Small Molecules Targeting p53 to Improve Antitumor Therapy

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Abstract: Small molecules targeting p53 represent an emerging group of potentially useful agents for the improvement of antitumor therapy. These modulators include agents that activate wild-type p53 or reactivate mutant p53 and inhibitors of p53 functions. Preclinical evidences support the interest of combination strategies with conventional antitumor agents.

Key Words: p53, nutlins, pifithrins, histone deacetylase inhibitors, ubiquitin ligase inhibitors, apoptosis, cancer cells, drug combination.

INTRODUCTION

The tumor suppressor protein p53 is a transcription factor which regulates cellular response to diverse forms of stress through a complex network that monitors genome integrity and cell homeostasis [1-3]. Activated p53 induces the transcription of multiple target genes leading to cell cycle arrest, DNA repair, apoptosis, senescence, or differentiation. Cell cycle arrest and apoptosis are the most relevant responses to DNA damage. The p53 pathway utilizes G1/S and G2/M checkpoint mechanisms to arrest cell cycle progression, thus preventing propagation of DNA damage while cells attempt to repair it. However, if the damage is too severe, activation of the p53 pathway results in apoptotic cell death to avoid the division of cells with unrepaired DNA and possible malignant transformation.

The interest of pharmaceutical research in p53 is due to the fact that p53 is the most frequently altered protein in human cancer. Approximately 50% of human malignancies harbor p53 gene mutations or deletions that disable its tumor suppressor function [4,5]. Moreover, in wild-type p53 tumors, increased levels of p53 negative regulators are a frequent event implicated in the control of p53 function. One of such regulators is the murine double minute-2 gene (MDM2), which controls p53 activity and stability [6-8].

The role of p53 in tumor response to cytotoxic treatment could vary depending on activation of distinct functions and specific molecular context. Wild-type p53 appears a major determinant of DNA damage-induced apoptosis and loss of p53 function in tumors is associated with an unfavorable prognosis in many forms of cancer [9-11]. In principle, wilde-type p53 tumors should be more sensitive to treatment through the induction of apoptosis, whereas p53 inactivation should result in drug-resistance. However, due to impairment of p53-dependent apoptosis (e.g., through overexpression of B-cell leukemia 2, Bcl-2), wild-type p53 could act as survival factor accounting for the prolonged treatment-induced arrest, aimed at facilitating DNA repair. Inactivation of p53 during tumor progression could render cells less capable of DNA repair and more sensitive to drug-induced mitotic catastrophe.

In tumors that lack p53-dependent apoptosis but retain p53-dependent growth arrest, p53 inactivation is expected to sensitize to DNA-damaging treatment *via* induction of mitotic catastrophe.

Therefore, p53 represents an important target for drug development. In principle, both activation and inhibition of p53 by small molecules has been exploited for improvement of cancer treatment. The former approach is expected to contribute to more efficient tumor cell killing by restoring p53 function, while the latter one should improve treatment outcome by reducing tissue injury by transient reversible conversion of normal tissues to a p53-deficient state. In addition, approaches incorporating molecules targeting p53 functions in combination with different conventional anticancer drugs have been proposed and are under preclinical evaluation. To date, no small molecules targeting p53 have reached clinical development.

Several classes of low molecular weight molecules able to modulate p53 functions have been reported, Fig. (**1**).

CHEMICAL ACTIVATORS OF P53

Among the approaches aimed at modulating the p53-dependent processes, gene therapy using p53 expression vectors has widely been explored, but the success has been limited by a number of drawbacks of delivery systems [12,13]. Thus, it appears of great interest the search for small molecules targeting p53.

The pharmacological activation of p53 by small molecules aimed at restoring its tumor suppressor function has been explored, using: a) inhibitors of p53-MDM2 binding, b) activators of mutant p53, c) inhibitors of ubiquitin ligase and d) inhibitors of histone deacetylase.

Inhibitors of p53-MDM2 Binding

There is a major interest in exploiting p53-MDM2 interaction to induce p53 accumulation and to enhance its proapoptotic function, because MDM2 is a negative regulator of p53 by promoting its ubiquitination [6-8]. This ap-

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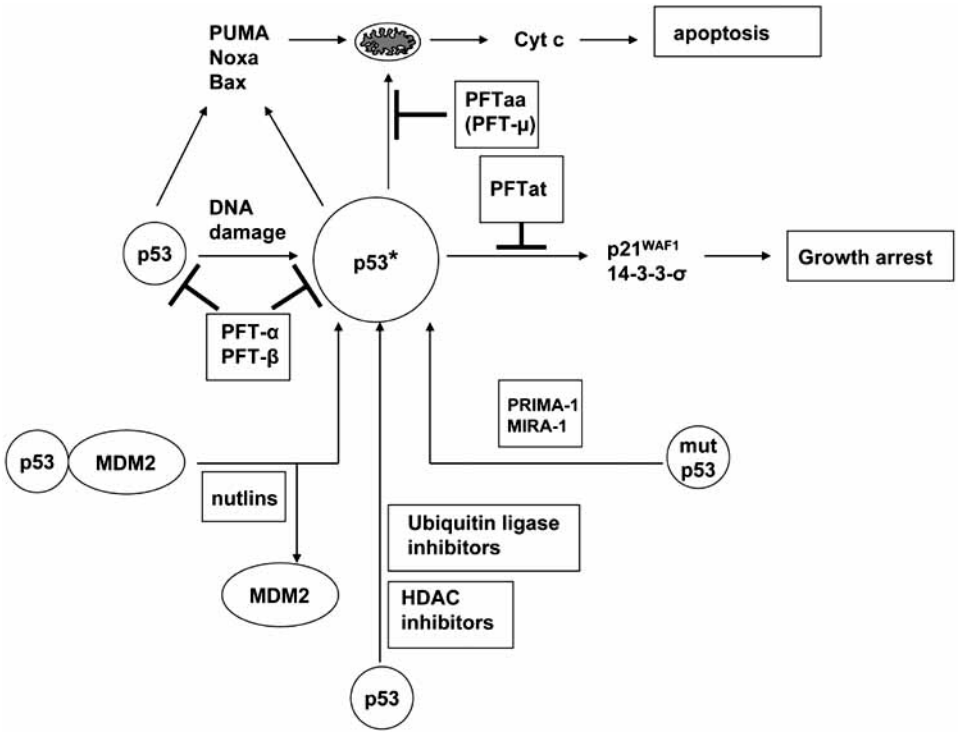


Fig. (1). Scheme of pharmacological dissection of p53 activities. The mechanisms of action of both small molecules activators and inhibitors of p53 are reported. PFTaa, anti-apoptotic PFT; PFTat, anti-transactivation PFT; p53*, activated p53.

proach for activation of p53 was stimulated by the definition of the hydrophobic p53-binding pocket on MDM2 (http://www.rcsb.org, PDB code 1YCR). The inhibitors of the p53-MDM2 interaction belong to different structural categories which are reported in Fig. (**2**) [14,15].

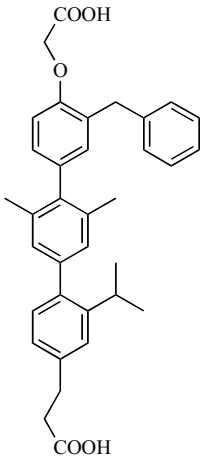
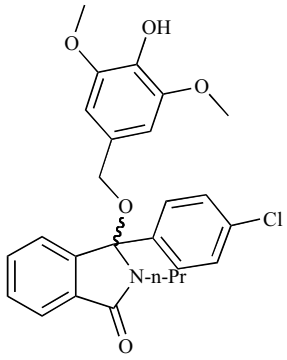
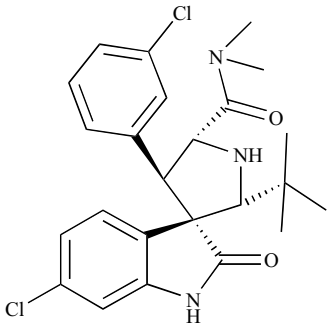
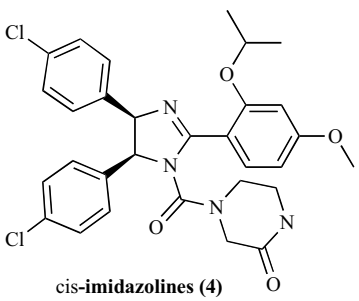
In an attempt to interfere with the MDM2 regulation on p53, the use of small molecules appears a promising strategy. The natural fungal cyclic nonapeptide chlorofusin was identified by screening a library of microbial extracts [16]. Also peptides which mimic p53 were synthesized to inhibit the p53-MDM2 interaction [17], but none of these molecules appeared a good candidate for clinical trials. The major drawback of chlorofusin and mimetic peptides was the low oral bioavailability.

