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Chiral Kinase Inhibitors

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Abstract

Small molecule kinase inhibitors are important tools for studying cellular signaling pathways, phenotypes and are, occasionally, useful clinical agents. With stereochemistry pervasive throughout the molecules of life it is no surprise that a single stereocenter can bestow a ligand with distinct binding affinities to various protein targets. While the majority of small molecule kinase inhibitors reported to date are achiral, a number of asymmetric compounds show great utility as tools for probing kinase-associated biomolecular events as well as promising therapeutic leads. The mechanism by which chirality is introduced varies but includes screening of chiral libraries, incorporation of chiral centers during optimization efforts and the rational installation of a chiral moiety as guided by structural and modeling efforts. Here we discuss several advanced chiral small molecule kinase inhibitors where stereochemistry plays an important role in terms of potency and selectivity.

1. Introduction

The beginning of our understanding of molecular chirality is often attributed to the work of Jean-Baptiste Biot who first used the term “optically active” to describe substances that rotated polarized light [1]. Boit’s pioneering studies on solutions of sugars that rotated polarized light in a concentration dependent manner instigated early pioneering work on the subject. Highlights include Louis Pasteur’s separation and study of tartaric acid crystals which spawned a molecular understanding of enantiomers [2]. Jacobus van’t Hoff introduced the chiral carbon in 1874 and Emil Fischer determined the 16 stereoisomers of the aldohexoses in 1894 [3, 4]. Fischer then described the lock and key model of binding that today permeates throughout the study of chemistry and biology [5]. As an appreciation for molecular chirality emerged, the realization that enantiomers can have different biological effects began to take hold at the turn of the 19th century. Landmark studies include the different biochemical oxidation rates for the isomers of tartaric acid, arabinose, and mannose; the different taste between D- and L-asparagine and between D- and L-glutamic acid; and the different biological and behavioral effects for dextro-cocaine and laevo-cocaine, atropine and scopolamine, as well as atropine and hyoscyamines [6]. The Easson-Stedman hypothesis marked a key recognition that crucial multi-point interactions between chiral small molecules and their chiral protein targets existed [7]. The successful high-resolution X-ray structure of sperm whale myoglobin gave the first detailed ‘snapshot’ of a large biomolecule where the effects of chirality on tertiary protein structure were displayed [8].

As synthetic approaches toward library development and advances in biological assay techniques were made, small molecules that perturbed specific biochemical events were

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discovered at an increasing pace. Even with the considerable history of chirality and its role in biology, most biologically active small molecules were synthesized, reported and studied as achiral entities or racemic mixtures (excluding natural products). Expectedly, these racemic and achiral compounds dominated the drug landscape for the better part of the 20th century. However, a recent and significant increase in fully synthetic drugs with defined stereochemical requirements has been documented [9, 10]. In large part, this is due to advances in large scale chiral separation techniques and asymmetric reactions. Currently, there are a growing number of optically pure chiral auxiliaries, catalysts and starting reagents available from commercial sources. As a result, more studies are emerging that describe the biochemical activity, pharmacokinetics and pharmacodynamics of small molecule stereoisomers. Many of these studies have established that one stereoisomer can have a desired pharmacological effect, while its enantiomer or diastereomer(s) can have a range of effects including: identical activity, lower activity, no activity and even fully opposing activity at the same target. To this end, in 1992 the US FDA stated that “to evaluate the pharmacokinetics of a single enantiomer or mixture of enantiomers, manufacturers should develop quantitative assays for individual enantiomers in *in vivo* samples early in drug development. This will allow assessment of the potential for interconversion and the absorption, distribution, biotransformation, and excretion (ADBE) profile of the individual isomers” [11]. This statement coincided with a significant increase in the worldwide approval of single enantiomer new molecular entities (NME) [9, 10].

