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Purine-Scaffold Hsp90 Inhibitors

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Abstract

Hsp90 is a molecular chaperone with important roles in regulating the function of several proteins with potential pathogenic activity. Because many of these proteins are involved in cancer and neurodegenerative promoting pathways, Hsp90 has emerged as an attractive therapeutic target in these diseases. Molecules that bind to the N-terminal nucleotide pocket of Hsp90 inhibit its activity, and consequently, disrupt client protein function. A number of these inhibitors from several chemical classes are now known, and some are already in clinical trials. This review focuses on the purine class of Hsp90 inhibitors, their discovery through rational design, and on efforts aimed towards their optimization and development into clinically viable drugs for the treatment of cancer. Their potential towards neurodegenerative diseases will also be touched upon.

Keywords

Heat shock protein 90; purine; cancer; neurodegeneration

INTRODUCTION

Heat shock protein 90 (Hsp90) is a molecular chaperone protein that is widely expressed and activated in cancers [1-3]. Its ability to stabilize client oncogenic proteins suggests a crucial role for Hsp90 in maintaining the survival of cancer cells [2]. Along these lines, Hsp90 can maintain a large pool of active and folded oncoproteins, for which its activated form has particular affinity; and as such, can serve as a protective “biochemical buffer” for cancer causing oncoproteins [2]. In this respect, degradation of a specific Hsp90 client in the appropriate genetic context (for example ER in a breast cancer cell with mutant ER or overexpressed HER2 in a HER2++ breast tumor) results in apoptosis and/or differentiation, whereas its degradation in normal cells, leads to little or no effect. This ability to interact and chaperone a large number of client oncogenic kinases and transcription factors, such as HER2, HER3, EGFR, ER, HIF1a, Raf-1, AKT and mutant p53, has led to the clinical development of Hsp90 inhibitors in a broad range of tumors [1-3].

Hsp90 is a family of chaperones which consists of Hsp90u and Hsp90B in the cytoplasm, GRP94 in the endoplasmic reticulum and TRAP1 in the mitochondria. Within the cell, Hsp90 exists predominantly as a dimer with each subunit comprised of three domains [4, 5];

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an N-terminal domain containing the ATP-binding pocket, a middle domain which functions in binding client proteins and a C-terminal domain as the site for dimerization and binding to various co-chaperones. The ATP-binding pocket is of high structural similarity among the four Hsp90s, and contains an unusual Bergerat fold. When bound to this pocket, the nucleotides adopt a distinctive C-bent shape found only in ATPases belonging to the GHKL family (G = DNA gyrase subunit B; H = Hsp90; K = histidine kinases; L = MutL). Because this pocket is so distinct from that of other ATPases [6] and because of the possibility of selective inhibition, it has become an attractive target for drug discovery efforts [7, 8].

Binding of ATP and ADP determines the conformation that Hsp90 adopts and affects its ability to interact with other chaperones and client proteins [9, 10]. The Hsp90 chaperoning cycle is a dynamic process in which client proteins are presented to it in an intermediate complex containing the co-chaperones Hsp70, Hsp40, HIP and HOP [2, 11]. Upon ATP binding and hydrolysis, Hsp90 forms a mature complex, containing p23, p50/cdc37 and immunophilins, which catalyzes the conformational maturation of Hsp90 client proteins. Molecules that can competitively bind to the nucleotide-binding pocket of Hsp90 interfere with the formation of functional protein complexes. As a result, the client protein is unable to attain its mature conformation and is subsequently ubiquitinated and targeted to the proteasome for degradation.

In cancer cells many of the client proteins of Hsp90 are oncogenic, and unlike drugs which target a single molecular target, inhibition of Hsp90 represents a combinatorial approach, whereby multiple targets are attacked simultaneously [1, 12]. Aside from the additive effect such an approach may have, it also can be expected to decrease potential for resistance. Additionally, cancer cells are more sensitive to the effects of inhibitors than normal cells. Despite the fact that Hsp90 is expressed in all cells, inhibitors selectively target and kill cancer cells, and a number of factors have been shown to contribute to this profile [13]. Hsp90 in tumor cells exists in a highly complexed state (superchaperone complex with Hsp70, Hsp40, Hop and p23) which has greater ATPase activity than that from normal cells, where it is present in a latent uncomplexed state, and has 100-fold higher affinity for certain Hsp90 inhibitors than that from normal cells or purified protein [14]. Certain Hsp90 inhibitors are also selectively retained in tumors, whereas they are rapidly cleared from normal tissue. For geldanamycin (GM, **1**) and 17-AAG (**2**), physiochemical properties have been proposed to contribute to this [15], but as a class, it reflects the fact that cancer cells are more dependent on stress response pathways for survival than are normal cells and thus, have a higher expression of the activated, complexed Hsp90.

