MISS MALIKOTSI QHOBOSHEANE (Orcid ID : 0000-0003-3005-0028)
PROFESSOR LESETJA J LEGOABE (Orcid ID : 0000-0003-2440-4993)
DR RICHARD MBI BETECK (Orcid ID : 0000-0002-6282-043X)

Article type : Research Letter

Synthesis and evaluation of C3 substituted chalcone-based derivatives of 7-azaindole as protein kinase inhibitors

Malikotsi A. Qhobosheane[1], Lesetja J. Legoabe[1,*], Béatrice Josselin[2,3], Stéphane Bach[2,3], Sandrine Ruchaud[2], Richard M. Beteck[1]

[1] *Centre of Excellence for Pharmaceutical Sciences, North-West University, Private Bag X6001, Potchefstroom 2520, South Africa*

[2] *Sorbonne Université, CNRS, UMR 8227, Integrative Biology of Marine Models Laboratory (LBI2M), Station Biologique de Roscoff, CS 90074, 29688 Roscoff Cedex, France*

[3] *Sorbonne Université, CNRS, FR2424, Plateforme de criblage KISSf (Kinase Inhibitor Specialized Screening facility), Station Biologique de Roscoff, CS90074, 29682 Roscoff, France*

* Correspondence should be addressed to LJL, email: lesetja.legoabe@nwu.ac.za

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/CBDD.13748

Abstract

Chalcones are a group of naturally occurring or synthetic compounds which possess a wide range of biological activities. In this paper, a series of twenty-three 7-azaindole-chalcone hybrids (**5a-w**) were synthesised and evaluated as potential protein kinase inhibitors. Analyses of structure activity relationships (SAR) revealed that some of these compounds exhibit significant activity against Haspin kinase; with compound **5f,** and **5q** exhibiting IC_{50} values of 0.47 μM and 0.41 μM respectively. Furthermore, **5f** also inhibits cyclin-dependent kinase 9 (CDK9/ CyclinT) in a micromolar potency (IC_{50} = 2.26 μM). This novel dual-target inhibitor is a promising lead for the development of chemopreventive/chemotherapeutic agents.

Keywords: 7-azaindole, chalcone, protein kinase, structure activity relationships, CDK9/CyclinT, Haspin, dual inhibitor.

1. Introduction

The protein kinase family is one of the super families of enzymes comprising of more than 500 proteins which are known to be highly evolutionary conserved across numerous eukaryotic species (Saxena *et al.*, 2017). These proteins regulate various cellular events, including cell survival, growth, differentiation and migration (London, 2013). They regulate these events by catalysing the transfer of phosphate(s) to serine, threonine, and, tyrosine residues of their substrate proteins (Drewry *et al.*, 2017). According to Tong *et al.* (2013), the protein kinase structure is made of a structurally well-defined ATP binding site and a structurally variable substrate binding site. The ATP binding site comprises of approximately 25 residues, some of which are nearly fully conserved for all kinases. However, some residues, particularly the "gatekeeper", exhibit higher variation.

Abnormal phosphorylation, hyperactivity or overexpression of protein kinases is implicated in a variety of human diseases; including cancer, diabetes and rheumatoid arthritis (Zhao & Bourne, 2018; Roskoski, 2019). As a result, this enzyme family has become one of the most important drugs target. Recent studies reported that approximately 20-30% of drug discovery efforts worldwide are directed towards the protein kinase superfamily (Roskoski, 2019). Zhang *et al.* (2009) reported that small molecule inhibitors of protein function are the most common and effective agents for molecularly targeted therapy aimed at treating human diseases and malignancies.

The concept "one drug multiple targets", coined as polypharmacology, is emerging as the next paradigm of drug discovery (Reddy & Zhang, 2013). According to Stankovic *et al.* (2018), inhibition of a single kinase often shows transient efficacy due to the development of resistance. To slow the onset of resistance, it has been suggested that multiple kinase inhibitors could be used as a therapeutic strategy for the treatment of human malignancies (Bhullar *et al.*, 2018). Dual targeting agents are better at overcoming transient activity and drug resistance, they therefore provide optimal effects in cancer treatment (Stankovic *et al.*, 2018). For example, a newly discovered drug candidate, fadraciclib (CYC065) (Figure 1), which is currently in phase I clinical trials has been found to potently inhibit CDK2 and CDK9, and it has shown promising activity against a variety of cancers including laryngeal tumours (Do *et al.*, 2018).

Several small molecule protein kinase inhibitors have been approved for clinical use or are currently in clinical trials (Griffith *et al.*, 2006). According to Roskoski (2019), the US Food and Drug Administration (FDA) has to date approved 48 small molecule protein kinase inhibitors for malignancies such as breast and lung cancer. Among these, 25 drugs target receptor protein

tyrosine kinases, ten target non-receptor protein tyrosine kinases while 13 target serine/threonine protein kinases (Roskoski, 2019).

