Novel Checkpoint 1 Inhibitors

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Abstract: Cell cycle ckeckpoints are activated in response to DNA damage. Their role consists in blocking the cell cycle to allow time for DNA repair. The activity of the G1 checkpoint is dependent on the p53 protein. In more than 50% of human tumor cells, the *p53* gene is mutated. In the *p53* mutated cells, the G1 checkpoint is lacking. In these cells, only the G2 checkpoint, although weaker than in healthy cells, provides cancer cells with the opportunity to repair the DNA after damage. Therefore, combining a G2 checkpoint inhibitor with a DNA damaging agent should force, selectively cancer cells, into a premature and lethal mitosis, due to an accumulation of DNA lesions. Among the regulators of the G2 checkpoint, Checkpoint 1 kinase (Chk1) plays a major role. A widespread interest has been recently devoted to the discovery of Chk1 inhibitors as potential useful compounds to enhance the antitumor efficiency of DNA damaging agents. This review article will summarize: (i) the chemical structures of the novel Chk1 inhibitors reported in the recent patents; (ii) their inhibitory activity towards Chk1; (iii) their effects on tumor cells in combination with DNA damaging agents; and (iv) the *in vivo* results on animal models.

Keywords: G2 checkpoint, Chk1 inhibitors, anticancer agents, cell cycle, benzimidazole quinolines, diazepinoindole derivatives, diarylurea derivatives, aminopyrazoles.

INTRODUCTION

Anticancer chemotherapy is a difficult challenge. Few therapies target specifically tumor cells. The ultimate goal for anticancer therapy is to kill cancer cells without causing damage to healthy cells. In this point of view, checkpoint 1 kinase (Chk1) inhibitors are especially interesting. The cell division cycle comprises four sequential phases G1, S, G2 and the mitosis M. The cell cycle is driven by ordered activation of kinases named cyclin-dependent kinases. The inhibition of these kinases can block the cell cycle and stop cell proliferation. However, the inhibition of cyclin-dependent kinases may induce effects on both cancer and healthy cells.

Three checkpoints in G1, S and G2 are activated in response to DNA damage. DNA lesions can be induced by some chemical agents or by radiation or during DNA replication. The role of the checkpoints is to delay the cell cycle progression when DNA damage occurs in order to provide time for DNA repair. The G1/S checkpoint is p53 dependent. In the presence of DNA damage, a rapid induction of p53 activity occurs, inducing cyclin-dependent kinases inhibition and cell cycle arrest to prevent the replication of damaged DNA during the S phase.

The *p53* gene is the most commonly mutated gene in human cancers. Thus, in the *p53*-mutated cells, the cascade of signals in response to DNA damage is inactivated and the G1/S checkpoint is abrogated. In this connection, the G2 checkpoint has triggered considerable interest over the past ten years. In the *p53*-mutated cells, in which the G1 checkpoint is lacking, only the G2 checkpoint is able to provide a delay in the cell cycle progression allowing the activation of DNA repair pathways [1-9]. Therefore, the G1 checkpoint defect distinguishes cancer cells from healthy cells and thereby provides a potential target for anticancer therapy. Among the G2 checkpoint effectors are ATM and ATR kinases and downstream Chk1 kinase [10]. Chk1 inhibitors abrogate the G2 checkpoint and sensitize, essentially *p53*-mutated cells, to DNA damaging agents. Several small molecules exhibiting inhibitory properties toward Chk1 have been previously described in the literature such as the bacterial metabolite UCN-01, natural compounds granulatimide and isogranulatimide, hymenialdisines, 13-hydroxy-15-oxozoapatlin, as well as synthetic compounds [10-21]. Some well-known Chk1 inhibitors are presented in (Fig. **1**). However, their non-selectivity and/or their weak inhibitory activity justified the need to have novel Chk1 inhibitors. Most of the above cited compounds interact with the ATP binding site of the kinase. Since the ATP binding site is similar in all the kinases, it was previously thought that it would be extremely difficult to obtain selective ATP-competitive kinase inhibitors. But with the resolution of more and more crystal structures of kinases, it appeared that close to the ATP-binding site, several pockets, different in the various kinases, could be exploited for the discovery of more selective kinase inhibitors. The three-dimensional structures of human Chk1 kinase domain and its binary complex with an ATP analog have been determined [22], as well as the crystal structures of Chk1 in complex with its inhibitors staurosporine and UCN-01 [12]. To identify new Chk1 inhibitors, a virtual screening was carried out on libraries of compounds by docking into the active site of Chk1. Thirty-six compounds on 103 that docked sucessfully into the active site, were found to inhibit the enzyme, among; them, oxindoles and pyrimidines inhibited Chk1 with IC_{50} values in the nano or micromolar range [23](Fig. **2**).

