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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

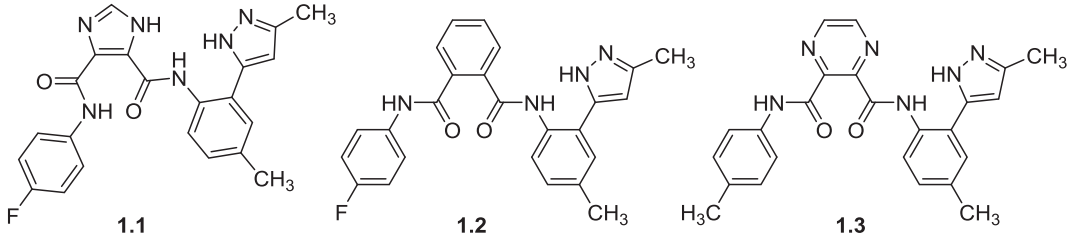

Fig. 1. Structures of the most active *anti*-DENV compounds from our previous work.