The breakthrough in the discovery of imatinib (Figure 1) as the first small molecule kinase inhibitor approved for cancer treatment motivated scientists to design newer protein kinase inhibitors with improved activity (Gao *et al.*, 2013; Bhullar *et al.*, 2018; Roskoski, 2019). Despite this fact, target promiscuity has largely limited the rational design strategies of these inhibitors (Gao *et al.*, 2013). Due to the high degree of conservation in the ATP binding pocket of a majority of protein kinases, specific inhibition remains a challenge (Hanson *et al.*, 2019). Furthermore, development of drug resistance due to the emergence of kinase target mutations has also proved to be a great challenge (Arslan *et al.*, 2006; Gross *et al.*, 2015). Mutations in the essential gatekeeper of several receptor tyrosine kinases have previously been studied, and they were reportedly overcome by small molecule inhibitors which do not require the gatekeeper for binding (Arslan *et al.*, 2006). These findings suggest that there is a need for the discovery of more novel and highly selective protein kinase inhibitors (Zhao & Bourne, 2018).

Figure 1: Structures of fadraciclib (CYC065) and imatinib

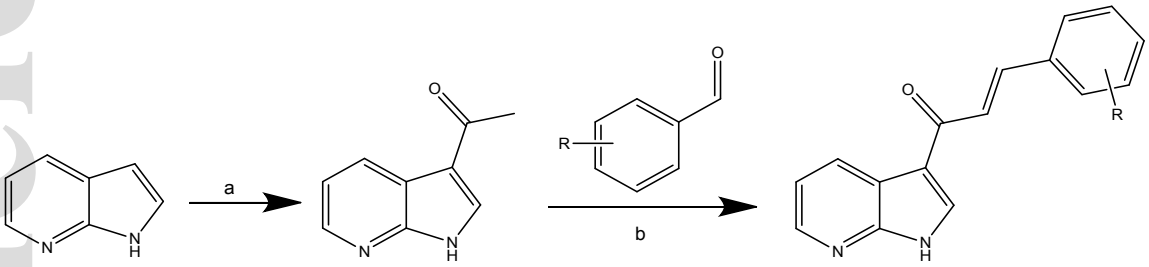
Chalcones have attracted considerable attention because of their promising therapeutic effects. Natural and synthetic chalcones have demonstrated a broad spectrum of therapeutic effects including antibacterial, antifungal, antiviral, anti-inflammatory, antioxidant, and most importantly, anticancer activities (Sangpheak *et al.*, 2019). According to Mphahlele *et al.* (2018), chalcone-based compounds have several mechanisms of action, among which are induction of apoptosis, DNA and mitochondrial damage, inhibition of angiogenesis and kinase inhibition. Mphahlele *et al.* (2018) synthesised a series of benzofuran-chalcone hybrids and evaluated their activity against several targets, including the epidermal growth factor receptor tyrosine kinase (EGFR-TK). The tested compounds inhibited EGFR-TK with sub micro molar IC_{50} values, and the most potent compound (**1**) exhibited an IC_{50} value of 0.09 ± 0.03 μM (Mphahlele *et al.*, 2018).

The 7-azaindole scaffold has attracted a great deal of interest for the synthesis of a variety of therapeutic agents due to its pivotal role in lead identification and optimization (Mushtaq *et al.*, 2008). Different acylated azaindoles have been reported for analgesic and anti-inflammatory activities (Mushtaq *et al.*, 2008). According to Mushtaq *et al.* (2008), azaindole analogues have been found to possess blood pressure lowering activities, and also act as effective coronary vasodilator, cardiovascular and hypotensive agents. Azaindole derivatives have also been found to be useful in treating disorders such as autoimmune disorders (Farina *et al.*, 2008), cancer (Heinrich *et al.*, 2015) and Alzheimer's disease (Sreenivasachary *et al.*, 2017).

Because of its outstanding ability to bind to the hinge region of multiple protein kinases, 7-azaindole has been acknowledged as a privileged fragment against kinases, and therefore it has been incorporated into many kinase inhibitors (Irie & Sawa, 2018). In an attempt to study their pharmacokinetic properties and potentially improve their pharmacological activity, Giles *et al.* (2019) designed a series of 7-azaindole-chalcones and studied their docking interactions with B-Raf kinase. It was interesting to discover that substituting positions -3 and -4 of 7-azaindole with hydroxyl and methyl groups (**2**), respectively, increased the inhibitor binding affinity for B-Raf (Giles *et al.*, 2019). This study suggested that conjugating 7-azaindole and chalcone has potential to increase the inhibitor-kinase binding affinity, and this may be a useful starting point for the development of protein kinase inhibitors. To this effect, in the present study, we synthesized numerous compounds containing the 7-azaindole-chalcone core. The 7-azaindole scaffold was acetylated at position C3 (**4**), after which it was treated with different substituted and unsubstituted aromatic aldehydes to yield a variety of chalcone derivatives (**5a-w**) as shown in scheme 1.

Figure 2: Chalcone-based protein kinase inhibitors reported in previous studies (Mphahlele *et al.*, 2018; Giles *et al.*, 2019).

