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QSAR analysis of pyrazolidine-3,5-diones derivatives as Dyrk1A inhibitors

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ABSTRACT

Individuals with Down syndrome (DS) suffer from mental retardation. Overexpression and the resulting increased specific activity of Dyrk1A kinase located on chromosome 21 cause a learning and memory deficit in Dyrk1A transgenic mice. To search for therapeutic agents with Dyrk1A inhibition activity, previously we obtained HCD160 as a new hit compound for Dyrk1A inhibition. In the present study, we synthesized 34 HCD160 derivatives to investigate the quantitative structure–activity relationship (QSAR). This analysis could provide important information for novel drug discovery for treatment of DS related learning and memory deficits.

Down syndrome (DS) results from an extra copy of human chromosome 21. With a frequency of one in roughly every 800 births, it is the most common genetic anomaly.[1] In addition to characteristic physical features, individuals with DS show a variety of phenotypes, including mental retardation, low muscle tone, congenital heart defects, gastrointestinal malformations, immune and endocrine system defects, a high incidence of leukemia, and early onset of Alzheimer's disease (AD). Among the phenotypes, mental retardation, such as learning and memory deficit and cognitive decline, is a major factor in preventing DS individuals from leading independent lives in their early to middle-age years.[2] After the third decade of their lives, individuals with DS also develop pathological hallmarks of AD, amyloid plaques and neurofibrillary tangles (NFTs).[3] In the process of searching for genes responsible for these phenotypes, the Dual specificity tyrosine (Y) phosphorylation Regulated Kinase 1A (Dyrk1A) gene was isolated from human chromosome 21.[4] Dyrk1A protein is a serine/threonine kinase known to play a critical role in neurodevelopment, and is activated by autophosphorylation at the Tyr-321 residue.[5] Transgenic mice overexpressing the Dyrk1A protein showed hippocampal-dependent learning and memory deficits.[6] Several recent reports suggested that Dyrk1A may also be involved in the pathological mechanisms of APP and Tau, and may accelerate the formation of amyloid plaques and NFTs which are insoluble deposits of β-amyloid and hyperphosphorylated Tau, respectively, causing the early onset of AD pathogenesis in DS.[7] Thus, the development of therapeutic agents with Dyrk1A inhibition activity will benefit individuals with DS in treating mental retardation and AD.

Harmine and epigallocatechin-3-gallate (EGCG), a major tea compound, were reported as potent Dyrk1A inhibitors in the course of searching for therapeutic agents.[8] Previously, as an initial attempt to develop a mechanism-based drug to treat Dyrk1A associated DS learning and memory deficits, we isolated a synthetic hit compound through a combination of in silico, in vitro, and in cell-based screening.[9] The hit compound, HCD160, appears to be a good starting template for further optimization in order to increase the inhibitory potency against Dyrk1A activity (Fig. 1). In the present study, we further investigated the quantitative structure–activity relationship (QSAR) for 34 pyrazolidine-3,5-diones derivatives of HCD160.

The synthetic strategy was designed to maintain the pyrazolidine-3,5-diones as a core scaffold based on the following observations: (i) the previous result showed that NH and C=O groups of HCD160 bind tightly to the backbones of Glu 239 and Met 240 of Dyrk1A; and (ii) amino acid groups in the ATP binding pocket of Dyrk1A have predominantly negative charges in the surface models as compared with other protein kinases, such as glycogen

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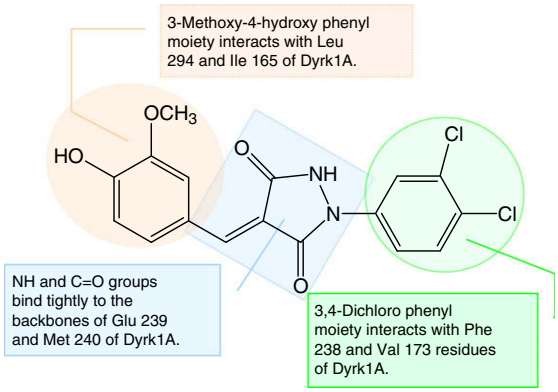

Figure 1. Schematic representation of the refined docking model of HCD160 with Dyrk1A using PharmoMap™.

synthase kinase-3 beta (GSK-3β), cycline-dependent kinase-2 (CDK-2) and casein kinase-2 (CK-2) (Fig. 2).[9,10] In silico screening for the ATP binding pocket was carried out using the PharmoScan™ system, a structure-based in silico screening tool developed by IDRTech Inc.[11] Modifications of the first series were performed to introduce various substitutions with stronger hydrogen bond interaction than that in the 3-methoxy-4-hydroxyphenyl moiety of HCD160 in the Dyrk1A ATP binding site (Table 1). The second series were modified to obtain various 1,4-disubstituted pyrazolidine-3,5-diones derivatives (Table 2). All the desired derivatives were prepared for testing via an in vitro Dyrk1A inhibition assay as previously described.[9] In order to evaluate the inhibitory potency against Dyrk1A activity, two types of inhibition assays were performed; autophosphorylation of Dyrk1A and kinase reaction on substrate phosphorylation.

