European Journal of Medicinal Chemistry 87 (2014) 529–539

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and evaluation of imidazole-4,5- and pyrazine-2,3-dicarboxamides targeting dengue and yellow fever virus☆

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ARTICLE INFO

Article history:
Received 22 May 2014
Received in revised form
17 September 2014
Accepted 18 September 2014
Available online 22 September 2014

Keywords:
Flavivirus inhibitors
Dengue virus
Yellow fever virus
Imidazole dicarboxylic acid
Pyrazine dicarboxylic acid

ABSTRACT

The results of a high-throughput screening assay using the dengue virus-2 replicon showed that the imidazole 4,5-dicarboxamide (I45DC) derivative (**15a**) has a high dengue virus inhibitory activity. Based on **15a** as a lead compound, a novel class of both disubstituted I45DCs and the resembling pyrazine 2,3-dicarboxamides (P23DCs) were synthesized. Here, we report on their *in vitro* inhibitory activity against dengue virus (DENV) and yellow fever virus (YFV). Some of these first generation compounds have shown activity against both viruses in the micromolar range. Within this series, compound **15b** was observed to display the highest antiviral potency against YFV with an EC_{50} = 1.85 μM. In addition, compounds **20a** and **20b** both potently inhibited replication of DENV (EC_{50} = 0.93 μM) in Vero cells.

1. Introduction

Dengue is the most common arthropod-borne viral infection in the world and is estimated to transmit 390 million new infections every year [3]. Dengue virus (DENV) belongs to the Flavivirus genus of the Flaviviridae. The genome of DENV is comprised of a 10.7 kb, single, positive-stranded RNA with at least four different circulating serotypes (DENV-1 to DENV-4) [4]. Reportedly, a fifth serotype now complicates vaccine development [4]. With increased levels of population growth, urbanization, and international travel, DENV illness has increased 30-fold in the last 50 years [5]. Most of the current research on dengue infections is focused on the treatment of symptoms, which often can be a tedious and intensive process. As there is neither a drug nor any vaccine available to combat DENV infections till date [6], it is highly important to explore and uncover small molecules that have potent anti-dengue activity. In the past half-decade, a number of antiviral agents with such inhibitory properties have been discovered. These include an adenosine analog **1** [7], a *N*-sulfonylanthranilic acid derivate **2** [8], the chlorophenyl-thiophene derivate **3** [9], and most recently some 2,4-diaminoquinazoline derivatives **4** [10] (see Fig. 1). Unfortunately, none of these substances have entered into clinical trials. Hence, further development of new chemical entities endowed with dengue inhibitory properties is warranted. Our previous efforts herein, focused on tritylated and alkylated nucleoside analogs, resulting in compounds endowed with strong anti-flavivirus inhibitory properties. However, no clear SAR could be determined [1,2]. Hence we now turned our attention to another lead molecule.

The five-membered imidazole ring is a structural unit found in many biologically active compounds. The strong therapeutic properties of imidazole containing drugs have encouraged medicinal chemists to synthesize a large number of novel chemotherapeutic agents comprising this entity. Amongst others, imidazole core structures are found in different carboxypeptidase, hemeoxygenase and lactamase inhibitors, as well as among anti-inflammatory, anticancer, antibacterial, antifungal, antitubercular, antidiabetic and antiviral products, further highlighted with some examples. Ramya et al. synthesized a series of novel 5-(nitro/bromo)-styryl-2-benzimidazole derivatives (**5**) and tested them for their antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and likewise evaluated the antifungal activity against *Candida albicans* and *Aspergillus fumigates* [11]. Biological activities proved comparable to ciprofloxacin. Kavitha C.S. et al. synthesized a series of 2-methylaminobenzimidazole derivatives in which compound **6** showed analgesic and anti-inflammatory activity comparable with the standard drug nimesulide [12]. Cenzo et al.

☆ This is part 3 in a series on Flavivirus inhibitors [1,2].

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http://dx.doi.org/10.1016/j.ejmech.2014.09.062

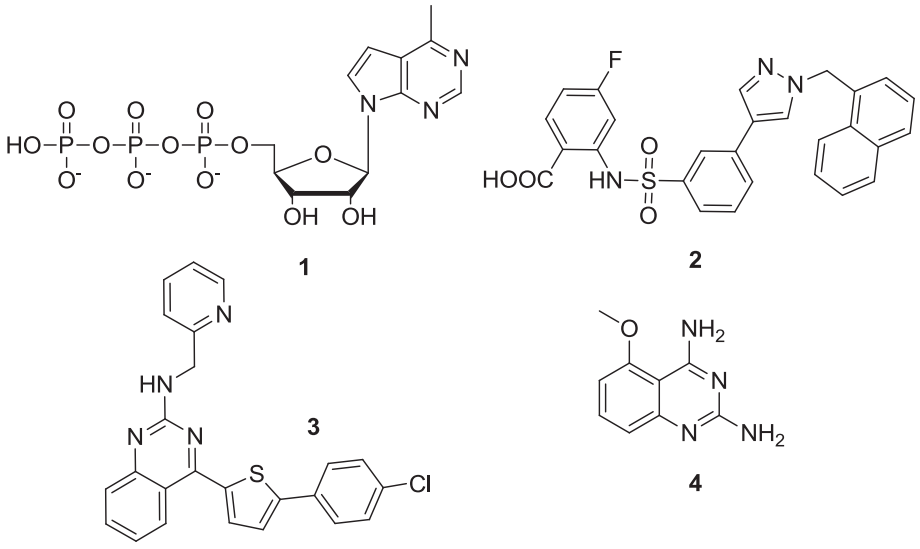

Fig. 1. Structures of some recently described antiviral compounds being inhibitory for dengue virus.