The role of chirality has permeated drug discovery efforts within all major target classes of the drugable genome. A major category of the drugable genome remains the kinome and kinase inhibitors represent an important class of small molecule tools and clinically explored agents. The majority of kinase inhibitors discovered to date are ATP-competitive inhibitors known as type I inhibitors. One of the first reported ATP-competitive inhibitors is the natural product staurosporine (Figure 1), known to be a potent pan-kinase active compound [12, 13]. While the lack of selectivity and high toxicity of this compound prevent it from becoming a useful drug, it has remained a benchmark control compound for a myriad of assays. The role of selectivity when targeting the kinome is an active area of research and debate [14, 15]. As there are over 500 kinases in the human genome it is important to state that selectivity plays a key role in the discovery of appropriate tool compounds to explore specific biological questions. The discovery and approval of imatinib (Figure 1) for treatment of chronic myelogenous leukemia (CML) validated the notion that selective agents can yield positive clinical results. There are currently over 70 kinase inhibitors in various stages of clinical development and each exhibits a different level of selectivity [16]. A second class of kinase inhibitors recognizes the inactive conformation of kinases and have been dubbed type II inhibitors. This variety of inhibitors, which include imatinib and sorafenib (Figure 1), often bind at locales with more structural divergence relative to the highly homologous ATP-binding sites. As a result, type II inhibitors can often be engineered to have higher selectivity profiles [17, 18]. Discovery of a type II or fully allosteric kinase inhibitor can be complicated and screening efforts typically yield a higher percentage of type I inhibitors. The incorporation of stereocenters is one strategy to confer selectivity to a type I inhibitor by taking advantage of the subtle three-dimensional differences found within the ATP binding domain. Given the preeminent role that kinases play in signal transduction pathways and the well characterized dysregulation of selected kinases within numerous diseases [14–16, 19–21] it is clear that there exists a need for novel kinase inhibitors. Here, we explore the imaginative ways that researchers have bestowed both potency and selectivity upon novel small molecule kinase inhibitors through the incorporation of chirality.

2. Discovery of the p38 α inhibitor PH-797804

The mitogen-activate protein kinases (MAPKs) are serine/threonine protein kinases that regulate numerous cellular responses to varied external stimuli. A prominent member of the MAPK family are the p38-isoforms α -, β -, γ -, and δ -. The p38 α isoform (also known as MAPK14) is encoded by the MAPK14 gene and is known to be widely expressed in various tissue types including leukocytes, epithelial cells and smooth muscle cells [22–26]. p38 α is among the most widely studied MAPK isoforms with over 50 disclosed X-ray structures containing a variety of bound ligands. MAP kinase kinases (MKKs), particularly MKK3 and MKK6, are responsible for the activation of p38 α in response to several characterized stimuli including proinflammatory cytokines and various environmental stresses. Activation of p38 α has several consequences including increased expression of TNF α , IL6, IL1, COX-2 and metalloproteinases [22,23]. Given its role as a key mediator of the inflammation process [23], p38 α has emerged as a key target within the study of a variety of diseases including rheumatoid arthritis, Crohn's disease, atherosclerosis, chronic obstructive pulmonary disease (COPD), severe asthma and psoriasis. As a result, numerous p38 inhibitors have been disclosed with a myriad of activities in preclinical disease models including significant mitigation of cytokine release within inflammation models, reduction of cardiac hypertrophy, protection against cardiac remodeling and treatment of COPD [27–36].

A recent addition to the p38 α inhibitor pipeline is **PH-797804**, an axially chiral, potent, selective and orally bioavailable p38 α inhibitor (Figure 2)[37]. This relatively unique chiral compound was purified by chiral chromatography to isolate both the *R*- and *S*-isomers. The ability to resolve the atropisomers arises from the high rotational energy barrier caused by the 6- and 6'-methyl substituents on the phenyl and pyridinone rings. The authors used molecular modeling to determine a barrier of >25 kcal/mol for rotation around the *N*-phenyl bond. The *S*-atropisomer (**PH-797804**) was determined to be a >100-fold more potent p38 α inhibitor than the *R*-isomer and an X-ray structure of the compound bound to p38 α has been reported (PDB:3HLL)(Figure 2). Examination of this crystal structure illustrates that the methyl amide group on the *S*-atropisomer is positioned in an open pocket.(referred to as the E₀ region). On the basis of this structure, it is likely that the methyl amide in the *R*-atropisomer encounters unfavorable steric interactions with Asp112 and Asn115.