As mentioned above, the N-terminal nucleotide-binding pocket of Hsp90 has such a distinct shape that it has been possible to selectively inhibit it selectively over all other ATPases and kinases which also bind ATP/ADP. GM (**1**) is an ansamycin antibiotic isolated from *Streptomyces hygroscopicus* [16] and was the first small molecule found to inhibit Hsp90. Even before Hsp90 was confirmed as its target, it was known to possess potent tumoricidal activity *in vitro* and *in vivo* [17]. However, it suffers from a number of drawbacks which have prevented its clinical development, including limited aqueous solubility and dose limiting hepatotoxicity. The latter is thought to stem from GM's benzoquinone moiety which has significant Michael acceptor activity. Analogs with reduced electrophilicity have

been developed, including 17-AAG (**2**) and 17-DMAG (**3**), and these have demonstrated proof of concept for Hsp90 inhibition. 17-AAG (**2**) was the first Hsp90 inhibitor to enter clinical studies, and has shown promising results in HER2-overexpressing tumors [18]. A number of drawbacks, including difficulty to formulate, cost of manufacture, and the difficulty to administer pharmacologically relevant doses without toxicity, has limited its development in other cancers. 17-DMAG (**3**) has similar *in vivo* and *in vitro* activity to 17-AAG (**2**) but is water soluble. This agent, as well as a reduced form of 17-AAG (**2**), IPI-504 (**4**) [19], have also entered clinical trials.

Because of the limitations of GM-based inhibitors, novel inhibitors of Hsp90 with more drug-like properties were actively sought. Structure-based design, high throughput screening, fragment-based design and virtual screening have all been utilized to identify small molecules that bind to the N-terminal ATP pocket of Hsp90. These efforts have identified a number of distinct chemotypes including purine (i.e. **16** and **26**), isoxazole (i.e. **5**) and 6,7-dihydro-indazol-4-one (i.e. **6**) as potent and selective Hsp90 inhibitors which have already or will soon enter into clinical trials [20-23]. The remainder of this review will focus on the purine class of inhibitors, with special emphasis on their discovery and development into clinical agents for the treatment of cancer, but will also touch upon their potential usefulness in neuro degenerative diseases.

PURINE-SCAFFOLD HSP90 INHIBITORS

1. Discovery of PU3

The first identified synthetic Hsp90 inhibitor was based on the purine (PU)-scaffold [24]. The unique structural features of the N-terminal nucleotide pocket as well as the shape adopted by ATP when Hsp90-bound, were used to rationally design a molecule to fit into this pocket. The initial lead molecule, PU3 (**7**, Fig. 2), bound to purified Hsp90 with an $EC_{50} = 15\text{--}20\text{ }\mu\text{M}$ ($1\text{ }\mu\text{M}$ for 17-AAG) and exhibited phenotypic effects in breast cancer cells similar to those observed for GM (**1**). In MCF-7 and SKBr3 breast cancer cells, **7** caused the degradation of HER2, HER3, Raf-1 and estrogen receptor (ER) onco-proteins at a concentration as low as $10\text{ }\mu\text{M}$ to $50\text{ }\mu\text{M}$. In a typical feed-back heat shock response due to Hsp90 inhibition, it induced the synthesis of Hsp90 and Hsp70 in these cells. **7** also exhibited anti-proliferative effects against genetically distinct breast cancer cells (i.e. MCF-7, ER+; SKBr3, HER2+; MDA-MB-468, ER- and HER2-) at low micromolar concentrations ($\sim 50\text{ }\mu\text{M}$) and caused G1 cell cycle arrest. G1-block was followed by morphological and functional differentiation.

The co-crystal structure of **7** bound to human N-terminal Hsp90u (see 1UY6.pdb) suggested that the purine ring binds in the same position as that of ADP, with the C6-NH₂ making a key interaction with Asp93 [25]. There is also a network of hydrogen bonds between N1, N7 and C6-NH₂ of **7** with Asn51, Ser52, Thr284 and Gly97 through three water molecules. The phenyl ring of **7** is stacked between the side chains of Phe138 and Leu107, and makes additional hydrophobic interactions with Met98 and Leu103. The methoxy groups make hydrophobic contacts with the aromatic rings of Trp162 and Tyr139 as well as with the aliphatic carbons of Ala111 and Val150. The first and second methylene groups of the N9-butyl chain provide additional hydrophobic contacts with Leu107 and Met98.

The discovery of PU3 (**7**) as an Hsp90 inhibitor, served to initiate medicinal chemistry efforts around the PU-scaffold in an effort to improve both potency and physical/ chemical properties. Major efforts have focused on probing the structure-activity relationship (SAR) of the aromatic moiety to the purine at C8-position, the nature of the linker between the PU-scaffold and the substituted aromatic ring, and the alkyl chain at N9 position. Substitutions to the 2-position of the adenine ring have also been investigated. The sum of these efforts to date have led to the development of a series of water soluble, low nanomolar potency compounds, which lack many of the limitations of 17-AAG (**2**) and 17-DMAG (**3**). In fact, one compound based on the PU-scaffold, BIIB021 (**26**), is currently in Phase I/II clinical trials, and another PU-H71 (**16**) is scheduled to enter clinical studies in the near future.

2. Structure-Activity Relationships

2.1. 8-Benzylpurines-Methylene Linker Series—In the first published report of a library of purine Hsp90 inhibitors, over 70 derivatives were synthesized and evaluated for their ability to bind purified Hsp90, inhibit the proliferation of MCF-7 cells and degrade HER2 [26]. This detailed report was the first investigation into the SAR of this class of compounds and served as an important reference for all future medicinal chemistry efforts. The effect of the 9-alkyl chain, substitution on C2 of the purine, addition of halogens on the trimethoxyphenyl moiety, and the nature and length of the bridge connecting the aromatic ring to the purine were investigated (Fig. 2).

