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(1*H*-Imidazo[4,5-*c*]pyridin-2-yl)-1,2,5-oxadiazol-3-ylamine derivatives: A novel class of potent MSK-1-inhibitors

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Abstract—A novel series of imidazo[4,5-*c*]pyridines bearing a 1,2,5-oxadiazol-3-ylamine functionality has been developed. These are potent inhibitors of mitogen and stress-activated protein kinase-1.

Mitogen and stress-activated protein kinases 1 (MSK-1) and MSK-2 (also named RSKB or RLPK) are localised to the nucleus and are activated by both mitogen (ERK) and stress-activated (p38) protein kinases to phosphorylate the transcription factors CREB and histone H3.[1,2] In macrophages and monocytes, MSK-1 is involved in CREB-mediated transcriptional regulation of IL-1β and COX2 in response to bacterial lipopolysaccharide. MSK-1 and -2 were also shown to be required for the stress-induced phosphorylation of both CREB and ATF1, and of histone H3 and HMG-14 in fibroblasts.[3,4] MSK-1 is also involved in the transcriptional activation of the NF-κB p65 subunit.[5] Thus, MSK-1 and -2 may have a critical role in linking cellular signalling pathways to chromatin modification and modulation of transcription factor complexes and have potential therapeutic utility. Indeed, it has been demonstrated that MSK-1 mediates excitotoxicity-induced death of hippocampal neurones[6] and that inhibitors of MSK-1 and -2 may, therefore, be of use in the treatment of diseases involving ischaemic injury.[7] Since inhibitors of the p38 pathway[8] and the ERK pathway[9,10] are reported to be neuroprotectants, MSK-1 and -2 inhibitors are also of interest in this regard.

To date only broad spectrum kinase inhibitors H89 (IC_{50} 120 nM) and Ro318220 (IC_{50} 8 nM) have been reported as MSK-1 inhibitors.[11] We recently conducted a screening programme with the aim of providing a novel MSK-1 inhibitor template. The 3-amino-1,2,5-oxadiazole compound **1** was identified as a tractable starting point for optimisation (MSK-1, IC_{50} 140 nM).

To aid the prioritisation of molecules for synthesis, protein docking studies were carried out. Although the X-ray crystal structure of the N-terminal domain of the inactive conformation of MSK-1 has been solved, this structure is likely to differ significantly from its active conformation.[13] Therefore, a computational homology model of active MSK-1 was built, based on the known[12] crystal structure of the closely related protein kinase A (PKA) complexed with H89 (an equipotent inhibitor of PKA, IC_{50} 135 nM).[11] Compound **1** was docked into the modelled ATP-binding site using the

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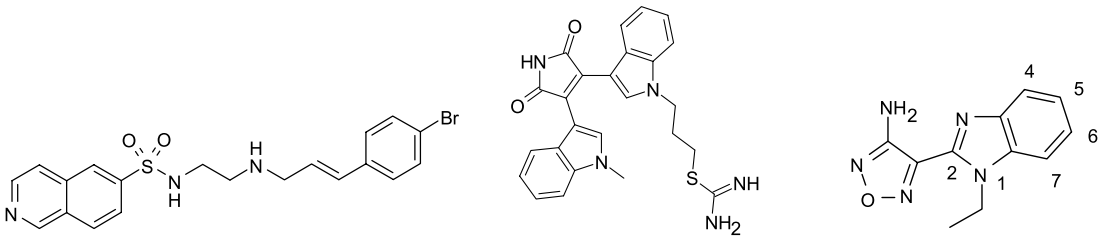


H89 **Ro318220** **1**

program GOLD and key features of the docking are shown in Figure 1.

This model suggests that the 3-amino-1,2,5-oxadiazole fulfils a key bifurcated hydrogen bonding interaction with the protein amide backbone of residues Asp132 and Ile134 in the conserved 'hinge' region. The model suggests restricted space around the 3-amino-1,2,5-oxadiazole due to 'gatekeeper' residue Leu131 adjacent to the amine functionality. Energy minimisation of the ligand indicates virtual co-planarity of the two heteroaryl rings, with possible intramolecular hydrogen bonding between the amino group and the benzimidazole ring nitrogen. The *N*1-ethyl group is suggested to bind into a lipophilic pocket constituted by Val53, Leu55, Phe348 and Leu184. Of particular interest to our initial chemical strategy, the protonated amine of conserved Lys81 is predicted to be in close proximity to C5. Thus, the first targeted analogues (Table 1) were those with substituents able to act as an H-bond

