Development of 2-(4-pyridyl)-benzimidazoles as PKN2 chemical tools to probe cancer

Fiona Scotta,⁎, Angela M. Fala,b,c, Lewis E. Pennicottb, Tristan D. Reuillonb, Katlin B. Massirerb,c, Jonathan M. Elkinsd, Simon E. Warda,e

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

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Abstract

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 IC₅₀ 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.1,2

Historically, the approval of imatinib3 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 research4 suggest that less than 20% of the human kinome has been well-studied,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), PKNγ, PAK2, PRO2, and STK7.6

Abbreviations: PKN, protein kinase N; Abl, Abelson murine leukemia viral oncogene; IC₅₀, half maximal inhibitory concentration; AGC, protein kinase A/G/C families; PKC, protein kinase C; PRK, protein kinase C-related kinase; PAK2, 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; CLK, CDC2-like kinase; SAR, structure activity relationship; CDI, 1,1′-carbonyldimidazole; TR-FRET, time resolved fluorescence resonance energy transfer; THF, tetrahydrofuran; EtOH, ethanol; HATU, hexafluorophosphate azabenzotriazole tetramethyl uronium; DiPEA, N,N-diisopropylethylamine; DCM, dichlormethane; AcOH, acetic acid; N,N-dimethyl-formamide; Kᵣ, dissociation constant; Kᵢ, 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, ethylenediaminetetraacetic acid; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; ATP, adenosine triphosphate; EGTA, egtaic 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).

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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 PKN3; 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,\(^17\) and prostate cancers.\(^18\) They are also reportedly involved in inflammation\(^19,20\) 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 ROCK\(^22–25\) 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 previously developed as a PARP inhibitor\(^27–29\) 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 library via the ChEMBL database when searching for PKN2 inhibitors. It had a reported \(K_i\) of 0.040 \(\mu\)M against PKN2 while only inhibiting two out of 137 other kinases (PKN1 and CLK4) with potencies lower than 0.100 \(\mu\)M.\(^31\) 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 conditions\(^32\) 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 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 IC\(_{50}\) values were measured (Table 1). Calculation of \(K_i\) values using the Cheng-Prusoff equation and the \(K_D\) 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 (\(K_i = 0.032 \, \mu\)M) with 17-fold selectivity over PKN1 (\(K_i = 0.500 \, \mu\)M) 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 IC₅₀ 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.

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Appendix A. Supplementary data

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

References

1. Blagg J, Workman P. Choose and use your chemical probe wisely to explore cancer biology. Cancer Cell. 2017;22(1):19–25. https://doi.org/10.1016/j.ccell.2017.06.005.

2. Arrowsmith GH, Audia JE, Austin C, et al. The promise and peril of chemical probes. Nat Chem Biol. 2015;11(8):536–541. https://doi.org/10.1038/nchembio.1867.

3. Gleevec: the Breakthrough in Cancer Treatment. http://www.nature.com/scitable/topicpage/gleevec-the-breakthrough-in-cancer-treatment-565. Accessed March 17, 2016.

4. Fedorov O, Müller S, Knapp S. The (un)targeted cancer kinase. Nat Chem Biol. 2011;6(9):166–169. https://doi.org/10.1038/nchembio.2979.

5. Fabbro D, Cowan-Jacob SW, Moebitz H. Ten things you should know about protein kinase 

6. Bauer AF, Sonzogni S, Meyer L, et al. Regulation of protein kinase C-related protein

7. Tanaka H, Bohno A, et al. Azole-Substituted Pyridine Compound. WO/2019/031618. 2019.

8. Van Steijvoort BF, Kaval N, Kulago AA, Maes BUW. Remote functionalization: palladium-catalyzed C5(sp3)-H arylation of 1-Boc-3-aminopiperidine through the use of B308412D.

9. Kock M, Lusich W, Jentzsch A, Use of Parp Inhibitors in Cosmetic Preparations. US7601666. 2005.

10. Amano M, Nakayama M, Kaibuchi K. Rho-kinase/ROCK: a key regulator of the cytoskeleton and cell polarity. J Biol Chem. 2010;285(23):17477–17484. https://doi.org/10.1074/jbc.M109.095307.

11. Calautti E, Grossi M, Mammucari C, et al. Fyn tyrosine kinase is a downstream mediator of Rho/PRK2 function in keratinocyte cell–cell adhesion. J Cell Biol. 2016;205(20):3543–3557. https://doi.org/10.1083/jcb.201605011.

12. Wallroth A, Koch PA, Marat AL, Krause E, Haucke V. Protein kinase N controls a cytokinin-induced cardiac dysfunction through phosphorylation of myocardial-related transcription factor A and disruption of its interaction with actin. Circulation. 2019. https://doi.org/10.1161/CIRCULATIONAHA.119.041019.

13. Shao J, Hollingworth G, Soldermann N, et al. Novel ROCK inhibitors for the treatment of pulmonary arterial hypertension. Bioorg Med Chem Lett. 2014;24(20):4812–4817. https://doi.org/10.1016/j.bmcl.2014.09.002.