PH-797804 is an ATP-competitive inhibitor and structural comparison of p38 γ -AMP-PNP and **PH-797804**-p38 α co-crystals illustrated that the pyridinone of **PH-797804** likely overlaps with the adenine moiety of ATP. **PH-797804** contains a hydrophobic 2,4-difluorophenyl group that extends into a lipophilic pocket of p38 α that is controlled by the Thr106 gatekeeper residue. This important hydrophobic interaction, along with two key hydrogen bonds between the pyridinone carbonyl of **PH-797804** and Met109 and Gly110 are presumed sources of the potency and selectivity of this kinase inhibitor. Interestingly, the Met109-Gly110 amide bond is inverted relative to its native conformation permitting this critical hydrogen bond. Importantly, the atropoisomerism of **PH-797804** helps govern the binding vector of the pyridinone moiety further facilitating this key hydrogen bond. Reasoning that the Thr106 gatekeeper and the Gly110 amide bonds rotation are keys to activity for this chemotype, a bioinformatics analysis was done and revealed that p38 β and Myt-1 enzymes contain the TXXXG motif in the active site. Importantly, no activity for **PH-797804** against Myt-1 was observed and a 10-fold higher IC₅₀ value was seen for p38 β compared to p38 α . **PH-797804** was screened again two kinase panels and showed high selectivity against other MAP kinase members as well as the JNK kinases. Importantly, **PH-797804** showed <20% inhibition (up to 10 μ M) against a number of kinases containing either a Thr106 or Gly110 homolog. Cellular assays demonstrated that **PH797-804** ablated p38 α signaling while having no appreciable inhibition of JNK and ERK or phosphorylation of c-Jun. Pfizer has now completed phase II trials with **PH-797804** for the treatment of

neuropathic pain associated with post-herpetic neuralgia and phase II clinical trials for the treatment chronic obstructive pulmonary disease are currently ongoing.

3. Discovery of the AKT inhibitors A-443654 and pyrimidine 3

The AKT family of kinases (also known as protein kinase B, PKB) are serine/threonine kinases that are important cellular signaling mediators and regulators of a myriad of cellular functions including protein synthesis, cell survival and proliferation, metabolism, neurological activity, and cardiovascular homeostasis [38–52]. AKT is a primary agent within PI3K signaling following phosphorylation by PDK1 and/or the mTORC2 complex [51]. The list of AKT interactions continues to grow and currently contains over 25 known roles including its phosphorylation of GSK3, FOXO transcription factors, MDM2, TSC1/2, and BAD. Because AKT regulates a large number of cellular functions and hyperactivation of AKT has been observed in many cancers, this protein has emerged as an important target for a variety of diseases. AKT's nodal role in a number of tumor-associated processes has spurred research that has shown blockage of AKT signaling results in reduced cell proliferation and induces apoptosis in cells overexpressing AKT.

Given the myriad of functions associated with the AKT family, numerous potent and selective inhibitors of this class of kinases have been discovered (including type I and fully allosteric inhibitors)[53–60]. Phosphatidylinositol analogs have been developed that interfere with the binding of the PH domain of AKT with phosphatidylinositol(3,4,5)-triphosphate [61–63]. The use of small peptides (≤ 20 -mer) resembling AKT's endogenous substrates have been examined and several show moderate potency and good selectivity [64, 65]. Attempts have been made to reduce the size of the amino acid sequence with little success. An amino-pyrimido-pyridazine with a chiral pentose-like appendage (**API-2/TCN**) was found to inhibit AKT2-transformed cells through screening of the NCI Diversity Set [66]. This molecule selectively inhibits phosphorylation of BAD, AFX and GSK-3 β and positive results in mouse xenograft model with aberrant AKT signaling have prompted examination in Phase I trials.