Studies on variations to the N9-chain revealed a preference for chains with the first two to three carbons of unbranched nature and a requirement that C1 be primary; no derivative which had a substituent on C1 had activity at concentrations as high as 250 μ M. From over forty-five diverse chains examined, pent-4-ynyl and 2-isopropoxy-ethyl were favored and resulted in improved binding to Hsp90 by almost an order of magnitude. Addition of cyano, vinyl, iodo, methoxy, ethoxy or amino at C2 of the purine, each resulted in decreased or no activity, whereas the introduction of fluorine increased both potency and water solubility. It is believed that these effects are the result of increased H-donor potential of the C6-NH₂ which makes critical interactions with Hsp90. The introduction of chlorine or bromine at C2' of the trimethoxy phenyl ring increased the potency, with chlorine being preferred, but an additional bromine at C6' abolished activity, likely due to steric effects. Substitution of the carbon linking the purine and trimethoxy-phenyl moieties to a nitrogen or oxygen or -OCH₂- resulted in inactive compounds, suggesting that the angle between these groups is critical. Combining the best evaluated substituents from this initial SAR resulted in PU24FCI (**8**, Fig. 2), which was 30 times more active (IC₅₀ = 2 μ M for HER2 degradation in MCF-7 cells) than **7** and exhibited a potency of binding to purified Hsp90 (EC₅₀ = 0.45 μ M), similar to 17-AAG (**2**).

PU24FCI (**8**) was highly specific for tumor Hsp90 (average EC₅₀ = 0.22 μ M) versus that from normal cells (average EC₅₀ = 8.8 μ M) and exhibited potent and selective antiproliferative effects against a broad panel of cancer cell lines (IC₅₀ = 2-7 μ M versus 43.5-63.5 μ M for normal cells) [27]. At similar concentrations in cancer cells, it led to the degradation of AKT, Raf-1, Bcr-Abl, HER2, ER, mAR and cMet, and it induced apoptosis. When administered *in vivo* to mice, **8** accumulated selectively into tumor tissue over blood,

liver and brain, for 6–48 h after administration. In mice xenografted with MCF-7 breast cancer tumors, **8** resulted in a 72% reduction in tumor volume when administered at 200 mg/kg on alternate days for 30 days.

The effects of modifying the number and position of the methoxy groups of PU3 (**7**) were also evaluated (Fig. 3). In one study, derivatives were evaluated for their ability to inhibit yeast Hsp90 ATPase activity in a malachite green assay [25, 28]. **7** was inactive ($IC_{50} > 200 \mu M$) in this assay, whereas 17-AAG (**2**) had $IC_{50} = 13 \mu M$. While a single methoxy at C4' remained inactive ($IC_{50} > 200 \mu M$), at C3' some inhibition of ATPase activity was shown ($IC_{50} = 75 \mu M$). Two methoxy groups at C2' and C5' were most active ($IC_{50} = 41 \mu M$), and this preference was also shown in a study that used HER2 degradation as a measure of activity [29]. Replacement of the 2'-OCH₃ with I (**10**) or Br led to increased potency, whereas Cl decreased activity of these 2',5'-disubstituted derivatives [29]. Interestingly, linking C3' and C4' by a methylenedioxy bridge (**9**) proved to be a favorable change with $IC_{50} = 15 \mu M$ in the ATPase assay, which was as potent as 17-AAG (**2**) [25].

2.2. 8-Arylsulfanylpurines-Sulfur Linker Series—While attempts to change the linking atom between the PU-scaffold and the aromatic moiety from carbon to nitrogen or oxygen or -OCH₂- led to derivatives with diminished biological activity [26], replacing it with sulfur has proven to be a much more tolerable modification [29–31]. The resulting 8-arylsulfanyladenines retained the necessary conformation required for binding to Hsp90. The SAR for this series closely correlated with that from the methylene linker series, and in this regard, a similar preference for pent-4-ynyl > 2-isopropoxy-ethyl > butyl, was observed for the 9N group (Fig. 4) [30]. However, unlike the methylene linker series, addition of fluorine to C2 of the purine ring did not have a significant effect on Hsp90 binding or cellular activity in HER2 degradation or inhibition of SKBr3 cells [29, 30]. While retaining the 9N group as pent-4-ynyl, a series of derivatives were prepared aimed at investigating the effects of various substituents on the 8-aryl ring on binding to Hsp90, ability to degrade HER2 and antiproliferative effects on SKBr3 cells. Similar to the methylene linker series, monosubstitution of OCH₃ in *meta*-position was favored over *para* or *ortho* (Fig. 4). While a 2'-hydroxy-methyl was not allowed, a 2'-OCF₃ group retained activity. There is a degree of tolerance for groups in the *para*-position as -Cl, -OCH₃, -COCH₃ retain binding affinity, however, introduction of the larger 1-pyrrolyl group abolishes binding.

