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Recent Progress in the Design, Study, and Development of c-Jun N-Terminal Kinase Inhibitors as Anticancer Agents

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The c-Jun N-terminal kinase (JNK) family, with its three members JNK1, JNK2, and JNK3, is a subfamily of mitogen-activated protein kinases. Involved in many aspects of cellular processes, JNK has been also associated with pathological states such as neurodegenerative diseases, inflammation, and cancers. In oncology, each isoform plays a distinct role depending on the context of the targeted tissue/organ, the tumor stage, and, most likely, the signaling pathway activated upstream. Consequently, the current challenge in finding new successful anti-JNK therapies is to design isoform-selective inhibitors of the JNKs. In this review, a particular focus is given to the JNK inhibitors that have been developed thus far when examining 3D structures of various JNK-inhibitor complexes. Using current data regarding structure-activity relationships and medicinal chemistry approaches, our objective is to provide a better understanding of the design and development of selective JNK inhibitors in the present and future.

Introduction

Given their role in many physiological and pathological cell signaling pathways, protein kinases have intensively been studied and described as ideal drug targets for cancer therapies. Over the past decade, many small molecule kinase inhibitors have been approved and have entered the market to treat different types of malignancies (Zhang et al., 2009). The mitogen-activated protein kinase (MAPK) family, which includes extracellular signal-regulated kinases (ERKs) and c-Jun N-terminal kinases (JNKs), is involved in the control of cell proliferation, differentiation, migration, inflammation, and apoptosis (Dhillon et al., 2007; Kim and Choi, 2010). The intracellular signaling pathways involving the MAPK family are often deregulated and activated in human cancers. Thus, much attention has been paid to the design of new inhibitors of this kinase family for the treatment of malignant disorders (Pratilas and Solit, 2010; Santarpia et al., 2012). JNK3 is predominantly expressed in the brain, testes, and heart (Bode and Dong, 2007), and its specific inhibition is able to inhibit neurodegenerative diseases in animal models (Parkinson and Alzheimer diseases) (Antoniou et al., 2011). JNK1 and JNK2 are ubiquitously expressed in tissues and can present with similar or different functions (Davis, 2000). JNK1 inhibitors would be of interest in metabolic diseases because animal models treated with anti-JNK1 become resistant to food-induced obesity (Sabio and Davis, 2010). JNK2, most often concomitantly with JNK1, is involved in autoimmune diseases such as rheumatoid arthritis and asthma (Bennett, 2006; Han et al., 2001). JNK2 also appears to be involved in vascular pathologies and atherosclerosis (Sumara et al., 2005).

Responsible for phosphorylating the protein encoded by the proto-oncogene *c-jun*, JNK was initially proposed to be the key mediator of cell transformation. Since this hypothesis, studies have contributed to a more complete understanding of the function of JNKs in oncogenic signaling. JNK signaling functions to induce defense mechanisms that protect organisms against a variety of situations by dually regulating stress-induced apoptosis and autophagy (Tournier et al., 2000). According to its positive regulation of apoptosis, a substantial body of evidence implicates JNK proteins in tumor suppression (Davis, 2000; Wagner and Nebreda, 2009). First, animal studies have demonstrated that JNK1-deficient mice are highly susceptible to tumor development (She et al., 2002; Tong et al., 2007), and more recently, impaired JNK signaling was shown to accelerate tumorigenesis in prostate, breast, or pancreatic cancer (Cellurale et al., 2012; Davies et al., 2014; Hübner et al., 2012). Second, JNK can sensitize cancer cells to genotoxic stress-induced cell death (Sau et al., 2012; Song et al., 2012; Xiao et al., 2012), and knockdown of both JNK1 and JNK2 by small interfering RNA impaired apoptosis in response to anticancer drugs (Oleinik et al., 2007). The information outlined earlier indicates that JNK can act as a tumor suppressor in different types of cancer.

On the other hand, hyperactivation of the JNK proteins has been reported in multiple cancer cell lines and tissue samples, and JNK can promote tumor formation or induce drug-resistance pathways in a wide range of human cancers, including hepatocellular carcinoma, cholangiocarcinoma, lung adenocarcinoma, colon carcinoma, or triple-negative breast cancer (Chen et al., 2014; Das et al., 2011; Feng et al., 2014; Hui et al., 2008; Leventaki et al., 2014; Nguyen et al., 2014; Sui et al., 2014). In this context, JNK can act as a tumor promoter, and its oncogenic action would be attributed to its role in positive regulation of autophagy processes. In normal cells and during early oncogenic transformation, autophagy is a tumor-suppressive process but may act as a critical survival pathway for

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established tumors (Choi, 2012). As many cancer cells have high apoptotic thresholds, the JNK-induced autophagy may serve as a survival mechanism in many tumor cells, allowing them to escape apoptotic or necrotic death in response to metabolic crisis. Thus, JNK can lead to an upregulation of autophagy that was associated with therapeutic resistance (Jia et al., 2014; Li et al., 2009). To further complicate the understanding of the pathological role of JNK in oncology, recent studies have shown that JNK1 and JNK2 present distinct, or even opposite, functions in different types of cancer. A hyperactivation of JNK1, but not JNK2, was observed in hepatocellular carcinoma and lung adenocarcinoma (Chang et al., 2009a, 2009b; Takahashi et al., 2010). In contrast, other studies have described an oncogenic function for JNK2 in various tumor-derived cell lines and animal models of cancer (Barbarulo et al., 2013; Ke et al., 2010). In addition, JNK1 and JNK2 exert a concomitant role in Ras-induced tumorigenesis in lung cancer (Cellurale et al., 2011), while JNK isoforms differentially regulate the metastatic cascade (Ebelt et al., 2013). Finally, the double-edged sword of JNK in tumor progression was reported in hepatocellular carcinoma, where JNK supports tumor development in the tumor microenvironment but inversely reduces the tumor burden in hepatocytes (Das et al., 2011).