In addition to these agents, Abbott Laboratories has disclosed a number of potent, pan-AKT inhibitors that show moderate to high selectivity over PKA [67]. A high-throughput screening (HTS) effort uncovered a chloropyridine (**1**, AKT IC₅₀ = 5 μ M) containing a chiral secondary amine. The subsequent optimization effort discovered **A-443654** (AKT1 IC₅₀ = 0.16 nM) that retained the chiral amine functionality and possessed impressive selectivity and cell-based activity (Figure 3). Continued optimization has led to a related inhibitor that retains the chiral amine and possesses improved kinase selectivity, a good safety profile and moderate oral bioavailability. An X-ray structure has been reported of **A-443654** bound to PKA, which is commonly used as a surrogate for AKT due to its relative ease of crystallization and homology with AKT at the ATP binding site (PDB:2UZU) (Figure 3). Astex Therapeutics has subsequently released a structure of **A-443654** bound to AKT2 (PDB:2JDR) and PKA (PDB:2JDS)(Figure 3)[68]. Interestingly, these structures illustrate moderately divergent binding orientations for **A-443654**. The methyl-indazole and pyridine adopt a matching binding mode whereby key hydrogen-bonds to the hinge region are found in both crystal structures. In contrast, the indole moiety is significantly divergent in its binding modality within the AKT2 and PKA structures. In PKA, the indole is oriented towards the glycine rich loop (cyan colored structure), while in AKT2 (purple colored structure), the indole ring is directed toward the ATP-binding pocket and a new hydrophobic pocket containing Met282, Phe439 and Val166 residues. The chiral primary amine occupies a similar position in both structures, forming key hydrogen bonds with Asp and Asn residues in an 'acidic pocket.' The chiral nature of the secondary amine imposes directionality on the indole moiety resulting in key hydrophobic interactions and hydrogen bonds. It can be

argued that the 40-fold selectivity for AKT over PKA arises from the orientation imposed by the chiral nature of the molecule as it dictates specific interactions with the divergent amino acid residues found in each binding pocket.

In 2006, Chiron Corporation published a potent AKT inhibitor that incorporated a chiral (2-aminoethyl)amide moiety (Figure 4)[69]. This agent derived from an achiral 2-amino-pyrimidine screening lead possessed a 3.0 μM IC_{50} value versus AKT. The lead structure evolved into a 2-pyrimidyl-5-amidothiophene core in which a variety of chirally pure analogues were evaluated including tertiary amines, terminal alcohols, esters, alkyl groups, and extended alkyl linkers. This effort revealed a preference for a 2-aminoethyl substituent with the *S*-configuration at the homobenzyl position (pyrimidine **3**). The *R* enantiomer was found to be ~100-fold less potent. An X-ray structure of **3** bound to PKA has been reported (PDB:2GU8)(Figure 4). Key hydrogen bonds between the primary amine and Asn171 and Asp184 make evident the importance of the *S*-configuration. A water-mediated hydrogen bond with Asp166 denotes a secondary binding construct that is enabled by the exact placement of the primary amine. The *S*-configuration also orients the dichlorophenyl group into a hydrophobic pocket created by the glycine rich loop. This example highlights the transformation of an achiral screening lead into a novel, chiral agent and underscores the importance of examining chirality during SAR explorations.

4. Discovery of the ERK inhibitors FR148083 and pyrimidine 7

The RAS/RAF/MEK/ERK signal transduction pathway is a well-studied and important cascade with relevance to numerous disease states with particular significance within many types of cancers [70–73]. The first FDA approved drug targeting this pathway is Sorafenib (Nexavar) [74], an inhibitor of multiple receptor protein kinases including RAFs (IC_{50} values for B-RAF and C-RAF are 6 and 22 nM, respectively), which is indicated for the treatment of renal cell carcinoma. Several MEK inhibitors have been advanced to clinical trials including PD0325901, AZD142886/ARRAY6244 and RDEA119 [75,76]. ERK lies downstream in the RAS/RAF/MEK cascade and is an important node for several signaling pathways. A primary phenotype affected by ERK is the activation of cell proliferation, survival and growth making ERK inhibitors highly sought after entities. Inhibitors of ERK activity are envisioned as potential therapeutics within cancer as well as other RAS/RAF/MEK/ERK pathway related diseases.