Again, similar to the methylene linker series, there was a clear preference for 2',5'-disubstitution over 2',4' or 3',5', with the most favorable substitution being 2'-iodo-5'-methoxy (**11**, Fig. 4), as had been previously reported for 9N = butyl. Substitution in this series at C2' followed the order I > Br > Cl ~ OCH₃ [29, 30]. Trisubstitution of the aryl ring with 3',4',5'-OCH₃ was also favorable and potency increased with addition of a 2'-Cl, but was diminished by a 2'-I. Connecting C4'-C5' by a methylenedioxy bridge, which was previously shown to increase the potency of PU3 (**7**), combined with substitution at C2' with halo groups, yielded some of the most potent compounds in this series with activity decreasing in the order I > Br > Cl [30, 31]. These compounds, as exemplified by **12** (Fig. 4), displayed nanomolar potency in both biochemical and cellular assays. Oxidation of the

linking sulfur to a sulfone or sulfoxide yielded derivatives which were either inactive or had diminished activity.

2.3. Water Soluble and Orally Available Derivatives-Incorporation of an Ionizable Amine on N9 Chain—Having previously optimized the benzene ring substituents with 2'-I-5'-OCH₃, Biamonte *et al.* incorporated an ionizable amino group on the N9 side chain of 8-arylsul-fanyladenines in an attempt to increase their water solubility and make these agents amenable for oral administration [29]. Amongst the most active compounds in this series was the *tert*-butyl amine **13** (IC₅₀ = 140 nM), neopentylamine **14** (IC₅₀ = 90 nM) and isobutylamine **15** (IC₅₀ = 100 nM) as determined in the HER2 degradation assay (Fig. 5). These were also active against the proliferation of MCF-7 breast cancer cells (IC₅₀ = 200 nM for **13**, 500 nM for **14** and 200 nM for **15**). As their phosphoric acid salts, these compounds displayed excellent water solubility (> 10 mg/ml) and when administered orally at 100 mg/kg to Balb/C mice were bioavailable (97% for **13**, 50% for **14**, and 14% for **15**) and attained pharmacologically relevant plasma concentrations. Following a single oral dose of **15** at 200 mg/kg to mice xenografted with A549 lung cancer, levels of HER2, pHER2, pAKT, pRaf-1 and pERK each decreased while levels of Hsp70 increased. When **14** was administered orally to mice xenografted with N87 stomach cancer at 200 mg/kg/day (2 × 100 mg/kg/day, 5 days/week) approximately 70% reduction in tumor volume was observed compared to untreated control.

He *et al.* reported potent water soluble inhibitors which incorporated the favorable 2'-halo-4',5'-methylenedioxy substitution on the 8-aryl ring with an ionizable 3-isopropylamino-propyl chain at N9 [31]. These compounds (**16-18**) were each potent inhibitors of Hsp90 from SKBr3 and MDA-MB-468 cells (IC₅₀ = 10-55 nM). PU-H71 (**16**) and PU-DZ8 (**17**), both containing 2'-I, were the most potent in inhibiting proliferation of SKBr3 and MDA-MB-468 cells (IC₅₀ = 50-90 nM) and in inducing HER2 degradation (IC₅₀ = 50-80 nM) (Fig. 5). **18**, the 2'-Br analog of **16**, exhibited a decreased potency in proliferation (IC₅₀ = 142-270 nM) and in inducing HER2 degradation (IC₅₀ = 205 nM). **16** and **17** also showed selectivity for Hsp90 from transformed cells (48-370-fold) and over 50-fold selectivity for inhibition of growth of SKBr3 cells over normal fibroblasts. **16** is a selective Hsp90 inhibitor; no significant inhibition was observed when screened against a panel of 359 kinases at 10 μM [unpublished results]. Interestingly, the closely related 2'-halo-4',5'-dimethoxy derivatives displayed a much diminished to no biological activity, suggesting that these bind within the hydrophobic pocket in a different manner. This is also supported by the observed order of potency of I > Br > Cl in the 2'-halo-4',5'-methylenedioxy series, which is reversed in the 2'-halo-3',4',5'-trimethoxy series. Indeed, the co-crystal structure of **16** bound to the N-terminal domain of human Hsp90a (see 2FWZ.pdb) revealed that the 2'-iodo substituent was oriented nearly 180° relative to the 2'-chloro of PU24FCI (**8**) [32]. One of the reasons given for such a drastic change in conformation is the necessity to avoid a steric clash with Tyr139. Whereas the methoxy groups of the 3',4',5'-trimethoxy and 4',5'-dimethoxy series can freely rotate away from the tyrosine ring, the fixed orientation of the 4',5'-methylenedioxy group prevents simple rotation as a means of alleviating steric strain. Instead, the whole 8-aryl ring system must rotate to avoid such a clash. As a result, the 2'-halogen in these series are oriented in completely opposite directions. The preference for

iodine in the 4',5'-methylene-dioxy series arises from the larger pocket that it is able to occupy as compared to that in either of the 3',4',5'-trime-thoxy or 4',5'-dimethoxy series.

