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(1*H*-Imidazo[4,5-*c*]pyridin-2-yl)-1,2,5-oxadiazol-3-ylamine derivatives: Further optimisation as highly potent and selective MSK-1-inhibitors

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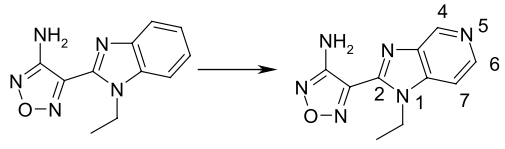
Abstract—The novel imidazo[4,5-*c*]pyridine 1,2,5-oxadiazol-3-yl template affords an excellent start point for identification of inhibitors of a number of protein kinases. Here we report on its optimisation for mitogen and stress-activated protein kinase-1 (MSK-1) inhibitory activity, and selectivity over other kinases.

Mitogen and stress-activated protein kinases 1 (MSK-1) and 2 are localised to the nucleus and are activated by both mitogen- (ERK) and stress-activated (p38) protein kinases. They phosphorylate a number of transcription factors including CREB, ATF1, HMG-14 and histone H3 in various cell types, and are involved in the transcriptional activation of the NF-κB p65 subunit.[1] Inhibitors may be of value as therapeutic agents since they are implicated in the regulation of inflammatory mediators and in excitotoxicity-induced neuronal death.[2,3]

We recently conducted a screening programme and identified the 2-[3-amino-1,2,5-oxadiazol-5-yl] benzimidazole compound **1** (MSK-1 IC_{50} 140 nM), which was modified to afford the highly potent (1*H*-imidazo[4,5-*c*]pyridin-2-yl)-1,2,5-oxadiazol-3-ylamine derivative **2** (MSK-1 IC_{50} 3 nM).[4]

Using the docking program GOLD, we have suggested a binding mode for **2** that explains its improved potency relative to **1** (Fig. 1) and rationalises our initial SAR.[4]



1 **2**

This model proposes H-bonding interactions of the amino-oxadiazole with the hinge region protein backbone, and of N5 of the imidazopyridine with Lys81. These are both common protein kinase features and, therefore, **2** represents an excellent start point for identification of inhibitors of this class of target. Indeed, Table 1 shows that **2** inhibits a number of representative kinases, including CDK2, GSK3β, MAPKAP-K1α, p70S6K and ROCK1. We now report on our studies to design analogues of **2** which are selective for MSK-1.

We used GSK-3 and MAPKAP-K1 (also known as RSK-1) to monitor selectivity as we explored the template further. Figure 1 suggests the vector of the C4 position is in the direction of a lipophilic pocket beyond the ''gatekeeper'' residue (Leu for MSK-1, GSK-3 and RSK-1). Access to this pocket affords enhancements in the activity and sometimes selectivity of inhibitors of other kinases and was our first area of investigation.

Keywords: MSK-1; Mitogen and stress-activated protein kinase; Imidazo[4,5-*c*]pyridine.
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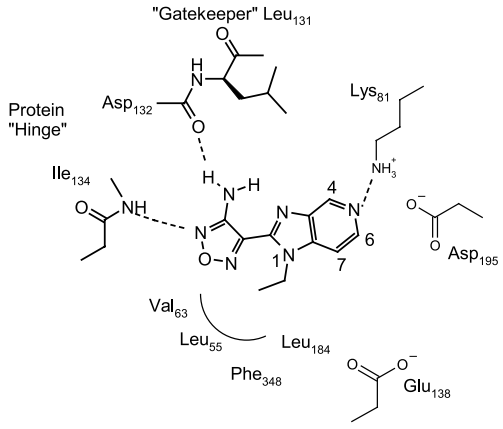


Figure 1. GOLD docking of compound **2** into an MSK-1 homology model.

Whilst substitution at C4 with the small lipophilic Me and Cl substituents in compounds **3** and **4**, respectively, was well tolerated (Table 2), increasing the size of the substituent even moderately (compounds **5**–**7**) produced a significant reduction in activity. Larger substituents (compounds **8**–**10**) either abolished or greatly reduced activity, presumably due to deleterious steric interactions with gatekeeper residue Leu131 which prevent access to the lipophilic pocket.

Interestingly, there was a moderate improvement in selectivity of compound **3** compared with compound **2** for MSK-1 over GSK-3 and RSK-1. The reasons for this are not clear since the gatekeeper residues for GSK-3 and RSK-1 are the same as for MSK-1 (Leu) and so other subtle requirements of the binding site must be invoked.

