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

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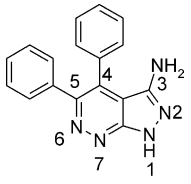
Abstract—A novel series of pyrazolo[3,4-*b*]pyridines has been identified that are potent inhibitors of glycogen synthase kinase-3 (GSK-3).

Glycogen synthase kinase-3 (GSK-3), so called because of its ability to phosphorylate glycogen synthase (GS),[1] is a serine/threonine kinase which exists as two isoforms (α and β) with molecular weights of 51 and 46 kDa, respectively.[2] The catalytic domains share ~90% identity, however GSK-3α possesses an additional ~60 amino acid sequence in the N-terminal kinase domain. Pharmacological differences between the isoforms have yet to be established. GSK-3 plays a key role in a number of diverse cellular processes. For example, insulin-stimulated glycogen synthesis is mediated by GS which considered to be the rate-determining enzyme of this process.[3] Phosphorylation of GS by GSK-3 leads to its deactivation.[4] In type 2 diabetics the rate of glycogen synthesis is impaired,[5] the activity of GS is reduced[6] while GSK-3 expression and activity are elevated, thus implicating GSK-3 in the disease process. A recent publication has demonstrated that selective small molecule inhibitors of GSK-3 have a positive impact on both GS activity and glucose uptake, including effects on insulin action, using mechanisms that differ from and are additive to those of insulin.[7] GSK-3 is also known to phosphorylate the microtubule associated protein tau in mammalian cells,[8] hyperphosphorylation of which is an early event in neurodegenerative conditions such as Alzheimers disease. Inhibition of GSK-3 with potent selective small molecule inhibitors has been shown to protect primary neurones from death induced by reduced PI 3-kinase pathway activity.[9] Bipolar disorder (manic depression) is commonly treated with lithium and the ability of this cation to inhibit GSK-3β has been proposed as a possible underlying therapeutic mechanism of action.[10] More recently, the X-ray structure of GSK-3β has been reported, enabling the possibility for structure based drug design.[11]

Pyridazine **1** was identified as a potent inhibitor of human GSK-3α (IC_{50} 250 nM), through pharmacophore searching of the SmithKline Beecham in house database.



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Automated ligand docking of pyridazine **1** into a GSK-3α homology model, [constructed using the crystal structure of the highly homologous cyclin dependant kinase-2 (CDK-2) (1 hcl)], using the program GOLD suggested a three point hydrogen bonding interaction with the hinge region of the ATP binding site (Fig. 1). Importantly, the phenyl ring at C-4 appeared to be orientated towards the front of the ATP binding site suggesting that this group could be removed to simplify our lead structure without significant loss of potency.

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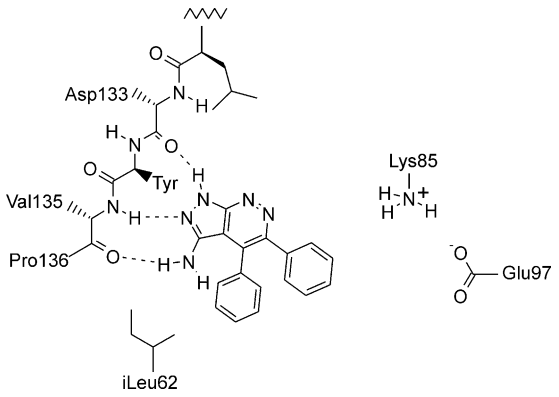
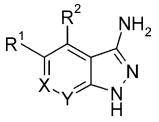


Figure 1. GOLD docking of pyridazine **1** into the GSK-3α homology model.

The amino group at C-3 was also orientated towards the front of the ATP binding site, indicating this position may be employed to introduce water solubilising groups if required. The nitrogen atoms at position 6 and 7 did not appear to be involved in binding.

