DOI: 10.1002/cmdc.200700131

Novel Inhibitors of Checkpoint Kinase 1

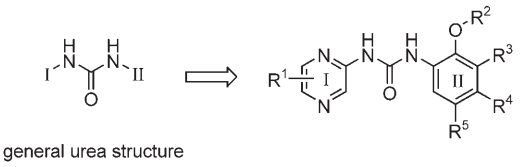
Kenneth L. Arrington* and Vadim Y. Dudkin[a]

To date, DNA-damaging chemotherapeutic agents constitute a basic tool set in the treatment of cancer. Whereas a number of such drugs have achieved widespread use, their shortcomings are well documented and include toxicity associated with lack of selectivity for tumors over normal proliferating cells, as well as efficacy limitations. Thus, widening the therapeutic window of DNA-damaging chemotherapies would lead to significant improvement in cancer care. Ideally, a dividing tumor cell would progress into mitosis after sustaining DNA damage inflicted by chemotherapy and subsequently undergo mitotic catastrophe and apoptosis. However, cells have the ability to halt the cell cycle in G1, G2, or S phases and pause to allow for DNA repair. Temporary arrest of the progression of the cell cycle therefore provides a survival mechanism for tumor cells and can be mediated through both p53 and Chk1 pathway activation. Whereas normal proliferating cells have an intact p53 pathway, it is compromised in many tumors, and for these Chk1-mediated G2/S arrest becomes a dominant defense mechanism from DNA-damaging chemotherapy.[1–7] Inhibition of Chk1 in such p53-deficient tumor cells with damaged DNA would abrogate the cell-cycle arrest and force the progression into mitosis resulting in cell death, thus selectively sensitizing these tumor types to chemotherapy. Accordingly, combination therapy comprising a DNA-damaging agent with a Chk1 inhibitor will potentially have a significantly higher therapeutic index than chemotherapy alone. Such realizations have driven great interest in the discovery of selective inhibitors of Chk1 kinase with a number of pharmaceutical companies and academic research labs contributing to the effort. This minireview will focus on the challenges and recent progress achieved in this area from a medicinal chemistry perspective.

In a majority of publications, inhibition of Chk1 enzymatic activity serves as a primary assay for compound screening. This can be followed by a number of experiments designed to evaluate functional activity in a cellular context, which typically measure effects of compound administration on either checkpoint abrogation or antiproliferative activity of a DNA-damaging agent. Cell cycle profile (FACS) analysis has been employed to observe changes in cell distribution from abrogation of checkpoint arrest and apoptosis following administration of a DNA damaging agent and Chk1 inhibitor.[8] A similar observation is made in the checkpoint escape assay[9] which utilizes an immunohistochemistry approach to measure the extent of mitotic marker expression. Antiproliferative effects of Chk1 inhibitors have been investigated with inhibition of colony formation[10] or cell viability (for example, MTS)[10] assays demonstrating sensitization of tumor cell lines to a DNA-damaging agent. Finally, measurement of Chk1 autophosphorylation provides a direct readout of a compound's inhibitory activity at the cellular level.[11] In recent years (2005–2007) both enzymatic and functional activity data have become available for novel[12] Chk1 inhibitors belonging to a number of structurally distinct series. Among these promising series, the diaryl ureas, fused-pyrazoles, quinolinones, aminopyrimidines, and others, are described below.

Diarylureas

Substituted ureas have become the most extensively investigated group of Chk1 inhibitors, with many patents and publications devoted to these series. Initially originating as hits from various high throughput screening campaigns, the cores belonging to this class have been the focus of several companies' Chk1 discovery efforts for some time. ICOS Corporation,[13] Millennium Pharmaceuticals,[14] and Abbott Laboratories[15] produced early (2003–2005) patent applications detailing the inhibitory activity of disubstituted ureas with similar embodiments. Each reported IC_{50} value is in the nanomolar range and the preferred embodiment usually contained a functionalized pyrazine as ring I and a disubstituted phenol as ring II (Figure 1). ICOS corporation has disclosed its latest discoveries



general urea structure

Figure 1. General ICOS, Millennium, and Abbott Diaryl Ureas.

in a series of patents[16–18] based on the general structure **18** (Figure 2). The most preferred configuration has W as a 4-methyl pyrazine, X^1 and X^2 as NH, and Y as O, with varying functionalities appended to the eastern phenyl ring. The compounds represented by this combination exhibit IC_{50} values ~100 nM in the cell-based Chk1 assay, although some are claimed to be <25 nM. These prototypical compounds are also at least 20-fold more selective for Chk1 over other protein kinases, including Chk2, CDK1, Akt-1, and p38MapK (some are

[a] *K. L. Arrington, V. Y. Dudkin*
Department of Medicinal Chemistry, Merck Research Laboratories,
P.O. Box 4
Sumneytown Pike
West Point, PA 19486 (USA)
Fax: (+1) 215-652-7310

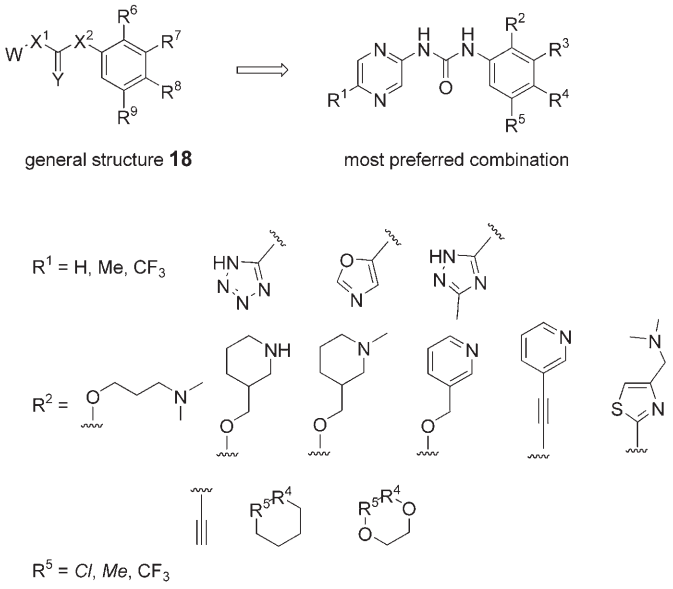
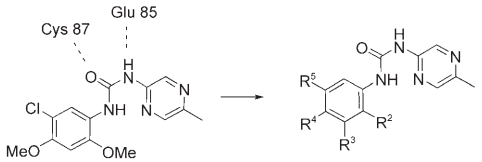


Figure 2. ICOS diaryl ureas (italics indicates preferred substituent).

claimed to have >75-fold selectivity). The synthesis of these molecules is rather facile, with the urea core being assembled by the displacement of the desired phenyl pyrazin-2-ylcarbamate with an appropriately substituted aniline. The aniline is optimally disubstituted at R^2 and R^5, with R^3 and R^4 being H. R^2, which is facing out toward the solvent, can tolerate a wide variety of functionality, including, optimally, ether linked alkyl chains of various lengths. R^5 is preferably halo (chloro) or alkyl (methyl).

Abbott Laboratories scientists have also recently unveiled new modifications to the *N*-aryl-*N*′-pyrazinylurea series. In one publication,[10] they report on extensive SAR research on the phenol ring involving cyclic ethers or branched aminoalkyl moieties (Table 1), with variations accomplished via a Mitsunobu reaction between the phenolic urea and the appropriate alcohol. A variety of substituents are tolerated at R^2, and X-ray crystallography has proven that these groups occupy a more spacious ribose pocket.[19] These efforts resulted in compounds with low nanomolar intrinsic potency and significant selectivity against a panel of serine/threonine kinases. Compounds **1** and **2** displayed IC_{50} values of 8 nM and 7 nM, respectively, and both demonstrated >200-fold selectivity over several closely related kinases, including Chk2, Akt1, and CDK1. In an attempt to improve the physical properties of the series, such as log*P* and solubility, the substitution at R^4 position was also investigated,[20] as X-ray analysis shows that attachments here point toward the solvent exposed area. Chk1 inhibition in vitro was evaluated initially, in a radiometric assay conducted in the presence of 5 μM ATP, then in the cell based MTS and FACS assays. This series had acceptable cellular potency, as exemplified by compounds **4** in the FACS assay (IC_{50} = 140 nM) and **5** in the MTS assay (IC_{50} = 580 nM) (Table 1), however, much of the class showed less than optimal cellular data, generally attributed to low cell permeability. A ratio was calculated by comparing the antiproliferative EC_{50} obtained when dosing HeLa cells with compound alone versus co-administration with the DNA-damaging agent doxorubicin (150 nM). Compound **2** had an MTS EC_{50} = > 59 μM when dosed singly, but with doxorubicin the value was 2.6 ± 1.3 μM, resulting in a ratio of >22. It also had an exemplary selectivity profile, so it was evaluated in antiproliferative studies with camptothecin and doxorubicin.[10] In SW620 cells, a human colon cancer cell line, a 20-fold potentiation of camptothecin was observed, registering an EC_{50} increase from 400 nM as a single agent, to 20 nM in the presence of 10 μM **2**. With doxorubicin, fivefold potentiation was observed in HeLa cells at the same concentration of **2**, with an EC_{50} increase from 490 nM to 90 nM. Compound **2** also showed promise in a soft agar colony growth assay,[21] augmenting (14-fold potentiation) the efficacy of camptothecin in sensitizing SW620 cells. Macrocyclic constrained diaryl ureas have also been researched by this group, and have proven to be more potent than the acyclic versions.[22] The size of the macrocyclic ring had only a modest impact on Chk1 inhibition, and a five-carbon linker was found to be optimal. Substitution at R^4 and R^5, both of which lie in the solvent exposed region, was tolerated, but was not well tolerated at R^3 (Table 2). These molecules were active in the MTS and FACS assays when dosed in combination with either doxorubicin

Table 1. Intrinsic and cellular potency of selected Abbott diaryl ureas.



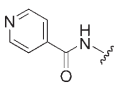
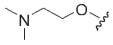
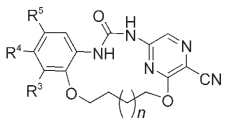
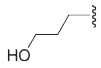
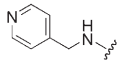
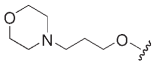
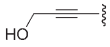
Compd.	R^2	R^4	R^5	Chk IC_{50} [nM]	MTS EC_{50} w/dox [nM]	FACS EC_{50} w/dox [nM]
1		H	Cl	8	400	–
2		H	Cl	7	2600	–
3	MeO		Cl	8	440	1710
4	MeO		Cl	12	760	140
5			Cl	10	220	580
6	MeO		H	10	4660	1450

Table 2. Biological data of selected Abbott macrocyclic diaryl ureas.



