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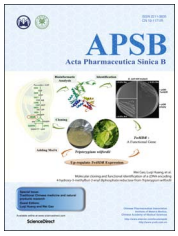

ORIGINAL ARTICLE

3,5-Bis(arylidene)-4-piperidones as potential dengue protease inhibitors

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KEY WORDS

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α,β-Unsaturated ketone;
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Abstract Dengue is a severe mosquito-borne viral infection causing half a million deaths annually. Dengue virus NS2B/NS3 protease is a validated target for anti-dengue drug design. A series of hitherto unreported 3,5-bis(arylidene)-4-piperidones analogues **4a**–**4j** were synthesized and screened *in silico* against DENV2 NS2B/NS3 protease to elucidate their binding mechanism and orientation around the active sites. Results were validated through an *in vitro* DENV2 NS2B/NS3 protease assay using a fluorogenic Boc-Gly-Arg-Arg-AMC substrate. Nitro derivatives of 3,5-bis(arylidene)-4-piperidones (**4e** and **4j**) emerged as promising lead molecules for novel protease inhibitors with an IC_{50} of 15.22 and 16.23 μmol/L, respectively, compared to the standard, panduratin A, having IC_{50} of 57.28 μmol/L.

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1. Introduction

Dengue virus (DENV) is a dreadful arboviral pathogen responsible for the tropical epidemic dengue fever (DF) causing high rates of global morbidity and mortality[1]. According to the World Health Organization (WHO), around 3.9 billion people are currently under high risk of dengue fever infection[2]. DENV infections have now become endemic in more than half of the world and recently an increased number of uncontrolled outbreaks with huge socio-economic implications have been reported[3]. DENVs exist as four closely related antigenic DENV, 1–4 serotypes, but the cross-immunity amongst each other after recovery is only partial and successive infection by different serotypes may worsen the severity due to an "antigen-dependent enhancement" effect (ADE). This ADE effect makes vaccine development against DENVs extremely difficult[4]. Recently, Sanofi obtained first approval for a long-anticipated tetravalent vaccine Dengvaxia® against dengue fever, but its efficacy against the different DENVs is still unclear[5]. Currently, there is no other vaccine or effective anti-viral therapy available in the market for the prevention or treatment of dengue fever. Therefore, there is a pressing need for development of new anti-dengue agents that are effective against all serotypes (Table 1).

The dengue virus genome is a single-stranded RNA encoding three structural proteins *viz*., capsid C, membrane M, and the envelope E along with the non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5 which are processed by trypsin-like NS2B-NS3 protease[6]). The catalytic triad (His51, Asp75, and Ser135) is within the NS3 protease domain, but a region of NS2B is also required for catalytic activity[7]. Protease complex NS2B/NS3 is essential for viral replication and therefore receives considerable attention as a therapeutic target for the development of novel dengue inhibitors[8].

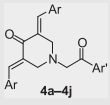
Previous studies have shown that the α,β-unsaturated ketone analogues isolated from *Boesenbergia rotunda* were potent inhibitors of DEN2 serine protease. Among these cyclohexenyl derivatives, 4-hydroxypanduratin A (K_i 21 μmol/L) and panduratin A (K_i 25 μmol/L) emerged as lead molecules for antidengue agents[9]. Subsequently, several 4-hydroxypanduratin analogues with potential dengue inhibitory activity have also been reported[10]. Moreover, 3,5-bis(arylidene)-4-piperidones, which are considered as a structurally distinct class of α,β-unsaturated ketones, possess marked inhibitory activities against viruses[11]. 3,5-Bis(arylidene)-4-piperidone derivatives constitute an important class of therapeutic agents exhibiting anticancer[12], antioxidant[13] and anticholinesterase[14] properties as well. Prompted by the above findings and in continuation of our interest in the biological activities of 3,5-bis(arylidene)-4-piperidone[15], we thought it worthwhile to incorporate the bioactive heterocycle piperidone moiety into α,β-unsaturated ketone mimics with the hope that the piperidone residue may serve as both the donor and acceptor in hydrogen-bonding interactions to improve binding affinity and also to verify its importance for the DEN2 serine protease inhibitory activity (Fig. 1).

2. Results and discussion

2.1. Chemistry

The target compounds 3,5-bis(arylidene)-1-(2-oxo-2-arylethyl) piperidin-4-ones **4a–4j** as depicted in Scheme 1 were obtained by the Claisen–Schmidt condensations of 4-piperidone (**1**) with different aromatic aldehydes in the presence of HCl and acetic acid followed by reaction with substituted phenacyl bromide[16]. The yields of titled compounds ranged from 68% to 87% after recrystallization with ethanol. The purity of the compounds was checked by TLC and elemental analyses. Both analytical and spectral data (NMR and IR) of all the synthesized compounds were in full agreement with the proposed structures. The IR spectrum of compound **4j** showed C=O and C–N stretching vibrations at 1670 and 1232 cm^{-1}, respectively. The ^1H NMR spectral data of compound **4j** showed three upfielded singlets at δ 3.85, 4.07 and 4.11 ppm due to OCH_3, $COCH_2$ and pipridine-methylene (CH_2) protons. The appearance of aromatic protons and disappearance of the NH signal of the piperidine moiety further confirmed the formation of the target compounds. The ^{13}C NMR spectral data of the compound showed three peaks in the aliphatic range of δ 54.23, 55.42 and 61.98 ppm due to pipridine-methylene (CH_2), methoxy and oxoethyl carbons, respectively, whereas two

Table 1 DENV2 NS2B/NS3 protease inhibition activities of 3,5-bis(arylidene)-4-piperidones (**4a–4j**).



4a–4j

Compd.	Ar	Ar'	Binding free energy (kcal/mol)	IC_{50} (μmol/L)[a]
4a	2-CH_3Ph	4-FPh	−10.00	ND[b]
4b	2-ClPh	4-FPh	−10.07	ND
4c	2,4-Cl_2Ph	4-FPh	−10.39	ND
4d	4-FPh	4-FPh	−9.49	ND
4e	4-NO_2Ph	4-FPh	−11.36	15.22±1.10
4f	2-CH_3Ph	4-OCH_3Ph	−10.53	ND
4g	2-ClPh	4-OCH_3Ph	−10.25	ND
4h	2,4-Cl_2Ph	4-OCH_3Ph	−10.11	ND
4i	4-FPh	4-OCH_3Ph	−9.81	ND
4j	4-NO_2Ph	4-OCH_3Ph	−11.09	16.23±1.30
Panduratin A	–	–	−10.10	57.28±1.30

–Not available.

[a]Values are indicated as means±standard deviations from 3 independent experiments performed in triplicate.

[b]ND, Not determined as <10% inhibition at 50 μmol/L.

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