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New polycyclic dual inhibitors of the wild type and the V27A mutant M2 channel of the influenza A virus with unexpected binding mode

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ABSTRACT

Two new polycyclic scaffolds were synthesized and evaluated as anti-influenza A compounds. The 5-azapentacyclo[$6.4.0.0^{2,10}.0^{3,7}.0^{9,11}$]dodecane derivatives were only active against the wild-type M2 channel in the low-micromolar range. However, some of the 14-azaheptacyclo[$8.6.1.0^{2,5}.0^{3,11}.0^{4,9}.0^{6,17}.0^{12,16}$]heptadecane derivatives were dual inhibitors of the wild-type and the V27A mutant M2 channels. The antiviral activity of these molecules was confirmed by cell culture assays. Their binding mode was analysed through molecular dynamics simulations, which showed the existence of distinct binding modes in the wild type M2 channel and its V27A variant.

1. Introduction

Influenza viruses cause the seasonal flu, which is worldwide associated with high socio-economic and medical burden [1]. Besides, influenza A virus possesses the capacity to cause sudden pandemics having higher mortality rates [2], such as the dreadful Spanish flu in 1918 [3,4] or the H1N1 pandemic in 2009 caused by a swine-origin influenza virus [5–8]. There is also increased vigilance that highly pathogenic avian influenza viruses such as H5N1 [9] or H7N9 [10] may potentially acquire human-to-human transmission, which may have devastating consequences. Though highly recommended for primary prophylaxis, the current influenza vaccines present several drawbacks [11], and hence antiviral drugs are equally vital for prevention and treatment of influenza infections. Only four anti-influenza drugs are available at this time: two neuraminidase inhibitors (oseltamivir and zanamivir) and two M2 channel blockers (amantadine, Amt, and rimantadine) [12]. Most of the circulating strains are Amt-resistant [13,14], which has compelled the Centers for Disease Control and Prevention to deter its use [15]. On the other hand, oseltamivir resistance has also been spreading [16,17] and dual resistance to both oseltamivir and Amt has been detected [18]. These reasons have prompted the quest for new antivirals as effective ways of fighting influenza.

The influenza A M2 polypeptide (A/M2) of 97 amino acids long, forms a homotetrameric proton channel that is critical for virus replication [19]. In the viral life cycle, A/M2 is crucial for acidification of the interior of the virus which is required for uncoating of the viral genetic material inside the host cell [20]. Furthermore, A/M2 regulates the intralumenal pH of the Golgi apparatus and in this way prevents the premature conformational change of hemagglutinin, another key viral protein [21]. The A/M2 protein is also the

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target of Amt and hence Amt-resistant influenza strains bear a mutated Amt-insensitive M2 channel. Although many mutations appear to be viable *in vitro* [22,23], only three mutants comprehend more than 99% of the reported resistance [24,25], namely L26F, V27A and S31N. Despite the fact that the S31N amino acid substitution is predominant in more than 98% of the mutated M2 channels found in H3N2 viruses [26], the V27A mutant has been isolated in 10–77% of influenza viruses, depending on the viral strain and season [24,27]. Furthermore, the V27A mutant has proved to be the only one among the three most prevalent mutations to be originated from drug selection pressure [25]. Finally, while the S31N and L26F mutants are sensitive, to some extent, to Amt, the latter is completely ineffective against the V27A mutant M2 channel. Therefore, there is great interest in the development of molecules capable of blocking multiple M2 channels to prevent the virus from finding an escape mechanism.

Wu et al. have very recently reported the synthesis of *N*-[(5-bromothiophen-2-yl)methyl]adamantan-1-amine, **1**, the first dual inhibitor of wild-type (wt) and S31N mutant M2 channels [28]. Our work has been focused on the V27A mutant M2 channel, describing pyrrolidine **2**, the most potent dual inhibitor of wt ($IC_{50} = 3.4\ \mu M$) and V27A ($IC_{50} = 0.29\ \mu M$) mutant M2 channels [29] (Fig. 1). Last year, we reported the triple inhibitor **3**, which is active against wt ($IC_{50} = 18.0\ \mu M$), V27A ($IC_{50} = 0.70\ \mu M$) and L26F ($IC_{50} = 8.6\ \mu M$) mutant M2 channels [30]. Good activities against both wt ($IC_{50} = 2.1\ \mu M$) and V27A ($IC_{50} = 22.6\ \mu M$) mutant M2 channels were found with polycyclic guanidine **4** [30] (Fig. 1), which inspired us to derivatize it in pursue of potent inhibitors of the V27A A/M2 mutant protein.

