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Evaluation of miscellaneous heat shock protein (Hsp90) inhibitors using different methodologies

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ABSTRACT

In this review, 100 diverse chemical derivatives were either designed, synthesized or naturally screened and tested as Hsp90 inhibitors, miscellaneous derivatives were synthesized that represent different scaffolds. Anti-Hsp90 activities of these compounds were examined and diverse activities were observed. Although Hsp90 inhibition had activities against cancerous cell line, there is no Hsp90 inhibitor as anticancer FDA approved drug yet, however some clinically investigated Hsp90 inhibitors are in phase I and phase II. Hsp90 inhibitors could act as a new strategy for treatment of cancer as a chronic disease rather than using typically aggressive chemotherapeutic agents.

Keywords: heat shock protein 90, cancer, ligand based design, structure based design, highthroughput screening

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INTRODUCTION

Heat shock protein 90 (Hsp90) belongs to a family of molecular chaperones which play a key role in the conformational maturation, stability and function of "client" protein substrates within the cell [1]. Amongst the client proteins of Hsp90 are many oncogenes which drive survival, proliferation, invasion, metastasis and angiogenesis of tumors [2] However Hsp90 was not considered as a drug target until the discovery and characterization of natural product inhibitors of Hsp90 function such as (**1**) geldanamycin [3-5] (GM) and (**2**) radicicol [6,7] (RD) (Fig. 1, table 1).

As a potential drug target, interest in Hsp90 has grown over the last few years and this is reflected in the numerous articles published recently [8-27]. Most importantly validation of Hsp90 as a target for drug discovery comes from both preclinical and emerging clinical studies with the (**3**) GM analogue 17-allylaminogeldanamycin (17-AAG, Fig.1, table 1) [28]. As the first Hsp90 inhibitor to enter into clinical development, the encouraging results in phase I trials [29-32] have led to additional clinical trials being initiated with 17-AAG (**3**) used as a single agent and in drug combinations in various cancer types. The development of the water soluble (**4**) ansamycin 17-dimethylaminoethylgeldanamycin (17-DMAG Fig. 1, table 1) [33] and (**5**) the pro-drug of 17-AAG, IPI-504 [34,35] (Fig. 1, table 1) which are reported to be in phase I/Ib and phase I/II clinical trials respectively. The inherent chemical complexity of these ansamycin inhibitors have led to significant efforts to identify novel small molecule inhibitors of Hsp90 using different techniques such as fragment based screening [36,37], X-ray based screening [38,39], ligand based design[40], structure based design[41-47], dbCICA analysis[48] and highthroughput screening[49].

2. Natural Hsp90 inhibitors

A biosynthetic medicinal chemistry approach was applied to the optimization of the natural product Hsp90 inhibitor macbecin (**6**). By genetic engineering, mutants have been created to produce novel macbecin analogues including a nonquinone compound (**7**) that has significantly improved binding affinity to Hsp90 (Kd 3 nM vs 240 nM for macbecin) and reduced toxicity (MTD g 250 mg/kg). Structural flexibility may contribute to the preorganization of (**7**) to exist in solution in the Hsp90-bound conformation [50].

Gedunin (**8**), a tetranortriterpenoid isolated from the Indian neem tree (Azadirachta indica), was recently shown to manifest anticancer activity via inhibition of the 90 kDa heat shock protein (Hsp90) folding machinery and to induce the degradation of Hsp90-dependent client proteins similar to other Hsp90 inhibitors [51].

The mechanism of action by which (**8**) gedunin induces client protein degradation remains undetermined, however, prior studies have demonstrated that it does not bind competitively versus ATP. In an effort to further probe the mechanism of action, 19 semi synthetic derivatives of gedunin (**9**-**28**) were prepared and their antiproliferative activity against MCF-7 and SkBr3 breast cancer cells determined. Although no compound was found to exhibit antiproliferative activity more effective than the natural product, essential functional group critical for antiproliferative activity have been identified [51].

