Deep Insight Section

Inhibitors of the heat shock protein 90: from cancer clinical trials to neurodegenerative diseases

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I) Introduction

The 90-kDa heat shock protein 90, Hsp90, belongs to the family of molecular chaperone responsible for the conformational maturation or reparation of other proteins, referred to as "clients", into biologically active structures (Pearl and Prodromou, 2006). Hsp90 exerts its essential ATP dependant chaperone function to more than three hundred client proteins involved in cell growth, differentiation and survival (Workman, 2004; Chiosis et al., 2004; Sreedhar et al., 2004b; Zhang and Burrows, 2004; Neckers, 2002). Many of them, more than forty, include overexpressed or mutant oncogenic proteins ErbB2/HER2 (Miller et al., 1994; An et al., 1997), Braf (Grbovic et al., 2006), Akt/PKB (Sato et al., 2000), muted p53

(Blagosklonny et al., 1996), transcription factors: hormone steroid receptors GR (Grad and Picard, 2007) ER and AR, angiogenic factors HIF-1α (Picard, 2006; Kuduk et al., 2000; Kuduk et al., 1999; Johnson and Toft, 1995), telomerase (Forsythe et al., 2001; Akalin et al., 2001) which are associated with the six hallmarks of cancer (Figure 1).

Under non-stress conditions the quaternary structure of Hsp90 is now well established to be a dimeric complex, and its abundance is approximately 1% of the total protein contents. Each monomer consists in three domains: the N-terminal domain (NTD), a middle domain (MD) implicated in client protein binding, and a C-terminal dimerization domain (CTD) (Figure 2) (Harris et al., 2004; Shiau et al., 2006).
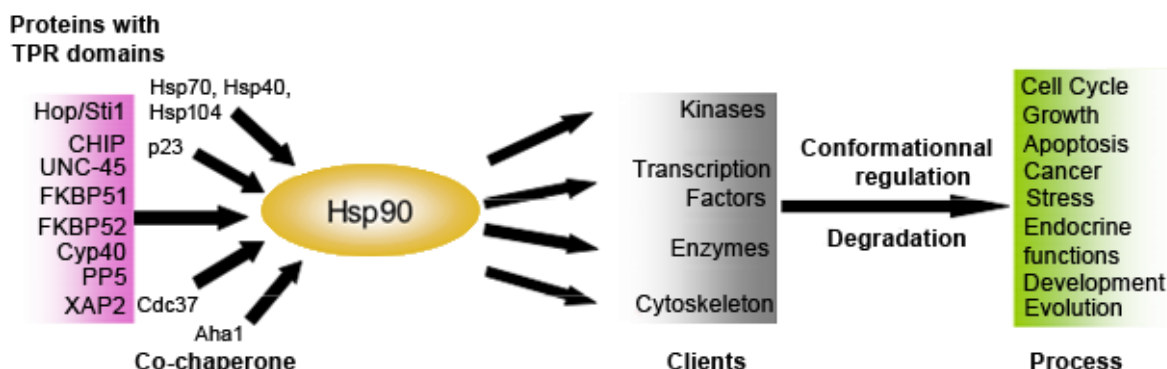

Figure 1: Hsp90 protein partners and clients destabilized by Hsp90 inhibition (Jackson et al., 2004).
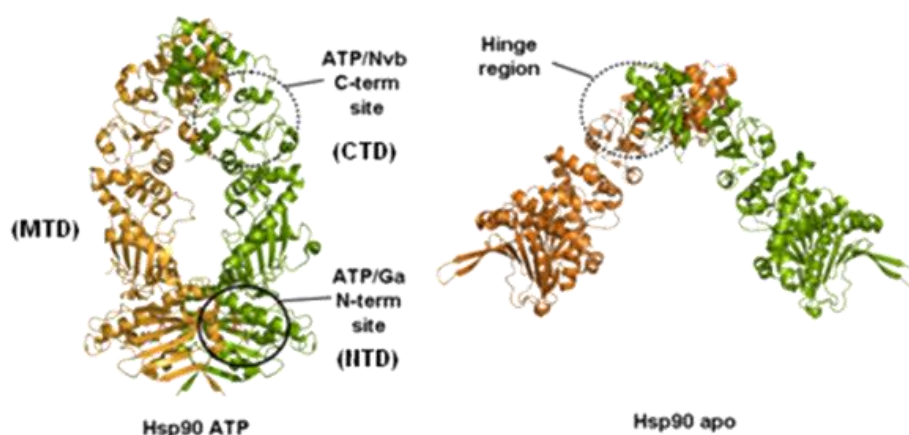
Figure 2: Structure of the full length yeast Hsp90, in complex with non hydrolysable ATP analogue (Hsp90 ATP) and structure of the full length E. coli Hsp90 without nucleotide (Hsp90 apo) (Shiau et al., 2006).

In the human proteome, several isoforms of Hsp90 have been isolated, Hsp90α (inducible form) and Hsp90β (constitutive form) localized in cytoplasm (Sreedhar et al., 2004a), while Grp94 (glucose-regulated protein) and TRAP-1 (HSP75/tumor necrosis factor receptor associated protein 1) are localized in the endoplasmic reticulum and in mitochondria respectively (Csermely et al., 1998; Maloney and Workman, 2002). To acquire its full active molecular chaperone activity, Hsp90 operates with molecular co-chaperones and partner proteins to form a series of multimeric protein complexes (Figure 3) including Hsp70, peptidyl-prolyl isomerases, immunophilins (FKBP51 and FKBP52) and the cyclophilin CYP40. Others co-chaperones such as p23, recently identified as a prostaglandine E2-Syntase, plays an important role in the activity of a number of transcription factors of the steroid/thyroid receptor family (Chan et al., 2008; Grad et al., 2006).

It is now well established, that Hsp90 needs to bind ATP in a pocket located in the N-terminal domain to exert its function. Thus, the Hsp90 protein function may be inhibited by molecules competing with ATP binding (such as geladanamycin: GA, Figure 3), thereby freezing the chaperone cycle, which in turn decreases the affinity of Hsp90 for client proteins and leads to 26S proteasome-mediated oncogenic client protein degradation (Sepp-Lorenzino et al., 1995). N-terminal domain Hsp90 inhibitors block cancer cell proliferation *in vitro* and cancer growth *in vivo* (Sharp and Workman, 2006).

To date, the full crystal structure of Hsp90 in complex with a non hydrolysable ATP analogue (Ali et al., 2006), and of the full length *E.Coli* Hsp90 without nucleotide (apo-Hsp90) (Shiau et al., 2006), have yet been reported (Figure 2). Furthermore, an interesting recent study investigated Hsp90 conformational changes in solution, shows a long range effects between Hsp90

domains, as the binding of co-chaperones (or inhibitors) at NTD induce conformational changes in the MD and CTD (Phillips et al., 2007). The C-terminal domain has been implicated biochemically as the site of a possible second, cryptic ATP-binding site on Hsp90. Its contribution to the overall regulation of chaperone function is not clear, but the antibiotic novobiocin (Nvb) (c.f. structure in Figure 15) has been reported to bind this site and alter the conformation of the chaperone (Yun et al., 2004).

Since pharmacological inhibition of Hsp90 by several families of small molecules leading to the degradation of oncogenic proteins, Hsp90 has become a target of interest against cancer and allowed the development of numerous small inhibitors (Biamonte et al., 2010).

II) Hsp90 health and cancer (Powers and Workman, 2007)

Hsp90 has probably been most widely acknowledged as a therapeutic target for the treatment of cancer (Mitsiades et al., 2007). Although there is no evidence of Hsp90 mutations in malignancy, there is increasing support for the view that this molecular chaperone plays an important role in the development, maintenance and progression of cancers.

