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6-Aryl-pyrazolo[3,4-*b*]pyridines: Potent Inhibitors of Glycogen Synthase Kinase-3 (GSK-3)

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Abstract—A novel series of 6-aryl-pyrazolo[3,4-*b*]pyridines has been identified that are potent inhibitors of glycogen synthase kinase-3 (GSK-3).

Glycogen synthase kinase-3 (GSK-3) is a serine/threonine kinase implicated in the control of several regulatory proteins.[1] It was first discovered by virtue of its ability to phosphorylate and inactivate glycogen synthase, the regulatory enzyme of mammalian glycogen synthesis. Since then a number of other substrates have been identified, implicating GSK-3 in the regulation of several physiological processes. Recently we identified pyridazine **1** through pharmacophore searching of the SmithKline Beecham in house database.[2a] Simplification of pyridazine **1** afforded a series of pyrazolo[3,4-*b*]pyridines[2a] and pyrazolo[3,4-*b*]pyridazines[2b] that were potent inhibitors of GSK-3 (Fig. 1).

The proposed rationale for this increased potency of the pyrazolo[3,4-*b*]pyridazines was confirmed by X-ray crystallography to be due to a hydrogen bond between the N-6 position of the inhibitor and a water lattice (Fig. 2a). Analysis of the binding orientation suggested to us that it maybe possible to displace the structural waters from the binding site via insertion of a suitably functionalised group at the 6-position of the pyrazolo[3,4-*b*]pyridine nucleus. In order to test this hypothesis, a small set of functionalised 6-aryl pyridines were designed and prepared (Table 1). The *para* phenol derivative **5**, demonstrated a dramatic improvement in potency compared to the parent phenyl analogue **4**. From modelling studies, it was predicted that the phenol moiety was involved in a H-bonding interaction with Glu97 and Asp200 (Fig. 2b). The *para* methoxy analogue **6** was inactive, possibly due to a steric clash with Glu97.

Interestingly, the *meta* phenol analogue **8** displayed comparable potency to the *para* phenol analogue **5**. Modelling studies predicted this also to be as a result of a hydrogen bonding network with Glu97 and Asp200 as proposed for the *para* phenol analogue **5**. Support for this rationale comes from the finding that analogue **7**, which has a hydroxyl moiety at both the 3- and 4-positions, displays comparable but not additive, potency to both **8** and **5**, suggesting these analogues do indeed interact with the same residues. Although the *ortho* phenol analogue **10** is more potent than the parent phenyl analogue **4** it is less potent than either the *para* or *meta* analogues. Having investigated the SAR around the C-6 position of the pyrazolopyridine nucleus, we sought to explore the C-5 position to see if additional potency could be achieved (Table 2). Interestingly, insertion of bromine at the C-5 position afforded an improvement in potency (cf **5** and **13**, **4** and **17**). Several hypotheses could explain the increase in potency observed. Firstly, the group at the C-5 position further occupies the ATP binding site and thus the inhibitor has greater surface contact with the enzyme. Secondly, there is a specific hydrophobic interaction with the bromine

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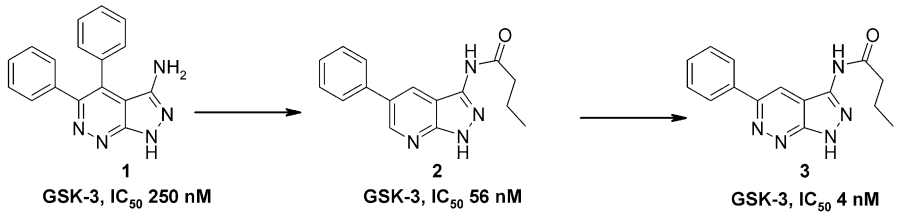


Figure 1. Optimisation of pyridazine 1.

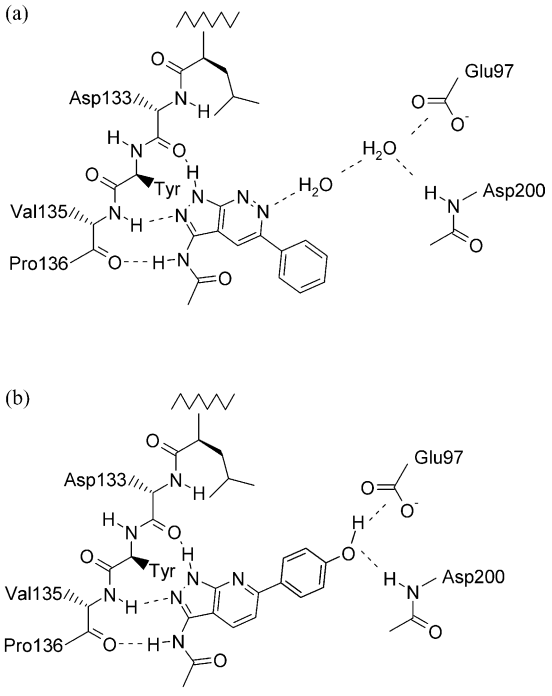
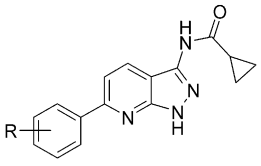


Figure 2. (a) X-ray co-crystallisation of pyrazolo[3,4-*b*]pyridazines with GSK-3; (b) model of 6-Aryl pyrazolo[3,4-*b*]pyridines.

atom at the C-5 position and the ATP binding site. Thirdly, the substituent at the C-5 position modulates the acidity of the pyrazolo ring, and thus further optimises the hydrogen bonding interaction with Asp133 of the hinge region of the ATP binding site. The SAR obtained to date may support the latter argument, given