Compiling all of these findings, it is now obvious that the role of JNKs in oncology signaling is tissue specific, cell type dependent, isoform specific, and dependent on the tumor stage, most likely, the signaling pathway activated upstream; and that it might vary according to stress signals and other circumstances. Given that, biomarkers should be discovered and used to distinguish and stratify patients who will be responders or nonresponders to anti-JNK therapy (Cellurale et al., 2011; Chin et al., 2012).

Consequently, designing new inhibitors of JNKs is now a very active area of research in academic and industrial laboratories. In this review, our objective is to provide the state of the art in the design and development of JNK inhibitors by analyzing published data in the fields of structural biophysics, medicinal chemistry, and pharmacology, as well as from the clinic. Primary focus is given to the role of the JNKs in cancer pathology and candidate JNK inhibitors that are or were in clinical development. Then, the medicinal chemistry strategies are developed using the support of X-ray crystallography data from the crystallized complexes of JNK members and their inhibitors. Finally, we reserve a section for describing the pharmacological and structural elements that highlight the challenge and importance of designing selective inhibitors that can discriminate between JNK isoforms.

Past and Current JNK Inhibitors in Development

Because deregulation of the JNK pathway is suspected to be causative of many disease states, tremendous efforts have gone into trying to inhibit this pathway, and unsurprisingly, a large variety of JNK inhibitors have been generated and evaluated in clinical phases (Table S1 available online). Among them are two major classes of compounds: the ATP-competitive and ATP-noncompetitive inhibitors and peptide inhibitors (Bogoyevitch and Arthur, 2008). The first type of inhibitor is the extensively tested reversible ATP-competitive inhibitor, such as SP600125 and CEP-1347 (KT7515). SP600125 has been used extensively in many in vitro and in vivo studies and has shown

efficacy in cell culture and in mouse models. Disease models, including pharmacodynamic models, highlight not only the efficacy of JNK inhibitors in controlling disease progression but also their tolerability (Assi et al., 2006; Gross et al., 2007; Minutoli et al., 2004). However, this class of inhibitors has varying degrees of toxicity and lacks the required specificity because ATP-competitive inhibitors would indiscriminately inhibit the phosphorylation of all JNK substrates. Consistent with these results, there are many reports of the failure of such competitive inhibitors in animal models. SP600125 development failed due to the lack of selectivity among the JNK family and because this compound was capable of inhibiting at least 13 other kinases (Tanemura et al., 2009). Therefore, second-generation ATP-competitive inhibitors such as CC-401 have been developed and have shown potential antineoplastic activity. CC-401 was developed based on the chemistry of SP600125 and is a potent inhibitor of all three forms of JNK (inhibition constant, K_i, = 25–50 nM); it has at least a 40-fold selectivity for JNK compared with other related kinases. In cell-based assays, CC-401 shows a specific JNK inhibition at concentrations of approximately 1–5 μM, and the compound has shown efficacy in renal injury models. Unfortunately, a phase 1 clinical trial using CC-401 for acute myeloid leukemia (NCT00126893) was discontinued, and the development of CC-401 was stopped. Another SP600125 derivative that has been developed is CC-930. The safety of CC-930, an antifibrotic inhibitor of JNK, was recently tested in two phase 2 clinical trials (Plantevin Krenitsky et al., 2012). These trials were terminated by the sponsor, citing the lack of support for study continuation based on the benefit/risk profile (NCT01203943 and NCT01466725). Semapimod (CNI-1493) has a structure that is completely different from those of other SP600125 derivatives. The mechanism of action of semapimod may be posttranscriptional, targeting the proinflammatory cytokine production regulated by MAPK (p38 and JNK) (Cohen et al., 1996). Moreover, in vitro kinase assays revealed that the direct molecular target of semapimod is the c-Raf protein kinase, an upstream modulator of the MAPK pathway. Moreover, a correlation between the patient response with semapimod treatment and the significant decrease in phosphorylation of JNK was observed clinically (Löwenberg et al., 2005). While promising positive results from an open-label phase 2 study had been reported, semapimod was ineffective in improving active Crohn's disease, with phlebitis presenting as a significant and dose-limiting side effect (Dotan et al., 2010; Hommes et al., 2002). Although semapimod is still under preclinical investigation with studies continuing its characterization, its clinical development is currently halted.

In addition, PGL5001 (bentamapimod, AS601245) is another ATP-competitive inhibitor with a half maximal inhibitory concentration (IC_{50}) of 80 nM, 90 nM, and 230 nM for JNK1, JNK2, and JNK3, respectively. Based on promising preliminary studies in animal models of autoimmune diseases and neuronal apoptosis, PGL5001 was first described to have therapeutic potential in multiple sclerosis and fibrosis (Carboni et al., 2004; Halazy, 2006). Today, PGL5001 is proposed as a potential first-in-class product for the treatment of endometriosis and is currently being evaluated in a Phase 2 clinical trial.

With the lack of tolerability and/or benefit observed in clinical phases in the past, the use of JNK inhibitors as therapeutic
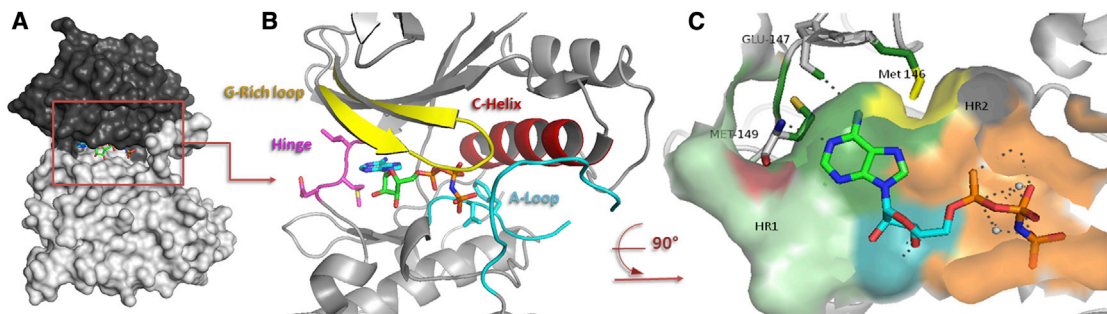
Figure 1. X-Ray-Resolved Structure of JNK3 in Complex with an ATP Analog, PDB ID: 1JNK
(A) Surface representation of the entire JNK3 structure illustrating its two kinase lobes: the N-terminal lobe in light gray and the C-terminal lobe in dark gray.
(B) Ribbon representation of JNK3 crystallized with a focus on the ATP-binding site with the G-rich loop in yellow, the C helix in red, the A loop in cyan, and the hinge in magenta.
(C) Schematic representation of the active site with domain separation. Pale green, hydrophobic region 1 (HR1); forest green, adenosine binding region; cyan, hydrophilic ribose region; orange, phosphate binding region; dark gray, hydrophobic region 2 (HR2); yellow, gatekeeper. All images were developed using PyMol.