In principle, the most desirable way to inhibit the p53-MDM2 interaction should be the use of small molecules that can selectively bind to the p53-MDM2 interface, thus allowing release of the negative regulator, p53 accumulation in cell nuclei and $p21^{WAF1}$ induction. Among such molecules, chalcones are the first reported low molecular weight inhibitors of the p53-MDM2 interaction. However, the chalcone derivatives exhibit low potency and specificity [18-20]. Syc7 and NSC279287 were identified using computer-aided design based on crystal structure data. Syc7 induced p53 accumulation, but its cytotoxicity appeared independent of p53 gene status [21]. The sulfonamide derivative NSC279287, showed a relatively weak inhibition of p53-MDM2 binding [22].

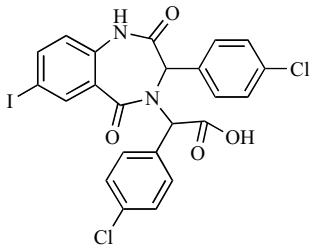
Another compound targeting the p53-MDM2 interaction is RITA (reactivation of p53 and induction of tumor cell apoptosis), identified by screening small-molecule compounds from the NCI library for ability to suppress cell growth in a p53-dependent manner using the isogenic pair of p53-proficient and -deficient HCT116 colon cancer cells [23,24]. In spite of the high cytotoxic potency in cells with wild-type p53, the mechanism by which RITA interferes with p53-MDM2 binding has not been fully understood. Overall, the small molecules described above have shown modest potency, lack of selectivity and inadequate pharmacological properties.

Spiro-oxoindoles and *cis*-imidazolines are the most potent inhibitors of the p53-MDM2 interaction and their potency correlate with the capability to inhibit cell growth. Among the generated compounds, the *cis*-imidazoline analogues (nutlins), first described in 2004, represent a promising class due to their potency and selectivity towards the p53-MDM2 complex, Fig. (**2**) and Fig. (**3**) [25]. Preclinical studies in cell systems and in tumor xenografts support their therapeutic interest for treatment of wild-type p53 tumors. A promising feature of the compounds appears the lack of significant toxicity on normal cells, in which late and reversible inhibition of proliferation after long-term exposure to high concentrations was documented [25].

Nutlins are synthetic *cis*-imidazoline analogues identified using a high-throughput and computer modelling approaches, in which different libraries of chemicals were tested for capability of binding to the hydrophobic cleft of the p53-MDM2 complex. The rationale for such screening came from structural studies of the p53-MDM2 complex, which suggested the possibility of disrupting p53-MDM2 binding. Indeed, the compounds were shown to displace the recombinant p53 protein from the complex, with IC_{50} values around

	Inhibition of p53-MDM2 interaction (IC_{50} μM)	**Cell growth inhibition (IC_{50} μM)**
 **α-helical mimetics (1)**	10-20	10-30
 **isoindolinones (2)**	5.3-200	
 **spiro-oxindoles (3)**	0.086-8.46	0.83 (wt p53) 22.5 (mut p53)
 cis-**imidazolines (4)**	0.09-0.26	1.4-1.8 (wt p53) 13-21 (mut p53)

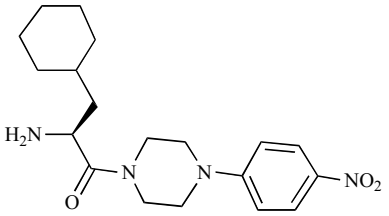
(Fig. 2. Contd….)



benzodiazepines (5)

0.22-0.7

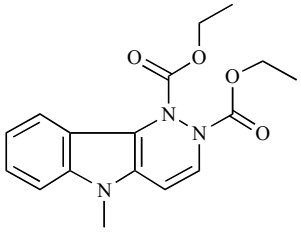
7-30



substituted piperazines (6)

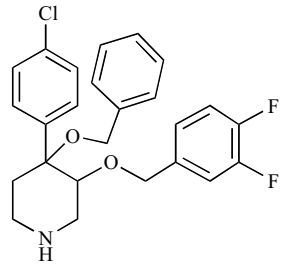
0.03-200

4



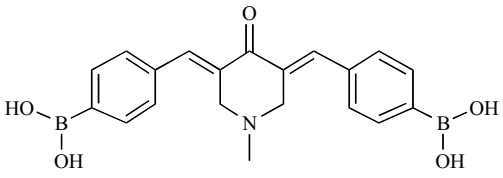
fused indoles (7)

10-26 (wt p53)
42-50 (mut p53)



substituted piperidines (8)

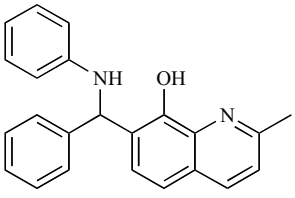
3-100



aryl boronic acids (9)

0.017

4.4-1.3



quinolinol (10)

<10

Fig. (2). Inhibitors of p53-MDM2 interaction. The reported IC_{50} values are from [14,15].

100 nM. As a consequence of binding inhibition, functional p53 is made available for triggering cellular effects [25].

Although the best candidate molecules, designated as nutlin 1, nutlin 2 and nutlin 3 – (±)4-[4, 5*bis*-(4-chlorophenyl)-2-(2-isopropoxy-4-methoxy-phenyl)-4, 5-dihydro-imidazole-1-carbonyl]piperazin-2-one - were initially synthetized as racemic compounds, studies using Biacore's surface plasma resonance technology have lead to the selection of active enantiomers. Indeed, nutlins have been proved to interact