We have previously shown[4] that substitution at N1 with lipophilic functionality such as cyclohexyl or cyclopropyl affords potent MSK-1 inhibitory compounds **11** and **12**, respectively (Table 3). Interestingly, whilst **11** had little selectivity over GSK-3, it showed a tendency towards selectivity over RSK-1. Cyclopropyl derivative **12** showed improved GSK-3 selectivity. Furthermore, introduction of substituents containing a basic nitrogen such as the 4-piperidin-1-yl and the 4-butylamino derivatives (**13** and **14**, respectively) retained MSK-1 activity and showed a trend towards improved selectivity over GSK-3 compared with that for **11** (Table 3). However, selectivity over RSK-1 showed no improvement or was reduced. These findings can be rationalised by our binding hypothesis (Fig. 1) which predicts the butylamine basic nitrogen to be in the region of Glu138. The corresponding residues in GSK-3 and RSK-1 are Thr and Asp, respectively.

Thus, further careful manipulation of the nature of the N1 substituent and its interaction with the lipophilic pocket in this region of the protein, together with careful positioning of a basic centre may be expected to offer both MSK-1 potency and enhanced selectivity. We next set out to further explore this possibility.

There was no MSK1 potency advantage from straight alkyl chain analogues **15** or **16** although the shorter chain **15** did show some improved selectivity over RSK-1. However, the more bulky 3-piperidinyl compound **17** combined the improved MSK-1 potency and modest RSK-1 selectivity of cyclohexyl derivative **11** with the modest GSK-3 selectivity of 4-piperidinyl analogue **13**. The selectivity over both GSK-3 and RSK-1 was further enhanced in the branched chain analogue **18**. Further evidence that an appropriately positioned basic centre is capable of affording selectivity is provided by the excellent potency and significantly enhanced GSK-3 selectivity of the 3-benzylamino derivative **20** when compared to the phenyl derivative **19**. This is in contrast to the 4-benzylamino derivative **21**. Thus, we were encouraged by these observations that access to lipophilic interactions close to N1 with branched alkyl or cycloalkyl functionality, together with a well positioned interaction with Glu138 may achieve the desired combination of potency together with selectivity over GSK-3 and RSK-1.

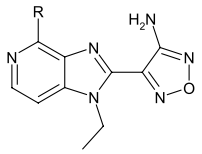
Examination of the MSK-1 homology model (Fig. 1) indicates that such interactions are also accessible through modification of the C7 position of compound **2**. We therefore next explored application of the above findings at this position. The bromo substituent in compound **22** (Table 4) retained good MSK-1 potency although, as expected, lacked selectivity over either GSK-3 or RSK-1. However, we were particularly pleased to find that introduction of hetero-cycloalkyl functionality (compounds **23**–**26**) afforded greatly enhanced selectivity over both GSK-3 and RSK-1. Of particular note, **26** (SB-747651-A) shows a 10-fold increase in MSK-1 potency and greatly enhanced selectivity over **23** suggesting an advantageous additional interaction with MSK-1, possibly through Glu138 as predicted by the MSK-1 homology model. Of interest to future studies will be the combination of this C7 substituent with the C4-methyl group of compound **3**.

Table 1. Kinase selectivity profile of compound **2**[a]

Compound	AMPK	CDK2	Chk1	CK2	DYRK1α	GSK3β	JNK1	Lck	MAPK2	MAPKAP-K1α	MAPKAP-K2	MKK1	MSK1	P70S6K	PDK1	PKA	PKCα	ROCK1	P38α	P38β	P38δ	SGK
2	59	84	−6	15	94	90	13	−16	41	97	12	17	96	95	32	75	56	90	−7	18	6	67

[a] Values are %*I* at 10 μM using 100 μM ATP (see Ref. 5 for kinases used and assay details).

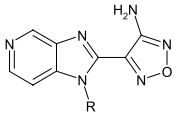
Table 2. MSK-1 activity and selectivity of C4 modified derivatives of compound **1**



Compound	R	MSK-1 IC_{50} (nM)	GSK-3/MSK-1[a].	RSK-1/MSK-1[b].
2	H	3	11	8
3	Me	8	104	21
4	Cl	4	13	19
5	Et	229	11	1.5
6	OMe	209	—	1
7	OEt	174	—	6
8	OPh	10,000	1	1
9	*N*-Pyrrolidine	2240	0.7	3
10	Ph	813	2	1

[a] Ratio of GSK-3 IC_{50} to MSK-1 IC_{50}.
[b] Ratio of RSK-1 IC_{50} to MSK-1 IC_{50}.

