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Synthesis and biological evaluation of 3,6-diamino-1*H*-pyrazolo[3,4-*b*]pyridine derivatives as protein kinase inhibitors

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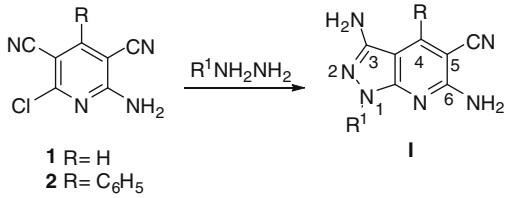
ABSTRACT

The synthesis and biological evaluation of a number of differently substituted 3,6-diamino-1*H*-pyrazolo[3,4-*b*]pyridine derivatives are reported. From the inhibition results on a selection of disease-relevant protein kinases [IC_{50} (μM) DYRK1A = 11; CDK5 = 0.41; GSK-3 = 1.5] we have observed that 3,6-diamino-4-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (**4**) constitutes a potential new and simple lead compound in the search of drugs for the treatment of Alzheimer's disease.

The pyrazolo[3,4-*b*]pyridine ring system[1] is present in a number of pharmaceutically important compounds targeted, for instance, to inhibit glycogen synthase kinase-3 (GSK-3).[2] Protein kinases occupy a central stage in signal transduction pathways, and kinase inhibition has become a major area for therapeutic discovery in view of the abnormal kinase regulation displayed in many diseases. Among the human kinases,[3] the cyclin-dependent kinases (CDKs),[4] and GSK-3[5] have been particularly explored. GSK-3 is a regulatory serine/threonine kinase implicated in the control of several proteins, which exists as two closely related isoforms (α and β) (84% overall identity −98% within their catalytic domains).[6] GSK-3 is known to play a key role in chronic inflammatory processes,[7] cancer,[8] and Alzheimer's disease (AD).[9] On the other hand, CDK5 inhibitors are expected to be active in the nervous system, via inhibition of neurotrophic pathways, and anti-apoptotic protection from neurofibrillary degeneration in AD.[10] In this context, and regarding a therapeutic strategy, it is nowadays generally accepted that inhibitors should be selected more on the basis of their inhibitory profile on several key kinases implicated in a particular disease rather than on potent inhibition of a single target.[11] Consequently, multitarget inhibitors acting on different kinases (GSK-3, CDK5, etc.) could therefore become of great therapeutic value.

In our search for new drugs for the treatment of AD,[12] we have now embarked in a project targeted to the discovery of new pyrazolo[3,4-*b*]pyridines able to inhibit GSK-3. We describe here that 3,6-diamino-4-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (**4**) is a dual GSK-3/CDK5 inhibitor, and a potential scaffold for the design of new compounds for treatment of Alzheimer's disease.[13]

The starting compounds in this project were the readily available 2-amino-6-chloropyridine-3,5-dicarbonitriles (**1**)[14] and (**2**).[15] The presence of the 2-chloropyridine-3-carbonitrile moiety in these precursors should allow us to carry out the reaction with hydrazines to prepare the pyrazolo[3,4-*b*]pyridine ring, leaving the amino group at C6 free for further transformation (Chart 1).



1 R= H
2 R= C_6H_5

I

Chart 1. General synthetic approach to 3-6-diamino-1*H*-pyrazolo[3,4-*b*]pyridines (**I**).

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Very interestingly, 3,6-diaminopyrazolo[3,4-*b*]pyridine (**I**) derivatives[16] have been scarcely investigated, and their potential biological interest as protein kinase inhibitors[2] has not been explored.