When these compounds were formulated as phosphoric acid or hydrochloric acid salts, they displayed better than 5 mg/ml solubility in PBS (pH 7.4) and a dramatically improved pharmacokinetic (PK) profile. PU-H71 (**16**) and PU-DZ8 (**17**) exhibited other favorable (PK) properties, including good biological membrane permeability ($P_e = 0.893 \times 10^{-6}$ and 0.927×10^{-6} cm/s in PAMPA, respectively), acceptable protein binding (12% unbound for mouse, rat and human), relative stability in mouse plasma (at 6h, 91% and 85% recovery, respectively) and human plasma (at 6h, 78% and 87%, respectively). Several cytochrome P450 enzymes in human liver microsomes (CYP3A4, CYP1A2, CYP2C19 and CYP2C9) were not inhibited by 25 μ M of these compounds, whereas IC_{50} values recorded for CYP2D6 were 0.89 and 0.162 μ M for **16** and **17**, respectively. After incubation with human liver microsomes for one hour, 37% and 30% of **16** and **17**, respectively, were degraded to only one major metabolite, the inactive diphenol derivative from cleavage of the methylenedioxy bridge.

In vivo activity of PU-H71 (**16**) was reported in small-cell lung carcinoma [33], hepatocellular carcinoma [34], triple-negative breast cancer [20] and diffuse large B-cell lymphoma [35]. These studies ascertained **16** as an Hsp90 inhibitor for which PK correlate with tumor Hsp90 pharmacodynamics (PD), and demonstrated that **16** exerted the most prolonged PD effect reported for an Hsp90 inhibitor to date. Namely, in the MDA-MB-468 triple-negative breast cancer (TNBC) xenografted tumors, pharmacologically relevant doses of **16** rapidly reached tumors and were retained at 48 h post-administration, with 10.5 and 1.8 μ g/g detected at 6 and 48 h, respectively (estimated as 20.6 μ M and 3.6 μ M); drug concentrations in non-tumorous tissues and plasma declined rapidly, being almost undetectable by 6 h. Because treatment of cultured MDA-MB-468 cells with 2.5 μ M **16** for 48 h elicited death in 80-90% of cancer cells, the 3.6 μ M concentration of **16** detected in tumors at 48 h, was a dose of high toxicity to tumors. Accordingly, high intratumoral PARP cleavage, as well as sustained down regulation of Hsp90 clients were recorded at this timepoint [20]. It was reported that while 17-DMAG (**3**) appeared very potent in TNBC cells *in vitro* and abrogated onco-kinase Raf-1 expression in cultured MDA-MB-231 cells, intravenous administration of 75 mg/kg 17-DMAG to mice bearing MDA-MB-231 xenografted tumors had only minimal effect on Raf-1, with 20% reduction recorded at 24 h post-administration [36, 37]. In contrast, **16** administered at similar doses abrogated the intra-tumoral Raf-1 and Akt proteins in this model. These effects were sustained for 36 and 48 h post-administration of the drug [20]. When mice with xenografted TNBC tumors were treated with **16**, potent antitumor activity was noted. At 75 mg/kg on an alternate day schedule it induced a complete response in 100% of mice in the MDA-MB-231 model and 50% of mice in the MDA-MB-468 model, the most potent targeted single agent anti-tumor effect yet reported pre-clinically in this tumor type. These long-term treatment periods resulted in no deaths and no apparent toxicities.

Because conventional GM-based Hsp90 inhibitors induce dose-limiting liver toxicity, PU-H71 (**16**) was tested in models of hepatocellular carcinoma (HCC) to evaluate whether it can elicit antineoplastic activity without causing significant liver damage [34]. **16** reduced the

viability of various HCC cell lines, induced the simultaneous degradation of numerous hepatocarcinogenic factors, and caused substantial cell cycle arrest and apoptosis. In contrast, nontumorigenic hepatocytes were less susceptible to Hsp90 inhibition. In HCC xenograft mouse models, **16** was retained in tumors at pharmacologically relevant concentrations while being rapidly cleared from nontumorous liver, showed potent and prolonged *in vivo* Hsp90 inhibitory activity and reduced tumor growth without causing toxicity.

In diffuse large B-cell lymphomas (DLBCL) the BCL6 transcriptional repressor is the main oncoprotein. The continued presence of BCL6 is required for survival of DLBCL. Because Hsp90 is overexpressed in DLBCL, and BCL6 and Hsp90 interact at the nuclear level, **16** was evaluated in both *in vitro* and *in vivo* models [35]. It selectively killed DLBCL cells that are dependent on the BCL6 transcriptional repressor and resulted in the degradation of BCL6. **16** was also active in 19 out of 21 primary DLBCL cells obtained from patient biopsies. When administered at 75 mg/kg/day to mice xenografted with Farage, Ly7 or SUDHL4 tumors it resulted in a 76, 95 and 95% inhibition of tumor growth, respectively, and induced the depletion of BCL6 protein and mRNA in the tumor xenografts. No toxicity was observed during treatment in these mice as well as in 50 additional normal mice as evidenced by a lack of microscopic organ damage and biochemical panels (including liver enzymes) and complete blood counts.