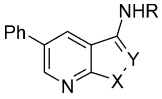
To validate the proposed binding mode and to simplify the lead template, a small set of analogues based around **1** were prepared (Table 1). Removal of the aromatic group at C-4 (cf. **1**, **2**) and the nitrogen at position 6 (cf. **2**, **3**) could indeed be tolerated with only minor loss of potency. Removal of the nitrogen at position 7 appears to be detrimental for potency (cf. **1**, **5**) although poor inhibitor solubility could account for this reduction in activity. However, the aromatic group at C-5 appears critical for potency (cf. **3**, **4**), consistent with binding in a hydrophobic pocket at the back of the ATP binding site.

Table 1. Inhibition of hGSK-3-α by selected pyrazolopyrid[*az*]line analogues



No.	R^1	R^2	X	Y	GSK-3α, IC_{50} nM[12]
1	Ph	Ph	N	N	250
2	Ph	H	N	N	530
3	Ph	H	CH	N	430
4	H	Ph	CH	N	>5000
5	Ph	H	N	CH	1260

Table 2. Inhibition of hGSK-3-α by selected heterocycles



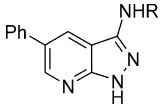
No.	X	Y	R	GSK-3α, IC_{50} nM[12]
3	NH	N	H	430
6	NMe	N	H	>5000
7	O	N	H	>5000
8	NH	CH	H	>5000
9	NH	N	COMe	291±73

With the initial SAR established we turned our attention to the validation of the proposed 3-point H-bonding pharmacophore (Table 2). Removal of either the H-bond donor at N-1 (**6** and **7**) or the H-bond acceptor at N-2 (**8**) led to a dramatic loss of potency, which is consistent with these atoms playing a critical role in the pharmacophore. Interestingly, acylation of the nitrogen at N-3 afforded a modest increase in potency (**9**, IC_{50} 291 nM) and from the predicted binding mode it appeared that a further increase in potency could be obtainable through a lipophilic interaction with iLeu62.

Amides at C-3 having more lipophilic substituents than methyl did indeed lead to dramatic improvements in potency, indeed the cyclopentyl analogue (**13**) displayed a ca. 60-fold improvement over the corresponding methyl analogue (**9**). By contrast the use of either a urea (**14**) or sulfonamide (**15**) moiety, as an amide isostere, reduced potency considerably, presumably due a six-membered intramolecular hydrogen bond between the N-2 atom of the pyrazole nucleus and the hydrogen of the urea, and an adverse conformational effect with the sulfonamide (Table 3).

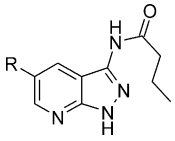
Having established small lipophilic amides as the optimal substituent at C-3, the SAR around C-5 was explored utilising Suzuki cross-coupling reactions with either the boronate **16** or the bromide **30** (Scheme 1) (Table 4). The fluorinated analogues **18**, **19** and **20** showed an increase

Table 3. Inhibition of hGSK-3-α for representative C-3 analogues

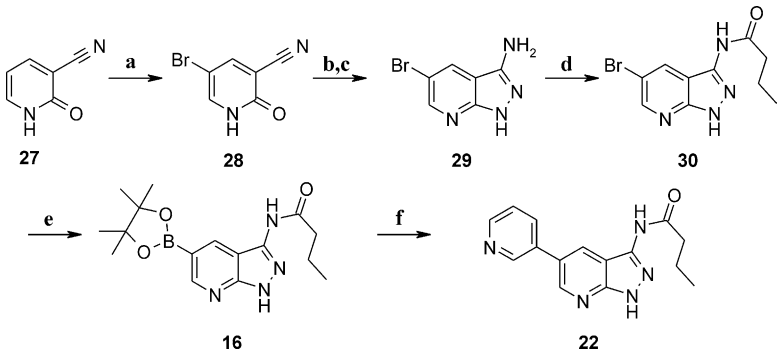


No.	R	GSK-3α, IC_{50} nM[12]
10	COEt	43±5
11	COnPr	56±6
12	CO*i*Pr	19±3
13	COcPent	5±1
14	CONHEt	2810
15	SO_2Me	3572