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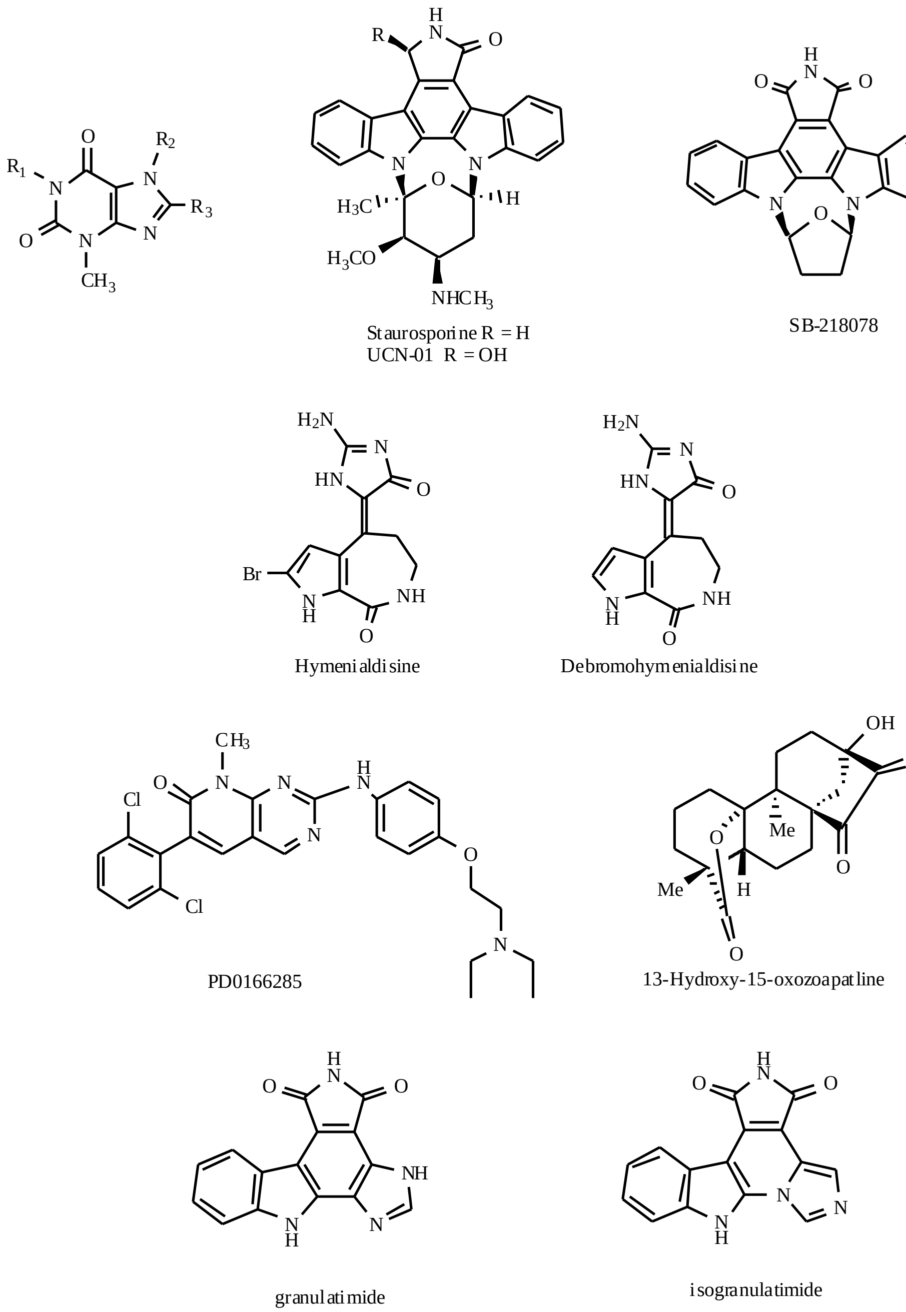


Fig. (1). Chk1 inhibitors.

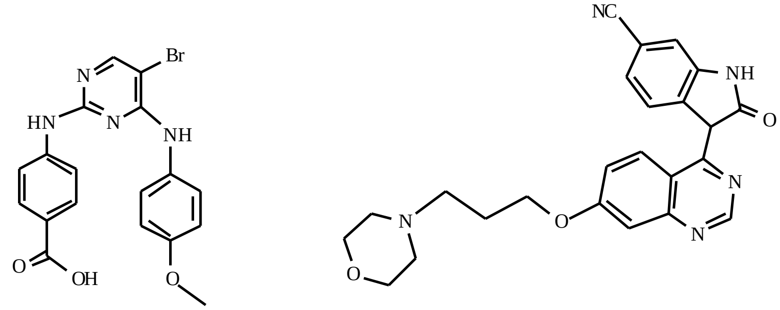


Fig. (2). Oxindole and pyrimidine Chk1 inhibitors.

This review is focused on the novel Chk1 inhibitors reported in the recent patents (2004-2005): their chemical structures are described as well as their biological properties as potential useful agents for the treatment of cancers.

CHEMICAL STRUCTURES OF NOVEL CHK1 INHIBITORS

Agouron Pharmaceuticals Inc. described aminopyrazoles bearing various substituents [24] (Fig. **3**). In these compounds, an aminopyrazole moiety is held in a fixed linear arrangement (L) with a resorcinol-like moiety. The amino group of the aminopyrazole can bear a benzylamino, a pyridine or a thiazole heterocycle substituent; the linear rigid linker can be a phenyl group disubstituted in para positions. Bristol-Myers Squibb Company also described several aminopyrazoles as potential Chk1 inhibitors [25]. The inhibitory activities of 3,5-disubstituted indazoles (Fig. **3**) are reported in two patents [26,27]. However, they did not exhibit selectivity toward Chk1. Abbott Laboratories described fused tri- and tetra-cyclic pyrazoles as Chk1 inhibitors [28] (Fig. **3**). New tricyclic Chk1 inhibitors in which an indole moiety is condensed to a seven-membered ring heterocycle are reported [29,30] (Fig. **3**). In the examples described, this seven-membered ring contains a hydrazone function. Among these compounds, compound **I** (Fig. **3**) has been considered as a prototype Chk1 inhibitor. Its biological activities have been extensively studied [31]. K.S. Keegan and collaborators [32] described a family of Chk1 inhibitors corresponding to the general formula shown in (Fig. **4**). In particular, aryl- and heteroaryl- substituted ureas **A** were reported in which the W heterocycle can bear one or several R' substituents. Other examples are described, such as carbamido-substituted heteroaryl compounds corresponding to the structural formula **B**. Other ureas, including macrocyclic compounds, were reported as Chk1 inhibitors by Millenium Pharmaceuticals, Inc. and Abbott Laboratories [33-36]. Their general chemical structures are shown in (Fig. **4**). Synthetic carbamates, diazepinones, pyrimides, and benzimidazole quinolones also showed inhibitory activities toward Chk1 [37-39] (Fig. **5**). Several recent patents described new molecules as potential Chk1 inhibitors, however their inhibitory activities toward this enzyme are not reported [40-51]. They will not be discussed here. Kawabe and Kobayashi [52] have synthesized various peptides and peptidomimetics inhibitors of Chk1, but they are not selective of Chk1 versus Chk2.