3. Highthroughput Hsp90 inhibitors

A high-throughput screen has been developed to identify small-molecule inhibitors that could be developed as therapeutic agents with improved pharmacological properties. A colorimetric assay for inorganic phosphate, based on the formation of a phosphomolybdate complex and subsequent reaction with malachite green, was used to measure the ATPase activity of yeast Hsp90. The assay was robust , reproducible and used to screen a compound collection of »56,000 compounds in 384-well format with Z factors between 0.6 and 0.8 [49]. In a high-throughput screening exercise, quinoline (**29**) was identified as a moderate inhibitor of Hsp90. Further hit identification, SAR studies, and biological investigation revealed several synthetic analogs in this series with micromolar activities in both fluorescent polarization (FP) assay and a cell-based Western blot (WB) assay. These compounds represent a new class of Hsp90 inhibitors with simple chemical structures [46]. Moreover high-throughput screening of a library of diverse molecules has identified derrubone (**30**), an isoflavone natural product from Derris robusta, as a potent Hsp90 inhibitor. Subsequent testing in several cellular-based assays established (**30**) as a low micromolar inhibitor *in vitro*. In addition, derrubone (**30**) induced the degradation of numerous Hsp90 client proteins, a hallmark effect resulting from Hsp90 inhibition. The identification of (**30**) as an Hsp90 inhibitor provides a new natural product scaffold upon which the development of novel Hsp90 inhibitors can be pursued [52].

Furthermore a series of 5-aryl-4-(5-substituted-2,4-dihydroxyphenyl)-1,2,3-thiadiazoles (**31**-**37**) were synthesized and their binding to several constructs of human Hsp90 chaperone measured by isothermal titration calorimetry (ITC). The most potent compound bound Hsp90 with the dissociation constant of about 5 nM [53].

4. Structure based derived Hsp90 inhibitors

Structure-based drug design was used to systematically synthesize scattered examples of the pyrrolo[3,4-d] pyrimidine scaffold, a straightforward approach for the synthesis of 4-amino-6-benzyl-6H-pyrrolo[3,4-d] pyrimidines has been developed and has been applied to the preparation of a small family of potential Hsp90 inhibitors. Among those prepared, a number of derivatives (**38**-**48**) proved to be able to bind to Hsp90 protein with IC_{50} values in the low micromolar range [54].

Crystallographic studies show that Hsp90 inhibitors bind in the ATP binding pocket interacting with the Asp93. Structure based optimization led to the identification of a novel class of 3-phenyl-2-styryl-3H-quinazolin-4-one Hsp90 inhibitors (**49**-**51**) with *in vitro* antitumor activity, they are identified by structure-based virtual screening of a chemical database with docking simulations in the N-terminal ATP-binding site, *in vitro* ATPase assay using yeast Hsp90, and cell-based Her2 degradation assay in a consecutive fashion. These results exemplify the usefulness of the structure-based virtual screening with molecular docking in drug discovery [55].

The purine-scaffold class, has been reported to be potent and selective against Hsp90 both *in vitro* and *in vivo* models of cancer. Here, a series of 8-arylsulfanyl, -sulfoxyl, and –sulfonyl adenine members of the purine class (**52**-**79**) was synthesized and evaluated as inhibitors of the chaperone. The results suggest that 8-arylsulfanyl adenine derivatives are good inhibitors of chaperone activity, whereas oxidation of the sulfides to sulfoxides or sulfones

leads to compounds of decreased activity. The study identifies derivative (**78**) as one of the most potent Hsp90 inhibitor of the purine-scaffold series (EC_{50}= 30 nM), and also as the compound of this class with highest selectivity for tumor vs normal cell Hsp90 (700 to 3000-fold) [56].

From Al-Sha'er *et al*, the anticancer activity of thiamine (vitamin B1) combined with its structural properties and docking studies suggested potential anti-Hsp90 activity for this vitamin. In experimental testing, thiamine illustrated anti-Hsp90 IC_{50} value of 12.5 µM. Therefore, in an attempt to capitalize on the simple structure of thiamine (**80**) and towards the development of new anti-Hsp90 inhibitors, 56 pyridinium-based structures tailored to thiamine were prepared and screened. The most potent (**81**, **82**) among the prepared compounds illustrated anti-Hsp90 IC_{50} values of 7.4 and 7.6 µM [44].