Compd.	*n*	R^3	R^4	R^5	IC_{50} [nM]	MTS EC_{50} [nM] + Dox	FACS EC_{50} [nM] + Dox	cdc25A Western EC_{50} [nM]
7	2	H	H	Cl	7	3040	2700	5400
8	1	H	H	Cl	6	430	240	70
9	2	H		Cl	5	860	190	850
10	2	H		Cl	13	1400	1200	230
11	2	H	H		7	–	–	–
12	2		H	Me	163	–	–	–

Agouron[27,28] and Abbott Laboratories[29] disclosed pyrazoles fused into either 3,5-disubstituted indazoles or tri- and tetracyclic cores, respectively (Figure 4). Recently, several companies have continued to focus on pyrazoles, and their derivatives, as potential Chk1 inhibitors. Abbott Laboratories has elaborated on the fused tri and tetra-cyclic pyrazole series,[29] featuring new functionalities which improve Chk1 potency.[30,31] This series was originally derived from an HTS hit (IC_{50} = 510 nM) (Figure 5). A typical synthesis of these inhibitors proceeds via the palladium-catalyzed cross coupling of a substi-

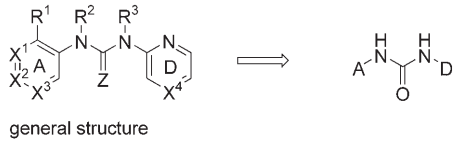
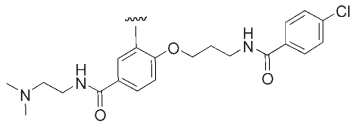
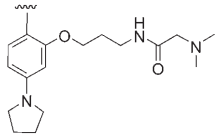
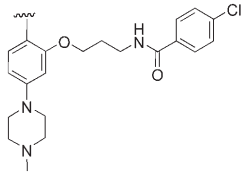
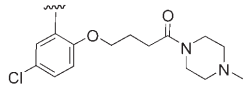
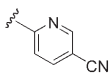
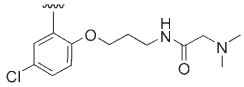
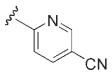


Figure 3. Millennium diaryl ureas.

or camptothecin in H1299 cells (Table 2). They have also shown the ability to protect cdc25A,[23] a key phosphatase protein which controls cell cycle progression through activation of CDK1, from degradation due to proteolysis.

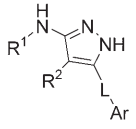
Researchers at Millennium Pharmaceuticals have also investigated the diaryl urea series,[24] producing molecules with more functionality on ring A. Of noted interest was the introduction of polarity on ring A (Figure 3). Another noted difference is the success achieved with 5-cyano pyrid-2-yl as ring D, with several of these analogues displaying >50% inhibition of Chk1 at a concentration of 1 μM in the Chk1 Flashplate® kinase assay. Most of the active compounds presented in this series bear a pyrazinyl functionality. A list of molecules which provide ≥50% inhibition in the aforementioned assay are shown (Table 3), but no cellular or selectivity data are given.

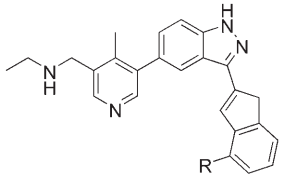
Table 3. Selected Millennium Chk1 inhibitors.

Compd.	Ring A	Ring D
13		
14		
15		
16		
17		

Fused pyrazole-based cores

Fused pyrazole-bearing systems have become well-recognized pharmacophores for numerous Chk1 inhibitors. For example, aminopyrazole cores have been reported by Agouron Pharmaceuticals[25] and Bristol-Myers Squibb Company,[26] whereas





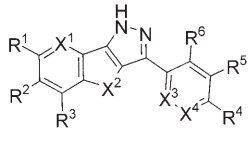


Figure 4. Early fused pyrazoles.

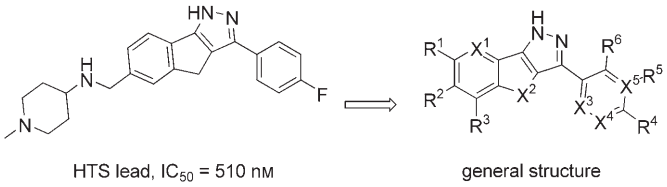


Figure 5. Agouron tricyclic pyrazole series.

tuted 3-iododihydroindenopyrazole with the desired boronic acid (or borane ester). The evolution of the series was aided by X-ray structural analysis, which suggested that H-bonding to the Glu 55 residue in the hydrophobic region of the ATP binding cleft could be advantageous, as evidenced by compound **22** (Table 4). Similar to some of the SAR established by Agouron Pharmaceuticals Inc. in the related aminopyrazole series,[25] it was discovered that optimized compounds contain a biphenyl-4-ol eastern portion and a substituted phenyl ring on the western side (Figure 5).[31] Although subnanomolar potency was achieved in vitro, only compound **22** exemplified desirable cellular potency when used in combination with doxorubicin in both the MTS assay (1 μM) with HeLa cells and the FACS assay (130 nM), with H1299 cells (Table 4).

Indazoles represent another important series of scaffolds containing fused pyrazoles.[32–35] A recent patent application from Merck KGaA[32] presents indazole squaric acid derivatives as inhibitors of Chk1, Chk2, and serum and glucocorticoid induced kinase proteins (SGK), which regulate ionic and water transport (Figure 6). Evaluating the SAR in this series established that R is typically just H, and B and B′ can be independently substituted with either a nitrogen or CH, with CH being particularly preferred. X is also optimally CH_2, although some compounds include a stereospecifically-appended methyl group to this carbon. R^1 is optimal as H. Various functional groups can also be tolerated at R^2, for example, halides, amines, and amides, but OH or alkyl ethers (for example, methoxy) are featured in many of the examples provided. R^3 was of particular interest and most derivatives produced displayed substitution at this position with different amides, optimally aryl or heteroaryl. Most abundant among the examples were the benzoyl and 3-chlorobenzoyl moieties (Figure 6). Compounds **A**, **B**, and **C** are representative of the series and, although no Chk1 inhibitory data was provided, these compounds exhibited low nM activity against SGK.

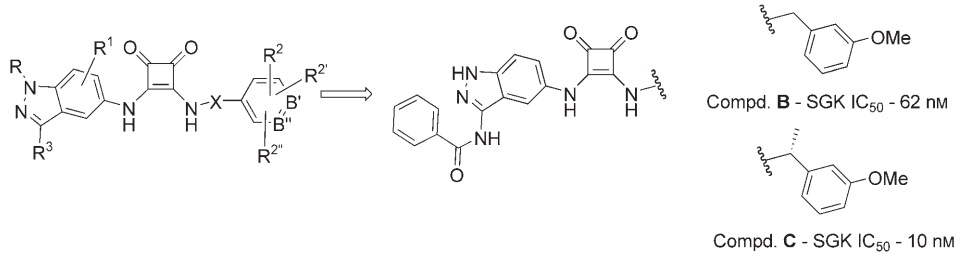
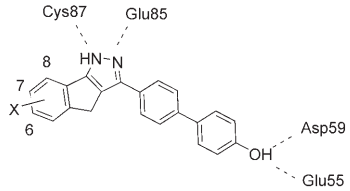
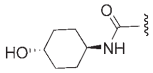
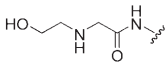
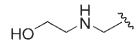
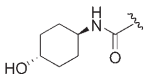


Figure 6. Merck KGaA indazole squaric acid Chk1 inhibitors.

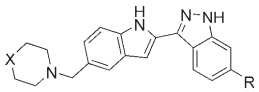
Table 4. In vitro profile of Agouron tricyclic pyrazoles.

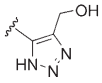


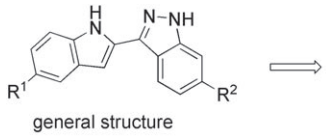
Compd.	6-X	7-X	Chk1 IC_{50} [nM]	MTS EC_{50} [nM] w/dox	FACS EC_{50} [nM] w/dox
18		H	6.2	1800	770
19		H	0.74	3300	2000
20	H		12	2500	3800
21		MeO	0.24	890	610
22	MeO	MeO	4.8	1000	130

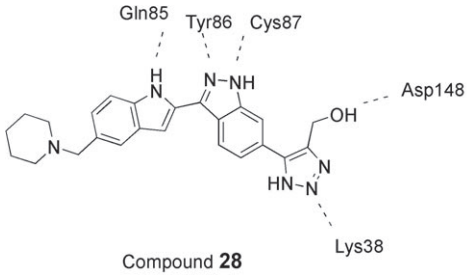
Merck and Co., Inc. has described indolyl indazoles as a novel means of inhibiting Chk1. These compounds are generally prepared by a palladium-catalyzed Suzuki cross-coupling reaction between the desired indole boronic acid and the functionalized 3-(indol-2-yl)indazole core.[33,34] The lead, originally prepared as a VEGFR2 (KDR) kinase inhibitor,[36] was identified from a direct screening campaign and contained a tetrazole at R (Table 5), which X-ray studies demonstrated to be engaged in an ionic interaction with Lys 38 (Figure 7). SAR studies (Table 5) identified compound **28**, with R^1 = benzylpiperidine and R^2 = hydroxymethyl triazole as being very potent, registering an IC_{50} value of 0.30 nM. X-ray studies further indicate that in addition to the known hydrogen bonds to the kinase

Table 5. In vitro data on selected indolyl-indazoles.



Compd.	X	R	Chk1 IC_{50} [nM]	CEA EC_{50} [nM]	Cdk7 IC_{50} [nM]
23	O	CN	30	> 50 000	11
24	O	$CONH_2$	12	980	1300
25	O		10	> 50 000	25
26	CH_2		2.6	350	57
27	CH_2		45	5000	7000
28	CH_2		0.30	690	56





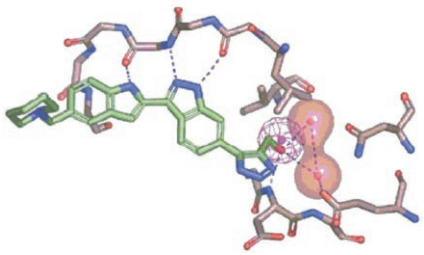
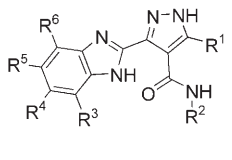


Figure 7. Merck ondolyl-indazoles.

domain backbone, the hydroxy group in the molecule displaces one of the three highly conserved water molecules, which may explain the enhanced potency. Many initial compounds in this series, however, had only modest activity in the functional checkpoint escape assay (CEA), despite showing acceptable cellular permeability, as evidenced by low shifts in the Chk1 autophosphorylation assay. This phenomenon was explained by strong inhibitory activity of these molecules versus the cyclin-dependent kinase Cdk7,[37,38] whose inhibition would enforce cellular arrest and counteract the effect of Chk1 inhibition.