One of the principal debates concerning the inhibition of the highly abundant Hsp90 is the selectivity of inhibitors for the chaperone protein in malignant cells (Kamal et al., 2003). Some works suggest that Hsp90 inhibitors could provide an exploitable therapeutic index (Banerji, 2005). Firstly, it has been reported that inhibitors were significantly more sensitive to Hsp90 in cancer cells (Neckers and Neckers, 2005; Powers and Workman, 2006; Chiosis, 2006; Whitesell et al., 1994; Neckers, 2006). In support to this surprising observation, Kamal et al. showed that the activity state of the Hsp90 chaperone machine was different in tumor cells.
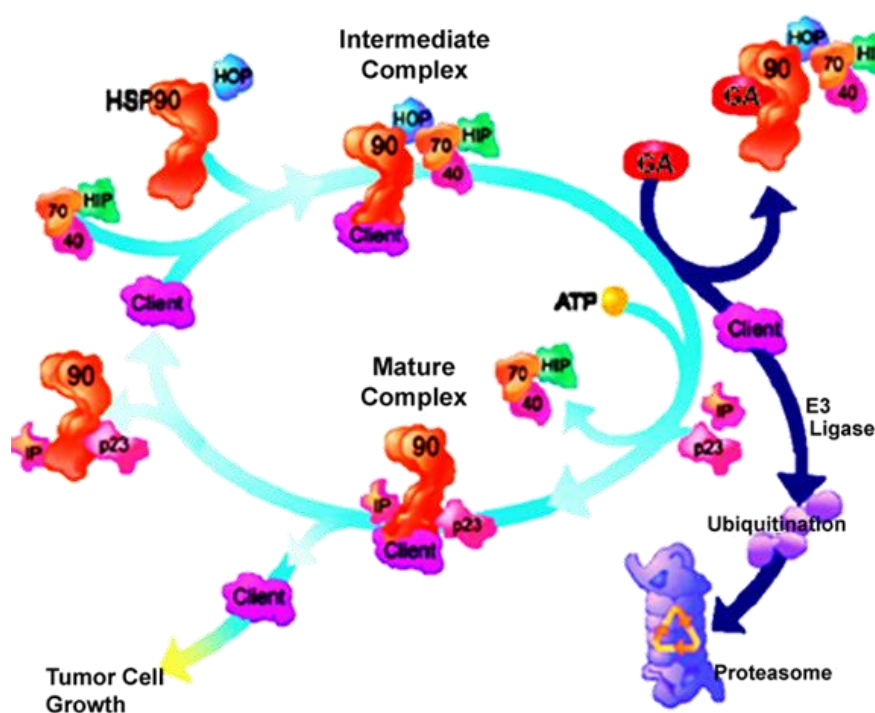
Figure 3: Hsp90 cycle: GA: geldanamycin analogue; 40=Hsp40; 70=Hsp70; IP: immunophillin; HIP: Hsp70-interacting protein; HOP=Hsp70/Hsp90 organizing protein (Biamonte et al., 2010).

Indeed, the Hsp90 is entirely bound in an active complex with co-chaperones, whereas most Hsp90 in normal tissues resides in a free, uncomplexed state (Workman, 2004; Kamal et al., 2003). Furthermore, Hsp90 is constitutively expressed at higher levels (2-10 fold) in tumor cells compared with their normal counterparts. This higher Hsp90 activity is probably due to the overexpression/amplification of mutated Hsp90 clients, and this is in correlation with the higher level of cochaperones of Hsp90 observed in cancerous cells.

Finally, the selective sensitivity of transformed cells for Hsp90 inhibitors may be partly due to the selective accumulation of these drugs in cancer cells since the *in vivo* observation of Hsp90 inhibitors in murine model system showed higher concentration in tumor tissue (Chiosis and Neckers, 2006).

Consequently, the Hsp90 has emerged as an exciting target for the development of cancer chemotherapeutics. However, despite the numerous molecules which have prompted a phase I clinical trial, it remains to be verified if Hsp90 inhibitors will provide adequate treatment in clinic.

III) Hsp90 inhibitors (Messaoudi, 2008)

The Hsp90 protein function may be inhibited with molecules that bind the ATP pocket, or its chaperone activity may be disturbed by small molecules binders interfering with domains in the C-terminus or median region. Although Hsp90 function provides an attractive target for the treatment of cancer, the feasibility and efficacy of the inhibitors approach has just begun to be explored in clinic.

Direct inhibitors of Hsp90 have been divided into two groups:

A) N-terminal domain binders

1) Ansamycin macrolactames

1.a) Quinone derivatives

Geldanamycin (Figure 4), was isolated from the broth of *Streptomyces hygroscopicus* in 1970s (De Boer et al., 1970). Further studies have shown that GA revert the phenotype of v-src oncogene transformed cells. However, this ability was not due to a direct action of the Src kinase activity, but to an inhibition of Hsp90. Subsequent immunoprecipitation and X-ray cristallographic studies have shown that GA competes with ATP and binds to the N-terminal domain site of Hsp90, leading the Hsp90 multichaperone complexes to the ubiquitin-mediated proteasome degradation (Roe et al., 1999; Stebbins et al., 1997). Since this observation, GA was used to identify additional Hsp90 substrates and to understand the role of Hsp90 in promoting malignant transformation. Although GA provided very promising antitumor effects, it showed several pharmacologic limitations as poor solubility, limited *in vivo* stability and high hepatotoxicity in some of the human tumor models (Neckers, 2006; Supko et al., 1995). Thus, the 17-position of GA has been an attractive focal point for the synthesis of GA analogues. Structure-activity relationship (SAR) studies have shown that structurally and sterically diverse 17-substituents
can be introduced without destroying antitumor activity. Then, further derivatives of GA, with similar

biological behaviour but a better toxicity profile, were synthesized (Schulte and Neckers, 1998). Therefore, new C-17 substituted derivatives 17-AAG (17-allyl-17-desmethoxygeldanamycin, also designed KOS953, CNF 1010, tanespimycin, Figure 4) and 17-DMAG (17-(2-dimethylaminoethylamino)-17-desmethoxygeldanamycin, KOS1022, alvespimycin, Figure 4) (Snader et al., 2002; Solit et al., 2007) were brought to the fore by displaying a significant enhancement of the chemical/metabolic stability.

17-AAG can be used in single agent or in combination with other cancer therapeutics (KOS953/bortezomib (Anderson, 2007; Richardson et al., 2007), KOS953/trastuzumab (Modi et al., 2007), 17-AAG/Paclitaxel (Sain et al., 2006), 17-AAG/cisplatin (McCollum et al., 2008)).

To enhance the pharmacokinetics and dynamics of 17-AAG, *Kosan Biosciences Incorporated* has developed a DMSO-free formulation (KOS953) contained cremophor, which is actually in Phase I clinical testing. Although 17-AAG and its numerous formulations have shown some encouraging clinical responses, they present important drawbacks (e.g.; liver toxicity and cumbersome formulation) that may limit their clinical applications whereas 17-DMAG exhibits a better water solubility and oral bioavailability (Ronnen et al., 2006). However, although clinical trials in myeloid leukemia seemed to be promising, the 17-DMAG was discontinued in 2008 (ClinicalTrials.gov).

1.b) Hydroquinone derivatives

In a different approach, *Infinity Pharmaceuticals* has developed IPI504 (retaspimycin or 17-AAG hydroquinone, Figure 4) (Adams et al., 2005; Sydor et al., 2006), a new GA analogue, in which the quinone moiety was replaced by a dihydroquinone one. Indeed,

the preclinical data suggested that the hepatotoxicity of 17-AAG was attributable to the ansamycin benzoquinone moiety, prone to nucleophilic attack. Furthermore, it was recently reported that the hydroquinone form binds Hsp90 with more efficiency than the corresponding quinone form (Maroney et al., 2006). In biological conditions, the hydroquinone form can interconvert with GA, depending on redox equilibrium existing in cell. It has been recently proposed, that NQ01 (NAD(P)H: quinone oxidoreductase) can produce the active hydroquinone from the quinone form of IPI504 (Chiosis, 2006). However, *Infinity Pharmaceuticals* showed that if the overexpression of NQ01 increased the level of hydroquinone and cell sensitivity to IPI504, it has no significant effect on its growth inhibitory activity. These results suggest that NQ01 is not a determinant of IPI504 activity *in vivo* (Douglas et al., 2009).

