained using a virus yield reduction assay determining the viral RNA load by real-time quantitative RT-PCR. As hoped for, the double modified target compounds **18a** and **18b** displayed low micromolar inhibitory effects albeit with low selectivity. The pyrazolyl containing monocarboxylated precursor purine **14** unexpectedly proved to be the most active of the present series with an EC_{50} of 1.9 μM and an SI of 109. Based on literature results, analogous molecular docking studies for our compounds confirmed the NS5 polymerase to be the probable target, highlighting the potential of bis-aminated purine and quinazoline scaffolds for further study as flavivirus inhibitors.

4. Materials and methods

4.1. Chemistry

General methods are largely as described before [18] and can be found in the supplementary section. Chromatographic purifications

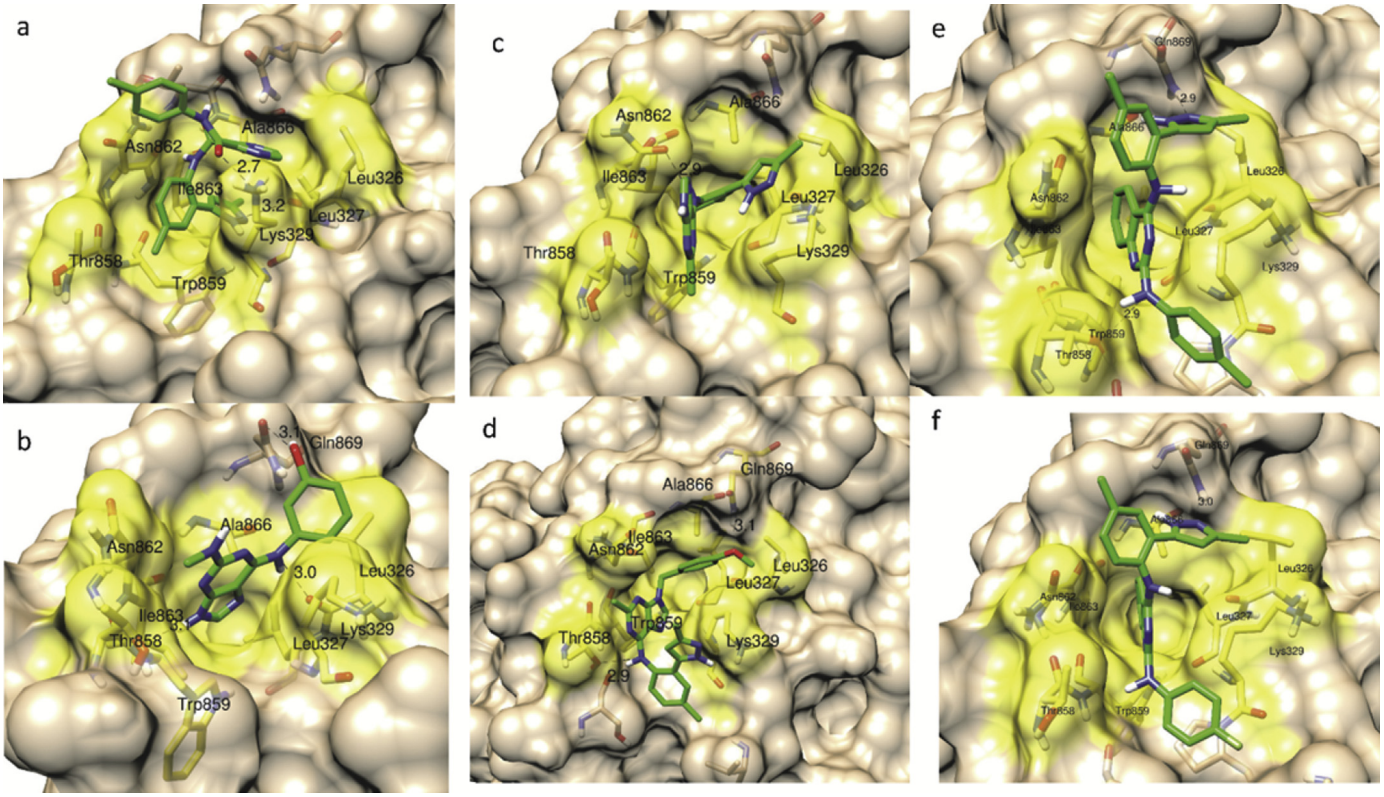


Fig. 4. Docking pose for different compounds in cavity B of DENV3 RdRp with residue numbering according to DENV-3 with panel A: **1.3** from Ref. [1] (Leu327, Lys329, Trp859 and Ile863); panel B: reference compound **1.9** [39] (hydrophobic contacts with Lys329, Trp859, Ile863); panel C: **15** (Leu327, Lys329, Trp859, Ile863); panel D: **14** (Lys329); panel E: **18a** (Trp859 and Ile863); panel F: **18b** (Lys329, Trp859 and Ile863).

on silica gel were carried out via slow methanol gradients in dichloromethane, unless indicated otherwise.

*4.1.1. N-(2-Bromo-4-methylphenyl)-2,2,2-trifluoroacetamide (**9**)*

Trifluoroacetic anhydride (6.38 mL, 45.16 mmol) was added dropwise to a stirring solution of aniline **5** (5.50 g, 31.1 mmol) and Et_3N (10.5 mL, 75.3 mmol) in DCM (100 mL) that was kept at 0 °C with the aid of an ice bath. After 15 min the solution was allowed to warm to room temperature (rt.) and was stirred for 3 h more, cooled again to 0 °C and quenched with saturated *aq.