Table 4. Inhibition of hGSK-3-α for representative C-5 analogues



No.	R	GSK-3α, IC_{50} nM[12]
11	Ph	56±6
16	$B(OCMe_2CMe_2O)$	356±129
17	H	2343±135
18	2-F-Ph	18±2
19	3-F-Ph	20±3
20	2,3-diF-Ph	7±1
21	2-Cl-Ph	27±6
22	3-Pyridyl	11±2
23	4-Pyridyl	443±50
24	4-Ph-Ph	851±51
25	2-Naphthyl	169±14
26	1-Naphthyl	241±8



Scheme 1. Preparation of pyrazolo[3,4-*b*]pyridine 22. Reagent and conditions: (a) Br_2, AcOH (59%); (b) $POCl_3$, PCl_5, reflux (94%); (c) $N_2H_4.H_2O$, EtOH, reflux (75%); (d) C_3H_7COCl, pyridine, reflux (80%); (e) bispinacolatodiboron, KOAc, $PdCl_2(dppf)_2$, DMSO, 100 °C (60%); (f) 3-bromo pyridine, KOAc, $Pd(PPh_3)_4$, $DMF/H_2O/EtOH$ (2:1:1), 100 °C (40%).

Table 5. Selectivity of pyrazolo[3,4-*b*]pyridines for GSK-3β[a]

No.	AMPK	Chk1	CKII	JNK	LCK	MAPK	RSK-2	MAPKAP-K2	MEK1	MSK1	P70S6K	PDK1	PHOS.K	PKA	PKBa	PKCA	PRAK	ROKa	SAPK2a	SAPK2b	SAPK3	SAPK4	SGK	CDK2/Cyclin A	GSK-3β
11	14	11	10	11	34	4	0	6	10	9	4	13	0	0	13	12	22	18	1	7	5	11	19	81	90
18	14	1	0	0	36	0	4	5	7	3	0	0	0	0	5	0	5	14	0	4	2	0	5	95	99
22	4	0	0	7	10	0	9	3	9	8	1	0	5	3	2	11	13	4	0	0	10	13	13	99	94

[a]Values are %I @10 uM using 100 uM ATP (see ref 13 for kinases used and assay details).

in potency relative to **11** as did the pyridyl analogue **22**. The potency gained through the fluorinated analogues could arise via either optimisation of lipophilic contact with the back of the ATP binding pocket, or from an H-bonding interaction with Lys85. From the predicted binding mode, the increase in potency observed for the pyridyl analogue could be due to interaction with the conserved salt bridge (Lys85/Glu97). Interestingly, the boronate **16** displays sub micromolar potency, indicating also that an aromatic group is not essential for activity. Analogues **24** and **25** were significantly less potent, suggesting that excess steric bulk around this position is detrimental, again supporting the postulated binding mode where the C-5 substituent is in close proximity to the conserved salt bridge.

Having identified a potent series of GSK-3α inhibitors, some of the above compounds were profiled against a panel of more than 20 kinases including GSK-3β (Table 5).[13] Although excellent selectivity is obtained against the majority of the kinases on the panel, significant inhibition of CDK-2 is observed, not unexpected given the high homology between the two kinases.

Chemistry[14,15]

The pyrazolo[3,4-*b*]pyridines were prepared as outlined in Scheme 1. Bromination of the commercially available pyridone **27** by bromine in acetic acid afforded bromide **28**. Treatment of this with phosphorus oxychloride and phosphorus pentoxide at reflux afforded the corresponding chloropyridine, which was treated with hydrazine hydrate in ethanol at reflux to yield the pyrazolopyridine **29**. Selective acylation of the C-3 amino group afforded the amide **30**, which underwent successful Suzuki cross-coupling with arylboronic acids to afford the desired analogues, for example, **22**. Alternatively, amide **30** could be converted to the boronate **16** utilising bispinacolatodiboranboronate and subsequently cross-coupled with a range of aryl bromides to provide the desired analogues, for example, **22**.