Acquired resistance to cisplatin is common in ovarian cancer, and presents a significant challenge to the prevention and treatment of relapse. When examined in HeyA8 and SKOV3 ovarian cancer cells alone or in combination with cisplatin, **16** blocked cell migration in wound-healing assays, as well as invasion through Matrigel. Inhibition of cell motility was associated with a flattened morphology, appearance of stress fibers and re-organization of actin cytoskeleton. Inhibition of cell migration was evident after a short (4 h) exposure to the drug, whereas longer exposure was required for inhibition of invasion [38]. A synergistic inhibition of cell growth was observed at low concentrations of both drugs. Because **16** inhibits tumor invasion and sensitizes ovarian cancer cells to the effects of cisplatin, it may be useful as a complement to platinum-based therapy in ovarian cancer.

As a consequence of its favorable PD and PK profile and potent anti-tumor activity, PU-H71 (**16**) is currently in late-stage IND evaluation for the treatment of cancer.

2.4. 8-Benzothiazolothio- and 8-pyridinothiazolothio- purines—A novel series of active purine derivatives were obtained by replacing the phenyl ring of 8-phenylsulfanyladenine with either benzothiazole or pyridinothiazole (Fig. 6) [39]. Activity of these compounds was dependent on substitution at the appropriate position of the heterocyclic ring. The unsubstituted benzothiazole derivative (**19b**) had $IC_{50} = 5000$ nM for HER2 degradation in MCF-7 cells. Substitution of chlorine in either the 4'-, 5'- or 6'-positions each resulted in less potent compounds (**19c-e**), whereas substitution at the 7'-position yielded a compound (**19a**) with an $IC_{50} = 180$ nM. In both the HER2 degradation assay and antiproliferation of MCF-7 cells, activity followed the order 7'-Cl $\gg$ H $>$ 6'-Cl $>$ 4'-Cl $>$ 5'-Cl. Substitution of the 7'-position with Br-, F-, CH₃-, or CH₃O- did not affect

HER2 degradation that much (IC_{50} = 190-330 nM), however, replacement with CH_3CH_2O - reduced activity 500-fold.

While maintaining the 7-chlorobenzothiazole ring, different N9-sidechains were evaluated for optimal potency. Analogs with short alkyl chains, (i.e. ethyl, *n*-propyl, *n*-butyl) had similar activities, though increasing to *n*-pentyl decreased activity. Substitution of the alkyl moiety with alcohol, ester and some amines did not significantly improve activity, however, the neopentylamine **20** and the ethylphosphonate **21**, were substantially more potent in both the HER2 degradation assay (35 and 30 nM, respectively) and proliferation assay (each 30 nM), suggesting that a two carbon linker is optimal in this series. The cyclopropylamine **22** had similar activity to the 9-butyl analog **19a** and was active *in vivo*; administered orally to mice xenografted with N87 tumors at 200 mg/kg, 5 days/week, resulted in 56% tumor growth inhibition after 40 days.

In the pyridinobenzothiazole series, compounds with 2-(diethylphosphonate) at 9N were most potent in both the HER2 degradation and proliferation assays, similar to the benzothiazole series, with **23** and **24** most potent. **24** was more soluble in simulated gastric and intestinal fluids as well as serum than the corresponding benzothiazole **21**, and despite this, was not detected in serum following oral administration of 100 mg/kg, suggesting poor permeability properties.

2.5. 2-Amino-6-halopurine Series—Each of the PU-scaffold Hsp90 inhibitors described thus far contain an aryl group connected to the purine ring at the 8-position via a methylene or sulfur spacer. Interestingly, a new class of purine Hsp90 inhibitors was obtained by the concomitant shift of the aryl substituent to the 9-position along with the NH_2 from the 6- to the 2-position [40]. They similarly bind to the N-terminal ATP pocket, although it is yet unclear whether in a fashion similar to the 8-substituted derivatives. Shifting solely the aryl substituent to the 9- position resulted in inactive compounds, suggesting that a six bond distance between the NH_2 and the aryl group is important for maintaining activity. In the 2-amino-6-chloro- 9-benzylpurine series, derivatives of good potency in a HER2 degradation assay were obtained that contained 2'- halo-3',4',5'- trimethoxybenzyl substitution (**25a-c**, Fig. 7). Substitution of the 2'-position with a halogen is important for obtaining derivatives of sub-micromolar potency in the HER2 degradation assay and followed the order Cl (0.05 μ M) ~ Br (0.07 μ M) > I (0.34 μ M) > H (2.0 μ M).

The necessity for 6-chloro substitution on the purine ring was demonstrated by the tremendous loss in potency observed for the unsubstituted analog (IC_{50} = 50 μ M) of **25b**. Replacement of the chlorine with a bromine was well tolerated, but with NH_2 , OCH_3 , OH, SH, or CH_3 resulted in decreased potency. The effect of an electron-withdrawing chlorine or bromine here was similar to the increase in potency observed for 2-fluoro substitution in the 6-amino-8- benzyl series described above, and the rational was similarly attributed to the enhanced hydrogen bonding potential of the 2- NH_2 group, which makes critical interactions with Hsp90.