* $NaHCO_3$ solution. The aqueous layer was extracted with DCM (3 × 100 mL). The organic layer was separated and dried over *anhydrous* Na_2SO_4, filtered and concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel to give 7.3 g (90%) of the title compound **9** as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.36 (brs, 1H, NH), 8.15 (d, J = 8.4 Hz, 1H), 7.42 (s, 1H), 7.18 (d, J = 8.4 Hz, 1H), 2.34 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 154.24 (q, J = 37.6 Hz), 137.3, 132.5, 130.3, 129.0, 121.6, 115.0 (q, J = 288.8 Hz), 113.8, 20.3; HRMS for $C_9H_8BrF_3NO$ ($[M+H]^+$) calcd.: 281.9736; found: 281.9742.

*4.1.2. N-Alkylation of 2,6-dichloropurine (**12a**, **12b**)*

A mixture of 2,6-dichloropurine **4** (3 g, 15.9 mmol) and *anhydrous* K_2CO_3 (6.57 g, 47.6 mmol) in dry DMF (100 mL) was stirred at rt. under N_2 atmosphere for 30 min *p*-Methoxybenzyl chloride (4.3 mL, 31.7 mmol) was slowly added to the reaction mixture at rt. and progress of the reaction was monitored by TLC. After stirring for 24 h, the reaction mixture was filtered and evaporated in *vacuo*. The mixture was diluted with EtOAc (100 mL) and washed with H_2O (100 mL) and the aqueous layer was back extracted twice with 100 mL of EtOAc. The organic phase was dried with Na_2SO_4 and evaporated. The product was purified by flash chromatography on silica gel to give the title compounds **12a** and **12b** as white powders.

2, 6-Dichloro-9-(4-methoxybenzyl)-9*H*-purine (12a): Yield: 2.71 g (55%); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.04 (s, 1H), 7.30 (d, J = 8.5 Hz, 2H), 6.92 (d, J = 8.5 Hz, 2H), 5.36 (s, 2H, $PhCH_2$), 3.82 (s, 3H, OMe); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.6, 159.8, 152.8, 151.5, 145.1, 130.4, 129.4, 125.6, 114.4, 55.0, 47.4; calcd.: 331.0124; found: 331.0124.