1.c) Clinical trials

In 2007, results of the phase I clinical trial of tanespimicyn (KOS953) with bortezomib in patients with relapsed refractory multiple myeloma were reported (Solit and Chiosis, 2008; Taldone et al., 2008). Dose escalations in the trial ranged from 100 to 340 mg/m^2 for tanespimycin, and from 0.7 to 1.3 mg/m^2 for bortezomib. Results showed that two patients, on the 41 enrolled, exhibited stable disease after two cycles, and 18 of them demonstrated a response to combination (Richardson et al., 2007). Moreover, the tanespimicyn was co-administrated with trastuzumab on 25 patients treated with up to 450 mg/m^2 of drug on a weekly schedule. This combination induced a regression of 21, 22 and 25% in three patients, which had failed trastuzumab therapy, with HER2-amplified breast cancer (Modi et al., 2007).
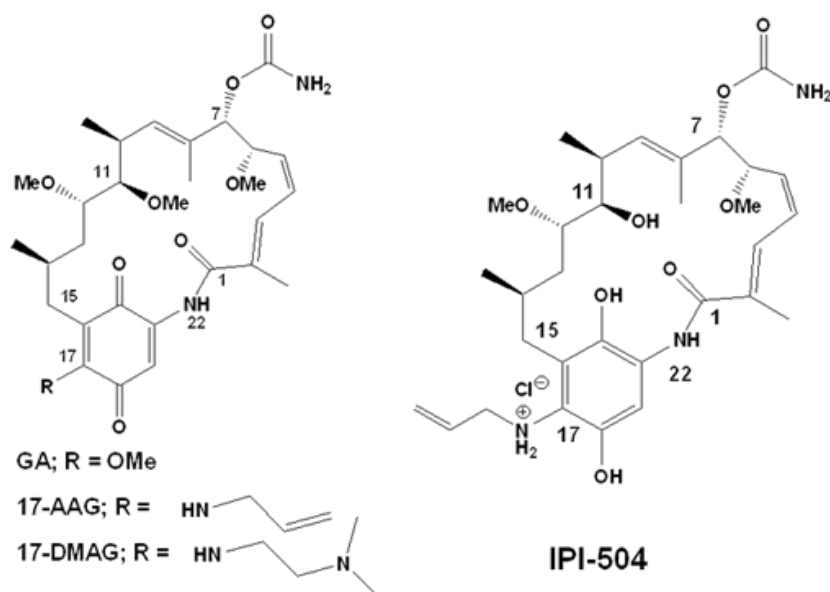

Figure 4: GA, 17-AAG, 17-DMAG and IPI504.

In 2008, *Infinity Pharmaceuticals* reported the results of a dose escalation Phase I/II clinical trial of retaspimycin hydrochloride in patients with metastatic and/or unresectable gastrointestinal stromal tumors (GIST) on a twice weekly schedule (400 mg/m^2). 4 of the 18 patients enrolled, achieved a partial response and 11/18 achieved stable disease. These results had initiated the phase III clinical trial of the study in 2008. However, *Infinity Pharmaceuticals* reported on April 2009, the decision to end its phase III study (RING trial) of IPI504 hydrochloride in patients with refractory gastrointestinal stromal tumors (Infinity Press Release). The trial was based on 46 patients whose tumors persist despite treatment with Gleevec (imatinib) and Sutent (sunitinib). Resulting data showed a higher than anticipated mortality rate. In this heavily pretreated population, IPI504 was not tolerated (400 mg/m^2 or placebo in 21 days cycles as a 30 min intravenous infusion twice weekly for 2 weeks followed by a 1 week rest) and the study was terminated early.

Nevertheless, the IPI504 is still evaluating in phase II trials in patients with non-small cell lung cancer, and in combination with herceptin (trastuzumab) in patients with HER-2 positive metastatic breast cancer.

In the same month, *KOSAN*, acquired by *BMS* in 2008, reported that the phase III clinical trial concerning the KOS953 or tanespimycin, in combination with Bortezomib in patients with multiple myeloma in first relapse has been suspended. This was probably a precaution as the metabolization of tanespimycin leads to IPI504.

Conforma Therapeutics/Biogen idec developed a hydroquinone form of the 17-AAG (CNF1010), trapped as HCl salt, which was in clinical phase I against chronic lymphocytic leukemia. However, to date, this program is terminated. Moreover, the CNF1010 had started a phase III trial against GIST in 2008. This study was also suspended due to the anticipated mortality rate of patients enrolled (ClinicalTrials.gov). Parallel efforts to improve the solubility and bioavailability of 17-AAG have led the *NCI* and *Kosan* to develop 17-DMAG (KOS1022) as a second generation alternative which has entered Phase I clinical testing (Santi et al., 2007). Promising results were obtained in patients with chemotherapy refractory acute myelogenous leukemia, as 3 of 17 patients had a complete response to therapy (Lancet et al., 2006). However, researches were given up in 2008, as the 17-DMAG presents an unusable toxicity profile.

1.d) Other analogues

Diverse derivatives of 17-AAG bearing non-redox-active phenol group designed by *Kosan Biosciences* were reported (Tian et al., 2007). Amongst them, KOSN1559 was claimed as the most potent Hsp90 inhibitor (e.g.; SKBr3 Cell Line IC$_{50}$=860 nM, Kd=16

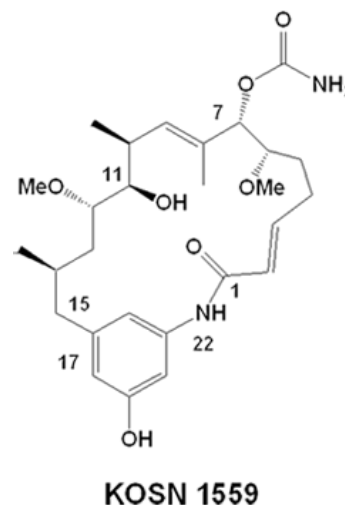nM) (Figure 5). To date, no clinical trial had been reported with this compound.

KOSN 1559

Figure 5: Structure of KOSN 1559.

2) Purines
2.a) Purines analogues

Limitations in the clinical use of 17-AAG and 17-DMAG have prompted the discovery of novel Hsp90 ATPase inhibitors with improved "drug-like" structural characteristics and better pharmacological profiles. To this end, structure-based design and high-throughput screening approaches performed at the Memorial Sloane Kettering Institute, have been taken to identify new chemotypes that inhibit Hsp90 ATPase activity. A significant breakthrough in the preparation of synthetic Hsp90 inhibitor was the PU3 (Figure 6). On the basis of X-ray analysis and molecular modelling, Chiosis's group, showed that PU3 was designed to place the purine moiety into the same spatial orientation as adenine ring of ATP in the nucleotide pocket of Hsp90 (Chiosis et al., 2001). PU3 presented molecular signature of Hsp90 inhibition, including the degradation of HER2, even if its affinity for Hsp90 is moderate.