Table 3. MSK-1 activity and selectivity of N1-modified derivatives of compound **2**



Compound	R1	MSK-1 IC_{50} (nM)	GSK-3/MSK-1[a]	RSK-1/MSK-1[b]
11	Cyclohexyl	3	1.5	13
12	Cyclopropyl	1.5	37	9
13	Piperidin-4-yl	17	12	17
14	$(CH_2)_4NH_2$	13	41	2
15	$(CH_2)_3NH_2$	22	23	33
16	$(CH_2)_3NMe_2$	617	6	15
17	(*R*,*S*)-piperidin-3-yl	2	17	33
18	(*R*,*S*)-$CH(Me)CH_2NH_2$	9	69	83
19	Ph	6	10	12
20	3-(CH_2NH_2)Ph	2.5	634	8
21	4-(CH_2NH_2)Ph	4	13	11

[a] Ratio of GSK-3 IC_{50} to MSK-1 IC_{50}.
[b] Ratio of RSK-1 IC_{50} to MSK-1 IC_{50}.

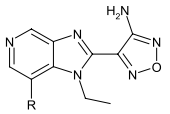
Compound **26** (SB-747651-A) was further profiled[5] (Table 5) across those enzymes for which compound **2** showed pronounced activity (>70% inhibition at 10 μM, Table 1). These data confirm the significant improvements in selectivity for **26** over these additional kinases.

The N1-modified analogues were prepared[6,7] as previously reported.[4] The C4-modified analogues were generated as indicated in Scheme 1 for compound **3**. Thus, an acetic acid solution of compound **2** was treated with 30% aqueous hydrogen peroxide and heated at 90 °C for 5 h to yield *N*-oxide **27** in moderate yield. This was quantitatively transformed into chloro derivative **4** by heating in phosphorus oxychloride for 6 h. Reaction with an appropriate nucleophile then afforded the desired C4-substituted products. For example, compound **4** in 1,4-dioxan was treated with a 2 M solution of trimethylaluminium in toluene and bis(triphenylphosphine)palladium (II) dichloride and then heated at reflux for 4 h to give compound **3**.

The C7 derivatives such as compounds **22** and **26** were obtained as indicated in Scheme 2. Thus, ethyl-(3-nitropyridine-4-yl)amine **28**[4] was brominated in hot acetic acid to afford the corresponding 5-bromo-analogue **29** in moderate yield. In analogous fashion to that employed for synthesis of compound **2**,[4] this was then converted in three moderately yielding steps to the key 7-bromo compound **22**. This versatile intermediate can be variously derivatised, including formylated by treatment with *n*-BuLi followed by addition of DMF to give the equally versatile 7-formyl intermediate **32**. This can be reductively aminated with an appropriate amine in the presence of polymer-bound cyanoborohydride to give amines such as **33**. Compound **33** was deprotected with TFA to give desired compound **26**.

The (1*H*-imidazo[4,5-*c*]pyridin-2-yl)-1,2,5-oxadiazol-3-ylamine template provides an excellent start-point for identification of inhibitors of a number of kinases. Potent and selective inhibitors of MSK-1 have been

Table 4. MSK-1 activity and selectivity of selected C7-modified derivatives of compound **2**



Compound	R	MSK-1 IC_{50} (nM)	GSK-3/MSK-1[a]	RSK-1/MSK-1[b]
22	Br	9	7	2
23	CH_2-(1-piperidin-1-yl)	7	242	93
24	CH_2-(1-piperazin-1-yl)	1.4	257	81
25	CH_2-(4-NH_2-piperidin-1-yl)	3	635	69
26 (SB-747651-A)	CH_2NH(4-piperidin-4-yl)	0.5	3800	309

[a] Ratio of GSK-3 IC_{50} to MSK-1 IC_{50}.
[b] Ratio of RSK-1 IC_{50} to MSK-1 IC_{50}.