Another example from Al-Sha'er *et al*, Eight selected sulfonamide drugs were investigated as inhibitors of Hsp90. The investigation included simulated docking experiments to fit the selected compounds within the binding pocket of Hsp90. The selected molecules were found to readily fit within the ATP-binding pocket of Hsp90 in low-energy poses. The sulfonamides torsemide (**83**), sulfathiazole (**84**), and sulfadiazine (**85**) were found to inhibit the ATPase activity of Hsp90 with IC_{50} values of 1.0, 2.6, and 1.5 µM, respectively. The results suggest that these well-established sulfonamides can be good leads for subsequent optimization into potent Hsp90 inhibitors [47].

5. Ligand based derived Hsp90 inhibitors

From Al-Sha'er *et al*, exploration of the pharmacophoric space of 83 Hsp90 inhibitors using six diverse sets of inhibitors to identify high-quality pharmacophores. Subsequently, genetic algorithm and multiple linear regression analysis were employed to select an optimal combination of pharmacophoric models and 2D physicochemical descriptors capable of accessing a self-consistent quantitative structure-activity relationship (QSAR) of optimal predictive potential ($r_{67}^{\ 2}$ = 0.811, F= 42.8, $r_{LOO}^{\ 2}$ = 0.748, $r_{PRESS}^{\ 2}$ (against 16 external test inhibitors) = 0.619). Three orthogonal pharmacophores emerged in the QSAR equation (1) suggesting the existence of at least three binding modes accessible to ligands within the Hsp90 binding pocket. Receiver operating characteristic (ROC) curves analysis established the validity of QSAR-selected pharmacophores. The pharmacophoric models and associated QSAR equation were employed to screen the national cancer institute (NCI) list of compounds and our in-house-built drugs and agrochemicals database (DAC). Twenty-five nanomolar and low micromolar Hsp90 inhibitors were identified. The most potent were formoterol (**86**), amodaquine (**87**), primaquine (**88**), and midodrine (**89**) with IC_{50} values of 3, 5, 6, and 20 nM, respectively [40].

QSAR equation : $\log(I/IC_{50}) = -1.47 + 2.24\ X\square 10^{-2}(Hypo1/7)^2 + 1.215\ X\square 10^{-2}(Hypo8/8)^2 + 2.7\ X\square^{10-2}(Hypo9/1)^2 - 9.0\ X10^{-4}(SsOH)^2 - 0.5446(AtypeN73)^2 + 8.3\ X^{10-2}(Jurs\text{-}RPCS)^2$

$r_{67}^{\ 2}$ = 0.811, F-statistic = 42.8, $r_{LOO}^{\ 2}$ = 0.748, $r_{PRESS(16)}^{\ 2}$ = 0.619 …….. (1)

6. dbCICA based Hsp90 inhibitors

From Al-Sha'er *et al*, docking 83 diverse Hsp90 inhibitors into the ATP-binding site of this chaperone using several docking–scoring settings was applied to develop new computational technique. docking-based comparative intramolecular contacts analysis (dbCICA) assess the different docking conditions and select the best settings.

dbCICA is based on the number and quality of contacts between docked ligands and amino acid residues within the binding pocket. It assesses a particular docking configuration based on its ability to align a set of ligands within a corresponding binding pocket in such a way that potent ligands come into contact with binding site spots distinct from those approached by low-affinity ligands, and vice versa. The optimal dbCICA models were translated into valid pharmacophore models that were used as 3D search queries to mine the National Cancer Institute's structural database for new inhibitors of Hsp90 that could potentially be used as anticancer agents. The process culminated in several micromolar Hsp90 ATPase inhibitors (**90**-**100**) [48].