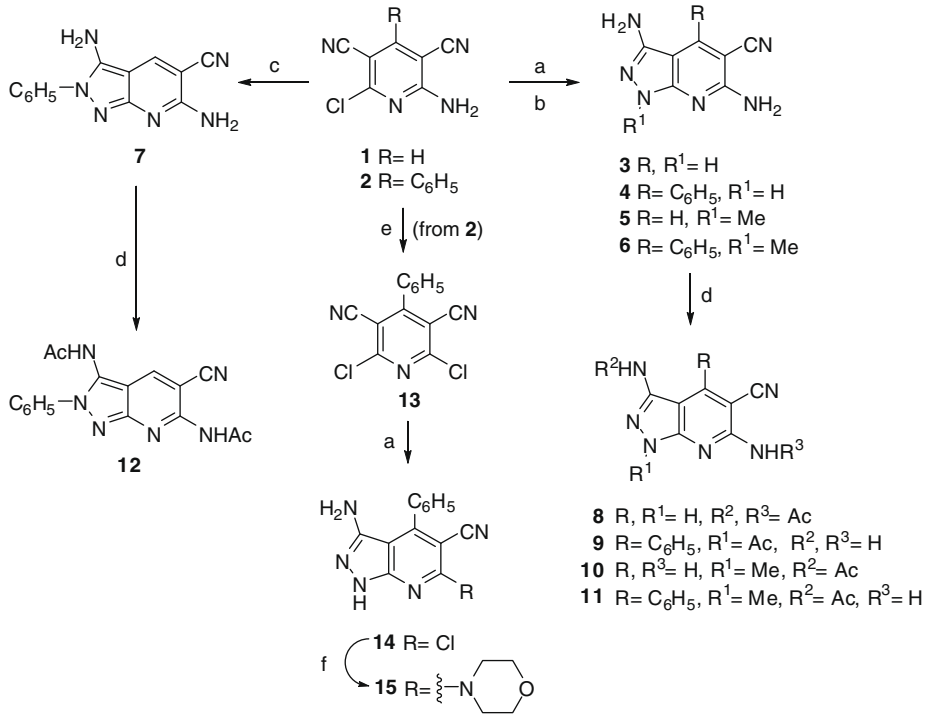
The reaction of compounds **1** and **2** with hydrazine hydrate gave compounds **3**[16a] and **4**,[16c,d] respectively, as expected (Scheme 1).[17] Using methylhydrazine, precursors **1** and **2** afforded the expected pyrazoles **5**[16a] and **6**, respectively (Scheme 1).[18] Similarly, the reaction of pyridine **1** with phenylhydrazine gave pyrazolopyridine **7** in good yield (Scheme 1).[18] Next, we submitted compounds **3**–**7** to acetylation to give mono- or diacetylated derivatives **8**–**12**[17] from low to good yields (Scheme 1).[19] The Sandmeyer-type reaction[20] of compound **2** gave known 2,6-dichloro-4-phenylpyridine-3,5-dicarbonitrile (**13**),[21a] which was further functionalized, leading to the 6-chloro-pyrazolopyridine **14**.[17] Reaction with morpholine provided derivative **15**[17] with a 71% yield (Scheme 1).

Finally, we prepared the known pyridine **17**,[21b] from readily available Soto's 2-aminopyridine **16**,[22] aiming at the synthesis of compounds with the pyrazole ring fused moiety bearing oxygenated groups at C6. The reaction of chloride **17** with hydrazine, under mild conditions, provided the 'open' hydrazine derivative **18**, which after acetylation gave molecule **19** (Scheme 2).[17]

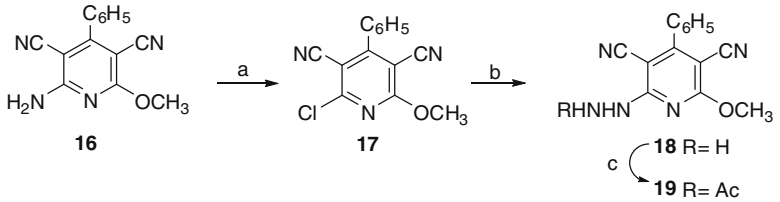
Compounds **3**–**12**, **14**, **15**, **18** and **19** were tested as potential inhibitors of three protein kinases relevant to AD: DYRK1A, CDK5/p25 and GSK-3α/β (see Supplementary data).[23] However, only compound **4** showed significant and interesting inhibitory activities [IC_{50}: 11 μM (DYRK1A), 0.41 μM (CDK5/p25) and 1.5 μM (GSK-3α/β)] (Table 1, Chart 2), while the other compounds were inactive at the highest concentration tested (10 μM) (Table 1).