Table 5. Comparison of potency of compound **2** with C7-modified compound **26** for selected kinases

Compound	ROCK-1 IC_{50} (nM)[6]	CDK2 IC_{50} (nM)[6]	P70S6K IC_{50} (nM)	DYRK1a IC_{50} (nM)
2	13	169	70	110
26	100	>10000	270	4900

identified through optimisation using insights provided by an MSK-1 protein homology model. In particular this has led to the identification of exceptionally potent MSK-1 inhibitor **26** (SB-747651-A) which has an excellent kinase selectivity profile, including >300-fold selectivity over RSK1 and >3000-fold selectivity over GSK-3.

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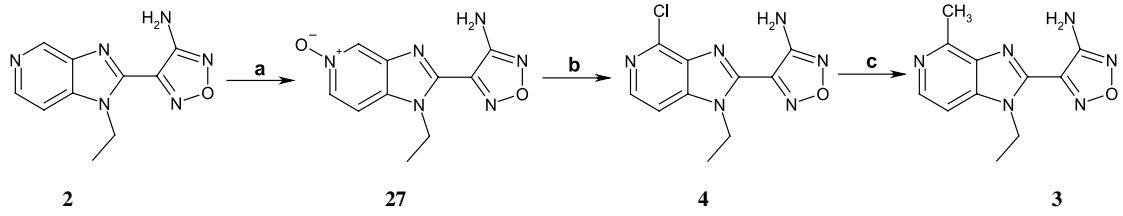
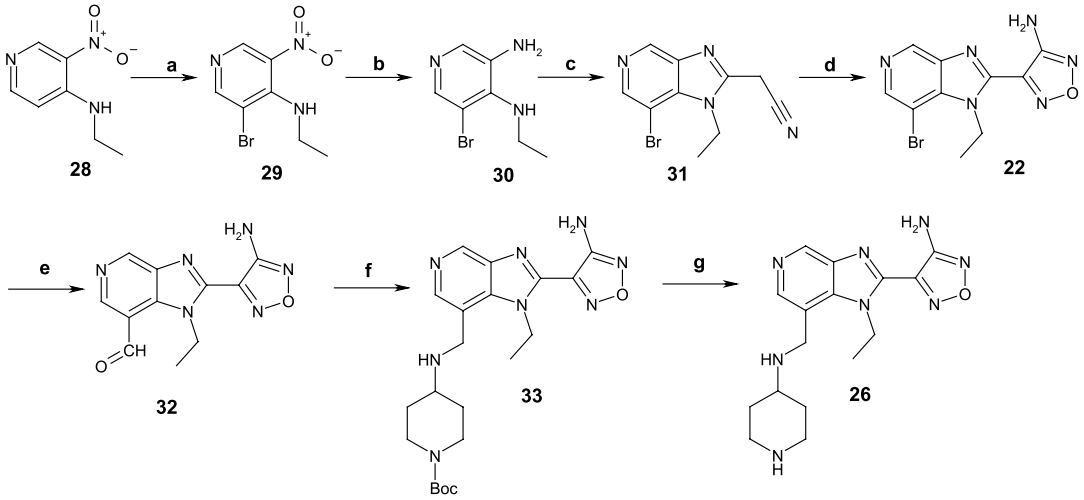


Scheme 1. Preparation of 4-substituted aza-benzimidazole derivatives **3** and **4**. Reagents and conditions: (a) 30% H_2O_2, AcOH, 90 °C (53%); (b) $POCl_3$, 120 °C (100%); (c) 2 M $Al(CH_3)_3$ in toluene, 1,4-dioxan, $Pd(PPh_3)_2Cl_2$, reflux (13%).



Scheme 2. Preparation of 7-substituted aza-benzimidazole derivatives **22** and **26**. Reagents and conditions: (a) Br_2, AcOH, 100 °C (43%); (b) sodium dithionite, ethanol/water, 60 °C; (c) Ethyl cyanoacetate, 190 °C (35%, two steps); (d) (i) $NaNO_2$, MeOH, HCl, rt; (ii) NH_2OH, NaOH, reflux (45%, 2 steps); (e) *n*-BuLi, DMF, THF, −78 °C (41%); (f) 4-amino-piperidine-1-carboxylic acid *tert*-butyl ester, (polystyrylmethyl)trimethylammonium cyanoborohydride, MeOH, AcOH, 21 °C (42%); (g) TFA, DCM, 21 °C (82%).

Assay Development & Compound Profiling for assay of the compounds.

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7. All novel compounds gave satisfactory ^{1}H NMR and LC/MS data in full agreement with their proposed structures.