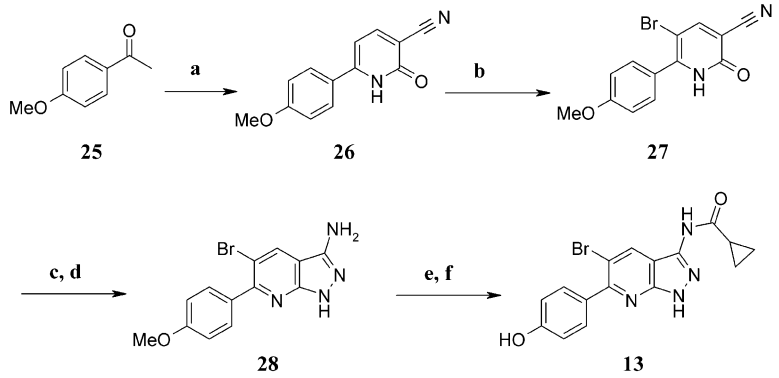
Table 1. Inhibition of hGSK-3α by selected 6-aryl-pyrazolo[3,4-*b*]pyridine analogues[3]



Compd	R	GSK-3α (IC_{50} nM)
4	Ph	425
5	4-OH	8±1
6	4-OMe	>5000
7	3,4-di-OH	8±1
8	3-OH	12±3
9	3-OMe	125±26
10	2-OH	36±11
11	2-OMe	1593

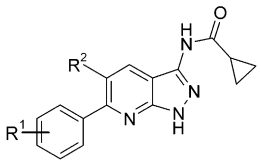
that a phenyl or methyl group at the 5-position does not lead to an increase in potency (cf **5** with **12**, **4** with **16**), while introduction of alternative electron withdrawing groups such as chlorine or a nitrile group also affords an increase in potency (cf **5** with **14**, **4** with **19**).

Previously, in the structurally related 5-aryl-pyrazolo[3,4-*b*]pyridazine series, we had identified the amide moiety as a position of the molecule that could tolerate the incorporation of tertiary amines and thus potentially increase aqueous solubility.[2b] Attachment of basic amines onto the side chain of the amide moiety was also in tolerated in this series without significant loss of potency (Table 3).



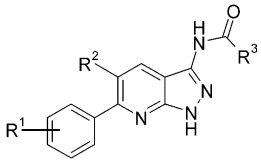
Scheme 1. Preparation 6-aryl-pyrazolopyrazolo[3,4-*b*]pyridine **13**. Reagents: (a) (i) DMF–DMA reflux, 12 h; (ii) 2-cyanoacetamide, NaOMe, MeCN, 80 °C (90%, two steps); (b) NBS, DMF, reflux (88%); (c) $POCl_3$, reflux (100%); (d) $N_2H_2{\cdot}H_2O$, EtOH, reflux, 12 h (77%); (e) BBr_3, 25 °C, 16 h (75%); (f) cyclopropyl carbonyl chloride, pyridine, reflux (90%).

Table 2. Inhibition of hGSK-3α by selected C-5 substituted 6-aryl-pyrazolo[3,4-*b*]pyridine analogues



Compd	R^1	R^2	GSK-3α, (IC_{50} nM)
4	H	H	425
5	4-OH	H	8±1
12	4-OH	Ph	24±3
13	4-OH	Br	0.8±0.4
14	4-OH	Cl	1±1
15	4-OH	Me	6±1
16	H	Ph	415±123
17	H	Br	75±10
18	H	Cl	234±32
19	H	CN	87±19

Table 3. Inhibition of hGSK-3α by 6-aryl-pyrazolopyridines containing basic side chains



Compd	R^1	R^2	R^3	GSK-3α, (IC_{50} nM)
20	H	Br	4-Piperidine-N-Me	383±59
21	4-OH	Br	$(CH_2)_3$piperazinyl-N-Et	4±1
22	4-OH	H	4-Piperidine-N-Me	12±2
23	4-OH	Br	4-Piperidine-N-Me	1±1
24	3-OH	H	$(CH_2)_3$piperazinyl-N-Et	21±3

Chemistry[4,5]

The pyrazolo[3,4-*b*]pyridine analogues were prepared following the general procedure outlined for analogue **13** in Scheme 1. Treatment of the commercially available ketone **25** with DMF–DMA at reflux afforded the intermediate dimethylaminopropenone, which was cyclised without purification to afford the pyridone **26**. Selective bromination at the 3-position of the pyridone employing NBS at reflux afforded **27** in excellent yield. Subsequent treatment with phosphorus oxychloride at reflux, followed by cyclisation of the intermediate chloronitrile with hydrazine afforded the amine **28**. Demethylation employing BBr_3 followed by subsequent selective N-3 acylation afforded the desired pyrazolo[3,4-*b*]pyridine **13** in excellent overall yield.

Introduction of a nitrogen atom into the 6-position of a series of pyrazolo[3,4-*b*]pyridines had previously been demonstrated to lead to a dramatic improvement in GSK-3 potency. Subsequent X-ray crystallography studies with these inhibitors indicated a hydrogen bond between the nitrogen at the 6-position and the water lattice. Utilising this structural knowledge, modification of the template to incorporate functionality at the 6-position capable of displacing these structural water molecules afforded a novel series of potent GSK-3 inhibitors.

Acknowledgements

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References and Notes

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3. Results are a mean of at least two determinations run in duplicate ($n=4$) and are given as mean. Mean±SEM are also quoted for compounds of specific interest.

4. Assay conditions described in ref 2a.

5. All novel compounds gave satisfactory analytical data in full agreement with their proposed structures.