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Regioselective synthesis of 5- and 6-methoxybenzimidazole-1,3,5-triazines as inhibitors of phosphoinositide 3-kinase

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ABSTRACT

Phosphoinositide 3-kinases (PI3K) hold significant therapeutic potential as novel targets for the treatment of cancer. ZSTK474 (**4a**) is a potent, pan-PI3K inhibitor currently under clinical evaluation for the treatment of cancer. Structural studies have shown that derivatisation at the 5- or 6-position of the benzimidazole ring may influence potency and isoform selectivity. However, synthesis of these derivatives by the traditional route results in a mixture of the two regioisomers. We have developed a straightforward regioselective synthesis that gave convenient access to 5- and 6-methoxysubstituted benzimidazole derivatives of ZSTK474. While 5-methoxy substitution abolished activity at all isoforms, the 6-methoxy substitution is consistently 10-fold more potent. This synthesis will allow convenient access to further 6-position derivatives, thus allowing the full scope of the structure-activity relationships of ZSTK474 to be probed.

The phosphoinositide 3-kinase (PI3K) pathway is an important cellular signalling pathway that functions to elevate levels of cell growth and proliferation. It is one of the most frequently activated pathways in cancer.[1,2] There are four highly homologous isoforms, designated PI3Kα, PI3Kβ, PI3Kγ and PI3Kδ, each having a distinct array of physiological functions. Activating mutations in PI3Kα have been found in about a quarter of breast and endometrial cancers, identifying PI3K as an important target for novel cancer therapeutics.[3]

Among a range of current clinical candidate drugs, ZSTK474 (**4a**) is a potent, pan-PI3K inhibitor that selectively inhibits PI3K over many other related kinases.[4] It is currently in Phase I trials for the treatment of advanced solid malignancies.[5] Like the majority of candidates it has comparable potency at the four Class I isoforms, which may lead to off-target effects that compromise therapeutic utility. Certainly, there is a need for more isoform-selective compounds to help delineate the roles played in cancer by individual isoforms.

The crystal structure of ZSTK474 bound to PI3Kδ was released in 2010.[6] Analysis of the binding mode of ZSTK474 to the p110δ ATP binding site showed that the difluoromethylbenzimidazole ring projects into what has been described as an affinity pocket. It also appeared likely that substitution of the benzimidazole ring might lead to altered potency and selectivity. Indeed, a recent paper detailed a limited number of 4-substituted and 4,6-disubstituted analogues that were effective in improving both solubility and potency.[7] For example, 4-methoxy substitution alone results in a 3-fold increase in potency and a shift towards PI3Kα-selectivity as compared to ZSTK474.

In a similar fashion, we were interested in investigating the influence of 5- and 6-methoxy substituents on potency and selectivity of ZSTK474 and analogues. The 2-difluoromethyl, 2-methyl and 2-isopropyl derivatives **4a–c** could be prepared readily through sequential substitution of cyanuric chloride as detailed in Scheme 1.[7] However, this synthetic scheme was unsuitable for the synthesis of 5- or 6-methoxy substituted benzimidazole derivatives, as use of the 5-methoxybenzimidazoles as reagents resulted in inseparable mixtures of the 5- and 6-methoxy regioisomers due to tautomerism at the ring nitrogens.

This limitation has impeded others working on substituted benzimidazoles relative to other variations such that relatively few examples have been published. In certain cases it may be possible to separate the mixture of such 5- and 6-substituted regioisomers via chromatography as was shown in the case of triazine based MAP kinase inhibitors and in other examples in the patent literature.[8–10] In our case, chromatographic separation couldn't be achieved readily, so a regioselective synthetic approach was necessary.

A recent review has identified a number of approaches to regioselective syntheses of N1-substituted benzimidazoles.[11] Palladium or copper catalysed reactions can be used in either intra- or intermolecular amination and cyclisation reactions using amidines or carbodiimides.[12–16] More recently, Ma and Buchwald have reported the development of a metal-catalysed cascade aryl amination and condensation process.[17–19] These reactions proceed via