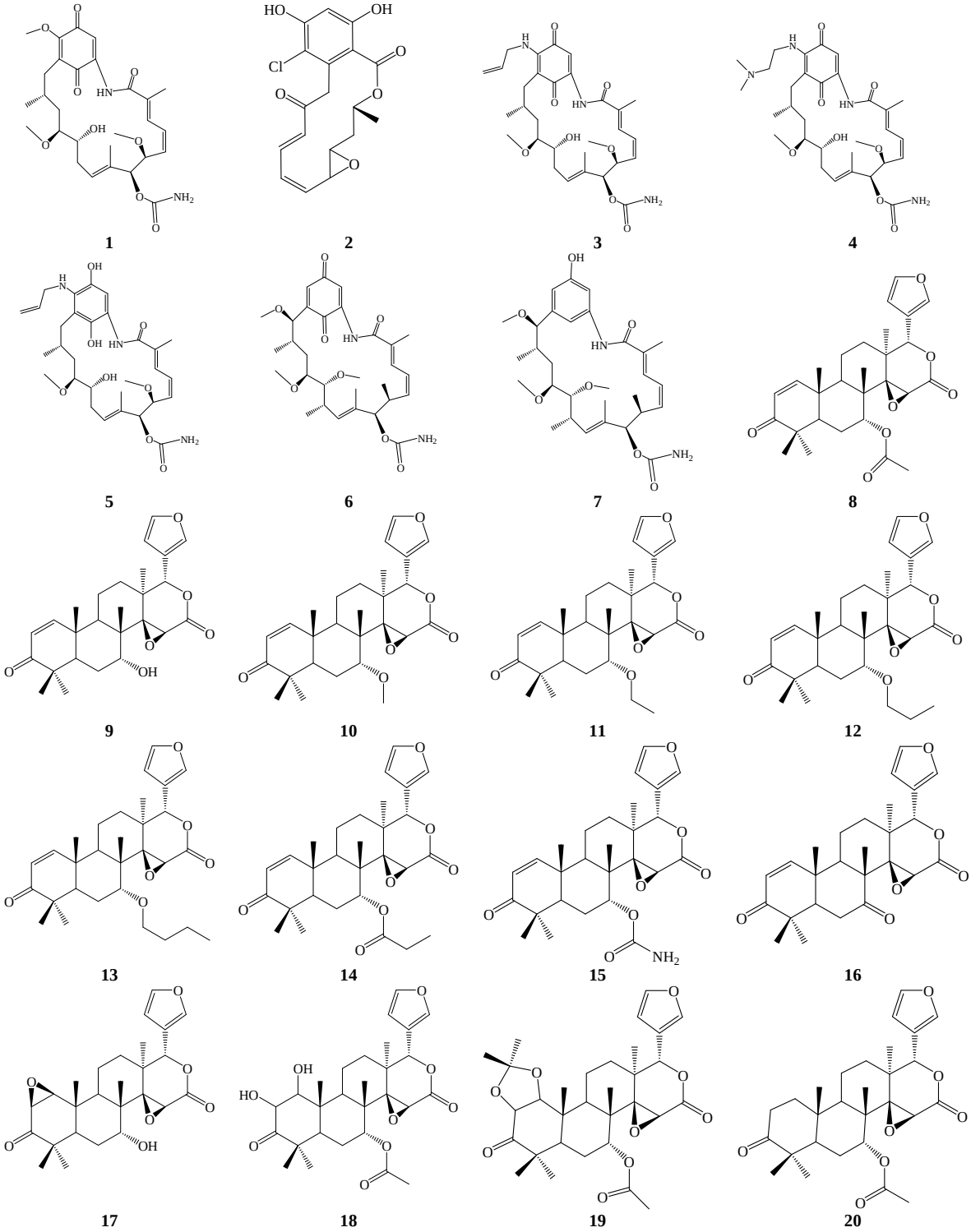


Figure 1: Chemical structure of selected Hsp90 inhibitors

