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# 4-(Benzimidazol-2-yl)-1,2,5-oxadiazol-3-ylamine derivatives: Potent and selective p70S6 kinase inhibitors

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ABSTRACT

We report herein the design and synthesis of 4-(benzimidazol-2-yl)-1,2,5-oxadiazol-3-amine derivatives as inhibitors of p70S6 kinase. Screening hits containing the 4-(benzimidazol-2-yl)-1,2,5-oxadiazol-3-ylamine scaffold were optimized for p70S6K potency and selectivity against related kinases. Structure-based design employing an active site homology model derived from PKA led to the preparation of benzimidazole 5-substituted compounds **26** and **27** as highly potent inhibitors ($K_i$ <1 nM) of p70S6K, with >100-fold selectivity against PKA, ROCK and GSK3.



The 70-kDa ribosomal S6 kinase (p70S6K; S6K1) is a Ser/Thr kinase that has been implicated in the control of cell growth and cell size.[1] As an effector kinase in the phosphoinositide-3-kinase (PI3K) pathway, p70S6K activity is regulated by the tuberous sclerosis complex proteins (TSC1/2) and the mammalian target of rapamycin (mTOR), in response to multiple types of signals.[2] Tuberous sclerosis complex (TSC) is an autosomal dominant disorder characterized by benign tumors (hamartomas) in which mutational inactivation of TSC1/2 leads to constitutive activation of mTOR and p70S6K. Constitutively active p70S6K is also found in many types of cancer cells due to dysregulation of the PI3K pathway.[3] In drosophila, knockout of TSC1/2 is lethal, but the lethality can be rescued by reduction of S6K signaling activity.[4] Taken together, these data suggest that small molecule inhibitors of p70S6K may reduce the oncogenic signal caused by activation of the PI3K pathway and may be an effective therapy for TSC or other types of cancer.

To identify inhibitor leads for p70S6K, we screened our compound collection and identified several 4-(benzimidazol-2-yl)-1,2,5-oxadiazol-3-amine derivatives (**1**) as inhibitors of p70S6K (Fig. 1).

Molecules bearing the 3-amino-1,2,5-oxadiazole moiety have been reported as inhibitors of glycogen synthase kinase (GSK3)[5] (e.g., **2**), as well as Rho kinase (ROCK1)[6] (e.g., **3, 4**) and mitogen and stress-activated protein kinase (MSK1)[7] (e.g., **5**), which, like p70S6K, are members of the AGC subfamily of Ser/Thr kinases.[8]

A homology model of the p70S6K active site was constructed starting from the X-ray crystal structure of PKAα (PDB ID: 1jbp).[9] Placement of the 3-amino-1,2,5-oxadiazole of **1** in its likely binding orientation, with hydrogen-bonding contacts between the amino group and the carbonyl of Glu173, and the oxadiazole N-2 and the backbone NH of residue Leu175 in the kinase hinge region showed an opportunity to improve the inhibitors by creating further interactions with two different binding pockets (Fig. 2). In this model, pocket A (lower left of Fig. 2) consists of the sidechains of residues Met225, Phe382, Glu179 along with the backbone carbonyl of Glu222 (lower middle of Fig. 2) and might be accessible from the benzimidazole nitrogen. Pocket B (upper right of Fig. 2) is a much smaller pocket, deeper in the active site cleft and is bounded primarily by the polar side chains of residues Lys123, Glu143, and Asp236 (upper right of Fig. 2). Access to this region would be made from the benzimidazole 5-position. The synthesis of compounds designed to interact with these two binding pockets is illustrated in Scheme 1.

In this scheme, readily available 2-nitro-4-substituted fluorobenzenes (**6**) were first treated with various amines to afford the substituted-amino nitrobenzenes in good yield. The products were reduced to diamines (**7**) by palladium-catalyzed hydrogenation. Treatment with the methyl 4-amino-1,2,5-oxadiazolyl-3-carbimidate (**8**)[10] gave the desired products (**9**–**27**) in good overall yield.