Scheme 1: Synthesis of 7-azaindole derivatives[a]



3 **4** **5a-w**

[a]Reagents and conditions: (a) $AlCl_3$, DCM, AcCl, rt, 2h; (b) HCl/MeOH, 100 ˚C, 24h

1-(1H-pyrrolo[2,3-b]pyridin-3-yl)ethanone (**4**) was prepared in a yield of 77% and used as key intermediate. The intermediate was prepared by Friedel-Crafts acylation following a method previously described by Zhang *et al.* (2002), and it was used in the next step without further purification. The target compounds (**5a-w**) were synthesised in poor to excellent yields (10-92%) using an acid catalysed aldol condensation method shown in Scheme 1 (Nel *et al.*, 2016; Amakali *et al.*, 2018). Confirmation of synthesis of the target compounds was done by NMR spectroscopic analysis. The absence of a singlet resonating around 2.6 ppm on the proton NMR spectrum of compounds **5a-w** confirmed the successful formation of target compounds. This peak is assigned to the $-CH_3$ of the acetyl unit, which is characteristic of compound **4**.

We screened a library of 23 synthetic 7-azaindole-chalcones against a panel of eight disease related protein kinases composed of cyclin-dependent kinases (CDKs): *Hs*CDK2/CyclinA, *Hs*CDK5/p25, *Hs*CDK9/CyclinT; *Hs*Haspin, human Proto-oncogene serine/threonine-protein kinase PIM1, porcine casein kinase 1 δ/ε (*Ssc*CK1δ/ε), *Lm*CK1 (from the intracellular parasite *Leishmania major*) and porcine Glycogen Synthase Kinase 3 (*Ssc*GSK3α/β). These compounds were tested at initial concentrations of 1 μM (Table 1) and 10 μM (Table 2a in supplementary data). Compounds that showed less than 30% residual kinase activity at 1 μM inhibitor concentration, for either CDK9/CyclinT or Haspin were considered active and were further tested over a wide range of concentrations on both kinases. Sigmoidal curves of kinase activity versus inhibitor concentration were constructed and from them IC_{50} values were determined. Figure 3 shows the dose-response curve of compound **5q**, the most potent Haspin inhibitor. The IC_{50} values are summarized in Table 2.

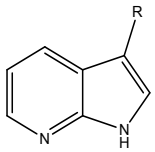
Figure 3: Haspin kinase activity versus increasing concentration of compound **5q**. Recombinant Haspin kinase was assayed in the presence of 10 μM ATP and increasing concentrations of 5q compound. Kinase activities are expressed in % of maximal activity, i.e., measured in the absence of inhibitor (mean ± SD = 2).

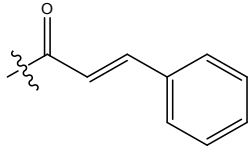
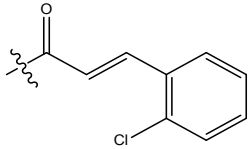
While most of the compounds screened did not inhibit the kinases studied here, **5f** and **5q** showed interesting activities against Haspin and/or CDK9/CyclinT. Compound **5q**, bearing a phenolic substituent displayed a high degree of selectivity, and it is noteworthy as the most potent Haspin inhibitor (IC_{50} = 0.410 μM). Interestingly, the corresponding unsubstituted compound, **5a,** showed no activity against Haspin. With the exception of **5q**, introducing electron donating group to the *para* position of the styrene ring led to complete loss of activity against all the tested kinases, as exemplified by **5g, 5h, 5j** and **5k**. A similar trend was observed in their electron withdrawing counterparts; no activity was reported for compounds bearing nitro, halogen or carboxyl groups. For example, compounds **5g, 5e, 5i, 5l, 5m, 5n, 5o, 5p, 5v** and **5w** were inactive against all tested protein kinases. Docking study shows that **5q** makes interactions with Hs_HASPIN through the NH of the 7-azaindole ring and Glu-165 and through the oxygen of the ketone moiety and H3O+ molecule in the enzyme. Replacing the OH group in **5q** with H, F, CH_3, OCH_3 groups generates analogues wherein the interactions seen with **5q** become weaker or lost completely. This observation suggests the OH as optimal substituent at this position.

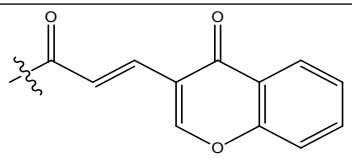
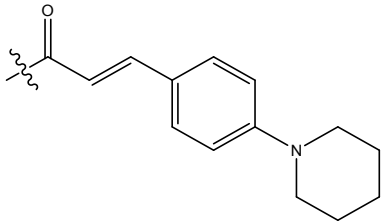
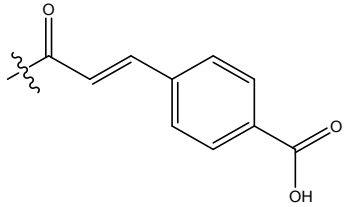
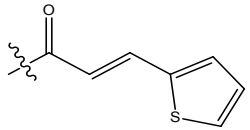
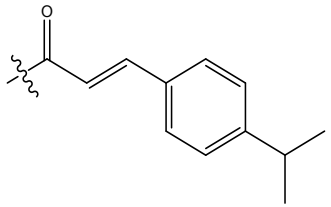
Larger groups in the 4-position of the phenyl ring such as piperidinyl (compound **5d**) resulted in a loss of activity, suggesting that bulky non-polar substituents may be undesirable. Furthermore, increasing the length of the linker between the 7-azaindole and the phenyl ring (**5s**) was accompanied by loss of activity.

