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

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Abstract—A series of 6-heteroaryl-pyrazolo[3,4-*b*]pyridines has been optimised to afford potent inhibitors of Glycogen Synthase Kinase-3 (GSK-3). These analogues display excellent selectivity over the closely related Cyclin Dependant Kinase-2 (CDK-2).

Glycogen synthase kinase-3 (GSK-3) a serine/threonine kinase was first discovered by virtue of its ability to phosphorylate and inactivate glycogen synthase, the regulatory enzyme of mammalian glycogen synthesis. A number of other substrates have since been identified, implicating GSK-3 in the regulation of several physiological processes.[1] As a result, GSK-3 inhibition has emerged as an attractive therapeutic target for the treatment of numerous serious pathologies, including Alzheimer's disease, stroke, bipolar disorders, chronic inflammatory processes, cancer and Type II diabetes.[2] In the preceding papers, we have discussed the rational design that led to the identification of a novel series of GSK-3 inhibitors based upon a 6-aryl-pyrazolo[3,4-*b*]pyridine nucleus (Fig. 1).[3]

Due to the high degree of homology between GSK-3 and Cyclin Dependant Kinase-2 (CDK-2) we decided to profile a range of a 6-aryl-pyrazolo[3,4-*b*]pyridines against this kinase (Table 1). Unfortunately whilst incorporation of a hydroxyl group at either the *para* or *meta* position of the C6-phenyl ring afforded an excellent improvement in GSK-3 potency (cf **3** with **5** and **10**), we also observed a concomitant increase in CDK-2 inhibition. Attempts to introduce CDK-2 selectivity by variation of the group at C-5 (cf **5** with **4** and **8**) or by flanking the phenol moiety (cf **5** with **6** and **7**) proved unsuccessful. Previously, in the structurally related 5-aryl-pyrazolo[3,4-*b*]pyridazine series, we had demonstrated that incorporation of a tertiary amine moiety into the amide side chain led to an excellent improvement in CDK-2 selectivity.[3b] Incorporation of this modification into the 6-aryl series did indeed afford inhibitors with improved selectivity (e.g., **9**), although intrinsic CDK-2 inhibitory potency was still high. From modelling studies the increase in both GSK-3 and CDK-2 potency by introduction of the hydroxyl group can be rationalised from the finding that Glu97 and Asp200, the proposed residues interacting with the hydroxyl moieties, are conserved in all kinases. Indeed from profiling of the phenols **4** and **5** against a panel of kinases a worsening in the overall selectivity profile was observed compared with the phenyl analogue **3** (Table 2).

Although we had demonstrated that selectivity against CDK-2 was a generic issue for the phenol analogues, the overall selectivity profile of the phenyl analogue **3** was excellent. We thus sought to identify an alternative C-6 substituent that would afford comparable potency to the phenol motif, but which was devoid of CDK-2 selectivity issues. It had been shown previously that replacement of the pyrazolo[3,4-*b*]pyridine nucleus with an indazole, afforded analogues with similar potency[3a] and for reasons of synthetic accessibility, we decided to explore the C-6 SAR further in this series (Table 3). To

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validate the strategy, the phenol **12** was prepared and, as predicted, showed increased potency against both GSK-3 and CDK-2 compared with the corresponding phenyl analogue **11**. Although the indolyl analogue **13** displayed good GSK-3 potency and CDK-2 selectivity, attempts to identify further phenol isosteres (e.g., **14**–**19**) or optimise the lipophilic interaction of the phenyl ring (e.g., **20**–**25**) proved generally unsuccessful. Interestingly, introduction of small heterocyclic substitutents at the C-6 position consistently afforded analogues (e.g., **26**–**30**) with greater potency than the corresponding phenyl analogue **11,** although CDK-2 selectivity was compromised in some cases.

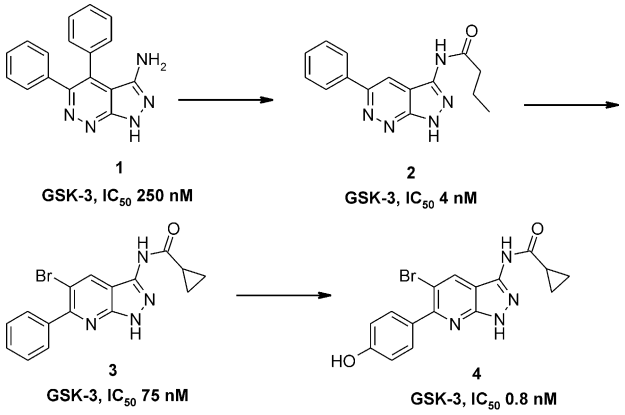
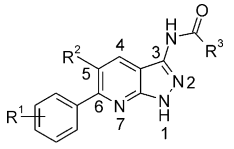


Figure 1. Identification of 6-aryl pyrazolo[3,4-*b*]pyridine 4.