intervention was suspended to obtain further validation. To this end, the current knowledge about the role of JNK signaling pathways in physiological and pathological states is progressing at a rapid pace (Sabapathy, 2012). Particularly in oncology, much work has been performed in solid and hematological cancers to offer perspectives on the challenges faced when developing drugs targeting JNKs.

Structural Insights into JNK Inhibition

With a large number of crystallized structures, the JNK family has been extensively studied and their structural description is biophysically possible. As in almost all other tyrosine kinases, the X-ray structure of JNKs is characterized by two distinct lobes, the N-terminal and the C-terminal lobes (Figure 1A), that are joined by a segment referred to as the hinge loop. The N-terminal lobe is mainly composed of β strands and two α helices, and the C-terminal lobe is composed of α helices. The cavity formed between the two lobes creates the kinase activation site or the ATP-binding site where the ATP docks. Typical kinase elements are present in JNK members (Figure 1B): the G-rich loop, which is a relatively small loop rich in glycine residues forming the active site roof; the C helix, which contains some of the residues that form the active site deep pocket or selectivity pocket; the activation loop (A loop), which plays a critical role in the activation/deactivation process; and the hinge loop, which is composed of five residues in all JNK members: Ala(113/151)-Asp(112/150)-Met(111/149)-Leu(110/148)-Glu(109/147) in JNK1&2/JNK3, respectively. The hinge region often plays a major role in inhibitor binding (Shaw et al., 2008). The ATP-binding site is composed of five distinct domains that are characterized by distinct chemical environments (Traxler and Furet, 1999) (Figure 1C): (1) a hydrophobic region 1 corresponding to the entrance pocket (HR1); (2) the adenine binding region where the nucleobase of the ATP makes hydrogen bonds with the hinge; (3) the hydrophilic ribose region where the sugar of the ATP binds; and (4) the hydrophilic solvent-exposed phosphate binding region, which is delimited from (5) the hydrophobic region 2 (HR2), which is called the selectivity pocket, as determined by the highly conserved aspartate-phenylalanine-glycine (DFG) motif (Krenitsky et al., 2012; Scapin et al., 2003; Szczepankiewicz et al., 2006).

New and improved JNK inhibitors are in the research and development pipeline and have a range of different inhibitor chemotypes. JNK inhibitors exist that belong to all of the known classes of kinase inhibitors, including reversible and irreversible inhibitors, ATP-competitive or -noncompetitive inhibitors, and allosteric inhibitors. Most of the new chemical entities targeting JNKs are type I inhibitors that are defined by their ability to compete with and replace ATP in the ATP-binding site of the kinase in its active form. A single residue named the gatekeeper (corresponding to a methionine either in position 108 in JNK1 and JNK2 or in position 146 in JNK3) restricts access to the HR2 selectivity pocket (Liu et al., 1998). The size and nature of this gatekeeper residue have been shown to control kinase sensitivity. In this regard, effective strategies for the design of selective type I inhibitors primarily consists of targeting the sequence variability at a given position of the ATP-binding site or exploiting the cavity (generally the selectivity pocket, considering the gatekeeper residue) of variable sizes in different kinases. Unfortunately, these strategies are not transposable to obtain selectivity among the JNK family itself. Indeed, while the substrate specificity for each isoform can be explained by the presence of particular and singular nonconserved residues (Xie et al., 1998), the ATP-binding sites of the three isoforms share a very high identity. Therefore, designing isoform-selective type I anti-JNK inhibitors remains a challenge.

ATP-Competitive Inhibitors of JNKs: Structure-Activity Relationship

The intense interest in developing and improving new ATP-competitive JNK inhibitors is exemplified by the development of the aminopyridine-based JNK inhibitor VIII (Szczepankiewicz et al., 2006), a trisubstituted thiophene with selectivity against JNK3 (Bowers et al., 2011a), and novel quinazoline JNK inhibitors (He et al., 2011). Because many different JNK inhibitors have been published, we have focused our analysis on the ATP-competitive inhibitors when crystallized with a JNK family member and the structure-activity relationship (SAR) studies around these structures. When analyzing the published data, we selected molecules and categorized them into two different groups based on their binding mode to the active site of the kinase and, more specifically, to the methionine residue of the
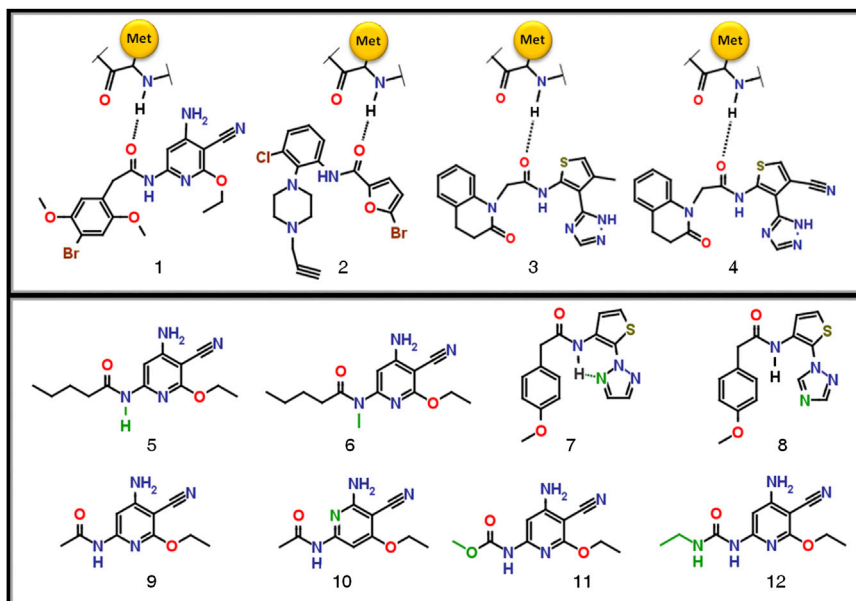
Figure 2. Representative Chemical Structures of the First Series of JNK Inhibitors