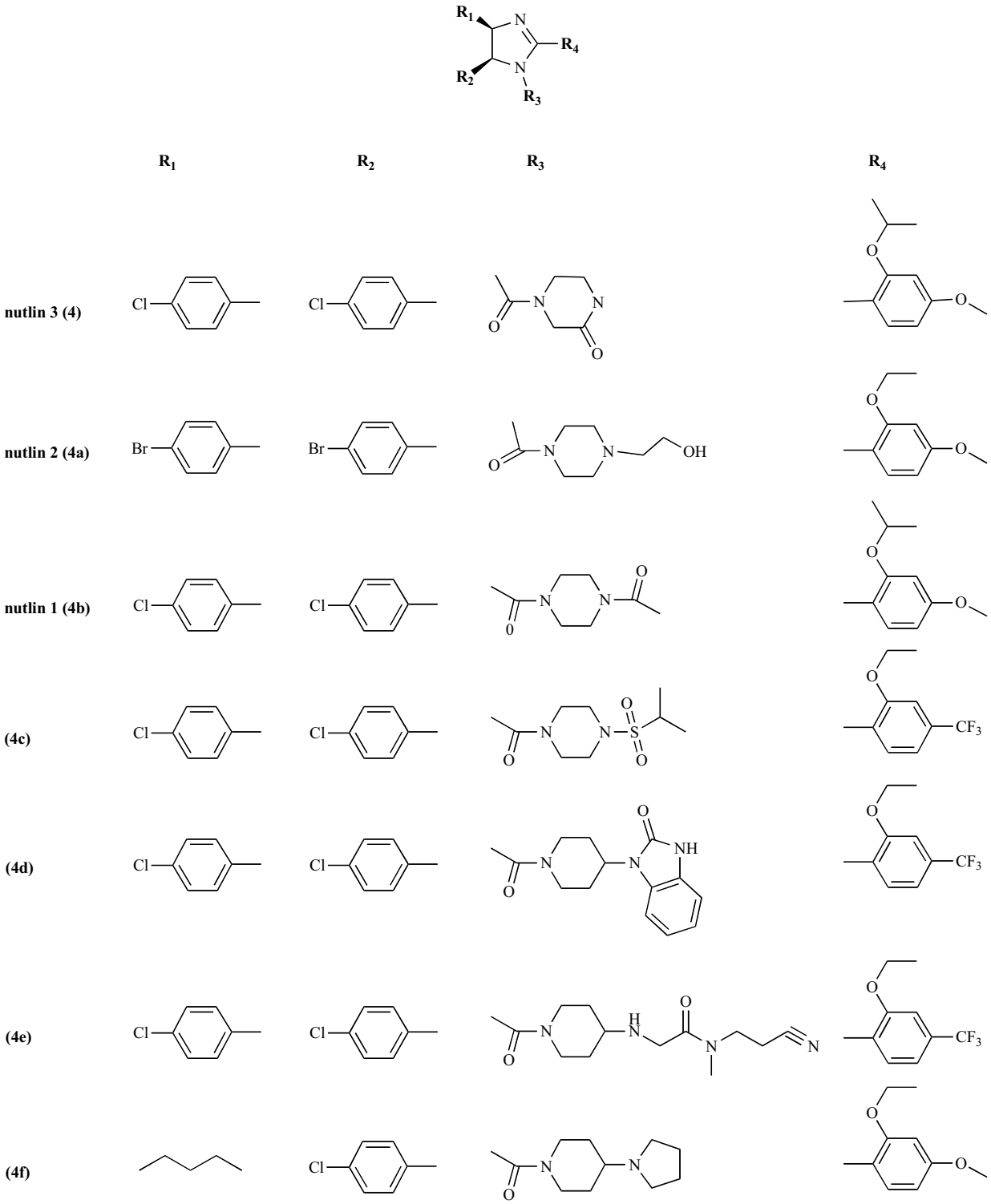


Fig. (3). *Cis*-imidazoline inhibitors of p53-MDM2 interaction. The most active compounds are reported.

with the hydrophobic cleft of MDM2, with nutlin 3 enantiomer a exhibiting the highest affinity. In fact, its IC_{50} value was around 0.1 μM, 150-fold lower than that of enantiomer b [25-27].

The mode of binding of nutlins to MDM2, determined according to the crystal structure of the MDM2-compound complex, supports that nutlins mimic the interaction of p53 because the scaffold of the antagonist reproduces features of the helical backbone of the p53 peptide. Indeed, the *cis*-imidazoline structure can be regarded as a molecular scaffold that directs three groups into the pockets usually occupied by the p53 aminoacids (Phe19, Trp23 and Leu26) (http://www.rcsb.org, PDB code 1YCR) [25]. In particular, the resolution of the crystal structure of MDM2-nutlin2 (http://www.rcsb.org, PDB code 1RV1) indicates that one bromophenyl moiety sits deeply in the Trp23 pocket, the other bromophenyl group occupies the Leu26 pocket, and the ethyl ether side chain directs towards the Phe19 pocket. In an attempt to improve the inhibitory potency, many compounds have been synthesized. In general, the new analogues fall in two categories, a) N-unsubstituted imidazolines and b) N-substituted imidazolines, Fig. (**3**) [14]. Although both series of derivatives appear active in reducing the p53-MDM2 interaction, the N-substituted compounds exhibited the best activity. This observation indicates the importance of a substituent in this position that appears critical in favouring the interaction with MDM2. In this regard, a forth aminoacid (Leu22) has been proposed to be important for p53-MDM2 binding [28]. X-ray data indicate that Leu22 is partially solvent exposed, as opposed to the main triad aminoacids, which are buried inside the binding pocket (http://www.rcsb.org, PDB code 1YCR). Thus, the introduction of polar substituents mimicking this moiety opened the way for further potency optimization, and N-substituted *cis*-imidazolines bearing polar groups could explain the increased potency in comparison to N-unsubstituted derivatives. In fact, none of them is more potent than nutlin 3, Fig. (**3**) [14].

The analysis of the cellular effects of nutlins in tumor cells has documented an activation of the p53 pathway in wild-type p53 cells, in the absence of p53-dependent responses typical of genotoxic agents i.e., lack of p53 phosphorylation [29,30]. Nutlins cause a dose-dependent increase in p53, $p21^{WAF1}$ and MDM2 levels [30]. The increase in p53 levels is due to a post-translational mechanism (i.e., reduced degradation) and does not involve up-regulation of gene expression. The effect on MDM2 itself is a rebound of the transcriptional activation by p53, that activates also an autoregulatory feedback loop helping to switch off p53 at the end of the response. In different cell systems, nutlins consistently induce p53-dependent cell cycle arrest with depletion of the S-phase fraction of cells and accumulation in G1 and G2 phases, together with triggering of p53-mediated apoptosis.

Many efforts have been performed in the search for more active compounds and recently, a spiro-oxindole derivatives named MI-63 has been found to be as potent as nutlin 3 [28].

The drug discovery effort resulted in the identification of a large number of chemically diverse small molecules capable of disrupting the p53-MDM2 interaction [14,15]. The specificity of the target inhibition and the precise mechanism of action remain to be elucidated in several cases (e.g., chalcones) [18,19,20].

Small Molecules Activators of Mutant p53

There are several types of small molecules that restore the p53 function. Here we focus on PRIMA-1 (p53-reactivation and induction of massive apoptosis) and MIRA-1 (mutant p53 reactivation and induction of rapid apoptosis), which act on mutant p53 protein. Since many tumors carry p53 mutations, the reactivation of p53-specific DNA binding is crucial to sensitize cells to p53-mediated apoptosis and strategies targeting mutant p53 should result in improvement of clinical outcome [31]. The great majority of p53 mutations in tumors are missense point mutations in the DNA binding core domain, thereby altering protein folding and abolishing specific DNA binding of p53.

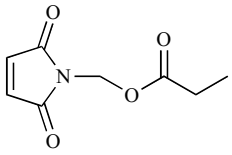
Small molecules identified by a chemical library screening have shown anticancer properties through restoration of the DNA binding activity of a mutant p53 [32]. The compound PRIMA-1 induces mutant p53-dependent apoptosis and inhibits *in vivo* tumor growth [33]. Recently, the maleimide analogues MIRA-1, have been reported to induce apoptosis in human tumors by restoring the normal function of mutant p53, Fig. (**4**) [31]. Although structurally unrelated, PRIMA-1 and MIRA-1, have similar activity profiles [31,33-35]. The compounds appear to affect mutant p53 through different mechanisms. PRIMA-1 induces expression of Heat Shock Protein 90 (HSP90) and enhances its binding to mutant p53, indicating that HSP90 mediates mutant p53 refolding [36]. Redox effects are apparently involved in the action of MIRA-1. The maleimide group contained in MIRA-1 potentially reacts with thiol and amino groups of the proteins. A reactive 3-4 double bond in the maleimide group has been proposed to be required for activity. Since the core domain of human p53 contains 10 cysteine residues, it is conceivable that covalent modification of these residues plays a role in conformational rescue leading to restoration of p53 functions [37,38]. Modification of cysteine residues has been proposed to inhibit disulfide bond formation and promote proper p53 folding. However, the mechanism of action of MIRA-1 and derivatives remains to be clarified.

Recently, a series of thiopyridine triazine derivatives was reported as promising p53 activators [39,40]. Although interesting, the mechanisms undergoing their action remain to be elucidated.

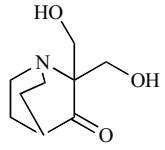
Stabilization of Wild-Type p53: Ubiquitin Ligase Inhibitors and Histone Deacetylase Inhibitors

Another approach to stabilize p53 cellular levels is by protecting p53 from MDM2-mediated ubiquitylation with ubiquitin ligase inhibitors. Small molecules, such as benzenesulfonamides, urea and imidazolone derivatives, inhibit E3 ligase activity of MDM2, thus preventing p53 ubiquitylation, Fig. (**4**) and Fig. (**1**) [41]. Another set of compounds, including 5-deazaflavin, have been found to inhibit MDM2 autoubiquitylation, thus leading to the activation of p53 function allowing the stabilization of both MDM2 and p53 [42]. The compounds only increase the amount of MDM2 and p53

Small molecules activators of mutant p53

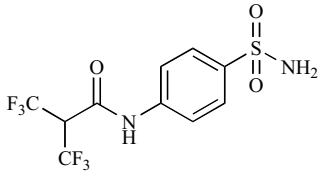


MIRA-1 (11)

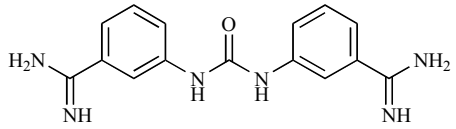


PRIMA-1 (12)

MDM2 ubiquitin ligase inhibitors

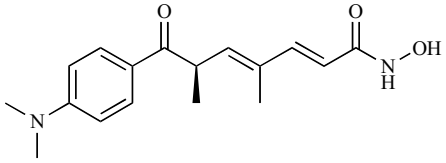


benzenesulfonamides (13)

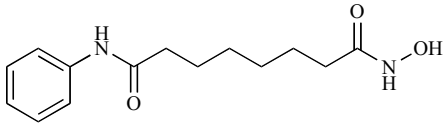


urea derivatives (14)

Histone deacetylase inhibitors



trichostatin A (15)



SAHA (16)

Fig. (4). Chemical structures of the most active small molecules activators of mutant p53, ubiquitin ligase inhibitors and histone deacetylase inhibitors.

but not that of other proteins, indicating that they are specific for E3 activity of MDM2.