INHIBITORY POTENCIES TOWARDS CHK1

Concerning the aminopyrazole compounds [24], C-terminally His-tagged full-length human CHK1 (FL-CHK1) was expressed using the baculovirus/insect cell system. Since the kinase activity induces a transfer of a phosphate from ATP to the substrate, the production of ADP from ATP that accompanies phosphoryl transfer to a synthetic substrate peptide was measured in the presence of several aminopyrazoles. The Ki values could be evaluated and the EC_{50} values in nanomolar could be determined in some cases (Table **1**).

The strongest inhibitory activities measured as EC_{50} values were observed with compounds **4**, **5**, **9**, **10**, and **11** corresponding to formula **I** and above all, with compound **22** (formula **III**). All these compounds bear a methylamino substituent (R_1) in the 4-position and no substituent on the resorcinol moiety except a fluorine atom in the 6-position of compound **9**. The selectivity toward various kinases was investigated with this series of compounds. The inhibitory activities of the aminopyrazoles toward tyrosine kinases such as VEGFR-2, FGFR-1 and other kinases such as phosphorylase kinase (PHK), Chk2, CDK1, Wee-1, SGK, AMPK, LCK, MAPK2, MSK1, PKB-α, ROCKII, p70 S6K, PKA, MAPK1, cSRC, PRK2, PDK1FYN, PKC-βII, and PKC-γ have been evaluated. Some aminopyrazoles were at least 20-fold more selective for Chk1 than for other kinases.

The Ki values toward Chk1 for some of indazoles [27] are in the nanomolar range (Fig. **6**).

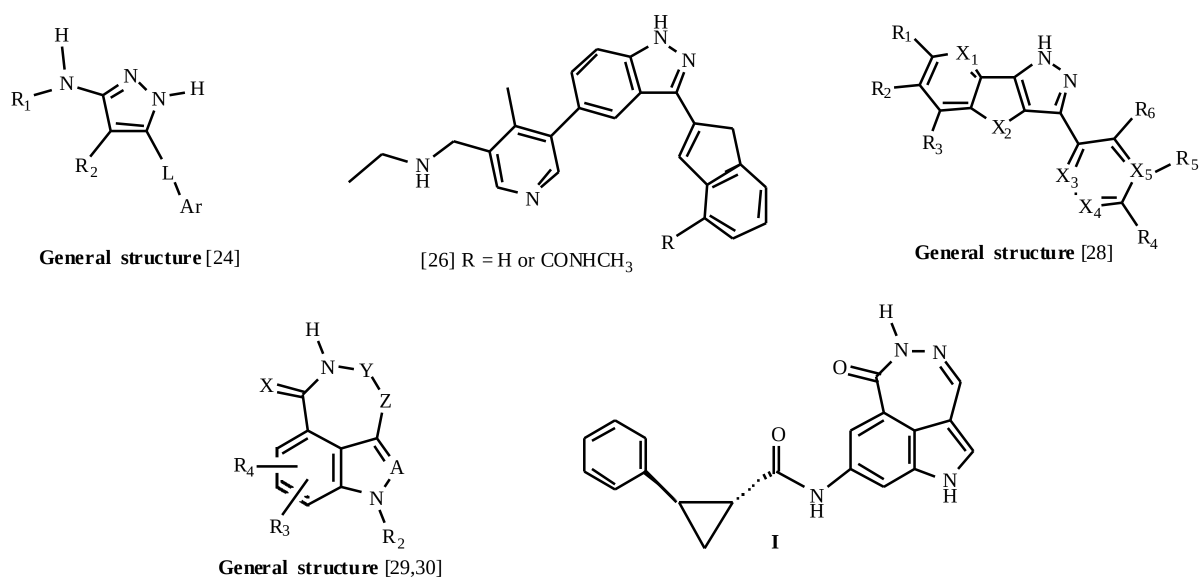


Fig. (3). Aminopyrazole, indazoles and tricyclic compounds.

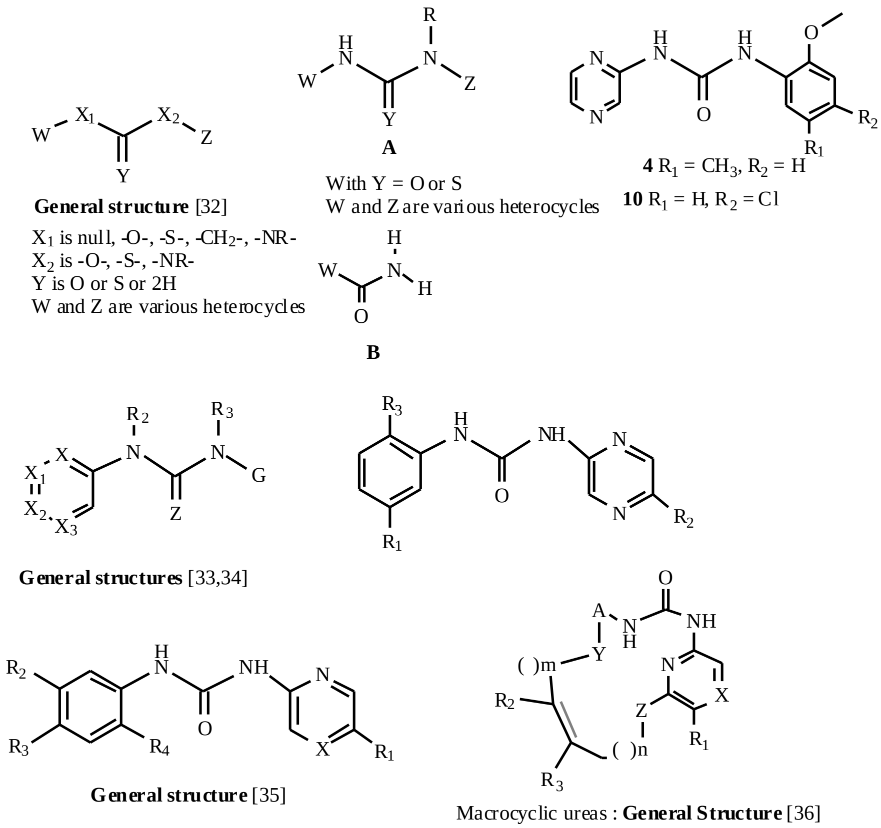


Fig. (4). Ureas as Chk1 inhibitors.