2, 6-Dichloro-7-(4-methoxybenzyl)-7*H*-purine (12b): Yield: 1.3 g (26%); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.19 (s, 1H), 7.14 (d, J = 8.7 Hz, 2H), 6.90 (d, J = 8.7 Hz, 2H), 5.59 (s, 2H, $PhCH_2$), 3.80 (s, 3H, OMe); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 163.4, 159.8, 152.9, 149.9, 143.6, 128.6, 125.4, 121.4, 114.5, 55.1, 50.3; HRMS for $C_{13}H_{10}Cl_2N_4O_1$ ($[M+Na]^+$) calcd.: 331.0124; found: 331.0122.

*4.1.3. N-(2-Bromo-4-methylphenyl)-2-chloro-9-(4-methoxybenzyl)-9H-purin-6-amine (**13**)*

To a solution of compound **12a** (60 mg, 0.19 mmol) and **9** (65 mg, 0.23 mmol) in DME (5 mL) was added *anhydrous* K_2CO_3 (80 mg, 0.58 mmol) at rt. and under N_2. The reaction mixture was slowly heated to 60–70 °C. Further work-up was as carried out for the synthesis of **8** using 10 mL of EtOAc, and purification by flash chromatography on silica gel gave 43 mg (48%) of the title compound **13** as a white solid while recovering some of the starting material **12a** (15 mg, 25%).

^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.47 (d, J = 8.4 Hz, 1H), 8.06 (s, 1H), 7.76 (s, 1H), 7.43 (s, 1H), 7.31–7.25 (m, 2H), 7.22 (d, J = 8.4 Hz, 1H), 6.91 (d, J = 8.4 Hz, 2H), 5.30 (s, 2H, $PhCH_2$), 3.82 (s, 3H, OMe), 2.35 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 159.5, 151.8, 150.6, 140.7, 134.7, 132.8, 32.4, 129.2, 128.7, 126.7, 121.8, 119.2, 114.2, 113.9, 55.0, 46.7, 20.2; HRMS for $C_{20}H_{18}BrClN_5O$ ($[M+H]^+$) calcd.: 458.0378;

found: 458.0378.

4.1.4. 2-Chloro-9-(4-methoxybenzyl)-N-(4-methyl-2-(3-methyl-1H-pyrazol-5-yl)phenyl)-9H-purin-6-amine (**14**)

Following the procedure for **13**, to a mixture of compounds **12a** (0.600 g, 1.95 mmol) and **11** (0.437 g, 2.33 mmol) dissolved in DME (60 mL) was added *anhydrous* K_2CO_3 (0.800 g, 5.85 mmol) at rt. under N_2. Progress of the reaction was monitored by TLC and after 24 h the solvent was evaporated *in vacuo*. The mixture was diluted with EtOAc (60 mL) and washed with H_2O (60 mL) and the aqueous layers were back extracted twice with EtOAc. The organic phase was dried with Na_2SO_4 and evaporated and the product was purified by on silica gel to afford the desired product **14** (0.33 g, 37%) along with partial recovery of the unreacted mixture of **12a** and **11**.

^{1}H NMR (600 MHz, DMSO) δ: 13.14 (s, 1H, NH), 12.16 (s, 1H, NH), 8.65 (d, J = 8.5 Hz, 1H), 8.42 (s, 1H), 7.58 (s, 1H), 7.29 (d, J = 8.7 Hz, 2H), 7.18 (d, J = 8.5 Hz, 1H), 6.92 (d, J = 8.7 Hz, 2H), 6.59 (s, 1H), 5.34 (s, 2H, $PhCH_2$), 3.72 (s, 3H, OCH_3), 2.34 (s, 3H, CH_3), 2.30 (s, 3H, CH_3); ^{13}C NMR (150 MHz, DMSO) δ: 159.0, 152.5, 152.1, 150.7, 150.4, 142.7, 139.8, 133.5, 132.0, 129.1, 128.6, 128.4, 128.3, 121.4, 120.7, 119.5, 114.2, 102.6, 55.2, 46.5, 20.6, 10.4; HRMS for $C_{24}H_{23}ClN_7O$ ($[M+H]^+$) calcd.: 460.16470; found: 460.1652.