Another crucial post-traslational modification of p53 is the acetylation process [43-48]. Acetylation of lysine residues at the C-terminal DNA binding regulatory domain of p53 by histone acetyltransferase (HAT) activates the expression of p53-target genes, whereas, histone deacetylase (HDAC) enzymes, which deacetylate p53, reduce p53 capability to transactivate target genes. Interestingly, MDM2 can promote p53 deacetylation by recruiting a separate complex containing HDAC [49]. Because lysine residues acetylated in p53 overlap with those that are ubiquitinated, p53 acetylation serves to promote protein stability. Unacetylated lysines then are ubiquitinated by MDM2, which ultimately leads to the disruption of p53.

Molecules that modulate p53 acetylation have been found to promote stability of the protein. In particular, small molecules inhibitors of HDAC increase the activation of p53 target genes, Fig. (**4**). HDAC inhibitors induce different effects in tumor cells, including growth arrest and cell death through diverse modes (apoptosis, autophagy, mitotic catastrophe and senescence) [50].

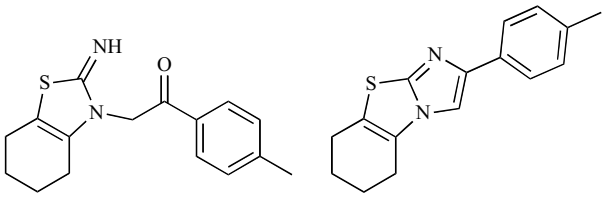
The main structural classes of available HDAC inhibitors include a) short-chain fatty acids (e.g., butyrates), b) hydroxamic acids (e.g., trichostatin A, TSA; suberoylanilide hydroxamic acid, SAHA), c) cyclic peptides and d) benzamides.

Phase I/II clinical trials with different HDAC inhibitors are ongoing and SAHA has been approved by FDA for the treatment of cutaneous T-cell lymphoma [50].

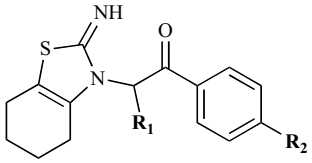
Other agents which could stabilize wild-type p53 have been identified. CP31398, a potent agent effective *in vitro*, exhibits significant antitumor activity [51]. Its mechanism of action is still unclear.

CHEMICAL INHIBITORS OF P53

Under some circumstances, the inhibition of p53 function may have therapeutic implications (e.g., chemoprotection of normal cells from apoptosis under acute stress conditions). Pifithrins (PFTs) are a new class of chemical inhibitors among which pifithrin-α, (PFT-α, 2-(2-imino-4,5,6,7-tetrahydrobenzothiazol-3-yl)-1-(4-methylphenyl)ethanone) has emerged as a leading compound, Fig. (**5**). PFT-α was isolated by screening of a chemical library in a cell-based system for its ability to reduce p53-dependent transactivation, and has been suggested to be effective in suppressing the side-effects of cancer treatment. In fact, although p53-mediated apoptosis is important for tumor suppression, it may contribute to side effects of therapy such as myelosuppression [52]. PFT-α reduces the activation of p53-regulated genes, including cyclin G, $p21^{WAF1}$, 14-3-3-σ and MDM2 and protects from genotoxic stress associated with cancer treatment [53], including apoptosis induced by a variety of stimuli in normal cells (i.e., doxorubicin-induced apoptosis in heart; camptothecin-, ischemia-, and dopamine-induced apoptosis in neurons; cisplatin-induced apoptosis in cochlear



Pifithrin a (17) **Pifithrin b (18)** **Pifithrin μ (19)**



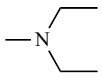
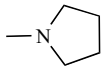
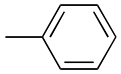
	R_1	R_2
(17)	H	CH_3
(17a)	H	H
(17b)	H	
(17c)	H	
(17d)	H	
(17e)	H	Cl
(17f)	H	
(17g)	CN	

Fig. (5). Chemical structure of pifithrins. The most active pifithrin-α derivatives are reported.

and vestibular hair cells; and endotoxin-induced apoptosis in liver tissue) [54-58]. The antiapoptotic effect of PFT-α is p53-dependent and involves suppression of caspase activation [57-62]. In neuronal cells, caspase inhibition by PFT-α correlates with suppression of mitochondrial dysfunction [63-66]. A direct inhibition of p53 translocation to mitochondria was found in kidney cells as a result of PFT-α action [67,68]. PFT- α lowers the levels of nuclear p53, but not those of cytoplasmic p53 protein after DNA damage [53,54,58,67-69]. These observations indicate that PFT-α might modulate the nuclear import/export (or both) of p53 and/or decrease stability of nuclear p53. Besides, it was shown that PFT-α caused an increase in diameter of functional nuclear pore [70]. Although the molecular mechanism through which these effects are achieved remains to be elucidated, PFTs could act by disrupting the hydrophobic interaction which drives p53-dimers association in stable tetramers [71].

A condensation product of PFT-α, from which water is spontaneously eliminated giving a ring closure, is referred to as pifithrin-β (PFT-β, 2-p-tolyl-5, 6, 7, 8-tetrahydrobenzo[d]-imidazo[2,1-b]thiazole), Fig. (**5**). Although less soluble, it is more stable than PFT-α in tissue culture medium and some of the inhibitory effects previously ascribed to PFT-α could be due to PFT-β or to a combination of the two compounds [72]. Since the conversion of PFT-α proceeds *via* an intramolecular cyclization reaction involving the imine and carbonyl groups, modification of the carbonyl function has been useful in synthesizing stable analogues of PFT-α exhibiting higher solubility [73]. Recently, a class of analogues belonging to the PFT-α and PFT-β bearing an exacyclic aromatic ring instead of a saturated ring was reported [74]. The aromatic analogues of PFT-β were highly active, whereas this modification in the aromatic PFT-α series dramatically diminished the activity. In addition, the absence of substituents at the N-position of the iminothiazole heterocy-

cle of PFT-α reduces the activity of the derivatives [75], while the introduction of lipophilic substituents (e.g., diethylamino, pyrrolidinyl, methoxy), large aromatic groups (e.g., phenyl) or chloro in para position of the phenyl ring showed similar and even greater activity than PFT-α, Fig. (**5**) [74,75]. Novel PFT-α analogues bearing different substituents on the methylene group on acetophenone moiety were reported. Some of these PFTs were more potent p53 inhibitors than the reference compound PFT-α [76]. In particular, the CN substitute derivative which also bears a metoxy group in para position of the phenyl ring has been found to be very active, Fig. (**5**). In addition, the introduction of an oxazole instead of a thiazole ring increases the potency of the PFT-α [75]. The most active PFT derivatives are reported in Fig. (**5**).

PFT-α and PFT-β act on p53 activities leading to inhibition of both apoptosis and growth arrest, Fig. (**1**). Growth arrest is mediated by activation of transcription of p53-responsive genes involved in cell cycle checkpoint control, such as $p21^{WAF1}$ and 14-3-3-σ. Apoptosis is triggered in most radiosensitive organs, such as thymus, by translocation of p53 into mitochondria and, in part, through transactivation of pro-apoptotic genes, such as Bax, Noxa or p53-upregulated modulator of apoptosis (PUMA), Fig. (**1**) [77]. Thus, different branches of the p53 pathway can be targeted separately by developing new classes of p53 inhibitors that are specific against either apoptotic or growth arrest functions of p53. This possibility has been recently supported by isolation of two new classes of PFTs [78]. These molecules can inhibit p53-dependent apoptosis in the absence of effects on p53-dependent transactivation and growth arrest (anti-apoptotic PFT series) or, on the other hand, can block p53-dependent transactivation without affecting p53-mediated apoptosis (anti-transactivation PFT series). Anti-apoptotic PFTs showed radio- and chemo -protective properties *in vivo*, while anti-transactivation PFTs may be used in combination with radio- and chemotherapy to sensitize tumors to treatment, Fig. (**1**) [78].