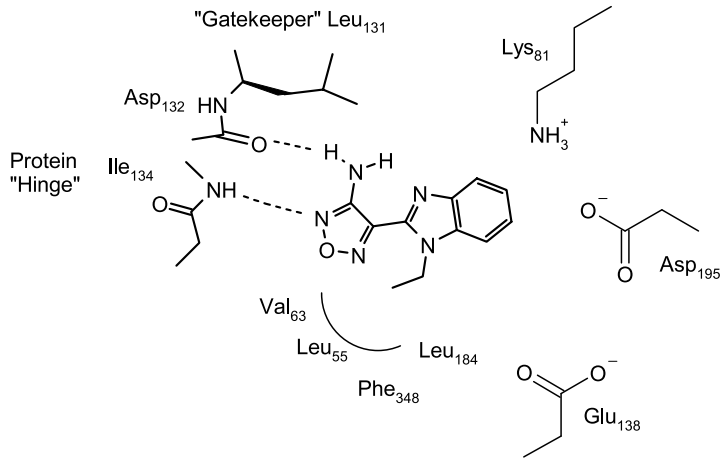
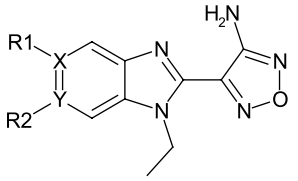


Figure 1. GOLD docking of compound **1** into MSK-1 homology model.

Table 1. Inhibition of hMSK-1 by 5- and 6-substituted benzimidazole analogues



Compound	R^1	R^2	X	Y	MSK-1 (IC_{50}, nM)
1	H	H	C	C	140
2	OMe	H	C	C	1200
3	$COCH_3$	H	C	C	4390
4	CH_2OH	H	C	C	974
5	F	H	C	C	38
6	OH	H	C	C	17
7	H	F	C	C	2120
8	H	OH	C	C	258
9	—	H	N	C	3
10	H	—	C	N	1540

acceptor in the region of C5, aimed at interaction with Lys81.

Whilst the more bulky substituents in compounds **2**–**4** gave a reduction in potency relative to **1**, the smaller fluoro and hydroxy functionality in compounds **5** and **6** gave significant enhancements to potency. Furthermore, we were extremely encouraged to find that the 5-aza-derivative **9** provided a 50-fold increase in potency over **1**. In contrast, when the same fluoro or hydroxy functionality was positioned at C6 to give compounds **7** and **8**, respectively, no improvement in potency was observed relative to unsubstituted **1**, and indeed fluoro compound **7** is significantly less potent than **1**. This reduction in potency was even more marked in the 6-aza-analogue **10**. These results are consistent with our original theory that H-bonding substituents at C5 would be favoured. Furthermore, the introduction of electron rich groups at C6 is likely to be disfavoured due to the proposed (Fig. 1) proximity of Asp195.

Having established the imidazopyridine compound **9** as a potent baseline compound, we next investigated the requirements of the 3-amino-1,2,5-oxadiazole moiety (Table 2).

Simple replacement of this moiety with a phenyl (**11**) or 3-furyl (**12**) group results in loss of all activity. There is also no activity in 2-, 4- and 3-pyridyl analogues **13**–**15**. However, the 2-amino, 3-pyridyl analogue **16** shows comparable potency to the original lead **1**, albeit still 35-fold less potent than direct comparator **9** despite having functionality capable of fulfilling the key bifurcated H-bond. Replacement of the 2-amino group in **16** with an hydroxyl group to give **17** or movement of the amino group to the 6-position as in **18** ablates all activity. The most effective 3-amino-1,2,5-oxadiazole replacement yet identified is the 2-aminopyrazine compound **19**. These data are consistent not only with a very specific H-bonding interaction with the protein as proposed in docking studies (Fig. 1) but also with the proposed role of the exocyclic amino group in intramolecular H-bonding. Whilst the 5-fold greater potency of pyrazine **19** over pyridine **16** may reflect differences in basicity, it is also reasonable to suggest that the CH group of the latter (X = CH) may have a detrimental steric effect on the proposed coplanarity of the pyridyl and imidazopyridine rings. Indeed, replacement of the N5 ring nitrogen in oxadiazole **9** with a methyl-bearing carbon in compound **20** completely ablates all MSK-1 activity. Unfortunately the desmethyl 3-amino-isoxazole analogue of **20** proved synthetically elusive. This steric effect may also be relevant to the 3-aminopyrazole derivative **21** which is 18-fold less potent than **9**, although this compound is also capable of existing in an alternative NH-tautomeric form. N-substitution of **21** to give **22** gives a further 30-fold reduction in activity, presumably due to methyl steric clash with the protein backbone adjacent to the H-bonding interaction. The importance of the 2-amino group in the amino-oxadiazole is further borne out by inactive compounds **23**, in which the amine is acylated so removing one H-bond donor and introducing steric clashes, and chloro compound **24** which has no H-bond donor and reduced H-bond acceptor capability. The corresponding des-amino compound proved synthetically elusive.