Conclusion

Through pharmacophore searching and the use of a GSK-3 homology model, potent inhibitors of hGSK-3α have been identified. Further optimisation of this novel scaffold, together with the kinase selectivity profile of compounds of interest is discussed in the subsequent papers.

Acknowledgements

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References and Notes

1. Cohen, P.; Yellowlees, D.; Aitken, A.; Donella-Deana, A.; Hemmings, B. A.; Parker, P. J. *Eur. J. Biochem.* **1982**, *124*, 21.
2. Woodgett, J. R. *Trends Biochem. Sci.* **1991**, *16*, 177.
3. (a) Leloir, L. F.; Olavaria, S. H.; Goldenberg, S. H.; Carminatti, H. *Arch. Biochem. Biophys.* **1959**, *81*, 508. (b) Mandarino, L.; Wright, K.; Verity, L.; Nichols, J.; Bell, J.; Kolterman, O.; Beck-Nielsen, H. *J. Clin. Invest.* **1987**, *80*, 655.
4. (a) Roach, P. *FASEB J.* **1990**, *4*, 2961. (b) Skurat, A. V.; Roach, P. J. *Biochem. J.* **1996**, *313*, 45. (c) Zhang, W.; DePaoli-Roach, A. A.; Roach, P. J. *Arch. Biochem. Biophys.* **1993**, *304*, 219. (d) Eldar-Finkelmann, H.; Argast, G. M.; Foord, O.; Fischer, E. H.; Krebs, E. G. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 10228.
5. DeFronzo, R. A.; Bonadonna, R. C.; Ferrannini, E. *Diabetes Care* **1992**, *15*, 318.
6. (a) Thorburn, A. W.; Gumbiner, B.; Bulacan, F.; Wallace, P.; Henry, R. R. *J. Clin. Invest.* **1990**, *85*, 522. (b) Beck-Nielsen, H.; Vaag, A.; Damsbo, P.; Handberg, A.; Neilsen, O. H.; Henriksen, J. E.; Thye-Ronn, P. *Diabetes Care* **1992**, *15*, 418. (c) Bogardus, C.; Lillioja, S.; Stone, K.; Mott, D. *J. Clin. Invest.* **1984**, *73*, 1185.
7. Nikoulina, S. E.; Ciaraldi, T. P.; Mudaliar, S.; Carter, L.; Johnson, K.; Henry, R. R. *Diabetes* **2002**, *51*, 2190.
8. Lovestone, S.; Reynolds, C. H.; Latimer, D.; Davis, D. R.; Anderton, B. H.; Gallo, J.-M.; Hanger, D.; Mulot, S.; Marquardt, B. *Curr. Biol.* **1994**, *4*, 1077.
9. Cross, D. A. E.; Culbert, A. A.; Chalmers, K. A.; Facci, L.; Skaper, S. D.; Reith, A. D. *J. Neurochem.* **2001**, *77*, 94.
10. Klein, P. S.; Melton, D. A. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 8455.
11. (a) Aoki, M.; Iwamoto-Sugai, M.; Sugiura, I.; Sasaki, C.; Hasegawa, T.; Okumura, C.; Sugio, S.; Kohno, T.; Matsuzaki, T. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2000**, *D56*, 1464. (b) Bax, B.; Carter, P. S.; Lewis, C.; Guy, A. R.; Bridges, A.; Tanner, R.; Pettman, G.; Mannix, C.; Culbert, A. A.; Brown, M. J. B.; Smith, D. G.; Reith, A. D. *Structure (London)* **2001**, *9*, 1143.
12. 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.
13. Davies, S. P.; Reddy, H.; Caivano, M.; Cohen, P. *Biochem. J.* **2000**, *351*, 95.
14. 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 μM substrate peptide (Biotin - KYRRAAVPPSPSLSRHSSPHQ (SP)EDEEE, where (SP) is a pre-phosphorylated serine) and 10 μM [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).
15. All novel compounds gave satisfactory ^{1}H NMR and LC/MS data in full agreement with their proposed structures.