Chiosis's group and *Conforma therapeutics/Biogen Idec* optimized this class of compounds leading to new analogues bearing a thioether bridge to connect the purine nucleus to substituted phenyl rings. Among them, the PUH58 (Figure 7) (Llauger et al., 2005), an 8-arylsulfanyl analogue of PU3, has been identified as the most potent and selective purine. Further efforts in optimization of this lead compound led to the development of PU24F-Cl, (Figure 7) which presents a higher affinity (30 times more than PU3) for the N-terminus of the Hsp90, and low micromolar activity in a cell proliferation assay (Chiosis et al., 2002; Vilenchik et al., 2004). In an *in vivo* experiment in MCF-7 tumor bearing mice, PU24FCl led to 70% inhibition when administered at a dose of 200 mg/kg every second day for 30 days (Vilenchik et al., 2004).
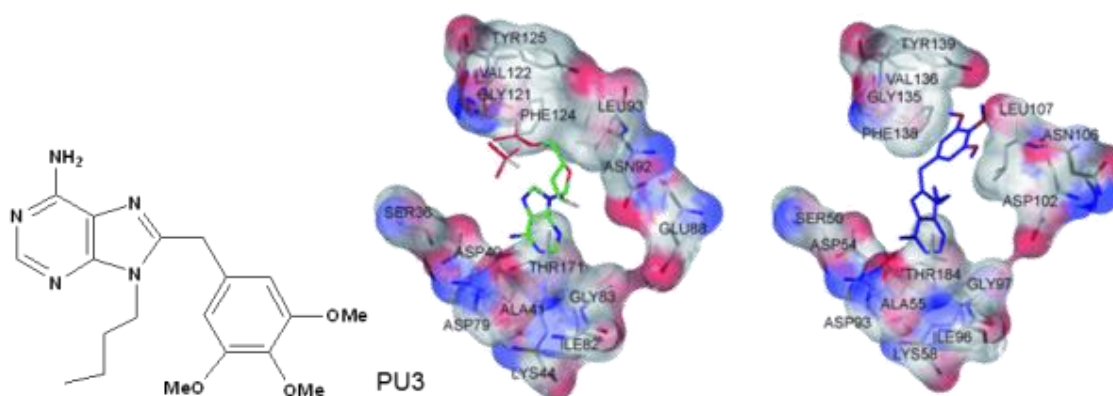
Figure 6: Co-crystal structures of ADP (left, in green) and PU3 (right, in magenta) with Hsp90.
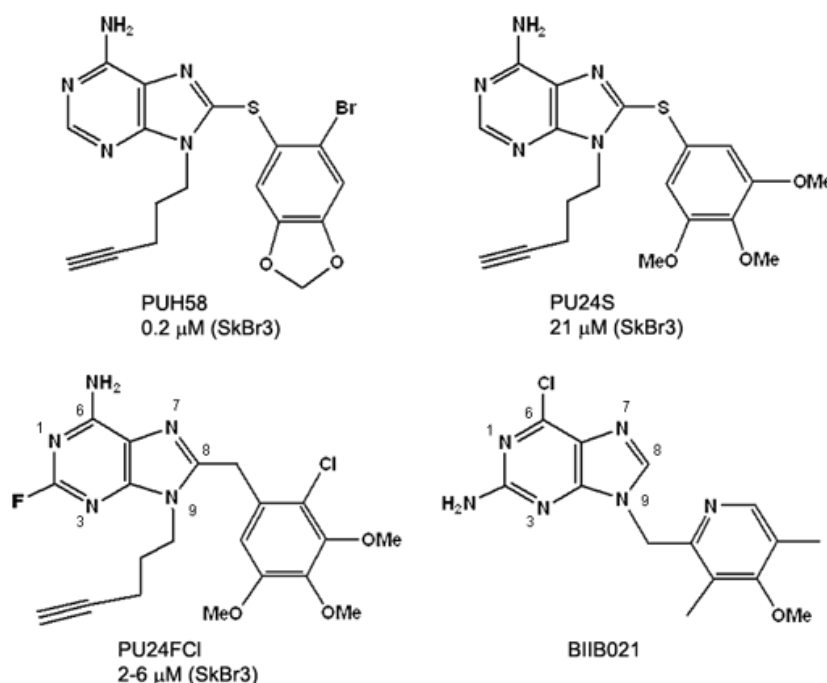

Figure 7: Structures of PU24FCl and analogues.

Additional investigations concerning the pharmacophore of this family were done. It was demonstrated that the presence of an amino group on the C2 position of the purine nucleus respects the global size of the molecule in regard to the parent one (PU3). In addition, it offers multiple possibilities for hydrogen bonding, and thus allowed the connection of the benzyl group on the N-9 rather than the C-8 of the purine. Thus, *Conforma-Biogen idec* have identifed the BIIB021 (originally named CNF 2024, Figure 7) (Kasibhatla et al., 2007) which displayed a binding affinity of 1.7 nM (4.6 nM for 17-AAG) and induces degradation of HER2 with an IC_{50} of 38 nM in MCF-7 cells (Lundgren et al., 2009). BIIB021 compound was entered in clinical trials in 2005.

2.b) Clinical trial

Currently, BIIB021 is the only purine analogue evaluated in phase I/II clinical trials in combination with trastuzumab (herceptin) against breast cancer, with an aromatase inhibitor (exemestane) in metastatic

HER2-advanced breast cancer, or alone in subjects with gastrointestinal stromal tumors. Results from the phase I trials showed that BIIB021 was well tolerated (800 mg twice weekly) and induced a significant inhibition of the HER2 (Elfiky et al., 2008).

3) Pyrazole and isoxazole derivatives

3.a) Pyrazole analogues

In 2004, CCT018159 (Figure 8), the first Hsp90 inhibitor in the pyrazole series, was identified by Workman et al. from a library of 60000 compounds, using a high throughput screening (HTS), in the Cancer Research UK Centre for Cancer Therapeutics (Rowlands, 2004). This compound inhibits the N-terminal ATPase activity of yeast and human Hsp90 with an IC_{50} of 7.1 and 3.2 μM, respectively (Cheung et al., 2005; Sharp et al., 2007a). Further HTS studies undertaken by Genomics institute of the Novartis Research Foundation (GNF) and based on time-resolved fluorescence resonance energy transfer (TR-FRET) had allowed identifying two leads, G3129 and

G3130 (Figure 8), amongst the one million molecules screened (Kreusch et al., 2005). However, both compounds exhibited relatively poor binding affinity to N-terminal domain of the Hsp90 (Kd=680 and 280 nM respectively). In SkBr3 breast cancer cells, G3130 caused the degradation of HER2 (IC$_{50}$=30 μM) while G3129 was ineffective.

In addition, the co-crystal structures of G3129 and G3130, bound to the N-terminus of human Hsp90α, were reported (Kreusch et al., 2005). From this study, it was showed that the resorcinol ring bound Hsp90 in a similar way than that of radicicol, a resorcylic lactone that inhibits Hsp90.
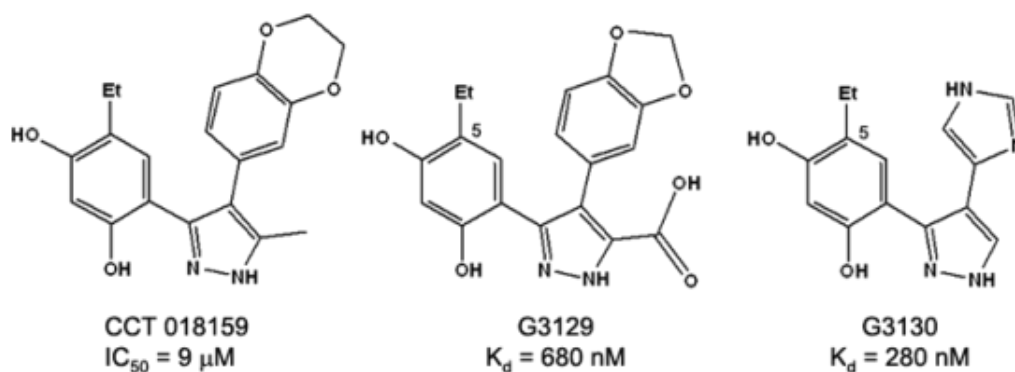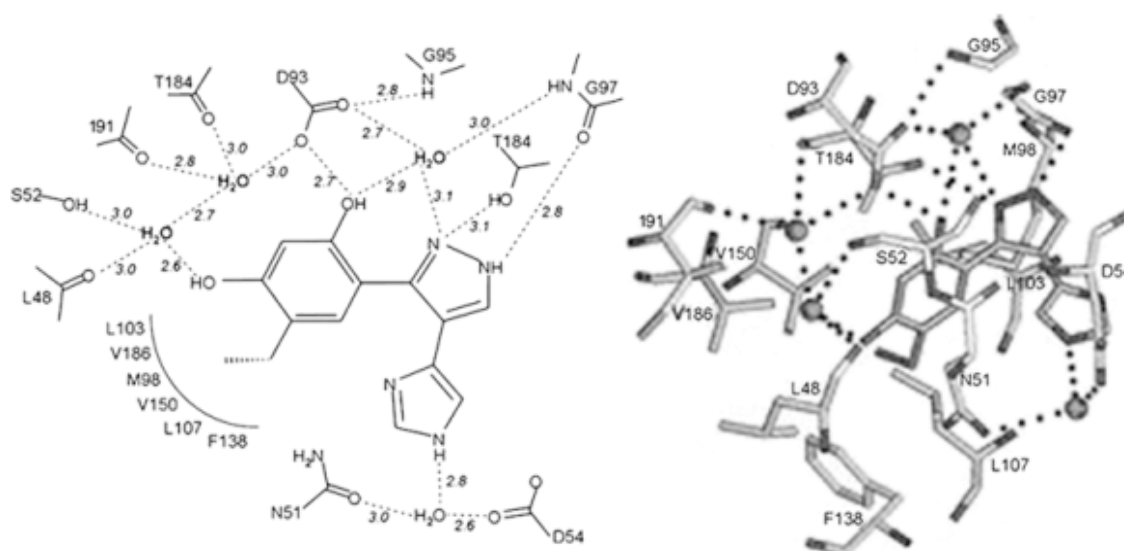

Figure 8: Structure of analogues G3129 and G3130.