It was envisaged, however, that because the group at C-6 was not interacting directly with Glu97 or Asp200, transferral of this promising SAR into the more potent 5-Br pyrazolopyridine series may lead to an increase in both GSK-3 potency and CDK-2 selectivity. To test this hypothesis, a range of C-6 heteroaryl pyrazolopyridines was prepared (Table 4). Gratifyingly, this led to an improvement in GSK-3 potency, without compromising CDK-2 selectivity (e.g., **32**, **34**, and **36**). Noteworthy is the importance of a C-5 Br substituent, which affords ca. 20-fold increase in GSK-3 potency without affecting selectivity (cf **33** with **34**). Lipophilic groups at C-3 are also preferred, thus replacing a cyclopropyl with a cyclopentyl can afford a ca. 5-fold improvement in potency (cf **31** with **32** and **35** with **36**). The rationale for the observed SAR at C-5 and C-3 has been discussed in previous communications.[3] Importantly, water solubilising groups are tolerated at the C-3 position to afford potent GSK-3 inhibitors with retention of excellent CDK-2 selectivity (e.g., **37** and **38**).

Cross screening of 6-heteroaryl-pyrazolo[3,4-*b*]pyridines against a panel of kinases indicated an excellent overall selectivity profile (Table 5).

Table 1. Inhibition of hGSK-3α and hCDK-2 by selected 6-aryl-pyrazolo[3,4-*b*]pyridine analogues[4]

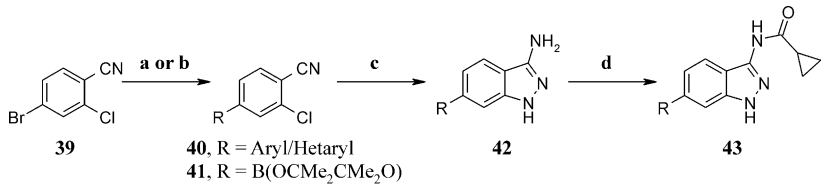


No.	R^1	R^2	R^3	GSK-3α, IC_{50} nM	CDK-2, IC_{50} nM
3	H	Br	cyPr	75±10	>1000
4	4-OH	Br	cyPr	0.8±0.4	5±1
5	4-OH	H	cyPr	8±1	13±2
6	3-Br-4-OH	H	cyPr	5±2	2±1
7	3-Cl-4-OH	H	cyPr	7±2	2±1
8	4-OH	Ph	cyPr	24±3	5±1
9	4-OH	Br	$(CH_2)_3$-4-piperazinyl-N-Et	4±1	60±1
10	3-OH	H	cyPr	12±3	62±3

Table 2. Selectivity profiling of pyrazolopyridines 3 and 4 and 5[a]

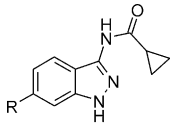
Compd	AMPK	Chk1	CKII	JNK	LCK	MAPK	MAPKAP-K2	MEK1	MSK1	P70S6K	PDK1	PHOS.K	PKA	PKBa	PKCA	PRAK	ROKa	SAPK2a	SAPK2b	SAPK3	SAPK4	DYRK1A	CDK2/Cyclin A	GSK-3α
3	0	28	31	0	49	0	12	10	5	0	20	3	3	0	0	21	8	**79**	11	1	0	28	25	**87**
4	**57**	**66**	3	15	**89**	45	19	5	**58**	14	**59**	30	19	17	**62**	1	4	41	**59**	**62**	**61**	**80**	**100**	**100**
5	**56**	0	3	12	**70**	29	14	12	**65**	8	31	**80**	20	17	**83**		41	15	10	**58**	**69**	**92**	**91**	**90**

[a]Values are % inhibition @10 μM using 100 μM ATP (see ref 5 for kinases used and assay details).



Scheme 1. Preparation of 6-aryl/heteroaryl indazoles. Reagents: (a) boronic acid, KOAc, $Pd(PPh_3)_4$; (b) (i) bispinacolatodiboran, KOAc, $PdCl_2(dppf)_2$, DMSO; (ii) arylbromide, KOAc, $Pd(PPh_3)_4$ 100 °C; (c) $N_2H_4{\cdot}H_2O$, EtOH, reflux; (d) C_3H_7COCl, pyridine, reflux.