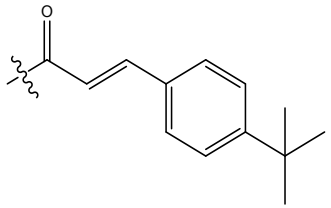
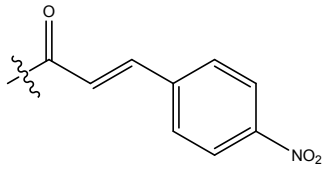
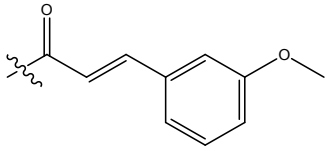
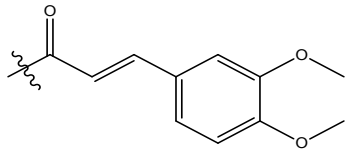
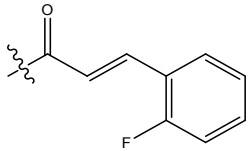
Most interestingly, replacing styrene as present in compound **5a** with 2-vinylthiophene to give compound **5f,** yielded a potent dual inhibitor for Haspin (IC_{50} = 0.470 μM) and CDK9/CyclinT (IC_{50} = 2.260 μM). It is notable that **5f** and **5q** exhibit similar potencies for Haspin (IC_{50} = 0.470 and 0.410 μM respectively) even though they contain different substituents. Also of note is the fact that replacing the thiophene ring of **5f** with an α-substituted nitrofuran (**5u**) led to decreased activity. These data led to an interesting observation that unsubstituted 5-membered heterocycles may be optimum substituents for the development of 7-azaindole derivatives as dual inhibitors of CDK9/CyclinT and Haspin. However, meaningful SARs could not be derived since most of the evaluated compounds did not inhibit the target kinases. It may therefore be concluded that compounds in this series constitute a promising starting point for the discovery on dual kinase inhibitors.

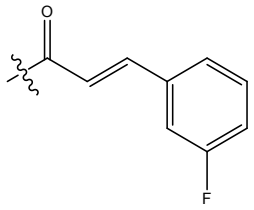
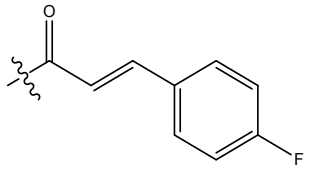
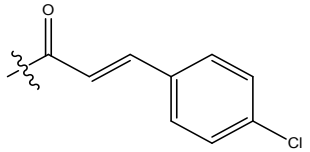
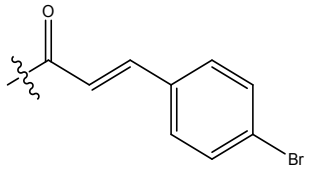
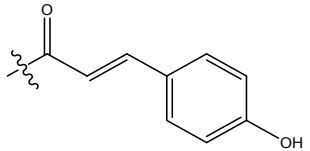
Table 1: Percentage of kinase inhibition of **4**, and **5a-w**. The table displays the residual activities detected after treatment with 1 µM of the tested compounds expressed in percentage. 100 % of residual activity is measured in the absence of inhibitor. ATP concentration used in the kinase assays was 10 µmol/L (values are means, n=2). Kinases are from human origin (*Homo sapiens*) unless specified: *Ssc*, *Sus scrofa*; *Lm*, *Leishmania major*.



Compound	R	Residual kinase activity in the presence of 1 µM of tested compound							
		CDK2/Cyclin A	CDK5/p25	CDK9/CyclinT	HASPIN	PIM1	*Ssc*_CK1δ/ε	*Ssc*_GSK3α/β	*Lm*_CK1
4		≥100	≥100	66	63	≥100	79	87	67
5a		≥100	94	47	34	≥100	74	65	61
5b		76	86	80	66	≥100	85	94	99

5c		93	75	90	79	50	67	63	94
5d		≥100	≥100	58	≥100	≥100	69	88	80
5e		85	78	51	88	81	72	91	66
5f		≥100	≥100	30	42	80	68	70	68
5g		91	92	63	56	90	72	91	55

5h		99	96	55	74	≥100	70	88	62
5i		≥100	≥100	56	69	≥100	76	86	76
5j		≥100	98	56	57	≥100	69	81	86
5k		≥100	≥100	74	69	94	81	72	77
5l		≥100	96	79	63	91	80	83	82

5m		68	96	73	81	95	89	98	78
5n		87	83	58	72	≥100	81	78	75
5o		84	94	52	53	93	63	92	47
5p		≥100	94	58	72	99	68	63	70
5q		≥100	≥100	45	23	90	76	84	63