Figure 9: Co-crystal structure of G3130 bound to the N-terminus of human Hsp90α.
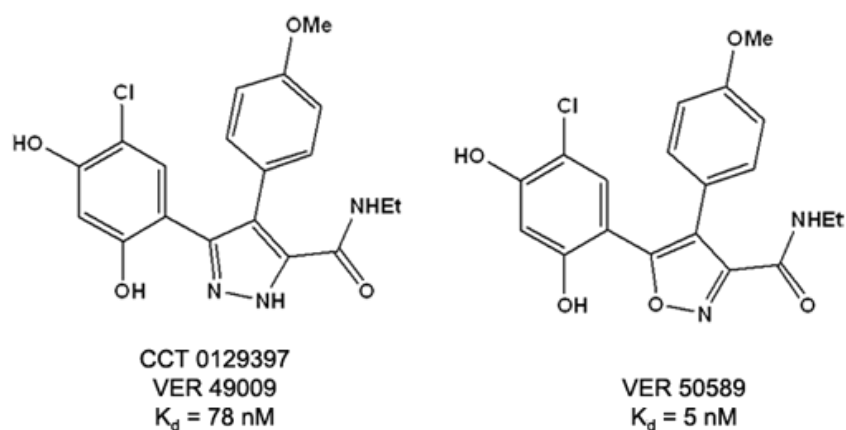

Figure 10: VER49009 and VER50589.

Furthermore, the 5-ethyl appendage projected into the aromatic pocket that accommodates the benzyl group of the purine analogues described before. The pyrazole provides hydrogen bound acceptor, and the imidazole (G3130) occupies the same pocket as the quinone of GA (Figure 9).

Based on these data, medicinal chemistry efforts led to the identification of the more potent analogue of CCT018159, the VER49009 (IC_{50} ATPase activity=0.14 μM, Figure 10), where the amide group was a key in forming a new interaction with the residue Gly97 of the protein (Dymock et al., 2005).

Synta Pharmaceuticals Corp. had reported another class of triazoles analogues as modulators of Hsp90 (Figure 11) (Ying et al., 2009). It has been shown that STA-9090, an unspecified new resorcinol-containing triazole compound (Lin et al., 2008), inhibits the activity of Hsp90 protein from 10 to 100 μM and thereby leading to degradation of Hsp90 client proteins such as HER2 gene product (Ying et al., 2009). More recently, it had been shown that the STA-1474, a highly soluble phosphate prodrug of STA-9090, exhibits very interesting biologic activity against osteosarcoma cell lines (McCleese et al., 2009).
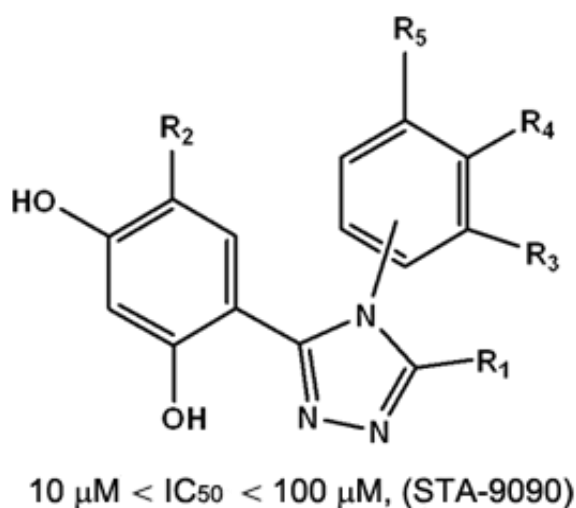

10 μM < IC_{50} < 100 μM, (STA-9090)

Figure 11: Pyrazoles reported by Synta Pharmaceuticals.

3.b) Isoxazole analogues

Further optimization of potency, pharmakinetic and pharmacodynamic properties of VER49009, were undertaken by Vernalis Ltd. (in collaboration with Novartis) to offer a series of isoxazole resorcinol inhibitors. One of these was the VER50589 (Figure 10) which exhibited a higher affinity (Kd=5 nM) than VER49009 (Kd=78 nM) (Sharp et al., 2007b). Thus, the pyrazole to isoxazole switch does not affect the critical hydrogen bound of the pyrazole resorcinol unit that anchors this class of inhibitors to the Hsp90 NH2-terminal ATP site (Brough et al., 2008). Moreover, VER50589 also showed improved cellular uptake over VER49009.

Brough et al. from *Vernalis*, reported recently the identification of new diarylisoxazole compound

VER52296 (Figure 12) (Brough et al., 2008; Eccles et al., 2008). The areas of interest for SAR studies were the 5' positon on the resorcinol ring, and the para substitution of the phenyl group on the isoxazole ring. It has been shown with the X-ray cristal structure, that the replacement of the chlorine, in regard to VER50589, by an isopropyl group, results in an additional hydrophobic interaction with Leu107 in the flexible lipophilic pocket of the N-terminal site of Hsp90. Additional hydrophobic interactions were also observed with Thr109 and Gly135 from the morpholine moiety present in VER52296/NVP-AUY922 (Figure 12). This latter, subsequently developed by Novartis, was found to be very potent in the Hsp90 Fluorescence Polarization binding assay (IC_{50}=21 nM) and displays an average GI50 of 2-40 nM in antiproliferation assays against different human tumor cell lines (Brough et al., 2008). In addition, as evaluated by cassette dosing to mice bearing subcutaneous HCT116 human colon cancer, VER52296/NVP-AUY922 was retained in HCT116 xenograft tumors when administered i.p., at concentrations well above the GI50. Further *in vivo* characterization in a human colon cancer xenograft model, VER52296/NVP-AUY922, also inhibits tumor growth by ~50% when dosed at 50 mg/kg i.p. daily. Moreover VER52296/NVP-AUY922 induces the degradation of HER2 with an IC_{50} of 7 nM. In addition, VER52296/NVP-AUY922 was tested in several xenografts (colon, glioblastoma, breast, ovarian, prostate) and a therapeutic response was observed in each case (Cheung et al., 2005; Jensen et al., 2008; Eccles et al., 2008).

VER52296
NVP-AUY922
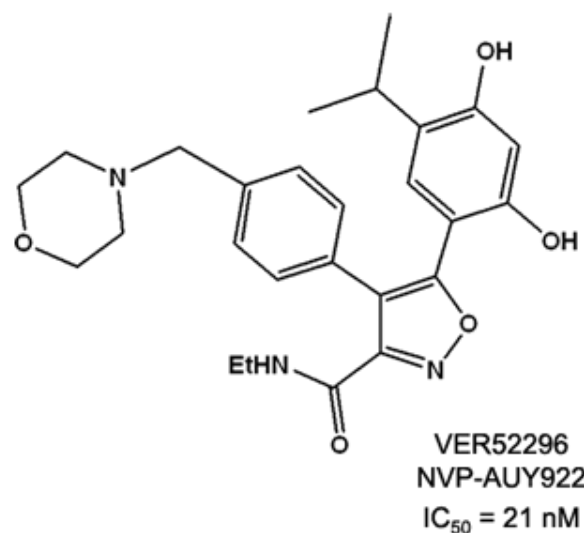IC_{50} = 21 nM

Figure 12: Structure of VER52296/NVP-AUY922.

3.c) Clinical trials

The VER52296/NVP-AUY922 is the sole isoxazole analogue currently in phase I/II clinical trials as a single agent or in combination with bortezomib or dexamethasone, in patients with relapsed or refractory multiple myeloma. AUY922 is also used as a single agent, in advanced solid malignancies and efficacy in

HER2$^+$ or ER$^+$ locally advanced or metastatic breast cancer patients. In this latter phase I/II trial, VER52296/NVP-AUY922 was intravenously administrated once a week schedule. The maximum dose reported is 54 mg/m^2. At 40 mg/m^2, VER52296 induces an up regulation (4-19 fold) of Hsp70 and a 20% reduction in soluble HER2 was achieved by 74% of patients.