14. Cheng Y, Zhu Y, Xu J, et al. PKN2 in colon cancer cells inhibits M2 phenotype polarization. Mol Cancer. 2018;17:13. https://doi.org/10.1186/s12943-017-0747-z.

15. Lin W, Huang J, Yuan Z, Feng S, Xie Y, Ma W. Protein kinase C inhibitor chelerythrine selectively inhibits proliferation of triple-negative breast cancer cells. Sci Rep. 2017;7(1):2022. https://doi.org/10.1038/s41598-017-02222-y.

16. Hopkins SR, Mgcgregor GA, Murray JM, Downes JA, Savic V. Novel synthetic lethality screening method identifies TIP60-dependent radiation sensitivity in the absence of BAFl80. DNA Repair (Amst). 2016;46:47–54. https://doi.org/10.1016/j.dnarep.2016.05.030.

17. Rajagopalan P, Nanjappa V, Patel K, et al. Role of protein kinase N2 (PKN2) in cigarette smoke-mediated oncogenic transformation of oral cells. J Cell Commun Signal. 2012;5(4):790–721. https://doi.org/10.1007/s12070-012-0042-2.

18. O’Sullivan AG, Mulvaney EP, Hyland PB, Kinsella BT. Protein kinase C-related kinase 1 and 2 play an essential role in thromboxane-mediated nesopirolide responses in prostate cancer. Oncotarget. 2015;6(28):26437–26456. https://doi.org/10.18632/oncotarget.6664.

19. Park YH, Wood G, Kastner DL, Chae JJ. Pyrin inflammasome activation and RhoA signaling in the autoinflammatory diseases FMF and HIDS. Nat Immunol. 2016;17(8):914–921. https://doi.org/10.1038/ni.3547.

20. Schnappauf O, Chae JJ, Kastner DL, Akseintjiech T. The pyrin inflammasome in health and disease. Front Immunol. 2019;10:1745. https://doi.org/10.3389/fimmu.2019.01745.

21. Sakuguchi T, Takefushi M, Wetscherek N, et al. Protein kinase N promotes stress-induced cardiac dysfunction through phosphorylation of myocardial-related transcription factor A and disruption of its interaction with actin. Circulation. 2019. https://doi.org/10.1161/CIRCULATIONAHA.119.041019.

22. Shen J, Hollingworth G, Soldermann N, et al. Novel ROCK inhibitors for the treatment of pulmonary arterial hypertension. Bioorg Med Chem Lett. 2014;24(20):4812–4817. https://doi.org/10.1016/j.bmcl.2014.09.002.

23. Pan J, Yin Y, Zhao L, Feng Y. Discovery of (3-6-methoxy-3-chroman-3-carboxylic acid)-(4-pyridin-4-yl)-amide as potent and isoform selective ROCK2 inhibitors. Bioorg Med Chem. 2019;27(7):1382–1390. https://doi.org/10.1016/j.bmc.2019.02.047.

24. Fabbro D, Cowan-Jacob SW, Moebitz H. Ten things you should know about protein kinase

25. Fabbro D, Cowan-Jacob SW, Moebitz H. Ten things you should know about protein kinase

26. Fabbro D, Cowan-Jacob SW, Moebitz H. Ten things you should know about protein kinase

27. Kock M, Lusich W, Jentzsch A, Use of Parp Inhibitors in Cosmetic Preparations. US7601666. 2005.

28. Lubisch W, Kock M et al. Heterocyclically substituted benzimidazoles, the production and application thereof. US6966437. 2004.

29. Takayama K, Koga Y et al. Benzimidazole Derivatives. WO/2001/02615. 2001.

30. Velik J, Baliharová V, Fínek-Greemms J, Bull S, Lamka J, Skálová L. Benzimidazole drugs and modulation of biotransformation enzymes. Res Vet Sci. 2010;89(2):395–108. https://doi.org/10.1016/j.resv.2009.05.005.

31. Metz JT, Johnson EF, Soni NB, Merta PJ, Kifle L, Hajdok PJ, Navigation the kine. Nat Chem Biol. 2011;7(4):200–202. https://doi.org/10.1038/nchembio.530.

32. Francis C, Rix, Smita Kacker, Sudhin Datta, Rul Zhao VRE. Olefin polymerization catalyst system and process for use thereof. US7061666. 2005.

33. Schmidt A, Shibalibian AG, Nieger M, Mariand M, Levillain P, Sense JM. On benzo[b][1,4]diazepinium-olates, -cholates and -carboxylates as anti-Hüeck mesomeric be-

34. Schwartz T, Kastner DL, Chae JJ. Pyrin inflammasome activation and RhoA signaling in the autoinflammatory diseases FMF and HIDS. Nat Immunol. 2016;17(8):914–921. https://doi.org/10.1038/ni.3547.