

Synthesis, Biological Evaluation and Molecular Docking Studies of 4-Arylidene-2-phenyloxazol-5(4H)-one Derivatives as Inhibitors of Dual-specificity Tyrosine Phosphorylation-regulated Kinases and Cdc2-like Kinases

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INTRODUCTION

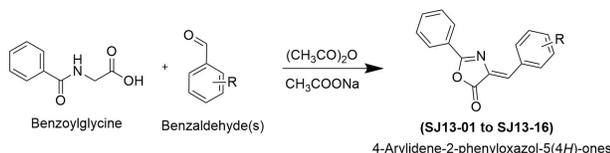
Reversible protein phosphorylation catalysed by protein kinases and phosphatases play an important regulatory role in majority of cellular pathways including metabolism, signal transduction, transcription, translation, cell growth and differentiation¹. Hyperactivity of protein kinases which has been shown to contribute to the pathogenesis of several diseases including cancer, diabetes and neurodegenerative disorders like Alzheimer's disease (AD), Down's syndrome (DS) etc. Inhibition of these kinases using small molecules is a valid therapeutic strategy for treatment of these diseases.

Although there are about 518 kinases in human kinome, our lab focuses on the small molecular intervention of two disease relevant kinases namely, cdc2-like kinase 1 (CLK-1), and dual-specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A). CLK1 and DYRK1A are involved in the regulation of alternative pre-mRNA splicing via SR-protein phosphorylation, and dysfunction of this tightly regulated process is linked to the progression of cancer, neurodegenerative diseases, and viral infections². Hence, targeting CLK1 and DYRK1A is an attractive approach for development of drugs for the treatment of neurodegenerative diseases, cancer, and viral infections.

MATERIALS AND METHODS

Synthesis of 4-Arylidene-2-phenyloxazol-5(4H)-ones (SJ13-01 to SJ13-16): The sixteen 4-Arylidene-2-phenyloxazol-5(4H)-ones were synthesized employing a one step synthetic protocol which involves condensation of benzoylglycine with substituted benzaldehydes in the presence of anhydrous sodium acetate and acetic anhydride (Scheme 1). The structures of the synthesized compounds were confirmed on the basis of their IR, ¹H NMR, and Mass spectral data.

SCHEME -1



Kinase Inhibition Assay

The inhibitory potency of the synthesized compounds were evaluated against CLK-1 and DYRK1A following an assay protocol described elsewhere³. In this protocol, the synthesized compounds were initially tested for *in vitro* kinase inhibition (%) at 10 μ M concentration. The compounds showing less than 50% inhibition were considered inactive ($IC_{50} > 10 \mu$ M) whereas those compounds displaying more than 50% inhibition at this concentration were considered active. The active compounds were further tested over a wide range of concentrations (usually 0.01-10 μ M) and their IC_{50} values were determined from the dose response curves (Sigma-Plot).

Molecular Docking Studies

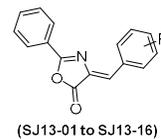
Molecular docking studies were performed to rationalize the effective CLK-1 and DYRK1A inhibition shown by compound 6f. The ATP binding site of CLK-1 (PDB id: 5J1W, 2.42 Å) and DYRK1A (PDB id: 24AZE, 3.15 Å) were used for docking calculations using FlexX docking module in LeadIT package (BioSolveIT, GmbH Germany (version 2.3.2)) using default settings.

CONCLUSION

We have designed and synthesized a series of 4-Arylidene-2-phenyloxazol-5(4H)-ones and evaluated their inhibitory potential against CLK-1 and DYRK1A. Among the 16 derivatives synthesized, compound **SJ13-12** with a 3-chloro and 4-hydroxyl substitution in the 4-arylidene ring demonstrated the best inhibitory profile against both DYRK1A and CLK-1 at submicromolar concentrations. Docking studies with the most potent analog **SJ13-12** showed that the molecule efficiently interacts with the ATP-binding site of both CLK-1 and DYRK1A. The findings from the study establish compound **SJ13-12** as a valuable lead molecule for the development of potent dual inhibitors of CLK-1 and DYRK1A.

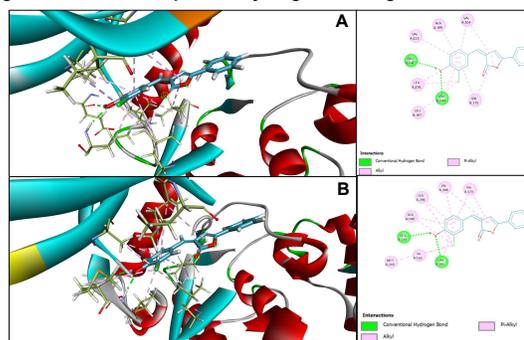
RESULTS

Table 1. Structural Data and Kinase Inhibition Data of 4-Arylidene-2-phenyloxazol-5(4H)-ones (SJ13-01 to SJ13-16).



Compound Code	R	CLK-1 IC_{50} (μ M)	DYRK1A IC_{50} (μ M)
SJ13-01	H	>10	>10
SJ13-02	4-F	>10	>10
SJ13-03	4-Cl	>10	>10
SJ13-04	4-OCH ₃	>10	>10
SJ13-04	4-NO ₂	>10	>10
SJ13-05	4-OH	>10	>10
SJ13-06	3-Cl	>10	>10
SJ13-07	3-NO ₂	>10	>10
SJ13-08	3-NO ₂	>10	>10
SJ13-09	3-Cl	>10	>10
SJ13-10	3-OCH ₃ , 4-OH	>10	>10
SJ13-11	3-OH, 4-OCH ₃	>10	>10
SJ13-12	3-Cl, 4-OH	0.31	0.3
SJ13-13	3-Br, 4-OH	>10	>10
SJ13-14	3,4-diOCH ₃	>10	>10
SJ13-15	3-OC ₂ H ₅ , 4-OH	>10	>10
SJ13-16	3-OCH ₃ , 4-OH, 5-Cl	>10	>10

Figure 1 (A) FlexX predicted binding mode of SJ13-12 in the ATP binding site of CLK-1. Important amino acids are depicted as sticks with the atoms colored as carbon – green, hydrogen – white, nitrogen – blue, oxygen – red, whereas the ligand is shown with the same color scheme as above except for carbon atoms which are represented in Turquoise. The green dotted lines represent hydrogen bonding. **(B)** FlexX predicted binding mode of SJ13-12 in the ATP binding site of DYRK1A. Important amino acids and the ligand are depicted as same color scheme as above. The green dotted lines represent hydrogen bonding.



References:

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