Noteworthy, the STA9090 (Figure 11) is currently enrolling patients in several phase I/II clinical trials against solid tumor, myeloid leukemia, non-small cell lung cancer and gastrointestinal stromal tumor.

4) Dihydroindazolone derivatives

4.a) SNX2112 and SNX5422

In 2007, Serenex Inc./Pfizer had started a phase I clinical trial program for the SNX5422 (Figure 13).

Using a new screening platform, the compounds retained on the ATP-affinity column were analyzed by mass spectrometry leading to the identification of a poorly soluble analogue, SNX2112, which will become the glycine prodrug SNX5422. The SNX2112 showed higher activity to bind Hsp90 (Ki=1 nM) than that of 17-DMAG and induced the degradation of HER2 with an IC$_{50}$ of 20 nM. Based on the observation that breast cancer cell lines with HER2 amplification are more sensitive to 17-AAG, the SNX2112 was tested using a panel of breast, lung, and ovarian cancer cell lines. In all cell lines studied, SNX2112 inhibited cell proliferation with IC$_{50}$ ranging from 10 to 50 nmol/L. In contrast to 17-AAG, the sensitivity of cancer cell lines to SNX2112 *in vitro* did not correlate with the level of HER2 expression (Chandarlapaty et al., 2008; Huang et al., 2006). This compound uniformly targets both the pro-proliferation pathways driven by HER2 and ERK as well as the anti-apoptotic Akt pathway (Okawa et al., 2009). Indeed, it exhibits potent *in vivo* antitumor activity that extends significantly the effects observed with GA analogues.

4.b) Clinical trial

SNX-5422 is currently in a phase I clinical trial in treating patients with solid tumor or lymphoma that has not responded to treatment. SNX5422 is equally tested to treat solid tumor cancer and lymphomas, and, in subjects with refractory hematological and solid tumor malignancies. In 2008, results of a phase I dose-escalation study of SNX5422 reported that this compound was well tolerated at 21 mg/m^2.

5) Other inhibitors

Another class of ATP Hsp90 inhibitors bearing a resorcinol moiety is radicicol (Rd) (Figure 14), a natural resorcylic lactone, isolated from the fungus *Monocillium nordinii* and *Monosporium bonorden*. Rd, also known as monorden, has been described to reverse the *Src*-transformed morphology of fibroblast (Whitesell et al., 1994). This effect was first attributed to the inhibition of the oncogenic Src (v-Src), and later proved to act as an inhibitor of Hsp90 despite its difference in structure to GA. Moreover, Rd was found to compete with GA for binding to the NTD of the chaperone, suggesting that Rd shares the geldanamycin binding site. This compound is a potent and specific inhibitor of the ATPase activity of Hsp90 with nanomolar affinity (Kd=19 nM). This causes destabilization of Hsp90 client proteins (v-Src, Raf-1, ErbB2 and Ras), many of which are essential for tumor cell growth. Although, the *in vitro* antitumoral activity of Rd is very promising however, its *in vivo* activity is very weak probably because of its chemical instability in serum and its rapid conversion into inactive metabolites due to the electrophilic nature of the dienone moiety.

Therefore, synthetic efforts have been directed to identify radicicol derivatives with improved *in vivo* activity (Proisy et al., 2006). To date, *Kyowa Hakko* described novel oxime-derivatives of Rd, including KF55823 and KF25706 (Soga et al., 2003) (Figure 14). Although these compounds exhibit potent antitumor activities in preclinical models and do not seem to cause hepatotoxicity, their clinical evaluations of these compounds has not been pursued.
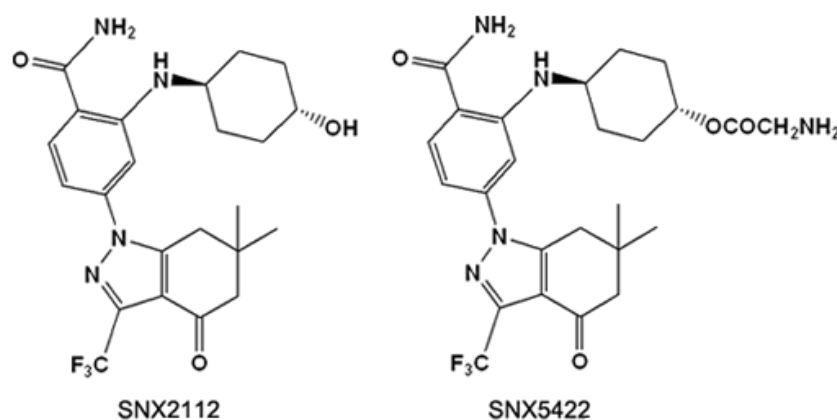

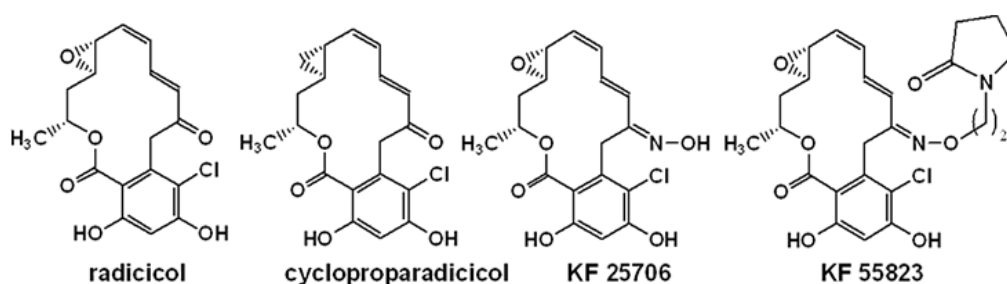**Figure 13:** Structure of SNX5422 and SNX2112.
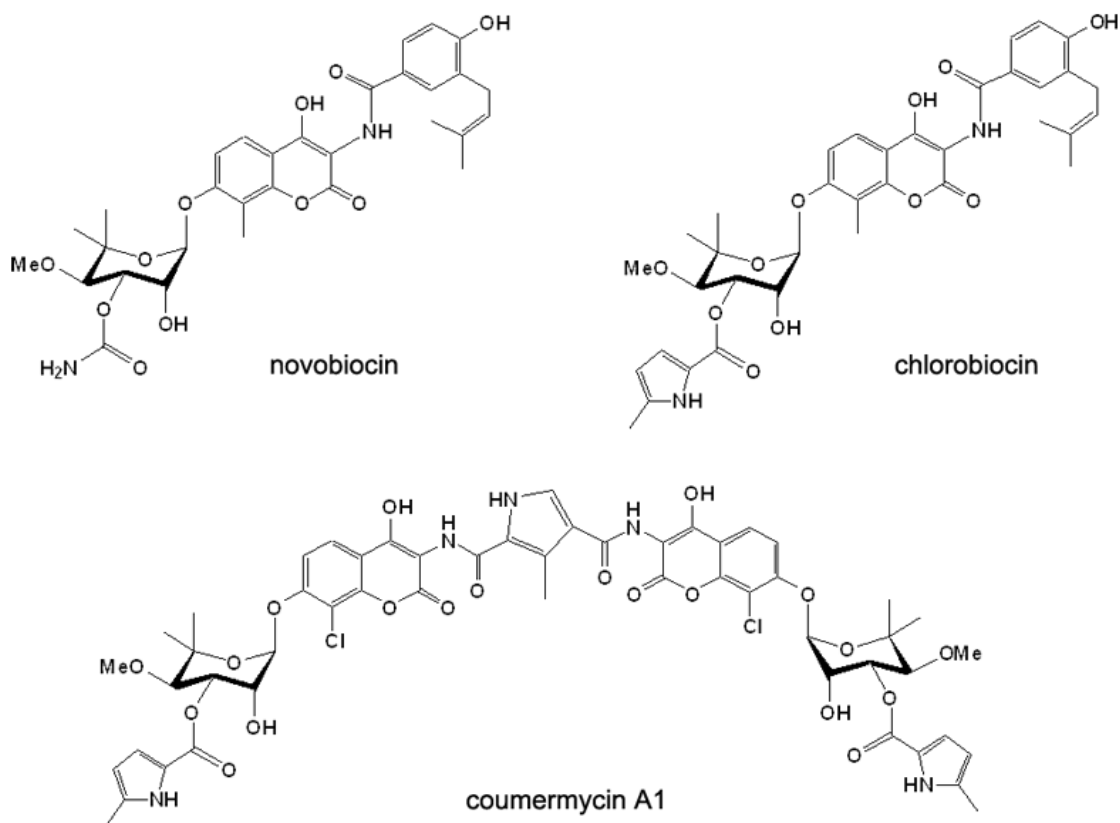
Figure 14: Resorcylic inhibitors.

