European Journal of Medicinal Chemistry 64 (2013) 137–147

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and biological evaluation of novel phosphatidylinositol 3-kinase inhibitors: Solubilized 4-substituted benzimidazole analogs of 2-(difluoromethyl)-1-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-1*H*-benzimidazole (ZSTK474)

Gordon W. Rewcastle [a,b,*], Swarna A. Gamage [a], Jack U. Flanagan [a,b], Jackie D. Kendall [a,b], William A. Denny [a,b], Bruce C. Baguley [a,b], Christina M. Buchanan [b,c], Mindy Chao [a], Philip Kestell [a], Sharada Kolekar [c], Woo-Jeong Lee [c], Claire L. Lill [a], Alisha Malik [c], Ripudaman Singh [a], Stephen M.F. Jamieson [a,b], Peter R. Shepherd [b,c]

[a] *Auckland Cancer Society Research Centre, School of Medical and Health Sciences, The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand*
[b] *Maurice Wilkins Centre for Molecular Biodiscovery, The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand*
[c] *Department of Molecular Medicine and Pathology, School of Medical and Health Sciences, The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand*

ARTICLE INFO

Article history:
Received 27 September 2012
Received in revised form
28 February 2013
Accepted 21 March 2013
Available online 6 April 2013

Keywords:
Anticancer
Antitumor
PI 3-kinase
Synthesis
ZSTK474

ABSTRACT

A range of 4-substituted derivatives of the pan class I PI 3-kinase inhibitor 2-(difluoromethyl)-1-[4,6-di-(4-morpholinyl)-1,3,5-triazin-2-yl]-1*H*-benzimidazole (ZSTK474) were prepared in a search for more soluble analogs. 4-Aminoalkoxy substituents provided the most potent derivatives, with the 4-$O(CH_2)_3NMe_2$ analog (compound **14**) being identified as displaying the best overall activity in combination with good aqueous solubility (25 mg/mL for the hydrochloride salt). This compound was tested in a U87MG xenograft model, but displayed less potency than ZSTK474 as a result of an unfavorable pharmacokinetic profile.

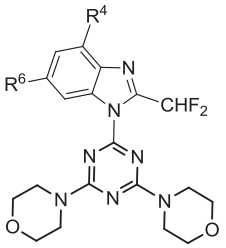
1. Introduction

Phosphoinositide 3-kinases (PI3Ks) are a family of three distinct classes (I, II and III) of lipid kinases that play key roles in cell and tissue physiology [1–3]. The three class-Ia PI 3-kinases (p110α/β/δ) and the sole class-Ib PI 3-kinase (p110γ) couple growth factor receptors and G-protein coupled receptors respectively to a wide range of downstream pathways [4–6]. These enzymes have different methods of activation and different kinetic properties [7], but all use phosphatidylinositol-4,5-diphosphate (PIP2) to produce phosphatidylinositol-3,4,5-triphosphate (PIP3). The cellular levels of PIP3 are tightly controlled by phosphatases including PTEN which dephosphorylates PIP3 back to PIP2 [8,9]. The importance of this pathway in cancer is highlighted by the fact that defects in both the kinase and phosphatase activities are commonly observed in tumors, and there is now increasing evidence that a high proportion of human cancers depend strongly on p110α for their survival and resistance to therapy [8–15]. Therefore the targeting of PI3K with small molecule inhibitors is one of the most promising new approaches to cancer treatment, and a number of programs to develop PI3K inhibitors are currently in progress [5,16–19], with several inhibitors in clinical trial [20–26].

2-(Difluoromethyl)-1-[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl]-1*H*-benzimidazole (ZSTK474) (**1**) (Fig. 1) is a potent ATP-competitive pan-class I PI3K inhibitor, with high selectivity over other classes of

* Corresponding author. Auckland Cancer Society Research Centre, School of Medical and Health Sciences, The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand. Tel.: +64 9 9236147; fax: +64 9 3737502.
E-mail addresses: g.rewcastle@auckland.ac.nz, grewcastle@orcon.net.nz (G.W. Rewcastle).

0223-5234/$ – see front matter © 2013 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.ejmech.2013.03.038



1; $R^4 = R^6 = H$
2; $R^4 = OH$, $R^6 = H$
3; $R^4 = OMe$, $R^6 = H$
4; $R^4 = NH_2$, $R^6 = H$
5; $R^4 = OMe$, $R^6 = NH_2$

Fig. 1. Structure of ZSTK474 (**1**) and substituted analogs.

PI3K and protein kinases [27–29], and demonstrated antitumor activity *in vivo* against human tumor xenografts [27,29].

We recently performed a structure activity relationship (SAR) study of this compound, and identified substitution at the 4- and 6-positions of the benzimidazole ring as having significant effects on the potency of substituted derivatives [30]. Electron donating substituents that increased the electron density on the benzimidazole 3-nitrogen were found to be particularly efficacious, and this was presumed to be due to enhanced H-bonding of the benzimidazole nitrogen to a specific lysine amino group in the ATP binding site of PI3K (Lys802 in p110α, Lys805 in p110β, Lys833 in p110γ and Lys779 in p110δ). 4-Substituents could also participate in H-bonding to the lysine amino group, and the 4-OH derivative **2** was particularly potent, although found to have poor pharmacokinetics, presumably as a result of rapid glucuronidation. The more stable 4-OMe derivative **3** was less potent than **2**, but showed greater selectivity for the p110α isoform of PI3K. Amino substituents were also efficacious, with the 4-NH_2 derivative **4** having similar potency to **3**, while the 4-OMe, 6-NH_2 derivative **5** was particularly potent. *In vivo* testing of **5** showed that it dramatically reduced cancer growth by 81% compared to untreated controls, although it suffered from solubility issues [30].