The inhibitory activities toward Chk1 of the tricyclic diazepinoindole derivatives [29] were evaluated using the same method as described in ref [24]. The most interesting Ki values (nM) are reported in (Table **2**).

Compounds of formula **II** have a considerably strong affinity for the enzyme, and especially diazepinoindoles with, in the 2-position, an unsaturated side chain bearing an amino function (compounds **21**, **22**).

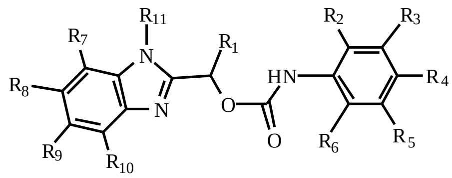
To get an insight into the enzyme selectivity, the inhibitory potencies toward the same enzymes as above described were evaluated. The selectivities are not described in the patent.

For the fused tri and tetracyclic pyrazole derivatives [28], the Chk1 enzymatic assay was carried out using recombinant Chk1 kinase domain protein (amino-acids residues from 1 to 289) and a polyhistidine tag at the C-terminal end. The best inhibitors in this series inhibited Chk1 at IC_{50} values in 0.1 nM range.

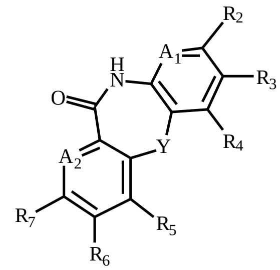
Substituted ureas have been reported as potent Chk1 inhibitors [33]. Their IC_{50} values toward this enzyme have been tested in a Chk1 FlashPlate® kinase assay (Table **3**). Compounds of formula **I** with a pyrazine heterocycle, a chlorine atom in R_4, and with an ether function in R_1 bearing an amino or a hydroxy substituent (compounds **1**, **2**, **24**) were potent Chk1 inhibitors. Compounds of formula **II** (**25-28**) bearing a methyl pyrazine, as well as compound **29** with two pyrazine moieties proved to be also potent Chk1 inhibitors. The replacement of the pyrazine aromatic heterocycle with a pyrimide (compare compounds **7** and **32**) strongly decreased the inhibitory potency toward Chk1. The replacement of the pyrazine heterocycle by a pyridine moiety substituted with an amido or cyano function (compounds **30** and **31**) retained the activity toward the enzyme. The selectivities toward other kinases are not reported in the patent.

In the urea series described in [32], the screen using FLAG.RTM-Chk1 identified a number of Chk1 inhibitors having IC_{50} values in the range of 1 to 100 µM. The selectivity of these substituted ureas against several kinases (DNA-PK, ATR, Cdc2, CKI, CAM KII, Chk2, MAP kinase, and PKA) has been evaluated. All the compounds tested showed at least 5-fold selectivity for Chk1 over the other enzymes. The inhibitory activities toward Chk1 of the urea derivatives reported by Li and collaborators [35] were evaluated using the same method as described in ref [28]. The IC_{50} values were found between 2 nM and 5 µM.

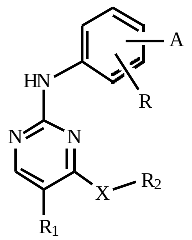
In the carbamate series [37], the IC_{50} values toward Chk1 were found in the micromolar range, and < 1 µM for the compound of the general formula (Fig. **5**) in which $R_7 = R_{10} = R_{11} = R_2 = R_5 = R_6 = H$, $R_8 = R_9 = CH_3$ and $R_3 = R_4 = Cl$. In the diazepinone series [38], the Chk1 enzymatic essay was carried out as described in ref [28]. The IC_{50} values for the tested compounds were between 0.2 nM and 280 µM. Among the pyrimidines Chk1 inhibitors [39], compound **A** (Fig. **5**) is one of the most efficient with an IC_{50} value of 20 nM.



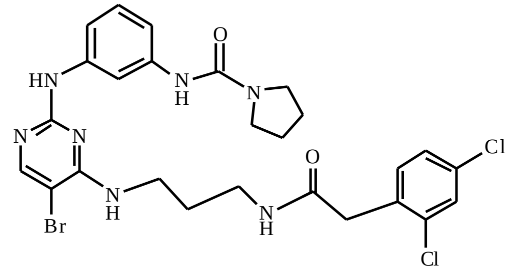
General structure [37]



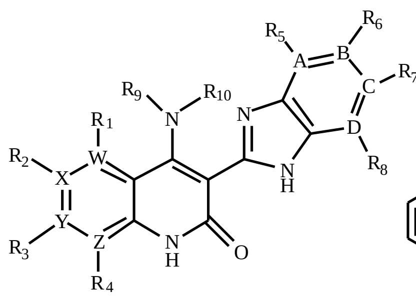
General structure [38]



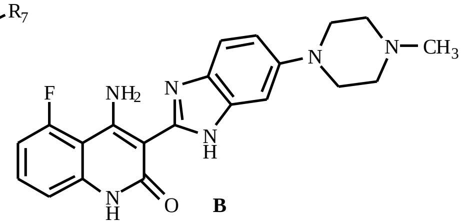
General structure [39]



A



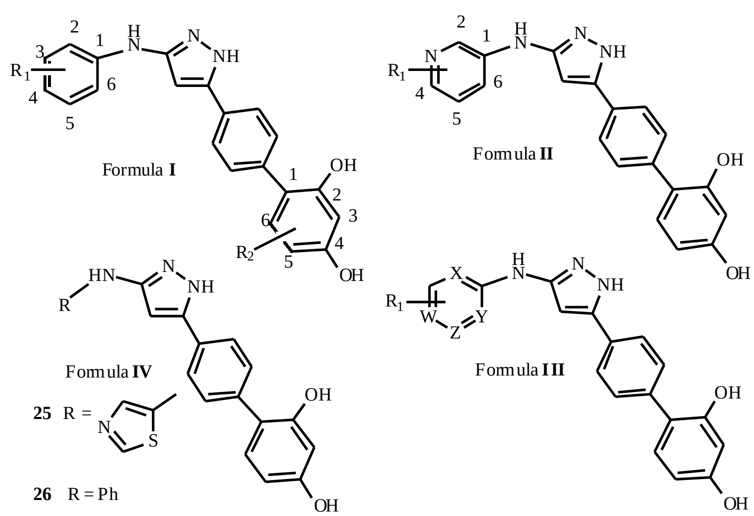
General structure [40]