Owing to their poor aqueous solubility, the 9-benzyl series had poor oral absorption properties. In an effort to improve this, the phenyl ring was replaced with either a 2'- or 3'-

pyridyl group. The 3',5'-dimethyl-4'-methoxy-2'-pyridyl derivative BIIB021 (**26**) and the similar 3'-pyridyl compound **27** (Fig. 7) had good solubility in biological fluids and demonstrated good oral exposure in mice. Furthermore, they retained potency in HER2 degradation ($IC_{50} = 0.03 \mu M$ for **26** and $0.02 \mu M$ for **27**) and inhibited the proliferation of both MCF7 and BT474 cells ($IC_{50} = 0.05$ - $0.15 \mu M$). In a competition binding assay using MCF7 cell lysates, **26** and **27** had $IC_{50} = 0.02$ and $0.018 \mu M$, respectively. Replacement of the 4'-OCH₃ of **26** with -OH, -OCH₂CH₃, -OCH(CH₃)₂, -CN, -NO₂, -NH₂ or -SOCH₃ decreased activity, whereas replacement with -I or -Br improved potency 2-3-fold. Both **26** and **27** were active *in vivo* when administered orally as their mesylate salt to N87 xenografted mice. **26** (125 mg/kg) and **27** (60 mg/kg) administered once daily, 5 days/week for 5 weeks demonstrated 87 and 83% tumor growth inhibition, respectively.

BIIB021 (**26**) was further evaluated against a variety of cancer cells and had an IC_{50} ranging from $0.06 \mu M$ to $0.14 \mu M$ [21]. In MCF-7 cells it induced the degradation of a variety of client proteins including HER2, ER, progesterone receptor, AKT and Raf-1. **26** also showed significant *in vivo* antitumor activity against a variety of different tumor models at doses ranging from 75-125 mg/kg administered orally once daily, five days per week for 4-5 weeks. A decrease in HER2, pAKT and cyclin D levels were observed in tumor after 6 h, and increased to control levels at 48, 24 and 24 h, respectively. Significantly, it can still be detected in tumor tissue at 48 h after administration, despite its short half-life in serum ($t_{1/2} = 0.5$ -1 h).

17-AAG (**2**) and other ansamycin derivatives are inactive in tumors that have upregulated efflux pumps or anti-apoptotic proteins or other genetic alterations. In contrast, BIIB021 (**26**) retained activity in these models. Accordingly, **26** was more active than 17-AAG against adrenocortical carcinoma, a tumor that naturally expresses P-gp, both *in vitro* and *in vivo*. These data potentially indicate that the new generation of synthetic anti-Hsp90 drugs, exemplified by **26** may have broader application against tumors with acquired multidrug resistance (MDR) or tumors located in organs protected by MDR proteins, such as the adrenal glands, brain and testis [41].

A recent report indicated that BIIB021 (**26**) enhanced the *in vitro* radiosensitivity of head and neck squamous cell carcinoma (HNSCCA) cell lines with a corresponding reduction in the expression of key radioresponsive proteins, increased apoptotic cells and enhanced G2 arrest. In xenograft studies, **26** exhibited a strong anti-tumor effect outperforming 17-AAG, either as a single agent or in combination with radiation [42]. Hsp90 inhibition by **26** also sensitized Hodgkin's lymphoma cells for natural killer cell-mediated killing via up-regulation of ligands engaging activating NK cell receptors [43].

BIIB021 (**26**) is currently in Phase I/II clinical trials and represents the first PU-scaffold Hsp90 inhibitor to be so.

2.6. PU3 Dimers—With the aim of taking advantage of the homodimeric nature of Hsp90, dimers related to PU3 (**7**) connected via a linker of varying length at N9 position were prepared [44]. This site was chosen because the co-crystal structure had indicated that the terminal alkyl moiety of **7** was exposed to solvent and did not participate in binding to

Hsp90. The PU3 dimers (**28a-e**, Fig. 8) were evaluated for cytotoxicity against MCF-7 and SKBr3 human breast cancer cells and revealed that activity increased with increasing length of the linker, with the C-20 analog **28e** most potent. Whereas the C-4 analog **28a** was inactive, **28e** had $IC_{50} = 1.46$ and $1.31 \mu M$ against MCF-7 and SKBr3 cells, respectively, and represents a 20-30 fold increase in cytotoxicity compared to **7** ($IC_{50} = 45.9$ and $31.8 \mu M$, respectively). **28e** also induced the degradation of HER2 and modestly increased Hsp70 levels.