In the present paper we investigate a wider range of benzimidazole 4-substituents, focusing particularly on oxygen or nitrogen-linked solubilizing moieties that might overcome the solubility problems seen with **5**, while still retaining the ability to assist in binding to the PI3K lysine amino group.

2. Chemistry

The 4-oxygen linked derivatives (**6**–**17**) of Table 1 were prepared by alkylation of phenol **2** [30,31] (Scheme 1). Compounds **6**–**9**, **11**, **12** and **15** were prepared directly from **2**, but the one-step synthesis of **14** occurred in too low a yield to be viable by this route. Accordingly, **14** was prepared from alcohol **8**, via mesylation and subsequent reaction with dimethylamine. Primary amines **10** and **13** were prepared from **2** via the respective phthalimides **18** and **19**, while the amino-alcohols **16** and **17** were prepared via amine addition to epoxide **20**.

The 4-nitrogen linked derivatives (**21**–**25**) of Table 1 were prepared from amine **4** [30,32] or its Boc derivative **26** [30] (Scheme 2). Alkylation of **26** followed by Boc removal gave amine **21**, while amino-amide derivatives **22**–**25** were prepared via acylation of **4**, and subsequent reaction of intermediates **28**–**31**. Thus primary amines **22** and **24** were prepared by TFA deprotection of their Boc derivatives **28** and **29**, while tertiary amines **23** and **25** were prepared by dimethylamine addition to chloroacetamide **29** or acrylamide **31** respectively.

Scheme 3 details an alternative (higher yielding) route to alcohol **8** which was subsequently used for a larger scale preparation of amine **14**. Thus, instead of proceeding through phenol **2**, whose prior synthesis involves a moderately yielding five-step procedure [30,31], the synthesis of **8** was achieved directly in just five steps starting from commercially available 2-amino-3-nitrophenol (**32**). Alkylation of **32** gave alcohol **33** which was converted to benzimidazole **34** by reduction and subsequent ring closure with difluoroacetic acid. Protection of the alcohol group of **34** as its TBDMS derivative, followed by reaction with 2-chloro-4,6-dimorpholino-1,3,5-triazine [33] in DMSO at 130 °C gave **36** which was then deprotected with TBAF to give **8** in 56% overall yield.

3. Results and discussion

3.1. Enzyme inhibition

All new compounds were tested for their enzyme activity against the p110α, β and δ isoforms of PI3K using a lipid kinase assay (Table 1).

Compounds **6**–**17** explored a variety of solubilizing substituents attached via a 4-oxygen linker with acidic carboxylate, neutral alcohol, and basic amine substituents being investigated. The oxyacetic acid derivative **6** did not show good enzyme or cellular potency, and was not studied further. The alcohols **7**–**9** retained good enzyme and very good cellular potency, but did not markedly improve solubility. The basic amino compounds **10**–**17** also retained good cellular potencies but had varying enzyme inhibitory effects, with **14** displaying the best enzyme potency against all three isoforms. Compounds **21**–**25** explored the use of a 4-nitrogen linker, but all were less effective than the oxygen linked analogs.

Overall, the 4-O$(CH_2)_3NMe_2$ analog **14** was the most active of the basic amino compounds, and displayed good aqueous solubility properties (25 mg/mL for the hydrochloride salt), and was therefore selected for further evaluation.

3.2. Cellular inhibition

The compounds were also evaluated using two human tumor cells lines, NZOV9 (Y1021C mutation of p110α enzyme) and NZB5 (wild-type p110α enzyme) and the data are shown in Table 1. The growth inhibitory IC_{50} values for some compounds were comparable with the IC_{50} values for isolated enzyme studies, while others were considerably higher, suggesting variable efficiency in cellular uptake. Growth inhibitory IC_{50} values for the NZOV9 line correlated significantly to enzymatic IC_{50} values for p110α IC_{50} values (Spearman rank correlation; $R = 0.60$; $p = 0.011$). However, NZOV9 IC_{50} values did not correlate significantly with p110β or p110δ IC_{50} values, and NZB5 IC_{50} values did not correlate to IC_{50} values of any of the enzyme assays. These results supported the hypothesis that the mutant p110α enzyme played a significant role in drug-induced inhibition of cell growth.

3.3. Inhibition of cell signaling

To determine whether compound **14** was capable of entering cells and attenuating signaling downstream from PI 3-kinase, the effect on phosphorylation of Akt/PKB was determined in HCT116 cells using previously described methods [34,35]. HCT116 cells were chosen for this experiment as they are commonly used in xenografts and have constitutive activation of the PI 3-kinase pathway due to activation of *PIK3CA*. We determined the IC_{50} for the inhibition of phosphorylation at not only the most commonly measured Ser473 site but also for Thr308 as this site is the one most directly linked to activation of PI 3-kinase in the cell. Compound **14**