Numerous efforts aimed at discovering ERK inhibitors have been reported including the discovery of the natural product **FR148083** (also known as LL-Z-1640-2). **FR148083** is reported to be an ATP competitive inhibitor of several kinases including MEK and ERK2 (IC_{50} values of 4 nM and 80 nM, respectively)(Figure 5) [77–81]. There are several key structural features of **FR148083** including three chiral centers, a trans alkene and a cis α,β -unsaturated ketone functionality. Ohori et al reported a crystal structure of ERK2 bound to **FR148083** which revealed a covalent bond between Cys166 and the α,β -unsaturated ketone functionality (PDB:2E14)(Figure 5)[77]. This structure further revealed that the two chiral hydroxyl groups form hydrogen bonds with Ser153 and Asn154 of ERK2 and the C10' methyl group is within the van der Waals range of a number of hydrophobic residues. This structure demonstrates that the stereochemistry of each chiral center and both double bonds imparts a unique three-dimensionality that plays a crucial role in the binding of **FR148083** to ERK2. Numerous structure activity studies on **FR148083** and the related natural product hypothemycin provide experimental data that confirms the roles of each of these stereocenters.

Researchers at Vertex Pharmaceuticals recently disclosed a small molecule ATP-competitive ERK2 inhibitor that relies heavily on a key chiral (2-hydroxyethyl)amide

moiety for its potent and selective binding. This agent was derived from a screening lead bearing a pyrazolylpyrrole scaffold (**4**, $K_i = 2.3 \mu\text{M}$) (Figure 6)[82,83]. A crystal structure of **4** bound to ERK2 indicated the pyrazolylpyrrole core maintained several pivotal hydrogen bonds to key residues within the kinase hinge region. Advancement of this lead included SAR explorations of the phenyl ring and dimethyl-amide moiety ultimately yielding **5** ($K_i = 86 \text{ nM}$). An undesired interaction of **5** with JNK3 ($K_i = 550 \text{ nM}$) prompted further evaluation. Crystal structures of **5** bound to ERK2 and JNK3 demonstrated an inversion of the binding alignment at JNK3 as compared to ERK2. The addition of a hydrogen bond donor at the benzylic methylene position was posited as means to engage hydrogen bond accepting residues within ERK2 while encountering adverse steric interactions within JNK3. The introduction of a chiral methyl group at the benzyl position gave a ~2-fold shift in potency. Incorporating a chiral hydroxymethyl (*S*-configuration) on the benzyl carbon and adjustment to a 3-chloro-4-fluoro substitution pattern yielded an analogue (**6**) with a >40-fold shift in potency ($K_i = 2 \text{ nM}$ for ERK2) and selectivity of JNK3 (>2000-fold). The corresponding analogue with the *R*-configuration was 75 times less potent. The crystal structure of ERK2 bound to **6** confirmed that the (*S*)-phenylglycinol engaged two key hydrogen bonds with the carboxylate of Asp165 and the carboxamide of Asn152 (PDB: 2OJJ)(Figure 6). A newer generation of these agents were recently reported that continue the use of the (*S*)-phenylglycinol amide motif [83]. An advanced derivative (**7**) possessed <2 nM ERK2 inhibition with >200-fold selectivity over GSK3, CDK2 and AuroraA and >500-fold selectivity over a large kinase panel. In HT29 cell proliferation assay **7** had an $\text{IC}_{50} = 48 \text{ nM}$ and showed good oral bioavailability in both rat and mouse models.