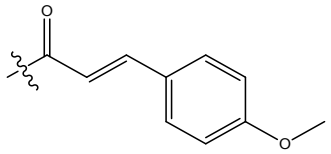
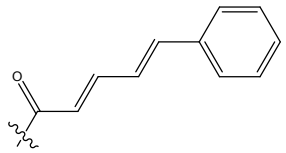
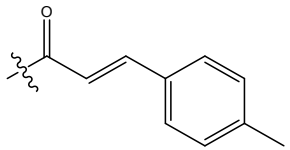
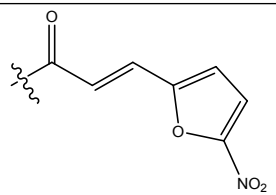
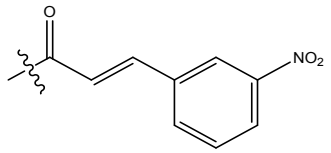
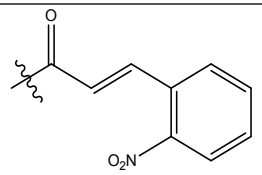
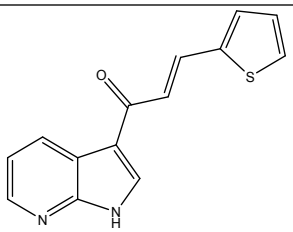
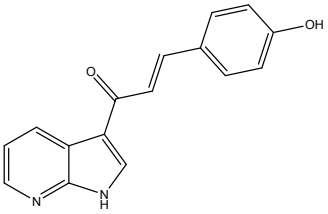
5r		≥100	84	65	48	≥100	91	89	78
5s		82	94	55	54	80	60	97	60
5t		96	≥100	61	49	85	≥100	84	87
5u		86	≥100	62	42	76	90	84	68
5v		≥100	95	94	63	96	≥100	≥100	98
5w		91	88	90	95	≥100	89	87	75

Table 2: Structures and IC_{50} (μM) of compound **5f** and **5q** against human CDK9/CyclinT and Haspin protein kinases.

Compound	**Structure**	**IC_{50} [a]**	
		***Hs*_CDK9/CyclinT**	***Hs*_HASPIN**
5f		2.260	0.470
5q		NP[b]	0.410

[a]IC_{50} values are reported in μM. Kinase activities were measured using 10μM ATP. Values are means, n=2.

[b]Not performed because of low activity at 1 μM (see Table 1).

The binding interactions of **5f** and **5q** with the crystal structure of Hs_HASPIN (PDB code: 6G34), as well as the binding interactions of **5f** with the crystal structure of Hs_CDK9/CyclinT (PDB code: 3TN8) were studied using Glide ligand docking as implemented in Maestro in the Schrödinger package. Enzyme crystal structures were obtained from protein data bank (PDB). The Protein-ligand interactions of **5f** and **5q** with Hs_HASPIN shows the presence of intermolecular hydrogen bonding between the NH of the 7-azaindole ring and Glu-165 (see entry A and B, Table 3). **5q** also interacted with Hs_HASPIN through hydrogen bonding between the oxygen of the ketone moiety and H_3O^+ molecule in the enzyme. Both **5f** and **5q** seems to be making the same essential interactions with Hs_HASPIN, this is also evident in their equipotent activity against the enzyme. The docking scores of **5f** and **5q** against Hs_HASPIN are -8.5801 and -6.9792, respectively. **5f** interacted with Hs_CDK9/CyclinT through distance interactions above 2 Å (see entry C, Table 3). The docking scores of **5f** against Hs_CDK9/CyclinT is -7.8810.

Table 3: Binding interactions of 5q and 5f against CDK9/CyclinT and Haspin protein kinases.

2. Conclusion

In summary, we investigated a series of chalcone-based 7-azaindole derivatives as potential protein kinase inhibitors. Although most compounds were inactive, 2 compounds (**5f** and **5q**) were identified as potent inhibitors of CDK9/CyclinT and Haspin kinase. **5f** inhibited both CDK9/CyclinT and Haspin in high micro molar potencies. SAR analysis suggests that the presence of an unsubstituted 2-thienyl group on the 7-azaindole framework may be necessary for designing multiple protein kinase inhibitors. This study demonstrates that 7-azaindole-chalcone-based compounds are good chemical scaffolds for the design of protein kinase inhibitors and have potential to be developed as anticancer drugs.

Conflict of interest

Stéphane Bach is a founder and member of the scientific advisory board of SeaBeLife Biotech (Roscoff, France). This company is developing novel therapies for treating liver and kidney acute disorders. The authours have no conflict of interest to declare.

Data Availability Statement

Detailed characterization data together with ^{1}H,^{13}C and HRMS spectra for target compounds 5a-w are available in the supplementary file. Samples of compounds can be obtained from corresponding authors upon request.