Among the anti-apoptotic PFTs, pifithrin-μ (PFT-μ, 2-phenylethynesulfonamide) was recently discovered as a selective inhibitor of mitochondrial branch of the p53 pathways, Fig. (**5**) and Fig. (**1**) [78]. PFT-μ inhibits p53 binding to mitochondria by reducing its affinity to antiapoptotic proteins Bcl-xL and Bcl-2 but has no effect on p53-dependent transactivation. PFT-μ has a high specificity for p53, protects cells from apoptosis and has been found to protect mice from doses of radiation that cause a lethal hematopoietic syndrome [78].

DRUG COMBINATION STRATEGIES TO IMPROVE CANCER THERAPY

In the search for more efficacious and less toxic antitumor therapy, p53 has long been a exploitable target. In this context, combination studies including molecules which target p53 functions with different conventional anticancer drugs have been proposed.

Nutlin 3 has been reported to synergize with Tumor Necrosis Factor α (TNF-α) in lung cancer cells [79]. The combination treatment decreases cell viability in a p53-dependent manner and strikingly inhibits the expression of Intercellular Adhesion Molecule 1 (ICAM-1) and Monocyte Chemotactic Protein-1 (MCP-1), which are involved in cancer cell invasion and metastasis. Thus, nutlin 3 has been proposed for lung cancer therapy [79]. Increased apoptosis and cell cycle arrest is observed following concomitant administration of nutlin and radiation in wild-type p53 lung cancer cells. The combination has also been found to decrease the ability of endothelial cells to form vasculature [80].

Since deletions and/or mutations of the p53 gene occur in only 5-10% of acute myeloid leukemia (AML) [81], recombinant Tumor Necrosis Factor α -Related Apoptosis-Inducing Ligand (TRAIL) and nutlin 3 have been combined in AML cell system [81]. The simultaneous combination of TRAIL and nutlin 3 results in caspase-dependent cleavage of $p21^{WAF1}$, and sensitization to apoptosis as a consequence of concomitant activation of extrinsic and intrinsic apoptotic pathways.

The pharmacological activation of the p53 pathway with nutlin 3 in Hodgkin lymphoma-derived cell lines leads to apoptosis induction and sensitizes the cells to other anticancer drugs. In particular, nutlin 3 synergizes with conventional chemotherapeutics and/or inhibitors of p53-independent survival pathways such as the HSP90 inhibitor geldanamycin, thus providing an attractive option for combination therapy in patients with Hodgkin lymphoma [82].

Although p53 mutations are rare in B-cell chronic lymphocytic leukemia (CLL), MDM2 overexpression is a frequent event resulting in p53 dysfunction. Nutlin 3 has been found to synergize with doxorubicin, chlorambucil, and fludarabine in these cells and, interestingly, the synergistic apoptotic effect was maintained in fludarabine-resistant cells [83,84].

Nutlins have been proposed to have utility in protecting normal proliferating tissues during antimitotic chemotherapy of tumors expressing mutant p53 [30]. Since normal cells possess wild-type functional p53, pretreatment with nutlins can arrest their proliferation and protect them from the toxicity of paclitaxel. As expected, cancer cells bearing mutant p53 are insensitive to nutlins but sensitive to paclitaxel-induced apoptosis because taxanes can activate p53-independent apoptosis [30]. This response is not a general feature since combination of nutlin and paclitaxel on mutant p53 MDA-MB-435 cells increases mitotic arrest and induces massive apoptosis. In contrast, the MDM2 antagonists, through activation of p53 pathway, may have a protective effect from mitotic inhibitors in wild-type p53 HCT116 and RKO colon cancer cells [85].

Cytosolic sequestration of hyperubiquitinated wild-type p53 is one of the several mechanisms that attenuate p53 function in neuroblastoma. Nutlin 3 has been observed to synergize with camptothecins by inducing p53 relocalization from the cytoplasm to the nucleus and promoting reactivation of p53 transcriptional and apoptotic function by inducing p53 deubiquitination [86].

Synergistic interaction has been observed between PRIMA-1 and adriamycin, cisplatin or flutarabine [31,87]. The effect appears to result from enhanced expression of

mutant p53 induced by chemotherapeutic drugs [31]. PRIMA-1 synergizes with adriamicyn in breast cancer, especially in cells with aberrant p53 function [36]. The restoration of the functions of p53 *via* PRIMA-1 has been found to reduce the migration, invasion and metastasis of breast cancer MDA-MB-231 cells by reducing the expression of the chemokine (C-X-C motif) receptor 4 (CXCR4) [88].

HDAC inhibitors have been reported to increase the cytotoxicity of several antitumor agents, including DNA-damaging agents which are known to activate p53 [89]. These synergistic combinations may have potentially clinical relevance. However, due to the pleiotropic effects of HDAC inhibition, the synergistic interaction could not be ascribed solely to modulation of p53 function.

TSA has been found to improve the efficacy of 5-fluorouracil, paclitaxel and SN38 in gastric cancer cell lines [90]. In addition, simultaneous treatment with TSA and doxorubicin increases cell death in androgen-receptor positive prostate cancer cells [91]. This synergistic effect of TSA has been ascribed to the up-regulation of p53. Pretreatment with TSA or SAHA increases the killing efficiency of VP-16, ellipticine, doxorubicin and cisplatin in human cancer cell lines of different tissue origin. Interestingly, the sensitization has been found in D54 cells, a brain tumor cell line intrinsically resistant to topoisomerase II inhibitors, and correlated with markedly induction of p53, $p21^{WAF1}$, and Growth Arrest and DNA Damage 45 protein (GADD45) levels [92].

The growth arrest function of p53 may have a protective effect against the cytotoxicity of a number of antitumor agents. On this basis, some combination strategies with the use of PFT have been designed. Recent evidence indicates that p53 is involved in androgen signaling in prostate cancer cells [93]. PFT-α reduces the transactivation of androgen-dependent protein expression mediated by p53 in prostate cancer cells, suggesting a possible use of PFTs during the androgen dependent growth of prostate cancers.

Particularly relevant are the results observed by combining topotecan with PFT-α in human glioblastoma cells [94]. Topoisomerase I has been found to be degraded upon topotecan treatment in p53 wild-type U87 cells but not in p53-deficient U138 cells, indicating that a wild-type protein is important for DNA damage repair leading to camptothecin resistance. PFT-α has been observed to synergize with topotecan by attenuating topoisomerase I degradation in wild-type p53 cells and reversing the resistance phenotype.

Exposure of astrocytic glioma U87MG cells to PFT-α before BCNU and temozolomide (cytotoxic drugs that are modestly helpful in the treatment of aggressive astrocytic gliomas) attenuates p53-mediated induction of $p21^{WAF1}$ protein resulting in sensitization of glioma cells to these drugs [95].

PFT-α has been found to overcome Ara-C resistance in a murine BXH-2 strain AML cell lines which exhibited an aberrant p53 protein. PFT-α induces apoptosis in both Ara-C-sensitive and -resistant cell lines, and decreases Ara-C resistance in cells with either normal or mutant p53. Thus, PFT-α could be useful in treatment of relapsed AML [96]. PFT-α was found to enhance chemosensitivity by a mechanism independent of p53 and involving p38 MAPK deregulation of Eukaryotic translation Initiation Factor 4E (eIF-4E) phosphorylation [97]. In colangiocarcinoma KMCH cells, which contain a functionally inactivating p53 mutation and increased levels of initiation factor eIF-4E, PFT-α proved to be active in enhancing chemosensitivity to gemcitabine in an eIF-4E dependent manner. Moreover, modulation of eIF-4E phosphorylation is an attractive therapeutic target for intervention in cancer treatment since this initiation factor is overexpressed in many cancers [98].

These observations, indicating a synergistic interaction in p53 defective cells, support a complex mechanism of action of PFTs, involving also p53-independent mechanisms.