4.1.5. N-(2-Bromo-4-methylphenyl)-2-chloro-9H-purin-6-amine (**6**)

To a stirred solution of compound **13** (30 mg, 0.065 mmol) in DCM (5 mL) was added TFA (40 μL, 0.65 mmol) at 0 °C under N_2 atmosphere. The reaction mixture was slowly heated to 60 °C and progress of the reaction was monitored by TLC. After 5 h, the mixture was cooled to 0 °C and neutralized with saturated aqueous K_2CO_3. The aqueous layer was extracted with DCM (3 × 10 mL). The organic layer was separated and dried over *anhydrous* Na_2SO_4, filtered and concentrated under reduced pressure and the residue was purified by column chromatography on silica gel to afford 11 mg (51%) of the title compound **6** as a white solid.

^{1}H NMR (600 MHz, DMSO) δ: 9.59 (s, 1H, NH), 8.20 (s, 1H), 7.54 (d, J = 1.1 Hz, 1H), 7.53 (d, J = 8.1 Hz, 1H), 7.24 (ddd, J = 8.1 Hz, 1.8Hz and 0.6 Hz), 3.66 (brs, 1H, NH), 2.34 (s, 3H, CH_3); ^{13}C NMR (150 MHz, DMSO) δ 153.6, 152.8, 152.2, 141.8, 137.4, 134.0, 133.0, 128.9, 128.0, 120.2, 117.3, 114.2, 20.2; HRMS for $C_{12}H_{10}BrClN_5$ ($[M+H]^+$) calcd.: 337.9803; found: 337.9797.

4.1.6. 2-Chloro-N-(4-methyl-2-(3-methyl-1H-pyrazol-5-yl) phenyl)-9H-purin-6-amine(**15**)

Analogous to the procedure for **13**, to a mixture of compound **4** (50 mg, 0.26 mmol) and **11** (60 mg, 0.32 mmol) dissolved in DME (5 mL) was added *anh*. K_2CO_3 (109 mg, 0.79 mmol) at rt. under N_2. After reaction for 24 h, the solvent was evaporated *in vacuo*, and the mixture was partitioned between EtOAc (10 mL) and H_2O (10 mL). The aqueous phase was back extracted twice with EtOAc. The organic phase was dried with Na_2SO_4 and evaporated. The product was purified by flash chromatography on silica gel to give 32 mg (35%) of the title compound **15** as a white solid.

Alternatively, deprotection of **14** with TFA as described for compound **6** afforded **15**.

^{1}H NMR (600 MHz, DMSO) δ: 13.36 (s, 1H, NH), 13.14 (s, 1H, NH), 12.10 (s, 1H, NH), 8.68 (d, J = 8.5 Hz, 1H), 8.26 (s, 1H), 7.57 (s, 1H), 7.17 (dd, J = 8.5, 1.4 Hz, 1H), 6.57 (s, 1H), 2.33 (s, 3H, CH_3), 2.30 (s, 3H, CH_3); ^{13}C NMR (150 MHz, DMSO) δ: 152.2, 152.0, 151.2, 150.7, 140.9, 139.7, 133.7, 131.8, 128.4, 128.3, 121.2, 120.5, 119.3, 102.5, 20.5, 10.4; HRMS for $C_{16}H_{15}ClN_7$ ($[M+H]^+$) calcd.: 340.1072; found: 340.1071.