N. Foloppe and colleagues at Vernalis Ltd have investigated the Chk1 ATP site by conducting X-ray crystallographic studies on a benzimidazol-2-yl-indazole core.[35] Similar cores were originally targeted by both Agouron Pharmaceuticals[39] and Aventis Pharmaceuticals[40] as potential



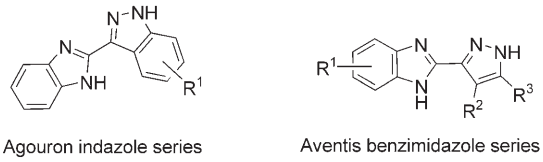


Figure 8. Early benzimidazolyl-indazoles.

protein kinase inhibitors (Figure 8). The synthesis of representative final molecules is completed by condensation of diaminobenzene on the substituted indazole-3-carbaldehyde. The X-ray analysis predicts that a phenyl ring substituted with relatively small hydrophobic groups are optimal, fitting into a buried hydrophobic pocket believed to be unique to Chk1, lined by Glu 55, Asn 59, and Val 68. This hypothesis is in consonance with the SAR already established by researchers from Pfizer/Agouron pharmaceutical on the same series.[39,41] Incorporation of a methoxyphenol on the indazole led to 330-fold increase in potency (IC_{50} = 42 nM), believed to be the result of increased hydrogen bonding interactions between the substituent and Asn 59, Glu 55, and Asp 148 located in the aforementioned conserved, buried pocket through the displacement of all three water molecules (Figure 9). These interactions were identified by computational modeling and confirmed by X-ray crystallography.[35] Based upon these discoveries, Vernalis R&D has recently introduced pyrazolo benzimidazoles[42] (Figure 10) as a

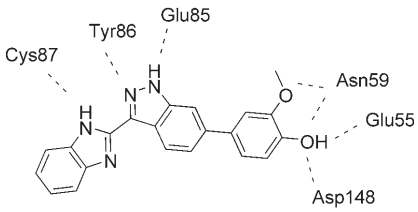


Figure 9. Proposed binding mode of benzimidazolyl-indazole series.

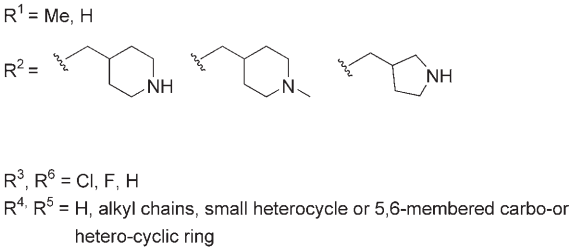
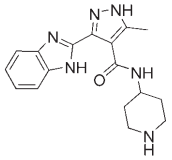
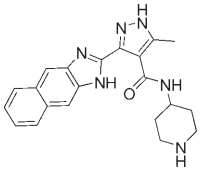
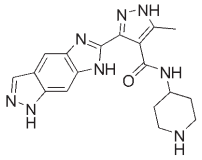
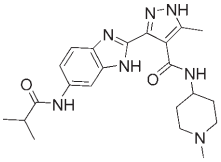
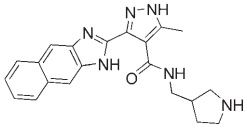


Figure 10. Vernalis pyrazolo benzimidazoles.

novel means of inhibiting Chk1 and 3′-phosphoinositide protein kinase-1 (PDK1), which has been found to promote apoptosis through the deactivation of the prosurvival PI-3 kinase-AKT pathway, and has also been implicated in T-cell function and proliferation, making it a viable target for autoimmune disorders. Extensive SAR has been established for this class, which is synthesized by the oxidative cyclization mentioned previously. R^1 is particularly preferred to be methyl, whereas R^2 provides the handle for basicity, and is currently preferred as piperidin-4-yl. R^3 and R^6 can tolerate small halogens such as fluoro and chloro, but is optimally substituted with just hydrogen. R^4 and R^5 seem to tolerate a wide variety of functionality, and the preferred structures contain these positions constrained into a five- or six-membered carbo- or hetero-cyclic ring (Table 6). All of the examples produced exhibited selectivity for both Chk1 and PDK1 over similar serine/threonine kinases, for example, Akt1 (>70-fold) and CDK-2 (>15-fold). Compounds **29**, **30**, and **31** also showed selectivity against a number of kinases in wide-range kinase screening conducted at Upstate. Some compounds, such as **5**, were proven to be selective for Chk1 over PDK1.

Table 6. Intrinsic potency and selectivity profile of Vernalis pyrazolo-benzimidazoles.

Compd.	Structure	CHK-1 IC_{50} [μM]	PDK-1 IC_{50} [μM]	AKT-1 IC_{50} [μM]	CDK2 IC_{50} [μM]
29		0.098	0.108	17.07	3.30
30		0.010	0.058	>50	4.54
31		0.006	0.010	26.84	5.11
32		0.006	0.074	>50	21.30
33		0.004	0.143	>50	6.24

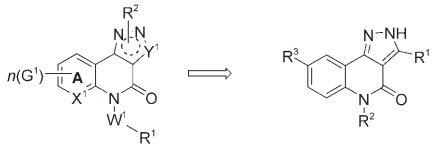
Studies on Chk1 inhibitors belonging to the tricyclic 2,5-dihydro-pyrazolo quinolinone class have been reported by Millennium Pharmaceuticals.[43,44] These molecules are synthesized by converting an amido-benzoate into a 4-hydroxy-3-carboxy-1,2-dihydroquinoline intermediate, followed by cyclization of the pyrazole with the appropriately substituted hydrazine. The pyrazole N and NH need to remain unsubstituted as they provide the essential hydrogen bonding interactions to the Cys87 and Glu85 residues of the kinase backbone. The R^1 position could only tolerate small moieties and was almost exclusively methyl. The R^2 position allowed greater variability, but was optimal as either an ethyl or amino propyl. There are also examples of fluorinated ethyl chains, as well as functionalized aminoalkyl chains presented among the most potent compounds. R^3 was optimal as either hydrogen, a halogen, a small heterocycle, or an unsaturated 3-carbon chain. Most of the preferred compounds bore either a chloro, prop-2-yn-1-amino side chain, or an amino-substituted small heterocycle, preferably 1-(2-thienyl)methanamino moiety. Representative preferred compounds are presented below, with some determined to have IC_{50} values of <500 nM (Table 7).

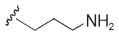
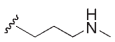
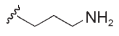
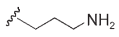
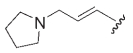
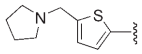
Merck and Co., Inc has reported a related pyrazoloquinolinone series,[45] which includes a fourth, often saturated, fused ring on the western portion of the molecule (Figure 11). Much of the SAR revolved around amending the aminopropyl side chain through functionalization, including conversion to various benzylic amines, sulfonamides, and amides. The side chain itself was also fluorinated and hydroxylated on the central (2) carbon. The Z ring system could be either carbocyclic or heterocyclic. Merck has also released the structurally similar imidazoylquinolinone series,[46] which can be synthesized by an intramolecular Heck coupling of the imidazolyl precursor. In all cases disclosed, R^1 was methyl and R^2 was a propyl amino side chain. The Z ring could be either a phenyl or naphthyl system, with functional groups appended to them. Some representative molecules from both of these series are presented, but no biological data was provided (Figures 11 and 12).

Aminopyrimidine structures

Aminopyrimidines and related analogues have been recognized as a common kinase pharmacophore, and many well-known Chk1 inhibitors contain this motif as an essential part of the core.[47] With the elucidation of the human Chk1 kinase domain structure, new pyrimidine-based inhibitors, such as di-

Table 7. 2,5-dihydro pyrazolo quinolinones by Millennium.



Compd.	R^1	R^2	R^3
34	Me		Cl
35	Me		H
36	Et		Cl
37	Me		
38	Me	Et	
39	Me		

dines.[49,50] Through receptor-based virtual screening,[51–55] electronic libraries containing small molecules were computationally docked into a three-dimensional structure of the ATP-binding site, and molecules of interest were then confirmed by X-ray crystallography. This effort identified ten novel Chk1 inhibitors, but the fused, adenine-type structures demonstrated the most potential,[49] with the unoptimized leads registering IC_{50} values in the nanomolar range (Figure 14, **A** = 31.8 nM and **B** = 15.6 nM). In further investigations,[50] medium throughput screening of a commercial library identified compound **C**, which, although structurally similar, was shown to bind in a slightly different configuration. This core is synthesized through a thermal cyclization of the derivatized 2-furylformamide thereby furnishing functionalized furylpyrimidin-4-amines. Alterations to the aniline side chain caused complete loss of affinity, and X-ray crystallographic studies concluded that the hydroxy-ethyl chain engaged in an intramolecular H-bond with one of the pyrimidine nitrogens, instead of the proposed interaction with Ser147 predicted by modeling. Structure-based design led to the replacement of the furan ring with pyrrole, with the assumption that the N-H would create an additional H-bond to the kinase backbone. This change indeed led to a tenfold increase in potency and also changed the binding conformation, placing the hydroxyl-ethyl side chain in the vicinity of the buried pocket, thereby allowing for derivatization to enhance the protein-ligand affinity. These modifications also caused a similar increase in the inhibition of cyclin dependent kinase 1 (CDK1).[56–58] CDK1 regulates G2 arrest, and its activation is required for DNA-damaged cells to pass through the G2 M checkpoint (Table 8).

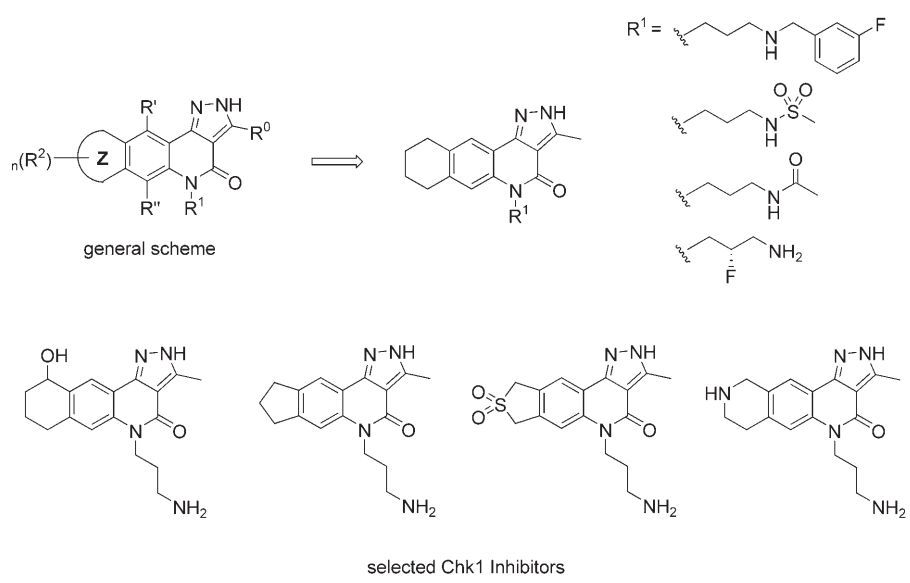


Figure 11. Merck pyrazoloquinolinone series.