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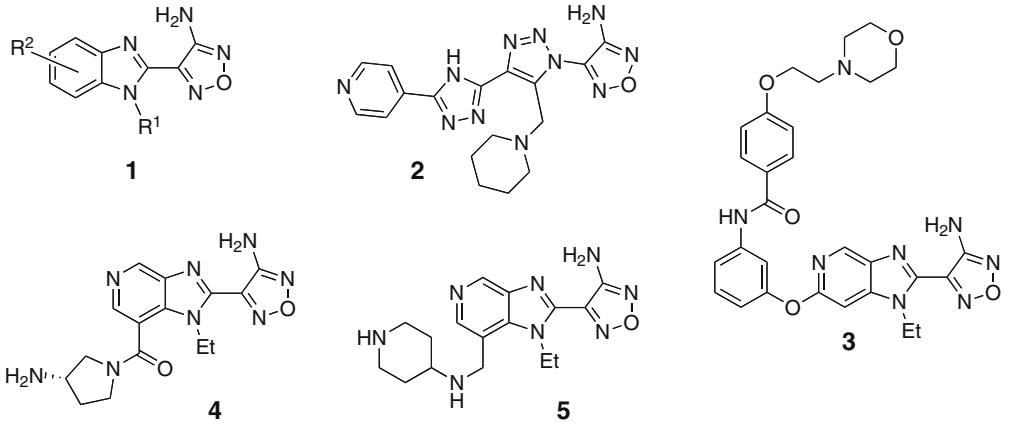



**Figure 1.** 3-Amino-1,2,5-oxadiazole kinase inhibitors.

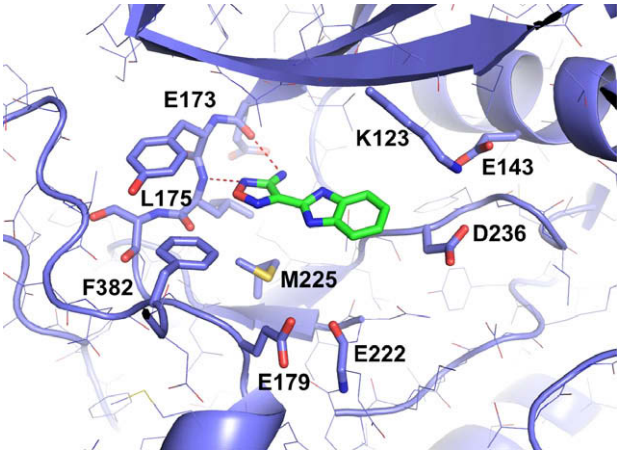
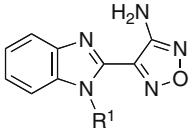



**Figure 2.** Homology model of the proposed binding mode of 4-(benzimidazol-2-yl)-1,2,5-oxadiazol-3-amine derivatives to p70S6K.

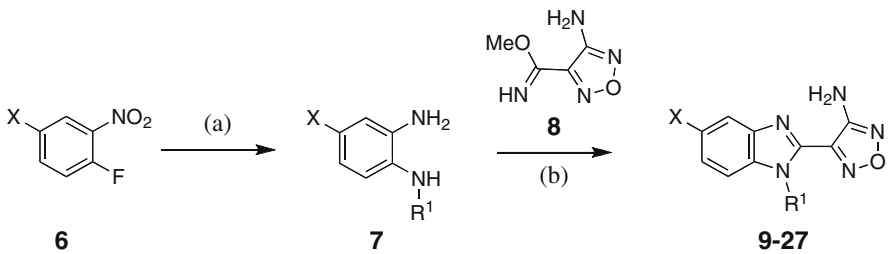
With respect to proposed pocket A, a survey of aliphatic benzimidazole N-1 substituents showed that the modest inhibition exhibited by the methyl group (**9**) could be improved significantly as larger substituents gave notably improved inhibition (Table 1). Directly attached cycloalkyl groups demonstrated the largest improvements with *N*-cyclobutyl (**13**), -cyclopentyl (**14**) and -cyclohexyl (**15**) compounds exhibiting <20 nM inhibition. More polar substituents generally showed significantly weaker binding affinity (data not shown). Aromatic benzimidazole N-1 substituents showed a strong dependency on substituent position. For example, *ortho*-aromatic substituents such as compounds **20** and **23**, were approximately 20-fold more potent than analogous *meta*- or *para*-substituted systems. In addition compounds **20**–**25** were much less active towards GSK3β or ROCK-1. Our model suggests that the conformational preferences for these *N*-aryl compounds (**20**–**25** in Table 1) do not affect the orientation of the 4-(benzimidazol-2-yl)-1,2,5-oxadiazol-3-ylamine relative to the unsubstituted parent compound (**19** in Table 1) but the Met225 sidechain can move to accommodate and make hydrophobic inter-

**Table 1**
Activity of N-substituted 4-(benzimidazol-2-yl)-1,2,5-oxadiazol-3-amine derivatives against p70S6K, PKAα, GSK-3β and ROCK-1[11]