4.1.7. N-(2-Bromo-4-methylphenyl)-2-chloroquinazolin-4-amine (**8**)

To a solution of compound **7** (3 g, 15.07 mmol) and the protected aniline **9** (5.08 g, 18.09 mmol) in dimethoxyethane (DME, 50 mL) was added *anhydrous* K_2CO_3 (6.23 g, 45.2 mmol) at rt. under N_2. The reaction mixture was slowly heated to 60–70 °C. Progress of the reaction was monitored by TLC and after 6 h, the mixture was cooled to rt. The solvent was removed *in vacuo* and the mixture was diluted with EtOAc (50 mL) and washed with H_2O (50 mL) and the aqueous layer was back extracted twice with 50 mL of EtOAc. The organic phase was dried with Na_2SO_4 and evaporated. The product was purified by flash chromatography on silica gel to give 4.7 g (88%) of the title compound **8** as a white solid.

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.57 (d, J = 8.5 Hz, 1H), 8.16 (brs, 1H, NH), 7.96–7.77 (m, 3H), 7.59 (dtd, J = 8.5, 6.8 and 1.7 Hz, 1H), 7.49–7.38 (m, 1H), 7.23 (dd, J = 8.5, 1.7 Hz, 1H). 2.35 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 157.7, 156.7, 151.0, 135.4, 133.6, 132.4, 132.3, 128.9, 128.0, 126.8, 122.2, 120.1, 114.3, 113.4, 20.3; HRMS for $C_{15}H_{12}BrClN_3$ ($[M+H]^+$) calcd.: 347.9898; found: 347.9900.

4.1.8. 2-Chloro-N-(4-methyl-2-(3-methyl-1H-pyrazol-5-yl)phenyl) quinazolin-4-amine (**16**)

To a solution of **7** (0.500 g, 2.5 mmol) and **11** (0.563 g, 3.0 mmol) in DME (50 mL) was added *anhydrous* K_2CO_3 (1.04 g, 7.5 mmol) at rt. under N_2 atmosphere. The reaction mixture was slowly heated to 60–70 °C for 24 h. Further work-up was as carried out for the synthesis of **8**, and purification on silica gel afforded 0.545 g (62%) of the title compound **16** as a pale yellow solid.

^{1}H NMR (500 MHz, DMSO) δ: 13.7 (brs, 1H, NH), 12.7 (brs, 1H, NH), 7.98 (d, J = 7.9 Hz, 1H), 7.87 (t, J = 7.9 Hz, 1H), 7.79 (s, 1H), 7.55 (d, J = 8.2 Hz, 1H), 7.42 (t, J = 6.9 Hz, 2H), 7.34 (d, J = 6.9 Hz, 1H), 6.46 (s, 1H), 2.45 (s, 3H, CH_3), 2.16 (s, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO) δ 159.4, 152.4, 147.8, 140.5, 139.2, 137.7, 136.2, 131.1, 130.3, 129.9, 129.5, 127.7, 125.6, 125.0, 116.7, 115.3, 102.7, 21.0, 10.3; HRMS for $C_{19}H_{17}ClN_5$ ($[M+H]^+$) calcd.: 350.1166; found 350.1165.

4.1.9. N4-(4-Methyl-2-(3-methyl-1H-pyrazol-5-yl)phenyl)-N2-(p-tolyl)quinazoline-2,4-diamine (**18a**)

A stirred solution of **16** (65 mg, 0.186 mmol) and **17a** (23 mg, 0.22 mmol) in 5 mL of isopropanol was heated to 100 °C in a closed tube. After 4 h, a pale yellow solid was filtered and the solid was washed with a minimum amount of cold isopropanol, and dried *in vacuo* to afford (52 mg, 66%) of the title compound **18a** as a pale yellow solid.