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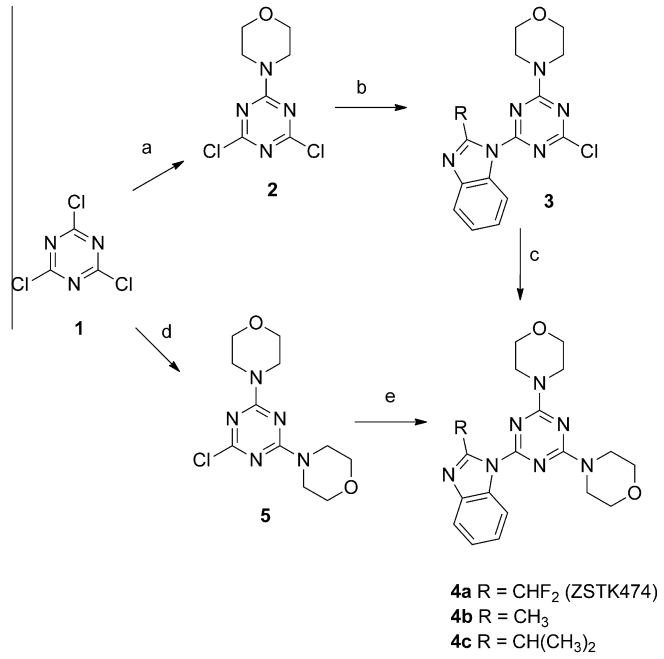


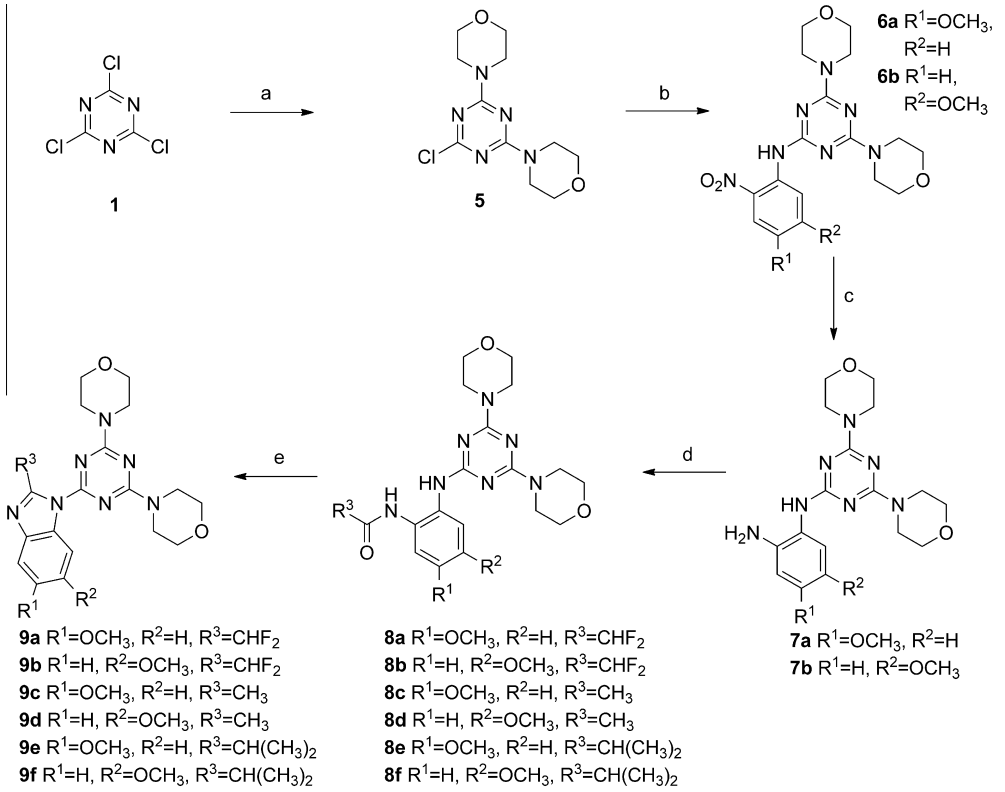
Scheme 1. Reagents and conditions: (a) morpholine, triethylamine, acetone, −20 °C; (b) 2-substituted benzimidazole, K_2CO_3, DMF, room temp; (c) morpholine, K_2CO_3, DMF, MW 140 °C; (d) morpholine, triethylamine, acetone, 0 °C to room temp; (e) 2-substituted benzimidazole, $Pd(OAc)_2$, Xantphos, Cs_2CO_3, 1,4-dioxane, MW 150 °C, 40 min.

o-aminoanilides as intermediates, before a condensation reaction produces the desired benzimidazole product. Metal catalysts can also be used to amidate *o*-halonitroarenes, followed by a reductive cyclisation process.[20,21] Alternatively, an appropriate *o*-nitroaniline can be used, with the free amine group functionalised, followed by a reduction and cyclisation to form the benzimidazole.[22] To our knowledge, there are no reports in the literature of these methods being applied to benzimidazole-substituted triazines.

We have developed a synthetic approach starting with appropriate 4- and 5-methoxy-2-nitroanilines for the synthesis of corresponding methoxy-substituted analogues of ZSTK474 derivatives. This efficient and general synthesis can be achieved under relatively mild conditions, provides regiospecific access to substituted benzimidazole–triazines and allows for further diversification through the 2-position of the benzimidazole (Scheme 2). It has allowed us to elucidate the specific influence of these substituents on PI3K affinity and isoform selectivity.