3. PU-Scaffold Hsp90 Inhibitors in Neurodegeneration

Recent evidence has suggested that Hsp90 inhibitors may have potential in treating tauopathies, neurodegenerative diseases characterized by aberrant phosphorylation and/or expression of tau protein, including Alzheimer's disease (AD) and frontotemporal dementia (FTD) [45, 46]. Hsp90 has been implicated in allowing for the accumulation of aberrant tau species which can result in neuronal death. Luo *et al.* have shown that the stability of p35, a protein required for the activation of cdk5 and subsequent pathogenic phosphorylation of tau, is maintained by Hsp90 through complex formation [45]. Inhibition of Hsp90 by PU24FC1 (**8**) or 17-AAG (**2**) in cellular models of tauopathy resulted in the degradation of p35 and decreased levels of mutant tau while leaving levels of wild type tau unaffected. A single dose of PU-DZ8 (**17**, 75 mg/kg) administered to tau transgenic mice resulted in the significant reduction of mutant tau levels as well as of p35. **17** is able to permeate the blood-brain barrier and attained a concentration $0.35 \mu g/g$ (~ 700 nM) at 4 h and a pharmacologically relevant dose was maintained for at least 12 h ($0.2 \mu g/g$, ~ 390 nM). Dickey *et al.* have shown that EC102 (**14**), a blood-brain barrier permeable PU-scaffold Hsp90 inhibitor, also resulted in the selective degradation of aberrant phosphorylated tau in transgenic mouse models of AD [46]. Both studies reported a parallel induction of Hsp70 in the brain of mice treated with **17** or **14**. In various cellular models of AD, increased levels of Hsp70 promoted tau solubility and tau binding to microtubules [47]. These findings suggest that in neurodegenerative diseases in general, and AD in particular, Hsp90 inhibition may offer a dual therapeutic approach. First, it may ameliorate protein misfolding by reduction of aberrant neuronal protein activity that leads to protein hyperphosphorylation and subsequent aggregation. Second, its therapeutic benefit may come from induction of Hsp70, a chaperone able of redirecting neuronal aggregate formation, and of protective potential against both Abeta and tau aggregate toxicity.

Hsp90 may also serve as a useful target to treat Parkinson's disease characterized by mutations to leucine-rich repeat kinase 2 (LRRK2) [48]. Mutations to LRRK2 results in a toxic gain of function and LRRK2 has been shown to form a complex with Hsp90 both *in vitro* [49, 50] and *in vivo* [48]. PU-H71 (**16**) affected the stability of LRRK2/Hsp90 complexes and reduced levels of LRRK2 in cultured neurons with greater potency against mutant ($IC_{50mut} = 26.9$ nM) LRRK2 than wild type ($IC_{50wt} = 387.0$ nM). **16** also rescued the axonal growth deficit of neurons derived from LRRK2 G2019S mutant mice.

Compared to normal cells, in which the Hsp90 complex remains latent and inactive, the Hsp90 complex in tumor cells exhibits a high binding affinity for Hsp90 inhibitors and a high ATPase activity [14]. This feature of tumor-associated Hsp90 reveals an indispensable

role of Hsp90 in protecting aberrant protein activities from cell homeostatic systems, thus facilitating malignant transformation. In a recent publication, Dickey *et al.* has demonstrated the presence of an activated Hsp90 complex in the AD brain as well [46]. In affected areas of AD brain, where pathologic protein accumulation is found postmortem, the Hsp90 complex had a significantly higher binding affinity (approximately 1,000-fold) for small molecule Hsp90 inhibitors than the Hsp90 derived from unaffected brain tissue of the same patients or from brain tissue of control cases. Taken together, these findings provide a strong rationale for the development of novel Hsp90-based therapeutic strategies in certain neurodegenerative diseases, such as tauopathies and Parkinson's disease.

CONCLUSION

Compounds based on the PU-scaffold were the first fully synthetic Hsp90 inhibitors to be discovered, and as such, to Purine-Scaffold Hsp90 Inhibitors Current Topics in Medicinal Chemistry, 2009, Vol. 9, No. 15 1445 address the liabilities of the first generation natural products, such as GM (1). PU3 (7) was the initial lead molecule, and since its discovery, more active and drug-like molecules were actively sought. Intensive medicinal chemistry efforts have led to molecules that selectively inhibit Hsp90 with nanomolar potency and display good PD and PK profile. These compounds effectively degrade Hsp90 client proteins important for initiating and driving the transformed phenotype *in vivo*, including HER2, Raf-1 and AKT. Furthermore, PU-H71 (16) and BIIB021 (26) are retained in tumor tissue for 48 h while being rapidly cleared from normal tissues. BIIB021 is currently in Phase I/II clinical trials and PU-H71 is scheduled to enter Phase I in the near future for the treatment of advanced cancer.

PU-scaffold Hsp90 inhibitors are also being evaluated for their usefulness in other diseases including neurodegeneration. Diseases such as AD, FTD and Parkinson's disease, which similar to cancer, can be caused by aberrant signaling processes, may be amenable to therapeutic intervention by Hsp90 inhibitors. Several brain-permeable PU-scaffold Hsp90 inhibitors, including PU-DZ8 (17) and EC102 (14) have already shown effectiveness in preclinical models of AD. These exciting new discoveries have prompted the development of these agents in diseases where only limited treatment options are currently available. Future medicinal chemistry efforts in the field will be aimed at tailoring these agents to more effectively penetrate the blood-brain-barrier and remain in the brain for an extended time at therapeutically useful concentrations.

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Fig. (1).
Structures of some non-purine Hsp90 inhibitors.

Fig. (2).
Structure of PU3 (**7**) and initial SAR of methylene linker series leading to PU24FCI (**8**).

Fig. (3).
Favorable substitutions to the phenyl ring of PU3 (7).

11 **12** PU-H58

Fig. (4).
SAR of sulfur linker series and structures of **11** and **12**.

Fig. (5).
Structures of PU-derivatives containing ionizable amino moieties at N9.

Fig. (6).
 Structures of 8-benzothiazolothio- and 8-pyridinothiazolothio-purines.

Fig. (7).
Structures of 2-amino-6-halopurines.

Fig. (8).
Structures of PU3 (7) dimers.