In the upper window, inhibitors crystallized with either JNK1 (structure 1; PDB ID: 2GMX) or JNK3 (structures 2, 3, and 4; PDB IDs: 3FV8, 3PTG, and 3RTP, respectively) are represented with the hydrogen bond allowing their interaction with the methionine residue of the hinge. In the lower window, selected chemical structures of series 1 are schematized with the singular structural elements (in green) impacting the binding with JNKs.

hinge. The first group is linked to the hinge by one hydrogen bond and can be divided into two subgroups based on the cyclic or acyclic character of the fragment (series 1 has an acyclic fragment, and series 2 has a cyclic fragment). The second group is able to bind to the hinge by forming two hydrogen bonds, which are adjacent for series 3 or nonadjacent for series 4.

ATP-Competitive Inhibitors that Interact with the Hinge with Only One Hydrogen Bond

Two main chemical fragments, either acyclic or cyclic, are able to bind with the hinge using a hydrogen bond with the oxygen atom of a carbonyl group.

ATP-Competitive Inhibitors Using One Hydrogen Bond and an Acyclic Fragment: Series 1

In this first series, four ATP-competitive inhibitors, presenting with the same binding mode to the hinge with a good IC_{50}, were cocrystallized with either the JNK1 or JNK3 isoforms. The binding mode involves one crucial hydrogen bond between the amidic carbonyl moiety present in the ligand and the hinge backbone (Figure 2). The residue involved in the hinge region is a methionine in either position 111 in JNK1 or position 149 in JNK3. The NH of the amidic motif of the inhibitor is oriented either inward (Figure 2, compounds 1, 3, and 4) or outward (Figure 2, compound 2), relative to the active site.

Using SAR studies (Bowers et al., 2011a, 2011b; Hom et al., 2010; Liu et al., 2007; Shin et al., 2009; Szczepankiewicz et al., 2006), the central structural elements of this series of compounds that influence the activity of the molecule can be highlighted (Table 1 in association with Figure 2). First, the N-methylation of the amidic nitrogen results in the total loss of the activity (compound 6 in comparison with compound 5 in Table 1). For some inhibitors, this hydrogen has another significant role: it intervenes in the intramolecular H-bond interaction between the amidic hydrogen and the triazol nitrogen, thereby inducing a partial planarity of the inhibitor structure (Bowers et al., 2011a) (structure 7 in comparison with structure 8 in

Table 1), which, in this case, seems to be crucial for the inhibitor activity. Most of the inhibitors possess one CH_2 group, linking the amidic carbonyl to a heteroaromatic ring (HAr), HAr-1. When this CH_2 group is replaced by a heteroatom such as oxygen or nitrogen (structure 9 in comparison with structures 11 and 12), the activity against JNK is lost. HAr-1 can make hydrophobic interactions, as observed between the substituted phenyl ring of the compound 1 and isoleucine in position 32 of JNK1 (Szczepankiewicz et al., 2006). It is interesting to note that additional residues on the protein surface offer the potential for hydrogen-bonding interactions with substituents in the 4- or 5-phenyl position (Szczepankiewicz et al., 2006). A second HAr, HAr-2, is also linked to the NH of the amidic function of the inhibitors. Although the heteroatom of this HAr-2 ring does not appear to interact with the protein surface, the position of this atom shows an impact on the inhibitory activity, as observed by comparing structure 9 with structure 10 (Figure 2).

ATP-Competitive Inhibitors Using One Hydrogen Bond and a Cyclic Fragment: Series 2

In this series, the hydrogen bond is made between a carbonyl group and the methionine backbone of the hinge region, as in series 1. Series 2 is distinct from series 1 because its carbonyl motif belongs to a ring. The crystal structure of the ligand-JNK complexes revealed that series 2 compounds present a typical binding mode with 1-carbonyl oxygen of the isoquinolone (compounds 13 and 14), azaquinolone (compound 15; Haynes et al., 2012), or 4-quinolone (compound 16) series interacting with the methionine of the hinge (Figure 3). The general structure of this series involves one CH_2 group substitution in position 2 of the central core by an aromatic ring or HAr. No interaction was indicated by the modification of substituents on the phenyl group in position 4 of the isoquinolone (Asano et al., 2008a). For isoquinolone, preferred substituents at position 6 are small lipophilic substituents such as chlorine or bromine (compound 18 is 100 times more potent than compound 19 on JNK1). Based on the information derived from the cocrystal structure of the compound 13 with JNK3 and SAR studies, further optimizations were conducted by introducing modifications mainly at position 2 of the isoquinolone ring and introducing a polar substituent on different positions of the benzyl moiety (Asano et al., 2008a). It was thus demonstrated in this series that the presence of a hydrogen bond accepting and/or donating groups in the para position of