The precursor 2-chloro-4,6-dimorpholino-1,3,5-triazine **5**[7] was subjected to Buchwald–Hartwig amination conditions with 4-methoxy-2-nitroaniline or 5-methoxy-2-nitroaniline to yield the corresponding substituted triazines **6a** and **6b** in 85% and 77% yields, respectively. The nitro group was reduced with $SnCl_2$ under mild, non-acidic conditions.[23] Due to differences in solubility of the two precursors, the use of ethanol was preferable for the 4-methoxy-2-nitroaniline derivative, giving a yield of 91% of **7a** while ethyl acetate gave enhanced reaction time and yield of 83% for the 5-methoxy-2-nitroaniline derivative **7b**. $SnCl_2$ reduction under acidic conditions was trialled initially, but it was found that the morpholine group was labile.

Quite specific conditions were required to form the desired benzimidazole products, **9a**–**f**. In the first instance, where the phenylenediamine derivative **7a** was refluxed with difluoroacetic acid and a catalytic amount of polyphosphoric acid, a 60:40 mixture of the 6- and 5-methoxy derivatives **9a** and **9b** resulted, a consequence of acid-catalysed isomerisation of the diamine species. Alternatively, refluxing **7a** or **7b** under non-acidic conditions with



Scheme 2. Reagents and conditions: (a) morpholine, triethylamine, acetone, 0 °C to room temp; (b) 4- or 5-methoxy-2-nitroaniline, $Pd(OAc)_2$, Xantphos, Cs_2CO_3, 1,4-dioxane, MW 150 °C; (c) $SnCl_2{\cdot}2H_2O$, ethanol or ethyl acetate, N_2, 70 °C; (d) acid chloride, DIPEA, DCM, room temp; (e) acetic acid, xylenes, 130 °C.

Table 1
Inhibition of PI3K isoforms

Compound	R	OCH_3 position	IC_{50} (μM)			
			PI3Kα	PI3Kβ	PI3Kγ	PI3Kδ
4a	CHF_2	—	0.006	0.006	0.038	0.003
9a	CHF_2	5	>5	>10	>10	>1
9b	CHF_2	6	0.375	0.214	>10	0.110
4b	CH_3	—	0.290	0.523	1.86	0.187
9c	CH_3	5	9.2	>10	>10	2.77
9d	CH_3	6	0.823	1.79	>10	0.458
4c	$CH(CH_3)_2$	—	0.431	0.419	2.53	0.128
9e	$CH(CH_3)_2$	5	>10	>10	>10	1.12
9f	$CH(CH_3)_2$	6	1.05	0.726	>10	0.423

IC_{50} values are the average of three independent experiments.

acetaldehyde in DMF in the presence of sodium metabisulfite did give the desired regioselective 2-methyl products **9c** and **d**.[24] However, a 2-desmethyl side-product was also present which made purification very difficult, resulting in low yields. It was thought to arise from thermal degradation or polymerisation of acetaldehyde during the reaction. Stirring **7a** and acetaldehyde at room temperature in the presence of sodium sulphate as a dehydrating agent gave no reaction after 48 h.[25]

Finally, success was obtained using a two-step approach. Monoacylation of **7a** and **7b** with difluoroacetylchloride was high yielding and gave the regioisomerically pure acetamides **8a** and **8b**, respectively. Cyclisation was completed by refluxing overnight in a mixture of acetic acid and xylenes to give **9a** and **9b**. The corresponding 2-methyl (**9c**, **d**) and 2-isopropyl derivatives (**9e**, **f**) were also obtained using acetyl chloride and isobutyryl chloride, respectively, illustrating the applicability of this method to the introduction of various substituents in the 2-position.

With the six methoxy-substituted derivatives **9a**–**f** and their unsubstituted counterparts **4a**–**c** in hand, they were then tested for activity against each of the four Class I PI3K isoforms. The results are summarized in Table 1.

The inclusion of a methoxy substituent at the 6- or 5-position of ZSTK474 results in analogues manyfold less potent than ZSTK474 (**4a**) itself. The 6-methoxy substituted analogue **9b** was at least 30-fold less potent against the PI3K isoforms and in fact activity was abolished against PI3Kγ. Substitution at the 5-position was even less favoured with compound **9a** unable to achieve 50% inhibition below 1 μM at any of the isoforms. This emphasizes the difference compared to the 4-methoxy substituted analogue reported by Rewcastle et al. which showed improved potency.[7] It is also consistent with their results, which revealed that the 4,5-dimethoxy derivative was significantly less active than both the 4-methoxy and the 4,6-dimethoxy derivatives.[7]

Interestingly, the 2-position benzimidazole substituent influenced the magnitude of potency lost when 5- or 6-methoxy substituents were introduced. Both the 2-methyl and 2-isopropyl substituted analogues of ZSTK474 are themselves somewhat less active than ZSTK474, but in these cases the drop in potency upon addition of a 6-methoxy substituent as in **9d** and **9f** was more marginal at around 2- to 3-fold, except for PI3Kγ which again failed to be inhibited.