The assay results of the first series of 19 HCD160 derivatives are summarized in Table 1. Seven compounds (**2**, **3**, **8**, **13**, **15**, **17** and **18**) show inhibitory activity against Dyrk1A autophosphorylation with IC_{50} values ranging from 0.6 to 20 μM (Table 1). Among them, compound **18**, with IC_{50} values of 0.6 μM, shows roughly fourfold increased inhibitory activity on autophosphorylation relative to that of HCD160, which showed IC_{50} values of 2.5 μM. However, only compounds **2** and **18** showed similar inhibition activity for autophosphorylation and kinase reaction, while other compounds exhibited no inhibition activity for kinase reaction at a concentration of 10 μM. This suggests the operation of different inhibition mechanisms between Dyrk1A autophosphorylation and substrate phosphorylation. Roscovitine, used as an internal control, showed less inhibition activity, with IC_{50} values of 5 μM for autophosphorylation and 63% inhibition of kinase activity.[8] The QSAR values for the second series of 15 HCD160 derivatives with 1,4-disubstituted pyrazolidine-3,5-diones are reported in Table 2. Compounds **21**, **30** and **34** exhibited inhibition activity against Dyrk1A autophosphorylation with IC_{50} values of 0.6, 1.2 and 2.5 μM, respectively, while the kinase activity was inhibited by 44–78%. Among the three compounds, compound **21** showed similar inhibition activity to that of compound **18** on autophosphorylation and kinase reaction. Therefore, we have described the synthetic methods of compounds **18** and **21** in the references and note paragraph.[12]

Most of the compounds tested in the present study showed less potent inhibition activity than that of HCD160. However, the effect of compounds **18** (IC_{50} = 0.6 μM) and **21** (IC_{50} = 0.6 μM) on autophosphorylation were very potent as compared with that of HCD160 (IC_{50} = 2.5 μM). And also, IC_{50} for compounds **18** and **21** on the substrate phosphorylation were 1.25 μM and 6 μM, respectively. Therefore, we further characterized these two compounds **18** and **21** by assessing the specificity of the inhibition and cytotoxicity. To evaluate the specificity, we performed comparative assay for them with other well-characterized kinases (Table 3). Selected kinases included a Dyrk family protein (Dyrk2) and neighboring proteins in the dendrogram constructed by Becker and Joost, such as CLK3 and GSK3β.[13] Other tested proteins represented kinases from the core panel described by Davies et al. and from kinase groups described by Vieth et al.[14] As expected from its close relationship to Dyrk1A, compound **18** showed a strong inhibition (83%) for Dyrk2 while compound **21** was much less inhibitory (21%). For GSK3β we have modeled the active site of Dyrk1A, compounds **18** and **21** showed no inhibition and 24% inhibition, respectively. For other tested kinases, two compounds showed a moderate (less than 30%) inhibition. The CC_{50} values (50% cytotoxic concentration) for compounds **18** and **21** obtained by WST-1 assay are >500 μM and >250 μM, respectively. We also tested if compounds **18** and **21** compete with ATP in the Dyrk1A ATP binding pocket. We observed that the inhibitory activities of these compounds were decreased when the reactions were performed at the increased ATP concentrations, indicating that inhibitors behave as competitors of ATP as we expected from the modeling (data not shown).

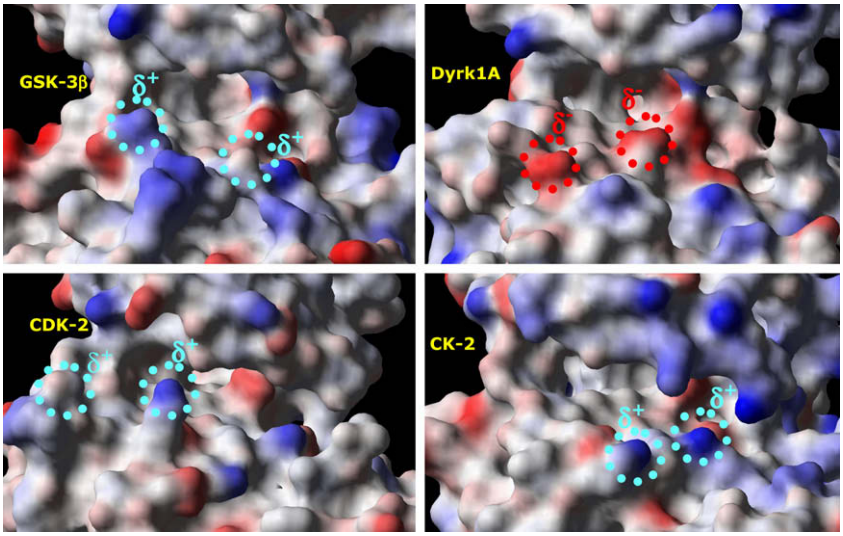

Figure 2. Surface models of the ATP binding sites of GSK-3β, Dyrk1A, CDK-2 and CK-2 (blue: positive charged region and red: negative charged region).