Online Research @ Cardiff

This is an Open Access document downloaded from ORCA, Cardiff University's institutional repository: https://orca.cardiff.ac.uk/id/eprint/132824/

This is the author’s version of a work that was submitted to / accepted for publication.

Citation for final published version:


Publishers page: http://dx.doi.org/10.1016/j.bmcl.2020.127040
<http://dx.doi.org/10.1016/j.bmcl.2020.127040>

Please note:
Changes made as a result of publishing processes such as copy-editing, formatting and page numbers may not be reflected in this version. For the definitive version of this publication, please refer to the published source. You are advised to consult the publisher’s version if you wish to cite this paper.

This version is being made available in accordance with publisher policies. See http://orca.cf.ac.uk/policies.html for usage policies. Copyright and moral rights for publications made available in ORCA are retained by the copyright holders.
Development of 2-(4-pyridyl)-benzimidazoles as PKN2 chemical tools to probe cancer

Fiona Scott\textsuperscript{h},\textsuperscript{a}, Angela M. Fala\textsuperscript{b},\textsuperscript{c}, Lewis E. Pennicott\textsuperscript{a}, Tristan D. Reuillon\textsuperscript{a}, Katlin B. Massirer\textsuperscript{b},\textsuperscript{c}, Jonathan M. Elkins\textsuperscript{d}, Simon E. Ward\textsuperscript{a,\textsuperscript{e}}

\textsuperscript{a} Sussex Drug Discovery Centre, University of Sussex, Sussex House, Falmer, Brighton BN1 9RH, United Kingdom
\textsuperscript{b} Centro de Química Medicinal (CQMED), Centro de Biologia Molecular e Engenharia Genética (CBMEG), Universidade Estadual de Campinas (UNICAMP), Campinas, SP 13083-875, Brazil
\textsuperscript{c} Structural Genomics Consortium, Nuffield Department of Medicine, University of Oxford, Oxford OX3 7DQ, United Kingdom
\textsuperscript{d} Centro de Química Medicinal (CQMED), Centro de Biologia Molecular e Engenharia Genética (CBMEG), Universidade Estadual de Campinas (UNICAMP), Campinas, SP 13083-886, Brazil
\textsuperscript{e} Structural Genomics Consortium, Nuffield Department of Medicine, University of Oxford, Oxford OX3 7DQ, United Kingdom
\textsuperscript{f} Medicines Discovery Institute, Cardiff University, Main Building, Park Place, Cardiff CF10 3AT, United Kingdom

\textbf{A R T I C L E  I N F O}

Keywords: Kinases Cancer Heart failure Inflammation AGC kinase PKN PRK2 Protein kinase N2 Benzimidazole Chemical probe Chemical tool

\textbf{A B S T R A C T}

Kinases are signalling proteins which have proven to be successful targets for the treatment of a variety of diseases, predominantly in cancers. However, only a small proportion of kinases (<20%) have been investigated for their therapeutic viability, likely due to the lack of available chemical tools across the kinome. In this work we describe initial efforts in the development of a selective chemical tool for protein kinase N2 (PKN2), a relatively unexplored kinase of interest in several types of cancer. The most successful compound, 5, has a measured IC\textsubscript{50} of 0.064 μM against PKN2, with ca. 17-fold selectivity over close homologue, PKN1.

Chemical tools/probes are drug-like compounds used to answer biological questions. They need not possess all the properties of a drug candidate, which can be dialled in at a later point in the drug development process. These compounds only need to be sufficiently stable, potent and selective towards their particular target.\textsuperscript{1,2}

Historically, the approval of imatinib\textsuperscript{3} as an effective Abl kinase inhibitor for treating chronic myeloid leukaemia stimulated efforts to better understand the 518 human protein kinases and their role in disease. Trends in research\textsuperscript{4} suggest that less than 20% of the human kinome has been well-studied,\textsuperscript{5} and selective inhibitors are only available for an even smaller fraction of those kinases.