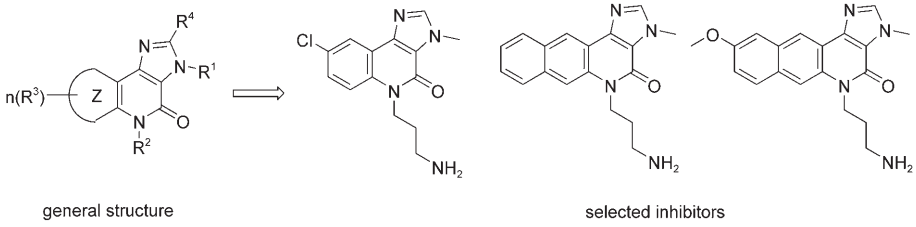


Figure 12. Merck imidazolyl quinolinones.

Recently, BioFocus Discovery Ltd has disclosed further advancement of the novel pyrimidin-4-yl-indazol-5-amines class, formed sequentially by 1) the amination and 2) the palladium-catalyzed Suzuki cross couplings of 4,6-chloropyrimidine.[59] Some of the disclosed compounds demonstrated potent inhibition of Chk1 activity ($IC_{50} = < 1$ μM), and the series also exhibited a unique affinity for Chk1 over other related kinases, especially CDK1, which was of particular importance (see above). Compound **A** (Figure 15) is a prototypical Chk1 inhibitor from this class, with an $IC_{50} < 1$ μM against Chk1and > 50 fold selectivity versus CDK1.

aminopyrimidine **1** and quinazoline **2**, were originally identified by virtual screening conducted by researchers at AstraZeneca,[48] with IC_{50} values of 4 μM and 450 nM, respectively (Figure 13). In light of recent discoveries, researchers at Vernalis Ltd. have also disclosed efforts involving aminopyrimi-

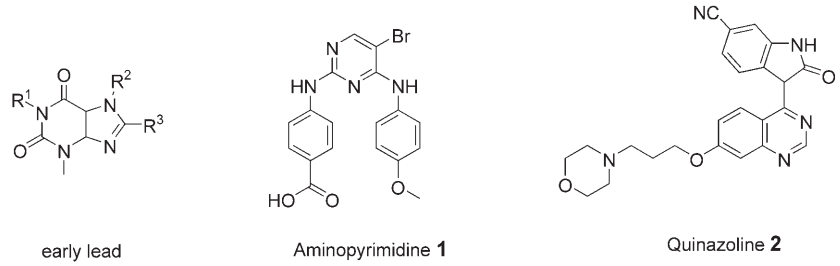


Figure 13. Early pyrimidine-type leads.

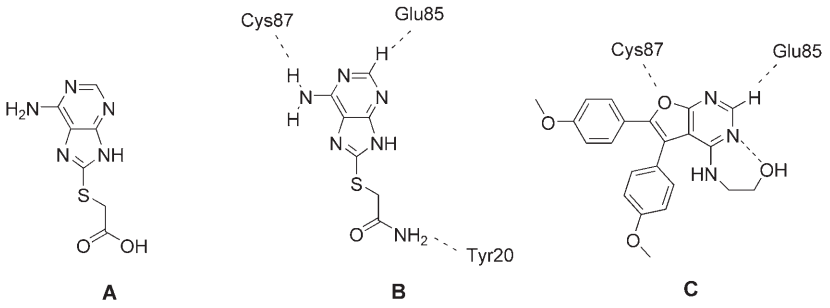
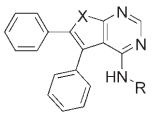


Figure 14. Vernalis aminopyrimidine leads.

Table 8. SAR and biological data.



X	R	Chk1 IC_{50} [nM]	CDK1 IC_{50} [nM]
O	H	>100000	>200000
O		20.9	>200000
O		>100000	>200000
NH		2.3	5.0
NH		1.4	1.5

Diaryl aminopyrimidines have also been reported as having efficacy against Wee1 kinase, another potential anticancer target involved in the regulation of the G2M checkpoint,[60–62] whose inhibition sensitizes tumor cells to DNA damage, similar to Chk1.[8,63,64] A collaboration between the University of Auckland, New Zealand, and Pfizer, Inc. has reported on the development of 2-amino-azaquinazolines[65] (Figure 16) as a class of Wee1 inhibitors which also demonstrates potency against Chk1. Recent research efforts have shifted to a new 4-phenylpyrrolo-[3,4-*c*]carbazole-1,3-(2*H*,6*H*)-dione core,[66] which has proven to effectively inhibit both Wee1 and Chk1 (Table 9). The core of this class can be synthesized by a Diels–Alder reaction with a substituted 2-vinylindole diene intermediate and maleimide, followed by aromatization with DDQ. Selectivity for either kinase could be enhanced by minor manipulations to the core: Chk1 selectivity was improved by the interchange of the phenyl substituent from the 4 to the 5 position of the central phenyl ring (compounds **42**, **43**), whereas Wee1 selectivity was enhanced through the incorporation of a 2-chlorophenyl functionality at the 4 position (compound **45**), or introduction of bulky substituents on the pyrrole ring (compound **50**). Low permeability ($P_{app} < 3 \times 10^{-6}$ cm s^{-1}) and low aqueous solubility (usually less than 3 μg mL^{-1}) have hampered the ability to analyze this class in vivo.

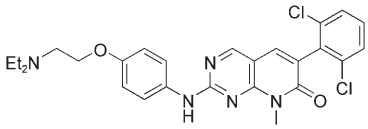


Figure 16. Pfizer Diarylaminopyrimidine Wee1 inhibitor.

Medicinal chemistry efforts at Exelixis, Inc. have spawned the 3-aminopyrazine class of Chk1 inhibitors, one of the earliest, and most advanced, series to date.[67] Of the hundreds of compounds presented in the patent, more than 50 molecules were found to have IC_{50} values of <50 nM. The central pyrazine serves as the anchor for substitution, almost exclusively at positions R^1 and R^2 (Figure 17). R^2 can be a methyl amide, although most preferred embodiments contained small saturated heterocycles, such as pyrrolidine

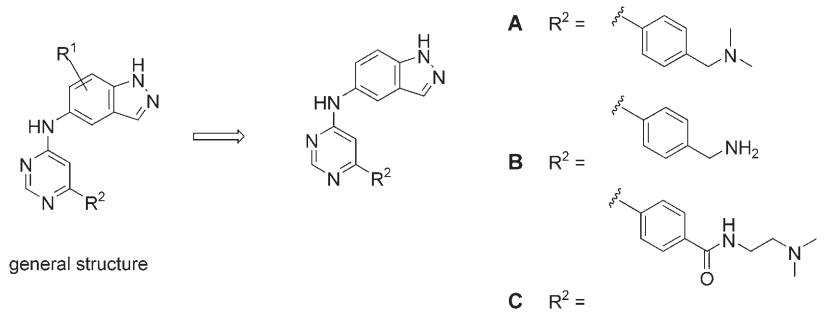
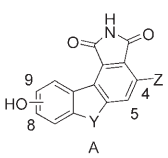
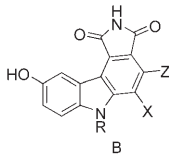


Figure 15. Pyrimidinyl-indazole amines by BioFocus.

Table 9. SAR and intrinsic data of 4-phenylpyrrolo-[3,4-c]carbazole-1,3(2H,6H)-dione dual Chk1/Wee1 inhibitors series.





Compd.	Structure	X	Y	Z	R	Chk1 (IC_{50} [nM])	Wee1 (IC_{50} [nM])
40	A	9-OH	NH	Ph	H	47	97
41	A	8-OH	NH	Ph	H	300	310
42	B	Ph	–	Me	H	32	9700
43	B	Ph	–	H	H	330	4000
44	A	9-OH	NH	2-ClPh	H	440	11
45	A	9-OH	O	2-ClPh	H	>10000	33
46	B	H	–	2-SMePh	H	>50000	33
47	B	H	–	4-CNPh	H	120	1800
48	B	H	–	2-Pyr	H	2300	580
49	B	H	–	2-ClPh	Et	120	50
50	B	H	–	2-ClPh	*n*Bu	27000	59

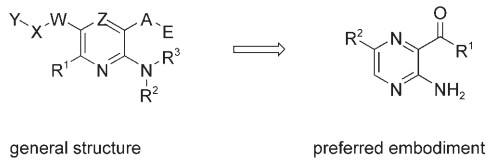


Figure 17. Exelixis aminopyrazines.

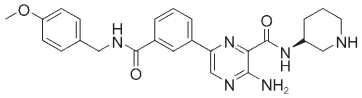
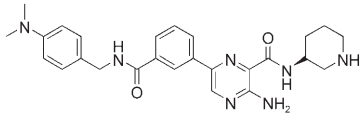
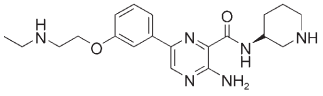
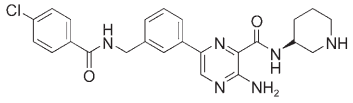
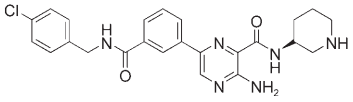
and piperidine, appended from the amide. R^2 was found to also tolerate carbonyl mimetics, and the substituted 1,2,4-triazol-5-yl and 1,2,4-triazol-3-yl systems produced many potent inhibitors. R^3 apparently could withstand the most derivatization, and most often consisted of a substituted phenyl ring directly attached to the pyrazine core by a Suzuki coupling. Functionality, generally, was appended at the 3 position of the phenyl ring, and the most potent constructs contained a substituted benzylic amine, although amide and ether-linked side chains resulted in compounds with low nanomolar potency. The selectivity of some of the best compounds was also evaluated, and many were found to be equipotent against Chk2 and KDR (Table 10). An undisclosed preferred compound (XL844) has been advancing through clinical trials (see http://www.exelixis.com/pipeline.shtml).