Acknowledgements

The NMR and MS spectra were recorded by Daniel Otto and Johan Jordaan of the SASOL Centre of Chemistry, North-West University. This work is based on the research supported by the National Research Foundation of South Africa (Grant specific unique reference numbers (UID) (96135). The authors also thank the Cancéropôle Grand Ouest ("Marines molecules, metabolism and cancer" network), IBiSA (French Infrastructures en sciences du vivant: biologie, santé et agronomie), Biogenouest (Western France life science and environment core facility network) for supporting the KISSf screening facility (FR2424, CNRS and Sorbonne Université, Roscoff, France) and “Ligue Contre le Cancer Grand Ouest” comity CIRGO (districts 29, 35, 22, 56 and 79). The Grant holders acknowledge that opinions, findings and conclusions or recommendations expressed in any publication generated by the NRF supported research are that of the authors, and that the NRF accepts no liability whatsoever in this regard. Molecular docking data were generated by Dr T. Tshiwawa, Rhodes University.

3. References

Amakali, K.T., Legoabe, L.J., Petzer, A. & Petzer, J.P. 2018. Synthesis and in vitro evaluation of 2-heteroarylidene-1-tetralone derivatives as monoamine oxidase inhibitors. *Drug research*, 68(12):687-695.

Arslan, M.A., Kutuk, O. & Basaga, H. 2006. Protein kinases as drug targets in cancer. *Current cancer drug targets*, 6(7):623-634.

Bhullar, K.S., Lagaron, N.O., McGowan, E.M., Parmar, I., Jha, A., Hubbard, B.P. & Rupasinghe, H.P.V. 2018. Kinase-targeted cancer therapies: progress, challenges and future directions. *Molecular cancer*, 17(1):48.

Do, K.T., Chau, N., Wolanski, A., Beardslee, B., Hassinger, F., Bhushan, K., Pruitt-Thompson, S., Scotton, A., Frame, S. & Zheleva, D.I. 2018. Abstract CT037: Phase I safety, pharmacokinetic and pharmacodynamic study of CYC065, a cyclin dependent kinase inhibitor, in patients with advanced cancers (NCT02552953): AACR.

Drewry, D.H., Wells, C.I., Andrews, D.M., Angell, R., Al-Ali, H., Axtman, A.D., Capuzzi, S.J., Elkins, J.M., Ettmayer, P. & Frederiksen, M. 2017. Progress towards a public chemogenomic set for protein kinases and a call for contributions. *Plos one*, 12(8):1-20.

Farina, C., Constantin, G., Laudanna, C. & Misiano, P. 2008. Indole and azaindole derivatives for the treatment of inflammatory and autoimmune diseases (Patent: WO/2007/048847).

Gao, Y., Davies, S.P., Augustin, M., Woodward, A., Patel, U.A., Kovelman, R. & Harvey, K.J. 2013. A broad activity screen in support of a chemogenomic map for kinase signalling research and drug discovery. *Biochemical journal*, 451(2):313-328.

Giles, D., Saiprabha, V. & Yeshna, G. 2019. Design of chalcones of 7-azaindole as raf-B inhibitors. *Journal of advanced chemical sciences*:606-608.

Griffith, R., Brown, M., McCluskey, A. & Ashman, L. 2006. Small molecule inhibitors of protein kinases in cancer-how to overcome resistance. *Mini reviews in medicinal chemistry*, 6(10):1101-1110.

Gross, S., Rahal, R., Stransky, N., Lengauer, C. & Hoeflich, K.P. 2015. Targeting cancer with kinase inhibitors. *The journal of clinical investigation*, 125(5):1780-1789.

Hanson, S.M., Georghiou, G., Thakur, M.K., Miller, W.T., Rest, J.S., Chodera, J.D. & Seeliger, M.A. 2019. What makes a kinase promiscuous for inhibitors? *Cell chemical biology*, 26:1-10.

Heinrich, T., Wucherer-Plietker, M. & Buchstaller, H.-P. 2015. 7-azaindole derivatives suitable for treatment of cancers (Patent: WO2010020308A1).

Irie, T. & Sawa, M. 2018. 7-azaindole: a versatile scaffold for developing kinase inhibitors (Patent: WO 2012175168A1).

London, C.A. 2013. Kinase dysfunction and kinase inhibitors. *Veterinary dermatology*, 24(1):181-e140.

Mphahlele, M., Maluleka, M., Parbhoo, N. & Malindisa, S. 2018. Synthesis, evaluation for cytotoxicity and molecular docking studies of benzo [c] furan-chalcones for potential to inhibit tubulin polymerization and/or EGFR-tyrosine kinase phosphorylation. *International journal of molecular sciences*, 19(9):2552.

Mushtaq, N., Saify, Z., Noor, F., Takween, S., Akhtar, S., Arif, M. & Khan, K.M. 2008. Synthesis and pharmacological activities of 7-azaindole derivatives. *Pakistan journal of pharmaceutical sciences*, 21(1):36-39.

Nel, M.S., Petzer, A., Petzer, J.P. & Legoabe, L.J. 2016. 2-Heteroarylidene-1-indanone derivatives as inhibitors of monoamine oxidase. *Bioorganic chemistry*, 69:20-28.