Table 1. SAR Summary for Each Series and the Described Compounds

Series	Compound	IC$_{50}$ (nM)		
		JNK1	JNK2	JNK3
1	1	77	160	NC
	2	140	NC	160
	3	32	31	25
	4	3	8	6
	5	350	690	NC
	6	>10,000	>10,000	NC
	7	830	5,000	1,500
	8	>10,000	>10,000	>10,000
	9	750	1,100	NC
	10	>10,000	>10,000	NC
	11	>10,000	>10,000	NC
	12	>10,000	>10,000	NC
2	13	10	NC	NC
	14	9	NC	NC
	15	49	NC	NC
	16	58	219	NC
	17	6	NC	NC
	18	8,800	NC	NC
	19	86	NC	NC
3	20	55	350	NC
	21	6	24	NC
	22	13	25	57
	23	NC	NC	270
	24	51	10	7
	25	809	1,140	709
	26	>10,000	>10,000	>10,000
	27	420	97	16
	28	NC	NC	3,000
	29	2,600	NC	NC
	30	>100,000	NC	NC
	31	48	NC	NC
4	32	101	NC	3
	33	NC	NC	550[a]
	34	400	NC	25
	35	NC	120	NC
	36	5,350	NC	30
	37	NC	NC	>4,000[a]
	38	73	NC	12

NC, not calculated.
[a]Inhibition constant, K_i.

the 2-benzyl group enhanced JNK1-inhibitory activity. It was proposed that this effect is more important when this substitution happens on a ramification of this position as in the terminal carboxyl group on the para-substituent of the benzyl group of compound 14. This could be explained by an unexpected electrostatic interaction between this second group and lysine in position 68 in JNK3 (corresponding to lysine in position 30 of JNK1). Hydrophilic substituents on this position would most

likely optimize the cell potency by positively regulating the lipophilicity of the compound.

It is interesting to note that, while the majority of compounds are derived from 2-benzyl isoquinolone structures, it was further proposed that a five-membered HAr such as a 5-carbonyl pyrazole group on the isoquinolone position 2 (compound 17) would be preferred relative to a six-membered ring (Asano et al., 2008b).

A structure-based drug design using compound 16 was used to guide the design and optimization of compounds derived from 4-quinolone (Gong et al., 2012). This study resulted in the identification of inhibitors that are able to inhibit 2- to 10-fold more JNK1 than JNK2.

ATP-Competitive Inhibitors that Interact with the Hinge with Two Hydrogen Bonds
ATP-Competitive Inhibitors Using Two Adjacent Hydrogen Bonds: Series 3

A large series of inhibitors crystallized with JNK1 or JNK3 mimics the two hydrogen bonds that are made by the adenine motif of ATP, a typical binding mode of kinase inhibitors (Scapin et al., 2003). The two hydrogen bonds are made between the methionine of the hinge and the two nitrogen atoms that are separated by a carbon in the core of an aminopyridine, aminopyrimidine, or purine derivative ring. The NH moiety on the backbone of the methionine of the hinge acts as the hydrogen bond donor, with the nitrogen atom (sp^2) of the pyridine derivative ring in the inhibitor acting as a hydrogen bond acceptor. The hinge methionine carbonyl interacts as a hydrogen bond acceptor, with the amine function (sp^3) substituting position 2 of the pyridine derivative ring that acts as a donor (Figure 4).

The importance of the amino group for the binding was also highlighted in two independent SAR studies. The first demonstrated that the replacement of the nitrogen with another atom, such as oxygen, by creating an ether function (compound 16 versus compound 25 in Figure 4 and Table 1, respectively) induced a complete loss of the activity of the inhibitor (Zhang et al., 2012). In the same way, a second SAR study showed that the addition of a substituent replacing the hydrogen atom on the amino group leads to a complete loss of activity (compound 30 compared with compounds 29 and 31) (Liu et al., 2007) or to a change in the orientation and the binding mode of the compound (compound 28 compared with compound 23) (Buckley et al., 2008). The X-ray crystal structure of the aminopyrimidine derivative 22 shows that the 5′-chlorine atom can sterically interact with the sulfur atom of the methionine at position 146 (the gatekeeper of JNK3) (Alam et al., 2007). The sulfur-π stacking interaction between the gatekeeper sulfide and a naphthalene ring was also well described on compound 27 (Protein Data Bank [PDB] ID: 3OY1; (Probst et al., 2011). It was further demonstrated that changing the size of the heterocycle substituting the amino group (4-piperidine in compounds 22 and 23 or 4-hydroxycyclohexyl in compounds 21 and 24) from six- to five- or four-membered rings induced a reduction in JNK activity, suggesting a less optimal fit of the smaller rings in the active site. Furthermore, it was shown that compound 21 is 10 times more active than compound 20 on JNK1 and JNK2. Palmer et al. (2013) concluded that the substitution of position 4 of the indazole, particularly with polar substituents such as
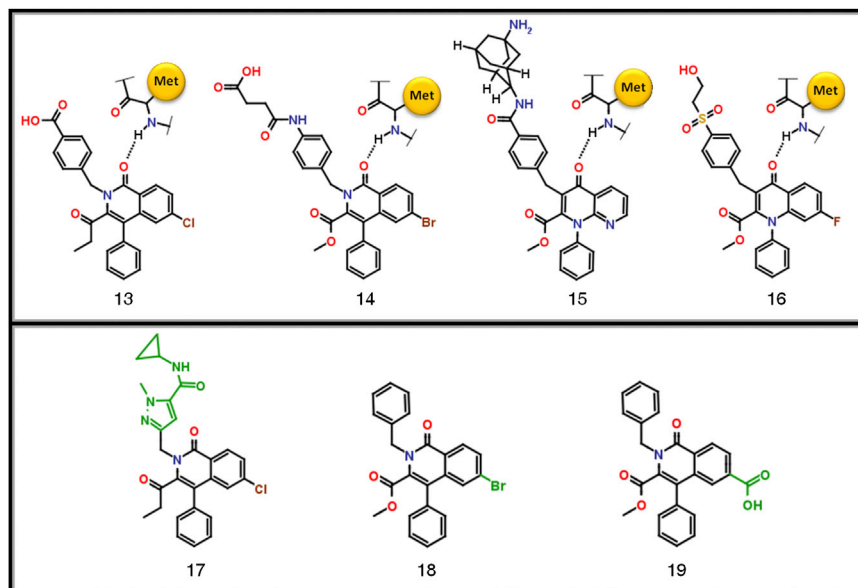
Figure 3. Representative Chemical Structures of the Second Series of JNK Inhibitors

