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Short communication

# Discovery of pyrido[3,4-*g*]quinazoline derivatives as CMGC family protein kinase inhibitors: Design, synthesis, inhibitory potency and X-ray co−crystal structure

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ABSTRACT

The design and synthesis of new pyrido[3,4-*g*]quinazoline derivatives is described as well as their protein kinase inhibitory potencies toward five CMGC family members (CDK5, CK1, GSK3, CLK1 and DYRK1A). The interest for this original tricyclic heteroaromatic scaffold as modulators of CLK1/DYRK1A activity was validated by nanomolar potencies (compounds **12** and **13**). CLK1 co−crystal structures with two inhibitors revealed the binding mode of these compounds within the ATP-binding pocket.



## 1. Introduction

Alzheimer's disease (AD) is expanding dramatically in association with population ageing. This neurodegenerative disorder is characterized by the apparition of intracellular neurofibrillary tangles containing microtubule associated Tau proteins and extracellular amyloid plaques that accumulate in brain. The control of microtubule stabilization is mediated by Tau interaction with tubulin and phosphorylation; abnormal phosphorylation of Tau could lead to neuronal cytoskeleton disruption. Additionally, increased phosphorylation of Tau leads to its aggregation into filaments that could be responsible for neuronal death [1,2]. Various protein kinases participate in the regulation of Tau by site-specific phosphorylation. CDK5, CK1, GSK3 and DYRK1A are involved in the formation of neurofibrillary tangles by abnormal Tau phosphorylation on AD-specific sites leading to the formation of filaments [3–7]. Moreover, CLK1 and DYRK1A participate in alternative pre-mRNA splicing, a physiological process that is altered in AD [8,9]. Due to the important physiological functions of these kinases, the development of multi-target directed ligands (MTDLs), inhibitors that could target more than one of them, is of high interest and may allow the development of new biological tools/therapies to better understand/treat AD [10].

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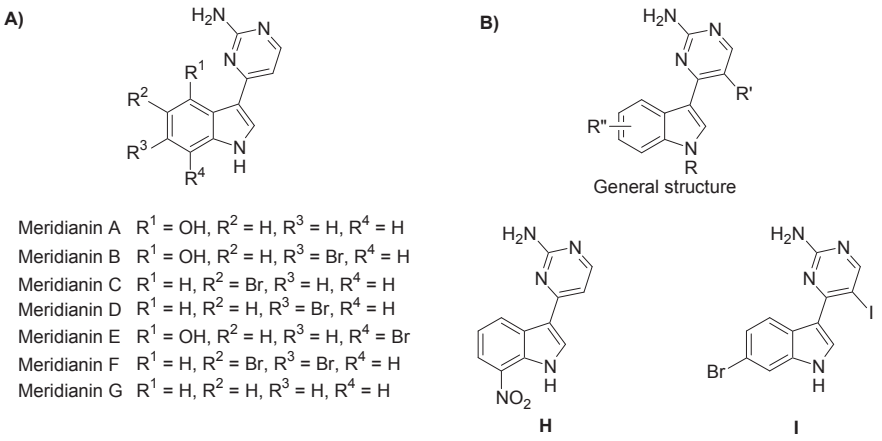


**Fig. 1.** A) Chemical structure of meridianins A–G, B) General structure of meridianin analogues and two DYRK1A/CLK1 inhibitors (**H**, **I**) described by our group.

Meridianins A–G (Fig. 1A), indolic derivatives substituted by a 2-aminopyrimidine ring, are natural products isolated from *Aplidium meridianum* ascidiae [11–13]. Recently, we described the synthesis of diversely substituted meridianin G analogues. In order to identify new tools to study AD, these compounds were evaluated on a panel of five AD-relevant members of the Ser/Thr kinases CMGC family (CDK5, CK1, GSK3, CLK1, DYRK1A) (Fig. 1B) [14–17].

The structure–activity relationship study performed on meridianin derivatives showed that the introduction of a bromine atom or a nitro group at the 6- or 7-position of the indolic moiety could result in DYRK1A/CLK1 inhibitors with nanomolar potencies (Fig. 1B, compounds **H**, **I**). Because of these interesting results, we decided to extend the structure–activity relationship study in this aminopyrimidine series. The putative binding mode between the ATP binding site of CLK1/DYRK1A and most active compounds of the series revealed that the aminopyrimidine moiety was oriented toward the bottom of the ATP-binding pocket, establishing two hydrogen bonds with the hinge [17]. In order to develop a second generation of inhibitors, we first decided to restrict the conformation between the aminopyrimidine and indole moieties. For this purpose these rings were connected by an additional phenyl ring. Next, we noticed that the 3-aminopyrimidinylindole chemical scaffold could be simplified by removing the pyrrole moiety that is not directly involved in the molecular interaction within the ATP-binding pocket. Finally, a pyridine moiety was introduced to reinforce the H-bonding network within the ATP-binding site (Fig. 2).

To validate this model, the synthesis of this novel pyrido[3,4-*g*] quinazoline series was undertaken. The inhibitory potency of new compounds on a panel of five kinases (CDK5/p25, CK1δ/ε, GSK-3α/β, DYRK1A and CLK1) was examined. To interact with targeted kinases, potential inhibitors should cross the blood–brain barrier (BBB) therefore physical properties that influence BBB permeability were also calculated. Finally the binding mode of this series within the CLK1 ATP-binding pocket was elucidated by X-ray crystallography.

## 2. Results and discussion

The preparation of the targeted molecules (Scheme 1) starts from commercially available 2-chloro-4-nitrobenzoic acid **1** that was first esterified using ethanol in the presence of sulfuric acid to give **2** in 98% yield. The reduction of the nitro group [18] of **2** led to amino derivative **3** that was iodinated by treatment with iodine and silver sulfate [19] to give **4**, as the major regioisomer, in 65% yield. The CuCN-mediated cyanation of **4** was performed in good yield utilizing a modified Rosenmund-von Braun procedure in the presence of L-proline according to Wang et *al.* [20]. Next, the amino group of **5** was substituted using a Sandmeyer type reaction to give its iodo analogue **6** in 84% yield [21]. The treatment of **6** by DIBAL-H led to **7** in 87% yield by ester and nitrile reduction [22]. Then, Sonogashira cross-coupling reaction of **7** with TMS-acetylene gave compound **8** in 87% yield [23,24]. The isoquinoline moiety was formed under microwave irradiation of **8** in the presence of ammonia in methanol [25]. Finally, oxidation of **9** using $MnO_2$ led to 6-chloroisoquinoline-7-carbaldehyde **10** in 88% yield (Scheme 1) [26].

The formation of the aminopyrimidine part was initially envisaged by reacting chloroaldehyde **10** with a guanidine salt. However, under the conditions tested, we never managed to get the expected aminopyrimidine. Therefore, in order to get a more activated product for the cyclization, a nitro group was regioselectively introduced on compound **10** leading to **11**.

Then, as anticipated the coupling reaction between **11** and

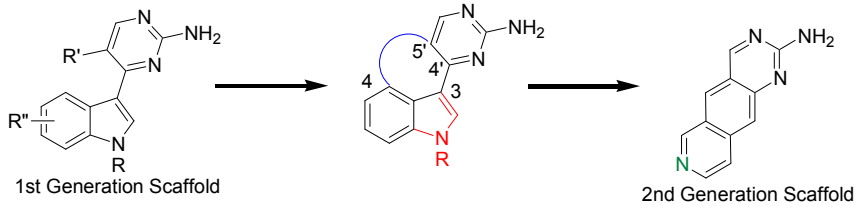


**Fig. 2.** Design of the second generation scaffold.