Quinolinones

Amides, both free and cyclically constrained, have demonstrated the ability to effectively inhibit Chk1. Many natural products and small molecule inhibitors, such as debromohymenialdisine,[68] and the heavily studied staurosporine (as well as its closely related derivative UCN-01),[8,69,70] which contain amides of varying types, have been identified (Figure 18). In addition to ureas, carbamates,[71] diazepinones,[72] and dihydro-diazepinoindoles[73,74] have also been described, the latter series being studied extensively by researchers at Pfizer (Figure 19). One prototypical diazepinone inhibitor (PF-00394691), which evolved from a similar HTS, has displayed dose-dependent potentiation of antitumor activity in combination with gemcitabine in a Colo205 mouse xenograph model, and has also shown efficacy when used in combination with other known DNA damaging agents, including camptothecin, and doxorubicin.[75,76] The clinical candidate which evolved from this series, PF477736, has been moved into phase I clinical development. It has shown efficacy in its ability to potentiate cells treated with docetaxel in vitro and in vivo, and significantly enhanced antitumor efficacy in Colo205 xenograft model.[77] It has also proven effective in conjunction with radiation.[78]

As of late, quinolinones have garnered much attention among pharmaceutical companies. Chiron corporation has fur-

Table 10. Biological data on selected amino pyrazines.

Compd.	Structure	Chk1 [nM]	Chk2 [nM]	KDR [nM]	cdc2 [nM]
51		<50	<50	50<X<1000	>10000
52		<50	<50	50<X<1000	>10000
53		<50	<50	50<X<1000	
54		<50	<50	<50	>10000
55		<50	<50	<50	1000<X<10000

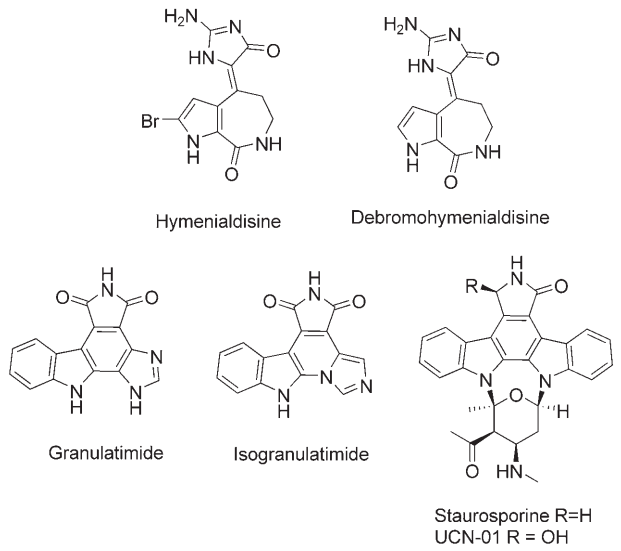


Figure 18. Various amide bearing early Chk1 inhibitors.

ther developed the benzimidazolyl-aminoquinolinone series,[79,80] with the core being assembled by condensation of ethyl-2-benzimidazole-acetate with PMB protected isatonic anhydride.[81] SAR was investigated for most positions of the molecule and R^2 on the western phenyl ring was most often a halogen or methyl, with chloro apparently being optimal. Earlier work on this template incorporated a fluorine at the R^1 position, but recent efforts have revealed that stereospecifically appending an amino alkyl group at R^9 can provide a dual benefit; enhancement of both potency and selectivity. It is believed that this is derived from interactions between the amine on the chain and the Glu 91 and Glu 134 residues of the earlier described acidic patch[50] (Figure 20). The positions substituted on the benzimidazole ring were R^6 and R^7, but R^6 was apparently more tolerant to derivatization (Figure 20). Compound **60** of this series (Table 11) has demonstrated synergy with camptothecin, significantly accelerating cell death when co-administered to MDA-MB-435 cells.[82] In a recent paper,[82] compound **60** has been reported to protect and restore the level of cdc25, which, as mentioned previously, is normally targeted by Chk1 for degradation following DNA damage. It also abrogated the G2M checkpoint and increased tumor apoptosis in an orthotopic breast cancer xenograft model conducted in combination with irinotecan.

Table 11. In vitro potency of benzimidazolyl quinolinones.

Compd.	R^1	R^2	IC_{50} [nM]
56	H		7.6
57	H		482
58	6-Me		1.4
59	6-Cl		0.5
60	6-Cl		0.32

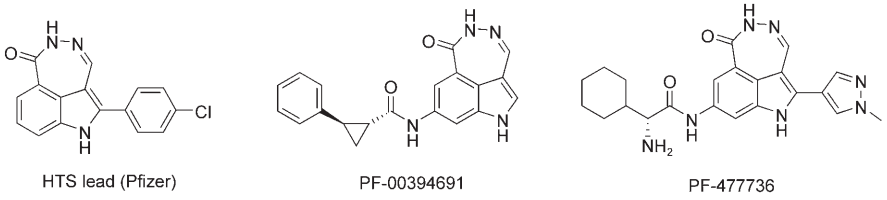


Figure 19. Evolution of the Pfizer dihydro-diazepinoindoles series.

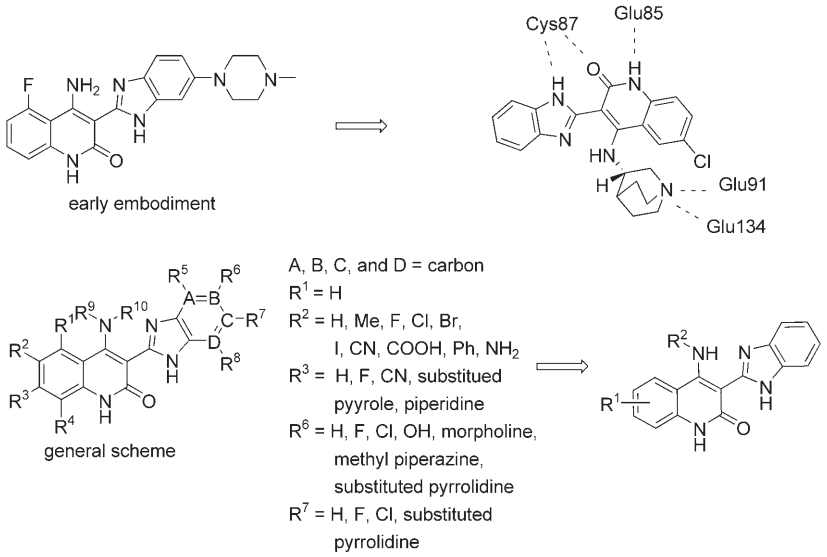


Figure 20. Benimidazolyl quinolinone series by Chiron.

Merck and Co., Inc, have described 6-substituted indolylquinolinones as a novel class of potent Chk1 inhibitors.[83,84] Similar to the indolyl indazoles, this series also originally developed for the KDR kinase inhibitor program,[36] and was also identified during HTS screening. Palladium-catalyzed Suzuki cross-coupling of the derivatized 3-iodoquinolin-2(1*H*)-one and the appropriately substituted BOC-protected-

indolyl boronic acid afforded the core (Figure 21). The SAR on the quinolinone can be analyzed by Suzuki couplings with the bromide or hydrolysis of a cyano group to the corresponding amides (Table 12). The SAR around the indole portion was studied by converting the indole aldehyde into a variety of benzyl amines. The quinolinone ring was optimally substituted at the 6 position, and small polar substituents, like pyrazole, were found to enhance the intrinsic potency. X-ray crystallography and docking models indicate that interactions between the pyrazole and water molecules in the hydrophobic pocket, as well as Glu 55, increase the affinity of these inhibitors (Figure 21). The high intrinsic potency of this class did not fully translate into functional cellular activity for many members. For these compounds, polar surface area (PSA) has been identified as the most uniform predictor of cellular permeability potential.[85] Compounds **63** and **65**, both bearing pyrazole and piperidine moieties, were found to have an optimal combination of biochemical potency and polarity, resulting in CEA EC_{50} values <100 nM. These molecules had moderate clearance in dog PK experiments.

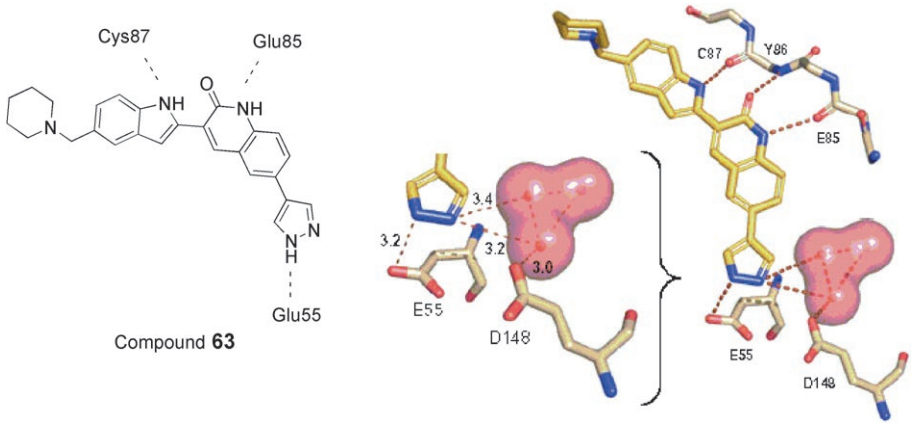
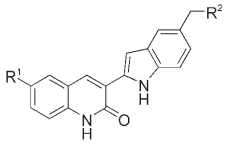
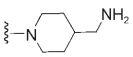
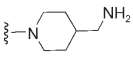
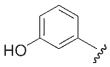
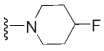
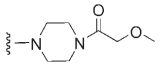


Figure 21. Merck indolyl quinolinone series.

Table 12. In vitro profile of selected indolyl quinolinones.



Compd.	R^1	R^2	Chk1 IC_{50} [nM]	CEA EC_{50} [nM]	PSA	dog PK Cl (mL min^{-1} kg^{-1}) (h)	$t_{1/2}$
61	CN		4	3700	–	–	–
62			0.70	3900	–	–	–
63			0.65	97	89	30	4.4
64			29	6300	79	–	–
65			1	120	89	22	2.5
66			2.1	1142	123	23	2.0

Triazolinones

AstraZeneca AB, and others, have reported on fused triazolones as potential Chk1 inhibitors.[86] This system can be readily formed by the intramolecular cyclization of a functionalized acetoacetanilide with concentrated sulfuric acid, followed by the condensation of the resulting 2-chloroquinolinone with ethyl carbazate. The R^1 position was often a small alkyl group (methyl), a heterocycle, or a handle for basic amines-either off of a methylene, carbonyl, or phenyl ring. Positions R^4 and R^5 were both mono- and disubstituted, but R^4 seems to be the preferred site for substitution, bearing either a small aryl or heteroaryl group, often affixed with a basic amine (Figure 22).

Benzoisoquinolines have been presented by Merck and Co. in a recent Chk1 inhibitor patent.[87] This class also features a wide array of molecules based on a fused, tricyclic isoquinoline core which can be synthesized by numerous schemes, such as an intramolecular photochemical cyclization with *E* or *Z* 2-phenyl-3-pyridin-4-ylacrylonitrile. R^1 was mostly H, with a few examples of aryl and heteroaryl substitution. R^2 was also typically H, although a few examples contained amine-bearing chains appended here. R^3 was heavily investigated, with numerous examples bearing esters, amides, benzylic alcohols, and benzylic amines. The most preferred substituent was either an ethyl or propyl amino side chain, possibly mimicking the basic side chain shared by most active Chk1 inhibitors, which reaches out toward the solvent. Both R^4 and R^5 could be functionalized, but derivatization at R^5 was more prominently featured among the examples provided, with halogens, small heterocycles, such as pyrazole, or phenyl rings substituted with benzylic amines, being used most often (Figure 23).