Reddy, A.S. & Zhang, S. 2013. Polypharmacology: drug discovery for the future. *Expert review of clinical pharmacology*, 6(1):41-47.

Roskoski, R. 2019. Properties of FDA-approved small molecule protein kinase inhibitors. *Pharmacological research*, 144:19-50.

Sangpheak, K., Tabtimmai, L., Seetaha, S., Rungnim, C., Chavasiri, W., Wolschann, P., Choowongkomon, K. & Rungrotmongkol, T. 2019. Biological evaluation and molecular dynamics

simulation of chalcone derivatives as epidermal growth factor-tyrosine kinase inhibitors. *Molecules*, 24(6):1092.

Saxena, A., Scaini, G., Bavaresco, D.V., Leite, C., Valvassoria, S.S., Carvalho, A.F. & Quevedo, J. 2017. Role of protein kinase C in bipolar disorder: a review of the current literature. *Molecular neuropsychiatry*, 3(2):108-124.

Sreenivasachary, N., Kroth, H., Benderitter, P., Hamel, A., Varisco, Y., Hickman, D.T., Froestl, W., Pfeifer, A. & Muhs, A. 2017. Discovery and characterization of novel indole and 7-azaindole derivatives as inhibitors of beta-amyloid-42 aggregation for the treatment of Alzheimer's disease. *Bioorganic and medicinal chemistry letters*, 27(6):1405-1411.

Stankovic, T., Dinic, J., Podolski-Renic, A., Musso, L., Buric, S., Dallavalle, S. & Pesic, M. 2018. Dual inhibitors as a new challenge for cancer multidrug resistance treatment. *Current medicinal chemistry*, 25:1-30.

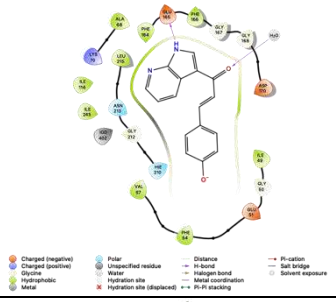
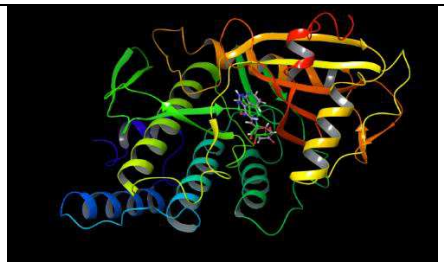
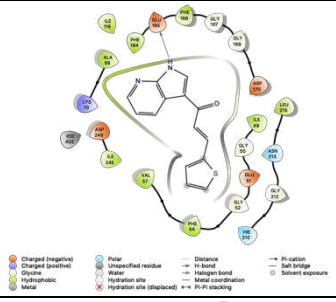
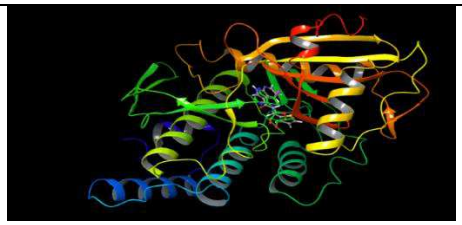
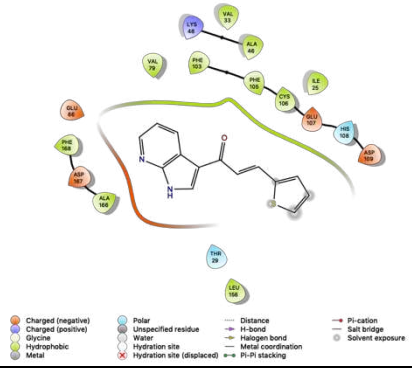
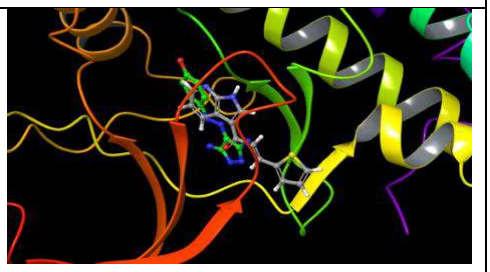
Tong, Y., Stewart, K.D., Florjancic, A.S., Harlan, J.E., Merta, P.J., Przytulinska, M., Soni, N., Swinger, K.K., Zhu, H. & Johnson, E.F. 2013. Azaindole-based inhibitors of Cdc7 kinase: impact of the Pre-DFG residue, Val 195. *ACS medicinal chemistry letters*, 4(2):211-215.

Zhang, J., Yang, P.L. & Gray, N.S. 2009. Targeting cancer with small molecule kinase inhibitors. *Nature reviews cancer*, 9(1):28-39.

Zhang, Z., Yang, Z., Wong, H., Zhu, J., Meanwell, N.A., Kadow, J.F. & Wang, T. 2002. An effective procedure for the acylation of azaindoles at C-3. *The Journal of organic chemistry*, 67(17):6226-6227.

Zhao, Z. & Bourne, P.E. 2018. Progress with covalent small-molecule kinase inhibitors. *Drug discovery today*, 23 (3):727-735.