Thus, the 3-amino-1,2,5-oxadiazole group and the 5-aza-modification are key to potency of the benzimidazole template. We next turned our attention to modification of the N1-substituent (Table 3).

The unsubstituted and methyl substituted analogues **25** and **26**, respectively, are approximately 650 and 40 times less potent than the parent ethyl derivative, supporting the proposed crucial lipophilic interaction in this region

Table 2. Inhibition of hMSK-1 by selected amino-1,2,5-oxadiazol-modified analogues of compound **1**

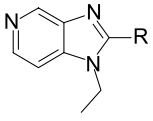
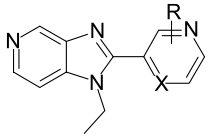
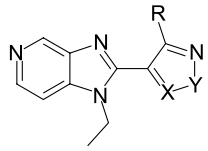
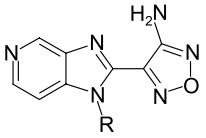
	Compound	X	Y	R	MSK-1 (IC_{50}, nM)
	**11**	—	—	Ph	>10,000
	12	—	—	3-Furyl	>10,000
	13	—	—	2-Pyridyl	>10,000
	14	—	—	4-Pyridyl	>10,000
	**15**	CH		H	>10,000
	16	CH	—	2-NH_2	107
	17	CH	—	2-OH	>10,000
	18	CH	—	6-NH_2	>10,000
	19	N	—	2-NH_2	20
	**9**	N	O	NH_2	3
	20	CMe	O	NH_2	>10,000
	21	CH	NH	NH_2	55
	22	CH	NMe	NH_2	1520
	23	N	O	NHAc	5097
	24	N	O	Cl	>10,000

Table 3. Inhibition of hMSK-1 by representative N1-substituted analogues



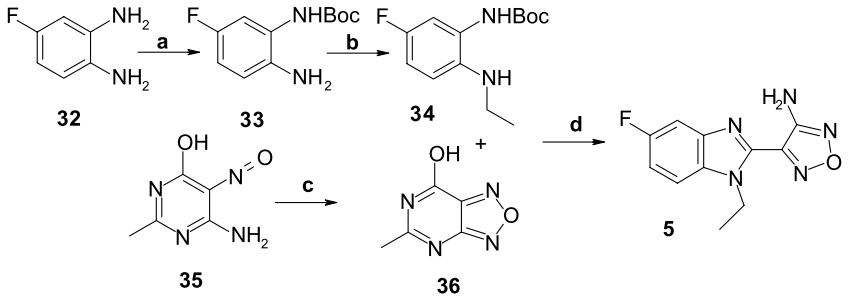
Compound	R	MSK-1 (IC_{50} nM)
9	Et	3
25	H	1960
26	Me	126
27	Cyclopropyl	1.8
28	Cyclohexyl	3
29	Ph	11
30	Piperidin-4-yl	17
31	Aminobut-4-yl	13

of the protein. Furthermore, the cyclopropyl derivative **27** and the cyclohexyl derivative **28** afford the greatest potency, being at least as potent as **9**. Lipophilic phenyl derivative **29** also affords good potency as do the more polar piperidin-4-yl and aminobutyl substituents in analogues **30** and **31**, respectively. The latter derivatives thus afford the opportunity for manipulation of the physicochemical properties of the template. Although entropically less favourable, the aminobutyl chain may be able to access a beneficial interaction such as with Glu138 (Fig. 1).