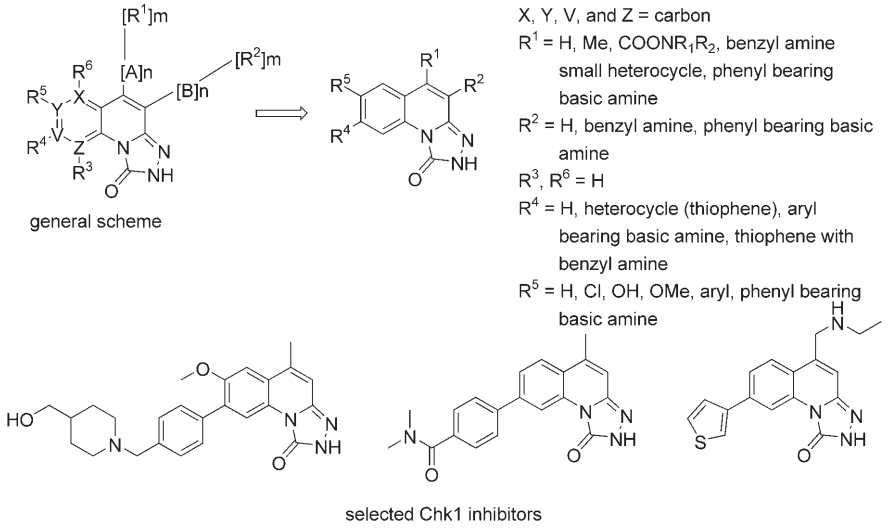


Figure 22. AstraZeneca fused triazoles.

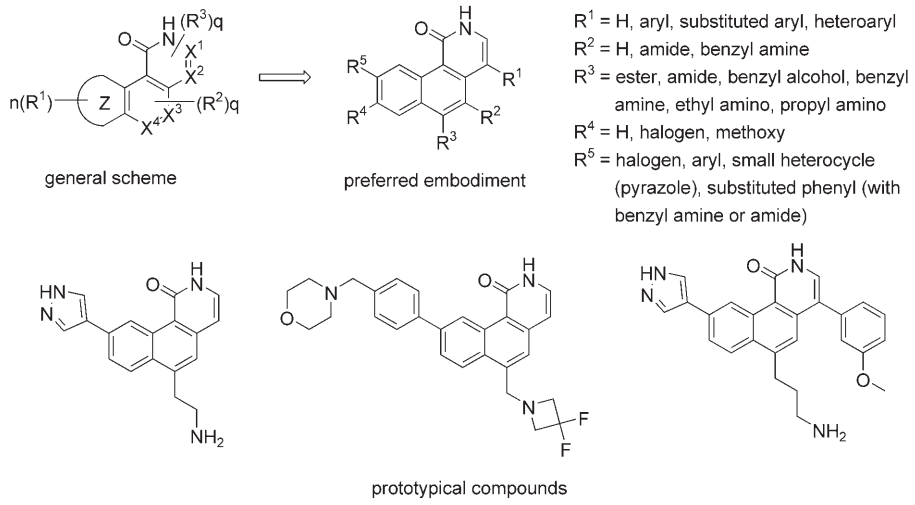


Figure 23. Benzoisoquinolines by Merck.

Thiophenes

AstraZeneca AB has recently released a patent describing a wide range of molecules featuring 5-memberred heterocycles as the core of a new class of Chk1 inhibitors, although all of the featured molecules specifically contain thiophene (Figure 24).[88] The R^1 position is typically a substituted phenyl ring, X is generally sulfur, and Y is usually CH. The location of the urea and carbonyl functionalities apparently can be interchanged between R^2 and R^3 without much effect in potency. The compounds presented typically inhibited Chk1 with IC_{50} or EC_{50} values $<100\ \mu M$ in vitro and in the cellular abrogation assay with HT29 cells, respectively, with two prototypical inhibitors shown below (Figure 24). Research in this series has culminated in the discovery of AZD7762,[89] a preclinical candidate currently in Phase I development. It is reported to be a potent and selective dual Chk1/2 inhibitor (5 nM and <10 nM, respectively) with an in vivo $EC_{50} = 10$ nM in HT29 cells. It has also shown the ability to potentiate the efficacy of gemcitabine and irinotecan.

AstraZeneca AB also recently introduced another class of Chk1 inhibitors featuring fused substituted heterocycles.[90] The class also shows inhibitory activity against PDK1, a family of serine/threonine kinases involved in the signaling cascade which influences cell growth,[91] and the Pak family of kinases, which are believed to play a role in cell survival, proliferation, transformation, and cell mobility.[92,93] Therefore, either one of these targets may also prove to be a viable chemotherapeutic area of interest. The preferred cores were either a 2-substituted-4-aminothienylpyridine-7-carboxamide or 2-substituted-4-amino-1*H*-indole-7-carboxamide. R^1 was optimal as either a substituted amine, such as a substituted piperazine, a sulfonamide, or a phenyl group. R^2 is tolerant to substitution with a variety of functionalities, although substituted piperidines were the most commonly used moieties. R^3 is optimally just hydrogen. Typical compounds in this series have achieved IC_{50} and/or EC_{50} values of $<100\ \mu M$ in either the intrinsic Chk1 assay, the in vitro Pak1

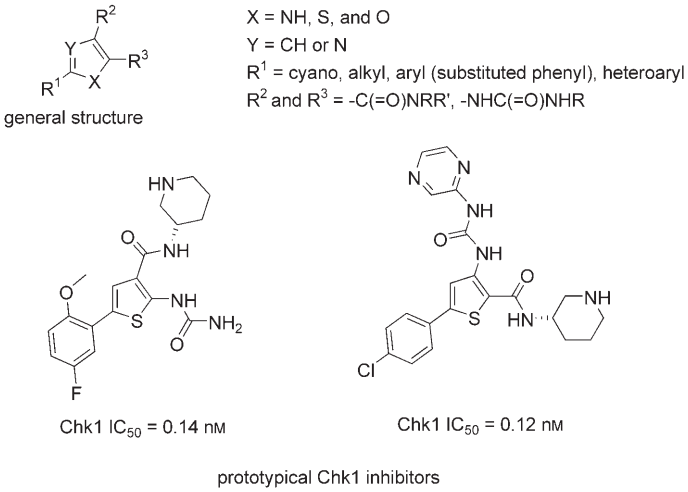


Figure 24. AstraZeneca thiophene Chk1 inhibitor class.

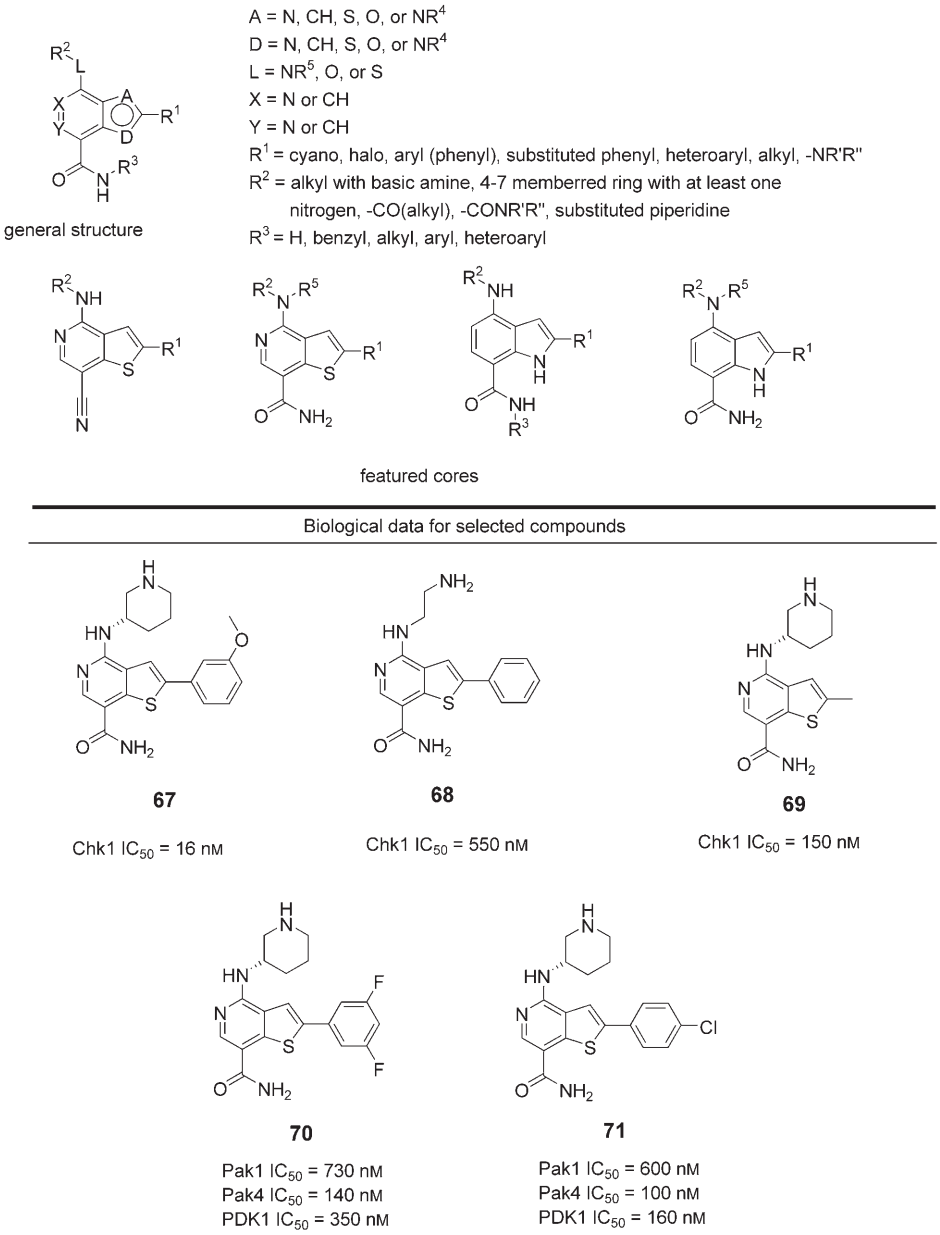


Figure 25. AstraZeneca fused heterocycles as Chk1 inhibitors.

enzyme assay, or both. A few representative compounds are shown below (Figure 25).