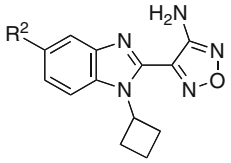
| ID | $R^1$ | p70S6K $K_i$ (μM) | PKAα $K_i$ (μM) | GSK-3β $K_i$ (μM) | ROCK-1 $K_i$ (μM) |
|---|---|---|---|---|---|
| **9** | Me | 0.54 | >4.0 | 7.3 | >3.3 |
| **10** | Et | 0.050 | 0.96 | 0.70 | 0.33 |
| **11** | *i*Pr | 0.040 | >3.3 | 1.5 | 1.9 |
| **12** | cPr | 0.095 | >3.3 | >3.3 | 0.45 |
| **13** | cBu | 0.004 | 0.54 | 0.38 | 0.18 |
| **14** | cPent | 0.013 | 0.72 | 0.21 | 0.34 |
| **15** | cHex | 0.012 | 0.60 | 0.24 | 0.72 |
| **16** | $cPrCH_2$ | 0.012 | 0.38 | 0.29 | 0.36 |
| **17** | $cBuCH_2$ | 0.069 | 1.4 | 0.69 | 0.66 |
| **18** | *i*Bu | 0.022 | 1.1 | 0.39 | 0.93 |
| **19** | Ph | 0.11 | 1.3 | 0.62 | 0.59 |
| **20** | 2-CN–Ph | 0.026 | >5.0 | >3.3 | 0.81 |
| **21** | 3-CN–Ph | 0.80 | >5.0 | >2.5 | >2.5 |
| **22** | 4-CN–Ph | 0.80 | >5.0 | 1.0 | >2.5 |
| **23** | 2-MeO–Ph | 0.031 | >5.0 | >5.0 | 1.3 |
| **24** | 3-MeO–Ph | 1.9 | ND[a] | >5.0 | >5.0 |
| **25** | 4-MeO–Ph | 0.55 | >4.0 | >5.0 | 2.1 |

[a] Result not determined.




**Scheme 1.** Synthesis of 4-(benzimidazol-2-yl)-1,2,5-oxadiazol-3-amine derivatives. Reagents and conditions: (a) (i) $R^1NH_2$, DMF, rt, 88–93%; (ii) $H_2$, Pd/C, EtOH, rt, 2 h, 95%; (b) AcOH, 110 °C, 15 min, 68–82%.

**Table 2**
Activity of 5-substituted 4-(benzimidazol-2-yl)-1,2,5-oxadiazol-3-amine derivatives against p70S6K, PKAα, GSK-3β and ROCK-1[11]

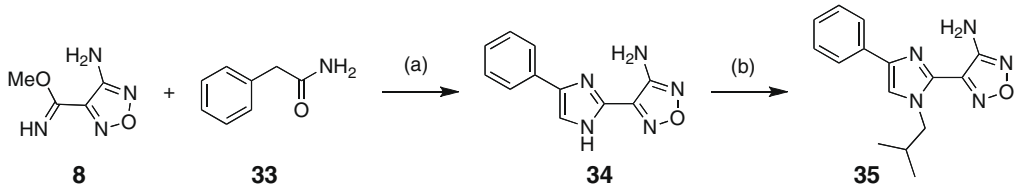


| ID | $R^2$ | p70S6K $K_i$ (μM) | PKAα $K_i$ (μM) | GSK-3β $K_i$ (μM) | ROCK-1 $K_i$ (μM) |
|---|---|---|---|---|---|
| **13** | H | 0.004 | 0.54 | 0.38 | 0.18 |
| **26** | OH | <0.001 | 2.8 | 1.1 | 2.2 |
| **27** | $NH_2$ | <0.001 | 0.034 | 2.1 | 0.085 |
| **28** | OMe | 0.039 | 2.5 | 0.46 | 0.94 |
| **29** | $CO_2Me$ | 0.41 | >3.3 | >3.3 | >3.3 |
| **30** | NHCOMe | 2.2 | >3.3 | >3.3 | >3.3 |
| **31** | Me | 0.019 | 2.6 | 3.2 | >3.3 |
| **32** | $CH_2OH$ | 0.016 | 1.5 | >3.3 | >3.3 |

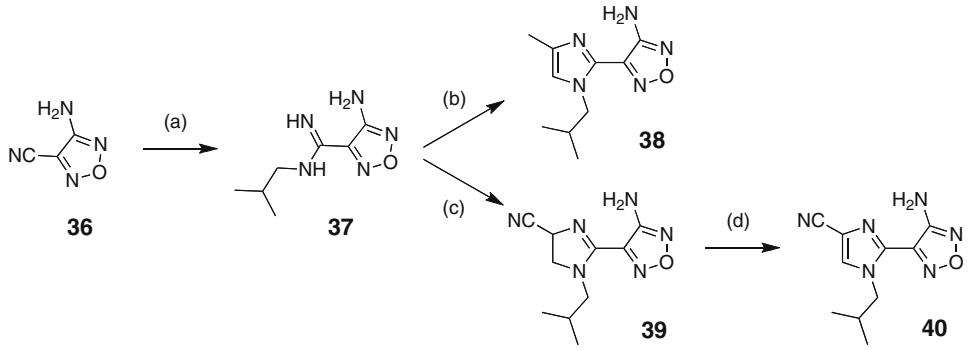
or Asp236 whereas PKAα and ROCK both require both corresponding acidic side chains to be coordinated with intermolecular H-bonds. This could be due to a reconfiguration of the Lys123 side chain. Experimentally determined ligand/kinase complex structures can help elucidate this potentially useful selectivity lesson.