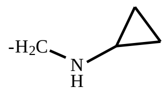
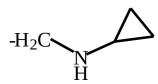
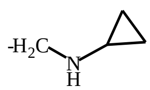
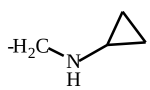
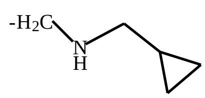
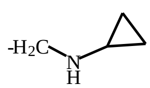
B

Fig. (5). Carbamates, diazepinones, pyrimidines, benzimidazole quinolones and macrocyclic compounds as Chk1 inhibitors.

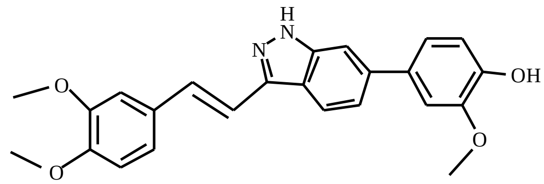
Table 1. Ki and EC_{50} Values (in nM) of Some Aminopyrazole Derivatives Toward Chk1 [24]



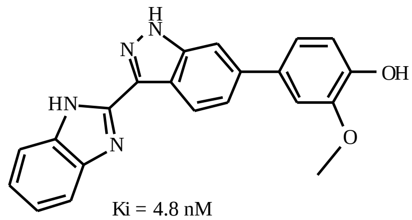
(Table 1) Contd….

Cpd	Formula	Substituents	Ki (nM)*	EC_{50} (nM)
1	I	R_1 = 5-CN, R_2 = H	B	7% at 0.5 μM
2	I	4-CN, R_2 = H	B	1% at 1 μM
3	I	R_1 = 3-  , R_2 = H	C	900
4	I	R_1 = 4 -  ,R_2 = H	C	60
5	I	R_1 = 4-CH_2 -NH-$CH(CH_3)_2$, R_2 = H	C	70
6	I	R_1 = 4-CH_2 -NH-$CH(CH_3)_2$, , R_2 = 5-CH_3	A	not available
7	I	R_1 = 4-CH_2 -NH-$CH(CH_3)_2$, , R_2 = 6-CH_3	C	380
8	I	R_1 = 4 -  , R_2 = 6-Cl	C	150
9	I	R_1 = 4 -  , R_2 = 6-F	B	88
10	I	R_1 = 4 -  , R_2 = H	C	75
11	I	R_1 = 4-CH_2-$NH(C_2H_5)CH_3$, R_2 = H	C	60
12	II	R_1 = 4 – 	B	165
13	II	R_1 = 4 -	B	1% at 1 μM
14	II	R_1 = 4-CH_2-NH-$CH(CH_3)_2$	C	560
15	II	R_1 = 4-CH_2-NH-C_2H_5	C	560
16	II	R_1 = 4-CH_2OH	A	5% at 0.5 μM
17	II	R_1 = 4-CS-NH-CH_3	B	10% at 0.5 μM
18	II	R_1 = H	C	540
19	III	X = Y = N, W = Z = CH, R_1 = H	B	not available
20	III	W = N, X = Y = Z = CH, R_1 = 5-CH_2OH	C	0% at 0.5 μM
21	III	Z = N, W = X = Y = CH, R_1 = 4-CH_2-NH-cyclopentyl	B	165
22	III	Z = N, W = X = Y = CH, R_1 = 4-CH_2-$N(CH_3)_2$	B	38
23	III	X = N, W = Y = Z = CH, R_1 = H	C	16% at 0.5 μM
24	III	W = N, X = Y = Z = CH, R_1 = H	B	14% at 0.5 μM
25	IV		C	10% at 0.5 μM
26	IV		C	500

*A = > 10; B = 1-10; C = <1.



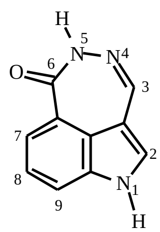
Ki = 5.2 nM



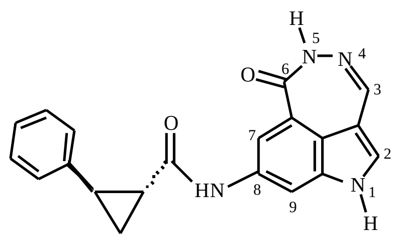
Ki = 4.8 nM

Fig. (6). Ki values toward Chk1 for a concentration of indazoles of 1 μM [27].

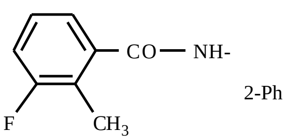
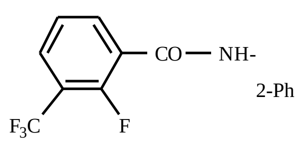
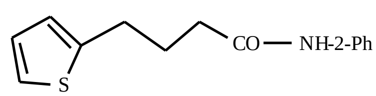
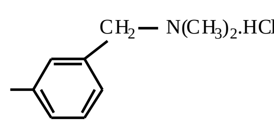
Table 2. Ki Values Toward Chk1 of Tricyclic Diazepinoindoles (in nM) [29]



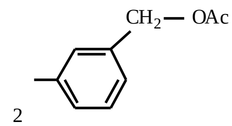
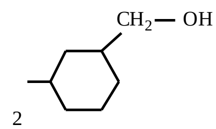
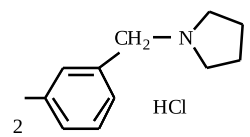
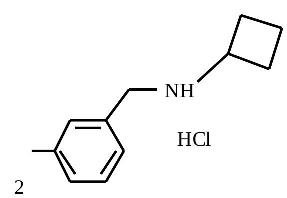
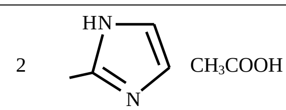
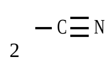
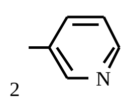
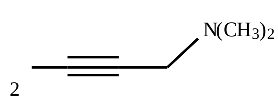
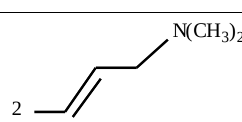
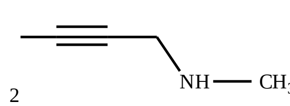
Formula **I**



Formula **II**

Cpd	Formula	Substituents	Ki (nM)
1	I	8- 	0.000964
2	I	8- 	0.000805
3	I	8- 	0.000359
4	II	2-Ph	0.000151
5	II	enantiomers 2-H	0.00095
6	II	2 	0.000587

(Table 2) Contd….