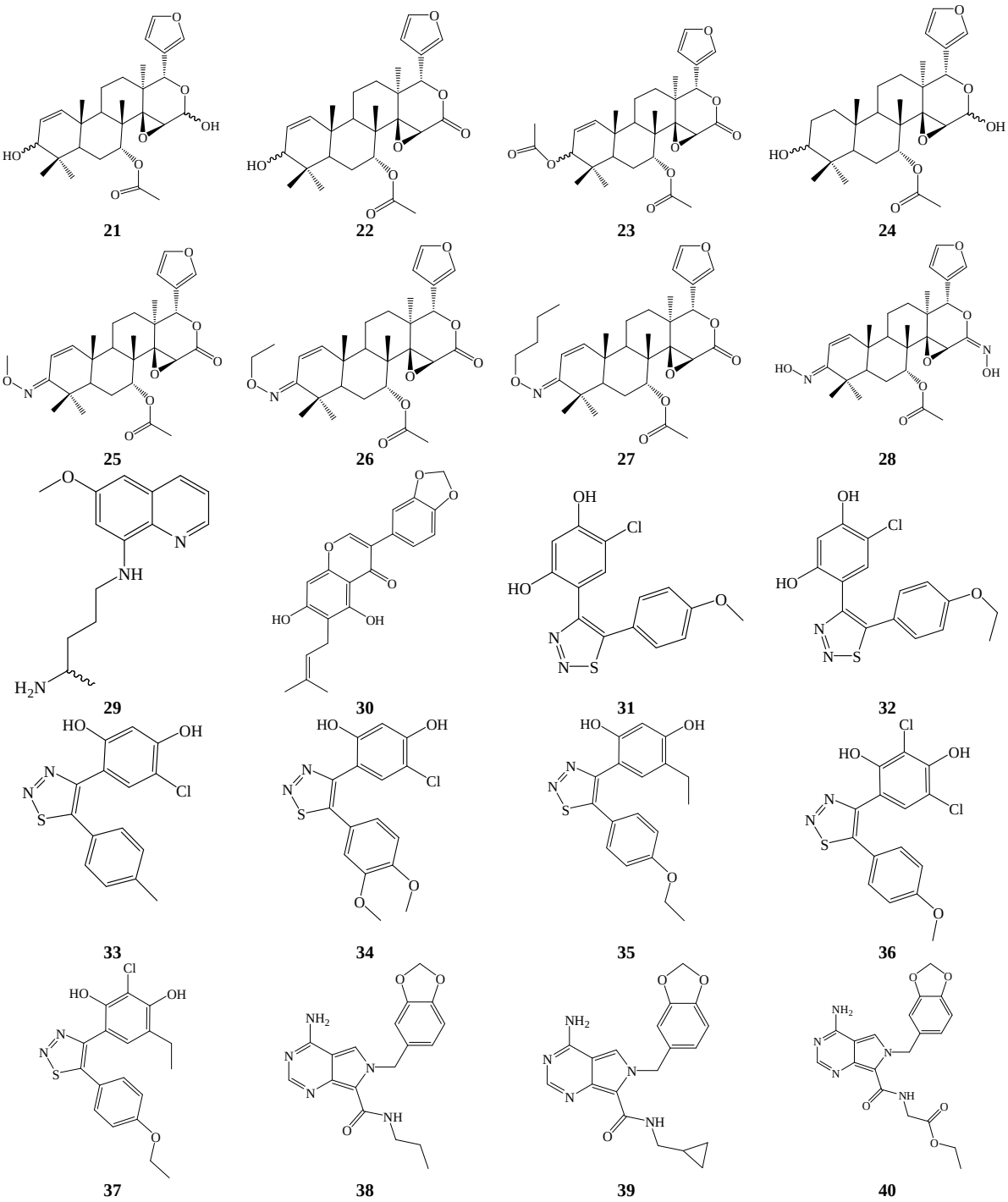
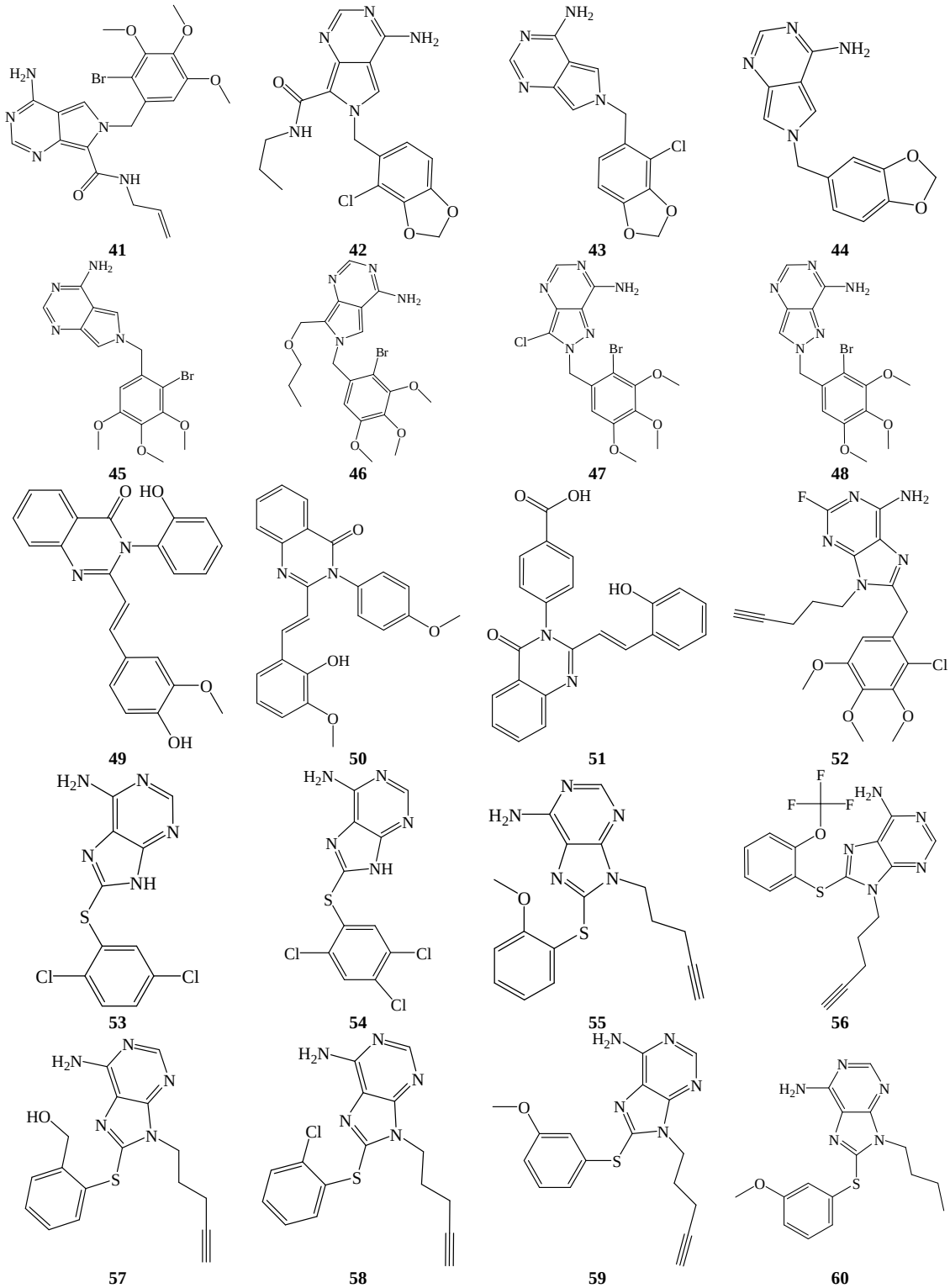