Protein kinase N2 (PKN2) (Fig. 1) is one of these understudied kinases. It is an AGC-type serine/threonine protein kinase. There are more than 60 AGC protein kinases in the human genome with 14 further classifications. PKN2 falls into the PKN sub-family, closely related to the PKC sub-family, and is one of three homologues (PKN1/2/3). It has a number of pseudonyms which include protein kinase C-related kinase 2 (PRK2), PKN\textsubscript{7}, PAK2, PRO2, and STK7.\textsuperscript{6}

\textbf{Abbreviations:} PKN, protein kinase N; Abl, Abelson murine leukemia viral oncogene; IC\textsubscript{50}, half maximal inhibitory concentration; AGC, protein kinase A/G/C families; PKC, protein kinase C; PRK, protein kinase C-related kinase; PRK2, p21 activated kinase 2; PRO2, glutamate 5-kinase Pro2; STK, serine/threonine kinase; PDB, protein databank; PARP, poly(ADP-ribose) polymerase; CHEMBL, European Molecular Biology Laboratory Chemical database; CLX, CDC2-like kinase; SAR, structure activity relationship; CDI, 1,1′-carbonyldiimidazole; TR-FRET, time resolved fluorescence resonance energy transfer; THF, tetrahydrofuran; EtOH, ethanol; HATU, hexafluorophosphate azabenzotriazole tetramethyl uronium; DIPPA, N,N-diisopropylethylamine; DCM, dichloromethane; AcOH, acetic acid; DMF, N,N-dimethyl-formamide; K_diss, dissociation constant; K_i, inhibitor constant; NMR, nuclear magnetic resonance; DMSO, dimethyl sulfoxide; MeOH, methanol; GST, glutathione S-transferase; DNA, deoxyribonucleic acid; SFM, scanning force microscopy; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; TCEP, tris(2-carboxyethyl)phosphine; EDTA, ethylendediaminetetraacetic acid; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; ATP, adenosine triphosphate; EGTA, egtazic acid; CV, column volumes

* Corresponding author.

E-mail addresses: f.scott@sussex.ac.uk (F. Scott), angelafala@gmail.com (A.M. Fala), l.e.pennicott@sussex.ac.uk (L.E. Pennicott), treuillon@its.jnj.com (T.D. Reuillon), kmassire@unicamp.br (K.B. Massirer), jon.elkins@sgc.ox.ac.uk (J.M. Elkins), wards10@cardiff.ac.uk (S.E. Ward).

https://doi.org/10.1016/j.bmcl.2020.127040

Received 15 November 2019; Received in revised form 28 January 2020; Accepted 14 February 2020

Available online 17 February 2020

0960-894X/ © 2020 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).
While the alkylation conditions given were said to be applicable to methylation of the benzimidazole N-H group, this proved unsuccessful; a dimethylated product formed instead, thought to be due to the susceptibility for the 4′-pyridyl to also alkylate after the benzimidazole N-H was capped using chemistry described by Tsukamoto et al. 36

The scope of this chemistry enabled the synthesis of 14 analogues using commercially available nitroanilines and di-anilines. Additional alkylation conditions allowed the capping of the benzimidazole N-H 36 (6) and alternative amide coupling conditions were used for preparing compound 11 37 and the penultimate amide intermediate used to make compound 19 38.

The potencies and selectivities of these compounds were tested using a TR-FRET binding-displacement assay in which the IC50 values were measured (Table 1). Calculation of Ki values using the Cheng-Prusoff equation and the K0 of the tracer (previously determined) allowed the affinity of the inhibitors for PKN2 and PKN1 to be compared (Table 1).

Compound 5 was validated as a PKN2 inhibitor (Ki = 0.032 μM) with 17-fold selectivity over PKN1 (Ki = 0.500 μM) which was not previously included in the Abbott library screen used in the Metz et al. study.31

The benzimidazole N=H was capped using chemistry described by Tsukamoto et al. 36 While the alkylation conditions given were said to be applicable to methylation of the benzimidazole using the corresponding methyl halide, this proved unsuccessful; a dimethylated product formed instead, thought to be due to the susceptibility for the 4′-pyridyl to also alkylate after the benzimidazole N-H. Repeating the specific reaction conditions used by the authors incorporated a methyl acetate ester at the 1-position (6) which led to loss of binding to PKN2.