5. Discovery of the JAK3 inhibitor CP-690,550

JAK3 is a non-receptor tyrosine kinase belonging to the JAK family that includes four homologous kinases: JAK1, JAK2, JAK3 and TYK2. JAK3 is a primary signaling component for cytokine receptors that respond to interleukin (IL)-2, IL-4, IL-7, IL-9, IL-15 and IL-21) [84]. JAK3 is phosphorylated in response to cytokine binding ultimately resulting in Stat phosphorylation and activation [85]. Because of JAK3's role in γc cytokine signaling regulation, a selective JAK3 inhibitor could potentially be useful as an agent for the treatment of autoimmune-related disorders and there are numerous reports of JAK3 inhibitors. In 2003, researchers from Pfizer reported **CP-690,550**, a potent and selective JAK3 inhibitor (Figure 7)[86]. While no relative or absolute configuration was given for the two chiral carbons, the report gave IC_{50} values of 1, 20 and 112 nM for JAK3, JAK2 and JAK1 respectively. The absolute configuration was disclosed as *3R,4R* for the piperidin-1-yl-3-oxopropanenitrile based drug in subsequent reports [87–91]. Jiang and coworkers developed a strategy allowing the synthesis of all four stereoisomers of **CP-690,550** by employing L- or D-serine as the starting material [92]. Cell-based assays utilizing all four stereoisomers uncovered that only **CP-690,550** was capable of disrupting JAK3 mediated Stat5 phosphorylation at the tested concentrations. This result highly suggests that alternative stereochemical configurations are deleterious to the inhibition activity at JAK3. A profile of a panel of 354 kinases was performed for all four stereoisomers and found that **CP-690,550** possessed similar binding affinities for JAK3, JAK2 and JAK1 (K_d values of 0.7, 2 and 3 nM, respectively). This contrasted the original report which detailed a modest degree of selectivity for JAK3 over JAK2 and JAK1. Notably, a significant potency drop for JAK2 and JAK3 was documented for stereoisomers **8**, **9**, and **10** (K_d values; **10**: JAK2= 600 nM, JAK3= 190 nM; **11**: JAK2= 270 nM, JAK3= 180 nM; **12**: JAK2= 420 nM, JAK3= 150 nM) (Figure 7). A recent patent detailed additional SAR for this agent distinctly detailing the importance of the chiral methyl group on C4 of piperidine ring. A series of sulfonamide analogues demonstrated that removal of the C4 methyl group caused a significant decrease in potency for JAK3 [93]. In 2009, Lucet and coworkers reported the crystal structures of JAK1 and JAK2 bound to **CP-690,550** (PDB:3EYG)(Figure 7)[94]. Based on the homology

of JAK1, JAK2 and JAK3 it is likely that **CP-690,550** adopts a similar binding pose at JAK3. Several structural features highlighted the role that chirality plays in the binding of **CP-690,550** to JAK1/JAK2. Similar to other purine-like inhibitors, the pyrrolepyrimidine ring forms two hydrogen bonds with Glu957 and Leu959 at the hinge region of JAK1. The 3*R*, 4*R* stereochemistry of piperidine ring orients the cyanoacetyl group toward a pocket formed by the glycine-rich loop. The remainder of the **CP-690,550** structure appears to engender binding affinity through space filling/van der Waals interactions and the chiral nature of this compound significantly governs this key aspect of **CP-690,550** binding.

6. Discovery of the TrkA inhibitors isothiazole 14 and AZ-23

The tropomyosin-receptor kinases (Trk) and their ligands are discreetly involved with neuronal cell growth and survival. Neurotrophins (growth factors) are typical ligands of the Trk receptors and are important proteins involved in the survival, development and function of neurons. TrkA, the first discovered tropomyosin-receptor kinase, mediates nerve growth factor (NGF) effects such as neuronal differentiation and survival [95–97]. Upon NGF binding, autophosphorylation of Trk increases the catalytic activity of the kinase domain and initiates the downstream signal transduction pathway [98]. Specifically, the Trk receptors have been discovered to have roles in malignant transformation, metastasis and survival signaling in tumors [99–101]. Over-expression of Trk and NGF has been found in many types of human cancers, particularly prostate and pancreatic cancers [102,103]. Development of TrkA inhibitors has drawn much attention as potential cancer treatments along with other therapeutic implications.

Researchers from Pfizer reported a series of isothiazole derivatives as potent TrkA inhibitors in 2006 [104]. A high-throughput screening effort uncovered the substituted isothiazole **11** as a lead with an IC_{50} values of 7 nM and 300nM against TrkA kinase and TrkA cell-based studies, respectively (Figure 8). Examination of this agents selectivity revealed that this compound possessed only modest selectivity over VEGFR2. A homology model of TrkA revealed a lipophilic pocket that was exploited to garner selectivity over VEGFR2. Introduction of a variety of substituents at the benzylic position uncovered the *R*-ethyl substituted **12** that possessed a 1300-fold selectivity for TrkA over VEGFR2. The corresponding *S*-isomer (**13**) had moderately good potency but only a 10-fold selectivity for TrkA over VEGFR2. Further SAR examinations led to the discovery of a highly potent and selective compound (**14**) that had sub-nanomolar potency in the biochemical assay and a 7 nM IC_{50} value in the cell based study. The importance of this chiral center was showcased by the fact that the *S*-isomer (**15**) was significantly less active versus TrkA and within the cell-based assay.