To better understand the value of the hydrophobic interactions provided by the benzene portion of the benzimidazole ring, we removed the ring and substituted the remaining imidazole at the 4-position. The imidazole derivatives were prepared as outlined in Schemes 2 and 3.

The 4-phenyl imidazole derivative (**35**) was prepared by initial condensation of phenylacetamide (**33**) with imidate (**8**) under microwave conditions to afford **34**, which was alkylated with isobutyl iodide to give the desired compound **35** (Scheme 2). The 4-methyl and 4-cyano imidazole derivatives **38** and **40** were prepared as shown in Scheme 3. 4-Amino-1,2,5-oxadiazole-3-carbonitrile[12] was treated with isobutylamine and $AlCl_3$ to afford the intermediate amidine which was used without characterization. Condensation with 1-chloropropanone afforded the 4-methyl



**Scheme 2.** Synthesis of 4-(1-isobutyl-4-phenyl-1*H*-imidazol-2-yl)-1,2,5-oxadiazol-3-amine. Reagents and conditions: (a) EtOH, cat. AcOH, 100 °C, μW, 5 min, 31%; (b) isobutyl iodide, NaH, DMF, 19%.

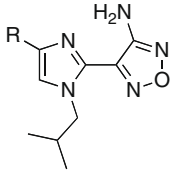


**Scheme 3.** Synthesis of 4-methyl and 4-cyano imidazoles. Reagents and conditions: (a) $^tBuNH_2$, $AlCl_3$, 1,2-DCE, rt, 20 min, 68%; (b) 1-chloropropanone, $K_2CO_3$, DMF, 180 °C, μW, 20 min, 25%; (c) 2-chloroacrylonitrile, $^iPrNEt_2$, THF, 160 °C, μW, 30 min, 50%; (d) $MnO_2$, toluene, 85 °C, 19%.

actions with small *ortho*-substituents. However, *meta*- or *para*-substituents are too large to be accommodated as effectively. The corresponding residues in each of GSK3β, ROCK-1 and PKAα are all leucines, which are more bulky and less able to accommodate the phenyl ring substituents.

Compounds designed to interact with pocket B are shown in Table 2: the benzimidazole substituent is fixed with cyclobutyl. Unsurprisingly, small polar substituents at the benzimidazole 5-position fared the best, with hydroxyl (**26**) and amino (**27**) groups going below the limit of detection of our assay (Table 2). However, larger polar groups such as acetamido (**30**) appear to be either too large for the pocket or possess unsuitable vectors for optimal interaction with the protein and displayed significantly poorer affinity. The off target activities of **26** and **27** raise interesting questions about how similar small polar substituents give rise to very different SAR. One possibility is that for p70S6K and GSK3β, the 5-substituent needs to donate a single H-bond to either Glu143

**Table 3**
Activity of 4-substituted imidazoles against p70S6K, PKAα, GSK-3β and ROCK-1[11]



| ID | R | P70S6K $K_i$ (μM) | PKAα $K_i$ (μM) | GSK-3β $K_i$ (μM) | ROCK-1 $K_i$ (μM) |
|---|---|---|---|---|---|
| **35** | Ph | 0.089 | 1.2 | >3.3 | >3.3 |
| **38** | Me | 0.34 | >3.3 | 0.69 | >3.3 |
| **40** | CN | 1.6 | >3.3 | >3.3 | >3.3 |

derivative (**38**), while condensation with 2-chloroacrylonitrile and subsequent oxidation gave the 4-cyano derivative (**40**).

As shown in Table 3, replacement of the benzimidazole system with a 4-phenylimidazole (compound **35**) maintained the activity and selectivity profile of the parent compound (cf. **18**, Table 1). Imidazoles bearing smaller 4-substituents (**38**, **40**) were significantly less potent.

In summary, a series of low molecular weight, potent and selective inhibitors of p70S6K have been identified and developed using a homology model derived from PKA. Selectivity relative to other AGC-family kinases is good. Compound **13** and the closely analogous benzimidazole 5-substituted analogs **26** and **27**, as well as the N-substituted 4-phenylimidazol-2-yl-1,2,5-oxadiazol-3-amines (e.g., **35**) are promising leads for further exploration of p70S6K and building an understanding of its in vivo role.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.07.022.

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