In the upper window, inhibitors crystallized with either JNK3 (structures 13 and 14; PDB IDs: 2ZDT and 2ZDU, respectively) or JNK1 (structures 15 and 16; PDB IDs: 4E73 and 4G1W, respectively) are represented with the hydrogen bond allowing their interaction with the methionine residue of the hinge. In the lower window, selected chemical structures of series 2 are schematized with the singular structural elements (in green) impacting the binding with JNKs.

alcohols and sulfonamides, improved the activity against JNKs and the physical properties of the molecule. Finally, SAR exploration of an aminopurine series led to the identification of the compound 24 (the development candidate CC-930), with an improvement in potency by introducing a tetrahydrofuranyl moiety in position 9 (Krenitsky et al., 2012; Plantevin Krenitsky et al., 2012).

ATP-Competitive Inhibitors Using Two Nonadjacent Hydrogen Bonds: Series 4

Several inhibitors were shown to benefit from two hydrogen bonds between two nonadjacent hydrogen bonding donors and acceptors with the methionine of the hinge (Figure 5). A first hydrogen bond is formed between the NH moiety of the methionine residue and an aromatic nitrogen atom on the inhibitor. A second hydrogen bond is formed between the carbonyl group of the methionine residue and an amidic NH motif on the inhibitor. As an example, the importance of the second hydrogen bond with the amide function of the inhibitors can be observed not only by X-ray crystallography but also by SAR analyses of the 6-anilinoindazole derivatives (compounds 32 versus compound 36; Swahn et al., 2005) and pyrrole carboxamide analogs (compound 33 compared with compound 37; Aronov et al., 2007). Through a crystallographic data study and SAR analyses, it was also illustrated that indazole-based and aminopyrazole-urea-based inhibitors interact with the hinge very similarly (compounds 34, 35, and 38). A particular case is observed with compound 35 (Shaw et al., 2008), which shares the two hydrogen bonds commonly established by this series with the methionine of the hinge and binds with a third hydrogen bond to the hinge (with the glutamate in position 109 of JNK2). This hinge binding mode is also similar to the one observed for 6-anilinoindazoles with JNK3 (PDB ID: 2B1P; Scapin et al., 2003).
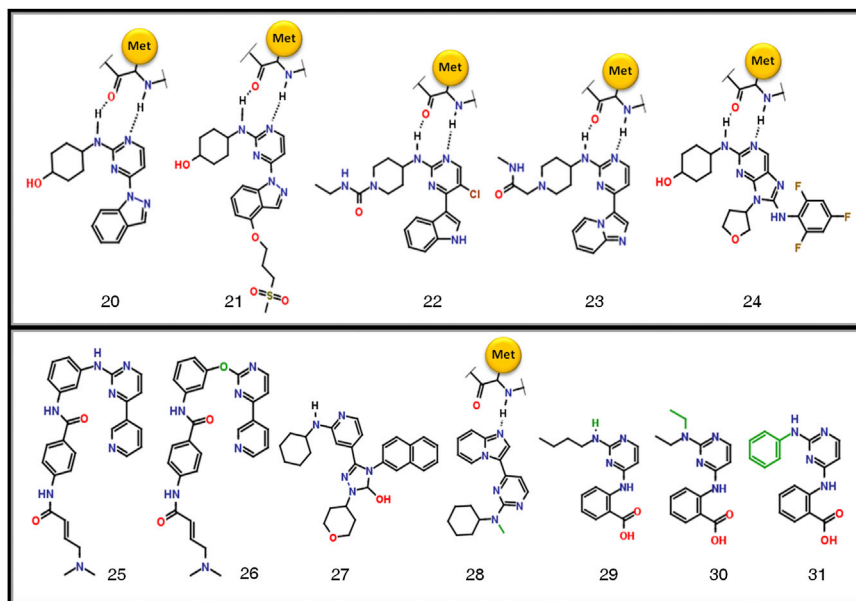
ATP-Competitive Inhibitors of JNKs: Summary of Structural Elements and Binding Modes for Each Series

From the structural and SAR analyses of the four chemical series, a common binding mode for the compounds in each series can be proposed; a summarized scheme is proposed in Figure 6. For the first series, a central amide function binds to the hinge by its carbonyl group and acts as a linker between two HArs (Figure 6, series 1). While the first HAr (HAr-1) lies outside the active site where it can make hydrophobic interactions, the second HAr (HAr-2) takes refuge in the adenosine pocket. For the second chemical series, all of the ligands share a central quinolone derivative ring substituted on positions 2, 3, and, 4 and less frequently on position 6 (Figure 6, series 2). The substituent in position 2, either a five- or a six-membered ring linked to the quinolone ring with a CH_2 linker, and the substituent in position 3 lie together in the HR1 region of the active site. Finally, position 4 is substituted by a phenyl ring that interacts with the hydrophilic sugar region. For the third series, the central entity is made of an amino group linked to a HAr-2 that resides in the adenine binding region. If the HAr-2 is an aminopyridine or aminopyrimidine ring, position 4 is usually substituted by a HAr-3 that constantly appears to take refuge in the sugar-binding region. If the HAr-2 is a purine derivative, the HAr-3 is linked in position 5. The substituents of the HAr-3 (R1 in the series 3 in Figure 6) come into contact with the selectivity pocket. The HAr-2 is also amino substituted (with or without a linker) by a HAr-1 that lies within the solvent-exposed region (Krenitsky et al., 2012). For the fourth series, it was more difficult to establish obvious SAR rules. A central common binding mode can be proposed with a common skeleton composed of a central aromatic ring or a HAr (five- or six-membered ring) supporting two substituents in the meta position relative to each other. The first substituent is commonly a pyrazole or an indazole ring that implicates the N_1 atom (sp^2) in the first hydrogen bond with the methionine of the hinge. The second substituent is a substituted amide function that involves the nitrogen in the second hydrogen bond with the methionine of the hinge. It is also interesting to note that the carbonyl group of the amide function can take two possible orientations (as described for the first series).