Molecular docking was used to study the binding of 5- and 6-methoxy derivatives compared to the crystallographically determined pose of ZSTK474 in PI3Kδ (PDB code 2WXL).[6] In ZSTK474, one morpholinyl oxygen atom forms a hydrogen bond with the backbone amide of V828 in the hinge region of PI3K; the benzimidazole substituent extends into the affinity pocket and the second morpholine group faces the solvent exposed region. Docking suggests that the 6-methoxy substitution should not be as disfavoured as the biochemical data suggests, as compound **9b** is able to adopt

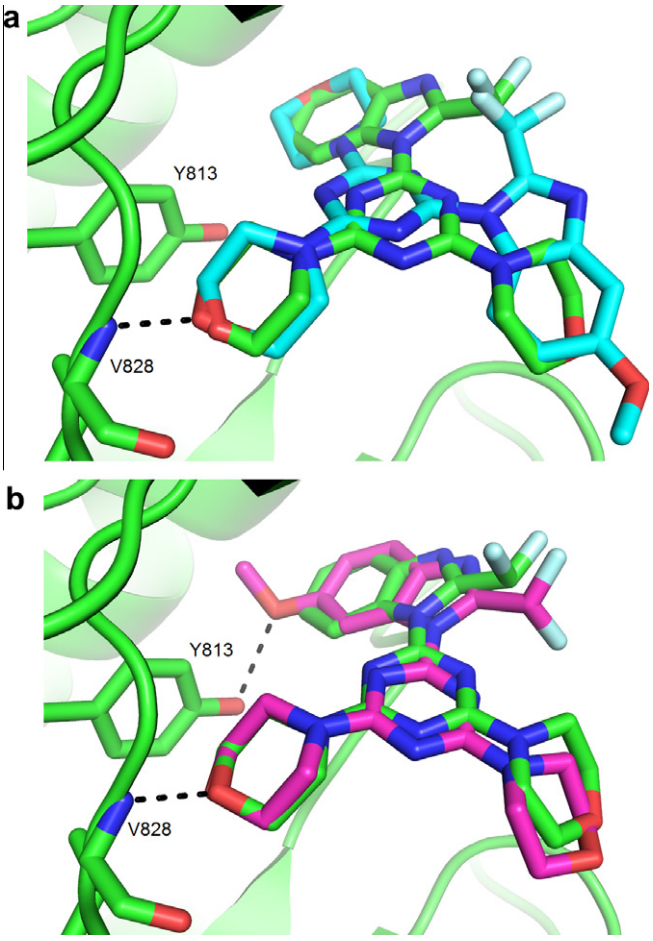


Figure 1. Compounds docked in the ATP-binding site of p110δ (2WXL PDB). (a) Comparison of the docked pose of **9a** (cyan) with the crystal structure binding mode of ZSTK474 (**4a**) (green); (b) comparison of the docked pose of **9b** (magenta) and ZSTK474 (**4a**) (green).

the expected pose (Fig. 1b). Compound **9a**, however, fails to assume the expected pose, instead the 5-methoxybenzimidazole group is facing the solvent exposed region, the second morpholine is in the affinity pocket and the triazine is rotated through the plane (Fig. 1a). The relative potency of the 6- and 5-methoxy substituents appears to be accounted for, however, as 6-methoxybenzimidazole derivatives can form a hydrogen bond with Y813 that the 5-methoxy derivatives cannot. The lost activity at PI3Kγ implies that this isoform is in some way more sensitive to substitution.

In conclusion, we have reported an efficient method for preparing 4,4′-(6-(5-methoxy-2-difluoromethyl-1*H*-benzo[*d*]imidazol-1-yl)-1,3,5-triazine-2,4-diyl)dimorpholine **9a**, its 6-methoxy regioisomer **9b**, and its 2-methyl **9c**,**d** and 2-isopropyl variants **9e**,**f**. Assay results indicate that 6-substituted benzimidazole rings were consistently more potent as PI3K inhibitors than the analogous 5-substituted benzimidazoles. These results illustrate both the synthetic accessibility of regioisomerically pure 6-substituted benzimidazole-1,3,5-triazines, and their utility as PI3K inhibitors.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.11.076.

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