PKNs have a fairly conserved primary sequence and they share the same architecture. The catalytic domain of PKN2 has 87% percent identity with PKN1; 70% with PKN2; and 50% with PKC kinases, while the N-termini regions are less conserved, sharing only 48% and 40% between PKN1/2 and PKN2/3, respectively.7,8

PKNs have been linked to various cellular roles, including cytoskeleton regulation,9 transport,10 cell adhesion,11 nutrient signalling,12 and cell cycle,13 as well as being a target of interest in colon,14 breast,15 renal,16 head,17 neck,18 and prostate cancers.19 They are also reportedly involved in inflammation19,20 and heart failure.21 So far, there is one X-ray crystal structure of PKN2 publicly available in the Protein Data Bank (PDB ID: 4CRS) (Fig. 2).

These previous studies have elucidated functions for PKN2 using molecular and cell biology techniques, and the conclusions would be greatly supported by validation through the use of small molecule inhibitors, especially to evaluate PKN2’s potential as a cancer drug target. Potent inhibitors are known for several AGC kinase family members, including ROCK22–25 and PKC,26 but currently there are no sufficiently selective inhibitors for PKN2.12

This work describes an initial effort to develop such compounds based around a benzimidazole core. Compound 5 was successfully developed as a PARP inhibitor27–29 but exhibited higher potency towards PKN2 than its desired target. Benzimidazoles are N-containing heterocycles that are prevalent in medicinal chemistry.30 The compound was found as part of a screen of the Abbott chemical library31 via the ChEMBL database when searching for PKN2 inhibitors. It had a reported Ki of 0.040 μM against PKN2 while only inhibiting two out of 137 other kinases (PKN1 and CLK4) with potencies lower than 0.100 μM.32 This was deemed a good starting point for repurposing the compound as a PKN2 inhibitor. We report the synthesis of that compound and subsequent SAR studies to determine its viability as a chemical tool for establishing the potential of PKN2 as a therapeutic target.

Compound 5 was successfully synthesised via a four step synthesis (Scheme 1). 2-Amino-3-nitro-benzoic acid (1) was treated with ammonia and CDI-coupling conditions32 to form amide 2. The 3-nitro group was reduced to aniline 3 with sodium dithionate,33 followed by the coupling of isonicotinic acid to the 3-position aniline to form amide 4,34 which was then heated in acetic acid to form benzimidazole 5.35

The potencies and selectivities of these compounds were tested using a TR-FRET binding-displacement assay in which the IC50 values were measured (Table 1). Calculation of Ki values using the Cheng-Prusoff equation and the K0 of the tracer (previously determined) allowed the affinity of the inhibitors for PKN2 and PKN1 to be compared (Table 1).

Compound 5 was validated as a PKN2 inhibitor (Ki = 0.032 μM) with 17-fold selectivity over PKN1 (Ki = 0.500 μM) which was not previously included in the Abbott library screen used in the Metz et al. study.31

The benzimidazole N=H was capped using chemistry described by Tsukamoto et al. 36 While the alkylation conditions given were said to be applicable to methylation of the benzimidazole using the corresponding methyl halide, this proved unsuccessful; a dimethylated product formed instead, thought to be due to the susceptibility for the 4′-pyridyl to also alkylate after the benzimidazole N-H. Repeating the specific reaction conditions used by the authors incorporated a methyl acetate ester at the 1-position (6) which led to loss of binding to PKN2.

PKNs have been linked to various cellular roles, including cytoskeleton regulation,9 transport,10 cell adhesion,11 nutrient signalling,12 and cell cycle,13 as well as being a target of interest in colon,14 breast,15 renal,16 head,17 neck,18 and prostate cancers.19 They are also reportedly involved in inflammation19,20 and heart failure.21 So far, there is one X-ray crystal structure of PKN2 publicly available in the Protein Data Bank (PDB ID: 4CRS) (Fig. 2).