Table 1
Inhibition activities IC_{50} (μM) for compounds **3**–**12**, **14**, **15**, **18** and **19**

Compound	DYRK1A	CDK5	GSK3
3	>10	>10	>10
4	11	0,41	1,5
5	>10	>10	>10
6	>10	>10	>10
7	>10	>10	>10
8	>10	>10	>10
9	>10	>10	>10
10	>10	>10	>10
11	>10	>10	>10
12	>10	>10	>10
14	>10	>10	>10
15	>10	>10	>10
18	>10	>10	>10
19	>10	>10	>10



Scheme 1. Reagents and conditions: (a) $NH_2NH_2{\cdot}H_2O$, 153 °C [**1**–**3** (DMF, 30 min, 87%); **2**–**4** (DMF, 1 h, 74%); **13**–**14** (EtOH, 1 h 68%)]; (b) NH_2NHMe, DMF, 153 °C [**1**–**5** (15 min, 99%); **2**–**6** (5 min, 80%)]; (c) $PhNHNH_2$, DMF, reflux, 1 h (70%); (d) Ac_2O [from **3**: 144 °C, 21 h, **8** (8%); from **4**: 0 °C, 20 h, **9** (10%); from **5**: rt, 3 h, **10** (82%); from **6**: rt, 8 h, **11** (21%); from **7**: 144 °C, 40 min, **12** (21%)]; (e) $(CH_3)_3CCH_2NO_2$, $CuCl_2$, CH_3CN, 65 °C, 8 h (68%); (f) morpholine, TEA, THF/EtOH (1/3), 70 °C, 5 d (71%).



Scheme 2. Reagents and conditions: (a) $(CH_3)_3CCH_2NO_2$, $CuCl_2$, CH_3CN, 65 °C, 4 h (80%); (b) $NH_2NH_2{\cdot}H_2O$, EtOH, reflux, 10 min (72%); (c) Ac_2O, 0 °C, 8 h; rt, 24 (84%).

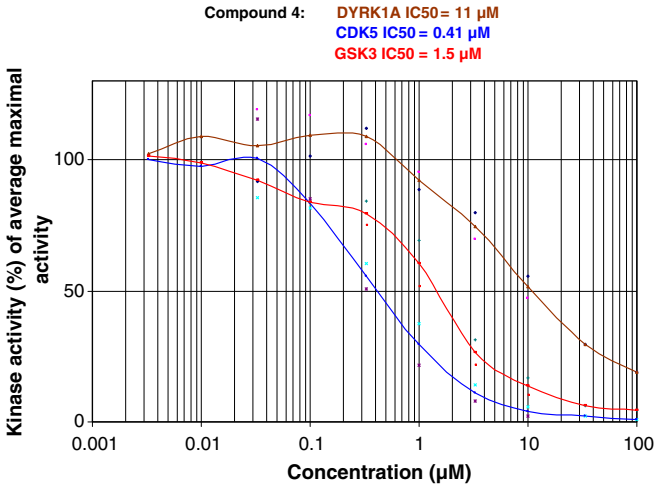


Chart 2. Kinase activity (%) of average max. activity for compound **4**.

In order to gain better insight into the binding mode of this family of compounds to the GSK-3β,[24] a docking study was undertaken for **4** and **6**, as active and inactive compounds, respectively, by means of the automated programme AutoDock. Docking results of compound **4** in the protein target (PDB code 1J1B) provided different solutions within an energy range of −11.4 to −7.9 kcal mol^{-1}, being the most populated cluster (68/100 solutions) in the range of −11.1 to −10.4 kcal mol^{-1} In this cluster, the ligand establishes three hydrogen bonding interactions within the ATP binding site. A related binding mode has been reported by Witherington et al.[2a] for pyrazolopyridazine **20**. However, important differences regarding the H-bonding pattern and ligand orientation in the binding site should be noted (Fig. 1). For **4**, the amino substituent at the pyridine ring drives the binding mode as it forms a hydrogen bond with the backbone CO of Asp133. This allows the pyridinic N and NH group of the pyrazolic ring to make two hydrogen bonds with the backbone NH and carbonyl of Val135, respectively (Fig. 2a). This region of protein kinases makes critical H-bonding contacts to the vast majority of inhibitory molecules that have been published to date. This orientation of the aromatic core places the phenyl substituent at C-4 in the hydrophobic pocket at the back of the ATP binding site, located between the two lobes N-terminal lobe, mostly consisting of β-sheets, and a large C-terminal lobe, essentially formed of α-helices. The presence of the aromatic ring appears to be critical as its removal results in a complete loss of activity (compare compound **3** with inhibitor **4**). This cavity can accommodate the aromatic ring through hydrophobic interactions; furthermore, it would be a potential optimisation site as some residues could make establish further interactions with polar groups.