Cpd	Formula	Substituents	Ki (nM)
7	II		0.000398
8	II		0.000336
9	II	2-Cl	0.0001
10	II	2-CO-NH-$(CH_2)_2$-$N(CH_3)_2$	0.000625
11	II	2-CO-NH-$(CH_2)_2$-OH	0.000553
12	II	2-CO-NH-CH_3	0.000358
13	II	2-$COOCH_3$	0.000115
14	II		0.000196
15	II		0.000605
16	II	2-H	0.000738
17	II		0.0002
18	II		0.0009
19	II		0.000102
20	II		0.000121
21	II		0.000064
22	II		0.000068

(Table 2) Contd….

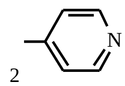
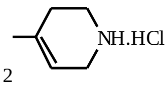
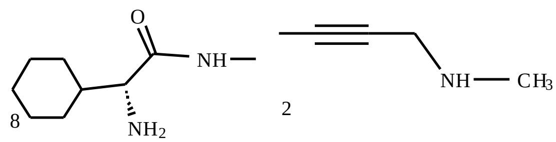
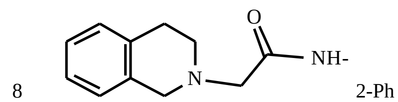
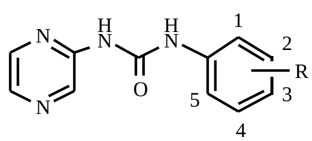
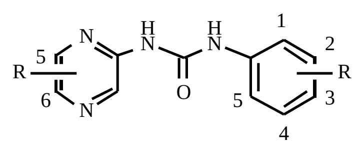
Cpd	Formula	Substituents	Ki (nM)
23	II		0.000219
24	II		0.00082
25	II	2-Br	0.000114
26	I		0.000529
27	I		0.000664

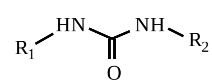
Table 3. Inhibitory Activities of Ureas Toward Chk1 (IC_{50} in μM) [33]



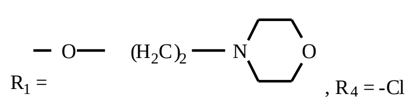
Formula **I**



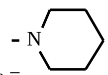
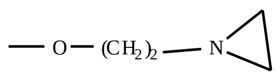
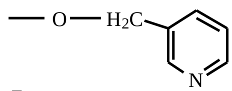
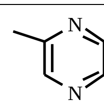
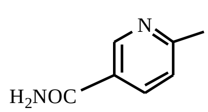
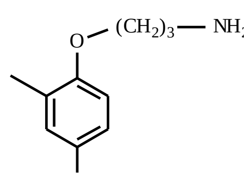
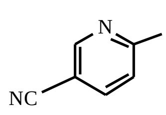
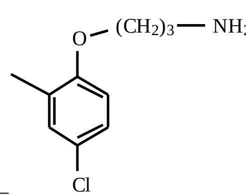
Formula **II**



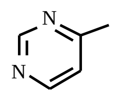
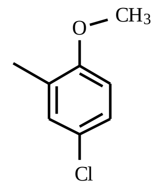
Formula **III**

Cpd	Formula	Substituents	IC_{50}*
1	I	R_1 = -O-$(CH_2)_3$-$N(CH_3)_2$, R_4 = -Cl	A
2	I	R_1 = -O-$(CH_2)_2$-$N(CH_3)_2$, R_4 = -Cl	A
3	I	R_1 = -O-$(CH_2)_2$-$CH(CH_3)_2$, R_4 = -Cl	B
4	I	R_1 = R_3 = -O-CH_3, R_4 = -Cl	A
5	I	R_1 = -O-CH_3, R_4 = CH_3	B
6	I	R_5 = -O-CH_3	B
7	I	R_1 = -O-CH_3, R_4 = -Cl	B
8	I	R_1 = -O-CH_3, R_4 = -$NHCOCH_3$	B
9	I	R_1 = -O-CH_3, R_4 = -$COOCH_3$	B
10	I		B
11	I	R_1 = -O-CH_3, R_4 = -CF_3	B
12	I	R_1 = -O-CH_3, R_3 = -OCH_3	B

(Table 3) Contd….

Cpd	Formula	Substituents	IC_{50}*
13	I	R_1 = -Cl, R_4 = $-OCH_3$	B
14	I	R_1 = $-CH_3$, R_4 = -Cl	B
15	I	R_3 = $-O-CH_3$, R_4 = -Cl	C
16	I	R_4 = -Cl	C
17	I	R_2 = $-O-CH_3$	C
18	I	R_3 = 	C
19	I	R_3 = $-O-CH_3$	C
20	I	R_3,R_4 = $-O-CH_2-O-$	C
21	I	R_1 =  , R_4 = -Cl	B
22	I	R_1 =  , R_4 = -Cl	B
23	I	R_1 = $-O-CH_3$, R_3 = -Cl, R_4 = $-CH_3$	B
24	I	R_1 = $-O-(CH_2)_3-OH$	A
25	II	R_1 = $-O-CH_2-C(CH_3)_2-CH_2-NH_2$, R_4 = -Cl, R_6 = $-CH_3$	A
26	II	R_1 = $-O-(CH_2)_2$-NH-cyclobutane, R_4 = -Cl, R_6 = $-CH_3$	A
27	II	R_1 = $-O-(CH_2)_3-N(CH_3)_2$, R_4 = -Cl, R_6 = $-CH_3$	A
28	II	R_1 = $-O-(CH_2)_2-N(CH_3)_2$, R_4 = -Cl, R_6 = $-CH_3$	A
29	III	R_1 = R_2 = 	A
30	III	R_1 =  R_2 = 	A
31	III	R_1 =  R_2 = 	A

(Table 3) Contd….