CONCLUSIONS

Despite the remarkable progress in understanding p53 functions, there are still many issues that need to be addressed before defining the therapeutic value and applications of p53 modulators. Although preclinical efforts on these small molecules are growing, the discovery and development of p53 modulators as therapeutic agents are still limited by the non-enzymatic nature of p53 as drug target. The best known p53 inhibitors belong to the emerging category of small-molecule modulators of protein-protein interaction targeting p53 protein or components of the p53 pathway. The development of pharmacological agents of this category is more problematic than the conventional enzyme inhibitors. A rational approach to optimize p53 modulators is still limited by a lack of detailed structural information concerning its interaction with other cellular proteins. The most promising modulators of p53 function appear MDM2 antagonists and the transcriptional inhibitors of p53. The possible applications of such modulators are quite different. Indeed, on the basis of different functions of p53 in response to various cytotoxic stresses, it may be useful to activate p53 or to inhibit its functionality, depending on the nature of cytotoxic injury and on the biological context.

The p53-MDM2 interaction has been validated as a novel therapeutic target. The available evidence supports the therapeutic interest of p53 activation by p53-MDM2 antagonists. In particular, the discovery of the nutlins strongly suggests that the p53-MDM2 interaction can be successfully manipulated with small molecules. Early studies with the nutlins have indicated that MDM2 antagonists may be effective as single agents if tumor cells possess wild-type p53 and relatively intact down-stream p53 signaling. In addition, nutlins appear to be effective in synergizing with different antitumor agents, thereby providing the rationale for novel combination approaches.

Due to the high frequency of mutation of the p53 gene observed in tumors, the compounds capable of reactivating the DNA binding activity of mutated p53 appear very interesting. However, these molecules have a low grade of specificity for p53. Indeed, their mechanism of action likely involves thiol and amino groups, which are widely represented in the cells.

The role of ubiquitin ligase inhibitors and HDAC inhibitors in reducing tumor growth is less specific, since they act

not only on p53. In this context, particularly relevant are HDAC inhibitors, which act by inducing chromatin remodeling and favoring gene expression. HDAC inhibitors are very active in reducing tumor growth and some of them have been approved for clinical treatment.

The understanding that the apoptotic and growth arrest functions of p53 can be targeted separately by developing specific p53 inhibitors has opened new perspectives. In this regard, PFT-μ represents the first reported compound able to inhibit p53-dependent apoptotic pathways and is emerging as a promising chemoprotective molecule. There is a need for new PFTs targeting only the growth arrest branch of the p53 pathways since they are expected to sensitize tumor cells when used in combination with radio- and chemo-therapy. To date, no molecules with such specificity are available. Relevant to this point is the observation that in contrast to their chemoprotection activity (e.g., reduction of side effects of chemo- and radiotherapy), PFTs could be useful in potentiate cancer cell killing mediated by different antitumor agents.

It is important to underline that the inhibition of p53 by PFTs in cell treated with DNA damaging agents and bearing wild-type p53 reduces apoptosis, while increases chromosomal aberration [99]. In addition, Lin and coworkers reported the suppression of high-fidelity double-strand break repair in mammalian cells treated with PFTs [100]. Thus, the temporary suppression of the function of p53 by PFTs aimed at increasing the survival of normal cells should be a promising approach to reduce the side-effects of cancer therapy but, the surviving cells could be genetically modified by genotoxic drugs and consequently the risk of secondary tumors may be increased.

In conclusion, since p53 has no enzymatic activity, the design of small molecules targeting p53 requires approaches more sophisticated than those used for inhibitors of catalytic activity of enzyme target. In addition to problems of target selectivity, the cell-based assay technologies (e.g., effects on intracellular signal transduction pathways, including intracellular protein translocation) may generate obvious difficulties in the interpretation of the mechanism of action of p53 modulators. It is well known that several cytotoxic agents or stress conditions can modulate p53 activity. Moreover, as observed with HDAC inhibitors, other non-specific agents, including redox modulators, may influence p53 function.

In spite of the complexity of methodological approaches, the relevance of p53 function supports its interst as a therapeutic target.

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ABBREVIATIONS

Bcl-2	=	B-cell leukemia 2
PUMA	=	p53-upregulated modulator of apoptosis
HSP90	=	Heat Shock Protein 90
TNF-α	=	Tumor Necrosis Factor α
ICAM-1	=	Intercellular Adhesion Molecule 1
MCP-1	=	Monocyte Chemotactic Protein-1
TRAIL	=	Tumor Necrosis Factor α-Related Apoptosis-Inducing Ligand
GADD45	=	Growth Arrest and DNA Damage 45 protein
eIF-4E	=	Eukaryotic translation Initiation Factor 4E
CXCR4	=	Chemokine (C-X-C motif) receptor 4
PFT	=	Pifithrin
MDM2	=	Murine double minute 2
AML	=	Acute myeloid leukemia
CLL	=	B-cell chronic lymphocytic leukemia
RITA	=	Reactivation of p53 and induction of tumor cell apoptosis
PRIMA-1	=	p53-reactivation and induction of massive apoptosis
MIRA-1	=	Mutant p53 reactivation and induction of rapid apoptosis
TSA	=	Trichostatin A
SAHA	=	Suberoylanilide hydroxamic acid
HAT	=	Histone acetyltransferase
HDAC	=	Histone deacetylase.

REFERENCES

[1] Fuster, J.J.; Sanz-Gonzalez, S.M.; Moll, U.M.; Andres, V. *Trends Mol. Med.,* **2007**, *13*, 192.
[2] Levine, A.J. *Cell,* **1997**, *88*, 323.
[3] Vogelstein, B.; Lane, D.; Levine, A. J. *Nature,* **2000**, *408*, 307.
[4] Hollstein, M.; Sidransky, D.; Vogelstein, B.; Harris, C.C. *Science,* **1991**, *253*, 49.
[5] Hainaut, P.; Hollstein, M. *Cancer Res.,* **2000**, *77*, 81.
[6] Fakharzadeh, S.S.; Trusko, S.P.; George, D.L. *EMBO J.*, **1991**, *10*, 1565.
[7] Oliner, J.D.; Kinzler, K.W.; Meltzer, P.S.; George, D.L.; Vogelstein, B. *Nature,* **1992**, *358*, 80.
[8] Oliner, J.D; Pietenpol, J.A.; Thiagalingam, S.; Gyuris, J.; Kinzler, K.W.; Vogelstein, B. *Nature,* **1993**, *362*, 857.
[9] Haupt, Y.; Maya, R.; Kazaz, A.; Oren, M. *Nature,* **1997**, *387*, 296.
[10] Leng, R.P.; Lin, Y.; Ma, W.; Wu, H.; Lemmers, B.; Chung, S.; Parant, J.M.; Lozano, G.; Hakem, R.; Benchimol, S. *Cell,* **2003**, *112*, 779.
[11] Dornan, D.; Wertz, I.; Shimizu, H.; Arnott, D.; Frantz, G.D.; Dowd, P.; O'Rourke, K.; Koeppen, H.; Dixit, V.M. *Nature,* **2004**, *429*, 86.
[12] Willis, A.C.; Chen, X. *Curr. Mol. Med.,* **2002**, *2*, 329.
[13] Fang, B.; Roth J.A. *Cancer Biol. Ther.,* **2003**, *2*, 115.
[14] Deng, J.; Dayam, R.; Neamati, N. *Expert Opin. Ther. Patents,* **2006**, *16*, 165.
[15] Dudkina, A.S.; Lindsley, C.W. *Curr. Top. Med. Chem*., **2007**, *7*, 952.
[16] Duncan, S.J.; Grüschow, S.; Williams, D.H.; McNicholas, C.; Purewal, R.; Hajek, M.; Gerlitz, M.; Martin, S.; Wrigley, S.K.; Moore, M. *J. Am. Chem. Soc.,* **2001**, *23*, 554.
[17] Chène, P.; Fuchs, J.; Bohn, J.; García-Echeverría, C.; Furet, P.; Fabbro, D. *J. Mol. Biol.,* **2000**, *299*, 245.
[18] Stoll, R.; Renner, C.; Hansen, S.; Palme, S.; Klein, C.; Belling, A.; Zeslawski, W.; Kamionka, M.; Rehm, T.; Mühlhahn, P.; Schu-