Designing Selective Inhibitors that Discriminate between JNK Isoforms: A Pharmacological and Structural Point of View

All of the intensive research and new findings regarding JNK functions make the limited success of JNK inhibitors that had entered clinical trials more understandable and predictable. In

fact, these inhibitors were used clinically without considering the context of tissue-dependent expression and role of the JNKs, or even the pathological significance of each isoform. Consequently, the lack of efficacy and the unpredicted side effects observed in these clinical trials are, most likely, due to the lack of an identification and selection of a subset of patients who would respond positively to anti-JNK therapy. Hence, while the identification of biomarkers that would allow for the stratification of patients still remains a challenge, the generation of JNK1-, JNK2-, and JNK3-specific inhibitors may be required to tackle the problem of inhibitor specificity. The beneficial effect of selectively targeting JNK is demonstrated by the positive results that have already been obtained with the peptide D-JNKI-1 (XG-102, AM-111) (Davis and Dickens, 2002; Dickens et al., 1997). Specifically designed to target JNK1, the inhibitory action of D-JNKI-1 peptide (a cell-permeable JNK inhibitor) is fundamentally different those that of classical small chemical inhibitors because it does not inhibit JNK's enzymatic activity but selectively blocks access to many of its substrates by a competitive mechanism (Borsello et al., 2003). This cell-permeable peptide inhibitor offers interesting possibilities for different therapeutic applications (Kersting et al., 2013; Sclip et al., 2014; Wang et al., 2003). After the successful completion of phase 1 and 1b clinical trials, a phase 2 study will be started for application in ophthalmology and in severe inner ear disorders. These very promising results suggest that, as expected, a higher compound selectivity for JNKs, relative to the other kinase families and the JNK family isoform specificity, results in higher clinical benefits. Thus, specificity is a key issue that needs to be properly evaluated for the development of successful JNK inhibition therapies and for JNKs to continue to be considered attractive therapeutic targets through the development of selective JNK inhibitory molecules.

In data from the literature, each compound of the series described in this review has shown a pan-JNK activity with selectivity against other MAPK family members and/or an obvious selectivity against one or two JNK isoforms relative to

the others. One of the fundamental bases of the pan-JNK selectivity against other kinases—and, more specifically, against MAPK—is the conformation of the hinge methionine carbonyl group, which is quite unique in JNKs relative to other kinases. At this position, the carbonyl residue is generally oriented toward the active site, clashing with the small molecule electronegative groups, whereas this carbonyl group is oriented outside the active site in JNKs, which allows small inhibitors with well-defined motifs to interact with this active site comfortably (Haynes et al., 2012). More challenging are the design and understanding of the selectivity that is observed against isoforms within the JNK family, despite the conservation of the ATP-binding site across these isoforms. In series 1, analogs of molecules 3 and 4 were shown to be five to ten times more potent inhibitors of JNK1 and JNK3 than of JNK2 (Bowers et al., 2011a, 2011b). In series 2, analogs of molecule 16 presented a reasonable selectivity for JNK1 compared with JNK2 (Gong et al., 2012). In series 3, molecules with high selectivity for JNK3 compared with JNK1 were described (Swahn et al., 2006). More specifically, the nature of the substituents and their orientation (R1 substituents in series 3 of Figure 6) influence the activity and the selectivity between JNK isoforms and against other kinases. As an example, by targeting the selectivity pocket, the naphthalene of compound 27 and its analogs are highly selective for JNK2 and JNK3, with four of them highly selective against only JNK3, relative to JNK1 (Probst et al., 2011). Similarly, analogs of compound 24 have shown some selectivity for JNK3 and JNK2 relative to JNK1 (Krenitsky et al., 2012; Plantevin Krenitsky et al., 2012). In series 4, molecules 32 and 38 exhibit a high potency for JNK3 inhibition relative to JNK1. It was proposed that some molecules of series 4 could cause a movement of the gatekeeper methionine (position 146 of JNK3) side chain (Kamenecka et al., 2009), allowing the N-phenyl urea group of the pyrazoles (compound 34) and the 5-anilinophenyl substituent on the indazoles (compound 38) to bind the hydrophobic region 2 (the selectivity pocket). According to this suggestion, it was demonstrated that, in JNKs, the main chain of the gatekeeper (Figure 2) is relatively labile for the three isoforms, particularly for JNK3, which demonstrated the leading capacity to accommodate inhibitors in its selective pocket by a shift of 2 Å. This induced fit binding to JNK3 would be more favorable than that
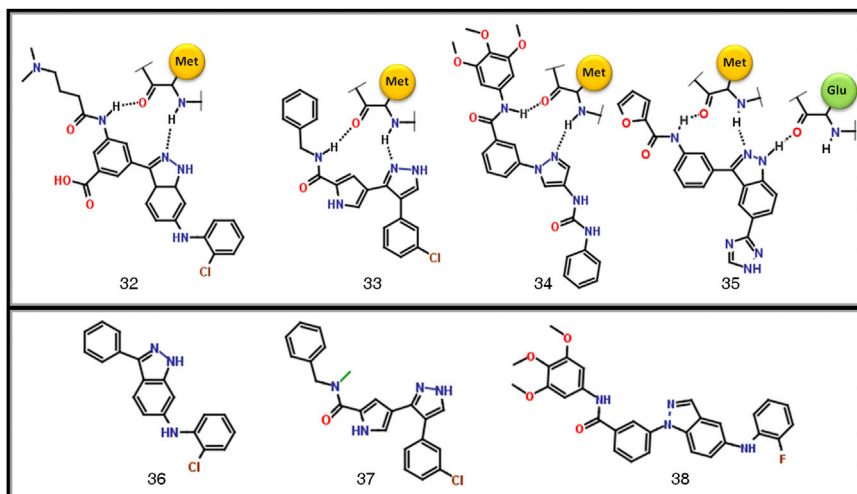
Figure 5. Representative Typical Chemical Structures of the Fourth Series of JNK Inhibitors
In the upper window, a selection of inhibitors crystallized with either JNK3 (structures 33, 32, and 34; PDB IDs: 2OK1, 2B1P, and 3FI2, respectively) or JNK2 (structure 35; PDB ID: 3E7O) are represented with the hydrogen bond allowing their interaction with the methionine residue of the hinge. In the lower window, selected chemical structures of series 4 were selected to illustrate the structural elements impacting the binding with JNKs (compound 38 was also crystallized with JNK3; PDB ID: 3FI3).