Figure 1: Chemical structure of selected Hsp90 inhibitors



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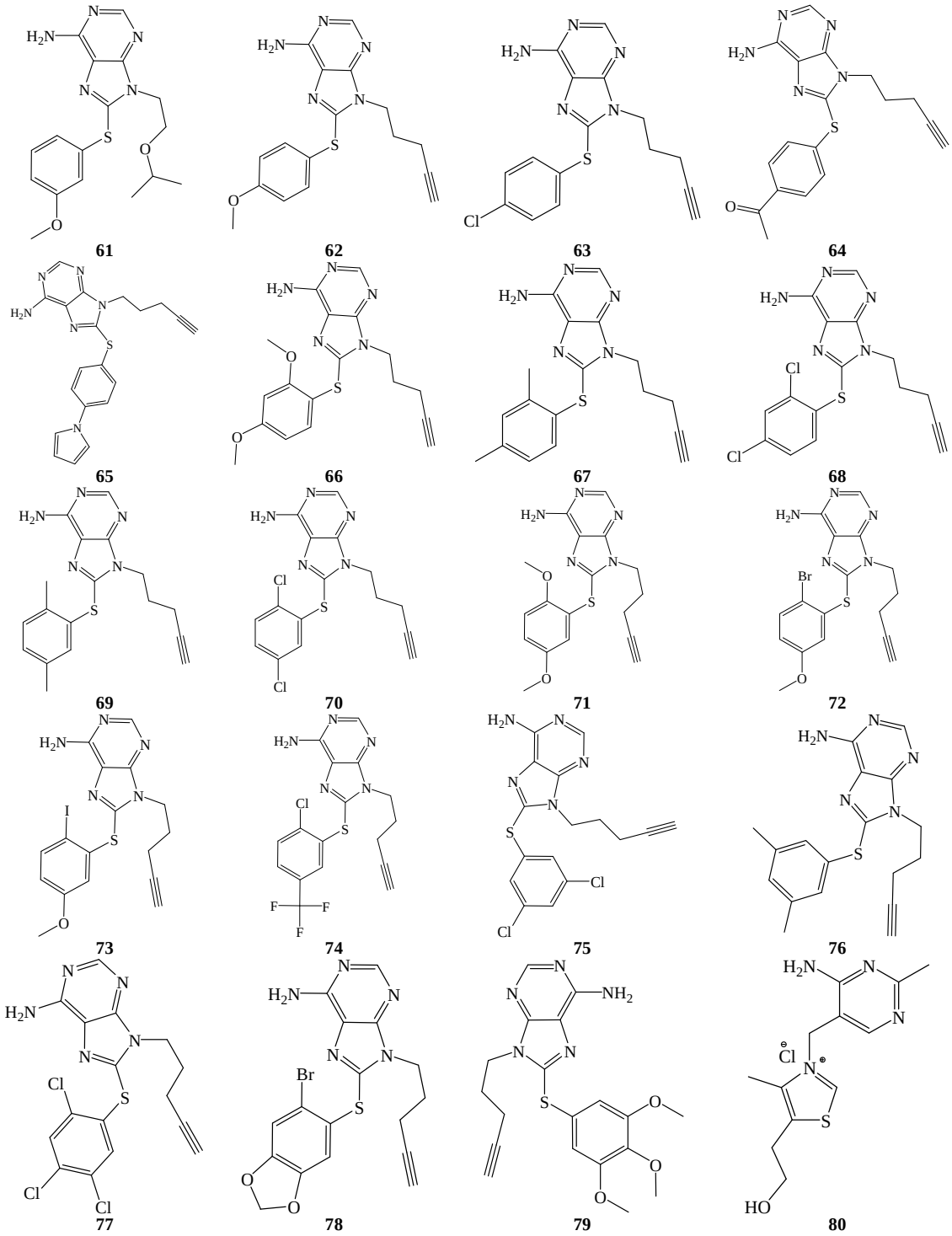

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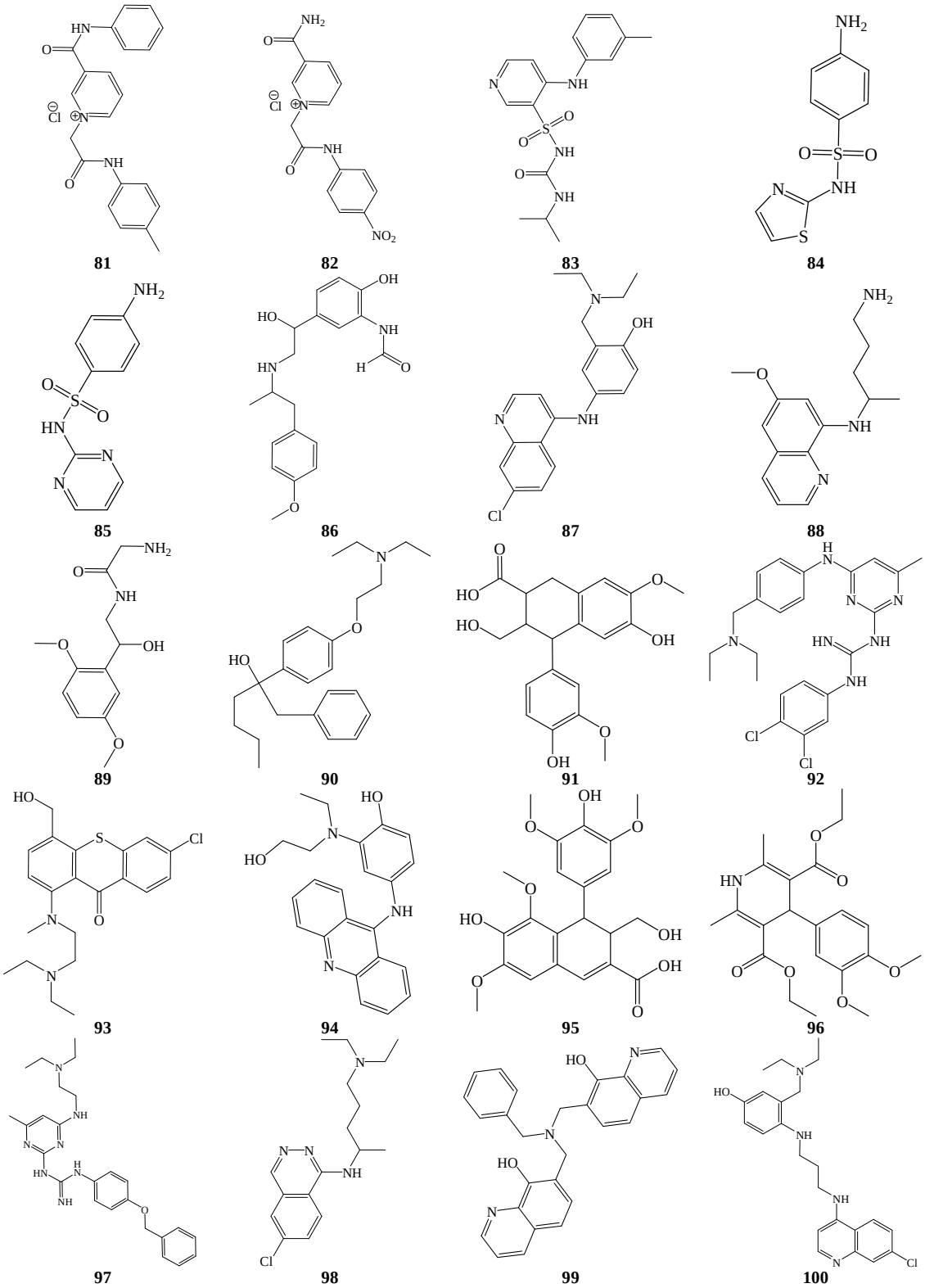