macher, R.; Hesse, F.; Kaluza, B.; Voelter, W.; Engh, R.A.; Holak, T.A. *Biochemistry,* **2001**, *40*, 336.
[19] Kumar, S.K.; Hager, E.; Pettit, C.; Gurulingappa, H.; Davidson, N.E.; Khan, S.R. *J. Med. Chem.,* **2003**, *46*, 2813.
[20] Modzelewska, A.; Pettit, C.; Achanta, G.; Davidson, N.E.; Huang, P.; Khan, S.R. *Bioorg. Med. Chem.,* **2006**, *14*, 3491.
[21] Zhao, J.; Wang, M.; Chen, J.; Luo, A.; Wang, X.; Wu, M.; Yin, D.; Liu, Z. *Cancer Lett.,* **2002**, *183*, 69.
[22] Galatin, P.S.; Abraham, D.J. *J. Med. Chem.,* **2004**, *47*, 4163.
[23] Issaeva, N.; Bozko, P.; Enge, M.; Protopopova, M.; Verhoef, L.G.; Masucci, M.; Pramanik, A.; Selivanova, G. *Nat. Med.,* **2004**, *10*, 1321.
[24] Bunz, F.; Dutriaux, A.; Lengauer, C.; Waldman, T.; Zhou, S.; Brown, J.P.; Sedivy, J.M..; Kinzler, K.W.; Vogelstein, B. *Science*, **1998**, *282*, 1497.
[25] Vassilev, L.T.; Vu, B.T.; Graves, B.; Carvajal, D.; Podlaski, F.; Filipovic, Z.; Kong, N.; Kammlott, U.; Lukacs, C.; Klein, C.; Fotouhi, N.; Liu, E.A. *Science,* **2004**, *303*, 844.
[26] Drakos, E.; Thomaides, A.; Medeiros, L.J.; Li, J.; Leventaki, V.; Konopleva, M.; Andreeff, M.; Rassidakis, G.Z. *Clin. Cancer Res.,* **2007**, *13*, 3380.
[27] Wang, Z.; Jonca, M.; Lambros, T.; Ferguson, S.; Goodnow, R. *J. Pharm. Biomed. Anal.,* **2007**, *45*, 720.
[28] Ding, K.; Lu, Y.; Nikolovska-Coleska, Z.; Wang, G.; Qiu, S.; Shangary, S.; Gao, W.; Qin, D.; Stuckey, J.; Krajewski, K.; Roller, P.P.; Wang, S. *J. Med. Chem.,* **2006**, *49*, 3432.
[29] Thompson, T.; Tovar, C.; Yang, H.; Carvajal, D.; Vu, B.T.; Xu, Q.; Wahl, G.M.; Heimbrook, D.C.; Vassilev, L.T. *J. Biol. Chem.,* **2004**, *279*, 53015.
[30] Vassilev, L.T. *J. Med. Chem.,* **2005**, *48*, 4491.
[31] Bykov, V.J.N.; Zache, N.; Stridh, H.; Westman, J.; Bergman, J.; Selivanova, G.; Wiman, K.G. *Oncogene,* **2005**, *24*, 3484.
[32] Foster, B.A.; Coffey, H.A.; Morin, M.J.; Rastinejad, F. *Science,* **1999**, *286*, 2507.
[33] Bykov, V.J.; Issaeva, N.; Shilov, A.; Hulcrantz, M.; Pugacheva, E.; Chumakov, P.; Bergman, J.; Wiman, K.G.; Selivanova, G. *Nat. Med.,* **2002**, *8*, 282.
[34] Bykov, V.J.N.; Issaeva, N.; Selivanova, G.; Wiman, K.G. *Carcinogenesis,* **2002**, *23*, 2011.
[35] Bykov, V.J.; Issaeva, N.; Zache, N.; Shilov, A.; Hultcrantz, M.; Bergman, J.; Selivanova, G.; Wiman, K.G. *J. Biol. Chem.,* **2005**, *280*, 30384.
[36] Rehman, A.; Chahal, M.S.; Tang, X.; Bruce, J.E.; Pommier, Y.; Daoud, S.S. *Breast Cancer Res.,* **2005**, *7*, 765.
[37] Buzek, J.; Latonen, L.; Kurki, S.; Peltonen, K.; Laiho, M. *Nucleic Acids Res.,* **2002**, *30*, 2340.
[38] Seo, Y.R.; Kelly, M.R.; Smith, M.L. *Proc. Natl. Acad. Sci. USA,* **2002**, *99*, 14548.
[39] Mandal, S.; Bérubé, G.; Asselin, E.; Mohammad, I.; Richardson, V.J.; Gupta, A.; Pramanik, S.K.; Williams, A.L.; Mandal, S.K. *Bioorg. Med. Chem. Lett.,* **2007**, *17*, 4955.
[40] Mandal, S.; Bérubé, G.; Asselin, E.; Richardson, V.J.; Church, J.G.; Bridson, J.; Pham, T.N.; Pramanik, S.K.; Mandal, S.K. *Bioorg. Med. Chem. Lett.,* **2007**, *17*, 2139.
[41] Lai, Z.; Yang, T.; Kim, Y.B.; Sielecki, T.M.; Diamond, M.A.; Strack, P.; Rolfe, M.; Caligiuri, M.; Benfield, P.A.; Auger, K.R.; Copeland, R.A.. *Proc. Natl. Acad. Sci. USA,* **2002**, *99*, 14734.
[42] Yang, Y.; Kitagaki, J.; Dai, R.M.; Tsai, Y.C.; Lorick, K.L.; Ludwig, R.L.; Pierre, S.A.; Jensen, J.P.; Davydov, I.V.; Oberoi, P.; Li. C.C.; Kenten, J.H.; Beutler, J.A.; Vousden, K.H.; Weissman, A.M. *Cancer Cell,* **2005**, *7*, 547.
[43] Luo, J.; Li, M.; Tang, Y.; Laszkowska, M.; Roeder, R.G.; Gu, W. *Proc. Natl. Acad. Sci. USA,* **2004**, *101*, 2259.
[44] Murphy, M.; Ahn, J.; Walker, K.K.; Hoffman, W.H.; Evans, R.M.; Levine, A.J.; George, D.L. *Genes Dev.,* **1999**, *13*, 2490.
[45] Luo, J.; Su, F.; Chen, D.; Shiloh, A.; Gu, W. *Nature,* **2000**, *408*, 377.
[46] Luo, J.; Nikolaev, A.Y.; Imai, S.; Chen, D.; Su, F.; Shiloh, A.; Guarente, L.; Gu, W. *Cell,* **2001**, *107*, 137.
[47] Vaziri, H.; Dessain, S.K.; Ng Eaton, E.; Imai, S.I.; Frye, R.A.; Pandita, T.K.; Guarente, L.; Weinberg, R.A. *Cell,* **2001**, *107*, 149.
[48] Juan, L.J.; Shia, W.J.; Chen, M.H.; Yang, W.M.; Seto, E.; Lin, Y.S.; Wu, C.W. *J. Biol. Chem.,* **2000**, *275*, 20436.
[49] Ito, A.; Kawaguchi, Y.; Lai, C.H.; Kovacs, J.J.; Higashimoto, Y.; Appella, E.; Yao, T.P. *EMBO J.,* **2002**, *21*, 6236.
[50] Xu, W.S.; Parmigiani, R.B.; Marks, P.A. *Oncogene,* **2007**, *26*, 5541.
[51] Takimoto, R.; Wang, W.; Dicker, D.T.; Rastinejad, F.; Lyssikatos, J.; el-Deiry, W.S. *Cancer Biol. Ther.,* **2002**, *1*, 47.
[52] Komarova, E.A.; Gudkov, A.V. *Biochem. Pharmacol.,* **2001**, *62*, 657.
[53] Komarov, P.G.; Komarova, E.A.; Kondratov, R.V.; Christov-Tselkov, K.; Coon, J.S.; Chernov, M.V.; Gudkov, A.V. *Science,* **1999**, *285*, 1733.
[54] Liu, X.; Chua, C.C.; Gao, J.; Chen, Z., Landy, C.L.; Hamdy, R.; Chua, B.H. *Am. J. Physiol. Heart Circ. Physiol.,* **2004**, *286*, 933.
[55] Culmsee, C.; Siewe, J.; Junker, V.; Retiounskaia, M.; Schwarz, S.; Camandola, S.; El-Metainy, S.; Behnke, H.; Mattson, M.P.; Krieglstein, J. *J. Neurosci.,* **2003**, *23*, 8586.
[56] Culmsee, C.; Zhu, X.; Yu, Q.S.; Chan, S.L.; Camandola, S.; Guo, Z.; Greig, N.H.; Mattson, M.P. *J. Neurochem.,* **2001**, *77*, 220.
[57] Zhang, M.; Liu, W.; Ding, D.; Salvi, R. *Neuroscience*, **2003**, *120*, 191.
[58] Schafer, T.; Scheuer, C.; Roemer, K.; Menger, M.D.; Vollmar, B. *FASEB J*., **2003**, *17*, 660.
[59] Jiang, M.; Yi, X.; Hsu, S.; Wang, C.Y.; Dong, Z. *Am. J. Physiol. Renal. Physiol.,* **2004**, *287*, 1140.
[60] Alves da Costa, C.; Mattson, M.P.; Ancolio, K.; Checler, F. *J. Biol. Chem.,* **2003**, *278*, 12064.
[61] Alvarez-Salas, L.M.; Arpawong, T.E.; DiPaolo, J.A. *Antisense Nucleic Acid Drug Dev*., **1999**, *9*, 441.
[62] Paitel, E.; Fahraeus, R.; Checler, F. *J. Biol. Chem.,* **2003**, *278*, 10061.
[63] Tamagno, E.; Parola, M.; Guglielmotto, M.; Santoro, G.; Bardini, P.; Marra, L.; Tabaton, M.; Danni, O. *Free Radic. Biol. Med.,* **2003**, *35*, 45.
[64] Nakaso, K.; Yoshimoto, Y.; Yano, H.; Takeshima, T.; Nakashima, K. *Neurosci. Lett.,* **2004**, *354*, 213.
[65] Duan, W.; Zhu, X., Ladenheim, B.; Yu, Q.S.; Guo, Z.; Oyler, J.; Cutler, R.G.; Cadet, J.L.; Greig, N.H.; Mattson, M.P. *Ann. Neurol.,* **2002**, *52*, 597.
[66] Proietti De Santis, L.; Balajee, A.S.; Lorenti Garcia, C., Pepe, G.; Worboys, A.M.; Palitti, F. *DNA Repair,* **2003**, *2*, 891.
[67] Dagher, P.C. *Kidney Int.,* **2004**, *66*, 506.
[68] Kelly, K.J.; Plotkin, Z.; Vulgamott, S.L.; Dagher, P.C. *J. Am. Soc. Nephrol.,* **2003**, *14*, 128.
[69] Lorenzo, E.; Ruiz-Ruiz, C.; Quesada, A.J.; Hernandez, G.; Rodriguez, A.; Lopez-Rivas, A.; Redondo, J.M. *J. Biol. Chem.,* **2002**, *277*, 10883.
[70] Feldherr, C.M.; Akin, D.; Cohen, R.J. *J. Cell Sci*., **2001**, *114*, 4621.
[71] Miller, M.; Lubkowski, J.; Rao, J.K.; Danishefsky, A.T.; Omichinski, J.G.; Sakaguchi, K.; Sakamoto, H.; Appella, E.; Gronenborn, A.M.; Clore, G.M. *FEBS Lett*., **1996**, *399*, 166.
[72] Walton, M.I.; Wilson, S.C.; Hardcastle, I.R.; Mirza, A.R.; Workman, P. *Mol. Cancer Ther.,* **2005**, *4*, 1369.
[73] Gary, R.K.; Jensen, D.A. *Mol. Pharm.,* **2005**, *2*, 462.
[74] Barchechath, S.D.; Tawatao, R.I.; Corr, M.; Carson, D.A.; Cottam, H.B. *J. Med. Chem.,* **2005**, *48*, 6409.
[75] Zhu, X.; Yu, Q.S.; Cutler, R.G.; Culmsee, C.W.; Holloway, H.W.; Lahiri, D.K.; Mattson, M.P.; Greig, N.H. *J. Med. Chem.,* **2002**, *45*, 5090.
[76] Pietrancosta, N.; Maina, F.; Dono, R.; Moumen, A.; Garino, C.; Laras, Y.; Burlet, S.; Quéléver, G.; Kraus, J.L. *Bioorg. Med. Chem. Lett.,* **2005**, *15*, 1561.
[77] Gudkov, A.V.; Komarova, E.A. *Biochem. Biophys. Res. Commun.,* **2005**, *331*, 726.
[78] Strom, E.; Sathe, S.; Komarov, P.G.; Chernova, O.B.; Pavlovska, I.; Shyshynova, I.; Bosykh, D.A.; Burdelya, L.G.; Macklis, R.M.; Skaliter, R.; Komarova, E.A.; Gudkov, A.V. *Nat. Chem. Biol.,* **2006**, *2*, 474.
[79] Dey, A.; Wong, E.T.; Bist, P.; Tergaonkar, V.; Lane, D.P. *Cell Cycle,* **2007**, *6*, 2178.
[80] Cao, C.; Shinohara, E.T.; Subhawong, T.K.; Geng, L.; Woon Kim, K.; Albert, J.M.; Hallahan, D.E.; Lu, B. *Mol. Cancer Ther.,* **2006**, *5*, 411.
[81] Secchiero, P.; Zerbinati, C.; di Iasio, M.G.; Melloni, E.; Tiribelli, M.; Grill, V.; Zauli, G. *Curr. Drug Metab.,* **2007**, *8*, 395.
[82] Janz, M.; Stuhmer, T.; Vassilev, L.T.; Bargou, R.C. *Leucemia,* **2007**, *21*, 772.