These previous studies have elucidated functions for PKN2 using molecular and cell biology techniques, and the conclusions would be greatly supported by validation through the use of small molecule inhibitors, especially to evaluate PKN2’s potential as a cancer drug target. Potent inhibitors are known for several AGC kinase family members, including ROCK22–25 and PKC,26 but currently there are no sufficiently selective inhibitors for PKN2.12

This work describes an initial effort to develop such compounds based around a benzimidazole core. Compound 5 was successfully developed as a PARP inhibitor27–29 but exhibited higher potency towards PKN2 than its desired target. Benzimidazoles are N-containing heterocycles that are prevalent in medicinal chemistry.30 The compound was found as part of a screen of the Abbott chemical library31 via the ChEMBL database when searching for PKN2 inhibitors. It had a reported Ki of 0.040 μM against PKN2 while only inhibiting two out of 137 other kinases (PKN1 and CLK4) with potencies lower than 0.100 μM.32 This was deemed a good starting point for repurposing the compound as a PKN2 inhibitor. We report the synthesis of that compound and subsequent SAR studies to determine its viability as a chemical tool for establishing the potential of PKN2 as a therapeutic target.

Compound 5 was successfully synthesised via a four step synthesis (Scheme 1). 2-Amino-3-nitro-benzoic acid (1) was treated with ammonia and CDI-coupling conditions32 to form amide 2. The 3-nitro group was reduced to aniline 3 with sodium dithionate,33 followed by the coupling of isonicotinic acid to the 3-position aniline to form amide 4,34 which was then heated in acetic acid to form benzimidazole 5.35

The potencies and selectivities of these compounds were tested using a TR-FRET binding-displacement assay in which the IC50 values were measured (Table 1). Calculation of Ki values using the Cheng-Prusoff equation and the K0 of the tracer (previously determined) allowed the affinity of the inhibitors for PKN2 and PKN1 to be compared (Table 1).

Compound 5 was validated as a PKN2 inhibitor (Ki = 0.032 μM) with 17-fold selectivity over PKN1 (Ki = 0.500 μM) which was not previously included in the Abbott library screen used in the Metz et al. study.31

The benzimidazole N=H was capped using chemistry described by Tsukamoto et al. 36 While the alkylation conditions given were said to be applicable to methylation of the benzimidazole using the corresponding methyl halide, this proved unsuccessful; a dimethylated product formed instead, thought to be due to the susceptibility for the 4′-pyridyl to also alkylate after the benzimidazole N-H. Repeating the specific reaction conditions used by the authors incorporated a methyl acetate ester at the 1-position (6) which led to loss of binding to PKN2.
Moving the 4′-pyridyl nitrogen in 7 and 8 resulted in loss of activity, as did introducing an electron-donating methoxy group at the 3′-position (9). This suggests the 4′-pyridyl ring acts as the hinge binder.

Attempts to make the 2′-pyridyl and 4′-pyrimidine analogues were unsuccessful (Scheme 2).

Capping the amide with one (10) or two (11) methyl groups led to increasing loss of activity respectively. Potency was lost when the amide was moved to the 5-position of the benzimidazole ring (12), Removing the amide completely (13) or exchanging the 4- or 5-position for another functional group (14–18) also led to loss of activity.

Introduction of a bromine at the 6-position (19) was hoped to provide a useful handle for incorporating various alkyl/aryl groups at that position using Suzuki coupling chemistry. This reaction was attempted at multiple stages of the synthetic route but was unsuccessful. Compound 19 was active against PKN2 but was nearly three times less potent than compound 5. Despite this reduction in potency, compound 19 is 26-fold selective over PKN1.

The SAR exploration around 5 confirms that the primary amide at the 4-position, 4′-pyridyl and free N–H at the 1-position are necessary for the compound’s activity against PKN2. Subsequent analogues prepared for this series did not improve potency for the target within the PKN family but did result in a slight improvement in selectivity over PKN1 in compound 19.

Chemical tools are needed to facilitate the exploration of lesser understood kinases such as PKN2 for its roles in healthy and cancerous cells. Benzimidazole 5 was validated as an inhibitor of PKN2 with IC\textsubscript{50} 0.064 μM and with ca. 17-fold selectivity over PKN1 with reported high selectivity across the wider kinome. Our efforts to develop a new compound to inhibit PKN2 resulted in compound 19 which was 26-fold selective for PKN2 over PKN1 despite having a near three-fold reduction in potency compared to compound 5.

**Acknowledgements**

This work was supported by a Continuing Excellence Fund from the Genome Damage and Stability Centre, University of Sussex. Thanks also to additional funding from the Wellcome Trust for initial assay experiments.

This work was also supported by the Brazilian agencies FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) (2013/
Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmbcl.2020.127040

References