Table 3: Binding interactions of 5q and 5f against CDK9/CyclinT and Haspin protein kinases.

Entry	Enzyme PDB code	Binding affinity	Protein-ligand interaction	Docking pose
A: 5q	**6G34**	-6.9792		
B: 5f	**6G34**	-8.5801		
C: 5f	**3TN8**	-7.8810		

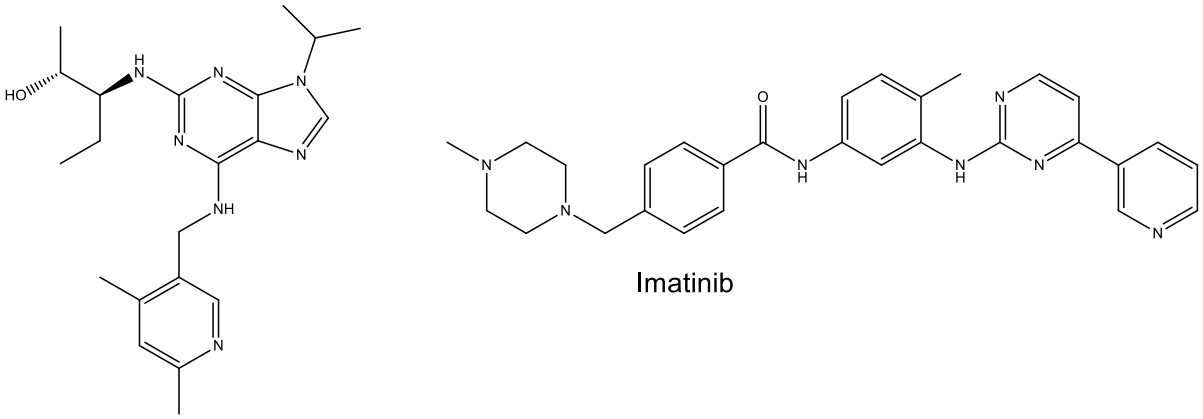
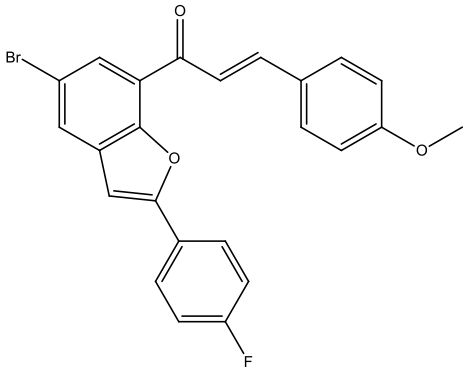
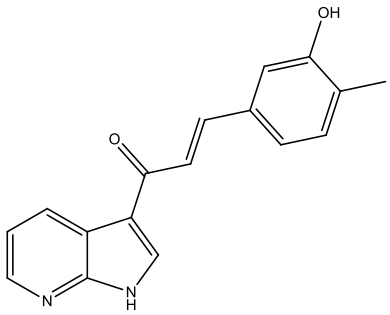


Figure 1: Structures of fadraciclib (CYC065) and imatinib



1 (EGFR-TK IC_{50} = 0.09 ± 0.03 μM)



2

Figure 2: Chalcone-based protein kinase inhibitors reported in previous studies (Mphahlele *et al.*, 2018; Giles *et al.*, 2019).

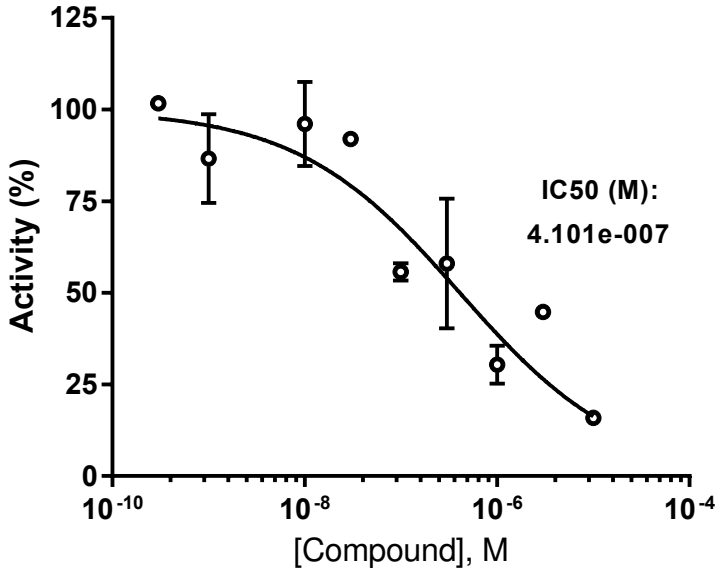


Figure 3: Haspin kinase activity versus increasing concentration of compound **5q**. Recombinant Haspin kinase was assayed in the presence of 10 µM ATP and increasing concentrations of 5q compound. Kinase activities are expressed in % of maximal activity, i.e., measured in the absence of inhibitor (mean ± SD = 2).