[83] Coll-Mulet, L.; Iglesias-Serret, D.; Santidrián, A.F.; Cosialls, A.M.; de Frias, M.; Castaño E.; Campàs, C.; Barragán, M.; de Sevilla, A.F.; Domingo, A.; Vassilev, L.T.; Pons, G.; Gil, J. *Blood*, **2006**, *107*, 4109.

[84] Kojima, K.; Konopleva, M.; McQueen, T.; O'Brien, S.; Plunkett, W.; Andreeff, M. *Blood,* **2006**, *108*, 993.

[85] Carvajal, D.; Tovar, C.; Yang, H.; Vu, B.T.; Heimbrook, D.C.; Vassilev, L.T. *Cancer Res*., **2005**, *65*, 1918.

[86] Becker, K.; Marchenko, N.D.; Maurice, M.; Moll, U.M. *Cell Death Differ.,* **2007** *14*, 1350.

[87] Nahi, H., Lehmann, S.; Mollgard, L.; Bengtzen, S.; Selivanova, G.; Wiman, K.G.; Paul, C.; Merup, M. *Br. J. Haematol.,* **2004**, *27*, 285.

[88] Mehta, S.A.; Christopherson, K.W.; Bhat-Nakshatri, P.; Goulet, R.J. Jr.; Broxmeyer, H.E.; Kopelovich, L.; Nakshatri, H. *Oncogene,* **2007**, *26*, 3329.

[89] Bolden, J.E.; Peart, M.J.; Johnstone, R.W. *Nat. Rev. Drug Discov.,* **2006**, *5*, 769.

[90] Zhang, X.; Yashiro, M.; Ren, J.; Hirakawa, K. *Oncol. Rep*., **2006**, *16*, 563.

[91] Rokhlin, O.W.; Glover, R.B.; Guseva, N.V.; Taghiyev, A.F.; Kohlgraf, K.G.; Cohen, M.B. *Mol. Cancer Res*., **2006**, *4*, 113.

[92] Kim, M.S.; Blake, M.; Baek, J.H.; Kohlhagen, G.; Pommier, Y.; Carrier, F. *Cancer Res.,* **2003**, *63*, 7291

[93] Cronauer, M.V.; Schulz, W.A.; Burchardt, T.; Ackermann, R.; Burchardt, M. *Oncogene*, **2004**, *23*, 3541.

[94] Tomicic, M.T.; Christmann, M.; Kaina, B. *Cancer Res.,* **2005**, 65, 8920.

[95] Xu, G.W.; Mymryk, J.S.; Cairncross, J.G. *Int. J. Cancer*, **2005**, *116*, 187.

[96] Yin, B.; Kogan, S.C.; Dickins, R.A.; Lowe, S.W.; Largaespada, D.A. *Exp. Hematol.,* **2006**, *34*, 631.

[97] Wehbe, H.; Henson, R.; Lang, M.; Meng, F.; Patel, T. *J. Pharmacol. Exp. Ther.,* **2006**, *19*, 1153.

[98] De Benedetti, A.; Graff, J.R. *Oncogene,* **2004**, *23*, 3189.

[99] Bassi, L.; Carloni, M.; Fonti, E.; Palma de la Peña, N.; Meschini, R.; Palitti, F. *Mutat. Res.,* **2002**, *499*, 163.

[100] Lin, Y.; Waldman, B.C.; Waldman, A.S. *DNA Repair,* **2003**, *2*, 1.

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