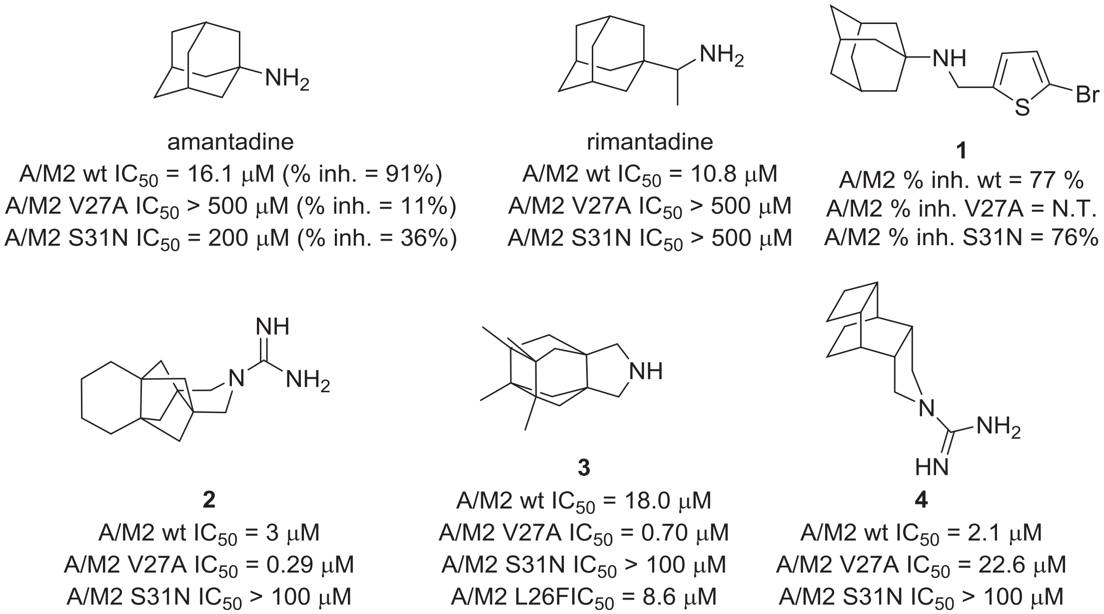
Herein we describe the syntheses and pharmacological evaluations of two families of polycyclic amines. One of them was designed with an enlarged scaffold with respect to **4** in order to explore the chemical space around this skeleton, and the other features a new small polycyclic structure that was also assessed. All the novel compounds were low micromolar inhibitors of the wt M2 channel, and one of them was found to be a submicromolar inhibitor. Besides, amine **10** and guanidine **12** were active blockers of the V27A mutant M2 channel, but strikingly the amine derivative was more potent than the guanidine.

2. Results and discussion

2.1. Chemistry

Our interest in modifying the polycyclic scaffold of Amt for developing novel M2 channel blockers has led to the syntheses of ring-contracted, ring-rearranged and 2,2-dialkyl derivatives of amantadine [31–34]. While exploring new carbon skeletons, we unveiled compound **4** as the only dual inhibitor of its family, with a low micromolar IC_{50} against wt M2 channel ($IC_{50} = 2.1\ \mu M$), and a mid-range IC_{50} against the V27A mutant ($IC_{50} = 22.6\ \mu M$) [30] (Fig. 1). From previous molecular dynamics studies [30], we assumed that both wt and V27A M2 channels may accommodate larger molecules than **4**. Since compound **4** was available in a few synthetic steps, we aimed at enlarging the molecule to evaluate its effects on the blockade of the M2 channels. On the other hand, we were also keen on the development of another small family of compounds easily available in a short synthetic sequence.

The synthesis of the first set of compounds, derivatives of **4**, started with known imide **5** easily accessed through the reaction of maleimide and cycloheptatriene [35]. The Diels–Alder reaction of **5** with 5,5-dimethoxy-1,2,3,4-tetrachlorocyclopentadiene furnished adduct **6** in 89% yield. This one was subjected to a one-pot dechlorination/deprotection reaction. The dechlorination was carried out by metallic sodium in liquid ammonia and an acidic deprotection of the acetal finally produced keto-imide **7** in 41% overall yield. Submitting **7**–180 °C in a sealed tube in the presence of hydroquinone as an antioxidant, produced polycyclic imide **8** through a one-pot decarbonylation coupled with an intra-molecular Diels–Alder reaction [36], in 44% yield over the two steps (Scheme 1). Of note, the reaction started with the retrocheletropic extrusion of a molecule of carbon monoxide followed by two disrotatory retroelectrocyclic rearrangements that provided a molecule with the suitable configuration for the intramolecular Diels–Alder reaction (Scheme 2). Imide **8** was further reduced to amine **9**, and was subsequently submitted to a catalytic hydrogenation to yield saturated amine **10**. Both amines were transformed into their corresponding guanidines **12** and **11** respectively, after being reacted with 1*H*-pyrazole-1-carboxamidine in moderate yields (Scheme 1).



amantadine
A/M2 wt IC_{50} = 16.1 μM (% inh. = 91%)
A/M2 V27A IC_{50} > 500 μM (% inh. = 11%)
A/M2 S31N IC_{50} = 200 μM (% inh. = 36%)

rimantadine
A/M2 wt IC_{50} = 10.8 μM
A/M2 V27A IC_{50} > 500 μM
A/M2 S31N IC_{50} > 500 μM

1
A/M2 % inh. wt = 77 %
A/M2 % inh. V27A = N.T.
A/M2 % inh. S31N = 76%

2
A/M2 wt IC_{50} = 3 μM
A/M2 V27A IC_{50} = 0.29 μM
A/M2 S31N IC_{50} > 100 μM

3
A/M2 wt IC_{50} = 18.0 μM
A/M2 V27A IC_{50} = 0.70 μM
A/M2 S31N IC_{50} > 100 μM
A/M2 L26FIC_{50} = 8.6 μM

4
A/M2 wt IC_{50} = 2.1 μM
A/M2 V27A IC_{50} = 22.6 μM
A/M2 S31N IC_{50} > 100 μM

Fig. 1. Structures of amantadine, rimantadine and some reported compounds with potent activities against the wt, S31N and/or V27A mutants. The IC_{50} values shown correspond to the 50% inhibitory concentrations determined by TEVC assay. The inhibition percentages shown were determined at 100 μM for 2 min by TEVC [28,29]. N.T: Not Tested.