Figure 15: Structures of novobiocin, chlorobiocin and coumermycin A1.

B) C-terminal domain binders

In 1991, Csermely, Kahn and co-workers reported the presence of a C-terminal ATP binding site on Hsp90 which becomes accessible when the N-terminal Bergerat pocket is occupied (Sõti et al., 2002). A decade later, it has been shown that Nvb interacts with an ATP-binding domain in the C-terminus of Hsp90 (Marcu et al., 2000a). Biochemical studies on the CTD of Hsp90 have identified an allosteric regulation process with the N-terminus site, where the occupancy of one site blocks the interaction of the ligand with the other site (Garnier et al., 2002). The structure of the full length and middle and C-terminal construction of Hsp90 with different nucleotide states (apo, ATP) have shown that there is a hinge region between the middle and C-terminal region of Hsp90. The conformation of this region is dictated by the status of the nucleotide at the N-terminal site. This observation is in accordance with the allosteric regulation of ATP binding. It suggests that the putative secondary ATP site could be located at the immediate proximity of the hinge.

IV) Coumarin inhibitors

Coumarin group antibiotics, such as novobiocin (Nvb), coumermycin A1 (Kd=10 nM) and clorobiocin (Figure 15), are potent inhibitors of the bacterial ATP binding gyrase B, a type II DNA topoisomerase (Gormley et al., 1996). Their affinity for gyrase is considerably higher than that of modern fluoroquinolones. These antibiotics have been isolated from various *Streptomyces* species (Lanoot et al., 2002) and all possess a 3-amino-4-hydroxycoumarin moiety as a key structural feature.

Nvb is licensed as an antibiotic for clinical use (*Albamycin; Pharmacia-Upjohn*) and for the treatment of infections with multi-resistant gram-positive bacteria such as *Staphylococcus aureus* and *S. epidermidis*

(Raad et al., 1995; Raad et al., 1998; Rappa et al., 2000). It had been demonstrated that the interaction of Nvb with Hsp90 induces alteration in the affinity of the chaperone for GA and Rd and causes *in vitro* and *in vivo* depletion of key regulatory Hsp90-dependant kinases including v-Src, Raf-1 and ErbB2 (e.g., ErbB2 in SkBr3 breast cancer cells ~700 µM). In addition, Nvb was found to bind the C-terminal nucleotide binding region of Hsp90, albeit with a lower affinity than with gyrase B. Moreover, Nvb disrupts the interaction of both the cochaperones p23 and HSC70 with the Hsp90 complex.

In 2005, the first attempt to improve the inhibitory activity of Nvb against Hsp90 was reported (Yu et al., 2005; Blagg et al., 2006). These authors have highlighted the crucial role of the noviose moiety at the 7-position of the coumarin ring for the biological activity. Compound A4 lacking the 4-hydroxyl of the coumarin moiety and containing an N-acetyl side chain in lieu of the benzamide was the most active compound. This compound was identified as Hsp90 inhibitor that induced degradation of Hsp90-dependent client proteins at 70-fold lower concentration than Nvb. Recently, in continuation of their structural modification studies, the same authors reported that 3'-descarbamoyl-4-deshydroxynovobiocin DHN2 (Figure 16) and compound KU135 (Shelton et al., 2009) proved to be a more effective and selective Hsp90 inhibitor (degradation of ErbB2 and p53 between 0.1 and 1.0 µM) (Burlison et al., 2006).

Our group reported a novel series of 3-aminocoumarin analogues (Le Bras et al., 2007a; Le Bras et al., 2007b) lacking the noviose moiety as a class of highly potent Hsp90 inhibitors. A representative example of this new class of inhibitors is 4TCNA (Figure 17) (Le Bras et al., 2007b).

In these analogues, the introduction of a tosyl substituent on C-4 position of coumarin nucleus (4TCNA) contributed to a significant extent for maximal activity despite weaker water solubility. Moreover, this lead has a particular implication in apoptotic process. Thus, 4TCNA promotes apoptosis through activation of caspases 7 and 8 in ER-positive MCF-7 human breast cancer cells, whereas in Ishikawa

endometrial adenocarcinoma cells, it induced apoptosis that was associated with caspase activation and cleavage of PARP. Furthermore, characterization of its mode of action revealed that 4TCNA induced-cleavage of the p23, recently identified as a prostaglandine E2-Syntase, which plays an important role in activity of a number of transcription factors of steroids/thyroid receptors family. These results demonstrate that this new denoviose compound presents originality in regard to Nvb osidic derivatives already known.

In another study based on a simplified 3-aminocoumarin scaffold, we also demonstrated that 4-tosyl-7-deshydroxycyclonovobiocic acid (4TDHCNA) (Figure 17) (Radanyi et al., 2008), exhibit increased inhibitory activity against the Hsp90 protein folding process (MCF7 IC_{50}=50 µM).

This result shows that removal of C7/C8 substituents is not detrimental for Hsp90 inhibitory activity and strongly enhances the capacity of 4DHTCNA to inhibit Hsp90. This compound was identified to be the most potent representative of the new family of simplified coumarins. Results from this study suggest that 4TDHCNA and 4TCNA, which exerted similar biological profile may be considered interesting compounds for the development of more potent novobiocin analogues.

More recently, results from our group allowed the identification of a new family of novobiocin analogues in which the coumarin unit has been replaced by a 2-quinoleinone moiety (unpublished results). The quinolone-scaffold represents a platform for the creation of easily synthesizable soluble molecules. Compound 4-tosyl-3[(chroman-6-yl) carboxylamino]-2-quinolon (4TCCQ, Figure 17) (IC_{50}=5-8 µM) is 100-fold more potent than the parent natural compound (novobiocin) and 6-fold more active than the synthetic analogue 4TCNA. Additionally, 4TCCQ induces the degradation of ERα and strongly induces the cell death in MCF-7 breast cancer cell line.

Overall, these data provides compelling evidence for the continued development of novobiocin-based C-terminal domain Hsp90 inhibitors as promising alternative to N-terminal domain inhibitors.
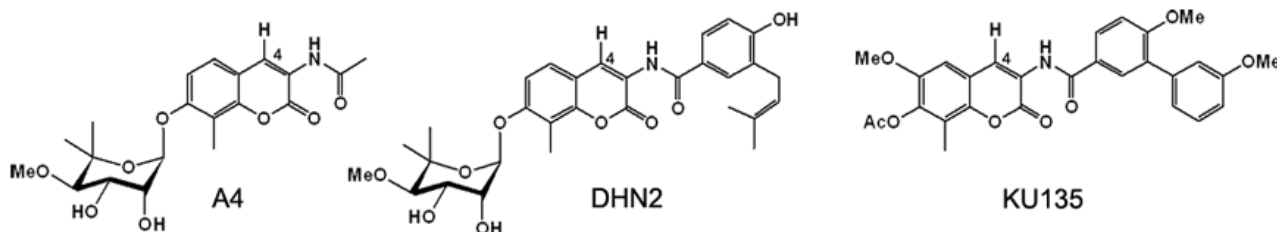

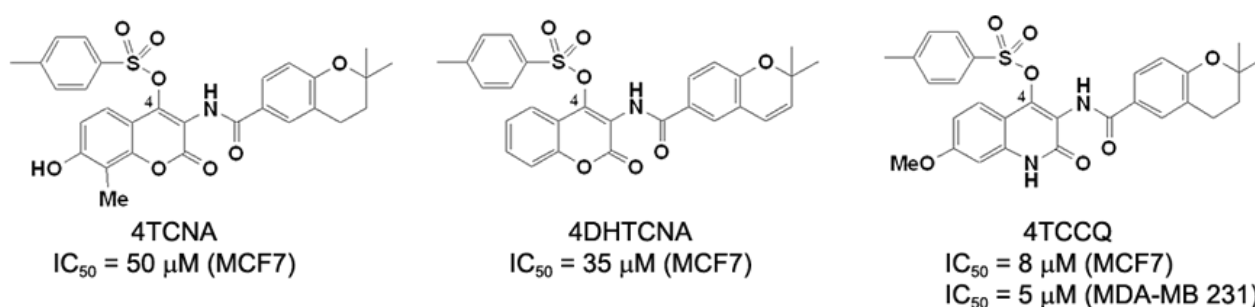**Figure 16:** Structures of A4, DHN2 and KU135.