In conclusion, there has been a great increase in the number of medicinal chemistry publications and patents devoted to Chk1 inhibitors, reflecting the significant interest in the target and potential that Chk1 inhibitors have for advancing the chemotherapy arsenal. Although clinical target validation has not yet been reported, several companies are advancing small-molecule Chk1 inhibitors toward the clinical development stage, including Exelixis, whose inhibitor XL844[94] has progressed into Phase I trials in chronic lymphocytic leukemia patients, and Pfizer, which is testing PF-00477736[95] in solid tumors. As more data on the effects of Chk1 inhibition is becoming available and more inhibitors are introduced, we look forward to the emergence of a new class of effective therapies for cancer treatment.

Keywords: cancer · chemotherapeutics · Chk1 · DNA damage

[1] D. Fisher, M. Méchali, *Nat. Cell Biol.* **2004**, *6*, 576–577.
[2] D. Shechter, V. Constanzo, J. Gautier, *Nat. Cell Biol.* **2004**, *6*, 648–655.
[3] R. G. Syljuåsen, C. S. Sørensen, L. T. Hansen, K. Fugger, C. Lundin, F. Johansson, T. Helleday, M. Sehested, J. Lukas, J. Bartek, *Mol. Cell. Biol.* **2005**, *25*, 3553–3562.
[4] M. B. Kastan, J. Bartek, *Nature* **2004**, *432*, 316–323.
[5] B.-B. S. Zhou, J. Bartek, *Nat. Rev. Cancer* **2004**, *4*, 216–225.
[6] A. N. Tse, G. K. Schwartz, *Cancer Res.* **2004**, *64*, 6635–6644.
[7] X. Huang, T. Tran, L. Zhang, R. Hatcher, P. Zhang, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 1065–1070.
[8] J. R. Jackson, A. Gilmartin, C. Imburgia, J. D. Winkler, L. A. Marshall, A. Roshak, *Cancer Res.* **2000**, *60*, 566–572.
[9] M. E. Fraley, R. M. Garbaccio, K. L. Arrington, W. F. Hoffman, E. S. Tasber, P. J. Coleman, C. A. Buser, E. S. Walsh, K. Hamilton, C. Fernandes, M. D. Schaber, R. B. Lobell, W. Tao, V. J. South, Y. Yan, L. C. Kuo, T. Prueksaritanont, C. Shu, M. Torrent, D. C. Heimbrook, N. E. Kohl, H. E. Huber, G. D. Hartman, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1775–1779.
[10] G. T. Wang, G. Li, R. A. Mantei, Z. Chen, P. Kovar, W. Gu, Z. Xiao, H. Zhang, H. L. Sham, T. Sowin, S. H. Rosenberg, N.-H. Lin, *J. Med. Chem.* **2005**, *48*, 3118–3121.
[11] K. L. Arrington, M. E. Fraley, R. M. Garbaccio, S. Y. Huang, C. W. Lindsley, J. T. Steen, F. Yang, WO2006086255A2, **2006**.
[12] For other Chk1 reviews see: a) M. Prudhomme, *Recent Pat. Anti-Cancer Drug Discovery* **2006**, *1*, 55–68; b) Z.-F. Tao, N.-H. Lin, *Anti-Cancer Agents Med. Chem.* **2006**, *6*, 377–388; c) M. Prudhomme, *Curr. Med. Chem.: Anti-Cancer Agents* **2004**, *4*, 435–438.
[13] K. S. Keegan, E. A. Kesicki, J. J. Gaudino, A. W. Cook, S. D. Cowen, L. E. Burgess, US20030069284A1, **2003**.
[14] R. G. Boyle, H. J. Imogal, M. Cherry, US20040014765A1, **2004**.
[15] G. Li, Q. Li, T. Li, N.-H. Lin, US20040259885A1, **2004**.
[16] L. E. Burgess, A. W. Cook, K. L. Fischer, J. J. Gaudino, S. T. Schlachter, WO2006012308A1, **2006**.
[17] A. W. Cook, J. J. Gaudino, WO2006014359A2, **2006**.
[18] F. S. Farouz, R. Holcomb, E. Thorsett, J. J. Gaudino, WO2006021002A2, **2006**.
[19] G. T. Wang, G. Li, R. A. Mantei, N.-H. Lin, C. Park, Z. Chen, P. Kovar, W. Gu, Z. Xiao, H. Zhang, H. L. Sham, T. Sowin, S. H. Rosenberg, *J. Med. Chem.* in press.