Cpd	Formula	Substituents	IC_{50}*
32	III	R_1 =  R_2 = 	C

*A < 0.1 µM; 0.1 µM < B < 1 µM; 1 µM < C < 20 µM.

Benzimidazole quinolinones [40] (Fig. **5**) were reported to be non-selective kinase inhibitors. A large majority of compounds in these series exhibit IC_{50} values of less than 10 µM toward VEGFR1, VEGFR2, VEGFR3, FGFR1, Chk1, Cdc2, GSK-3, CDK2, CDK4, MEK-1, Chk2, NEK-2, Raf, Fyn, Lck, Rsk2, PAR-1, c-Kit, c-Abl, p60src, FGFR3, FLT-3, PDGFRα, and PDGFRβ. The IC_{50} values for many of the exemplary compounds are in the nanomolar range. However, no table of the IC_{50} values according to the substituents is described in the patent. One of the compounds, 4-amino-5-fluoro-3-[5-(4-methylpiperazin-1-yl)-1*H*-benzimidazol-2-yl]quinolin-2(1*H*)-one **B** (Fig. **5**) has been extensively studied *in vivo*.

IN VITRO EFFECTS OF THE CHK1 INHIBITORS

The *in vitro* effects of the aminopyrazole and tricyclic Chk1 inhibitors were determined using an ELISA assay by monitoring the abrogation of DNA damage-induced checkpoint control. The cancer cells were treated with a DNA damaging agent such as the topoisomerase I inhibitor camptothecin. The Chk1 inhibitors were then added at increasing concentrations. In parallel, control cells were prepared with only Chk1 inhibitors to assure that the inhibitors alone have no effect on the cell cycle. The IC_{50} values (concentration of the Chk1 inhibitor inducing 50% cell growth inhibition) were determined for Chk1 inhibitors treatment alone and for treatment with Chk1 inhibitors in combination with a DNA damaging agent. The ratio between IC_{50} Chk1 alone/IC_{50} in combination represents the Potentiation Factor PF_{50}. The PF_{50} values determined for the tricyclic Chk1 inhibitors [30] tested ranged from 2 to 8.

To investigate how Chk1 inhibitors can enhance the killing of cells, twelve tumor cell lines were incubated with increasing concentrations of DNA damaging agents in the presence of a fixed concentration of the aminopyrazole inhibitors. The concentrations of DNA damaging agent causing 50% growth inhibition with and without the Chk1 inhibitor were determined. The ratio between IC_{50} Chk1 alone/IC_{50} in combination treatment represents the Potentiation Factor PF_{50} and is a measure of the potency of the combination treatment. With cisplatin as the DNA damaging agent, at concentrations less than 0.5 µM, the killing of the cells was enhanced from 2-5 fold.

In similar conditions, the substituted ureas **4** and **10** (Fig. **4**) were found by K. Keegan and collaborators [32] to enhance from 2- to 10 fold the killing of the cells treated with 5-fluorouracil at concentrations less than 100 µM. Moreover, in HeLa cervical adenocarcinoma cells, compounds **4** and **10** were found to enhance killing by irradiation 2-3 fold.

In HT29 and HCT116, colon carcinoma cells were incubated with increasing concentrations of a DNA damaging agent (camptothecins, 5-fluorouracil and etoposide). With a fixed concentration of other ureas [32], it has been shown that the Chk-1 inhibitors substituted in R_1 with a -$(CH_2)_n$-NR_2 side chain significantly enhanced the activity of DNA damaging agents (Table **4**).

This kind of experiments was not reported with benzimidazole quinolinones [40]. Only the cytotoxicities were evaluated in the absence of DNA damaging agents. In this case, the cytotoxicities observed were probably essentially due to the inhibition of other kinases than Chk1, such as tyrosine kinases.

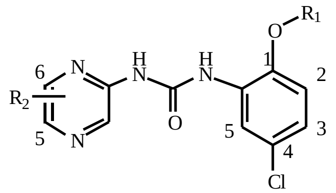
IN VIVO TUMOR MODEL STUDIES

Combination therapy studies have been successfully carried out using benzimidazole quinolinone **B** (Fig. **5**) [40] in combination with irinotecan (CPT-11) in the KM12L4a colon tumor model. A cyclic dosing regimen of compound **B** at 50 mg/kg in combination with CPT-11 gave remarkable results as shown in (Fig. **7**). Another study combining compound **B** and ZD1839 (Iressa) in the A431 epidermoid tumor model led also to significant tumor regressions as shown in (Fig. **8**).

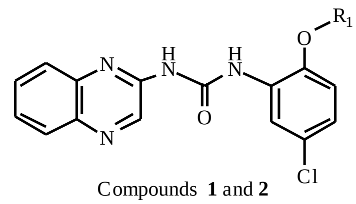
CURRENT & FUTURE DEVELOPMENTS

In conclusion, several new series of potent Chk1 inhibitors have been discovered during these past few years. Important structure-activity relationship studies allowed to point out the more efficient substituents to be introduced on the general structures. Combining DNA damaging agents and Chk1 inhibitors is a attractive strategy to fight cancer [53]. Since Chk1 inhibitors are expected to enhance the antitumor properties of DNA damaging agents, combination therapies could allow to decrease the amounts of these agents required for the chemotherapies and thereby minimize the toxicity in healthy cells and the resistance to these cytotoxic agents. Exciting results begin to be published [31]. PF-00394691 (Compound **16**, Table **2**) is a potent and selective Chk1 inhibitor exhibiting an IC_{50} value of 0.4 µM toward this kinase. Combination therapies using PF-00394691 and gemcitabine, irinotecan or cisplatin enabled dose-dependent