4TCNA
$IC_{50} = 50 \mu M$ (MCF7)

4DHTCNA
$IC_{50} = 35 \mu M$ (MCF7)

4TCCQ
$IC_{50} = 8 \mu M$ (MCF7)
$IC_{50} = 5 \mu M$ (MDA-MB 231)

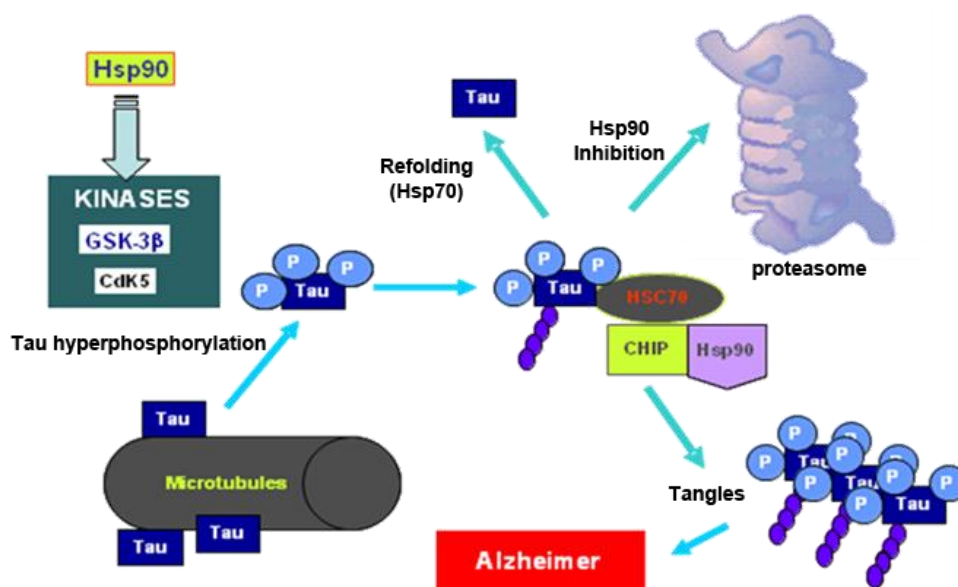**Figure 17:** Structures of 4TCNA, 4TDHCNA and 4TTCQ.

Figure 18: Tau-Hsp90 in AD.

V) HSP 90 in Alzheimer's disease

Hsp90, a molecular chaperone, has come into its own as a tantalizing target for cancer therapies. However, its important functions of stabilization, rematuration, disaggregation of many client proteins could be exploitable in others diseases.

Indeed, neurodegenerative diseases are characterized by the accumulation of misfolded proteins that results in plaque formation. These proteins rely upon HSP's for their refolding and viability. Recently, it was suggested that Hsp90 may play a crucial role in maintaining pathogenic changes that lead to neurodegenerative diseases (Luo et al., 2008). Furthermore, the inhibition of Hsp90 by 17-AAG derivatives and geldanamycin, induces the HSP induction via HSF-1 activation, resulting in neuroprotective activities. In the Alzheimer's disease, the most common tauopathy, in addition to β-amyloid deposition, there is an accumulation of abnormal species of hyperphosphorylated protein tau which leads to the formation of toxic neurofibrillary tangles (Luo et al., 2007; Dickey et al., 2007). This hyperphosphorylation is caused by abnormal kinases (CdK4, GSK-3β) activities resulting in dissociation of transformed tau from microtubules, aggregation and formation of neurofibrillary tangles which can block the synaptic transmission (Figure 18).

Thus, the decrease of hyperphosphorylated tau levels through refolding or degradation may provide a possible therapeutic strategy against AD. In this purpose, Dickey and Luo have presented evidence that the stability of p35, (neuronal activator of CdK4) and P301L mutant (most common mutation in Alzheimer disease) are maintaining by Hsp90.

Dou et al. 2007 reported that Hsp90 associates with GSK-3β, regulating its stability and function, preventing its degradation by the proteasome and so allowing the increase of tau hyperphosphorylation. Thus, the use of Hsp90 inhibitors leads to a destabilization of GSK-3β and to a decrease of hyperphosphorylated tau protein.

Dickey et al. 2007 demonstrated that CHIP (a tau ubiquitin ligase) is intimately linked to tau degradation following Hsp90 inhibition and that this process is specific for promoting degradation of only aberrant phosphorylated tau due to the fact that the Hsp90 complex, in AD brain, presents higher affinity for inhibitors than in unaffected brain tissue.

Recently, compound A4 (Figure 16) was found to exhibit significant protection against the Aβ-induced

toxicity at low concentrations (Lu, 2009). These results suggest that novobiocin analogues may represent an effective class of novel compounds for treatment of AD.

VI) Conclusion

Since the first discovery of natural analogues, GA and RD, the search for inhibitors of Hsp90 has generated considerable interest as evidenced by the number of compounds in clinical evaluations (Table 1).
However, if the first clinical results were very

encouraging, it seems that currently the development of Hsp90 inhibitors experiencing some difficulties, especially due to their toxicity. Stopping clinical trials of IPI504, which represented the most advanced HSP90 inhibitors, is the unfortunate illustration of that. Thus, many efforts are still needed in the understanding of the administration of these agents but also in the synthesis of new molecules. Moreover, the involvement of Hsp90 in other non-oncological diseases such as Alzheimer's disease shows the importance of acquiring new and more potent inhibitors with suitable pharmacological and pharmacokinetic profiles.

Drug		Drug Name	Licence	Combination	Condition	World status	Therapy
17-AAG	KOS953	tanespimycin	Kosan-BMS	Bortezomib	S	III	MM
					C	II/III	MM
	CNF1010	tanespimycin	Conforma-Biogen		T	I	CLL
17-AAG hydroquinone	IPI504	restapimycin	Infinty		T	III	GIST
					NYR, ANR	II	ADL, PC
				Trastuzumab	R	II	BC (Her2$^+$)
					T	II	MetM
	IPI493				R	I	AM
17-DMAG	KOS1022	alvespimycin	Kosan-BMS		T	II	BC (Her2$^+$)
				Trastuzumab, Paclitaxel	C	I/II	BC
					ANR	I	ST, AST, UST
BIIB021			Conforma-Biogen	Aromasin	R	II	BC, GIST
					R	I	AST, BC
					T	I	CLL
VER52296/ NVP-AUY922			Vernalis-Novartis		NYR	II	AGC
				Alone and Bortezomib	R	I/II	RRMM
					R	I/II	BC
					R	I/II	AST
SNX5422			Serenex-Pfizer		R	I	ST
STA9090			Synta Pharmaceuticals		R	I/II	AML, CML, ALL, NSCLC, GIST
					R	I	ST
Other Inhibitors (Structure not known)							
MPC-3100			Myriad Genetics		R	I	Cancer
KW-2478			Kyowa Hakko		NYR	I/II	MM
					ANR	I	MM, CLL
AT-13387			Astex Therapeutics		R	I	ST

Table 1: Current clinical trials.

Condition: R: recruiting, ANR: active, not recruiting, C: completed, NYR: not yet recruiting, S: suspended, T: terminated.
Therapy: ADL: Advanced Dedifferentiated Liposarcoma, AGC: Advanced Gastric Cancer, AM: Advanced Malignancies, AML: Acute Myeloid Leukemia, AST: Advanced Solid Tumors, BC: Breast Cancer, CLL: B-Cell Chronic Lymphotic Leukemia, CLL: Chronic Lymphocytic Leukemia, CML: Chronic Myelogenous Leukemia, ALL: Acute Lymphoid Leukemia, MM: Multiple Myeloma, MetM: Metastatic Myeloma, NSCLC: Non Small Cell Lung Cancer, PC: Prostate Cancer, GIST: Gastrointestinal Stroma Tumor, RRMM: Relapsed or Refractory Multiple Myeloma, ST: Solid Tumors, UST: Unresectable Solid Tumors.

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