of JNK1 and JNK2, and this, most likely, can be explained by the sequence differences in their selectivity pocket (Swahn et al., 2005). The leucine in position 144 of JNK3 and JNK2, instead of the bulky isoleucine in position 106 of JNK1, should play an especially crucial role in conferring a more favorable induced fit to JNK3 and JNK2 (Probst et al., 2011; Swahn et al., 2006).

To conclude, with crystal structures and SAR analysis, three main inhibition profiles are obviously observed: first, compounds that concomitantly inhibit JNK1 and JNK3 with selectivity against JNK2; second, a concomitant inhibition of JNK2 and JNK3 with selectivity against JNK1; and third, an inhibition profile that concerns compounds with a unique JNK3 selectivity (Probst et al., 2011).

Conclusions
It is essential to take into close consideration which JNK proteins are beneficial targets and, more important, what effects small molecule inhibitors of JNKs have on physiological processes. Accumulating evidence supports a role for the JNK proteins in the pathogenesis of different solid and hematological malignancies and highlights the many challenges and scientific oppor-

tunities in the targeting of JNKs in cancer (Bubici and Papa, 2014). A number of ATP-competitive and ATP-noncompetitive JNK inhibitors have been developed, but they have several limitations, such as a lack of specificity and cellular toxicity. With the understanding that JNK isoforms act differently depending primarily on the tissue and the pathological state, most of the drug design efforts have thus been undertaken to find isoform-selective compounds. An analysis of the literature and X-ray crystal structures illustrated, as in other kinase inhibitors, the role of the binding moiety to the kinase hinge region. By also using SARs, several binding modes of JNK inhibitors can be drawn involving either one or two hydrogen bonds within the hinge. Regardless of the binding mode observed, all of these inhibitors are highly potent against the JNK family members. Therefore, some research has demonstrated that finding isoform selectivity is possible, notably by targeting the HR2 region. Most of the efforts directed at selectivity were focused on the inhibition of JNK3 because this isoform is primarily expressed in the brain and is an attractive target for neurodegenerative diseases. Because it has been proven that selectivity for JNK3 is possible, many studies have attempted to determine how to find selectivity for the two other isoforms. In most of the oncology indications, the inhibition of JNK3 should be avoided. The decision to inhibit and target either JNK1 or JNK2 should be taken in a manner that is dependent on
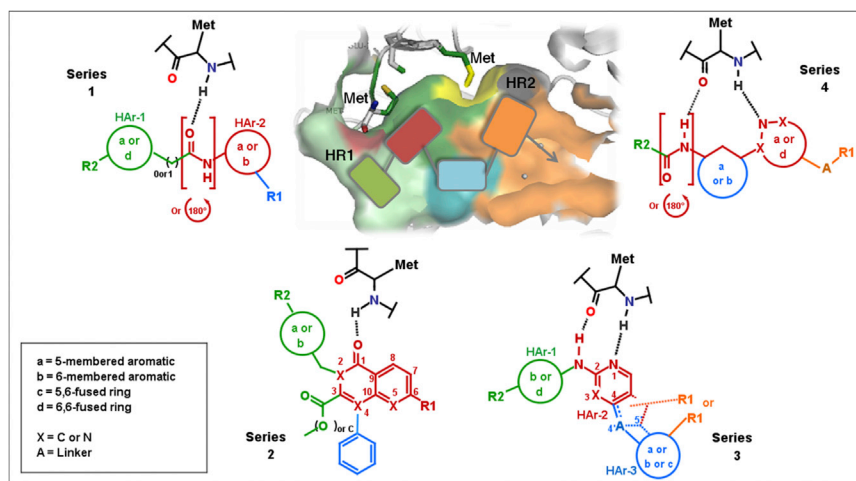
Figure 6. Schematic Representation of the Four Different Series of Categorized ATP-Competitive JNK Inhibitors
The binding mode of the four series of compounds to the methionine of the hinge is represented using hydrogen bonds that are symbolized by a dotted line. The different parts of each structure are color coded. The central scaffold that interacts with the hinge and the adenine binding region is represented in red. The part of the molecule in blue occupies the ribose region. The green and optional orange parts of the ligand lie in the hydrophobic regions 1 and 2 (HR1 and HR2, respectively).

the knowledge of mutation, expression, and activation states of both isoforms in the targeted cancer. While this review was focused on ATP-competitive inhibitors, it is well known that other strategies would also allow for the development of isoform selectivity. We can mention inhibitors that are able to bind to the ATP-binding site of JNK in its inactive form (also known as DFG-out state) and the development of peptides that bind to the substrate pocket or covalent inhibitors that benefit from the presence of a particular cysteine in the kinase site as the success of such alternative approaches.

SUPPLEMENTAL INFORMATION

Supplemental Information includes one table and can be found with this article online at http://dx.doi.org/10.1016/j.chembiol.2014.09.007.

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