[20] G. Li, L. A. Hasvold, Z.-F. Tao, G. T. Wang, S. L. Gwaltney II, J. Patel, P. Kovar, R. B. Credo, Z. Chen, H. Zhang, C. Park, H. L. Sham, T. Sowin, S. H. Rosenberg, N.-H. Lin, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2293 – 2298.
[21] "A-641397, a highly selective Chk1 inhibitor augments DNA damaging agents against human cancer cells": Z. Chen, Z. Xiao, W. Gu, M. H. Bui, P. J. Merta, G. T. Wang, G. Li, N.-H. Lin, P. Kovar, H. L. Sham, S. H. Rosenberg, T. J. Sowin, H. Zhang, Manuscript in preparation.
[22] Z.-F. Tao, L. Wang, Y. Tong, G. Li, M. Przytulinska, G. Sullivan, Z. Chen, Z. Xiao, W. Gu, J. Xue, M.-H. Bui, P. Merta, P. Kovar, H. Zhang, C. Park, K. D. Stewart, H. L. Sham, S. H. Rosenberg, T. Sowin, N.-H. Lin, *Presented at 232nd ACS National Meeting* (San Francisco, CA, United States) Sept. 10 – 14, **2006**, MEDI-128.
[23] K. Kristjánsdóttir, J. Rudolph, *Chem. Biol.* **2004**, *11*, 1043 – 1051.
[24] R. G. Boyle, H. J. Imogal, M. Cherry, N. M. Khan, WO2005072733A1, **2005**.
[25] M. D. Johnson, M. Teng, J. Zhu, WO05009435A1, **2005**.
[26] A. J. Dyckman, J. Das, K. Leftheris, C. Liu, R. V. Moquin, S. T. Wrobleski, US20040248853, **2004**.
[27] S. E. Kephart, I. J. McAlpine, S. H. Reich, US20050026960A1, **2005**.
[28] R. S. Kania, S. L. Bender, A. J. Borchardt, S. J. Cripps, C. L. Palmer, A. M. Tempczyk-Russell, M. D. Varney, M. R. Collins, US20050124662A1, **2005**.
[29] Y. Tong, A. K. Claiborne, G. Li, N.-H. Lin, H. L. Sham, T. J. Sowin, Z.-F. Tao, US20040259904A1, **2004**.
[30] Y. Tong, A. K. Claiborne, G. Li, N.-H. Lin, H. L. Sham, T. J. Sowin, Z.-F. Tao, WO2004080973A1, **2004**.
[31] Y. Tong, A. K. Claiborne, Z.-F. Tao, P. Kovar, C. Park, Z. Chen, R. B. Credo, K. D. Stewart, H. Zhang, S. H. Rosenberg, H. L. Sham, T. J. Sowin, N.-H. Lin, *Presented at 232nd ACS National Meeting* (San Francisco, CA, United States) Sept. 10 – 14, **2006**, MEDI-126.
[32] W. Mederski, R. Gericke, D. Dorsch, M. Klein, N. Beier, F. Stieber, F. Lang, WO2006105865A1, **2006**.
[33] M. E. Fraley, J. T. Steen, E. J. Brnardic, K. L. Arrington, K. L. Spencer, B. A. Hanney, Y. Kim, G. D. Hartman, S. M. Stirdivant, B. A. Drakas, K. Rickert, E. S. Walsh, K. Hamilton, C. A. Buser, J. Hardwick, W. Tao, S. C. Beck, X. Mao, R. B. Lobell, L. Sepp-Lorenzino, Y. Yan, M. Ikuta, S. K. Munshi, L. C. Kuo, C. Kreatsoulas, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 6049 – 6053.
[34] J. T. Steen, M. E. Fraley, E. J. Brnardic, K. L. Arrington, G. D. Hartman, Y. Yan, S. Munshi, M. Ikuta, L. C. Kuo, C. Kreatsoulas, M. Reilly, E. Mahan, S. Stirdivant, B. Drakas, E. S. Walsh, K. Hamilton, C. Buser, K. Rickert, W. Tao, J. Hardwick, X. Mao, S. Beck, R. B. Lobell, L. Sepp-Lorenzino, *Presented at 232nd ACS National Meeting* (San Francisco, CA, United States) Sept. 10 – 14, **2006**, MEDI-127.
[35] N. Foloppe, L. M. Fisher, G. Francis, R. Howes, P. Kierstan, A. Potter, *Bioorg. Med. Chem.* **2006**, *14*, 1792 – 1804.
[36] M. E. Fraley, K. L. Arrington, C. A. Buser, P. A. Ciecko, K. E. Coll, C. Fernandes, G. D. Hartman, W. F. Hoffman, J. J. Lynch, R. C. McFall, K, Rickert, R. Singh, S. Smith, K. A. Thomas, B. K. Wong, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 351 – 355.
[37] R. P. Fisher, D. O. Morgan, *Cell* **1994**, *78*, 713 – 724.
[38] J. W. Harper, S. J. Elledge, *Genes Dev.* **1998**, *12*, 285 – 289.
[39] R. S. Kania, S. L. Bender, A. J. Borchardt, J. F. Braganza, S. J. Cripps, Y. Hua, M. D. Johnson, T. O. Johnson, Jr., H. T. Luu, C. L. Plamer, S. H. Reich, A. M. Tempczyk-Russell, M. Teng, C. Thomas, M. D. Varney, M. B. Wallace, WO200102369A2, **2001**.
[40] M. L. Edwards, P. J. Cox, S. Amendola, S. D. Deprets, T. A. Gillespy, C. D. Edlin, A. D. Morley, C. J. Gardner, B. Pedgrift, H. Bouchard, D. Babin, L. Gauzy, A. Le Brun, T. N. Majid, J. C. Reader, L. J. Payne, N. M. Khan, M. Cherry, WO2003035065A1, **2003**.
[41] Q. Li, G.-D. Zhu, *Curr. Top. Med. Chem.* **2002**, *2*, 939 – 971.
[42] D. L. Walmsley, M. J. Drysdale, C. J. Northfield, C. Fromont, WO2006134318A1, **2006**.
[43] R. G. Boyle, H. J. Imogal, M. Cherry, A. J. Humphries, E. F. Navarro, D. R. Owen, N. A. Dales, M. J. LaMarche, C. Cullis, A. E. Gould, P. D. Greenspan, WO2005118583A1, **2005**.
[44] R. G. Boyle, H. J. Imogal, M. Cherry, A. J. Humphries, E. F. Navarro, D. R. Owen, N. A. Dales, M. J. LaMarche, C. Cullis, A. E. Gould, WO2005028474, **2005**.
[45] E. J. Brnardic, M. E. Fraley, R. M. Garbaccio, J. S. Steen, WO2006074281A2, **2006**.
[46] M. E. Fraley, J. S. Steen, WO2007015837A2, **2007**.
[47] J. Bryant, M. J. Kochanny, S. Yuan, S.-K. Khim, B. O. Buckman, D. O. Arnaiz, U. Bomer, H. Briem, P. Esperling, C. Huwe, J. Kuhnke, M. Schafer, L. Wortmann, D. Kosemund, E. Eckle, R. J. Feldman, G. B. Phillips, US20040186118A1, **2004**.
[48] P. D. Lyne, P. W. Kenny, D. A. Cosgrove, C. Deng, S. Zabludoff, J. J. Wendoloski, S. Ashwell, *J. Med. Chem.* **2004**, *47*, 1962 – 1968.
[49] N. Foloppe, L. M. Fisher, R. Howes, A. Potter, A. G. S. Robertson, A. E. Surgenor, *Bioorg. Med. Chem.* **2006**, *14*, 4792 – 4802.
[50] N. Foloppe, L. M. Fisher, R. Howes, P. Kierstan, A. Potter, A. G. S. Robertson, A. E. Surgenor, *J. Med. Chem.* **2005**, *48*, 4332 – 4345.
[51] G. Schneider, H. J. Böhm, *Drug Discov. Today* **2002**, *7*, 64 – 70.
[52] R. Abagyan, M. Totrov, *Curr. Opin. Chem. Biol.* **2001**, *5*, 375 – 382.
[53] X. Barril, R. E. Hubbard, S. D. Morley, *Mini-Rev. Med. Chem.* **2004**, *4*, 779 – 791.
[54] D. B. Kitchen, H. Decornez, J. R. Furr, J. Bajorath, *Nat. Rev. Drug Discovery* **2004**, *3*, 935 – 949.
[55] H. J. Böhm, M. Stahl, *Rev. Comput. Chem.* **2002**, *18*, 41 – 87.
[56] P. Jin, Y. Gu, D. O. Morgan, *J. Cell Biol.* **1996**, *134*, 963 – 970.
[57] R. Y. C. Poon, W. Jiang, H. Toyoshima, T. Hunter, *J. Biol. Chem.* **1996**, *271*, 13283 – 13291.
[58] N. Rhind, B. Furnari, P. Russell, *Genes Dev.* **1997**, *11*, 504 – 511.
[59] V. Birault, C. A. Woodland, WO2005103036A1, **2005**.
[60] L. L. Parker, P. Worms, *Science* **1992**, *257*, 1955 – 1957.
[61] C. H. McGowan, P. Russell, *EMBO J.* **1993**, *12*, 75 – 85.
[62] C. H. McGowan, P. Russell, *EMBO J.* **1995**, *14*, 2166 – 2175.
[63] R. G. Syljuåsen, C. S. Sørensen, J. Nylandsted, C. Lukas, J. Lukas, J. Bartek, *J. Cancer Res.* **2004**, *64*, 9035 – 9040.
[64] H. Zhao, J. L. Watkins, H. Piwnica-Worms, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 14795 – 14800.
[65] B. D. Palmer, J. B. Smaill, G. W. Rewcastle, E. M. Dobrusin, A. Kraker, C. W. Moore, R. W. Steinkampf, W. A. Denny, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1931 – 1935.
[66] B. D. Palmer, A. M. Thompson, R. J. Booth, E. M. Dobrusin, A. J. Kraker, H. H. Lee, E. A. Lunney, L. H. Mitchell, D. F. Ortwine, J. B. Smaill, L. M. Swan, W. A. Denny, *J. Med. Chem.* **2006**, *49*, 4896 – 4911.
[67] C. A. Buhr, T.-G. Baik, S. Ma, Z. Tesfai, L. Wang, E. W. Co, S. Epshteyn, A. R. Kennedy, B. Chen, L. Dubenko, N. K. Anand, T. H. Tsang, J. M. Nuss, C. J. Peto, K. D. Rice, M. A. Ibrahim, K. L. Schnepp, X. Shi, J. W. Leahy, J. Chen, L. E. Dalrymple, T. P. Forsyth, T. P. Huynh, G. Mann, L. W. Mann, C. S. Takeuchi, WO2003093297A2, **2003**.
[68] D. Curman, B. Cinel, D. E. Williams, N. Rundle, W. D. Block, A. A. Goodarzi, J. R. Hutchins, P. R. Clarke, B.-B. Zhou, S. P. Lees-Miller, R. J. Anderson, M. Roberge, *J. Biol. Chem.* **2001**, *276*, 17914 – 17919.
[69] B. Zhao, M. J. Bower, P. J. McDevitt, H. Zhao, S. T. Davis, K. O. Johanson, S. M. Green, N. O. Concha, B.-B. S. Zhou, *J. Biol. Chem.* **2002**, *277*, 46609 – 46615.
[70] P. R. Graves, L. Yu, J. K. Schwarz, J. Gales, E. A. Sausville, P. M. O'Connor, H. Piwnica-Worms, *J. Biol. Chem.* **2000**, *275*, 5600 – 5605.
[71] E. Y. Rui, T. O. Johnson, Jr., J. H. Kellum, US20050148643A1, **2005**.
[72] L. A. Hasvold, L. Hexamer, G. Li, N.-H. Lin, H. L. Sham, G. M. Sullivan, L. Wang, P. Xia, US20040254159A1, **2004**.
[73] S. Benedict, M. Bennett, S. Ninkovic, M. Teng, Y. Rui, F. Wang, US20050075499A1, **2005**.
[74] S. Ninkovic, M. J. Bennett, Y. Rui, F. Wang, S. P. Benedict, M. Teng, WO2004063198A1, **2004**.
[75] A. Blasina, E. Chen, J. Kornmann, K. Anderes, Presented at Protein Kinases: Signaling Success—First RSC-SCI Symposium on Kinase Inhibitor Design (Part 1), (London, UK) May 9 – 10, **2005**.
[76] K. Anderes, A. Blasina, R. Castillo, E. Chen, L. Deaton, M. Gregory, J. Herrera, J. Kornmann, E. Kraynov, M. Stempniak, Presented at Protein Kinases: Signaling Success—First RSC-SCI Symposium on Kinase Inhibitor Design (Part 1) (London, UK) May 9 – 10, **2005**.
[77] Pfizer Inc., *Poster presented at American Association for Cancer Research (AACR) Annual Meeting* (Los Angeles, CA, United States) April 14 – 18, **2007**, Poster # 4373.
[78] Pfizer Inc., *Poster presented at American Association for Cancer Research (AACR) Annual Meeting* (Los Angeles, CA, United States) April 14 – 18, **2007**, Poster # 5386.
[79] T. G. Gesner, P. A. Barsanti, S. D. Harrison, Z.-J. Ni, N. M. Brammeier, Y. Zhou, V. P. Le, US20050256157A1, **2005**.

[80] Z.-J. Ni, P. Barsanti, N. Brammeier, A. Diebes, D. J. Poon, S. Ng, S. Pecchi, K. Pfister, P. A. Renhowe, S. Ramurthy, A. S. Wagman, D. E. Bussiere, V. Le, Y. Zhou, J. M. Jansen, S. Ma, T. G. Gesner, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3121 – 3124.
[81] J. C. Sircar, T. Capiris, S. J. Kesten, D. J. Herzig, *J. Med. Chem.* **1981**, *24*, 735 – 742.
[82] A. N. Tse, K. G. Rendahl, T. Sheikh, H. Cheema, K. Aardalen, M. Embry, S. Ma, E. J. Moler, Z. J. Ni, D. E. Lopes de Menezes, B. Hibner, T. G. Gesner, G. K. Schwartz, *Clin. Cancer Res.* **2007**, *13*, 591 – 602.
[83] S. Huang, R. M. Garbaccio, M. E. Fraley, J. T. Steen, C. Kreatsoulas, G. D. Hartman, S. Stirdivant, B. Drakas, K. Rickert, E. S. Walsh, K. Hamilton, C. A. Buser, J. Hardwick, X. Mao, M. Abrams, S. Beck, W. Tao, R. Lobell, L. Sepp-Lorenzino, Y. Yan, M. Ikuta, J. Z. Murphy, V. Sardana, S. Munshi, L. Kuo, M. Reilly, E. Mahan, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5907 – 5912.
[84] S. Huang, R. M. Garbaccio, M. E. Fraley, J. Steen, C. Kreatsoulas, G. D. Hartman, S. Stirdivant, B. Drakas, K. Rickert, E. S. Walsh, K. Hamilton, C. A. Buser, J. Hardwick, X. Mao, M. Abrams, S. Beck, W. Tao, R. Lobell, L. Sepp-Lorenzino, Y. Yan, M. Ikuta, J. Z. Murphy, V. Sardana, S. Munshi, L. Kuo, M. Reilly, E. Mahan, *Presented at 232nd ACS National Meeting* (San Francisco, CA, United States) Sept. 10 – 14, **2006**, MEDI-129.
[85] C. Papageorgiou, G. Camenisch, X. Borer, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1549 – 1552.
[86] S. Ashwell, J. Ezhuthachan, P. D. Lyne, N. J. Newcombe, M. Pass, V. Oza, M. Su, D. Toader, D. Yu, Y. Yu, WO2004081008A1, **2004**.
[87] K. L. Arrington, V. Y. Dudkin, M. E. Fraley, R. M. Garbaccio, G. D. Hartman, S. Y. Huang, C. Kreatsoulas, E. S. Tasber, WO2007008502A2, **2007**.
[88] S. Ashwell, T. Gero, S. Ioannidis, J. Janetka, P. Lyne, M. Su, D. Toader, D. Yu, Y. Yu, WO2005066163A3, **2005**.
[89] S. Ashwell, Presented at American Association for Cancer Research (AACR) Annual Meeting (Los Angeles, CA, United States) April 14 – 18, **2007**.
[90] K. Daly, N. Heron, A. Hird, S. Ioannidis, J. W. Janetka, P. Lyne, J. Scott, D. Toader, M. Vasbinder, D. Yu, Y. Yu, WO2006106326A1, **2006**.
[91] P. Blume-Jensen, T. Hunter, *Nature* **2001**, *411*, 355 – 365.
[92] G. M. Bokoch, *Annu. Rev. Biochem.* **2003**, *72*, 743 – 781.
[93] R. Kumar, M.-C. Hung, *Cancer Res.* **2005**, *65*, 2511 – 2515.
[94] See www.exelixis.com; XL844.
[95] See www.clinicaltrials.gov; trial NCT00437203.

Received: May 31, 2007
Revised: June 28, 2007
Published online on September 13, 2007