synthesized a series of 1, 4-diarylimidazole-2(3H)-one derivatives **7** and their 2-thione analogs and found antitumor activity [13]. Finally, Sharma et al. synthesized imidazole derivatives for antiviral screening and different [2-(substituted phenyl)-imidazol-1-yl]-benzamides like **8** and **9** were selected as the most promising antiviral agents [14] (see Fig. 2).

Imidazole-4,5-dicarboxylic acid (I45DC) and its derivatives such as those bearing primary or secondary amides have previously been reported to be active against HIV-1 protease [15]. Likewise, some derivatives of this scaffold were uncovered as potential kinase inhibitors with antiproliferative activity against HL-60 cells [16]. In addition, inhibition of protein–protein interaction between Hepatitis C glycoprotein E2 and CD81 has been reported previously [17]. The I45DCs are known as well to be a structural component of some antibiotics [18] and also to affect memory [19]. Substitution on the imidazole moiety herein has been reported, both in solution phase as well as in a combinatorial fashion on solid support.

High throughput screening of a compound library led to the identification of N^5-(4-fluorophenyl)-N^4-(4-methyl-2-(3-methyl-1H-pyrazol-5-yl)phenyl)-1H-imidazole-4,5-dicarboxamide **15a** (see Fig. 3) with an EC_{50} of 2.50 μM and 3.47 μM against DENV and YFV, respectively. In search for new compounds with potential for clinical use as antiviral agents, a series of compounds based on this I45DC scaffold were synthesized with regiospecific attachment of the substituents and these analogs were investigated for their inhibitory properties against DENV and YFV. In addition, the imidazole core was substituted for a pyrazine ring leading to a series of pyrazine-2,3-dicarboxylic acids (P23DC). The intended substitution of the central imidazole ring would result in slightly different orientation of the attached amide groups, and in a change of the hydrogen bonding pattern exchanging the combination of a hydrogen donating and a hydrogen accepting nitrogen into two hydrogen accepting nitrogen atoms. In this communication, we report on the synthesis and biological activity of both new series of dissymmetric I45DCs and P23DCs, which we studied for their inhibitory properties against YFV and DENV.

2. Results and discussion

2.1. Synthetic aspects

The general procedure employed for the synthesis of dissymmetrically disubstituted I45DCs is shown in Scheme 1. Imidazole-4,5-dicarboxylic acid **10** was allowed to reflux with $SOCl_2$ in toluene with catalytic DMF to afford pyrazinedione diacid dichloride **11** in 92% yield [20]. The literature route for amide formation suggests hydrolysis of the acid chlorides to carboxylic acids by the addition of water. Amines are then added to open both acyl imidazole bonds affording two identical imidazole analogs. Baures et al., replaced the water with phenol, thereby modulating the reactivity of the acid chloride in comparison with an acyl imidazole bond for the subsequent addition of two different amines [20]. The difficulty in preparing such analogs is that their synthesis is highly dependent upon the substituents to be introduced. Primary benzyl amines, for example, are too reactive to provide selectivity in the reaction, whereas anilines can be added in a proper stoichiometric ratio in order to provide a pyrazine dione intermediate **12** which can often be purified by crystallization. In the case of aniline derivatives, the resulting pyrazine intermediates are often insoluble in the reaction solvent and can be isolated simply by vacuum filtration. In parallel, different bromoanilines or benzylamines were subsequently coupled with commercially available heterocyclic boronic acids through palladium catalyzed Suzuki reaction to give the desired second aniline in 60–75% yield. Addition of this second aniline to the pyrazine intermediates **12a**–**c** resulted in opening of the acyl imidazole bond to obtain the expected product. Based on this approach, a series of novel molecules was prepared within this I45DC family containing various substituted phenyl rings. For some of the final products **15**, the reaction sequence was changed with prior attachment of the aniline carrying a heterocycle.

Pyrazines are important pharmacophores present in a number of biologically active compounds such as antimycobacterial, antibacterial, antidiabetic, and hypnotic agents. To further explore previous structure–activity relationship, we planned to substitute the five-membered imidazole ring with a pyrazine ring. Hereto, regioselective functionalization of the pyrazine started from commercial pyrazine-2,3-dicarboxylic acid (P23DA) and a four-step synthesis of the target compounds has been described in Scheme 2. P23DA was converted to its corresponding anhydride **17** by treatment with acetic anhydride at room temperature.

Reaction with *p*-fluoroaniline or *p*-toluidine resulted in opening of the five-membered ring with concomitant formation of the first amide bond (**18a**,**b**). Reaction with trifluoroacetic anhydride at 0 °C afforded the anhydric activated product **19a** or **19b** allowing introduction of the second amide. Hereto, the intermediate was reacted with preformed anilines or benzyl amines **14a**–**m** to give