^{1}H NMR (600 MHz, DMSO) δ: 13.28 (brs, 1H, NH), 12.84 (brs, 1H, NH), 10.46 (brs, 1H, NH), 8.41 (d, J = 8.2 Hz, 1H), 8.28 (brs, 1H), 7.89 (t, J = 7.6 Hz, 1H), 7.64 (d, J = 8.7 Hz, 2H), 7.56 (t, J = 7.6 Hz, 1H), 7.36 (d, J = 7.9 Hz, 2H), 7.21 (d, J = 7.3 Hz, 2H), 6.99 (s, 1H), 6.56 (s, 1H), 2.35 (s, 2 × 3H, 2 × CH_3); 2.30 (s, 3H, CH_3); ^{13}C NMR (150 MHz, DMSO) δ 158.4, 152.0, 149.7, 140.3, 139.6, 135.4, 135.0, 133.9, 132.1, 129.5, 128.4, 128.2, 125.4, 123.9, 123.8, 123.8, 123.6, 118.0, 111.3, 102.9, 20.7 (2 × C), 10.5; HRMS for $C_{26}H_{25}N_6$ ($[M+H]^+$) calcd.: 421.2135; found: 421.2132.

4.1.10. N2-(4-fluorophenyl)-N4-(4-methyl-2-(3-methyl-1H-pyrazol-5-yl)phenyl)-quinazoline-2,4-diamine (**18b**)

A stirred solution of **16** (100 mg, 0.286 mmol) and **17b** (38 mg, 0.344 mmol) in 5 mL of isopropanol was heated in a closed vessel for 6 h at 100 °C. The precipitate formed was filtered and the solid was washed with a minimum amount of cold isopropanol and dried under vacuum to afford 56 mg (46%) of the title compound **18b** as a pale yellow solid.

^{1}H NMR (600 MHz, DMSO) δ 13.27 (brs, 1H, NH), 12.80 (s, 1H, NH), 10.45 (s, 1H, NH), 8.45 (d, J = 8.1 Hz, 1H), 8.30 (brs, 1H), 7.91 (t, J = 7.6 Hz, 1H), 7.67 (d, J = 6.1 Hz, 2H), 7.61–7.49 (m, 3H), 7.26 (s, 2H), 7.05 (s, 1H), 6.60 (s, 1H), 2.37 (s, 3H, CH_3), 2.30 (s, 3H, CH_3); ^{13}C NMR (150 MHz, DMSO) δ 160.6, 159.0, 158.6, 152.4, 152.3, 149.9, 140.0, 135.4, 135.0, 133.0, 132.1, 128.4, 128.2, 125.9, 125.4, 123.9,

123.6, 118.3, 118.2, 115.8, 115.7, 111.5, 103.0, 20.6, 10.5; fluorine coupled spectrum and missing quaternary signals; HRMS for $C_{25}H_{22}FN_6$ ($[M+H]^+$) calcd.: 425.1884; found: 425.1875.

4.2. Antiviral activity determination for DENV and YFV

Antiviral activities were determined as described in detail before by Vincetti et al. [39] (and reflect the activities as determined versus Dengue virus serotype 2 in Vero B cells [African Green monkey kidney cells obtained from the European Collection of Cell Cultures (ECACC)]. Basically, in CPE-reduction assays, cells are incubated for 7 days with serial compound dilutions, both in presence and in absence of virus. Afterwards, the 50% effective concentrations (EC_{50}) and the 50% cytotoxic concentrations (CC_{50}), which are defined as the compound concentration that is required to inhibit the virus-induced cytopathogenic effect (CPE), are calculated based on microscopic scoring data. Indeed, besides quantification of toxicity by the MTS/PMS method [39] all assay wells were checked microscopically for minor signs of virus-induced CPE or alterations of host cell morphology caused by the compound, and these results sometimes deviate from the MTS/PMS method [44]. More accurate activity data are obtained with a virus yield reduction assay, which determines the viral RNA load by real-time quantitative RT-PCR.

4.3. Molecular modeling

Using AutodockTools [45] a box of 60 × 60 × 60 units of 0.375 Å was centered on atom CB of the Lys329 sidechain. Polar hydrogens were added to enzyme and ligands as well as Gasteiger charges. Docking using autodock 4.2 was performed 100 times for every molecule to explore exhaustively the conformational space. From the top 5 scores, dockings were selected based on maximal interaction with the cavity residues (manual inspection via UCSF Chimera [46]).

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Appendix A. Supplementary data

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