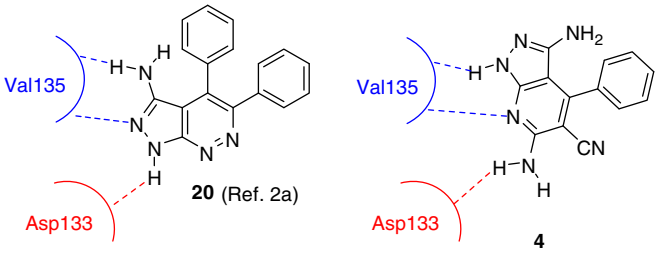


Figure 1. Schematic representation of the binding mode of **20** and **4** inside the GSK-3β binding site. To note that the 4-phenyl substituent in **4** places in the same orientation as the 5-phenyl ring in the Whiterington's model.

It can be envisaged that the absence of one of these key three points of H-bonding lead to a loss of potency. Thus, docking results for **6** revealed a lack of consistent binding mode (only 8/100 solutions placed the ligand inside the binding site, energy range −9.4 to −8.6 kcal mol^{-1}). The removal of the pyrazolic proton reduces the binding energy and induces a displacement of the skeleton from the binding site, as compared with **4** by superposition (Fig. 2b), to avoid steric repulsions between the methyl substituent and the backbone carbonyl of Val135. This would account for the low inhibitory activity of **6** and **9**.

A binding mode similar to the Whiterington's model (Fig. 1), in which the 3-amino group binds to the hinge region of the kinase and the 4-phenyl group keeps close to the lipophilic Gatekeeper residue, could also explain the lack of activity in absence of the 4-aromatic substituent. Nevertheless, this alternative binding mode has not been observed from our docking analysis.

To sum up, we have reported for the first time the protein kinase inhibition of 3,5-diamino-1*H*-pyrazolo[3,4-*b*]pyridine derivatives. For the active compound **4**, the molecular modelling predicts a new paradigm,[2] where the C(6)-NH_2 substituent at the pyridine ring drives the binding mode as it firmly binds to Asp133, allowing the nitrogen on the pyridine and NH group of the pyrazole ring to make two hydrogen bonds with the backbone NH and carbonyl of Val135. In addition, this analysis explains the inhibitory profile observed regarding the N(1) –Me and C(4)-Ph substituents, and suggests that the aromatic ring and C(5) would be potential positions for further optimizations of this family of compounds. Finally, new derivatives of inhibitor **4** functionalized at C(3)NH_2 should be also prepared in order to test the effects of the amino group of the pyrazolic ring.[25] Work is now in progress to prepare such molecules, and results will be reported in due course.

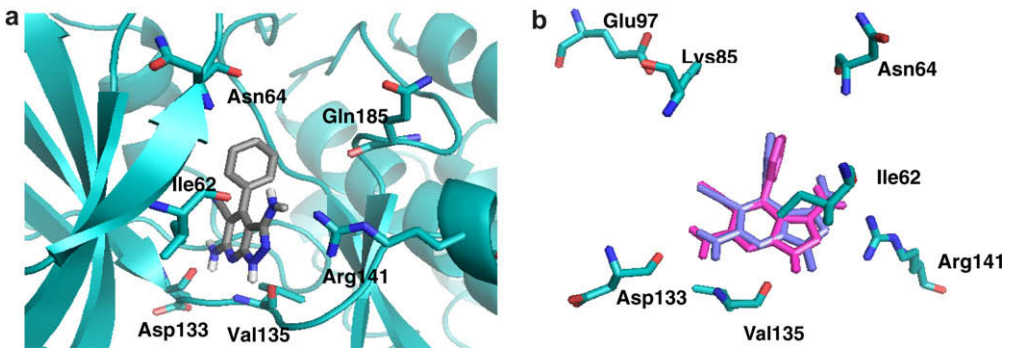


Figure 2. (a) Compound **4** docked pose into the binding site of GSK-3β. Relevant residues of the site are shown by sticks. (b) Superimposition of the docking solutions for compound **4** (pink) and **6** (violet) in the binding site.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.06.099.

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25. We thank one the reviewers for this suggestion. The preparation of these new molecules needs a new synthetic plan, now in progress in our laboratory, based on the reaction of compound **2** with benzylhydrazine, pyrazole formation, regioselective N-acylation, and N1 deprotection.