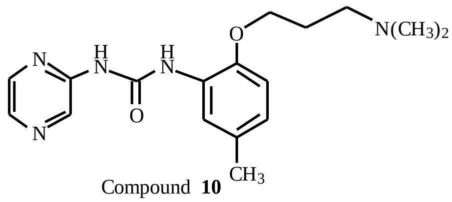
Table 4. IC_{50} Values (in μM) of Ureas Versus Chk1. Increased Cytotoxicity of DNA Damaging Agents in the Presence of Various Concentrations of Chk1 Inhibitors [32]



Formula **I**



Compounds **1** and **2**



Compound **10**

Cpd	Formula	Substituents	IC_{50} toward Chk1 (μM)	Shift at 1.25 μM	Shift at 2.5 μM
1		R_1 = -$(CH_2)_2$-$N(CH_3)_2$	0.019	1	1.4
2		R_1 = -$(CH_2)_3$-NH_2	0.005	4.4	6.2
3	I	R_1 = -$(CH_2)_2$-$N(CH_3)_2$, R_2 = H	0.008	1.9	2.6
4	I	R_1 = -$(CH_2)_2$-NH_2, R_2 = H	0.016	1.2	1.4
5	I	R_1 = -$(CH_2)_3$-$N(CH_3)_2$, R_2 = 5-CH_3	0.0045	3.3	4.4
6	I	R_1 = -$(CH_2)_3$-NH_2 , R_2 = 5-CH_3	0.004	toxic	toxic
7	I	R_1 = -$(CH_2)_3$-$N(CH_3)_2$, R_2 = H	0.0035	2.2	3.4
8	I	R_1 = -$(CH_2)_3$-NH_2, R_2 = H	0.0025	toxic	toxic
9	I	R_1 = -$(CH_2)_4$-NH_2, R_2 = 5-CH_3	0.003	toxic	toxic
10			0.016	2.1	2.9
11	I	R_1 = -$(CH_2)_2$-$N(CH_3)_2$, R_2 = 6-OCH_3	0.020	2.4	3.03

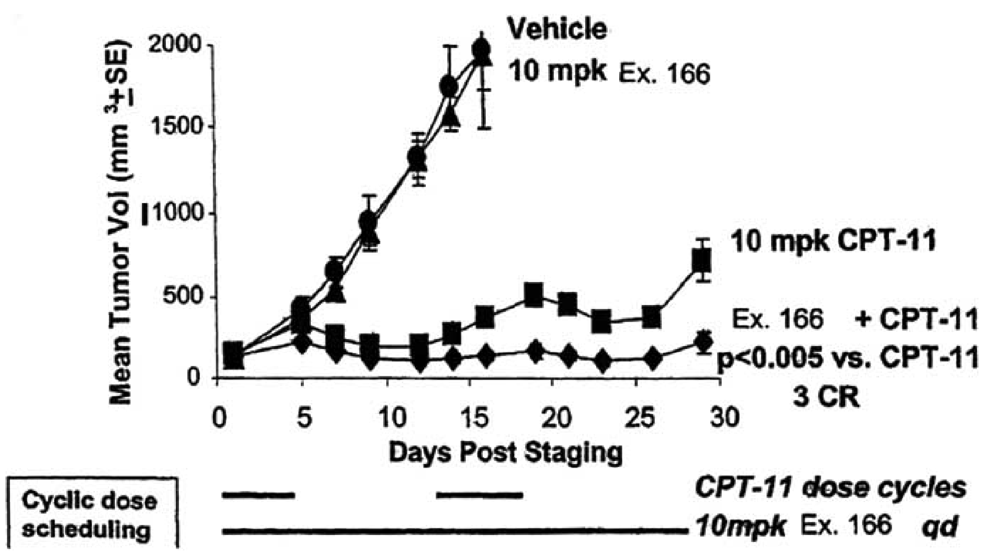


Fig. (7). Tumor responses with a cyclic dosing regimen of Ex.166 (compound **B**, Fig. **5**) at 50 mg/kg in combination with CPT-11 in the KM12L4a colon tumor model.

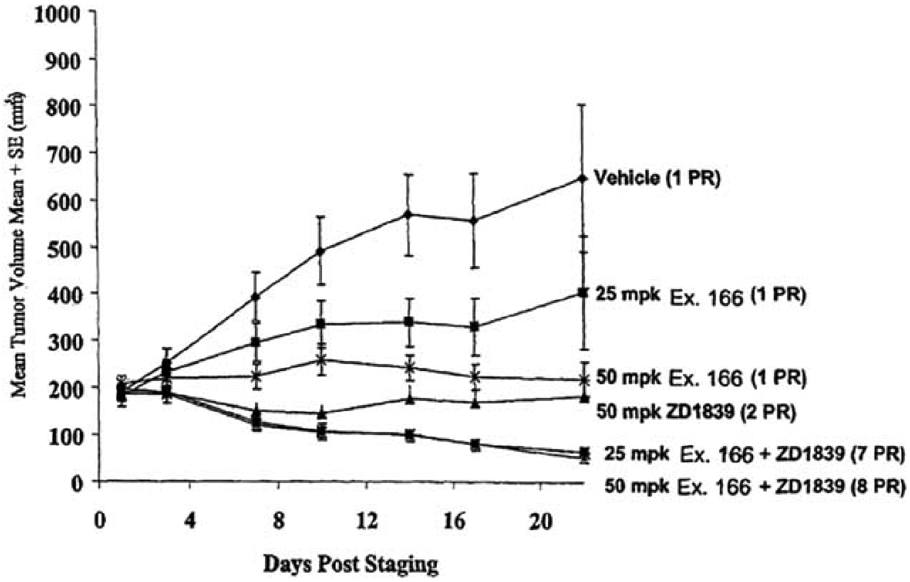


Fig. (8). Tumor responses with Ex.166 (compound **B**, Fig. **5**) in combination with Iressa (ZD1839) in the A431 epidermoid tumor model.

potentiation of antitumor activity in PC3M-Luc-C6 xenograft [31]. Very likely, the interesting chemosensitization induced by Chk1 inhibitors will be confirmed in the next few years.

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