Table 3. Inhibition of hGSK-3α and hCDK-2 by selected indazole analogues



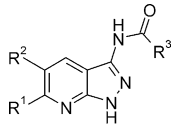
No.	R	GSK-3α, IC_{50} nM	CDK-2, IC_{50} nM
11	Ph	498±30	>1000
12	4-OH	15±1	5±1
13	5-Indolyl	42±5	>1000
14	6-Quinolyl	>1000	>1000
15	Ph-4-SO_2NH_2	>1000	>1000
16	Ph-3-SO_2NH_2	481±25	>1000
17	Ph-3-$NHSO_2Me$	>1000	>1000
18	Ph-4-$NHSO_2Me$	>1000	>1000
19	5-1*H*-pyrid-2-one	>1000	>1000
20	2-F-Ph	>1000	>1000
21	2,5-diF-Ph	>1000	>1000
22	3,4-diF-Ph	>1000	>1000
23	2,3-diF-Ph	>1000	>1000
24	3-F-Ph	828	>1000
25	4-F-Ph	>1000	>1000
26	2-Pyrrolyl	320±61	497±33
27	2-Furanyl	50±15	631±55
28	3-Furanyl	35±2	>1000
29	2-Thienyl	215±29	341±21
30	3-Thienyl	329±73	484±26

Chemistry[6,7]

The 6-heteroaryl-pyrazolo[3,4-*b*]pyridines were prepared according to the same general procedure previously described.[3c] Synthesis of the 6-aryl/heteroaryl indazoles were prepared according to the procedures outlined in Scheme 1. Suzuki cross-coupling of bromide **39** with a range of arylboronic acids afforded the corresponding nitrile analogues, **40**. Alternatively, the bromide **39** could be converted to the corresponding boronate **41**, utilising bispinacolatodiboran. Subsequent Suzuki cross-coupling with a range of aryl bromides afforded the 6-substituted analogues **40**. Treatment of the nitriles with hydrazine hydrate at reflux afforded the indazole analogues **42** which underwent selective C-3 acylation with cyclopropyl carbonyl chloride in pyridine at reflux to give the desired analogues **43** in excellent overall yield.

Profiling a series of 6-arylpyrazolo[3,4-*b*]pyridines revealed that introduction of a phenol group into the C-6 phenyl group afforded an excellent improvement in GSK-3 potency but also a dramatic reduction in CDK-2 selectivity. This finding is most likely due to the high

Table 4. Inhibition of hGSK-3α and hCDK-2 by 6-heteroaryl-pyrazolo[3,4-*b*]pyridines



No.	R^1	R^2	R^3	GSK-3α, IC_{50} nM	CDK-2, IC_{50} nM
3	H	Br	cyPr	75±10	>1000
4	4-HO-Ph	Br	cyPr	0.8±0.4	5±1
31	2-Thienyl	Br	cyPr	39±18	639±78
32	2-Thienyl	Br	cyPent	7±1	>1000
33	2-Furyl	H	cyPr	141±18	>1000
34	2-Furyl	Br	cyPr	7±2	>1000
35	2-Thiazoyl	Br	cyPr	99±55	>1000
36	2-Thiazoyl	Br	cyPent	16±4	>1000
37	2-Thienyl	Br	CH_2-4-Piperidine-N-Et	18±3	>1000
38	2-Furyl	Br	(±)-3-Pyrrolidine-N-Bn	14±1	>1000

Table 5. Selectivity profiling of pyrazolopyridine **34**[a]

AMPK	Chk1	CKII	JNK	LCK	MAPK	MAPKAP-K2	MEK1	MSK1	P70S6K	PDK1	PHOS.K	PKA	PKBa	PKCA	PRAK	ROKa	SAPK2a	SAPK2b	SAPK3	SAPK4	DYRK1A	CDK2/Cyclin A	GSK-3β
5	8	4	2	8	6	2	19	3	1	8	3	0	1	0	18	13	9	4	7	10	23	31	87

[a]Values are % inhibition @10 μM using 100 μM ATP (see ref 5 for kinases used and assay details).

degree of homology between the two kinases and the suggestion from molecular modelling studies that the phenol moiety interacts with two conserved residues. Optimisation of the C-6 position, avoiding functionality capable of H-bonding to these residues, led to the identification of a series of 6-hetero-aryl-pyrazolo[3,4-*b*]pyridines that displayed excellent GSK-3 potency and CDK-2 selectivity.

Acknowledgements

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

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6. Using microtitre plates, GSK-3 was assayed in 50 mM MOPS buffer, pH 7.0 containing 5% glycerol, 0.01% Tween-20, 7.5 mM 2-mercaptoethanol, 10 mM magnesium actetate, 8 uM substrate peptide (Biotin-KYRRAAVPPSPSLSRHSSPHQ (SP)EDEEE, where (SP) is a pre-phosphorylated serine) and 10 uM [g-33P]-ATP. After incubation for 1 h at room temperature, the reaction was stopped by addition of 50 mM EDTA solution containing Streptavidin coated SPA beads (Amersham) to give a final 0.2 mg of beads per assay well in a 384 microtitre plates were counted in a trilux 1450 microbeta liquid scintillation counter (Wallac).

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