Reports in 2008 and 2009 from AstraZeneca detailed a series of pyrimidine-2,4-diamines as potent TrkA inhibitors [105,106]. The bromopyrimidine-2,4-diamine **16** was discovered from an HTS effort to possess an IC_{50} of 270 nM against TrkA and 1.1 μ M against TrkB (Figure 9). During optimization several key structural changes were made including alteration from 3-methylisoxazole to phenyl and alteration of the benzyl position. The benzyl position was presumed to be prone to metabolic oxidation. To address this issue the authors examined several moieties at this position including methyl group which were examined as pure enantiomers **17** and **18**. The *S*-isomer (**18**) was found to possess a significantly lower IC_{50} value than the *R*-isomer in a cell-based assay of TrkA. However, this analogue suffered from poor solubility and selected PK properties. Continued modifications resolved these issues resulting in the discovery of **AZ-23**, which possess an EC_{50} of approximately 2 nM for TrkA in a cell based analysis. **AZ-23** was reported to possess good aqueous solubility (100 μ M), oral bioavailability and appropriate PK properties warranting advanced studies. **AZ-23** also has a promising selectivity profile

versus a large panel of kinases including FGFR1, Flt3, Ret, MuSK, Lck, EphA2, FGFR3, IR, and JAK2 [103]. This ATP-competitive inhibitor blocked tumor growth in an engineered TrkA-driven allograft model as well as a xenograft model.

8. Conclusions and Perspectives

Chirality is playing an ever-increasing role in pharmacology and drug discovery and chiral small molecules are rapidly establishing themselves as attractive probe compounds and clinical reagents. The kinome is a major segment of the drugable genome and kinase inhibitors are an established division of the pharmacopeia and chiral kinase inhibitors are beginning to appear at an increased pace. A single chiral center can instill otherwise unachievable subtlety toward the binding interactions of a ligand at highly homologous domains of kinases bestowing selectivity and potency that often eludes achiral small molecules. Here, we have highlighted several examples whereby chirality has altered the potency, selectivity, cell-based efficacy and even DMPK properties of a kinase inhibitor. Given these successes and continued advances in asymmetric synthetic and separation technologies it is likely that stereochemistry will no longer be avoided during efforts to discover and optimize novel ligands targeting the kinome and beyond.

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Figure 1.
Chemical structures of the well studied kinase inhibitors staurosporine, imatinib and sorafenib.

PH-797804p38 α IC₅₀ = 5.8 nMp38 β IC₅₀ = 40 nMp38 γ IC₅₀ = >200 μ Mp38 δ IC₅₀ = >200 μ M **Figure 2.**

Chemical structures of **PH-797804** and a reproduction of the X-ray structure of **PH-797804** bound to p38 α (PDB:3HLL).

Figure 3. Chemical structures showing the progression of chloropyridine **1** to **A-443654** and reproductions and overlay of the X-ray structures of **A-443654** bound to PKA (PDB:2JDS) (cyan colored structure) and AKT2 (PDB:2JDR)(purple colored structure). Note; residue numbering reflects PKA structure.

Figure 4. Chemical structures showing the progression of a lead AKT inhibitor **2** and the optimized **3** and a reproduction of the X-ray structure of **3** bound to PKA (PDB:2GU8).

FR148083MEK IC_{50} = 4 nMERK2 IC_{50} = 80 nM

Figure 5.
Chemical structure of **FR148083** and a reproduction of the X-ray structure of **FR148083** bound to ERK2 (PDB:2E14).

Figure 6. Chemical structures showing the progression of a lead ERK2 inhibitor **4** to **7** and a reproduction of the X-ray structure of **6** bound to ERK2 (PDB:2OJJ).

Figure 7.
Chemical structure of **CP-690,550** and a reproduction of the X-ray structure of CP-690,550 bound to JAK1 (PDB:3EYG).

Figure 8.
Chemical structures showing the progression of the lead TrkA inhibitor **11** to **14**.

Figure 9.
Chemical structures showing the progression of the lead TrkA inhibitor **16** to **AZ-23**.