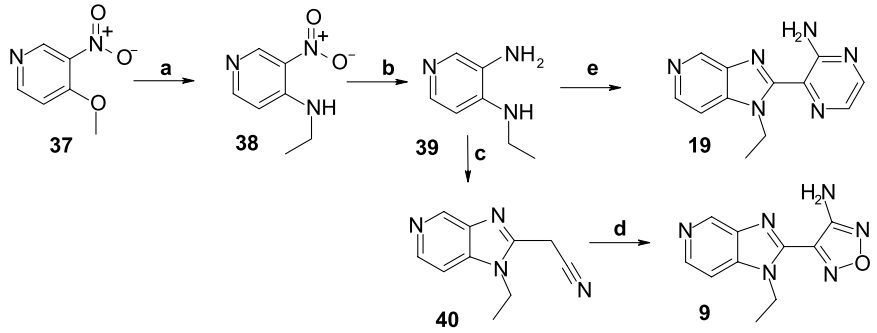
Thus, the nature of the N1-substituent is also crucial to the potency of the template. In keeping with the model, the binding pocket shows a preference for small, branched alkyl substituents. However, there is scope for introduction of functionality capable of significantly altering the overall properties of the molecule. This bodes well for the future optimisation of this template.

The benzimidazole derivatives, such as compound **5**, were prepared[14,15] as outlined in Scheme 1. Thus, 4-fluorobenzene-1,2-diamine **32** was selectively mono-protected to give **33** by warming in di(*t*-butyl)dicarbonate until homogeneous. This was then deprotonated with potassium *t*-butoxide and treated with ethyl iodide to give **34**. The amino-1,2,5-oxadiazol synthon **36** was prepared in moderate yield by treatment of 4-amino-6-hydroxy-2-methyl-5-nitrosopyrimidine **35** in acetic acid with lead tetra-acetate. Heating **34** and **36** together in acetic acid then yielded the desired 5-fluoro-benzimidazole derivative **5** in moderate yield.

The 5-aza-benzimidazole derivatives were prepared as outlined in Scheme 2 and is representative of the approach taken to this class of compound. Thus, commercially available 4-methoxy-3-nitropyridine hydrochloride **37** was stirred with the appropriate primary amine in ethanol at reflux to afford good yields of the substituted amino-nitropyridines, for example, **38**. Catalytic hydrogenation then afforded the corresponding diaminopyridine **39**. Condensation with neat ethylcyanoacetate at high temperature provided acceptable yields of the cyanomethyl benzimidazole intermediate **40**. This was then cyclised in a moder-



Scheme 1. Preparation of substituted benzimidazole derivatives. Reagents and conditions: (a) di(*t*-butyl)dicarbonate, 50 °C (73%); (b) KOtBu, THF then EtI, 21 °C (38%); (c) Pb(OAc)4, AcOH, 21 °C (32%); (d) AcOH, reflux (17%).



Scheme 2. Preparation of substituted aza-benzimidazole derivatives. Reagents and conditions: (a) ethylamine, ethanol, reflux (88%); (b) H_2, 10% Pd/C, 21 °C (94%); (c) ethylcyanoacetate, 190 °C (37%); (d) (i) $NaNO_2$, methanol, HCl, 21 °C, (ii) NaOH, NH_2OH, water, 90 °C (43%, two steps); (e) 2-aminopyrazine-3-carboxylic acid, polyphosphoric acid, 130–195 °C (6%).

ately yielding, two-step reaction sequence consisting of nitrosylation with sodium nitrite in methanolic HCl, followed by heating with a basic aqueous solution of hydroxylamine to give the 3-amino-1,2,5-oxadiazol derivative **9**. Alternatively, diaminopyridine **39** was added as a mixture with an appropriate aryl carboxylic acid to hot polyphosphoric acid and further heated to afford benzimidazole derivatives, for example, **19**.

A new MSK-1-inhibitory template has been identified by high-throughput screening. An initial understanding of the SAR has been established and potent analogues developed using an MSK-1 protein homology model. Further optimisation will be discussed in subsequent papers.

Acknowledgments

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14. MSK-1 was assayed at a concentration of 2 nM in 50 mM HEPES buffer, pH 7.5, containing 2 μM biotinylated peptide (biotin-GRPRTSSFAEG-OH); 20 μM ATP; 25 Bq/pmol ATP^{33}; 10 mM $MgCl_2$; 0.1 mM EDTA; 0.0025% Tween 20; 5 mM β-mercaptoethanol. After incubation for 1 h at 20 °C, the reaction was stopped by addition of 200 mM EDTA solution containing streptavidin-coated SPA beads (Amersham) to give a final 0.2 mg of beads per assay well. The plates were shaken for 10 min before centrifugation at 2500 rpm for 10 min. ^{33}P incorporation was quantified by scintillation counting in a Wallac Trilux.
15. All novel compounds gave satisfactory ^1H NMR and LC/MS data in full agreement with their proposed structures.