Figure 1: Chemical structure of selected Hsp90 inhibitors

Table 1: Anti-Hsp90 activities of selected inhibitors using different bioassay techniques

Compound	Activity µM	Compound	Activity µM	Compound	Activity µM
1	0.24	**21**	82.38	**41**	204.8
2	0.019	**22**	47.70	**42**	8
3	0.006	**23**	>100	**43**	60
4	0.024	**24**	>100	**44**	647
5	0.0038	**25**	>100	**45**	29.2
6	0.240	**26**	>100	**46**	147.9
7	0.003	**27**	>100	**47**	6
8	3.22	**28**	26.49	**48**	2
9	21.09	**29**	5.8	**49**	31.8
10	12.99	**30**	0.23	**50**	20
11	12.06	**31**	0.013	**51**	22
12	18.14	**32**	0.0063	**52**	0.23
13	>100	**33**	0.017	**53**	85.1
14	>100	**34**	0.034	**54**	90.7
15	7.05	**35**	0.042	**55**	15.4
16	4.49	**36**	>20	**56**	34.9
17	>100	**37**	>20	**57**	5.6
18	>100	**38**	21	**58**	85.1
19	>100	**39**	40.8	**59**	42.2
20	>100	**40**	235.6	**60**	0.97

Table 1: Anti-Hsp90 activities of selected inhibitors using different bioassay techniques

Compound	Activity µM	Compound	Activity µM
61	2.8	**81**	7.4
62	19.1	**82**	7.6
63	3.4	**83**	1.0
64	2.7	**84**	2.6
65	100	**85**	1.5
66	2.0	**86**	0.003
67	8.6	**87**	0.005
68	37.5	**88**	0.006
69	13.4	**89**	0.020
70	2.1	**90**	68% @10µM
71	0.19	**91**	70% @10µM
72	0.07	**92**	65% @10µM
73	0.05	**93**	55% @10µM
74	3.7	**94**	53% @10µM
75	30.4	**95**	48% @10µM
76	10.6	**96**	47% @10µM
77	8.5	**97**	43% @10µM
78	0.03	**98**	40% @10µM
79	1.5	**99**	31% @10µM
80	12.5	**100**	30% @10µM

CONCLUSION

Miscellaneous Hsp90 inhibitors are crucially interesting as shown by the diverse discovery methodologies. Diversity of chemical structures of Hsp90 inhibitors indicates the flexability of Hsp90 ATP-binding pocket that fit both small and large molecules. The emergence of huge number of Hsp90 inhibitors indicates the necessities of preclinical evaluation, pharmacokinetics analysis, toxicity studies in order to continue the clinical evaluation of most safe and effective agents.

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